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DEVELOPMENT OF AN ANALYTICAL METHOD AND PRELIMINARY ASSESSMENT OF HEALTH RISKS OF ORGANOPHOSPHATE ESTERS IN VARIOUS MARINE FISH SPECIES IN VIETNAM

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LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

1. **Thanh-Thien Tran-Lam**, Phuong Thi Pham, Minh Quang Bui, Yen Hai Dao, Giang Truong Le, *Organophosphate esters and their metabolites in marine fish from Vietnam: Analytical method development and validation*, *Journal of Food Composition and Analysis*, 2024, 1-13, SCIE; IF – 4.0.
2. **Thanh-Thien Tran-Lam**, Phuong Thi Pham, Minh Quang Bui, Yen Hai Dao, Giang Truong Le, *Organophosphate esters and their metabolites in silver pomfret (Pampus argenteus) of the Vietnamese coastal areas: Spatial-temporal distribution and exposure risk*, *Chemosphere*, 2024, 1-14, SCIE; IF – 8.1.
3. **Tran Lam Thanh Thien**, Pham Thi Phuong, Bui Quang Minh, Dao Hai Yen, *Lifetime bioaccumulation and tissue distribution of organophosphate triesters in silver pomfret (Pampus argenteus) of the Northern coastal areas of Vietnam*, *Vietnam Journal of Science and Technology*, accepted on 09/23/2024, Scopus category.

INTRODUCTION

Justification for the thesis

In recent years, the rapid advancement of the worldwide chemical industry has resulted in a notable rise in the use of flame retardants, particularly organophosphate esters (OPEs). OPEs are extensively used in the manufacturing of plastics, textiles, electronics, building materials, and many consumer items due to their efficient flame-retardant characteristics and versatile chemical structure. The manufacturing, use, and disposal of items containing OPEs lead to the emission of these chemicals into the environment. Recent research in several Asian and European nations has identified OPEs in wastewater, sludge, sediments, and notably in aquatic life. Preliminary survey findings in Vietnam have shown the presence of OPEs in surface water, air, and marine fish, which constitute a significant component of the food chain for the population. Research conducted by Bui Quang Minh et al. (2023) identified ΣOPE levels between 39.0 and 181.5 ng/g dry weight in marine fish samples obtained from local markets in Hanoi. The results indicate that OPEs are prevalent in the environment and have a tendency to bioaccumulate in marine organisms.

Numerous OPEs possess the capacity for bioaccumulation and dissemination along the food chain. Consumption of marine fish contaminated with OPEs may lead to their absorption in the human body, resulting in detrimental consequences including endocrine disruption, neurological damage, metabolic problems, and an increased risk of cancer. Specifically, once entering the body, OPEs may undergo biotransformation into metabolites (di-OPEs), for which toxicity and human health impact data remain few and have not been adequately included into existing risk assessments.

Despite brominated flame retardants, including polybrominated

diphenyl ethers (PBDEs), being a focal point for research due to their significant accumulation and toxicity, numerous countries have prohibited or limited their use following the inclusion of PBDEs in the control list of the Stockholm Convention in 2009, resulting in a shift towards OPEs as preferred substitutes. Although the toxicity of PBDEs has been extensively researched, the toxicity profile and long-term effects of OPEs, particularly di-OPE metabolites, remain little understood. This is an immediate need for study on OPEs, particularly with the limited data on their contamination levels and effects in Vietnam.

At present, Vietnam has not established a threshold level for OPE concentration in food, nor are there defined analytical procedures for monitoring these substances in biological samples. Concurrently, research on OPE in food and seafood, particularly marine fish, remains disjointed and unstructured. In light of the aforementioned deficiencies and scientific voids, the researcher chooses to research the thesis topic: "*Development of an analytical method and preliminary assessment of health risks of organophosphate esters in various marine fish species in Vietnam*".

The research objectives

- Development of an analytical method and validation of the analytical method for OPEs and di-OPEs in various marine fish species using UHPLC-HRMS.
- Assessment of the occurrence of OPE and di-OPE in various marine fish species obtained from major fishing ports throughout three regions of Vietnam, along with an investigation of the correlation between OPE concentration and the growing environment of each species.
- Preliminary health risk assessment of OPE in humans via marine fish consumption.

The content of the thesis

- Optimization of the method for the simultaneous extraction of OPE and di-OPE compounds in fish tissue, followed by analysis using high-performance liquid chromatography combined with high-resolution mass spectrometry.
- Method validation for the analysis of OPEs and di-OPEs in fish muscle tissue.
- Investigation of OPE and di-OPE concentrations in acquired fish specimens. The analytical findings assessed the distribution of OPE in marine fish obtained from fishing ports in the three research locations, as well as the correlation between OPE and di-OPE in marine fish.
- Preliminary assessment of consumer health risks associated with exposure to OPE via the gastrointestinal system.

The scientific and practical significance of the thesis

The investigation into the analysis and initial assessment of health risks associated with OPEs in certain marine fish species is a novel research avenue in Vietnam. The thesis focuses on optimizing and developing a methodology to determine OPE and di-OPE concentrations in marine fish, so establishing a foundation for evaluating the disparities in OPE and di-OPE contamination patterns across fish species and assessing the implications for human health.

Novel contributions of the thesis

- This study is the first investigation to develop and validate a methodology for the simultaneous analysis of OPEs and di-OPEs in marine fish tissue using ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry, thereby enhancing efficiency and expediting the analytical process.
- This thesis is the first study to examine the occurrence and

distribution of OPEs and di-OPEs in various marine fish species gathered from various fishing ports in the northern, central, and southern regions of Vietnam. The fish species exemplify several ecological strata (surface, midwater, and benthic layers), enabling an initial evaluation of the distribution of OPEs based on their habitat.

