Toxic Potency of 3,3',4,4',5-pentachlorobiphenyl relative to 2,3,7,8-tetra-chlorodibenzo-*p*-dioxin in the Japanese medaka early life stage assay.

Youngchul Kim and Keith R. Cooper, Department of Environmental Science, Rutgers-The State University, New Brunswick, NJ 08903 USA

Abstract - Studies were carried out to compare the toxicity of 3,3',4,4',5-pentachlorobiphenyl (PCB 126) with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in newly fertilized Japanese medaka (*Oryzias latipes*) eggs and one-month-old larvae. PCB 126 caused the same type and sequence of lesions as TCDD. The TEF for PCB 126 based on the LD_{50} value ranged from 0.017 to 0.024. The inhibition of swim bladder inflation (SBI) was the most sensitive and clearly defined end point measured. The TEF based on inhibition of SBI was 0.030. The inhibition of SBI resulted in 100% death in post-hatch larvae. The wasting-type syndrome was the most sensitive toxicological end point measured in the one-month-old larvae and the TEF for PCB 126 was 0.022.

Introduction

The toxic equivalency factor (TEF) method has been proposed for use in ecological and human health risk assessment of PCDDs, PCDFs, and PCBs[1]. TEFs describe the fractional potency of a congener relative to TCDD, which is assigned a value of 1.0. Once the TEF for a congener is known, it may be multiplied by the congener's concentration in animal tissue to determine the toxic equivalent quantity (TEQ) contributed by that congener. In a complex mixture, the individual TEQ can be added for all congeners to determine the total TEQ. The total TEQ may then be compared with the laboratory-determined toxicity of TCDD to assess the risk to exposed organisms. Because of accumulation in the food chain and high TEF value of PCB 126, PCB 126 has been considered the most toxicologically significant PCB congener present in environmental biota. PCB 126 is often the greatest contributor to the total TEQ in feral fish tissue, accounting for 40 to 90% of the total toxic potency of PCBs having a "dioxin-like" activity[2-3]. Recent studies have shown that the relative toxic potency of PCB 126 in fish may differ significantly from the toxic potency in rodents[4-5]. This study was undertaken to evaluate toxic potency of PCB 126 in Japanese medaka (*Oryzias latipes*) using several different toxicological end points.

Experimental Methods

Chemicals. [1,6-^{3}H] 2,3,7,8-Tetrachloro dibenzo-*p*-dioxin (34.7 Ci/mmol, radiochemical purity of 98%) was purchased from Chemsyn Science Laboratories (Lenexa, Kansas), and (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).

Animal Husbandry. Adult Japanese Medaka (*Oryzias latipes*), golden strain, brood stock were obtained from Gulf Coast Research, Gulfport, MS. Approximately 50 to 70 fish were housed in 10 or 15 gallon glass aquaria. Incoming water was filtered in-line by a sand filter, two 25 μm particle filters, and an activated carbon filter. Filtered water flowed through an eight watt ultraviolet sterilizer and was distributed to individual aquria at a flow rate of up to two gallons per hour. The water temperature was maintained at 25 ± 2°C using a central heater and a individual heater. The fish were fed Tetramin® tropical fish food (Tetra Werke, Melke, Germany) and newly hatched brine shrimp, *Artemia salina*, three to four times a day, and were maintained on an artificial photoperiod of 16-hour light and 8-hour dark cycle.

Test Organisms and Exposure Systems. Egg collection and handling followed the method by Kirchen and West[6]. A static non-renewal exposure assay system was used for embryo toxicity tests. Eighty to ninety blastula stage eggs were placed individually into five different doses of [^{3}H]-TCDD (1.0-11.5 pg/ml) and [^{14}C]-PCB 126 (30.8-845.0 pg/ml) dissolved in 1ml of rearing solution within a 2 ml Teflon capped vial. The exposed eggs were kept in an incubator at 25 ± 0.5°C. Thirty two eggs for [^{3}H]-TCDD and sixteen eggs for [^{14}C]-PCB 126 were examined for toxic effects under a dissecting microscope daily until death or 3 days post-hatch, and the rest of eggs were used for the measurement of tissue dose on day eleven after exposure. In larval toxicity tests, forty one one-month-old larvae, standard length; 8.0 ± 0.7 mm, and wet weight; 5.9 ± 2.0 mg (N=10), were statically exposed to five different doses of [^{3}H]-TCDD (0.08-3.83 pg/ml) and [^{14}C]-PCB 126 (3.0-185.7 pg/ml) dissolved in 200 ml of dechlorinated water within a 450 ml glass rearing dish for 96 hours. Following the exposure, ten larvae were removed for tissue dose measurements and the rest of the larvae were transferred to a two and half gallon glass aquaria, set up as a flow-through system, and were observed for 60 days. The water temperature was maintained at 17 ± 2°C using a central heater. The larvae were fed newly hatched brine shrimp once or twice a day. In both embryo and larval toxicity tests, a vehicle solvent control (0.02 % acetone) was used.

