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일본송사리에서 TCDD 및 PCB 126의 흡수 및 배설

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Uptake and Elimination of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin(TCDD) and 3,3′,4,4′,5-pentachlorobiphenyl(PCB 126) in Japanese Medaka(*Oryzias latipes*)

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ABSTRACT

초기생애단계의 일본송사리를 실험어류로 사용하여 TCDD와 PCB 126의 생물농축정도를 비교 측정하였고, TCDD와 PCB 126의 체외 배설에 대한 동태학적 특성을 비교 분석하였다. 생물농축실험은 초기생애단계의 새끼치어 및 어린 물고기에 TCDD와 PCB 126을 여러 농도로 물에 용해시켜 96시간 동안 정체된 상태로 폭로시킨 후 생물농축계수(BCF)와 지방-표준화한 생물농축계수(BCF_L)를 측정하였다.

TCDD와 PCB 126의 일본송사리 초기생애단계의 새끼치어 및 어린 물고기에서 측정된 96-h BCF 값 및 96-h BCF_L값은 폭로된 농도와 상관없이 비슷하게 나타난 반면, 생체크기가 클수록 그 값은 작게 나타났다. 일본송사리 새끼치어, 어린 물고기, 성어의 생체 내 총 지방함량은 각각 5.7, 4.2, 4.6%로 측정되었다.

체외배설 동태실험은 어린 물고기에 TCDD와 PCB 126을 3가지 농도로 물에 용해시켜 96시간 동안 정체된 상태로 폭로시킨 후, flow-through장치로 공급되는 맑은 물로 옮겨 42일간 사양하면서 0, 7, 14, 21, 28, 42일에 생체 내 TCDD와 PCB 126 잔류량을 측정하여 분석하였다. 배설은 1차 반응속도론적으로 이루어졌으며 한 구획모형보다 두 구획모형이 더 적합한 것으로 분석되었다. 두 구획모형을 사용한 분석에서 TCDD와 PCB 126의 체외배설 반감기($t_{\frac{1}{2}}$) 및 배설속도상수(β)는 각각 27.2일과 32.3일 및 0.025/d와 0.021/d로 측정되었다.

Keywords: BCF, $t_{\frac{1}{2}}$, Medaka, TCDD, PCB 126

I. INTRODUCTION

TCDD and PCB 126 are highly lipophilic and resistant to metabolic transformation. These chemicals are of great concern in aquatic toxicology due to their high toxic potentials, additive toxic interactions, and significant contributions to the total toxic equivalents(TEQs)[1-5]. The major route of uptake for waterborne chemicals in fish is across the gill epithelium, but cutaneous uptake also plays a significant role in small fish[6]. Bioconcentration of lipophilic chemicals occurs largely through passive diffusion. Absorbed

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lipophilic chemicals are distributed throughout the body by being bound to serum lipids and lipoproteins. Mammals have a dioxin binding protein(CYP1A2) in the liver that increases the hepatic level.

Fish accumulate highly lipophilic compounds mostly in the fatty tissues, not preferentially in the liver[7-8]. The uptake, storage, and elimination of lipophilic chemicals are known to be influenced by the life stage, size, and lipid content of fish. This study was undertaken to compare the bioconcentrations of TCDD and PCB 126 in different sizes of Japanese medaka, and to examine the whole body elimination kinetics of TCDD and PCB 126 in juvenile Japanese medaka.

II. EXPERIMENTAL METHODS

1. Chemicals

[1,6-^{3}H] 2,3,7,8,-tetrachlorodibenzo-*p*-dioxin (34.7 Ci/mmol, radiochemical purity of 98%) was purchased from Chemsyn Science Laboratories (Lenexa, Kansas). [3,4,5,-phenyl-ring -UL-^{14}C] 3,3',4,4',5-pentachlorobiphenyl (12 mCi/mmol, radiochemical purity of 97%) was purchased from Sigma chemical Company (St. Louis, Mo.). HPLC grade hexane and isopropanol were purchased from Fisher Scientific (Springfield, NJ). ScintiVerse®BD was purchased from Fisher Scientific (Springfield, NJ).

2. Test organisms and exposure systems

For bioconcentration studies, three different sizes of fry and juvenile Japanese medaka were used. Forty to fifty animals were statically exposed to varying doses of [^{3}H]-TCDD (1.02~5.34pg/㎖) and [^{14}C]-PCB 126 (62.3~271.7 pg/㎖) dissolved in 200㎖ of dechlorinated water within a 450㎖ glass rearing dish for 96 hours. Following the exposure, ten animals were used for the tissue dose measurement.

