

ENDOCRINE DISRUPTING CHEMICALS BIOACCUMULATION
THROUGH FOOD CHAIN AND THEIR EFFECTS ON FISH

(魚類による内分泌攪乱化学物質の食物連鎖経由生物濃縮と
その影響に関する研究)


NURULNADIA BINTI MOHD YUSOFF

2014

1100092183



tesis
RA 1224.2 .N8 2014



1100092183
Endocrine Disrupting Chemicals bioaccumulation through food chain and their effects on fish / Nurulnadia Mohd Yusoff.

PUSAT PEMBELAJARAN DIGITAL SULTANAH NUR ZAHIRAH
UNIVERSITI MALAYSIA TERENGGANU (UMT)
21030 KUALA TERENGGANU

1100092183		

Lihat Sebelah

United Graduate School of Agricultural Sciences
Kagoshima University
Korimoto, Kagoshima City 890-0065 Japan

List of Tables	IV
List of Figures	VI
List of Abbreviations	VII
Abstract	1
Abstract in Japanese	3

In fulfillment of the requirements for the

DOCTOR OF FISHERIES

Degree

CHAPTER 1: GENERAL INTRODUCTION	1
Background of EDC study	1
What is EDC?	1
Target EDCs	2

ENDOCRINE DISRUPTING CHEMICALS BIOACCUMULATION THROUGH
FOOD CHAIN AND THEIR EFFECTS ON FISH

(魚類による内分泌攪乱化学物質の食物連鎖経路生物濃縮とその影響に関する研究)

Bioaccumulation and Toxicity of EDCs	12
Axonal transport efficiency	16
Vitellogenin	17
Metabolism of EDCs by glucuronidation	18
Objectives of this study	20
Overview of this study	20

NURULNADIA BINTI MOHD YUSOFF

CHAPTER 2: BIOACCUMULATION IN POLYCHAETE - DIETARY ROUTE	22
---	----

Under the supervision of

DR. JIRO KOYAMA

(Professor)

Bioaccumulation of dietary endocrine disrupting chemicals (EDCs) by the polychaete, <i>Paraprionospio pinnata</i>	22
Abstract	22
Introduction	23
Materials and Methods	23
Results and Discussion	23

September 2014

TABLE OF CONTENTS

List of Tables	IV
List of Figures	VI
List of Abbreviations	VII
Abstract	1
Abstract in Japanese	3

CHAPTER 1: GENERAL INTRODUCTION..... 4

Background of EDC study	4
What is EDC?	4
Target EDCs	6
Potential sources of exposure	10
Potential environmental transports	12
EDCs transfer in aquatic organisms.....	13
Bioaccumulation and biomagnification	15
Assimilation efficiencies.....	16
Vitellogenin	17
Metabolism of EDCs by glucuronidation	18
Objectives of this study.....	20
Overview of this study	20

CHAPTER 2: BIOACCUMULATION IN POLYCHAETE – DIETARY ROUTE..... 22

Bioaccumulation of dietary endocrine disrupting chemicals (EDCs) by the polychaete, *Perinereis nuntia*

Abstract	22
Introduction.....	22
Materials and Methods.....	23
Results and Discussion	33

Conclusion	39
------------------	----

CHAPTER 3: BIOACCUMULATION IN POLYCHAETE – FIELD STUDY

.....	40
-------	----

Accumulation of endocrine disrupting chemicals (EDCs) in the polychaete *Paraprionospio* sp. from the Yodo River mouth, Osaka Bay

Abstract.....	40
Introduction.....	40
Materials and Methods.....	42
Results and Discussion	46
Conclusion	55

CHAPTER 4: BIOACCUMULATION IN FISH – DIETARY ROUTE..... 56

Bioaccumulation of dietary endocrine disrupting chemicals (EDCs) by the benthic fish, *Pleuronectes yokohamae*

