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There are no actual or potential conflicts of interest to declare for any author.

**Abstract**

In this work we have evaluated the potential carcinogenic and acutely toxic risks associated to the exposure to highly prevalent organic and inorganic contaminants through the consumption of fishery products by the Spanish population. The concentrations of 8 organochlorine pesticides (OCPs), 18 polychlorinated biphenils (PCBs), 7 polycyclic aromatic hydrocarbons (expressed as benzo[a]pyrene toxic equivalents ($B[a]P_{eq}$)), and three inorganic toxic elements [arsenic (As), cadmium (Cd), and mercury (Hg)] were determined in 96 samples of the most consumed species of white fish, blue fish, cephalopods and seafood species, which were acquired directly in markets and supermarkets in the Canary Islands, Spain. The chemical concentration data were combined with the pattern of consumption of these foodstuffs in order to calculate the daily intake of these contaminants, and on this basis the risk quotients for carcinogenicity and acute toxicity were determined for Spanish adults and children. Our results showed that the daily intake of OCPs, PCBs and $B[a]P_{eq}$ , which is associated to blue fish consumption was the highest within the fish group. The estimated intake of pollutants can be considered low or very low for the individual contaminants, when compared to reference values, except in the case of HCB and As. All the estimated intakes were below the reported Tolerable Daily Intakes. Considering the additive effects of multiple contaminants, the risk of acute toxic effects can also be considered as low or very low. However, our results reflect that the current consumption of white fish in adults and children, and also the blue fish in the case of adults, poses a moderate carcinogenic risk to Spanish consumers, mainly related to their concentrations of As. The conclusions of this research may be useful for the design of appropriate risk communication campaigns.

## 1. - INTRODUCTION

Organic and inorganic contaminants, such as legacy pesticides, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), mercury (Hg), arsenic (As), or cadmium (Cd) are commonly targeted contaminants for research and in monitoring programs. In the last decades, efforts have been made to raise knowledge about the adverse effects on humans and animals, worldwide distribution pattern, and new methods are developed to analyze these compounds in very different matrices and various environmental media (Luzardo et al., 2013b; Sharma et al., 2014). Thus, numerous studies have revealed that these toxic compounds, individually and in combination, may contribute to the development of severe health problems such as cancer, immune suppression or genotoxic effects in humans, even with long-term low-dose exposure (Bergman et al., 2012; Jarvis et al., 2014; WHO, 2003), and many of them have demonstrated endocrine disrupting effects in both animals and humans (Camacho et al., 2014; Kortenkamp et al., 2011). In fact, the use of organochlorine pesticides (OCPs) and PCBs is now banned in most developed countries, but they are still widespread in the environment (Almeida-González et al., 2012; Kakuschke et al., 2010; Luzardo et al., 2014).

Although there are different routes of exposure for humans to these pollutants, it has been established that ingestion of food contributes more than 90% of total human exposure, and that the fatty fraction of food represents the main entrance to the human body (Darnerud et al., 2006; Vazquez et al., 2015). In the last decade, studies on human dietary exposure to persistent pollutants have been carried out in various countries over the world and it has been reported that the dietary intakes vary considerably between countries. The dietary intakes are mainly influenced by the specific dietary habits of each country

(Domingo and Bocio, 2007; Storelli et al., 2011). The daily intake of contaminants needs to be calculated on the basis of the typical food basket consumed in the country obtained from surveys on consumers. The dietary exposure to a wide range of persistent organic and inorganic pollutants of Spanish consumers has been investigated by several authors in the past years for different food groups, such as milk and cheese (Almeida-González et al., 2012; Luzardo et al., 2012), eggs (Luzardo et al., 2013a), yogurt (Rodríguez-Hernández et al., 2015c), meat and processed meat (Rodríguez-Hernández et al., 2015a; Rodríguez-Hernández et al., 2015b), and seafood (Bocio et al., 2007; Domingo and Bocio, 2007; Falcó et al., 2006). Also several basket market studies have been performed in Spain including the major food groups (Bocio and Domingo, 2005; Bocio et al., 2005; Falco et al., 2003; Llobet et al., 2003a; Llobet et al., 2003b; Llobet et al., 2003c), and even the consumption of foods of animal origin has been investigated as a determinant of contamination by OCPs and PCBs (Boada et al., 2014). However, to date only few studies have estimated the carcinogenic risk associated to the exposure to contaminants associated to certain food groups in the Spanish population (Rodríguez-Hernández et al., 2015a; Rodríguez-Hernández et al., 2015b), and to our knowledge none has been developed for the seafood group.

Fish is an important supplier of high quality nutrients such as omega 3 fatty acids, which have been proven reduce the risk of stroke, lower blood pressure and improve arterial integrity, and even decrease the risks of certain cancers (Kris-Etherton et al., 2002). However, fish is also one of the main contributors of the total dietary intake of environmental pollutants (Bocio et al., 2005; Falco et al., 2003; Llobet et al., 2003b; Llobet et al., 2003c). Thus, on the one hand, the health benefits of sea foodstuff consumption have been proven but on the other hand there also exist an increasing

concern of the potential risk arising from exposure to toxic pollutants through the intake of fishery products. Because of the growing public concern about the health effects of food borne diseases related to chemical pollutants, there exists the need carrying out studies on particular food groups (such as fish), based on their current pattern of consumption by a given population. In some guidance documents for environmental risk assessment, a reference point from toxicity testing is divided by a default assessment factor and the result compared to the predicted exposure by computing their ratio, which is known as the risk quotient (RQ) (EFSA, 2015; USEPA, 2000). It has been proposed that RQ is a good method to estimate the risk to carcinogenic and acutely toxic effects associated to food contaminants in a population and that is useful to establish exposure limits to those chemicals.

