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Baseline

Persistent organic pollutants in tissues of farmed tuna from the Adriatic Sea

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ABSTRACT

This study investigated the levels and distribution of polychlorinated biphenyls (PCBs) and organochlorine pesticides in three tissue types of farmed Bluefin tuna (*Thunnus thynnus*): muscle, liver and branchiae. Seven adult species were caught in 2015 at a tuna farm in the Croatian Adriatic. The organochlorine compound levels decreased in the following order: liver > muscle > branchiae while contaminant distribution in all three tissues followed the same order: ΣPCB ≫ ΣDDT > ΣHCH ~ HCB. The found POP levels indicated moderate pollution of farmed tuna and were below all limits set by current laws. Furthermore, no cytotoxic effect of the POP mixture extracted from tuna liver samples on human neuroblastoma cells was observed.

Tuna is one of the most valued fish species in the human diet. Its meat is nutritionally extremely rich; tuna contains a high percentage of protein which makes it an excellent choice for a healthy diet and prevention of cardiovascular diseases. The tuna is a top predator of the benthic-pelagic trophic web and a highly migratory species that can travel long distances and is found in diverse regions around the globe. It is one of the most important commercial fish with a global annual consumption of several million tons. The meat of farmed Bluefin tuna (*Thunnus thynnus*) is one of the world's most expensive food products and is used almost exclusively for Japanese specialties sushi and sashimi. Bluefin tuna has been farmed in the Adriatic Sea since 1996 and is nowadays one of the most important Croatian export products. The technology of farming (fattening) tuna is based on capturing wild tuna (usually around 10 kg weight) and transporting it to cages where it feeds mostly on sardines until it gains the desired biomass.

Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) belong to a group of chemicals known as persistent organic pollutants (POPs). Due to their lipophilic nature and chemical stability they are prone to bioaccumulation and biomagnification along the food chain, reaching the highest levels in organisms like tuna that are at the top of the food chain.

Following findings with regard to their adverse effects both on the environment and humans, series of measures (*Stockholm Convention* as one of the most important) have been undertaken in order to protect the environment and human health, but despite efforts to minimize their further release into the environment, these contaminants are still present in all environmental compartments, including the aquatic. In water, these compounds tend to adsorb on particulate matter and deposit in sediment, which acts as a sink but also source of further exposure for the surrounding biota (Storelli and Perrone, 2010). Seafood examination for toxic contaminants is therefore essential in order to limit consumer's exposure. Tuna muscle is an appropriate matrix for monitoring contamination and obtaining information about food safety (Chiesa et al., 2016).

The purpose of this study was to investigate the levels and contamination patterns of the most studied POPs, PCBs and OCPs, in Bluefin tuna farmed in the Adriatic Sea. We examined POP concentrations and composition in three tissues: muscle - commonly used as human food; liver – an organ with an important role in the distribution and detoxification/transformation of xenobiotics and with high lipid content ideal for POP accumulation; branchiae – an organ in constant contact with water and dissolved particulate matter containing organic contaminants. Additionally, since human neurons are highly sensitive to xenobiotics we wanted to check if there was a possible cytotoxic effect of POPs extracted from tuna liver on the human neuroblastoma cell line.

Tunas were sampled in January 2015 at a tuna farm in the coastal area of Zadar County located in the Croatian Adriatic (Fig. 1). Seven

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Fig. 1. Sampling location – sea area of Zadar county (red indicated within Croatia). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

adult species were caught, average weight 65 kg and 155 cm in length. From each individual, 3 tissues were taken - branchiae, liver and muscle (white). Liver samples from one tuna were not available for analysis.

In the tuna tissues we analysed 7 OCPs: HCB (hexachlorobenzene), α-HCH, β-HCH, γ-HCH (α-, β-, γ-hexachlorocyclohexanes), *p,p′*-DDE, *p,p′*-DDD and *p,p′*-DDT, and 17 PCB congeners [(six indicator PCB congeners (IUPAC number: 28, 52, 101, 138, 153, 180), as well as 11 toxicologically relevant congeners: 8 mono-*ortho* substituted dioxin-like (DL) PCBs (IUPAC numbers: 105, 114, 118, 123, 156, 157, 167, 189), and 3 toxicologically relevant non-dioxin-like (NDL) congeners (IUPAC numbers: 60, 74, 170)]. We also calculated summary data for several major groups of contaminants: ΣIndPCBs as the sum of 6 indicator PCBs, ΣToxRelPCBs as the sum of 11 PCB congeners that represents the more toxic fraction of PCBs in samples, ΣHCHs as the sum of the α-, β-, and γ-HCH, ΣDDTs as the sum of *p,p′*-DDT, *p,p′*-DDD, and *p,p′*-DDE.