For elimination studies, the juvenile Japanese medaka (standard length: 11.2±0.6 ㎜, wet weight: 20.8±5.3㎎) were exposed to three different doses of [^{3}H]-TCDD and [^{14}C]-PCB 126 for 96hours and transferred to a two and half gallon glass aquaria, set up as a flow-through system. Incoming water was filtered in-line by a sand filter, two 25㎛ particle filters, and an activated carbon filter. Filtered water was flowed through an eight watt ultraviolet sterilizer and was distributed to individual aquarium at a flow rate of up to two gallons per hour. The water temperature was maintained at 20±2℃ using a central heater. The fish were fed Tetramin® tropical fish food (Tetra Werke, Melke, Germany) and newly hatched brine shrimp, *Artemia salina*, two to three times a day, and were maintained on an artificial photoperiod of 16-h light and 8-h dark cycle. Six to seven animals were removed to measure the tissue dose at day 7, 14, 21, 28, and 42.

3. Total lipid content analyses

Fry, juvenile, and adult Japanese medaka were analyzed for the total lipid content using a modified U.S. Environmental Protection Agency method[9]. Three composites per each life stage of animals, total wet weight of 0.8~1.1g, were homogenized in a 50㎖ round bottom centrifuge tube containing 30㎖ of hexane : isopropanol(3 : 2) extraction solution, then centrifuged for 10 minutes at 2000 rpm. The supernatant was washed with 30㎖ of 40℃ 0.47 M Na_2SO_4 using a 125㎖ of separate funnel to remove isopropanol and water. The hexane layer was dried and weighed for the total lipid content.

4. Liquid Scintillation Counting

Radioactivity in the water or tissue were measured by a Tracor Mark III liquid scintillation counter (Tracor Analytic, ELK Grove Village, Ill. Disintegrations per minute(DPM) of samples were converted to concentration equivalents after subtraction of background level. Each animal was weighed, then digested with 1㎖ of 1N sodium hydroxide for 24 hours followed by neutralization with 50㎕ of glacial acetic acid. Ten ㎖ of ScintiVerse® BD were added to the water or the digested tissue samples prior to liquid scintillation counting.

5. Whole body elimination half-life calculation

Because growth effect during the experimental period leads to reduction in the concentration of chemicals in an organism(e.g., pg/mg), the whole body dose of chemicals in an organism(e.g., pg) was used in this study. A Simusolv® modeling and simulation software (version 3.0, Dow Chemical Company) was used for the whole body elimination kinetics. A two compartment model was used to calculate the whole body elimination half-life($t_{\frac{1}{2}}$).

The equations are presented below[10] :

$$C(t)=Ae^{-\alpha t} + Be^{-\beta t}$$

$$t_{\frac{1}{2}}=0.693/\beta$$

Where

$C(t)$ = the whole body tissue dose at the time of t (pg)

A, B= intercepts for the central compartment elimination and the peripheral compartment elimination, respectively (pg)

α,β = slopes for the central compartment elimination and the peripheral compartment elimination, respectively (d^{-1})

t = the time (d).

III. RESULTS AND DISCUSSION

The 96-h bioconcentration studies of TCDD and PCB 126 in three different sizes of fry and juvenile Japanese medaka are summarized in **Table 1**. The 96-h BCFs for TCDD and PCB 126 were similar each other, and relatively independent of exposure concentrations in three different sizes of Japanese medaka.

Table 1. Summary of the bioconcentration studies using the fry and juvenile Japanese medaka exposed to two or three different doses of waterborne TCDD and PCB 126 statically and nonrenewally for 96 hours

Life stage	Standard length(mm)	Wet weight (mg)	Chemicals	96-h BCF[a]	96-h BCF_L[b]
Fry	7.4±0.6	4.1±1.1	TCDD PCB 126	1388±55 1355±89	24350±965 23772±1561
	8.1±0.5	6.3±0.9	TCDD PCB 126	650±44 836±56	11400±772 14670±982
Juvenile	11.2±0.6	20.8±5.3	TCDD PCB 126	277±6 238±16	6595±143 5667±381

Each value represents a mean±standard deviation.
[a]Bioconcentration factor.
[b]Lipid normalized bioconcentration factor.