As fish is a staple food of the Spanish diet, with an average consumption of 26.4 kg/person/year (MAGRAMA, 2015) we have designed this study in which we assess the toxic potential of the current pattern of consumption of this food group by the Spanish population. We have acquired seafood samples directly at points of sale to the consumer, and the sampling was designed to follow the Spanish consumers' preferences. We have assessed two types of health risks associated with the consumption of seafood: the carcinogenic risk, and the acute toxicity potential. In this research we have calculated the RQs considering multiple contaminants in fishery products for both carcinogenic and acutely toxic effects, and on this basis we calculated the number of healthy meals per month for a safe consumption in the Spanish population. Obviously, the results of this study need to be considered in the context of the proven health benefits of the nutrients of fish, but may serve for the design of appropriate risk communication campaigns in order to reduce the consumption of certain types of seafood with the aim of optimizing

169 the risk-to-benefit balance.

170

171 **2. - MATERIAL AND METHODS**

172

173 **2.1. Sampling**

174

175 We selected for this study the most consumed species of seafood: fish (white fish and
176 blue fish), cephalopods, crustaceans and bivalve molluscs in Spain, according to the data
177 available (AECOSAN, 2006; AECOSAN, 2011). A total of 93 samples from the main
178 commercial species (MAGRAMA, 2015; Martín Cerdeño, 2010) were randomly acquired
179 from multinational retailers settled in the Canary Islands (Spain) between September and
180 November of 2014.. The samples purchased were transported to the Laboratory of
181 Toxicology of the University of Las Palmas de Gran Canaria (ULPGC) and processed
182 immediately upon arrival at the laboratory. We processed and analyzed only the edible
183 parts of seafood (muscle + skin, depending on how the species are consumed). Each
184 sample was constituted by five individual subsamples for each species of fish and
185 cephalopods (fillets, small fishes, or parts of octopus and squids), and six subsamples of
186 each species of crustaceans and mollusks to give pooled samples (using a stainless steel
187 domestic food processor). Thus, 5 to 6 of these composites were used to obtain the data
188 of each species. After that, all samples were frozen at – 80ºC (until analysis).

189

190 The species of white fish included in this study were: wreckfish (*Polyprion americanus*),
191 megrim *(Stephanoiepis hispidus*), sole (*Solea vulgaris*), seabass (*Dicentrarchus labrax*),
192 hake (Merluccius merluccius), toothed sparus (*Dentex dentex*), parrot fish (*Sparisoma*
193 *cretense*), gilt head fish (*Sparus aurata*) and iridiscent shark (*Pangasius hypophthalmus*).

194 The selected species of blue fish were: tuna (*Thunnus thynnus*), salmon (*Salmo salar*),
195 sardine (*Sardina pilchardus*), and trout (*Salmo trutta*). Additionally, we included those
196 most consumed species of crustaceans, cephalopods, and mollusks: shrimp (*Parapenaeus*
197 *spp.*), prawn (*Penaeus spp.*), mussel (*Mytilus galloprovincialis*), octopus (*Octopus*
198 *vulgaris*), and squid (*Theutida spp.*).
199
200 **2.2. Chemicals, reagents and analytes of interest**
201
202 All the organic solvents (dichlorometane, hexane, ethyl acetate, and cyclohexane) were
203 of mass spectrometry grade (VWR International, PA, USA). Ultrapure (UP) water was
204 produced in the laboratory using a Milli-Q Gradient A10 apparatus (Millipore, Molsheim,
205 France). The inert desiccant (Celite ® 545) was purchased from Sigma-Aldrich (St.
206 Louis, USA). Bio-Beads SX-3 were purchased from BioRad Laboratories (Hercules,
207 USA). Standards of OCPs, PCB congeners, and internal standards (ISs, PCB 202,
208 tetrachloro-m-xylene, p,p'-DDE-d8, heptachloro epoxide cis, and phenanthrene-d10),
209 were purchased from Dr Ehrenstorfer, Reference Materials (Augsburg, Germany).
210 Standards of PAHs were purchased from Absolute Standards, Inc. (Connecticut, USA).
211 All standards were neat compounds. Stock solutions of each compound at 1 mg/mL were
212 prepared in cyclohexane and stored at -20 °C. Diluted solutions from 0.05 ng/mL to 40
213 ng/mL were used for calibration curves (9 points).
214
215 All samples were screened for the presence of the following anthropogenic contaminants:
216 (a) 8 OCPs: the four isomers of hexachlorocyclohexane (α-, β and γ-, and δ- HCH), p,p′-
217 DDT and its metabolites (p,p′-DDE, and p,p′-DDD) and hexachlorobenzene (HCB); (b)
218 a total of 18 congeners of PCBs: the six marker PCBs (M-PCBs), and the 12 dioxin-like