The analytical procedure was previously described in Kljaković-Gašpić et al. (2015). Briefly, 5 g of tissue was cold-extracted with 40 mL of *n*-hexane, repeatedly cleaned with 96% sulphuric acid and analysed with dual-column gas chromatography with electron capture detector (s) (GC-ECD) on a CLARUS 500 chromatograph (Perkin Elmer, USA). The oven temperature was programmed from 100 °C to 110 °C at 4 °C min^{-1} (isothermally 5 min at 110 °C) and then to 240 °C at 15 °C min^{-1} (50 min isothermally at 240 °C). Qualitative and quantitative analyses were done using external standard containing all of the determined pollutants.

The average recoveries were between 75% and 89% (RSD 1–11%) Method blanks showed no interference with the pollutants of interest. The limits of detection (LOD) varied depending on the compound: 0.020 ng g^{-1} wet weight (ww) for γ-HCH, DDE, PCB-138, PCB-153, PCB-180 and 0.010 ng g^{-1} ww for the other compounds. The performance of the analytical procedure was validated through analysis of reference material IAEA-406 (fish homogenate) supplied by the International Atomic Energy Agency-Marine Environment Laboratory (IAEA-MEL), Monaco. The produced data on OCP and PCB values were within acceptable range according to the associated reference material sheet (Villeneuve et al., 2006).

Tuna liver sample extracts (prepared previously for chemical analysis) were evaluated for their potential cytotoxic effect on human neuroblastoma cell line SH-SY5Y (ECACC 94030304). SH-SY5Y cells were grown and maintained in DMEM F12 (Sigma–Aldrich, Steinheim, Germany) supplemented with 15% (*v*/v) fetal bovine serum (FBS, Sigma-Aldrich, Steinheim, Germany), 2 mM glutamine and 1% (v/v) non-essential amino acids (NEAA, Sigma-Aldrich, Steinheim, Germany), at 37 °C in a 5% CO_2 atmosphere according to the standard supplier's protocol. Two days prior to the experiment, cells were detached using 0.25% Trypsin/EDTA solution (Sigma-Aldrich, St. Louis, MO, USA), re-suspended and seeded in 96-well plates. The assay was performed in 120 μL/well media volume with 20.000 or 40.000 cells (confluent monolayer). The tuna liver extracts prepared previously in *n*-hexane (concentrations ranged from 128 ng mL^{-1} to 321.5 ng mL^{-1}) were evaporated to residues under a gentle stream of nitrogen after which the residues were dissolved in 50 μL of DMSO and mixed thoroughly. To this mixture, 50 μL of fetal bovine serum (FBS, Sigma-Aldrich, Steinheim, Germany) was added to be suitable for a cell-culture application. The concentration of OCP and PCB in these stock solutions ranged from 1280 to 3215 ng mL^{-1} (recalculated from concentrations determined by chemical analysis). Cells were exposed to extracts for 24 h in a concentration range from 0.78–100 ng mL^{-1} made in a serial dilution. This range corresponds to a maximum of 50 ng of POPs per well. For sample extracts II and VI, 100 ng mL^{-1} was excluded from evaluation either to low solubility in aqueous cell medium or to DMSO concentration exceeding the 3%, which influences cell viability

Table 1
Concentrations of organochlorine contaminant (ng g^{-1} wet weight) in tissues of farmed tuna from the Adriatic Sea.