It has been known that the fish BCF is significantly correlated with the chemical's octanol/water partition coefficient (*kow*)[11]. The similar 96-h BCF values for TCDD and PCB 126 in this study could be attributable to the similar log *kow* values for TCDD(6.80) and PCB 126(6.89)[11·12]. In a juvenile fathead minnow study, Adams *et al.*[13] also found that the bioconcentration factors (BCFs) for TCDD were dependent upon length of exposure, but independent of exposure concentration.

The 96-h BCFs for TCDD and PCB 126 in this study exhibited a tendency to decrease with increasing animal body weight. Opperhuizen and Sijm[14] found that the uptake rate constants for TCDD in larger fish were lower than those in smaller fish. In fish, the gill is the major site of uptake of waterborne chemicals. Ventilation volume and gill surface area increase with increasing body size, but decrease per unit body weight with increasing body size[15]. Gill perfusion per unit body weight appears to be relatively independent of body size[16]. In contrast to larger fish, the cutaneous surface area in small fish(<4g) is often greater than the branchial surface area[17]. The surface area to volume ratios for branchial and cutaneous surfaces in a 1kg fish are approximately 2 : 1 and 1 : 1, respectively, but in fathead minnow they are 15 : 1 and 25 : 1, respectively[6]. Lien and McKim[6] suggested that the combined effects of a greater proportion of cutaneous surface area compared to branchial area and a shorter diffusion distance across the skin in small fish could result in a greater contribution of cutaneous absorption to total uptake in small fish. Indeed, Saarikoski *et al.*[18] reported that the uptake across the skin in small fish may contribute as much as 25~40% of total absorbed dose, whereas in large fish(≈ 1 kg), cutaneous absorption contributed only 2~5% in rainbow trout and 5~10% in channel catfish[19].

Total lipid content analyses in the fry, juvenile, and adult Japanese medaka are summarized in **Table 2**. The average total lipid content in the fry(5.7%) was slightly higher than those in the juvenile(4.2%) and adult animals(4.6%).

Table 2. Total lipid content in the fry, juvenile, and adult Japanese medaka

Life stage	Wet weight(mg)	Total lipid content(%)*
Fry	6.3±0.9	5.7±0.3
Juvenile	20.8±5.3	4.2±1.3
Adult	397.9±57.2	4.6±0.2

Each value represents a mean±standard deviation.
*The value is the mean of 3 measurements±standard deviation.

Because highly lipophilic compounds are mostly accumulated in the fatty tissues, the lipid normalized BCF (BCF_L) is used to reduce the intra- and inter-species variability. In this study, the 96-h BCF_L values for both TCDD and PCB 126 exhibited a tendency to decrease with increasing animal sizes(Table1).

The elimination studies of TCDD and PCB 126 in the juvenile Japanese medaka are summarized in **Table 3** and **Table 4**, respectively. Animals exposed to TCDD and PCB 126 did not show any toxic responses throughout the experimental period.

Table 3. Summary of the elimination studies using the juvenile Japanese medaka exposed to waterborne TCDD statically and nonrenewally for 96 hours followed by 42 days rearing in the flow-through system

Water conc. (pg/ml) (N=5)	Tissue dose* pg(pg/mg)					
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 42
1.02±0.08	3.01±1.03 (0.29±0.03)	1.89±0.79 (0.11±0.02)	1.35±0.57 (0.05±0.02)	1.27±0.49 (0.04±0.01)	1.01±0.57 (0.02±0.01)	0.90±0.30 (0.01±0.01)
2.43±0.05	8.49±2.59 (0.66±0.04)	5.44±1.70 (0.27±0.05)	4.18±0.98 (0.17±0.02)	3.77±1.50 (0.11±0.03)	2.62±1.09 (0.06±0.02)	2.39±0.62 (0.03±0.01)
3.89±0.04	13.21±3.94 (1.07±0.13)	9.47±4.12 (0.51±0.12)	6.11±0.45 (0.23±0.03)	5.74±1.71 (0.16±0.04)	4.79±2.21 (0.10±0.03)	3.93±0.50 (0.06±0.01)

Each value represents a mean±standard deviation.
*Six to seven animals were used for the tissue dose measurement.

