Title

Investigation of persistent organic pollutants (POPs) in sharks from Indonesia: Species-specific accumulation and implication for human exposure

Principle Investigator

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**Member of the project stated in the proposal*

Aim

- To determine the concentration and species-specific accumulation of PCBs in the muscle of different shark species from Indonesia
- To estimate the exposure to health risks derived from the consumption of sharks using the target hazard quotient (THQ) and the carcinogenic risk (CR)

Analytical Procedures

Samples. 54 **s**hark muscles, consisting of 20 shark species, were collected from landing ports in Tanjung Liar, West Nusa Tenggara in November 2021 and May 2022, and Aceh in September 2022 (**Table 1).** Muscle samples were collected from each animal and stored at -20 °C in a plastic ziplock bag until further analysis. For each specimen, sex and length (total length (TL) in cm) were recorded.

Chemical Analysis. The samples were then freeze-dried and stored in plastic ziplock. The freeze-dried sample (2-3 g) was transferred into a 50mL tube and extracted using a homogenizer (T 25 Digital Ultra-Turrax®; IKA Japan K.K.) with 20mL of acetone, 20mL of acetone/hexane (1:1, v/v, 2 times), and 20mL hexane.[1,2] The crude extract was concentrated, exchanged with hexane, and stored in an amber glass tube containing 10mL of hexane. Recovery checks for PCBs in shark samples and NMIJ CRM 7404-a—Organic Pollutants in Japanese Seabass Tissue, AIST, Japan, were performed using the standard laboratory method. Briefly, 1 mL of blank, shark, and CRM extracts were added to a different 10-mL test tube and spiked with surrogate standard PCBs. For the matrix spike standard, 20 µL and 200 µL of native PCBs were added to the low spike standard (LSS) and middle spike standard (MSS), respectively. All samples were analyzed in duplicate. Subsequently, all the extracts were cleaned using a multilayer silica gel column (44% and 22% sulfuric acid-impregnated silica gel) with elution solvents as a mixture of 10% dichloromethane in hexane[2]. The extracts were concentrated to 300 µL and purified using a semi-automated cleanup device with two cartridge columns: the First RAPiANA column (44% sulfuric acid-impregnated silica gel and activated silica gel) and the second column (alumina and 10% silver nitrate-impregnated alumina) (RAPiANA®; Miura Co., Ltd., Matsuyama, Japan).[3–6] Targeted PCBs were eluted from the concentration column (alumina and 10% silver nitrate-impregnated alumina) with 1.2 mL toluene, evaporated under a gentle nitrogen flow, and added syringe spike standard (PCB-ISS-H, Wellington Laboratories) before GC/MS analysis.

Instrumental analysis of PCBs. Using high-resolution GC-MS, 209 PCB congeners were identified (Agilent GC 6890N; Agilent Technologies, USA, and JMS-800D; JEOL, Japan). Anh et al. (2019) provided all details of the instrumental analysis for PCBs. The target compound concentrations were determined using the isotope dilution method and are presented in ng g^{-1} dry weight (dw).

Results and discussion

Recovery check of PCBs. We performed several analyses to ensure data quality, including analyses of procedural blanks, replicate standards, replicate samples, spiking surrogates, native standards, and NMIJ CRM 7404-a—Organic Pollutants in Japanese Seabass Tissue, AIST, Japan. The relative standard deviations (RSDs) for all detected compounds in the replicate samples were below 20%. Surrogate recovery ranged from 50% to 110% (**Figure 1**). While matrice spiked recoveries for the lower and middle concentrations of native PCBs were between 75–105% and 83–106%, respectively, except for PCB 194, 199, and 206, which were higher than 120% (**Figure 2**). The concentrations of the targeted PCB congeners in NMIJ CRM 7404-a, determined using our method, were comparable to the certified values (**Table 2**). The values of the analytical results in this study are shown in two significant digits based on the QA/QC.