219 PCBs (DL-PCBs), which were numbered according to the International Union of Pure
220 and Applied Chemistry (IUPAC): IUPAC numbers # 28, 52, 77, 81, 101, 105, 114, 118,
221 123, 126, 138, 153, 156, 157, 167, 169, 180, 189; (c) the 7 PAHs listed as carcinogens by
222 the United States Environmental Protection Agency: benzo[a]anthracene, chrysene,
223 benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene
224 and indeno[1,2,3,-cd]pyrene. Finally, we also included the analysis of 3 inorganic toxic
225 elements, which have been reported to be very abundant in fish: arsenic (As), cadmium
226 (Cd), and mercury (Hg).
227
228 **2.3. Extraction and clean-up**
229
230 Prior to the extraction procedure, samples were lyophilized for 72 hours. For the
231 extraction of organic pollutants from fishery products samples, we firstly extracted the
232 fat because all these chemicals are completely lipid-soluble and therefore found bound to
233 the lipid fraction. 5 g of each lyophilized sample were spiked with the ISs mix (10 µg/ml)
234 in acetone to yield a final concentration of 20 ng/g and mixed with 30 grams of Celite®
235 to absorb all humidity. The method of extraction and purification followed that
236 recommended by the European Standard for the determination of pesticides and PCBs in
237 fatty food (EN, 1996a; EN, 1996b), whose validity has been previously proven in our
238 laboratory for fatty samples (Camacho et al., 2014; Camacho et al., 2013a; García-
239 Álvarez et al., 2014a). This method combines an automated Soxhlet extraction method
240 (FOSS Soxtec Avanti 2055) with a purification step using gel permeation
241 chromatography (GPC), and gives acceptable recoveries that range between 74.5 % and
242 104.7 %. Briefly, the Soxtec™ 2055 Auto Fat Extraction (Foss® Analytical, Hilleroed,
243 Denmark) apparatus consisted of an extraction unit, a control unit and a drive unit. The

244 samples, prepared as described above, were inserted into the extraction unit, 40 ml of
245 solvent (dichloromethane) were added to the extraction cups in a closed system and the
246 cups were heated with an electric heating plate. The three-step extraction consisted of
247 boiling, rinsing and solvent recovery. The recovered solvent was evaporated in a rotary
248 evaporator (Hei-VAP Advantage™, Heidolph Instruments®, Schwabach, Germany) at
249 40 °C to prevent analytes loses. Using a precision balance, the fat obtained was carefully
250 weighted into a zeroed glass tube in order to be able of correcting the results and express
251 them against fresh weight of product. 100 mg of the Soxhlet extracted fat were dissolved
252 in 2 ml of cyclohexane/ethyl acetate (1:1) and subjected to purification by gel permeation
253 chromatography (BioBeads SX-3) using cyclohexane/ethyl acetate (1:1) at a constant
254 flow of 2 ml/min as the eluent. The first 25 minutes of elution, containing the great
255 majority of lipids (> 98%), were discarded. The 25-90 minutes elution volume (130 ml),
256 containing all of the analytes that were co-extracted with the fat, was collected. The
257 sample was concentrated using a rotary evaporator, and finally the solvent was evaporated
258 to dryness under a gentle nitrogen stream. The analytes were re-dissolved in 1 ml of
259 cyclohexane without any further purification and these extracts in cyclohexane were used
260 for the gas chromatography/triple quadrupole mass spectrometry (GC-MS/MS) analysis.
261
262 For the analyses of inorganic contaminants, 0.5 g aliquots of lyophilized samples were
263 mineralized with 6 mL of nitric acid ($HNO_3$) and 50 µl of Yttrium (Y) was added as an
264 internal standard. Vessels were then placed inside a microwave oven (Milestone ETHOS
265 ONE) and heated up to 190°C for 50 minutes. All of the reagents used were of high
266 quality, for analysis of trace elements (Suprapur, Merk, Darmstadt, Germany). After
267 cooling, digested samples were filtered with 1 µm strainer and diluted to a final volume
268 of 50 mL with distilled water into a conical polypropylene tube.

269

270 **2.4. Procedure of chemical analysis, quality assurance (QA) and quality control**
271 **(QC)**

272

273 Gas chromatography analyses of organic contaminants were performed in a single run on
274 a Thermo Trace GC Ultra equipped with a TriPlus Autosampler and coupled to a Triple
275 Quadrupole Mass Spectrometer Quantum XLS (Thermo Fisher Scientific Inc., Waltham,
276 MA, USA), as previously described (Bucchia et al., 2015; Formigaro et al., 2014), and
277 identifications were done using an electron ionization (EI)-MS/MS based on the retention
278 time and the relative ion ratios of each of the analytes. Quantifications were performed
279 against calibration curves as mentioned above. The LOQs of organic pollutants ranged
280 from 0.008 to 0.028 ng/g wet weight, as previously described (García-Álvarez et al.,
281 2014b) (Supplementary Table 1).

282

283 All the measurements were performed in triplicate, and we used the means for the
284 calculations. In each batch of samples, four controls were included for every 18 vials (6
285 samples): a reagent blank consisting of a vial containing only cyclohexane; a vial
286 containing 2 ng/ml of each of the pollutants in cyclohexane; and an internal laboratory
287 quality control sample (QCs) consisting of fish oil spiked at 20 ng/ml of each of the
288 analytes, which was processed using the same method as the seafood samples. The results
289 were considered to be acceptable when the concentration of the analytes determined in
290 the QC sample was within 15% of the deviation of the theoretical value.

291

292 Inorganic elements (As, Cd, and Hg) were quantified with inductively coupled plasma-
293 optic emission spectrometry technique (ICP-OES) using a Perkin Elmer Optima 2100 DV