Table 4. Summary of the elimination studies using the juvenile Japanese medaka exposed to waterborne PCB 126 statically and nonrenewally for 96 hours followed by 42days rearing in the flow-through system

Water conc. (pg/ml) (N=5)	Tissue dose* pg(pg/mg)					
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 42
62.3±16.6	160.5±44.5 (13.9±2.1)	102.9±78.8 (5.1±1.9)	78.5±40.6 (2.8±1.3)	62.0±48.5 (1.8±1.6)	52.5±27.4 (1.1±0.5)	50.1±41.1 (0.6±0.5)
130.9±22.4	375.4±98.8 (33.3±4.6)	255.2±140.5 (15.0±2.1)	201.7±80.2 (7.9±3.0)	178.5±65.4 (5.4±1.8)	137.9±32.6 (3.1±0.5)	121.0±48.2 (1.9±0.7)
202.5±14.1	553.2±231.4 (47.7±9.7)	471.7±138.4 (26.7±4.5)	403.4±99.9 (15.9±4.4)	337.8±53.0 (9.9±4.1)	236.7±60.2 (6.2±2.3)	190.2±74.4 (2.9±0.9)

Each value represents a mean±standard deviation.
*Six to seven animals were used for the tissue dose measurement.

The average uptake doses(pg/mg) of TCDD and PCB 126 after 96-h exposure ranged from 0.29 to 1.07 and from 13.9 to 47.7 respectively. These tissue doses are lower than the lowest observed adverse effect levels (LOAELs) for TCDD(1.4pg/mg) and PCB 126(60.8pg/mg) in one-month-old Japanese medaka larvae[4]. The whole body elimination was plotted using the Simusolv® program (version 3.0) and the curves are shown in **Figures 1** and **2**. Animals exposed to the highest doses of both TCDD(1.07pg/mg) and PCB 126(47.7pg/mg) were not used for data analyses since their body burdens were higher than most environmental levels.

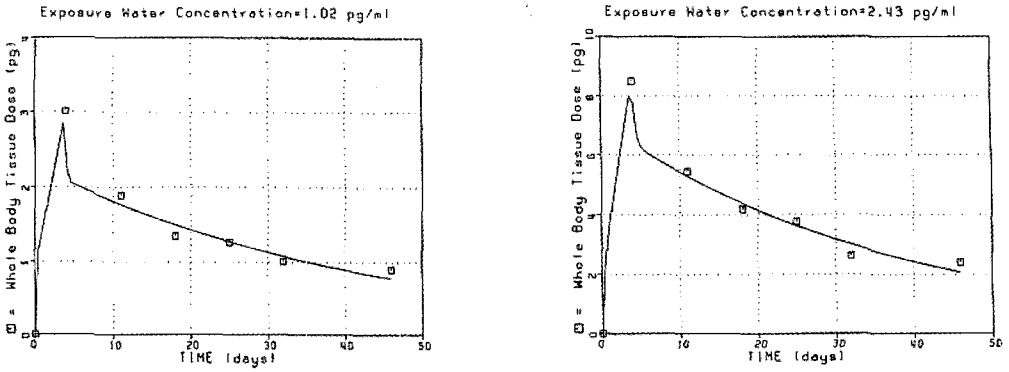

Fig. 1. Uptake and elimination phases in the juvenile Japanese medaka exposed to waterborne TCDD statically and nonrenewally for 96 hours followed by 42days rearing in the flow-through system.

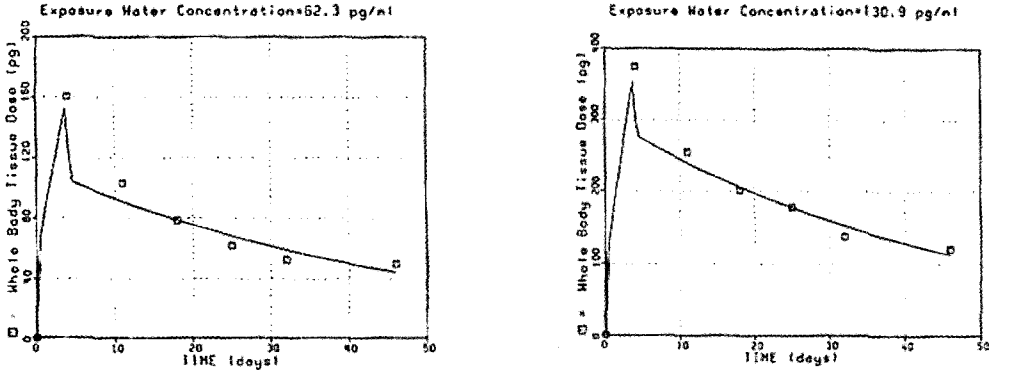

Fig. 2. Uptake and elimination phases in the juvenile Japanese medaka exposed to waterborne PCB 126 statically and nonrenewally for 96 hours followed by 42days rearing in the flow-through system.