- Initial assessment of health risks associated with OPE exposure via marine fish intake. This aids in supplying scientific evidence to assist regulatory agencies in establishing OPE residue limits in food and overseeing food safety.

II. CONTENT OF THESIS

CHAPTER 1. RESEARCH BACKGROUND

The literature review section consolidates prior research on the topics.

1.1. Introduction of flame retardants

1.2. Organophosphate flame retardants

1.2.1. Application as flame retardant

1.2.2. Application as plasticizer

1.2.3. Utilization of OPE flame retardants

1.2.3.1. Global state of OPE utilize

1.2.3.2. Vietnam's state of OPE utilize

1.3. Physical and chemical properties of OPE and applications

1.4. Toxicity of OPE

1.5. Metabolites

1.6. Environmental presence of OPE

1.6.1. Sources of OPE emissions in the environment

1.6.2. Occurrence and factors affecting the distribution/metabolism of OPEs in the marine ecosystem

1.6.3. Origin and metabolism of OPEs in marine organism

1.7. Current state of marine fishing and consumption in Vietnam

+ Southern region: Ba Ria - Vung Tau, Ho Chi Minh City, Tien Giang

2.2. Research methodology

2.3. Chemicals, instruments and apparatus

2.4. Sample collection and preservation

Marine fish specimens (223 samples) were obtained from ports and major seafood markets in three areas of Vietnam. The three fish species used for this experiment were silver pomfret (*Pampus argenteus*, n = 172), yellowstripe scad (*Salaroides leptolepis*, n = 32), and flounder (*Psettodes erumei*, n = 19), obtained from the aforementioned sites between 2021 and 2023. The fish samples were meticulously covered in aluminum foil, preserved in a cooler with dry ice, and transported to the laboratory. Subsequently, the fish specimens were weighed, skinned, and filleted to exclude bones and heads. The fish fillet samples were dried using dust-free paper, frozen at -20°C, and freeze-dried until a consistent mass was achieved. Subsequent to freeze-drying, the materials were pulverized and preserved in opaque glass containers at -20°C until analysis.

2.5. Experimental research content

2.5.1. Development of a UHPLC-HRMS analytical methodology

Examine the optimal parameters for the analysis of OPE and di-OPE, including electrospray ionization, flow rate, and column oven temperature, using UHPLC-HRMS. Assess the stability of the analytical signal in UHPLC-HRMS. Ascertain the instrument detection limit and instrument quantification limit of OPE and di-OPE using UHPLC-HRMS.

2.5.2. Development of sample preparation method

2.5.2.1. Prepare blank sample

A blank sample was generated utilizing silver pomfret tissue after homogenizing the extracted sample using a cold acetonitrile solvent to

eliminate analytes, minimally affecting the complex properties while preserving the features of the sample matrix.

2.5.2.2. Examination of preliminary sample processing techniques

Figure 2.1 illustrates the phases in the sample preparation approach for quantifying OPE and di-OPE concentrations in fish.

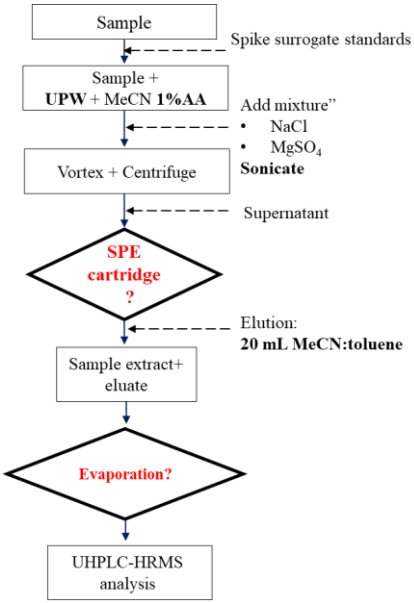

Figure 2.1. All stages in the sample preparation neeed modification

2.5.2.3. Optimizing solvent evaporation conditions

The impact of solvent vaporization conditions was examined in three scenarios: (i) rotovap, (ii) vaporization under N_2 flow at ambient temperature, and (iii) a combination of vacuum centrifugation and vaporization under N_2 flow at 1°C.

2.5.2.4. Assessment of pure extract materials

The effectiveness of 10 commercial purifying substances was examined and assessed. The EMR-WAX material was employed to assess the impact of sample matrix in fish with varying fat content.

2.5.3. Method validation

Following the optimization of the sample processing procedure, the method parameters including the method detection limit (MDL), method quantification limit (MQL), accuracy, reproducibility, repeatability and matrix effect were assessed to validate the method for the analysis of OPE and di-OPE.

2.5.4. Analysis of OPE and di-OPE content in marine fish samples

The marine fish samples collected in section 2.4 were analyzed to determine the OPE content in the samples. Specifically, 223 fish samples (172 silver pomfret samples, 19 flounder samples and 32 yellowstripe scad samples) were analyzed to determine OPE and di-OPE in fish muscle samples.