294 instrument coupled with a CETAC U5000AT + ultrasound nebulizer for mercury. A
295 calibration curve and two blanks were run during each set of analyses to check purity of
296 the chemicals, and the blank reading was subtracted from all of the experimental readings.
297 The sample readings (two replicates for each sample and three readings for each replicate)
298 were performed using axial plasma, which provides increased sensitivity, lower
299 background, and improved the limits of detection (LODs) compared to traditional radial
300 plasma. This sensitivity enhancement results in a 5- to 10-fold improvement in the
301 detection limits compared with radially viewed plasma. The concentration values were
302 obtained from the mean of each three readings. The accuracy of the method was verified
303 using reference materials (CRM 278: lyophilized mussel, Community Bureau of
304 Reference, BCR, Brussels). All values of reference materials were within the certified
305 limits. LODs, expressed by wet weight (w.w.), were 0.1 ng/g for As; 1.8 ng/g for Cd; and
306 0.061 ng/g for Hg. The LODs were determined following the protocol described by
307 Perkin Elmer ICP application study number 57.
308
309 **2.5. Dietary intake estimates and calculations**
310
311 For the assessment of the contaminants' exposure through the consumption of fishery
312 products, we first grouped the results of contaminants in food as white fish, blue fish,
313 cephalopods, and seafood (mean values, expressed in ng/g fresh product), and then
314 multiplied these values by the average daily consumption rate of each one of these types
315 of food (expressed in grams/day). Following the recommendations of the EFSA we have
316 used also the percentile 97.5$^{th}$ of consumption to calculate the estimated daily intakes
317 (EDIs) using the upper-bound approach. These assessments (middle-bound (MB) and
318 upper bound (UB)) were done for both adults and children (average body weight: 68.48

319 and 34.48 kg, respectively). A zero value was assigned to all the compounds below the
320 LOD, and for those compounds below the limit of quantification (LOQ) but above the
321 LOD, the value was assumed to be ½ LOQ (Camacho et al., 2013b; Luzardo et al., 2013b).
322 Food consumption data of the Spanish population were obtained from the Spanish Diet
323 Model for the Determination of the Consumer's Exposure to Chemicals of the Spanish
324 Agency for Consumer Food Safety and Nutrition (AECOSAN, 2006; AECOSAN, 2011).
325
326 In this research, for the calculations we considered the total value of DDTs (ΣDDTs) as
327 the sum of the measured values of p,p′-DDT, p,p′-DDE and p,p′-DDD; the total value of
328 HCH residues (ΣHCH) as the sum of the 4 HCH isomers (α-,β-,γ- and δ-HCH); the HCB
329 as an independent contaminant; the value of the PCB congeners that are considered
330 markers of exposition (ΣM-PCB: #28, 52, 101, 138, 153 and 180); the value of the PCB
331 congeners that are similar to dioxins (ΣDL-PCBs: #77, 81, 105, 114, 118, 123, 126, 156,
332 157, 167, 169 and 189). For the risk estimation, we calculated the potential toxicity for
333 the DL-PCBs (in terms of toxic equivalence to dioxins; $\sum TEQ_{DL\text{-}PCBs}$) using the OMS
334 2005 TEQs (Van den Berg et al., 2006). Finally, we also considered the total content of
335 carcinogenic PAHs ( Σ c-PAHs) following the EFSA recommendations (EFSA, 2008).
336 Benzo[a]pyrene is the most widely known and studied compound of this group due to its
337 importance as one of the most potent carcinogenic hazards. Thus, the carcinogenic risk
338 of a PAH mixture is often expressed by its BaP equivalent concentration ($B[a]P_{eq}$). Thus,
339 for the risk estimation, we used toxic equivalency factors (TEFs), which are established
340 for the carcinogenic PAHs (Nisbet and LaGoy, 1992), to express the results in the form
341 of benzo[a]pyrene toxic equivalents ($B[a]P_{eq}$).
342
343 **2.6. Risk characterization**

We applied a risk quotient (RQ) to estimate whether the intake of contaminated sea foodstuff. We calculated this intake RQ as the ratio between the consumption of a given foodstuff (in this case seafood expressed in grams/day, $R_{fish}$) and the maximum tolerable consumption of that foodstuff ($CR_{lim}$), which is calculated taking into account their concentrations of contaminants. We have used this index both, for the calculation of the carcinogenic risk, and also for the risk of acutely toxic effects associated to the consumption of that food.

Thus, in the present study, the carcinogenic effects of multiple contaminants were evaluated using the methodology previously used for different food groups (Rodríguez-Hernández et al., 2015a; Rodríguez-Hernández et al., 2015b; Yu et al., 2014), according to the following formulas:

(Equation 1)

$$RQ = \frac{R_{fish}}{CR_{\lim single}} \text{ (for a single contaminant)}$$

(Equation 2)

$$RQ = R_{fish} \cdot \sum_{m=1}^{x} \frac{1}{CR_{\lim multiple}} \text{ (for multiple contaminant groups)}$$

(Equation 3)

$$CR_{\lim(single\ or\ multiple)} = \frac{ARL \cdot BW}{\sum_{m=1}^{X} C_m \cdot CSF_m}$$

Where $CR_{lim}$ is the maximum allowable consumption rate for a particular fishery product (kg/day), and may be calculated either for a single contaminant or for various chemicals belonging to the same chemical group, and assuming they share the toxicological properties; ARL is the maximum acceptable individual lifetime risk level, which is dimensionless and a value of $10^{-5}$ (one-in-100.000) was used in this study, base on the literature (Yu et al., 2014); BW is the body weight (kg); $C_m$ is the median concentration of contaminant *m* in a particular fishery product (mg/kg); and $CSF_m$ is the cancer slope factor of contaminant *m* for a carcinogenic hazard (mg/kg/day)-1. In the case of multiple contaminants with the same CSF, their concentrations in a particular type of seafood were summed (from m = 1 to m = X).

In addition, we evaluated the acutely toxic effects of multiple contaminants using the following equation:

(Equation 4)

$$CR_{lim} = \frac{BW}{\sum_{m=1}^{X} \frac{C_m}{RfD}}$$

where $RfD_m$ is the reference dose of contaminant *m* for acute toxic effects (mg/kg/day).

The RfD and CSF values of contaminants for carcinogenic and toxic effects were taken from the Integrated Risk Information System (IRIS) of the USEPA (http://www.epa.gov/IRIS/).

