

1 **DISTRIBUTION OF PERSISTENT ORGANIC POLLUTANTS (POPs) IN WILD**
2 **BLUEFIN TUNA (*Thunnus thynnus*) FROM DIFFERENT FAO CAPTURE**
3 **ZONES**

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24 **Abstract**

25 Residues of environmental contaminants in food represent a concern in food safety
26 programs. In this study, the distribution of persistent organic pollutants (POPs) were
27 evaluated in 79 tuna samples from FAO areas 51 (Indian Ocean), 71 (Pacific Ocean), 34
28 (Atlantic Ocean), and 37 (Mediterranean Sea). 6 polychlorinated biphenyls (PCBs), 16
29 organochlorines (OCs) and 7 polybrominated biphenyl ethers (PBDEs) were selected as
30 representative compounds according to EFSA POPs monitoring guidelines. An
31 analytical method, based on Accelerated Solvent Extraction (ASE), with an “in-line”
32 clean-up step and GC-MS/MS detection, was developed, validated and applied. PCBs
33 were detected in all FAO areas, with a prevalence of 100% for most of them. In the
34 FAO area 37, only, all PBDEs were detected. Only 5 OCs were detected. The results
35 showed that POPs contamination of tuna reflects FAO area contamination; in particular
36 FAO area 37 was the most polluted. Moreover, tuna muscle was an appropriate matrix
37 for monitoring contamination and for obtaining information about food safety.

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42 **Keywords:** FAO zones, Bluefin tuna; Triple Quadrupole, Accelerated Solvent
43 Extraction (ASE), Persistent Organic Pollutant (POPs)

45 **1. Introduction**

46 Since the second half of the past century, a particular care has been devoted to the
47 analysis of various essential elements and toxic contaminants in seafood in order to
48 limit exposure of consumers to contaminants while maximizing the benefits of seafood
49 consumption. (Herceg-Romanic', Kljakovic'-Gašpic', Klincic', & Ujevic, 2014). Fish
50 possess clear nutritional benefits providing high quality protein, minerals, essential trace
51 elements, fat-soluble vitamins (Vitamin D) and essential fatty acids (Da Cuña et al.,
52 2011). However, fish is also known to bioaccumulate contaminants, such as toxic
53 metals and Persistent Organic Pollutants (POPs), which can represent a risk for human
54 health. Anthropogenic inputs of POPs into the marine environment have increased their
55 levels to large extent within past a few decades. The waters of estuaries, coastal areas
56 and "enclosed" seas as the Mediterranean Sea are often characterized by high
57 concentrations of variably toxic POPs among which are commonly found pesticides and
58 heavy metals (Di Bella et al., 2006; Ansari, Marr, & Tariq, 2004). POPs represent the
59 best-known contaminants; they are mostly man-made chemicals that might accumulate
60 in the environment for a significant time (Gui et al., 2014) and bioaccumulate in
61 organisms (due to their highly stability, low volatility and lipophilic nature), leading to
62 the contamination of foodstuffs, even those not directly treated (Panseri et al., 2014). In
63 fact, concerning seafood, once in the marine water, these compounds become
64 distributed between water phase and particulate matter, which acts as a sorbent and
65 transports them into sediment, which serves both a sink and a source of contamination
66 to the surrounding biota (Storelli & Perrone, 2010). So, marine organisms occupying a
67 top trophic position in the marine ecosystem accumulate great concentrations of these
68 lipophilic contaminants and can become more vulnerable to their toxic effects. Among