Liquid Scintillation Counting. Tissue doses were measured by a Tracor Mark III liquid scintillation counter (Tracor Analytic, ELK Grove Village, IL). Disintegrations per minute (DPM) of samples were converted to concentration equivalents after subtraction of background level. To measure the tissue dose of [^{3}H]-TCDD, five composites of chorionated embryos and seven composites of newly hatched larvae were used. For the tissue dose measurement of [^{14}C]-PCB 126, five composites of chorionated embryos and six composites of newly hatched larvae were used. On day eleven after exposure, composites of embryos and newly hatched larvae were weighed, then digested with 1 ml of 1 N sodium hydroxide for 24 hours followed by neutralization with 50 μl of glacial acetic acid prior to liquid scintillation counting. In larval toxicity tests, ten individual larvae were measured for the tissue doses of both [^{3}H]-TCDD and [^{14}C]-PCB 126.

Results and Discussion

The toxic doses and TEFs for PCB 126 in the Japanese medaka embryo tests are summarized in Table 1. The same type and sequence of lesions were observed in the Japanese medaka embryos and newly hatched larvae after exposure to PCB 126 as those observed after TCDD exposure.

Lesions did not develop until the liver rudiment formed in both TCDD and PCB 126 exposed animals[7]. There was a dose-dependent increase absorption of TCDD and PCB 126 both in chorionated embryos and newly hatched larvae ($r^2 > 0.97$). Uptake doses (%) of TCDD and PCB 126 in chorionated embryos/newly hatched larvae on day 11 after exposure were 33.6/25.9 and 37.8/27.7, respectively. Embryos exposed to lethal doses died with lesions of multifocal hemorrhage, pericardial and yolk-sac edema, craniofacial malformation, and inhibition of swim bladder inflation (SBI). LD_{50} (pg/mg) of chorionated embryos/newly hatched larvae for TCDD and PCB 126 were 2.7/4.7 and 111.2/225.1, respectively. Embryos exposed to relatively high doses developed a tube-shaped heart, and died prior to or at hatching, ED_{50} (pg/mg) of chorionated embryos/newly hatched larvae for TCDD and PCB 126 were 3.7/6.0 and 213.9/376.7, respectively. ED_{50} (pg/mg) of haching inhibition for TCDD and PCB 126 were 7.0/10.0 and 243.2/407.1 as a tissue dose basis of chorionated embryos/newly hatched larvae, respectively. Embryos exposed to sub-lethal doses developed an inhibition of SBI, ED_{50} (pg/mg) of chorionated embryos/newly hatched larvae for TCDD and PCB 126 were 1.9/3.5 and 59.2/125.9, respectively. SBI normally occurred within 2 days after hatch, and the percentage inhibition of SBI in controls was less than 10%. The larvae which did not inflate their swim bladder by 3 days post-hatch rarely inflated the swim bladder (< 5%). All larvae showing inhibition of SBI died, even though they had lived until 3 days post-hatch. Inhibition of SBI was dose related for both TCDD and PCB 126 ($r^2 > 0.95$). Inhibition of SBI was the most sensitive and easily observed end point measured. Harris et al.[8] reported that lack of SBI is possibly due to the inhibition of lactate dehydrogenase and carbonic anhydrase, which are important enzymes in the gas gland that control the inflation of the teleost swim bladder. TEFs for PCB 126 in the Japanese medaka embryos based on inhibition of SBI and mortality in this study were 0.030 and 0.022, respectively. Our values are lower than 0.06 and 0.09 reported by Harris et al. The difference may be due to the fact that our data is based on tissue dose and Harris et al. was based on exposure concentration.