2.5.5. Assessment of OPE exposure risk in fish consumption

2.5.5.1. Estimated daily exposure

Estimated daily intake (EDI, ng/kg/day) and lifetime average daily dose (LADD, ng/kg/day) through consumption of marine fish was calculated using the formula 2.1 and 2.2:

$$EDI = \frac{C_i \times IR}{BW} \quad (2.1)$$

$$LADD = \frac{C_i \times IR \times RF \times ED}{BW \times AT} \quad (2.2)$$

2.5.5.2. Non-carcinogenic risk assessment

The hazard quotient (HQ) and hazard index (HI) are used to describe the non-carcinogenic risks of OPEs via ingestion for each specific compound and the sum of the OPE compounds. The HQ and HI are calculated as formula 2.3.

$$HI = \sum_{i=1}^{n} HQ_i = \sum_{i=1}^{n} \frac{EDI_i}{RfD_i} \quad (2.3)$$

Where, R*f*D is the oral reference dose (ng/kg/day). If HI > 1, OPE exposure in fish has adverse health effects. Conversely, HI < 1 indicates that the potential adverse health effects on humans from consuming marine fish are negligible.

2.5.5.3. Cancer risk assessment

Incremental lifetime cancer risk (ILCR) is often used for chemicals recognized as carcinogenic due to prolonged exposure, including TCEP and TnBP. ILCR is computed via formula 2.4.

$$ILCR = OSF \times LADD \quad (2.4)$$

2.5.6. Data analysis

This study utilized the R program (version 4.3.2) to process data, evaluate statistics, and present results visually. Calculations, statistical analyses, and package programs were utilized in R.

CHAPTER 3. RESULTS AND DISCUSSION

3.1. Optimization of UHPLC-HRMS conditions

3.1.1. Optimizing electrospray ionization conditions

Utilizing Central Composite Face-Centered (CCF) experimental design to optimize the flow rates for sheath gas, auxiliary gas, and sweep gas. K-fold cross-validation (k = 10) was used to validate and verify the model's consistent performance. The ideal parameters for sheath gas, auxiliary gas, and sweep gas at the mass spectrometer's ionization source were 20 arb, 15 arb, and 5 arb, respectively.

3.1.2. Optimize flow rate and temperature

This study determined that a column temperature of 45°C and a flow rate of 0.4 mL/min are appropriate conditions for liquid chromatography analysis, based on the evaluation of the effects of flow rate and temperature on the resolution and asymmetry coefficient of chromatographic peaks.

3.1.3. Optimal conditions for UHPLC-HRMS

This study presents the UHPLC-HRMS conditions, detailed in Tables 3.1 and 3.2, derived from surveys on the most optimal circumstances for analyte molecule ionization in ESI mode and UHPLC parameters.

Table 3.1: UHPLC operating conditions

Components	**Parameter**			
Column	Hypersil Gold™ aQ (150 mm × 2,1 mm, 3,0 µm particle size)			
Flow rate	0.40 mL/min			
Oven temp.	45°C			
Injection volume	3.0 µL			
Injection solvent	MeOH/UPW = 1/1 (*v*/*v*)			
Mobile phase	(A) UPW containing 0.1%FA (pH_w^w=2.68)			
	(B) MeOH			
Gradient	**Time (min)**	**%A**	**%B**	**Curves**
	0.0	100	0	
	0.5	100	0	5
	2.0	60	40	1
	22.0	0	100	5
	22.1	100	0	5
	25.0	100	0	5

Table 3.2: HRMS operating conditions

Ion source		**Mass analyzer**	
Parameter	***Value***	***Parameter***	***Value***
Spray voltage (kV)	+4.9, -3.5	*Q1 resolution (FWHM)*	35,000
Sheath gas (arb)	20	*Q3 resolution (FWHM)*	17,500
Auxiliary gas (arb)	15	*AGC target*	1×10^5
Sweep gas (arb)	5	*Maximum IT*	50 ms
Transfer tube temp. (℃)	320	*Collision gas*	Ar (5.0) Rate: 2 arb
Solvent evap temp.(℃)	300		

Table 3.3: OPE and di-OPE mass-spec conditions and retention time

Compound	Surrogate	Retention time (min)	Mode	Precursor (Da)	Fragmentation ion (Da) with collision energy (V)	
					Quantitative ion (Da)	Confirm ion (Da)
TMP	TEP-d_{15}	2.97	+	141.1	79.1 (22)	109.0 (16)
TEP	TEP-d_{15}	4.74	+	183.1	99.0 (18)	127.1 (12)
TnPrP	TnPrP-d_{21}	9.42	+	225.2	99.1 (17)	141.1 (10)
TIPrP	TnPrP-d_{21}	8.56	+	225.2	99.1 (17)	141.1 (10)
TnBP	TnBP-d_{27}	13.88	+	267.1	99.0 (17)	155.0 (10)
TiBP	TnBP-d_{27}	13.58	+	267.1	99.0 (17)	155.0 (10)
THP	TEHP-d_{51}	19.14	+	351.2	99.1 (19)	267.1 (10)
TBOEP	TnBP-d_{27}	14.60	+	399.2	199.0 (15)	299.1 (13)
TEHP	TEHP-d_{51}	21.48	+	435.2	99.0 (38)	113.3 (38)
TPhP	TPhP-d_{15}	12.80	+	327.2	77.1 (38)	152.1 (38)
CDP	DPhP-d_{10}	12.64	+	341.1	152.0 (35)	299.0 (26)
EHDPHP	TnBP-d_{27}	14.01	+	363.1	174.0 (35)	251.0 (8)
TCP	TEHP-d_{51}	16.12	+	369.0	166.1 (29)	243.1 (28)
TCEP	TEP-d_{15}	5.63	+	285.0	63.0 (24)	99.0 (22)
TDCIPP	TnPrP-d_{21}	12.15	+	430.8	99.0 (26)	209.0 (15)
DMP	BCEP-d_{8}	2.90	–	125.0	79.0 (25)	63.0 (18)
BCEP	BCEP-d_{8}	4.61	–	221.0	35.2 (10)	103.0 (10)
DPhP	DPhP-d_{10}	9.11	–	249.1	93.1 (30)	155.0 (24)
BDCIPP	BBOEP-d_{8}	8.55	–	319.0	35.2 (10)	283.0 (10)
DnBP	DnBP-d_{18}	8.72	–	208.5	78.5 (56)	152.9 (50)
BBOEP	BBOEP-d_{8}	8.12	–	297.2	79.0 (30)	197.1 (20)
BEHP	BEHP-d_{34}	15.02	–	321.3	209.1 (15)	79.0 (39)