Abstract.....	56
Introduction.....	56
Materials and Methods.....	58
Results and Discussion	63
Conclusion	70

CHAPTER 5: ASSIMILATION EFFICIENCIES 71

Assimilation efficiencies of dietary endocrine disrupting chemicals (EDCs) by the benthic fish, *Pleuronectes yokohamae*

Abstract.....	71
Introduction.....	71
Materials and Methods.....	72

Results and Discussion	78
Conclusion	82
CHAPTER 6: GLUCURONIDATION ACTIVITY.....	83
Glucuronidation activity of bisphenol A (BP) in <i>Pleuronectes yokohamae</i> intestine and liver	
Abstract.....	83
Introduction.....	83
Materials and Methods.....	85
Results and Discussion	87
Conclusion	90
CHAPTER 7: GENERAL DISCUSSION & CONCLUSION.....	91
REFERENCES.....	93

LIST OF TABLES

Table 1.1	Common name, abbreviation, IUPAC name, CAS No., chemical structure, molecular formula, molecular mass, water solubility, n-octanol/water partition coefficient ($\log K_{ow}$), soil organic carbon-water partitioning coefficient ($\log K_{oc}$).....	7
Table 1.2	EDCs in wastewater effluent, surface water, sediment, groundwater and drinking water, (<i>Source</i> : Campbell et al. 2006).....	13
Table 2.1	Nominal concentrations of mix EDCs spiked onto the diet.....	27
Table 2.2	Precursor and daughter ions of EDCs and limit of detection by LC-MS/MS-ESI	32
Table 2.3	EDCs concentration, mean (SD), ng/g dry weight in control and spiked diet, n=3	33
Table 2.4	BMF, k ₂ , kd and t _{1/2} of EDCs in low and high exposure groups	38
Table 3.1	Precursor and daughter ions of EDCs and limit of detection by LC-MS/MS-ESI	46
Table 3.2	NP, OP and BP concentrations in various invertebrates.....	51
Table 3.3	EDCs concentration in sediment (ng/g w.w.) collected around Yodo River-mouth.....	52
Table 3.4	EDCs concentrations in sediment samples collected in Japan	52
Table 3.5	EDCs concentrations in water samples collected in Japan.....	53
Table 3.6	EDCs concentration in sediment and invertebrates	53
Table 4.1	Nominal concentrations of individual EDC spiked onto the diet.....	58
Table 4.2	Precursor and daughter ions of EDCs and limit of detection by LC-MS/MS-ESI	61
Table 4.3	Mean EDCs concentration, ng/g dry weight, in control and spiked diet, n=2	63
Table 4.4	BMF in low, medium and high exposure groups.....	67
Table 4.5	BMF of NP and OP measured from ratio of predator to prey	67
Table 5.1	Nominal concentration of mix EDCs spiked to the diet.....	73
Table 5.2	Precursor and daughter ions of EDCs and limit of detection by LC-MS/MS-ESI	77
Table 5.3	Mean concentration of EDCs, ng/g dry weight (d.w.) in spiked diet, n=378	

Table 5.4	Mean concentration of EDCs, ng/g d.w. in feces, n=3	79
Table 5.5	Assimilation efficiency of EDCs by <i>P. yokohamae</i> , n=3	80
Table 5.6	Assimilation efficiency of EDCs (%) in various test subjects.....	80
Table 6.1	Mean microsomal UGT activities (nmol/min/mg) toward BP by <i>P. yokohamae</i> intestine and liver.....	88

