ENDOCRINE DISRUPTING CHEMICALS BIOACCUMULATIO 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)

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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	17β-estradiol
HNO_3	Nitric acid
$HClO_4$	Perchloric acid
k2	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）の食物連鎖経由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）、17β-エルトラジオール（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はなく、生物濃縮することはなかった。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由での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も、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由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の食物連鎖経由あるいは底質経由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。