3.1.4. Signal stability of OPE and di-OPE assessment via UHPLC-HRMS

The assessment results indicated that the UHPLC-HRMS apparatus employed for OPE and di-OPE analysis produced robust analytical signals with significant stability.

3.1.5. Limit of detection and limit of quantification of UHPLC-HRMS

The calculated limits of detection and limits of quantification of the device were relatively small and stable across substances, ranging from 0.06 to 0.09 pg and 0.20 to 0.30 pg, respectively.

3.1.6. Standard curves of OPE và di-OPE

Standard curves for OPE and di-OPE were established by correlating substance concentrations (1.0-100.0 ng/mL) with peak areas, resulting in standard curve equations with linear regression coefficients above 0.995. The average relative bias was less than 5.0%, and the substantial slope coefficient indicated the method's excellent sensitivity.

3.2. Development of a sample preparation method for analyzing OPE

3.2.1. Optimizing solvent evaporation conditions

The study's results indicated that the solvent vaporization technique markedly influenced analyte recovery, yielding average recoveries of 66.3% (rotovap at 10 mbar and 40°C), 80.8% (N_2 gas at ambient temperature), and 97.2% (vacuum centrifugation combined with N_2 gas at 1°C) (figure 3.1); this variation was statistically significant (ANOVA, $p < 0.05$).

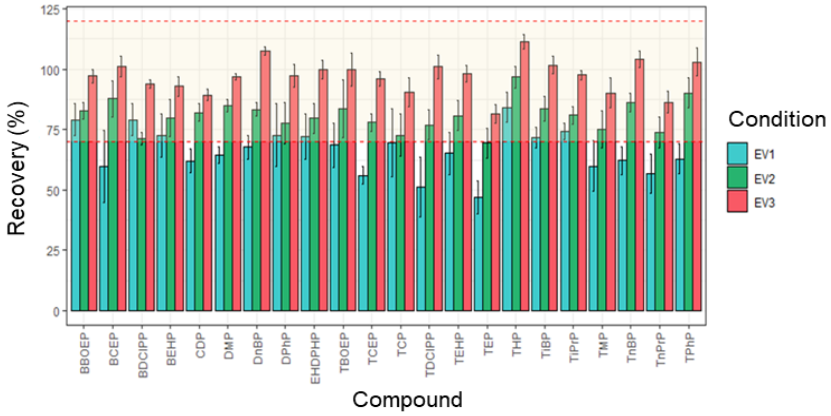

Figure 3.1: Recoveries of compounds under vaporization conditions

Vacuum evaporation results in sample loss due to "bumping" during phase transition, while EV2 (N_2 blowing at ambient temperature) mitigates this issue but extends evaporation duration and poses a danger of analyte oxidation. Conversely, EV3 – using vacuum centrifugation at 10 mbar and 40°C alongside N_2 gas flow at 1°C – proficiently regulates the evaporation process, minimizes sample loss, and decreases processing duration, so yielding the best and most uniform recoveries. EV3 was shown to be the most effective evaporation technique for precise quantitative analysis.

3.2.2. Results of selection of pure metarials

ANOVA analysis indicated that the disparity in compound recovery using the examined adsorbent materials was statistically significant ($p = 1.8\times10^{-21} < 0.05$). All compounds exhibited recoveries within the acceptable range, indicating that WAX-EMR was an appropriate adsorbent material for the extraction purification process in this investigation, with recoveries of OPE and di-OPE substances ranging from 84.6% to 109.4%.

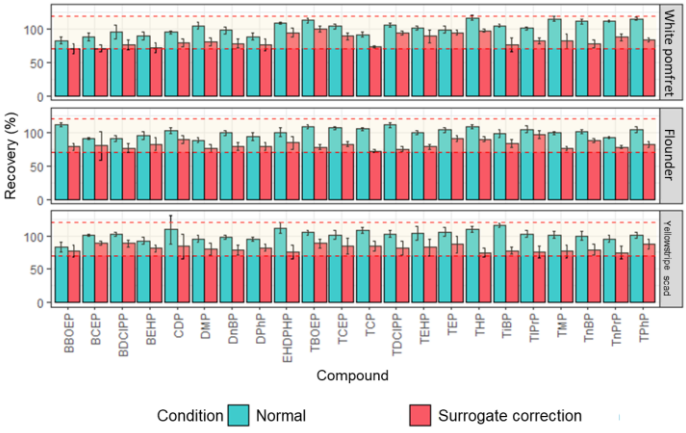

Figure 3.2: Recovery of OPE and di-OPE in fish using the EMR-WAX

The findings indicated that the EMR-WAX effectively purified extracts from fish samples with varying lipid matrix compositions (Figure

3.2). The recoveries of OPE compounds varied from 69.7% to 130.0%, contingent upon the sample matrix and the ionization characteristics of each drug (e.g., TPhP experienced inhibition, while TCP was increased in ESI). The di-OPE group exhibited a statistically significant disparity in recovery between white pomfret and yellowstripe scad ($p < 0.05$), indicating the impact of matrix composition on extract purification. Following correction with surrogate standards, the recoveries of the compounds fell within the permitted range (80–120%), so affirming the essential function of surrogate standards in quantitative analysis. The EMR-WAX system is adept at processing complex biological materials.