LIST OF FIGURES

Figure 1.1	Possible route of exposition for emergent estrogenic compounds (Esplugas et al. 2007)	11
Figure 2.1	Experimental set-up for control, low and high test groups.....	27
Figure 2.2	Accumulation and elimination of NP, OP, BP and E2 (ng/g wet weight) by <i>P. nuntia</i> in low and high exposure groups. Error bars indicate the standard deviation (n=3).	37
Figure 3.1	Polychaete and sediment sampling stations around Yodo river-mouth, Osaka Bay. Sediment samples were collected at ST17, ST18 and ST21 only.	44
Figure 3.2	Mean concentrations of NP, OP, BP, E1 and E2 (ng/g wet weight) in <i>Paraprionospio</i> sp. (n=2).	48
Figure 4.1	Tanks set-up for individual EDC exposure	60
Figure 4.2	Accumulation of NP and OP (ng/g wet weight) in <i>P. yokohamae</i> in control, medium, low and high exposure groups. Error bars indicate the standard deviation (n=3).	64
Figure 4.3	Induction of vitellogenin C in <i>P. yokohamae</i> at day 0, 2, 7, 14, 21 and 28 in control and exposed groups	68
Figure 5.1	Cr (III) oxide mixed with ground diet and pelletized	73
Figure 5.2	Glass aquariums in water bath set-up for assimilation rate experiment .	74
Figure 6.1	Chromatograms of BP in control sample (liver/intestine) without activation by UDP-glucuronic acid and incubation (a), liver sample with activation by UDP-glucuronic acid and incubation (b), intestine sample with activation by UDP-glucuronic acid and incubation (c)	88
Figure 6.2	Microsomal UGT activities toward BP by intestine of carp and <i>P. yokohamae</i> ; (a) Daidoji et al. 2006, (b) Yokota et al. 2002, (c) this study	89

LIST OF ABBREVIATIONS

AE	Assimilation efficiency
APnEO	Alkylphenol(n)polyethoxylate
BAF	Bioaccumulation factor
BCF	Bioconcentration factor
BMF	Biomagnification factor
BP	Bisphenol A
CAS No	Chemical Abstracts Service registered number
DCM	Dichloromethane
d.w.	Dry weight
EDCs	Endocrine disrupting chemicals
E1	Estrone
E2	17 β -estradiol
HNO ₃	Nitric acid
HClO ₄	Perchloric acid
k ₂	Elimination rate
kd	Uptake rate
log K _{ow}	Octanol–water partitioning coefficient
log K _{oc}	Organic carbon-water partitioning coefficient
n.d	Not detected
n.a	Not available
N ₂	Nitrogen gas
NaCl	Sodium chloride
NP	Nonylphenol
NPnEO	Nonylphenol(n)ethoxylate
OP	Octylphenol
OPnEO	Octylphenol(n)ethoxylate
t _{1/2}	Half-life
UDPGT	Uridine diphospo-glucuronosyltransferase
v/v	Volume over volume
w.w.	Wet weight

ABSTRACT

Endocrine Disrupting Chemicals (EDCs) are known to impair the reproduction in fish by intersex, altered mating behavior and reduced testicular growth. Target EDCs in this study consist of natural (E1 and E2) and estrogenic chemicals (NP, OP and BP) which were reported as potentially estrogenic to aquatic organisms.

This study consists of five experiments examining the bioaccumulation of EDCs through food chain which representing by commercial diet, polychaete and benthic fish. In experiment 1, bioaccumulation of EDCs was determined in polychaete through dietary exposure. Biomagnification factor (BMF) values indicated EDCs were not biomagnified in polychaete. Besides, E1 concentration was below detection limit and speculated to be biotransformed. In experiment 2, EDCs concentration was measured in wild polychaete collected from Osaka Bay. EDCs concentrations were unexpectedly high in polychaete compared to sediment; thus, predicted to biomagnify the compounds from the sediment and possibly transfers through food chain. Therefore, bioaccumulation of EDCs was determined in benthic fish, *Pleuronectes yokohamae* through dietary exposure in experiment 3. BMF values also demonstrated no biomagnifications and this finding were verified by no induction of vitellogenin in fish serum. In each exposure experiments, higher EDCs concentration were observed in exposed groups compared to control suggesting the assimilation in *P. yokohamae*. This assumption has been affirmed in experiment 4 by the high assimilation efficiencies (AE) computed in *P. yokohamae* by dietary exposure with percentage of over 88–96% (except NP). Therefore, low bioaccumulation of EDCs in homogenate fish tissues and presence of compounds concentration below detection limit (BP, E1 and E2) in this study were probably due to intensive metabolism. EDCs had been reported to be metabolized and biotransformed into glucuronide conjugates in fish; hence