69 POPs, polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCs), are two
70 groups of the most studied contaminants. Although the production and usage of these
71 compounds, in most industrialized countries, some of them, as DDT, were banned in
72 the 1970s, but they still persist in all parts of the environment because they are resistant
73 to environmental degradation (Boethling et al., 2009). In effect, although PCBs and OCs
74 levels in the environment are steadily declining (Albaiges, Murciano, & Pon, 2011),
75 they continue to bioaccumulate in human and animal tissues and biomagnify in food
76 chains, and may have potentially significant impacts on human health and the
77 environment (Kljaković-Gašpić, Herceg Romanić, Klinčić, & Tičina, 2015). All these
78 compounds are regulated by Stockholm Convention (2001), which aims to eliminate or
79 restrict the production and use of POPs. In terms of emerging classes of POPs, it is
80 interesting to pose the attention to the presence of polybrominated diphenyl ethers
81 (PBDEs), also known as brominated flame retardants (BFRs) that share a number of
82 chemical features characteristics as well as bioaccumulation mechanisms similar to
83 PCBs. They are widely used industrial chemicals added to various materials important
84 in manufacture of electronic equipment, upholstered furniture, construction materials,
85 textiles to minimise or even suppress the combustion process. Thus given the ubiquity
86 of plastics in the modern world, it is not surprising that PBDEs are being found in all
87 environmental compartments, including aquatic ecosystem. Not only the capacity of
88 PBDEs to bioaccumulate in biotic fatty tissues and biomagnify up the food chain
89 (several studies demonstrated their occurrence in wildlife and human tissue) in
90 combination with their resistance to degradation, but also their toxicity make this class
91 of chemicals of a high concern to the environment and human health. Furthermore the
92 European Commission has asked Member States to monitor the presence of BFRs in
93 food over the next two years. The move is in response to EFSA's recommendation that

94 more data on the levels of BFRs in food should be gathered. (Bragigand et al., 2006;
95 McDonald, 2002; Commission Recommendation 3 March 2014)

96 The Bluefin tuna, *Thunnus thynnus* (Linnaeus 1758), has a relevant importance for the
97 sea ecosystems not only from an economic but from an ecological point of view as well.
98 Bluefin tuna shows interesting and peculiar features that may affect their contaminant
99 bioaccumulation. In fact, Bluefin tuna are the best example of a fast-growing, long-
100 lived, wide-ranging fish, capable of migrating from the Mediterranean Sea to the
101 Atlantic Ocean and back. Then, they are top predators of the benthic-pelagic trophic
102 web from the time they are yearlings, feeding on several species of small fish,
103 crustaceans, and cephalopods; once adults, their diet becomes more specific, relying on
104 large cephalopods and pelagic fish.

105 On the basis of above mentioned considerations the purpose of the present research was
106 to evaluate the presence of different POPs (PCBs, OCs and PBDEs), in Bluefin tunas
107 arising from four different FAO catch areas, in order to have an overview and mapping
108 of their distributions. Tuna was chosen as fish species because is principally distributed
109 from the offshore waters to the open seas in tropical and temperate regions almost all
110 over the world, as in the Pacific, Atlantic, and Indian Oceans (Wilson et al., 2005) This
111 species represent an important commercial fish product, and its ecology and biology has
112 been well-studied (Fromentin, & Powers, 2005). Then, the obtained values can be used
113 to fill the database of levels of organic contaminants in seafood, in particular for flame
114 retardants presence about which scarce literature exists and used for future risk
115 assessment of the Italian population. Lastly the paper describe a rapid, accurate and
116 sensitive method to determine multi-residues by GC-MS/MS (PCBs, organochlorines
117 (OCs) and PBDEs) by using the Accelerated-Solvent-Extraction sample preparation

118 method with “in-line” clean up purification approach. The attention regarding the
119 sample preparation method should increase the overall sample laboratory throughput by
120 decreasing time and cost requirements and at the same time be environmentally
121 friendly.

122

123 **2. Experimental procedure**

124 *2.1 Chemicals and reagents*

125 Mix solution of PCBs congeners (PCB 28; PCB 52; PCB 101; PCB 138; PCB 153 and
126 PCB 180), PCB 209 (internal standard (IS) for PCBs), mix solution of PBDEs (PBDE
127 28; PBDE 33; PBDE 47; PBDE 99; PBDE 100; PBDE 153 and PBDE 154) and fluoro-
128 bromodiphenyl ether (FBDE), IS for flame retardants, were purchased from
129 AccuStandard (New Haven, USA). Standard solution of 16 OCs (α -HCH;
130 Hexachlorobenzene; β -BHC; Lindane; Heptachlor; Aldrin; Heptachlor epoxide; Trans
131 Chlordane; 4,4'-DDE; Endosulfan I; 2,4'-DDT; Endrin; 4,4'-DDD; Endosulfan II; 4,4'-
132 DDT and Endosulfan sulfate) was purchased from Restek (Bellefonte, PA, USA). Silica
133 gel 60 (0.063–0.200 mm) was purchased from Merck (Darmstadt, Germany). Hexane,
134 isooctane, acetone (special grade for pesticide residue analysis (Pestanal)) and 4-
135 nonylphenol (IS for OCs) were purchased from Fluka (Sigma-Aldrich, St.Louis, MO,
136 USA). However, since a wide range of contaminants were included in the study, for
137 some the Maximum Levels (MLs) were still below this concentration and for others
138 they were well above this concentration.