Compound	Liver (n = 6)			Muscle (n = 7)			Branchiae (n = 7)		
	Median	Min	Max	Median	Min	Max	Median	Min	Max
% Lipid	10.1	3.4	12.6	3.0	1.8	6.5	0.8	0.4	1.5
Polychlorinated biphenyls									
Indicator PCBs (Ind PCBs)									
PCB-28	0.68	< 0.01	0.83	0.69	< 0.01	1.19	0.47	0.27	0.87
PCB-52	1.39	0.78	2.47	1.58	1.10	2.39	1.83	0.31	3.33
PCB-101	1.02	0.41	1.92	0.56	0.30	1.06	0.28	< 0.01	0.34
PCB-138	8.48	3.71	9.81	4.90	2.68	13.06	1.17	0.63	1.58
PCB-153	13.99	7.29	17.90	8.40	4.59	16.21	1.99	0.99	4.57
PCB-180	5.74	2.89	6.99	3.10	1.39	4.85	0.67	0.47	0.79
ΣInd PCBs	31.40	15.85	38.84	19.39	10.54	36.75	6.66	3.99	10.09
Toxicologically relevant PCBs (ToxRel PCBs)									
PCB-60	0.11	< 0.01	0.23	0.40	0.19	0.71	< 0.01	< 0.01	0.41
PCB-74	0.46	0.28	0.72	0.30	< 0.01	0.44	0.17	< 0.01	0.89
PCB-105	0.78	< 0.01	1.23	0.63	0.30	0.92	0.21	< 0.01	0.42
PCB-114	0.10	< 0.01	1.30	0.41	< 0.01	0.83	0.11	0.05	0.32
PCB-118	2.22	1.03	3.01	1.10	0.70	2.46	0.34	0.14	0.72
PCB-123	2.16	0.87	2.81	0.60	0.36	1.29	0.28	0.12	0.51
PCB-156	0.70	0.18	1.10	0.30	0.18	0.74	0.09	0.06	0.75
PCB-157	0.17	0.05	0.33	0.15	0.07	0.39	0.34	0.30	0.55
PCB-167	0.15	< 0.01	0.46	0.29	0.15	1.15	0.40	0.03	1.52
PCB-170	1.89	0.91	2.95	0.98	0.56	2.24	0.19	0.12	0.53
PCB-189	0.42	< 0.01	0.58	0.06	< 0.01	0.53	0.08	< 0.01	0.18
ΣToxRel PCBs	10.03	4.33	11.32	6.33	3.62	8.81	2.93	1.51	3.92
ΣPCB	41.43	20.18	50.16	24.43	14.16	45.55	9.34	6.45	13.69
Organochlorine pesticides									
α-HCH	0.22	0.16	0.28	0.18	0.04	0.56	0.16	0.07	0.21
β-HCH	0.33	< 0.01	0.63	0.24	< 0.01	0.87	0.13	0.07	0.31
γ-HCH	0.11	< 0.02	0.17	0.07	< 0.02	0.22	0.11	0.07	0.17
ΣHCH	0.67	0.37	0.90	0.72	0.08	1.43	0.41	0.28	0.66
p.p'-DDE	6.54	3.26	8.14	3.86	2.44	8.22	0.90	0.44	2.04
p.p'-DDD	1.30	0.59	1.62	0.78	0.36	2.27	0.20	0.04	0.45
p.p'-DDT	1.85	0.86	2.71	0.88	0.61	2.48	0.25	0.14	0.35
ΣDDT	10.05	4.71	12.41	5.60	3.40	12.97	1.26	0.87	2.74
HCB	0.74	0.40	0.97	0.30	0.17	0.58	0.12	0.05	0.17

significantly *per se*. After 24 h incubation at 37 °C in a 5% CO_2 atmosphere, cells were washed with PBS buffer (1 ×) and the cytotoxicity profile was determined by MTS assay (Mosmann, 1983) using predefined kit (CellTiter 96® AQueous One Solution Cell Proliferation Assay, Promega, Madison, WI, USA). The procedure followed a protocol described by the manufacturer in which 120 μL of MTS reagent mix in PBS was added in each well in a 1:6 ratio, incubated 0.5–3 h after which the absorbance was read at 492 nm on Infinite M200PRO plate reader (Tecan Austria GmbH, Salzburg, Austria). Data was evaluated from at least two experiments (each in duplicate) using IC_{50} nonlinear fit equation predefined in GraphPad Prism6 software and presented as percentage of inhibition to untreated control cells.