According to the previous reports it is considered that if the RQ value is equal or less than 1 then it can be considered that the risk is low (< $10^{-5}$) via fishery products consumption. However, the population is considered to be at health risk when RQ is greater than 1. (Yu et al., 2014).

**2.7. Meal suggestions for the consumption of seafood.**

Once we determined the concentrations of pollutants in seafood we considered very useful for the consumer and the health authorities to calculate the maximum intake of these foods that can be considered safe. The USEPA notes that daily fish consumption limits may be more conveniently expressed as the allowable number of fish meals (of a specified meal size) that may be consumed over a given time period (USEPA, 2000; Yu et al., 2014). For the consumer to express this as the number of allowable meals per month is more practical. Therefore, we calculated the number of allowable meals per month considering multiple contaminants for carcinogenic and acute toxic effects according to the following equations:

(Equation 5)

$$C_{mm} = \frac{R_{fish} \cdot TP}{MS}$$

(Equation 6)

417 $$RC_{mm} = \frac{C_{mm}}{RQ}$$

418

419 where $C_{mm}$ is the current number of meals per month for each type of fishery product;
420 MS is the meal size (225 g for fish, and 120 g for cephalopods and seafood); TP is the
421 averaged time period (month = 30.44 days); and $RC_{mm}$ is the recommended maximum
422 number of serving of each food per month.

423

424 **2.8. Statistical analysis**

425

426 Database management and statistical analysis were performed with PASW Statistics v
427 20.0 (SPSS Inc., Chicago, IL, USA). Because the data did not follow a normal
428 distribution, the statistical analyses involved the use of non-parametric tests. The
429 differences of contaminants between two independent groups were tested with the Mann–
430 Whitney U-test and Kruskal Wallis test. *P* values of less than 0.05 (two-tailed) were
431 considered statistically significant.

432

433 **3. RESULTS AND DISCUSSION**

434

435 ***3.1. Occurrence of chemical pollutants in fishery products***

436

437 Table 1 shows the concentrations of the toxic contaminants included in this study in the
438 different groups of fishery products: blue fish, white fish, cephalopods and other seafood
439 (crustaceans and molluscs). We also present in this table the statistical comparison
440 between the two classes of fish, and also the comparison between cephalopods and
441 seafood. In addition, we also considered interesting to present the comparison between

442 total seafood (including cephalopods) in a graphical manner as supplementary material
443 (Suppl. Figure 1).
444
445 We found great differences in the levels of contaminants among the different groups of
446 fishery products (Table 1), and also among the different species within each group (data
447 not shown). This is logical, because the distribution of the pollutants in the aquatic
448 organisms is highly dependent on the environment that they live, as well as on other many
449 factors, such as the trophic levels, the feeding habits of the species, differences in
450 metabolism due to different abilities of biotransformation, the excretion rate of these
451 compounds from the body, etc… (Liao et al., 2016). Moreover, it is well know that most
452 of the contaminants included in this study are lipid soluble and therefore it is reasonable
453 to find a direct relationship between their concentration and the lipid content of each
454 species. Thus, as seen in Table 1, we found that blue fish (which contains at least 5% of
455 lipids in the edible part) has higher levels of organic pollutants than white fish: ΣDDTs
456 (median: 1.5 vs. 0.21 ng/g); ΣHCHs (median: 0 ng/g in both groups; mean: 0.4 vs. 0.1
457 ng/g); HCB (median: 0.6 vs. 0.1 ng/g); M-PCBs (median: 2.6 vs. 0.3 ng/g); $\sum TEQ_{DL\text{-}PCBs}$
458 (0.006 vs. 0.0009 pg/g), and $B[a]P_{eq}$ (0.2 vs. 0.03 ng/g). These findings are consistent
459 with other studies that found that higher levels of contamination occur in blue fish
460 (Mezzetta et al., 2011). We also found that fish in general (blue and white fish) presented
461 higher levels of organic pollutants than cephalopods, molluscs, and crustaceans, which
462 may be also related with the lower percentage of fat of these foods. This is also consistent
463 with the data published previously (Bayarri et al., 2001; Carubelli et al., 2007). The only
464 group in which these differences were not observed was PAHs, (expressed as $B[a]P_{eq}$),
465 as we found that the levels in cephalopods were similar to those in blue fish. Other authors
466 have also previously reported high levels of PAHs in molluscs, even higher than in fish

467 (Martí-Cid et al., 2007), probably due to the fact that most edible sea molluscs are filter
468 feeders.
469
470 With regards to inorganic pollutants, we included in this study the determination of As,
471 Cd, and Hg due to the concerns on human health of these elements, and that it has been
472 reported these metals are the most abundant in sea foodstuff (EFSA, 2009a; EFSA,
473 2009b; EFSA, 2012). There are many studies, which have determined their contents in
474 the edible parts of commercial seafood species, since the monitoring of metal
475 concentrations in fish meat is very important to ensure compliance with food safety
476 regulations and consequent consumers' protection (Bosch et al., 2016). In the present
477 study the pattern of contamination observed for organic pollutants in which blue fish
478 species are the most contaminated is not maintained. Except in the case of Hg, we found
479 that cephalopods, crustaceans, and molluscs exhibited the highest levels of these elements
480 (Table 1), which probably relates with their different feeding habits. White fish also had
481 higher concentrations of As than those detected in blue fish species. We considered this
482 especially of concern since white fish is the most consumed fish by the Spanish
483 population, and several studies have shown that the intake of As, particularly the
484 inorganic forms of this metalloid, is related with an increase incidence of cancer (Carlin
485 et al., 2015; Di Lorenzo et al., 2015). Although we could not perform the speciation of
486 As and only the total content of As was measured, it is accepted that in the edible parts
487 of marine fishes, ~ 10% of As is generally present in inorganic forms (Rahman et al.,
488 2012). Assuming that this ratio is maintained in the samples of aquatic organisms
489 included in this study we considered only 10% of the values depicted in Table 1 in the
490 further risk assessment, which is detailed in the following sections.
491

492 ***3.2. Daily intake of toxic contaminants through the consumption of fishery products***

493

494 The estimation of the daily intake (EDI) of pollutants through the consumption of fishery
495 products was obtained by combining the results of contamination of the samples and the
496 pattern of consumption of these products as reported by the Spanish authorities (median
497 and percentile 97.5$^{th}$, in ng/day) (AECOSAN, 2006; AECOSAN, 2011). The results of
498 these estimations (MB and UB approaches) for both adults and children are presented in
499 Table 2.