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141 *2.2 Sample collection*

142 A total of 79 Bluefin wild tunas (*Thunnus thynnus*) originating from different FAO
143 catch areas were selected for this study. Details of sampling and biometric data are
144 reported in Table S1. All tuna samples were provided by the most important tuna
145 industry at the national level and by the Fish Market of Milan, from different FAO catch
146 areas. All samples were captured and collected during April-May 2015. An overview of
147 the sampling areas according to its FAO capture zone was shown in Fig. 1.
148 Representative sample from each tuna was obtained by sampling fish tissue from 3
149 different anatomic zones (proximal, ventral and caudal); each sample was then stored at
150 -22 °C until the analyses.

151

152 *2.3 Accelerated Solvent Extraction (ASE) procedure with clean-up “in line”*

153 In order to analyse a large number of pesticides from different classes, a simple
154 extraction and clean up in single step (“in-line”) method was optimised to expand range
155 applicability. The extraction was performed using an ASE 350 (Thermo-Fisher
156 Scientific, Waltham, MA, USA). A 33 mL cells for accelerated solvent extraction
157 (ASE) were used for the analysis. A representative portion (300 g) of tuna was obtained
158 from each fish and minced, then 3 g were homogenised with an equal weight of
159 Diatomaceous earths, sodium sulfate and transferred into the cell. 1 mL of isooctane
160 solution containing the three ISs was added (20 ng g⁻¹ PCB 209; 2 ng g⁻¹ FBDE and 50
161 ng g⁻¹ 4-nonylphenol). To fill the remaining empty part of the cell diatomaceous earths
162 were added. The cells were packed with one cellulose filter at the bottom followed by
163 the fat retainer (10 g silica gel). The dried samples were transferred to the ASE cells.
164 Temperature (80°C), pressure (1500 psi), number of static cycles (3 min each), purging

165 time (90 s with nitrogen) and rinse volume (90%) were fixed throughout the study. The
166 extraction solvent was a mixture of hexane/acetone (4:1, v/v). Organic extracts were
167 finally collected in 66 mL vials and treated with sodium sulphate to remove any
168 possible humidity. Afterwards, the extract was collected and dried under vacuum in a
169 centrifugal evaporator at a temperature of 30°C. The residue was dissolved in 200 µL of
170 isooctane and submitted to analysis by GC/MS-MS.

171 An uncontaminated tuna sample (previously checked for the presence of POPs and
172 considered blank with a concentration of compounds < LOD) used as control was
173 selected for all procedure's optimization steps. For fish fortification, 3 g of the control
174 sample was spiked by adding an appropriate volume of the standard working solution to
175 cover the concentration range from 1 to 100 ng g⁻¹ (six calibration points: 1, 10, 20, 40,
176 80, 100 ng g⁻¹) for PCBs; from 0.5 to 10 ng g⁻¹ (five calibration points: 0.5, 1, 2, 5, 10
177 ng g⁻¹) for PBDEs and from 5 to 1000 ng g⁻¹ for OCs (eight calibration points: 5, 10,
178 25, 50, 100, 200, 400, 1000 ng g⁻¹), in relation to pesticide maximum residue levels
179 (MRLs) to realise the matrix-matched calibration curves.

180

181 *2.4 GC-MS/MS analysis of POPs*

182 Triple quadrupole mass spectrometry (QqQ) in electronic impact (EI) mode was
183 employed for the simultaneous detection and quantification of POPs in tuna samples.

184 A GC Trace 1310 chromatograph coupled to a TSQ8000 triple quadrupole mass
185 detector, (Thermo Fisher Scientific, Palo Alto, CA, USA), was used to confirm and
186 quantify residues in fish samples by using a fused-silica capillary column Rt-5MS
187 Crossbond-5% diphenyl 95% dimethylpolysiloxane (35 m x 0.25 mm i.d., 0.25 µm film
188 thickness, Restek, Bellefonte, PA, USA). The oven temperature program was: initial