3.2.3. Optimal sample preparation

After optimizing the experimental conditions, the complete sample treatment process used in this study is shown in figure 3.3.

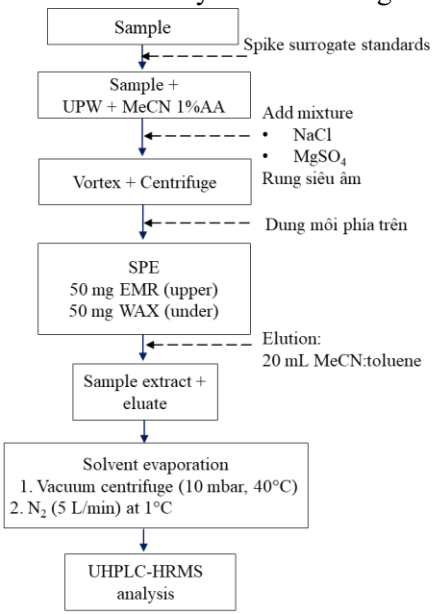

Figure 3.3: OPE analysis sample processing method after optimization

3.3. Method validation

The results of accuracy, repeatability, reproducibility, MDL, MQL, and matrix effect are shown in tables 3.4 and 3.5. The recoveries of the compounds at the three tested concentrations were from 83.1% to 106.1%, 83.3% to 108.6%, and 83.9% to 107.9%, respectively, according to the criteria of SANTE/11312/2021. The method of analysis exhibited a repeatability with an RSD% of 1.4% to 7.7%. The analytical approach demonstrated reproducibility with a relative standard deviation (RSD%) of 1.9% to 6.3% (n = 18). The RSD% for both repeatability and reproducibility were all below 20%. Ten duplicate samples were analyzed to ascertain the method detection limit (MDL) at a concentration of 0.2 ng/g into the fish flesh sample matrix, following the optimized process (Figure 3.3). The estimated MDL findings for the analytes varied from 0.020 ng/g to 0.050 ng/g, with the calculated R values ranging from 4.0 to 9.9, hence satisfying AOAC standards. The MQL of the compounds varied from 0.07 ng/g to 0.18 ng/g. This research also assessed the matrix impact. The matrix impact values for the analytes varied from -17.7% to -4.0%. The result indicated the presence of contaminants in the sample matrix that influenced the analytes throughout the ionization process using the ESI method. Nonetheless, these values fall within ±20%, so they satisfy the criteria of SANTE/11312/2021. The findings indicate that the analytical approach satisfactorily fulfills the criteria for quantitative determination of trace OPE levels in fish flesh samples.

3.4. OPE content in marine fish

3.4.1. OPE and di-OPE concentration in fish

The concentrations of OPE compounds in this study were calculated on a dry basis. The concentrations of OPE compounds are presented in Table 3.6.

Table 3.4: Recovery, repeatability, reproducibility, limit of quantification and matrix effect of the analytical method for OPE in fish

TT	Compound	Recovery (%)			Repeatability (RSD%)			Reproducibility (RSD%)			MQL (ng/g)	ME(%)
		1 ng/g	50 ng/g	100 ng/g	1 ng/g	50 ng/g	100 ng/g	1 ng/g	50 ng/g	100 ng/g		
1	TMP	87.7	85.6	85.7	3.5	3.0	2.3	3.3	4.5	3.6	0.08	-16.2
2	TEP	87.2	85.9	83.9	3.0	3.1	4.3	3.3	3.0	3.3	0.11	-11.0
3	TnPrP	97.1	95.0	101.5	3.9	4.8	1.7	3.4	4.5	2.3	0.07	-4.0
4	TIPrP	106.1	102.8	101.1	5.5	7.7	4.7	5.3	5.9	6.3	0.12	-9.7
5	TnBP	92.3	97.5	91.7	1.5	2.6	1.9	3.8	3.1	2.5	0.09	-13.0
6	TiBP	85.8	97.6	105.3	3.6	4.6	1.8	3.7	3.5	3.1	0.11	-14.6
7	THP	93.8	106.7	103.6	5.1	3.3	3.6	4.9	3.3	3.1	0.13	-17.6
8	TBOEP	87.8	85.4	86.9	5.4	2.6	4.4	4.4	2.4	3.8	0.16	-11.7
9	TEHP	84.0	86.3	86.6	5.9	3.5	3.5	5.4	3.8	3.8	0.13	-9.1
10	TPhP	86.7	83.3	86.5	4.0	2.6	5.0	4.6	2.8	4.8	0.13	-7.5
11	CDP	96.5	106.5	97.8	5.8	1.5	2.9	5.6	1.9	3.2	0.15	-11.9
12	EHDPHP	85.4	108.6	94.2	4.4	4.4	2.9	5.2	4.1	3.6	0.14	-12.5
13	TCP	93.7	97.4	107.9	4.1	4.4	3.1	4.9	4.5	2.9	0.15	-17.7
14	TCEP	105.8	103.1	106.4	4.7	3.3	2.1	5.6	3.4	3.0	0.15	-15.0
15	TDCIPP	93.9	88.4	92.7	4.0	2.1	3.1	5.5	2.5	2.9	0.15	-7.0

