Form 3

Annual Report

LaMer, Ehime University

Date (12, March, 2019)

To Director of LaMer

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Include the report on the result of the project~~/meeting~~ in a separate sheet.

1. Project / ~~Meeting~~ title

Analysis of persistent organic pollutants in coelacanths (*Latimeria chalumnae*) from Indonesia

2. Members of project / ~~meeting~~

Name	Affiliation	Position	Contribution part
PI Dede Falahudin	Research Center for Oceanography, Indonesian Institute of Science (LIPI)	Researcher	Analysis samples and write a report
Members Agus Sudaryanto	Agency for the Assessment and Application of Technology (BPPT)	Researcher	Prepared proposal of project
LaMer Faculty member in charge Shin Takahashi	CMES and Graduate School of Agriculture, Ehime University	Professor	Prepared for a report

3. Contents (please write in separate sheet, A4-size, within 5 pages including figures and tables. Itemize “Title, members’ names and affiliations, aim, procedure, result, publication/conference presentation, perspectives in future”).

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Project Report

Analysis of Persistent Organic Pollutants in the Coelacanths (*Latimeria chalumnae*) from Indonesia

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**Member of project stated in the proposal*

1. Introduction

During the last few decades, contamination by anthropogenic chemicals such as persistent organic pollutants (POPs) has spread all over the world. As evidence, they were detected in various environmental components and biota including those far from human activities. Particularly, research efforts on field observations and numerical models of global fate of POPs have revealed oceanic water bodies to be a global reservoir and final sink for these toxic contaminants that undergo transport from emission sources and partition between air and water and scavenge to deep-sea layers by various biogeochemical and geophysical processes (Takahashi *et al.*, 2014). Various classes of POPs such as organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and other brominated flame retardants (BFRs) have been detected in deep-sea organisms from different parts of the world (de Brito *et al*., 2002, Lee *et al.*, 1997, Takahashi *et al*., 1998, Jamieson *et al.*, 2017).

There were several reasons on investigation of persistent organic pollutants in the deep-sea organisms such as to know their fate, transport and the specific accumulation characteristics these toxicants. Afterward, we will able to measure their transport along oceanic system and observe deep-sea environment as final location for these persistent contaminants. Further, to implement and evaluate the effectiveness of international agreements to protect the marine environment from the deleterious effects of POPs, interdisciplinary approaches including studies on biogeochemical and geophysical processes in the ocean as well as field observations are required to delineate the global and regional

fate of POPs (Takahashi *et al*., 2014).

2. Aim of Study

The aim of this study is to determine the occurrence of POPs and related compounds, comprising PCBs, PBDEs, and selected novel brominated flame retardants (NBFRs) in different tissues of coelacanth (*Latimeria menadoensis*) obtained from Indonesia.

3. Analytical Procedures

Samples. Three tissue samples (muscle, liver, and ovary) were sampled from the specimen of female sea king fish (Indonesian Coelacanth, *Latimeria menadoensis*) with CCC No. 299 that caught by gill net on November 5, 2014 around Gangga Island, North Sulawesi. This specimen was had a body length of 130cm and weight of around 22kg.

Chemical analysis. Tissue samples were freeze-dried and grinded into fine powder by using mortar and pestle. The sample (2 to 3 g) was transferred into a 50mL tube and subsequently extracted with 20mL acetone, 20mL a mixture of acetone/hexane (1:1, v/v) and 20mL hexane by using a homogenizer (T 25 Digital Ultra-Turrax®; IKA Japan K.K.). The crude extract was concentrated and solvent-exchanged into hexane. A portion of extract corresponding to 1 g of sample was used for BFR and PCB analysis. Before purification, the extract was spiked with surrogate standards of PBDEs (monofluorinated FBDE-15, -99, -183, -208, and $^{13}C_{12}$-BDE-209) and PCBs (Congener, $^{13}C_{12}$-PCB-1, -3, -4, -8, -15, -19, -28, -52, -54, -70, -77, -81, -95, -101, -104, -105, -114, -118, -123, -126, -138, -153, -155, -157, -167, -169, -170, -180, -188, -189, -202, -205, -208, and -209). The extract was cleaned up by passing through a multilayer silica gel column and an activated silica gel column with elution solvents as mixtures of 10% and 5% dichloromethane in hexane, respectively. The eluate was concentrated and spiked with internal standards of PBDEs (FBDE-154) and PCBs ($^{13}C_{12}$-PCB-9, -37, -79, -111, -162, -194, and -206) before GC/MS quantification. Chemicals and solvents used in this study were reagent grade for the determination of PCBs and purchased from Wako Pure Chemical Industries, Ltd.