189 temperature 80 °C, hold 3 min, increased to 170 °C at 10 °C min⁻¹, then from 170 °C to
190 190 °C at 3 °C min⁻¹, then raised to 240 °C at 2 °C min⁻¹, then ramped to 280 °C at 3 °C
191 min⁻¹ and finally from 280 °C to 310 °C at 10 °C min⁻¹ and held at this temperature for 5
192 min. The carrier gas (helium, purity higher than 99.999%) was in constant flow mode at
193 1.0 ml min⁻¹. A volume of 1 µL was injected using programmed temperature vaporizer
194 injection (PTV) in splitless mode with a 1-min splitless period and the following inlet
195 temperature programme: 80 °C (0.05 min), 14.5 °C s⁻¹ to 200 °C (1 min) and 4.5 °C s⁻¹
196 to 320 °C (12 min – cleaning phase). A baffle liner (2 mm × 2.75 mm × 120 mm, Siltek-
197 deactivated; Thermo Fisher Scientific) was used. The transfer line was maintained at
198 270 °C and the ion source at 250° C. The electron energy and the emission current were
199 set to 70 eV and 50 µA, respectively. The scan time was 0.3 s and the peak width of
200 both quadrupoles was 0.7 Da full width at half maximum. Argon was used as a collision
201 cell gas at a pressure of 1.5 mTorr. The QqQ mass spectrometer was operated in
202 selected reaction monitoring mode (SRM) detecting two-three transitions per analyte,
203 which are listed together with the particular collision energies in Table 3. Identification
204 of pesticides was carried out by comparing sample peak relative retention times with
205 those obtained for standards under the same conditions and the MS/MS fragmentation
206 spectra obtained for each compound.

207 The Xcalibur™ processing and instrument control software program and Trace Finder
208 3.0 for data analysis and reporting (Thermo Fisher Scientific) were used.

209

210 *2.5 Validation parameters and quality control*

211 The method was evaluated for its repeatability, linearity, recovery, limit of detection
212 and quantification. The limits of detection (LOD) and quantification (LOQ) were
213 calculated from the calibration curve in the concentration range corresponding to the

214 lower concentration levels according to MRL for each pesticide. LOD was calculated
215 using the equation $LOD = 3.3 SD_0/slope$, where SD_0 is the residual standard deviation.
216 The limit of quantification was calculated as $LOQ = 3 LOD$. Working solution were
217 prepared by diluting the stock solution in hexane for pesticides and then stored at
218 $-40^{\circ}C$. Mixed compound calibration solution, in hexane, was prepared from the stock
219 solutions ($10\mu mL^{-1}$) and used as spiking solutions as well. Recovery of the analytes
220 studies were carried out at fortification level of $10 ng g^{-1}$, while the method repeatability
221 (expressed as coefficient of variation, CV, %) was evaluated analysing six replicates
222 each by adding known quantities of POPs standard solution ($50 ng g^{-1}$) to 3 g of
223 homogenized fish (SANTE/11945/2015; Panseri, Soncin, Chiesa, & Biondi, 2011).

224 *2.6 Statistical analyses*

225 All statistical analyses performed used SPSS 15.0 (SPSS Inc., Chicago, Illinois).
226 Because of the skewed distribution of all measured parameters, the results are presented
227 with range, the 25th, the 50th (median), and the 75th percentile values (Table 6). Based
228 on the examination of normal scores plots of residuals, most of contaminant
229 concentration data were transformed to achieve normality prior to statistical analysis.
230 Natural log-transformations achieved best normal approximation for organic
231 contaminants presented in Fig. S1. Wilcoxon matched pairs test was used to test for
232 differences of POPs levels among FAO capture zone. Significance was accepted at
233 probabilities of 0.05 or less. Also, Spearman correlation analyses were used to assess
234 the relationship between $\sum PCBs$ and $\sum OCs$ and the lipid percentage of tuna from
235 different zones. Results were considered significant at a 5% critical level ($p < 0.05$).

236

237 **3. Results and discussion**

238 *3.1 Validation parameters*

239 The proposed method has been optimised for the multi-residue analysis of 29 persistent
240 organic pollutants. A GC-MS/MS chromatogram of tuna sample naturally contaminated
241 was shown in Fig. S2. An overview of the quantitative and confirmation MS/MS
242 transitions and the collision energies selected for each compound in EI mode is given in
243 Table S2. Notwithstanding that a highly selective QqQ mass spectrometer is used, since
244 GC-MS instruments are generally rather intolerant to non-volatile matrix impurities, the
245 choice of an appropriate sample preparation strategy is also important to avoid poor
246 ionization, background noise and contamination of the whole GC-MS system. All
247 results obtained for all compounds confirm the efficacy of the present method for the
248 determination of multi-residue pollutants in fish tissue.