Table 3.5: Recovery, repeatability, reproducibility, limit of quantification and matrix effect of the analytical method for di-OPE in fish

		Recovery (%)			Repeatability (RSD%)			Reproducibility (RSD%)				
TT	**Compound**	**1 ng/g**	**50 ng/g**	**100 ng/g**	**1 ng/g**	**50 ng/g**	**100 ng/g**	**1 ng/g**	**50 ng/g**	**100 ng/g**	**MQL (ng/g)**	**ME(%)**
1	DMP	83.1	90.6	85.4	3.7	4.9	1.4	4.4	4.1	3.1	0.10	-16.0
2	BCEP	90.5	86.9	93.4	5.1	4.7	3.7	6.2	4.6	3.8	0.14	-8.7
3	DPhP	91.6	93.7	95.8	3.8	6.7	3.8	3.2	5.7	3.3	0.17	-6.0
4	BDCIPP	90.9	92.2	91.4	3.7	5.0	4.6	3.7	5.3	4.5	0.16	-9.3
5	DnBP	87.9	91.5	91.5	4.7	3.6	3.5	4.5	3.9	4.1	0.13	-13.3
6	BBOEP	87.8	92.8	86.7	3.6	2.2	3.3	3.2	3.5	2.7	0.14	-13.5
7	BEHP	90.1	93.4	96.4	5.2	3.8	4.2	4.6	3.8	5.3	0.18	-11.3

Table 3.6: OPE concentration (ng/g dry weight) in marine fish

Compound	DF (%)	Conc. range	Mean	Median
TMP	58	0.1 - 3.0	1.1	0.9
TEP	56	0.1 - 1.8	0.8	0.8
TnPrP	53	0.1 - 1.2	0.5	0.4
TIPrP	49	0.1 - 1.3	0.4	0.3
TnBP	66	0.1 - 1.4	0.6	0.6
TiBP	60	0.1 - 2.7	0.9	0.8
THP	64	0.1 - 2.9	1.1	0.9
TBOEP	66	0.3 - 16.8	5.2	5.1
TEHP	65	0.1 - 15.4	4.6	3.4
TPhP	63	0.1 - 3.0	1.1	1.0
CDP	50	0.1 - 1.8	0.6	0.5
EHDPHP	48	0.1 - 2.3	0.7	0.5
TCP	55	0.1 - 3.1	1.2	1.1
TCEP	68	0.1 - 24.3	3.7	2.8
TDCIPP	54	0.1 - 11.2	3.5	3.5
Σalkyl OPEs	100	0.5 - 28.1	9.6	8.8
Σaryl OPEs	95	0.1 - 6.6	2.1	1.7
ΣCl–alkyl OPEs	87	0.1 - 32.4	5.0	4.3
ΣOPEs	100	2.1 - 39.3	16.0	15.2
DMP	91	0.1 - 165	23.9	12.3
BCEP	39	0.4 - 29.7	6.5	4.9
DPhP	79	0.3 - 28.6	5.7	3.7
BDCIPP	59	0.2 - 25.3	5.5	3.2
DnBP	50	0.1 - 4.3	1.0	0.9
BBOEP	60	0.1 - 33.1	9.5	7.4
BEHP	78	0.6 - 167.6	34.6	21.0
Σdi-OPEs	100	3.2 - 263.4	65.4	51.3
Total	100	5.2 - 291.8	81.4	66.9

The concentrations of total OPEs (∑OPEs) in fish samples varied from 2.1 to 39.9 ng/g dw (mean 16.0 ± 8.2 ng/g dw), equating to 0.48-11.04 ng/g wet weight, which is lower than in several global areas. TCEP was the main component, exhibiting the greatest quantities owing to its stability and capacity for bioaccumulation via protein binding. Simultaneously, aryl-

OPEs like TPhP and TCP were detected at low quantities, despite their elevated log KOW, indicating their restricted environmental prevalence. TBOEP and TEHP were detected at elevated amounts as a result of emissions from consumer items.

3.4.1.1. Variations in OPE content between fish species

Seventy-two fish samples from three species, obtained in August 2022, were tested to evaluate the disparities in OPE contamination levels, namely alkyl, aryl, and Cl-alkyl OPEs. The findings indicated that white pomfret exhibited the greatest concentration of alkyl OPEs (9.2 ± 5.3 ng/g dw), yellowtail had a predominant level of Cl-alkyl OPEs (8.3 ± 7.3 ng/g dw), while flounder was characterized by a predominance of aryl OPEs (2.3 ± 1.4 ng/g dw) (Figure 3.4.a). The concentrations of ∑OPEs varied substantially, with the greatest level found in yellowstripe scad (17.0 ± 9.5 ng/g dw), exhibiting a statistically significant difference compared to flounder ($p < 0.05$, figure 3.4.b).