500

501 *3.2.1 Organic contaminants*

502

503 According to our results the greater contribution to the EDI of organochlorine compounds
504 (in both adults and children) occurs through the consumption of blue fish (68.4% and
505 50.8%, respectively) followed by white fish (25.3 and 40.1%, respectively), seafood
506 (4.2% and 5.0%, respectively), and cephalopods (2.3 and 4.0%, respectively). This
507 pattern was observed for all the individual compounds, and this had been also reported
508 for these pollutants by several authors (Mezzetta et al., 2011; Moon et al., 2009).
509 According to our calculations the EDI of ΣM-PCBs was the highest, followed by ΣDDTs.
510 To adequately evaluate the exposure to contaminants by means of the consumption of a
511 given food group it is necessary to compare the values with the previously calculated
512 reference values, such as the Tolerable Daily Intake (TDI). Regarding this we have to
513 note that none of the OCPs exceeded their respective TDIs (JECFA, 2000), and even did
514 not surpass 1% of those values, nor in the MB nor in the UB approach (TDI ∑DDTs =
515 10000 ng/kg b.w., TDI ∑HCHs = 5000 ng/kg b.w., TDI HCB = 160 ng/kg/day) (ATSDR,
516 2002; Luzardo et al., 2013a). To be able of comparing the exposure to PCBs with some

517 reference values it is necessary to use the approximation of toxic equivalence to dioxins
518 as defined by the WHO (Van den Berg et al., 2006), as the TDI for PCBs has been set in
519 the context of dioxin exposure (2 pg WHO-TEQ/kg b.w/day (SCF, 2000)). Once the
520 results were transformed using the corresponding TEFs, our results indicate that the
521 exposure to dioxin-like PCBs through the consumption of fishery products only accounts
522 for 1.08% of that TDI in the worst scenario (adults, UB approach, Table 2).
523
524 Regarding to the other group of organic pollutants included in this research – the PAHs
525 – the EDI of $\Sigma B[a]P_{eq}$ was estimated to be 9.34 ng/day and 5.30 ng/day in Spanish adults
526 and children respectively, and fivefold when the UB approach is considered. Similarly to
527 organochlorine pollutants, blue fish species were the main contributors to the exposure to
528 these carcinogenic pollutants within this food group (57.4% in adults and 46.9% in
529 children, Table 2). Although the TDI for the carcinogenic PAHs has not yet officially
530 established, we used the TDI for $B[a]P_{eq}$ of 20 ng/kg b.w. day, as recommended for the
531 Contaminated Land Exposure Assessment of UK (CLEA-UK, 2008). The EDIs of
532 $B[a]P_{eq}$ calculated in this study account for less than 3% of this reference value in both
533 adults and children, in the worst-case scenario (Table 2).
534
535 *3.2.2. Inorganic contaminants*
536
537 When we consider the intake of inorganic contaminants, contrary to what is described
538 above, we found that white fish is the main contributor. Arsenic is considered one of the
539 most dangerous elements for health and all the studies conducted so far show that the
540 foods that are the richest in inorganic arsenic are seaweed, fish, other seafood and cereals
541 (EFSA, 2009b). According to our estimations the daily intake of total As through fishery

542 products could be as much as 1.96 μg/kg/day (adults, worst case scenario (UB approach),
543 Table 2), which would represent almost 94% of the established TDI (2.1 μg/kg/day,
544 (JECFA, 2010)), which is of very much concern. If we take into account that the most
545 dangerous As is that which is in inorganic form, and we assume that 10% of total As in
546 fishery products is inorganic As (Rahman et al., 2012), the average intake would represent
547 around 14%-60% of the estimated average inorganic As exposure from food and water
548 across 19 European countries (0.13 to 0.56 μg/kg b.w./day, (EFSA, 2009b)). Moreover,
549 the EFSA CONTAM Panel has identified a range of values for the 95% lower confidence
550 limit of the benchmark dose of 1% extra risk ($BMDL_{01}$) for each endpoint of a wide range
551 of key epidemiological studies (0.3 to 8 μg/kg/day, (EFSA, 2009b)), and recommended
552 that the overall range is used as reference instead of a single reference value. Thus, the
553 lowest values, which correspond with the risk of lung cancer, are well below the MB-EDI
554 of 0.78 μg/kg/day reported in this study, which would mean that theoretically the current
555 pattern of fish consumption in Spain would not be exempt of risk (even more if the UB
556 approach is taken into account, Table 2).
557
558 The Cd has also been extensively studied due to its toxic properties (EFSA, 2009a), being
559 considered primarily toxic to the kidney, where it accumulates over time and may cause
560 renal dysfunction. Besides, the International Agency for Research on Cancer has
561 classified Cd as a probable human carcinogen on the basis of occupational studies, and
562 recently epidemiological studies have revealed an increased risk of lung, endometrium,
563 bladder, and breast cancer in relation with the environmental exposure to this metal
564 (EFSA, 2009a; Menon et al., 2015; Vilahur et al., 2015; Weidemann et al., 2015).
565 However, basically all the carcinogenicity data available are related to inhalation
566 exposure, and there are no studies of orally ingested cadmium suitable for quantitation,