249 The method showed a good linearity with determination coefficients equal or higher
250 than 0.99 for all the compounds investigated and good repeatability confirming the
251 present method as useful to monitor compounds belonging to different chemical classes
252 (Table S3). The recoveries ranged from 108 to 119 % for PCBs; from 91 to 102 % for
253 PBDEs and from 75 to 96. % for OCs. The CVs were all in the range from 4 to 14 %.
254 The one-step ASE method using silica as fat retainer is both rapid and cost-effective and
255 minimizes waste generation compared to the classic methods. The time required in the
256 laboratory is reduced to half by combining the extraction and the two clean-up steps
257 (i.e., GPC and SPE) in one single ASE step. Silica impregnated with sulphuric acid is
258 the most frequently used fat retainer for integrated extractions of organic contaminants
259 but florisil and neutral alumina have also been used (Muller, Bjorklund, & von Holst,
260 2001). A recent study of the fat-retention capacity of sulphuric-acid- impregnated silica,
261 florisil, and basic, neutral, and acidic alumina showed that all fat retainers, except basic

262 alumina (1.4%), yielded fat-free or nearly fat-free extracts (<1%) (Sun, Ge, Lv, &
263 Wang, 2012; Ghosh et al., 2011). So the final selection of neutral-silica was preferred in
264 order to minimise the laboratory waste. Our results are then in accordance with Zhang,
265 Ohiozebau & Rhind, (2011) that used neutral silica as fat retainer to extract and clean-
266 up polybrominated diphenyl ethers and polychlorinated biphenyls from sheep liver
267 tissue obtaining good validation parameters in term of recovery and precision for all
268 investigated compounds.

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270 *3.2 Application to tuna sample from different FAO catch areas*

271 The method developed was applied to the analysis of 79 tunas from different FAO
272 areas, in order to evaluate the occurrence of persistent organic pollutants (POPs) to have
273 an overview and mapping on their distribution. The results of detection frequency and
274 concentration levels of POPs residues, found in tuna samples, are presented in Tables 1
275 and 2. Because of the skewed distribution of all measured parameters, the results are
276 presented with range, the 25th, the 50th (median), and the 75th percentile values. Spatial
277 distribution of PBDEs and PCBs among FAO catch areas is shown in Fig. S1 and an
278 overview of the profile of detected POPs in tuna samples are presented in Fig. 2.

279 All the PCBs investigated were detected in all tuna samples, with the exception of the
280 PCB 153, which tends to be always present in the FAO 37 area, while in the other three
281 areas was only detected in five samples (two in FAO 34 area, three in FAO 51 area).

282 In this study, we found a positive correlation between \sum PCBs and lipid percentage of
283 tunas from all investigated FAO zones. Due to the lipophilic nature of PCBs, they are
284 generally well correlated to the lipid content in biota samples (Xia, Lam, Wu, Xie, &
285 Lam, 2012). In particular the correlation coefficients calculated were $R^2=0.71$ in FAO

286 zone 51; $R^2=0.73$ in FAO zone 71; $R^2= 0.79$ in FAO zone 34 and $R^2=0.83$ in FAO zone
287 37; P value was lower than 0.05 for all FAO areas. The relationship between Σ PCBs (ng
288 g^{-1} wet weight) and lipid percentage among FAO investigated zones is showed in Fig.
289 S3.

290 The concentrations of PCBs in the samples from the FAO 37 area were much higher
291 than those of the other three areas; in fact they range from 25.07 to 1649.64 ng g^{-1} lipid
292 weight , while in the other areas ranged from 5.09 to 36.12 ng g^{-1} lipid weight. Being a
293 semi-closed basin, the Mediterranean Sea has limited exchange with the open ocean
294 (Giménez, Gómez-Campos, Borrell, Cardona, & Aguilar, 2013) and this facilitates the
295 accumulation of these pollutants.