glucuronidation activity was analyzed in the microsomal of intestine and liver of *P. yokohamae* in experiment 5. High UGT activity in the microsomes of intestine and liver suggesting efficient metabolism and elimination of BP form the *P. yokohamae* body. Thus, it can be justified that BP was not bioaccumulated/biomagnified in the previous dietary exposure due to glucuronidation. The other target EDCs were assumed to be glucuronidated as well based on the verification by other authors who conducted studies specifically on fish.

In the present study, EDCs were not bioaccumulated through the food chain. This finding has been verified by high glucuronidation activities in intestine (first-pass metabolism organ) of *P. yokohamae*.

ABSTRACT

魚類の内分泌を攪乱し、再生産に影響を及ぼす可能性のあり、海底質中に残留する内分泌攪乱化学物質（以下EDCs）の食物連鎖経路生物濃縮とその影響について、5つの実験を実施して研究した。

第1の実験では人口底質で飼育した底生動物（イソゴカイ、*Perinereis nuntia*）に、EDCs（ノニルフェノール（NP）、オクチルフェノール（OP）、ビスフェノールA（BP）、17 β -エストラジオール（E2）およびエストロン（E1））を添加した餌を投与し、それらの生物濃縮を調べた。その結果、ゴカイ中EDCs濃度は上昇するものの餌中の濃度を上回ることとはなく、生物濃縮することとはなかった。第2の実験では底質中EDCs濃度が既知の大阪湾で採取した底生動物中EDCs濃度を調べた。その結果、多毛類の1種（*Paraprionospio* sp.）で底質中濃度を上回るEDCsが検出され、底質→底生動物→底生魚類の食物連鎖経路でのEDCs生物濃縮の可能性が示唆された。そこで第3の実験では底生魚種であるマコガレイ（*Pleuronectes yokohamae*）稚魚にEDCsを添加した餌を投与し、餌からのEDCsの生物濃縮を検証した。しかし、EDCs濃度の若干の上昇は観察されたものの、いずれの魚体中濃度とも餌のそれらを上回ることとはなく、餌からのEDCs生物濃縮は認められなかった。これは同実験のマコガレイ中に、メス特有の卵黄前駆物質でEDCs暴露のバイオマーカーであるvitellogenin（Vg）の誘導がほとんど認められなかったことから、EDCsの体内へほとんど蓄積しなかったことがうかがえる。さらに第4の実験として、EDCsを添加した底質を敷いた水槽でマコガレイを飼育し、底質からのEDCs移行を確かめた。その結果、餌投与実験と同様にEDCsの体内濃度上昇はほとんど認められなかった。一方、第5の実験でマコガレイ消化管からの餌中EDCs消化吸収率を測定した結果、NPで50%、その他のEDCsで88～96%の消化吸収率が得られた。

以上の結果、底質中EDCsは一部の底生動物中に生物濃縮されることから、これらを餌とする底生魚類に食物連鎖経路で移行することが考えられた。一方、EDCs添加餌のマコガレイへの投与実験およびEDCs添加底質での飼育実験から、EDCsの食物連鎖経路あるいは底質経路での魚類への生物濃縮の可能性の低いことが明らかとなった。マコガレイによる餌中EDCs消化吸収率の高かったことから考えて、消化管から吸収される際にEDCsは何らかの代謝を受け、それらの代謝物として体内に吸収されたと考えられる。さらにこれらの代謝物にはVgを誘導するような女性ホルモン作用がほとんどなかったことが実験で確認された。