Instrumental analysis for BFRs. Thirty eight PBDEs (di- to decabrominated congeners, including BDE-7, -10, -15, -17, -28, -30, -47, -49/71, -66, -77, -85, -99, -100, -119, -126, -138, -139, -140, -153, -154, -156/169, -171, -180, -183, -184, -191, -196, -197, -201, -203, -204, -205, -206, -207, -208, -209) and four NBFRs (such as PBEB, BB-153, BTBPE, DBDPE) were quantified using a gas chromatograph connected to a quadrupole mass spectrometer (GCMS-QP2010 Ultra, Shimadzu). BFRs were separated on a fused-silica capillary column (DB-5ht, 15 m × 0.25 mm × 0.1 μm, Agilent Technologies). Helium was used as carrier gas. Temperature of injection port was 260°C. Initial column oven temperature was 135 °C for 1 min, increased to 215 °C (10 °C min^{-1}), to 275 °C (5 °C min^{-1}), to 295 °C (20 °C min^{-1}, held 0.5 min) and finally raised at 20 °C min^{-1} to 310 °C and held for 4 min. The mass spectrometer

was operated in electron capture negative ionization (ECNI) mode. Methane was used as moderating gas. The temperature of the interface and ion source was 310 °C and 250 °C, respectively. Twelve ions were selectively monitored, including: m/z = 79.0/81.0 and 158.8/160.8 (Br^- and HBr_2^-, for all compounds); 406.6/408.6 and 486.5/488.5 ($C_6HBr_4O^-$ and $C_6Br_5O^-$, for hepta- to decaBDEs); 426.5/428.5 ($C_6FBr_4O^-$, for FBDE-208); and 496.6/498.5 ($^{13}C_6Br_5O^-$, for $^{13}C_{12}$-BDE-209).

Instrumental analysis for PCBs. PCBs (209 mono- to decachlorinated congeners) were quantified using a 6890N gas chromatograph (Agilent Technologies) connected to a JMS-800D high resolution mass spectrometer (JEOL). The separation was performed on a HT8-PCB capillary column (60 m length × 0.25 mm internal diameter × 0.25 μm film thickness, Kanto Chemical). Helium was used as carrier gas at a constant flow of 1 mL min^{-1}. Inlet temperature was 280 °C. A sample volume of 1 μL was injected in splitless injection mode. Column oven temperature was programmed from 120 °C, increased to 180 °C (20 °C min^{-1}), to 260 °C (2 °C min^{-1}), and ramped to 300 °C (5 °C min^{-1}, hold 4 min). Mass spectrometer was operated in positive electron ionization (EI) mode at a resolution of ≥ 10,000 at 10% valley. Ionization energy was 38 eV and acceleration voltage was 10 kV. Temperature of interface and ion source was 280 °C. Data were acquired in selected ion monitoring (SIM) mode using two molecular ions for each native and $^{13}C_{12}$-PCB congener.

Lipid content. Sub samples (1mL) of extract were added to the pre-weighed porcelain crucible and record the weight. After that, the solvent was evaporated and the porcelains were re-weighed again to calculate total lipid content (%) for each sample.

Quality assurance and quality control (QA/QC). All methods for PBDEs and PCBs analysis were validated by the replicate analysis of standards and samples, spiking surrogate standard and analysis of SRM (Table 1).

Table 1. Parameter of QC/QA analysis of coelacanth samples

Parameter	PBDEs	PCBs
Results of SRM Mussel NIST 2974a	Accuracy 57–101%	Accuracy 55–98%
Results of CRM Seabass NMIJ 7404a	–	Accuracy 71–95%
Results of duplicate samples	RSD < 15%	RSD < 15%
Recoveries of surrogate standards	70–120%	60–110%
Method detection limits	0.01–0.1 ng/g wet wt.	0.003–0.3 pg/g wet wt.

4. Results and discussion

Concentration of BFRs in Coelacanth. Percentages of lipid content varied in different tissue of coelacanth, with liver tissue having higher percentage of lipid content (71.79%) than muscle (10.57%) and ovary (1.40%). These results agree with other study that deep-sea fish have high percentage of lipid on their liver where the processes of metabolism and storage of xenobiotic compounds occurred (Covaci *et al.*, 2020). Therefore, on this report, concentration

of PBDEs and NFBRs were normalized with lipid content and reported on lipid weight, l.w., basis. In general, concentration of Σ_9PBDE in coelacanth ranged from 0.93 to 1.94 ng/g l.w. with average of 1.5 ng/g l.w (Table 2). Variation of residue value of PBDE in different tissue of coelacanth was influenced by several factor including physical and chemical properties of congeners, biotransformation processes, metabolism and also permeability of membrane (Ma *et al*., 2013).

Due to different on the number of PBDEs congener determined, sample types, and the methods of samples analyzed, comparison of PBDEs concentrations to other study were limited and quite challenging. However, previous study reported that composition of PBDE congener in biota is similar over the world with the most detected are BDE-47, BDE-99, BDE-100, BDE-153, and BDE-154, which all of congener was as flammable agent on the commercial penta-BDE mixture consumer products (Covaci *et al.,* 2020). Therefore, to understand the status of PBDEs pollution in coelacanth from Indonesia, residue levels of PBDE found in the present study were compared only limited on congener basis including BDE-17, -28, -47 and BDE-99 (Table 3). The sum of Σ_4PBDE (1.36 ng/g l.w.) in muscle tissue of coelacanth from this study were slightly higher than the level reported for deep-sea fishes from NW Mediterranean (Koenig *et al.*, 2013) and the Sulu Sea (Ramu et al., 2013), except species *Bathygadus sp* and *Lamprogrammus niger*. In contrast, the result from this study was lower than Σ_3PBDE from two Mediterranean deep-sea fishes *Trachyrinchus trachyrinchus* and *Coelorhynchus coelorynchus* (Covaci *et al.*, 2020).