567 so we did not further considered this metal as a carcinogen in the present study.
568 Nevertheless, as many other toxic effects (other than cancer) have been described for Cd,
569 a Provisional Tolerable Weekly Intake (PTWI) of 7 μg/kg has been established.
570 According to our estimations the average intake in Spanish population through the
571 consumption of fishery products does not reach 2% of its PTWI (9% when the UB
572 approach is considered). The EFSA has determined from the analyses of more than
573 140000 food samples that seafood are the commodities where the highest Cd levels are
574 detected, and besides it has also been determined that only 3-5% of this metal is absorbed
575 after dietary exposure (EFSA, 2009a). Considering this and the estimations done in this
576 research, we can conclude that the dietary exposure to Cd in Spain is currently very low,
577 and very far away from being worrying.
578
579 Finally, regarding to the Hg, it has also been established the foods in the group "Fish and
580 other seafood" have the highest values of this highly toxic heavy metal in comparison to
581 all other food groups, although the different surveys available indicate that the total Hg
582 content varies widely among different fish species, and is highest in predatory fish
583 (JECFA, 2004; JECFA, 2006). The toxic properties of Hg are well known, especially for
584 kidney and the developing nervous system. Therefore the EFSA's CONTAM Panel has
585 established a PTWI of 4 μg/kg (EFSA, 2012) for this metal. According to our results, the
586 dietary exposure to total Hg from fishery products of an average Spanish consumer is
587 0.37 μg/kg/week in adults and 0.53 μg/kg/week in children (Table 2). These values are
588 more than tripled for both age groups when the UB approach is considered. As is
589 estimated that approximately 90% of the total mercury in fish and shellfish is present in
590 the form of methyl mercury (MeHg) (EFSA, 2005), our results would indicate that
591 Spanish adults would be exposed to 0.33 μg/kg/week, and Spanish children to 0.48

592 μg/kg/week of this extremely toxic form of Hg, in the Mb approach (1.13 μg/kg/week
593 and 1.47 μg/kg/week, respectively in the UB approach). However, it should be also noted
594 that one of the major risks that have been associated to Hg, and in particular to MeHg, is
595 developmental toxicity, where a brief exposure to the foetus can lead to permanent
596 damage. Various organizations have estimated the daily intake of mercury (as MeHg) that
597 is unlikely to be harmful. The World Health Organization has estimated that 0.22
598 μg/kg/day is unlikely to be harmful, with pregnant women identified for concern (Wise,
599 2004). Considering this, our estimates indicate that in the upper bound approach a Spanish
600 pregnant woman could be exposed to 73% of this reference value (0.16 μg/kg/day), only
601 via seafood consumption, and the children, which are high consumers of seafood would
602 almost reach this threshold (96%). These results can be considered of very much concern.
603
604 The estimates of this study regarding Hg are consistent with the exposure estimates in the
605 European Union (EU) as calculated by the EFSA using the middle bound approach, which
606 range from the lowest minimum of 0.14 μg/kg/week in very elderly to the highest
607 maximum of 5.05 μg/kg/week in adolescents. If we additionally consider that it has been
608 estimated that Hg in fish would represent approximately 37% of total dietary intake
609 (36.8% of food product coverage) (EFSA, 2012), a bulk calculation indicate that Spanish
610 adults would be exposed to 25% of the PTWI through their total diet (9.2% from fishery
611 products), and that this exposure would reach 35.7% of PTWI in the case of children
612 (13.2% from fishery products). Therefore, the estimated exposure to total Hg in Spain
613 from the diet alone would not exceed the PTWI, as it has also been reported for the rest
614 of EU's countries (EFSA, 2012).
615
616 ***3.3. Health risk assessment via multiple contaminants associated to the consumption***

617 ***of fishery products in Spain***

618

619 Although according to the above calculations none of the individual TDIs are exceeded
620 for any of the contaminants, the consumption of fish implies the exposure of the consumer
621 to multiple contaminants, and antagonistic, synergistic, and additive interactions among
622 the contaminants can occur. For the adequate human health risk assessment the USEPA
623 recommends that the additive model be used for multiple contaminants that cause similar
624 toxicological effects (USEPA, 2000; Yu et al., 2014). Using the calculated acute reference
625 doses (RfDs) and cancer slope factors (CSFs) for the contaminants included in this study
626 (USEPA, 2014) we have considered two types of health risks: acute toxicity and
627 carcinogenic (genotoxic) potential of fish consumption. For each of these endpoints, we
628 first calculated the individual $CR_{lim}$ to estimate the exposure limits to these chemicals
629 through the consumption of fishery products, as previously reported (Yu et al., 2014).
630 Secondly, from the calculated $CR_{lims}$ we calculated the individual RQs. The RQ
631 evaluation has been proposed as a convenient method of estimating population risk and
632 to provide a plausible worst-case scenario for initial screening of potential risk (USEPA,
633 2000; Yu et al., 2014). Finally, the RQs of each type of pollutant were summed and
634 presented as the overall risk associated to each subgroup of food (blue fish, white fish,
635 cephalopods, and other seafood) (Figure 1).