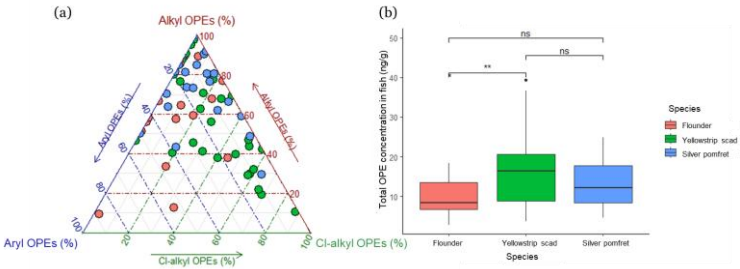

Figure 3.4: Ternary diagram showing alkyl OPE, aryl OPE and Cl-alkyl OPE and (b) the distribution of ΣOPE level among fish species

PCA analysis indicated that TCEP, TBOEP, and TDCIPP were the main chemicals influencing data dispersion; nevertheless, a distinct separation across fish species or sample locations was not evident owing to the overlap in pollutant profile features (Figure 3.5). The buildup of OPEs is contingent not only upon the source of environmental contamination but

also on the biological traits, including absorption capacity, metabolism, and feeding behavior, of each fish species.

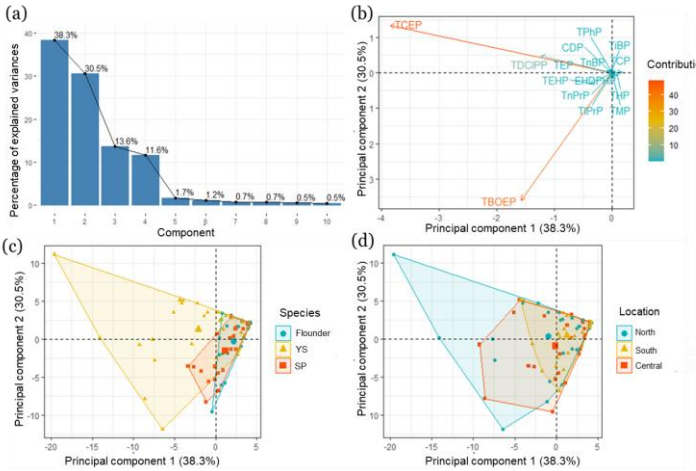

Figure 3.5: PCA results based on OPE concentrations in fish samples

3.4.1.2. Spatiotemporal variation in OPE content in silver pomfret

The distribution of OPE groups in pompano indicated that alkyl OPEs were predominant, followed by Cl-alkyl and aryl OPEs. From 2021 to 2023, the contamination profile of silver pomfret was mostly characterized by alkyl OPEs, particularly TBOEP, which constituted 66.9% (Figure 3.6.a). There were notable disparities in OPE concentrations throughout sample periods ($p < 0.05$), with decreased levels seen in 2021, influenced by the COVID-19 lockdown. The use of social distancing measures and the closure of factories reduced industrial emissions, thereby lessening environmental damage. Subsequent to 2022, the concentrations of OPE in silver pomfret have shown a tendency to rise once again. This result demonstrated a distinct correlation between industrial activity and OPE contamination levels in pompano (Figure 3.6.b).

The PCA findings per year indicated that chemicals including TBOEP, TEHP, TCEP, and TDCIPP were the primary variables influencing the variance of OPE profiles in pompano (Figure 3.7). Specifically, fish samples from the Northern area exhibited elevated levels of TEHP and

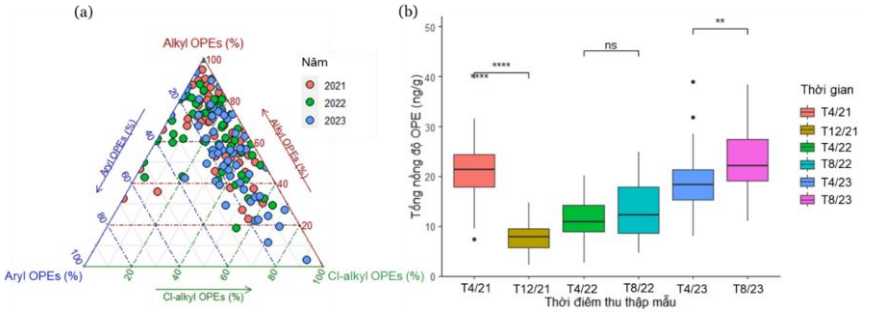

Figure 3.6: Ternary diagram showing the proportions of alkyl OPE, aryl OPE and Cl-alkyl OPE in fish meat samples and (b) the distribution of ΣOPE concentrations in pomfret samples

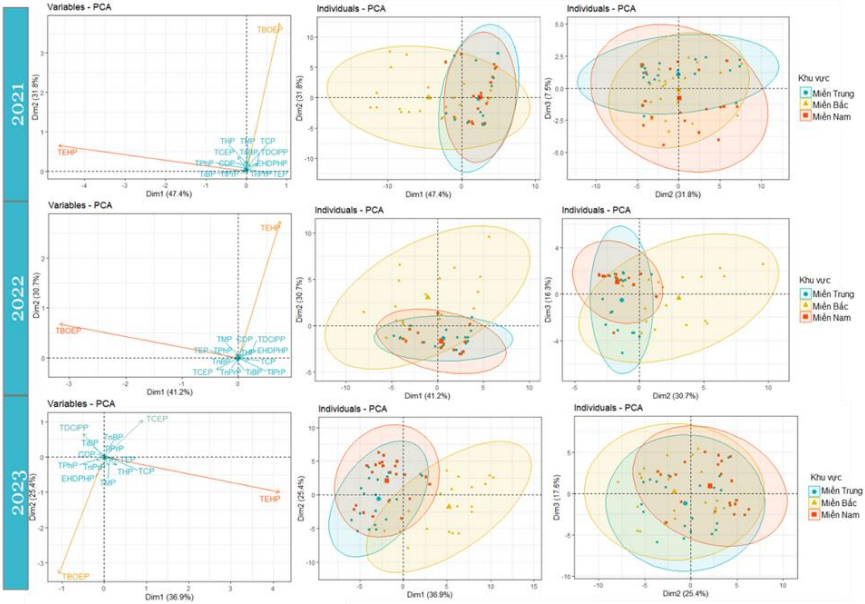

Figure 3.7: PCA results based on the concentrations of OPE compounds in silver pomfret collected during 2021-2023

and TBOEP, suggesting the impact of anthropogenic activities in this area. The findings indicate that OPE accumulation in fish is regulated not only by the emission source but also by temporal, spatial, and chemical aspects of each molecule.