Table 2. Concentrations of PBDEs and NBFRs in different tissue samples of coelacanth (ng g^{-1} lipid weight) collected Indonesia

Compound	MDL	Muscle	Liver	Ovary
BDE-7	0.01	< 0.01	ND	0.03
BDE-10	0.01	ND	ND	ND
BDE-15	0.01	0.09	0.17	ND
Σ_3 Di-BDEs		**0.10**	**0.17**	**0.03**
BDE-17	0.01	0.26	0.14	0.71
BDE-28	0.01	0.18	0.13	ND
BDE-30	0.01	ND	ND	ND
Σ_3Tri-BDEs		**0.44**	**0.28**	**0.71**
BDE-47	0.02	0.63	0.27	1.19
BDE-49/71	0.02	0.19	0.14	ND
BDE-66	0.02	ND	ND	ND
BDE-77	0.02	ND	ND	ND
Σ_4Tetra-BDEs		**0.82**	**0.41**	**1.19**
BDE-85	0.04	ND	ND	ND

BDE-99	0.03	0.29	ND	ND
BDE-100	0.03	ND	ND	ND
BDE-119	0.05	ND	ND	ND
BDE-126	0.05	ND	ND	ND
Σ_5 Penta-BDEs		**0.29**	**0.00**	**0.00**
BDE-138	0.04	ND	0.07	ND
BDE-139	0.02	< 0.02	ND	ND
BDE-140	0.02	ND	ND	ND
BDE-153	0.03	ND	ND	ND
BDE-154	0.05	ND	ND	ND
BDE-156/169	0.05	ND	ND	ND
Σ_7 Hexa-BDEs		**0.00**	**0.07**	**0.00**
Σ_5 Hepta-BDEs		ND	ND	ND
Σ_6 Octa-BDEs		ND	ND	ND
Σ_3 Nona-BDEs		ND	ND	ND
BDE-209		ND	ND	ND
Total Σ_{36} PBDEs		**1.65**	**0.93**	**1.94**
PBEB	0.01	ND	ND	ND
BB-153	0.04	ND	ND	ND
BTBPE	0.03	ND	0.15	ND
DBDPE	0.10	ND	ND	ND
Total Σ_4 NBFRs		**0.00**	**0.15**	**0.00**
Lipid content (%)		10.57	71.79	1.40

Congener profiles and potential sources of BFRs. The distribution pattern of PBDE congeners in coelacanth tissue was different to the typical pattern of deep-sea organism investigated from other location in the world, where higher congener (more than hexa-BDEs) usually detected (Koenig *et al.*, 2013). In addition, undetected higher congener of PBDE in this study was potentially due to characteristics of higher PBDE that have low bioaccumulation potential and easily to degrade into lower brominated PBDE congeners via debromination (Letcher *et al*., 2014). Based on results from this study, of the 36 PBDE congeners evaluated, only nine congeners (*BDE-7, -15, -17, -28, -47, -49/71, -99 and -138)* and one (BTBPE) of four NFBR's congener analyzed were detected in coelacanth tissues. All detected congener mainly lower brominated congener (di- to tetra-BDEs) with BDE-17 and BDE-47 were the most detected congener up to 24% and 45%, respectively. This result coherence with previous studies that also have observed high concentration of BDE-47 in the

liver of Mediterranean deep-sea fish up to 50% and also in the amphipod from Mariana Trench and the Kermadec Trench, showed predominance of BDE-47 account for 71% of the ΣPBDE concentration (Jamieson *et al*., 2017).

Furthermore, to observe the origins or biodegradation capacity of coelacanth on PBDE contamination, further analysis is needed including using ratio between BDE-99/BDE-100, BDE-99/BDE-47, BDE-153/BDE-154 and BDE-183/BDE-154 (Ma *et al.*, 2013).

5. Perspectives in future

We have found clear evidence of the expansion of contamination by emerging POPs including PBDEs and NBFRs into the habitat of coelacanth. Even though the concentration was smaller than other concentration of POPs found in deep-sea organism, presence of POPs accumulation in different part of coelacanth tissue would be as alarm for us to stop release of such chemical into the sea. Furthermore, due to specific congener already found in the coelacanth, further analysis is needed to investigate fate and sources of PBDEs in the coelacanth fish. In addition, including biogeochemical and geophysical processes on study of marine contaminant in the depp-sea region are required to delineate the global and regional fate of POPs.

Chemical analysis is continued to determine PCBs and other potential emerging organic contaminants.

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