The whole body elimination phases for both TCDD and PCB 126 obeyed first-order kinetics and fit a two compartment model better than a single compartment model. The coefficients of determination (γ^2) for TCDD/PCB 126 in a single compartment model analyses and two compartment model analyses were 0.919±0.026/0.921±0.015 and 0.991±0.002/0.992±0.001, respectively. In the two compartment model analyses, the calculated $t_{\frac{1}{2}}$ values for TCDD and PCB 126 were 27.2±0.7 days and 32.3±1.1 days, respectively. The elimination rate constants of the peripheral compartment(β) for TCDD and PCB 126 were 0.025/d and 0.021/d, respectively. A single compartment model regards an organism as one homogeneous compartment, and assumes that all of the chemical in the animal is rapidly available for elimination. However, a single compartment model does

not correctly estimate the elimination kinetics for animal systems having a storage compartment. The need for multicompartment model arises from the large tissue-to-tissue differences in partitioning and specific blood perfusion rates. The absorbed chemicals are distributed initially into the lipids of highly perfused tissues and then distributed into the lipids of less perfused tissues. In TCDD uptake and elimination studies in medaka, approximately 90% of the TCDD was in the extracted lipid[20]. The transfer of chemicals between various tissues is rate limited by blood flows and partitioning into lipid-rich compartments. The blood flows to adipose tissues and skeletal muscles are only a few percent of those to highly perfused organs[21]. The biphasic elimination kinetics observed in this study could be explained by an initial rapid elimination of chemicals from the lipids in highly perfused tissues(central compartment) followed by the slower elimination from the lipids in less perfused tissues(peripheral compartment). The major elimination routes of chemicals in fish are urinary excretion through the kidneys, transport across respiratory surfaces of gills, and secretion into the bile from the liver for subsequent fecal excretion.

Using a single compartment model, the $t_{\frac{1}{2}}$ for TCDD in juvenile fathead minnow(0.5~1.0g) was reported to be 14.5 days[13], and the $t_{\frac{1}{2}}$ for TCDD in fry rainbow trout(0.38±0.09g) ranged from 15 to 17 days[22]. These values are lower than the $t_{\frac{1}{2}}$ value in this study(27.2days) using a two compartmental model. Schmieder *et al.*[20] reported 47% for TCDD was eliminated after 6 months in medaka(175mg, 7.5% lipid content) exposed to does of 2.4pg/mg at day 12 of exposure. However, the animals used in their studies are much larger than the animals used in this study, and some animals showed toxic effects during the exposure and depuration period. Because of the sequestration of highly lipophilic chemicals in compartments distant from the elimination sites and the decrease of the surface areas to volume ratios for the elimination sites, elimination rates for highly lipophilic compounds generally decrease with increasing body weight[23]. The lower elimination of TCDD in the medaka studies by Schmieder *et al.*[20] could be attributable to the larger fish size and toxic effects.

IV. SUMMARY

Studies were carried out to compare the bioconcentrations of TCDD and PCB 126 in different sizes of Japanese medaka, and to examine the whole body elimination kinetics for TCDD and PCB 126 in juvenile Japanese medaka.

For bioconcentration studies, three different sizes of fry and juvenile medaka were exposed statically to varying doses of waterborne TCDD and PCB 126 for 96hours. The 96-h bioconcentration factors and their lipid normalized values for TCDD and PCB 126 were similar, and decreased with increasing body size. The average total lipid content(%) in the fry, juvenile, and adult Japanese medaka were 5.7, 4.2, and 4.6, respectively.

For elimination studies, the juvenile medaka were exposed to three different doses of water-borne TCDD and PCB 126 for 96 hours, and

then depurated for 42days in a flow-through system. The elimination phases followed first-order kinetics and fit a two compartment model better than a single compartment model. The calculated whole body elimination half-life($t_{\frac{1}{2}}$) values for TCDD and PCB 126 using a two compartment model were 27.2 days and 32.3 days, respectively. The elimination rate constants of the peripheral compartment(β) for TCDD and PCB 126 were 0.025/d and 0.021/d, respectively. The $t_{\frac{1}{2}}$ value for PCB 126 was higher than that of TCDD even though they have similar log Kow values.

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