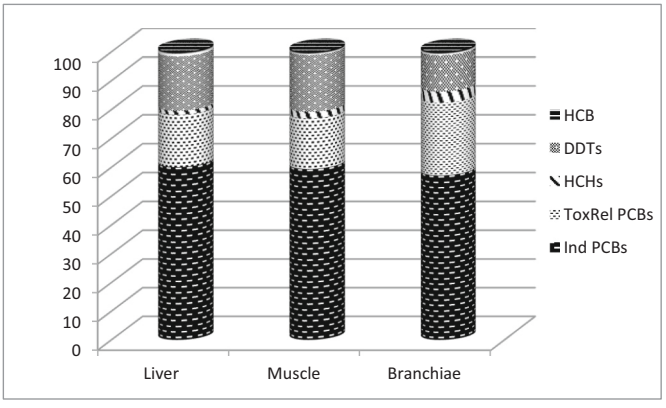

Fig. 2. Percentage contribution of different POP groups to total concentration found in samples of tuna liver (n = 6), muscle (n = 7), and branchiae (n = 7).

The data analysis was performed using STATISTICA (data analysis software system, StatSoft Inc., 2013, version 13). Because of the skewed distribution of all of the measured parameters, the results are presented with range and median values. Due to a small number of samples, we used non-parametric statistics to explore possible correlations, with the level of significance set at $p < 0.05$. Spearman correlation analyses were used to assess the relationship between different groups of pollutants and the lipid percentage in tissues of tuna, and Kruskal-Wallis test to explore differences in contaminant levels found in tissues. For evaluation of cytotoxicity data, we used GraphPad Prism6 software, one-way ordinary ANOVA Dunnett's multiple comparisons test to untreated control with $p < 0.05$.

The organochlorine compound distribution in all three tissues followed the same order: ΣPCB ≫ ΣDDT > ΣHCH ~ HCB (Table 1, Fig. 2). Median concentrations of ΣPCB were 41.4, 24.4 and 9.3 ng g^{-1} ww in liver, muscle and branchiae, respectively. A ΣPCB/ΣDDT ratio higher than 1 indicated that the investigated area was to a higher extent exposed to industrial over agricultural organochlorines. Also, other factors like exposure period, bioavailability and pollutant distribution patterns can enhance PCB uptake in aquatic organisms (Nicklisch et al., 2017). We also found PCB domination in various marine organisms from the Adriatic Sea (Kljaković-Gašpić et al., 2015; Kožul et al., 2011; Herceg Romanić et al., 2014a) including wild tuna sampled 1996 (Klinčić et al., 2020). Studies analysing tuna from the Mediterranean Sea revealed the same profile (Kannan et al., 2002; Corsolini et al., 2005; Maisano et al., 2016; Chiesa et al., 2016).

Among OCPs, the highest concentrations in all tissues were found

Table 2
POP concentrations (ng g^{-1} lipid or ng g^{-1} wet* weight) and DL-PCBs TEQs (in parenthesis TEQ for mono-*ortho* PCBs if available) in muscle tissue (M) and liver (L) of different tuna species worldwide.