296 The Mediterranean marine environment has been exposed to a handful of adverse
297 events, which greatly threaten marine organisms. One of the most significant occurred
298 in the 1990s, when tens of thousands of striped dolphins died in the Mediterranean.
299 Analyses revealed high levels of polychlorinated biphenyls in the fish's tissue as well as
300 in liver and other organs (Kannan et al., 1993; Borghesi et al., 2009). The POPs
301 pollution of Mediterranean Sea ecosystem is attributable to the many sources of
302 agricultural, municipal, and industrial contamination in the adjacent regions. In
303 particular, these chemicals mainly arrive in the sea as a consequence of evaporation,
304 atmospheric fallout, surface run-off, and wastewater discharges from the intensively
305 cultivated areas, the densely populated urban centres, the large industrial complexes,
306 and the many waste dumps clustered along the coasts. This hypothesis is confirmed by
307 the presence of the highest concentrations of organochlorine and PCBs pollutants in the
308 sea bass and the grey mullet, two strictly resident and benthic species, which inhabit
309 nearshore marine areas (Bailey et al., 2001; Naso et al., 2005).

310 Moreover, in FAO 37 area, PCBs 101, PCB 138, PCB 153 and PCB 180 are at higher
311 concentrations compared to PCB 28 and PCB 52; the abundance of these congeners is
312 consistent with their high prevalence in technical mixtures, high lipophilicity, stability
313 and persistence, which facilitate adsorption to sediments and accumulation in the
314 aquatic ecosystem, and to their molecular structure. PCBs 101, 138, 153 and 180, being
315 refractory to metabolic attack by monooxygenases, tend to be more slowly eliminated
316 because of their high degree of chlorination and the lack of adjacent unsubstituted H-
317 atoms in ortho–meta and/or meta–para position on the aromatic ring. (Storelli, et al.,
318 2009; Masci, Orban, Navigato, 2014). In fish, PCBs decreased growth; caused ionic
319 imbalance, hyperglycemia, anemia, toxicopathic lesions in tissues, such as gill, liver,
320 and spleen; disrupted reproduction; and ultimately affected population levels (Khan,
321 2011; Miranda et al., 2008). The fate of individual PCB congeners is determined by
322 both environmental processes and physical-chemical properties of individual congeners,
323 and differential rates of uptake, metabolism and elimination will influence the congener
324 profile to which target tissues are ultimately exposed. Except for dioxins and dioxin like
325 PCBs , EU regulation on maximum permissible levels (MPL) for organochlorine
326 compounds in fish for human use (EFSA, 2010; Decision (EC) No 2455/2001)
327 prescribes only the concentrations of six indicator PCBs in fish and mussels ($<75 \text{ ng g}^{-1}$
328 fresh tissue), while concentrations of OCs are not regulated by any law. The sum of the
329 six indicator PCBs can be used as an appropriate marker for occurrence and human
330 exposure to NDL-PCBs because this value represents about 50% of the total NDL-
331 PCBs in food (EFSA, 2010). Since the sum of indicator PCBs in our study (2.49-38.25
332 ng g^{-1} wet weight; 55.33 to 910.71 lipid weight) was lower than proposed MPL, results
333 of this research suggested that the consumption of analysed tunas does not pose a health
334 risk when considering exposure to NDL-PCBs even if the concentration in tuna from

335 FAO 37 was closer to MPL. Concerning PBDEs, the 47, 100, and 154 congeners were
336 detected in all samples with concentrations between 0.06 ng g⁻¹ and 139.76 ng g⁻¹ lipid
337 weight; PBDE 99 and PBDE 153 were found in the FAO 51 area and FAO 37 area,
338 while the remaining congeners (28 and 33) were only detected in FAO 37 area. These
339 data show that, as for PCBs, all the PBDEs investigated have been detected in the
340 Mediterranean Sea, probably because of the reasons mentioned previously. Another
341 interesting aspect is that the prevalence of PBDEs in the FAO 37 area is higher than the
342 other areas, in fact it ranges from 25 to 100 %, while in the other three ones the
343 frequency is between 5 and 65 % except for PBDE 154, which was detected with a
344 prevalence of 85% in the FAO 37 area. Unfortunately, there are no many studies
345 regarding the concentration of PBDEs in foodstuff, so few data are available. A study of
346 Corsolini, Guerranti, Perra, & Focardi (2008), focused on the presence of PBDEs in
347 different swordfish tissues in the Mediterranean Sea, shows that PBDEs were detected
348 in the swordfish muscles in a range from 4 pg g⁻¹ to 1.91 ng g⁻¹, concentrations lower
349 than tuna samples. These results are in accordance with ours because tuna has a fat content
350 greater than the swordfish, and being their lipophilic character responsible for their
351 bioaccumulation in fatty tissues, this involves in a higher concentration in tuna samples.