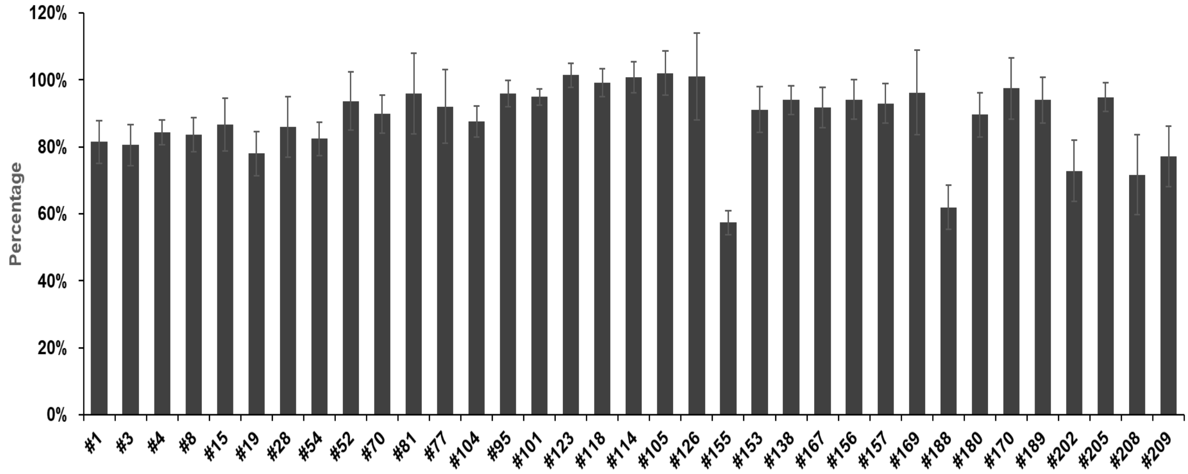

Figure 1. Recovery of $^{13}C_{12}$-PCBs mixture surrogate standard (n: 10)

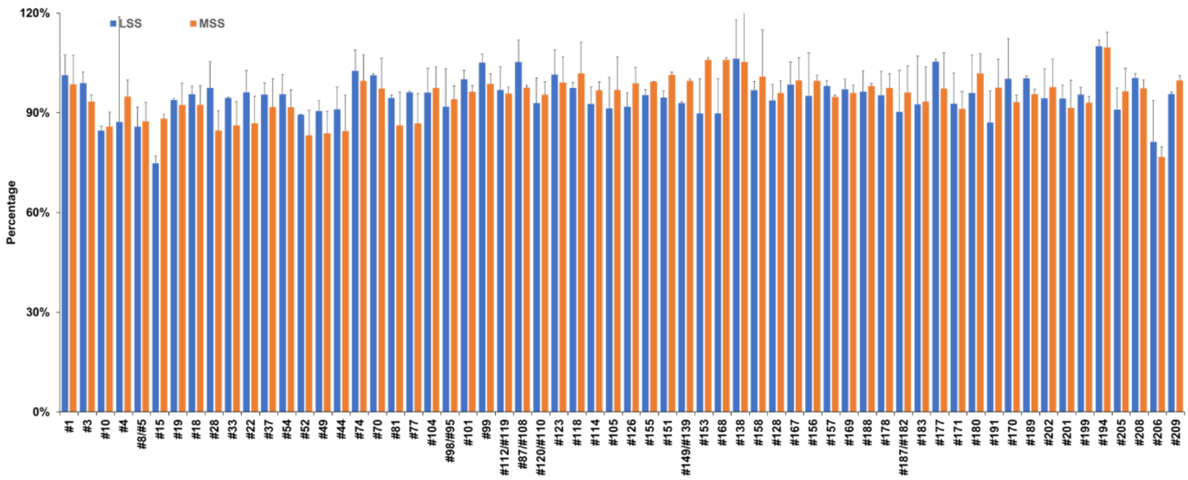

Figure 2. Recovery of matrice spike with native PCB standards. Low surrogate spike (LSS; n: 2) and Middle surrogate spike (MSS, n:2)

Table 2. Recovery of NMIJ CRM 7404-a (ng g^{-1} dw), (Mean±SD)