The toxic doses and TEFs for PCB 126 in the one-month-old Japanese medaka larvae tests are summarized in Table 2. There was a dose-dependent increase in the absorption of TCDD and PCB 126 in the exposed larvae, $r^2 = 0.99$ and $r^2 = 0.89$, respectively. The BCF for TCDD and PCB 126 in this study were 714 and 765, respectively. The lesions of larvae exposed to PCB 126 were similar to those exposed to TCDD. The most significant lesions were the following wasting-type syndromes: weight loss; shortened length; deformation of the lower jaw; multifocal hemorrhages in the head, body and caudal area and/or fin erosion with bent or broken fin rays; and epidermal edema on the head. The larvae exposed to lethal doses showed erratic swimming and a tendency to remain on the bottom of the aquaria. There was a marked decrease in food consumption at one week after transfer to clean water. The LD_{50}/ED_{50} (pg/mg) for one-month-old larvae exposed to TCDD and PCB 126 were 2.3/2.0 and 138.0/89.7, respectively. Wasting-type syndrome for both TCDD and PCB 126 exposed animals was dose related ($r^2 > 0.95$), and a more sensitive end point than mortality. The wasting-type syndrome is a useful toxicological end point for animals exposed to sub-lethal doses. TEFs for PCB 126 based on LD_{50} and ED_{50} were 0.017 and 0.022, respectively. The LOAELs (pg/mg) for TCDD and PCB 126 were 1.4 and 60.8, respectively. The standard lengths and wet-weights of the larvae exposed to the LOAELs were significantly different ($p < 0.05$) from the control.

In conclusion, PCB 126 caused similar lesions as those following TCDD exposure in the Japanese medaka (*Oryzias latipes*). TEFs for PCB 126 based on LD_{50} in a Japanese medaka

(0.017-0.024) are much higher than those for trout species (0.003-0.005)[4-5], and in the range reported for rodents (0.01-0.1) based on either the NOAEL or the LOAEL from a subchronic feeding study[9]. Inhibition of swim bladder inflation (SBI) was a sensitive toxicological end point, and the TEF for PCB 126 based on inhibition of SBI was 0.030.

Table 1. Toxic potencies of PCB 126 relative to 2,3,7,8-TCDD on the Japanese medaka embryos and newly hatched larvae based on different toxicological end points.

End point[a]	2,3,7,8-TCDD	PCB 126	TEF
NOAEC(pg/ml)	1.0	54.7	-
NOAEL(pg/embryo)	0.3	26.0	0.012
NOAEL(pg/mg embryo)	0.3	21.2	0.014
NOAEL(pg/larva)	0.3	16.3	0.018
NOAEL(pg/mg larva)	0.7	37.9	0.018
LOAEC(pg/ml)	3.4	83.5	-
LOAEL(pg/embryo)	1.0	35.8	0.028
LOAEL(pg/mg embryo)	1.0	28.4	0.035
LOAEL(pg/larva)	1.0	32.9	0.030
LOAEL(pg/mg larva)	1.8	63.6	0.028
LC_{50}(pg/ml)[b]	8.1(7.2-9.1)[f]	411.6(282.3-598.9)	-
LD_{50}(pg/embryo)	2.9(2.5-3.3)	133.6(96.3-188.9)	0.022
LD_{50}(pg/mg embryo)	2.7(2.3-3.1)	111.2(79.6-159.3)	0.024
LD_{50}(pg/larva)	2.0(1.8-2.2)	99.0(72.5-132.5)	0.020
LD_{50}(pg/mg larva)	4.7(4.1-5.3)	225.1(161.3-309.5)	0.021
EC_{50}(pg/ml)[c]	6.2(5.1-7.1)	207.8(110.7-321.6)	-
ED_{50}(pg/embryo)	2.1(1.6-2.4)	72.8(44.1-106.1)	0.029
ED_{50}(pg/mg embryo)	1.9(1.5-2.3)	59.2(35.4-87.4)	0.032
ED_{50}(pg/larva)	1.6(1.3-1.8)	59.8(34.5-83.5)	0.027
ED_{50}(pg/mg larva)	3.5(2.8-4.1)	125.9(70.7-183.4)	0.028
EC_{50}(pg/ml)[d]	10.6(9.6-12.6)	765.2(567.1-2568.7)	-
ED_{50}(pg/embryo)	4.0(3.5-5.0)	249.3(180.6-514.3)	0.016
ED_{50}(pg/mg embryo)	3.7(3.3-4.5)	213.9(152.2-443.8)	0.017
ED_{50}(pg/larva)	2.4(2.2-2.7)	162.3(127.3-353.0)	0.015
ED_{50}(pg/mg larva)	6.0(5.5-7.0)	376.7(295.9-1130.5)	0.016
EC_{50}(pg/ml)[e]	18.2(12.9-109.8)	843.6(643.7-4270.5)	-
ED_{50}(pg/embryo)	7.7(5.2-73.3)	281.1(204.9-855.0)	0.027
ED_{50}(pg/mg embryo)	7.0(4.6-63.8)	243.2(173.8-730.0)	0.029
ED_{50}(pg/larva)	3.5(2.7-20.2)	176.2(140.7-568.4)	0.020
ED_{50}(pg/mg larva)	10.0(7.2-106.0)	407.1(328.1-1548.7)	0.025