Sampling location	Year of sampling	Species	Tissue	*P,p´*-DDE	ΣDDT	HCB	ΣPCB	TEQ_{1998} (pg g^{-1} ww)	Reference
Adriatic Sea	1996	Tt (w)	L	362–823	458–900	2.7–3.0	1149–1980	(2.84–5.08)	Klinčić et al. (2020)
			M	256–706	347–778	2.50–7.50	545–1800	(0.67–2.58)	
Palizzi, southern Italy	1999	Tt (w)	L	61–110*			5670–14,400		Kannan et al. (2002)
			M	30–67*			280*		
Ionian coast, Sicily, Italy	1999	Tt (w)	L	45–110*		0.1–0.7*	117–357*		Corsolini et al. (2005)
			M	5-97*		0.05–0.9*	12–229*	1.97 (0.96)	
Japan, 7 regions	2003, 2007	To (f)	M-*akami*	160–2250		14–26	340–980		Hisamichi et al. (2012)
			M-*toro*	190–5480		11–32	430–1630		
Straits of Messina (Sicily, Italy)	2004	Tt (w)	L	< 1000–4558			< 97–9140		Di Bella et al. (2006)
			M	< 1000–3480			< 97–2324		
Australia	2004	Tm (w)	M				24–200	0.1–0.21	Padula et al. (2008)
		Tm (f)					7.7–240	0.17–3.5	
Mediterranean Sea (Ionian Sea)	2005	Tt (w)	L	275–645	289–702		325–812	0.55	Storelli et al. (2008)
South Australia	2005	Tm(f)	M				0.08–1.58*	0.11–1.18	Phua et al. (2008)
Favignana (western Sicily, Italy)	2005	Tt (w)	L	279		6.6	337		Vizzini et al. (2010)
			M	2923		31.7	2752		
Gulf of Castellammare (NW Sicily, Italy)		Tt (f)	L	265		15.1	372		
			M	2540		35.7	1917		
Barbate coast, Spain, Atlantic Ocean	2006	Tt (w)	L				12.0 ± 3.3*	4.8 (1.6)	Sprague et al. (2012)
			M				5.2 ± 0.9*	2.2 (0.7)	
West Indian Ocean	2007	Talb (w)	L	5.1–34.8	6.6–63	17.6–83	52–621		Torres et al. (2009)
			M	2.3–24.1	4.4–43	121–602	44–382		
Straits of Messina, central Mediterranean Sea	2011	Tt (w)	L		18.7–24.3*		61–148 *	0.14–0.3	Maisano et al. (2016)
Reunion Island, SW Indian Ocean	2013	Ta (w)	M		8.9–83.8		2.2–33	0.085[a]	Munschy et al. (2016)
South Africa, SE Atlantic Ocean					8.9–63		2.7–21.8	0.147 [a]	
Indian Ocean, western	2015	Tt (w)	M	< LOD	< LOD-171	< LOD	50–93		Chiesa et al. (2016)
Pacific Ocean, western central				< LOD	< LOD	< LOD	47–65		
Atlantic Ocean, eastern central				< LOD	< LOD-153	< LOD	50–73		
Mediterranean Sea				< LOD −785	< LOD-1110	< LOD	118–3713		
Adriatic Sea	2015	Tt (f)	L	**12.1–18.7 (3.3–8.1)***	**18.0–27.0 (4.7–12.4)** *	**1.2–2.3 (0.4–1.0)** *	**78.4–116.0 (20.2–50.2)** *	**(0.13–1.48) (0.09–0.24)** [a]	This study
			M	16.3–37.1 (2.4–8.2) *	23.1–53.9 (3.4–13.0) *	1.2–2.7 (0.2–0.6) *	93.7–234.9 (14.2–45.6) *	(0.55–0.99) (0.08–0.19) [a]	

Species: Tt-*Thunnus thynnus*; Tm-*Thunnus maccoyii*; To-*Thunnus orientalis*; Ta-*Thunnus alalunga*; Talb-*Thunnus albacares*; w-wild tuna; f-farmed; [a]calculated based on Toxicity Equivalence Factors defined by World Health Organization in 2005 ($WHO\text{-}TEFs_{2005}$) (Van den Berg et al., 2006).

for DDTs whose median concentrations ranged from 1.3 in branchiae to 10.1 ng g^{-1} ww in liver. In liver and muscle tissues, DDTs in average accounted for more than 85% of ΣOCP, and 71% in branchiae. *p,p'*-DDE, a very persistent main *p,p'*-DDT metabolite, was generally the most abundant individual compound in the OCP group, accounted for 58% of ΣOCP in liver and muscle, and 49% in the branchiae. This suggests that the DDT contamination of the investigated tuna was mostly due to the historical use and/or remote sources and that no new sources of contamination had emerged. The same *p,p'*-DDE domination was found in wild Bluefin tuna sampled in the Adriatic almost two decades earlier (Klinčić et al., 2020).

A common dominance of indicator PCB congeners within PCB congeners was found in all analysed tissues, the average contribution to ΣPCB ranging from 69% in branchiae to 77% in liver. Higher chlorinated congeners (PCB-153, PCB-138, and PCB-180) alone contributed to ΣPCB from 43% in branchiae to 69% in liver. These congeners mainly dominated the PCB pattern due to their high stability, lipophilic nature, and metabolic resistance. ΣIndPCBs can be used as an appropriate marker for the occurrence and human exposure to NDL-PCBs because this value represents about 50% of the total NDL-PCBs in food (EFSA, 2010), and even more in the case of the tuna muscles analysed in our study, on average 76%. The maximum ΣIndPCB value found in tuna muscles was two times lower than the value of 75 ng g^{-1} ww set as the maximum permissible level by the European Commission in fish (European Commission (Decision (EC) No 1259/2011), 2019) suggesting that the consumption of analysed tuna farmed in Adriatic Sea does not pose a health risk with consideration to NDL-PCBs.