Compound	Certified value	Measured value 2018	Measured value 2023
CB-28	4.73 ± 0.58	4.41 ± 0.57	4.51
CB-70	5.7 ± 0.6	4.8 ± 0.5	5.76
CB-105	2.62 ± 0.27	2.24 ± 0.20	2.65
CB-138	14.0 ± 0.5	9.35 ± 0.96	12
CB-202	1.05 ± 0.06	1.01 ± 0.10	1.05

Future plan

As this study only analyzed PCBs in shark samples, we will continue our analysis of shark samples for other contaminants, including organic chlorinated pesticides (OCPs), brominated flame retardants (BFRs), and polycyclic aromatic hydrocarbons (PAHs). In addition, stable isotope δ13C and δ15N analyses will be beneficial for determining feeding habits. Finally, we used the target hazard quotient (THQ) and carcinogenic risk (CR) to estimate the exposure health risks derived from the consumption of sharks.

Reference

(1) Takahashi, S.; Oshihoi, T.; Ramu, K.; Isobe, T.; Ohmori, K.; Kubodera, T.; Tanabe, S. *Mar. Pollut. Bull.* **2010**, *60* (2), 187–196.

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(3) Hong, J.; Miki, Y.; Honda, K.; Toita, H. *Chemosphere* **2012**, *88* (11), 1287–1291.

(4) Hoang, A. Q.; Aono, D.; Kawashima, A.; Hamada, N.; Falahudin, D.; Watanabe, I.; Tsugeki, N. K.; Kuwae, M.; Takahashi, S. *Chemosphere* **2021**, *281*, 130867.

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(6) Takahashi, S.; Anh, H. Q.; Watanabe, I.; Aono, D.; Kuwae, M.; Kunisue, T. *Sci. Total Environ.* **2020**, *743*, 140767.

Table 1. Shark samples from Tanjung Luar, NTB and Aceh, Indonesia, TL: Total Length, SL: Standard length

No	Spesies	Tanjung Luar, NTB, Eastern of Indonesia				Aceh, Western of Indonesia				
		Date	Sex	#	TL/SL (cm)	Date	Sex	#	TL (cm)	SL (cm)
1	*Sphyrna lewini*	17/11/21	F	3	210–260 (231)		F	1	102	67
2	*Alopias pelagicus*	19/11/21	F	2	/110-263 (186)		M	3	243-290 (267)	131-228 (196)
3	*Prionace glauca*	19/11/21	F	2	223-285 (254)		M	1	239	215
		30/05/22	F	1	316	01/09/22				
4	*Carcharinus falciformis*	17/11/21	F	2	220-242 (231)		F	2	NA	NA
		30/05/22	F	1	233					
5	*Galeocerdo cuvier*	21/11/21	M	2	232-264 (248)		F	3	243-251 (248)	165-168 (166)
			F	1	232					
6	*Alopias superciliosus*						F	3	203-302 (246)	
7	*Carcharhinus obscurus*	17/11/21	F	2	267-318 (293)					
8	*Centrophorus lusitanicus*	12/10/21	F	4	120-158 (139)					
9	*Carcharinus leucas*	20/11/21	F	1	237					
10	*Dalathias licha*	12/10/21	F	1	127					
11	*Isurus oxyrinchus*	19/11/21	F	2	195-198 (197)					
12	*Isurus paucus*	19/11/21	F	1	176					
13	*Carcharhinus obscurus*	29/05/22	F	1	270					
14	*Isurus oxyrinchus*	30/05/22	F	1	197					
15	*Squalus montalbani*	05/06/22	F	4	71-86 (76)					
16	*Squalus nasutus*	05/06/22	F	3	51-62 (57)					
17	*Cephaloscyllium pictum*	05/06/22	F	3	63-72 (68)					
18	*Squalus hemipinnis*	05/06/22	F	1	75					
19	*Squalus edmundsi*	05/06/22	M	2	50-55 (53)					
20	*Mustelus stevensi*	05/06/22	M	1	71					