636

637 *3.3.1. Acute toxicity potential of the consumption of fishery products*

638

639 When the acute toxic effects of the contaminants were considered, the maximum
640 allowable consumption rates ($CR_{lims}$) (Table 3) of blue fish in children were from 350
641 times higher (for $\sum TEQ_{DL\text{-}PCBs}$) to 5185 times higher (for $B[a]P_{eq}$) than the current

642 consumption rate of this type of food (Table 2), and these values were more than double
643 in adults. For white fish the $CR_{lims}$ were from 7 times higher (for As) to 15801 times
644 higher (for $B[a]P_{eq}$); for cephalopods the $CR_{lims}$ ranged from 25 (As) to 127000 times
645 higher (ΣHCHs); and for seafood from 12 (As) to 91145 times higher (HCB) than the
646 current consumption rates of these food subgroups by Spanish children. Again, in all the
647 cases the estimations of maximum allowable consumption for Spanish adults were more
648 than double than in children (Table 3). Therefore, the individual RQs ranged from 0 to
649 0.06 in adults and 0 to 0.13 in children for the individual contaminants (Table 3), and at
650 most 0.2 for all contaminants (white fish, children) (Figure 1A). Thus, as all the RQ
651 values were much lower than 1 we can conclude that the consumption of the fishery
652 products would not pose risk of producing acute toxicity associated to their content in
653 chemical contaminants.
654
655 *3.3.2. Carcinogenic potential of the consumption of fishery products*
656
657 In a similar manner we also calculated the maximum allowable consumption limits and
658 the RQs associated to the current consumption of this group, but considering the
659 carcinogenic potential (Table 4).
660
661 Based on the contamination and the consumption data of fishery products, our
662 calculations indicate that again all the $CR_{lim}$ of the individual pollutants were higher than
663 the pattern of current consumption (which would not indicate obvious health risks due to
664 the intake or uptake of contaminants via fish consumption would be experienced) except
665 in the case of inorganic As (using the current CSF value of 1.5 mg/kg/day on IRIS,
666 (USEPA, 2014)), for which the current consumption of all the subgroups of fishery

667 products would exceed the maximum allowable rate. When we considered the additive
668 effect of all contaminants by food subgroups (Figure 1B) the RQs were higher than 1 for
669 blue fish, white fish, and seafood in Spanish adults, and for white seafood in Spanish
670 children, mainly due to the contribution of As. This means that the current dietary intake
671 of fishery products would represent a risk of carcinogenicity, especially associated to the
672 consumption of white fish. These results are consistent with those recently reported in the
673 Mediterranean region, where the highest risk of carcinogenicity of the fish consumption
674 pattern was associated with the content in As of these foods (Copat et al., 2013). In that
675 study Copat et al. (2013) suggested a modification of the pattern of consumption of these
676 foods, as we also do in the following section.
677
678 ***3.4. Meal recommendation for consuming fishery products***
679
680 The USEPA has suggested that the $CR_{lim}$ for carcinogenic and acute toxic effects
681 (whichever value is lower) should be used to calculate the maximum number of meals of
682 fishery products per month, and thus be able of giving advise to consumers to protect the
683 human health (USEPA, 2000; Yu et al., 2014). As in this study we found that the $CR_{lims}$
684 for carcinogenic effects were lower than those of acute toxicity, we used these values to
685 calculate the maximum number of meals of each food subgroup that would no pose
686 obvious health risks due to the intake or uptake of contaminants via fish consumption
687 (this is, consumption which that would allow a RQ $\leqq$ 1 for all products). In Table 5 we
688 summarize these recommendations for adults and children ($RC_{mm}$), and compare these
689 recommended maximum number of meals with the current pattern of consumption ($C_{mm}$).
690 According to our calculations, and strictly considering the results of our study, the
691 Spanish population should reduce the consumption of fishery products in general terms,

692 but more importantly in adults. Since the white fish involves greater risk, as detailed in
693 this research, its consumption should be further reduced, to around one-third of the
694 current consumption rate (that is, no more than one meal every two weeks). Adults should
695 also slightly reduce consumption of blue fish and cephalopods, crustaceans and mollusks
696 (Table 5). However, it is also necessary to consider that the health benefits of the high
697 value nutrients from seafood have been deeply studied (PUFa as well as vitamin $D_3$,
698 iodine, vitamin B12, etc.), and Therefore, it is not advisable to recommend abruptly
699 reducing fish consumption (EFSA, 2014). Nevertheless, the results of this study should
700 be taken into account for the design of appropriate risk communication campaigns aimed
701 to reduce the consumption of certain types of seafood; the aim should be an optimal risk-
702 to-benefit balance.
703
704 **4. CONCLUSIONS**
705
706 In this research we have estimated the daily intake of contaminants through the
707 consumption of fishery products. When these intakes are individually considered we
708 found that none of the reference values (tolerated daily intakes) were exceeded, although
709 the case of As, HCB, and $B[a]P_{eq}$ could be somewhat of concern. However, when we
710 estimated the risk associated to multiple contaminants acting together we found a
711 moderate risk of carcinogenicity. Therefore, a decrease in the consumption of fish and
712 seafood is recommended to avoid the carcinogenic risk associated to these pollutants,
713 especially in the case of white fish, whose consumption should be reduced to one-third
714 of the current level. It seems necessary to maintain surveillance programs that monitor
715 the trend of persistent pollutants in sea foodstuffs, and especially of the concentrations of
716 toxic elements, such as arsenic. The results of this study may be taken of utility for risk

managers in the design of appropriate risk communication campaigns aimed to reduce the consumption of certain types of seafood with the aim of obtaining an optimal risk-to-benefit balance of fish consumption.

## 5. FIGURE CAPTIONS

**Figure 1.** Hazard ratios of the contaminants for acutely toxic effects **(A)** and carcinogenic effects **(B)** in adults and children via consumption of fishery products. The red line indicates the threshold for toxic effect (RQ = 1).

**Supplementary Figure 1.** Comparison of the levels of organic pollutants (A) and inorganic pollutants (B) in fish (blue + white fish) and seafood ((cephalopods + seafood) * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$

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