Among the toxicologically relevant PCBs in tuna liver and muscle, PCB-118, PCB-123, and PCB-170 were found at the highest levels, while in branchiae their levels were not elevated in comparison to other toxic PCBs. PCB-118 and PCB-123 are mono-*ortho* PCBs with DL toxicity and an assigned toxic equivalent factors (TEF). In contrast, PCB-170 is a di-*ortho* PCB congener, as are PCB-138, PCB-153, and PCB-180, and while not posing DL toxicity, it is highly persistent and among the most prevalent PCBs found in various aquatic organisms (Kannan et al., 2002; Corsolini et al., 2005; Vizzini et al., 2010; Herceg Romanić et al., 2014a, 2014b; Kljaković-Gašpić et al., 2015; Klinčić et al., 2020).

To evaluate the toxicity of measured PCB levels, toxic equivalents (TEQ) for eight mono-*ortho* PCB congeners were calculated (TEQ = ΣC_i x TEF); both with TEF values determined in 1998 (Van den Berg et al., 1998) and revised values from 2005 (Van den Berg et al., 2006). They are presented in Table 2.

Mono-*ortho* PCBs contribute to DL-PCB TEQ to a minor extent compared to non-*ortho* congeners (approximately with 30%) due to lower toxicity and assigned TEF. With the assumption that the maximum TEQ value obtained for tuna muscle (0.19 pg g^{-1} ww) in our study is only one third of the total DL-PCB TEQ, the value would be around 0.6 pg g^{-1} ww, which is still considerably below the value set by the European Commission for TEQ_{2005} of DL-PCBs (3 pg g^{-1} ww) in fish muscles. Very similar TEQ values based on mono-*ortho* PCBs were reported by Corsolini et al., 2005 and Sprague et al., 2012 for wild tuna caught near Italy and Spain, respectively.

As visible from Table 1, the contaminant levels decreased in the following order: liver > muscle > branchiae, in the same way as the percentage of tissue lipid content. This was expected due to the known hydrophobic nature of POPs whose concentrations are strongly influenced by the lipid content of tissue. In liver, all contaminant groups were strongly correlated with % lipid (r = 0.714–0.829, $p < 0.05$), and the similar was found for muscle samples ($r > 0.857$, $p < 0.05$) with the exception of ΣHCH for which correlation with % lipid was not statistically significant. Low lipid content in branchiae was the probable reason for low binding of POPs and only HCB levels correlated with lipid content with statistical significance (r = 0,891, $p < 0.05$).

By exploring differences in contaminant levels we found that concentrations of all contaminant groups (except of ΣHCH) in branchiae are statistically significantly lower than in muscles (z = 2.3–2.8, p = 0.0005–0.001) and liver (z = 3.4–3.9, p = .0005–0.001), while between muscle and liver concentrations there is no significant difference.

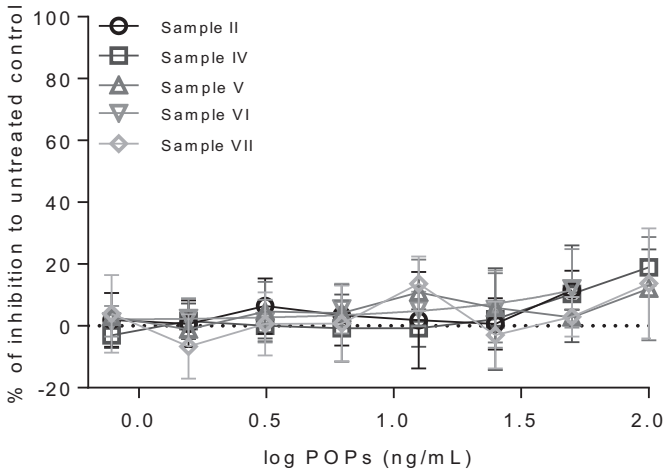

Fig. 3. Cytotoxicity of POPs from tuna liver samples extracts on SH-SY5Y determined by MTS assay. Each point presents the mean (± S.E.) of 4 different experimental values. No statistically significance difference ($p > 0.05$) was observed compared to the untreated control cells.