352 Also for OCs a positive correlation between Σ OCs and lipid percentage of tunas from
353 all investigated FAO zones was found. The correlation coefficients obtained were
354 R²=0.73 in FAO zone 51; R²=0.86 in FAO zone 71; R²=0.87 in FAO zone 34 and
355 R²=0.92 in FAO zone 37; *P* value was lower than 0.05 for all FAO areas. This result
356 was in accordance with Erdogrul, Covaci, & Schepens (2005) that investigated the
357 levels of organochlorines, polychlorinated biphenyls and polybrominated diphenyl
358 ethers in fish species from Kahramanmaras, Turkey. The relationship between Σ OCs

359 (ng g⁻¹ wet weight) and lipid percentage among FAO investigated zones is showed in
360 Fig. S4.

361 Regarding OCs, only five compounds were detected in tuna samples. Endosulfan sulfate
362 was detected in all FAO areas, with a mean concentration of about 156,67 ng g⁻¹ lipid
363 weight in each area; the prevalence for this OC was between 65 and 89 %; $p < 0.05$.

364 Endrin was present in FAO 51, 71 and 34 areas, with a concentration ranges from 40.72
365 to 928.81 ng g⁻¹ lipid weight and with a frequency range from 5 to 30 %. No studies
366 showed the presence of Endosulfan sulphate and Endrin in tuna samples, therefore this
367 is the first study to indicate their possible presence. pp-DDT (one of the two congeners
368 of DDT investigated) was found in all areas, except the 71; op-DDT (the second
369 congener) was only detected in the Mediterranean Sea. The prevalence of pp-DDT was
370 higher than that of op-DDT, in fact it ranged from 15 to 89 %, while for op-DDT the
371 frequency was 5 % (it was found in just one sample of FAO 37 area). In addition to
372 DDT, also its metabolite pp-DDE was detected, but only in the FAO 37 area, where its
373 concentration ranged from 30.75 to 785.13 ng g⁻¹ lipid weight and its prevalence was
374 47%. These data are in according to many other studies, in which DDT and its
375 metabolites were detected in different marine organisms. Storelli et al. (2009) studied
376 the presence of OCs in deep-sea from the Mediterranean Sea, and they found both DDT
377 (op' and pp') and DDE (pp') in their samples. Also Ueno et al. (2003) demonstrated the
378 presence of DDT in Skipjack tuna. All these data show that DDT and its metabolites,
379 due to hydrophobic properties, are absorbed by aquatic organisms and bioaccumulate,
380 leading to the final contamination of foodstuffs. The organochlorines pollution is
381 attributable to many sources: atmospheric fallout, intensive agriculture, densely
382 populated urban centres and large industrial complexes; these factors probably play a
383 key role in pollution of FAO areas, especially for the Mediterranean Sea.

384 This study shows that, investigating three different classes of POPs, is possible to have
385 an overview and mapping on their presence in four FAO areas. Furthermore, much
386 information was provided for further studies, especially for PBDEs, for which many
387 data are not yet available in literature.

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394 **4. Conclusions**

395 An analytical method was developed and applied to evaluate the POPs residues in tuna
396 samples from different FAO areas. The method proved to be simple and rapid, requiring
397 small sample sizes, minimizing solvent consumption, due to the ASE with an “in line”
398 clean up step. MS/MS detection provides both quantitative information and
399 confirmation of POPs residues in tuna confirming the one-step ASE method a valid
400 alternative to classical extraction methods because the analytical quality is comparable.
401 The determination of POPs in foods is necessary to ensure that human exposure to
402 contaminants does not exceed tolerable levels for health. The results of this study show
403 that POPs contamination of tuna is strictly related to the FAO area of origin, reflecting
404 the specific pollution of a given environment, as most stressed for the Mediterranean
405 Sea. Moreover, as expected, it was possible to obtain an accurate profile of persistent
406 organic pollutants in order to have an overview and to map the distribution of POPs in

407 fish for the consumer's food safety purpose. Indeed further experimental plans will be
408 designed extending the analyses to other compounds belonging to flame retardant
409 chemical class to add new knowledge about contamination and presence of these
410 emerging contaminants in fish.

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417

418 **5. References**

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