3.4.2. Correlation between OPE and di-OPE in fish sample

∑di-OPEs concentrations varied from 3.2 to 263.4 ng/g dw (mean 63.9 ± 48.2 ng/g dw), with BEHP, DMP, and DPhP being the predominant components. All OPE/di-OPE couples exhibited $R_{di/tri} > 1$, with the exception of BBOEP/TBOEP, suggesting a propensity for substantial di-OPE metabolism and storage in fish tissues. Certain couples, including TMP–DMP, TPhP–DPhP, and TnBP–DnBP, had statistically significant positive relationships. The elevated $R_{di/tri}$ values of DMP, DPhP, and BEHP indicate the concurrent existence of OPEs and di-OPEs in both biological entities and the environment.

3.5. Risk of OPE exposure in fish consumption

3.5.1. Estimated daily exposure

The mean ΣEDI in children was greatest for yellowstripe scad (46.27 ng/kg/day) and white pomfret (45.19 ng/kg/day), while flounder exhibited a much lower EDI (28.74 ng/kg/day). The same tendency was seen in adults, indicating that yellowstripe scad and white pomfret may possess elevated total OPEs compared to flounder under the surveyed circumstances. Conversely, the ΣLADD in children for white pomfret was 8.45 ng/kg/day, substantially twice that of flounder at 4.68 ng/kg/day and yellowstripe scad at 3.97 ng/kg/day. In adults, the ΣLADD from yellowstripe scad (19.83 ng/kg/day) and white pomfret (19.37 ng/kg/day) surpassed that of flounder (12.32 ng/kg/day).

3.5.2. Health risk assessment

3.5.2.1. Non-carcinogenic risk assessment

In the case of calculations based on positive samples, the HI for OPEs from marine fish consumption was 20.04×10^{-4} for children and 5.01×10^{-4} for adults at the median level. At the 95th percentile, the HI values rose to 44.33×10^{-4} and 11.08×10^{-4}, respectively. All HI values were below 1, indicating that exposure to OPEs from marine fish intake in this investigation did not provide a substantial non-carcinogenic risk to either children or adults.

3.5.2.2. Cancer risk assessment

The incremental lifetime cancer risk (ILCR) was estimated for four receptor groups, namely children and adults at mean exposure and at the 95th percentile, under two exposure scenarios (positive samples only and all analyzed samples). For TCEP, ILCR values in the positive-sample scenario ranged from 1.08×10^{-8} (children, mean) to 1.31×10^{-7} (adults, 95th percentile), whereas in the all-samples scenario the corresponding values were 7.20×10^{-9} and 1.15×10^{-7}. In contrast, TnBP yielded ILCR values nearly one order of magnitude lower in all cases: only 7.36×10^{-10} to 7.88×10^{-9} for positive samples, and 5.52×10^{-10} to 6.90×10^{-9} when all analyzed samples were considered. Thus, TCEP consistently emerged as the major contributor to cancer risk, with ILCR values approximately 15-17 times higher than those of TnBP for both children and adults, regardless of the exposure scenario. Monte Carlo simulations were also applied to ILCR for both age groups. The results showed P50 ILCR values in the range of 7.71×10^{-9}- 1.42×10^{-8} and P95 ILCR values in the range of 1.12×10^{-8} - 2.08×10^{-8} across the four groups (children/adults and the two data-treatment scenarios). Even at the 95th percentile, the ILCRs remained well below the commonly used threshold of 10^{-6} for indicating a potentially significant cancer risk. All ILCR readings in this investigation were within the level of concern advised by the US Environmental Protection Agency.

CONCLUSION

The thesis has completed the set objectives and achieved the following main results:

- Development and validation of a simultaneous analysis procedure for 15 OPEs and 7 di-OPEs in marine fish muscle using ultra-high performance liquid chromatography coupled to high resolution mass spectrometry (UHPLC-HRMS) with a detection limit of 0.020–0.050 ng/g and recovery of 83.1%-108.6%, ensuring the criteria according to the SANTE/11312/2021 guideline.
- Employing the methodology for quantifying the levels of OPE and di-OPE in the muscle of three types of fish obtained from various fishing port locales throughout three regions. The ΣOPEs levels in fish ranged from 2.1 to 39.3 ng/g dw, whereas di-OPEs varied from 3.2 to 263.4 ng/g dw. No variation in concentration was observed across species; however, the OPE profile in silver pomfret from the northern area notably differed from that in other locations.
- The initial health risk evaluation of OPEs in marine fish gathered in this research was conducted for children aged 1-6 years and adults. The ΣEDI of OPEs in children was around fourfold greater than the exposure value for adults. The LADD for adults was fivefold greater than that of children. The HI, HQ, and ILCR values were all below 1, even under the most adverse conditions.

PROPOSAL

To further understand OPE pollution in marine fish in Vietnam, research should be expanded by time and fish species, particularly higher-trophic species, to evaluate biomagnification. Research on di-OPE will also help establish pollution management measures and safeguard public health by revealing its buildup and effects.