An interesting finding were the very strong correlations (r = 0.66–1.00) between concentrations of contaminant groups, most prominent in liver and muscle (with exception of ΣHCH). This undoubtedly indicated a mutual contamination source, which along with the influence of the breeding location contamination, in case of farmed tuna could have been the contaminated feed used for tuna diet. Vizzini et al., 2010 also highlighted the crucial requirement for rigorous criteria for site selection and appropriate feed choice for farmed tuna in order to reduce risk to consumers.

In order to put our results into perspective, in Table 2 we included studies whose results completely or partially could be compared to ours. In the 1990s in the Mediterranean Sea, tens of thousands of striped dolphins died and analyses revealed high levels of polychlorinated biphenyls in fish tissue as well as in liver and other organs (Kannan et al., 1993). Such an event would certainly affect the overall marine ecosystem. Our results on wild tunas sampled in 1996 in the Adriatic Sea (Klinčić et al., 2020) revealed PCB levels (range from 545 to 1800 ng g^{-1} lw) among the highest reported in literature. Chiesa et al., 2016 reported concentrations of PCBs in tuna samples from the Mediterranean (range from 25 to 1650 ng g^{-1} lw) much higher than those from the Indian, Pacific and Atlantic Ocean (range from 5 to 36 ng g^{-1} lw). High levels of pollutants in the Mediterranean Sea have been attributed to its shape of a semi closed basin, large population and many sources of agricultural, municipal, and industrial contamination. According to the data from Table 2, the ΣPCBs found in this study were lower than those reported for the Mediterranean area. Data for farmed tuna are scarce and the only study reporting levels in farmed tuna from any area close to the Croatian Adriatic is that by Vizzini et al., 2010 whose reported values for DDE, HCB and ΣPCBs considerably exceeded those found in our study.

To evaluate the potential negative effects of a POP mixture on human neuroblastoma cells, we used tuna liver samples extracts in which the highest POP concentrations were measured. This well-accepted *in vitro* model involves neurons as highly sensitive and one of the most important cells of our body (Yu et al., 2004; Xia et al., 2008; Kovalevich and Langford, 2013; Xie et al., 2010; Cheung et al., 2009). As the results indicate, under our experimental condition no cytotoxic effect was observed (Fig. 3) up to 100 ng mL^{-1}. This is in accordance with previously published studies on SH-SY5Y cells exposed to a PCB mixture reporting IC_{50} values from 3 to 5 μg mL^{-1} (Cocco et al., 2015;

Canzoniero et al., 2006), and the similar was reported for studies on other human cells as well (Mizukami-Murata et al., 2018; Rodriguez et al., 2018). In other words, toxicity effects such as damage to cell membranes and mitochondrial dysfunction (Tan et al., 2004; Ghosh et al., 2010; Cocco et al., 2015), occurred at concentrations that are generally much higher than the PCB concentrations commonly reported in fish populations (Vizzini et al., 2010; Kljaković-Gašpić et al., 2015).

From all of the mentioned results and comparisons, we can conclude that the Adriatic Sea farmed tuna investigated in this study was moderately contaminated with POPs, mainly PCBs, but the levels found were below all legal limits and do not pose risk for humans moderately consuming tuna meat. However, one should bear in mind that for assessing true human health risk it is crucial to monitor POP levels in human biological samples because food is only one of several routes of exposure.

CRediT authorship contribution statement

Darija Klinčić: Investigation, Validation, Writing - original draft. **Snježana Herceg Romanić**: Resources, Supervision, Funding acquisition. **Maja Katalinić**: Funding acquisition, Formal analysis, Writing - review & editing. **Antonio Zandona**: Methodology. **Tena Čadež**: Methodology. **Marijana Matek Sarić**: Formal analysis, Visualization. **Tomislav Šarić**: Formal analysis, Writing - review & editing. **Dejan Aćimov**: Conceptualization, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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