a. calculated by the EPA probit analysis program (version 1.4).
b. death before 3 days post-hatch.
c. inhibition of swim bladder inflation (SBI).
d. severe lesion resulting in death prior to or at hatching.
e. inhibition of hatching.
f. 95% lower and upper confidence limits.

Table 2. Toxic potencies of PCB 126 relative to 2,3,7,8-TCDD on the one-month-old Japanese medaka larvae based on different toxicological end points.

End point[a]	2,3,7,8-TCDD	PCB 126	TEF
NOAEC(pg/ml)	0.9	51.9	-
NOAEL(pg/larva)	4.5	221.0	0.020
NOAEL(pg/mg larva)	0.6	31.6	0.019
LOAEC(pg/ml)	1.8	123.4	-
LOAEL(pg/larva)	8.1	443.3	0.018
LOAEL(pg/mg larva)	1.4	60.8	0.023
LC_{50}(pg/ml)[b]	3.4(2.9-4.3)[d]	176.1(157.7-215.6)	-
LD_{50}(pg/larva)	14.4(12.4-17.9)	833.1(694.2-1108.1)	0.017
LD_{50}(pg/mg larva)	2.3(2.0-2.9)	138.0(110.1-194.2)	0.017
EC_{50}(pg/ml)[c]	2.9(2.5-3.5)	143.2(127.7-158.2)	-
ED_{50}(pg/larva)	12.4(10.8-14.7)	589.2(505.4-695.3)	0.021
ED_{50}(pg/mg larva)	2.0(1.8-2.4)	89.7(74.9-110.2)	0.022

a. calculated by the EPA probit analysis program (version 1.4).
b. death during 60 days rearing.
c. wasting-type syndrome.
d. 95% lower and upper confidence limits.

Literature Cited

(1) Safe, S. *Crit. Rev. Toxicol.* **1990**, 21, 51-88.
(2) Smith, L. M.; Schwartz, T. R.; Feltz, K.; Kubiak, T. J. *Chemosphere.* **1990**, 21, 1063-1085.
(3) Hong, C. S.; Bush, B.; Xiao, J. *Ecotoxicol. Environ. Saf.* **1992**, 23, 118-131.
(4) Walker, M. K.; Peterson, R. E. *Aquat. Toxicol.* **1991**, 21, 219-238.
(5) Zabel, E. W.; Cook, P. M.; Peterson, R. E. *Environ. Toxicol. Chem.* **1995**, 14, 2175-2179.
(6) Kirchen, R. V.; West, W. R; *The Japanese medaka, Its care and development*, Carolina Biological Co.; Burlington, **1976**; PP. 36.
(7) Wisk, J. D.; Cooper, K. R. *Environ. Toxicol. Chem.* **1990**, 9, 1159-1169.
(8) Harris, G. E.; Kiparissis, Y.; Metcalfe, C. D. *Environ. Toxicol. Chem.* **1994**, 13, 1405-1413.
(9) Van Birgelen, A. P. J. M.; Van Der Kolk, J.; Fase, K. M.; Bol, I.; Poiger, H.; Brouwer, A.; Van Den Berg, M. *Toxicol. Appl. Pharmacol.* **1994**, 127, 209-221.