Perinatal exposure to dioxins perturbs learning performance of the rat in a dose-specific fashion.

Rieko Hojo[1], Hojo Rieko [1], Kakeyama Masaki[1], Yonemoto Junzo [1], Tohyama Chiharu[1]

[1]National Institute for Environmental Studies, Tsukuba

Introduction

Dioxins (chlorinated dibenzo-p-dioxin congeners and related compounds including coplanar PCBs) are transferred transplacentally and lactationally from mothers to the developing brain of offspring. Maternal exposure to dioxins are suspected to cause adverse effects on the advanced brain function of offspring, because Previous studies indicate that the most toxic dioxin congener, 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD), affected the advanced brain function of rats, even when mothers had been exposed to a relatively low level of dioxins that would not affect themselves [1, 2]. In coplanar PCBs, which are dioxin-like, toxic equivalency factors (TEFs) are based on similar toxicity to TCDD and on a common mechanism of action, mediated by the aromatic hydrocarbon receptor (AhR)[3]. However, non-coplanar PCBs, which are considered to be non-dioxin-like PCBs, also show adverse effects on the learning and memory functions of offspring [4, 5]. In the present study, we hypothesize that coplanar PCBs have two types of toxicities, one is the similar to TCDD and the other is the specific toxicity of PCB itself. To address this hypothesis, effects of maternal exposure to one of the coplanar PCBs, 3,3',4,4',5-pentachlorobiphenyl (PCB126, 1997 WHO TEF = 0.1), on learning and behavioural performance of rats assessed by schedule-controlled operant behavior (SCOB) were examined and compared to TCDD.

Methods and Materials

***Subjects and exposure*:** Long-Evans Hooded rats were purchased from Charles River (Chicago, USA). Administration of a single dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) or 3,3',4,4',5-pentachlorobiphenyl (PCB126) were performed on gestational day (GD) 15. Pregnant dams were randomly dosed with 50, 200 and 800 ng TCDD/kg (T50, T200, T800 groups, respectively), or 500, 2000 and 8000 ng PCB126/kg (P500, P2000, P8000 groups, respectively), or vehicle (Control group). On postnatal day (PND) 80, pups from exposed-dams were randomly selected for the experiment.

***Behavioral procedure*:** Behavior was evaluated in operant chamber boxes (Muromachi Kikai, Tokyo) containing two levers along one wall. Rats responding correctly to the lever were provided with a diet pellet (Noyes Co., Inc., Lancaster, NH), and the pellet feeder was located between the levers. A house light, which was mounted in the center of the ceiling, was turned on during the

sessions. Two cue lights, were mounted above each of the lever, were used as a discriminative stimulus. Schedule control and data acquisition were accomplished by a ComPACT ops/w operant behavioral test control system (Muromachi Kikai).

In the SCOB, the multiple FR20 DRL20s schedule (MultFRDRL), was continuously conducted for 30 days (30 sessions). In this schedule, FR20 comprised as one component. In FR20 schedule, the 20th of the lever-pressing were reinforced. The DRL20s schedule comprised as the second component. In DRL20s, each interval between lever presses had to have at least 20 seconds for acquiring a pellet. MultFRDRL schedule lasted 49 min.

***Statistical methods*:** All statistical analyses were carried out by using the Stat View 4 statistical analysis program (Abacus Concepts, Berkeley, USA). A difference was considered significant at $p<0.05$. Data for dam's body weights, pup's weights and the number of pups per dam were analyzed by two-way analysis of variance (ANOVA). Other dose-response data were analyzed by one-way ANOVA followed by Scheffé's post-hoc test. For each component in MultFRDRL, the response rates (number of responses/min) and reinforcement rates (number of reinforcements/min) were analyzed independently by two-way ANOVA with repeated measurement.

Results and Discussion

***Maternal and postpartum data*:** Dam's body weights, pup's weights and the number of pup per dam were not different between experimental groups. There was no statistical significance in the ratio of male and female pups per litter among groups.

***Control group*:** Response and reinforcement rates (responses/min and reinforcements/min, respectively) in each group in the last 5 sessions of SCOB are shown in Figure 1 (during the FR component) and Figure 2 (during the DRL component). In the FR component, the mean ± S.E.M. of response and reinforcement rates in Control group (open bars) were 24.2 ± 3.09 (Figure 1a) and 1.0 ± 0.16 (Figure 2b). In the DRL component, response and reinforcement rates in this group were 8.2 ± 0.40 (Figure 2a) and 0.014 ± 0.0007 (Figure 2b).

***TCDD-exposed groups*:** Exposure to TCDD altered the learning and behavioral performance of offspring in the SCOB (shown as solid bars in figures). During the FR component, animals in T50 group responded at significantly lower rate than those in Control-animals ($p<0.01$, Figure 1a). On the other hand, T200-animals responded at significantly higher rates than Control-animals ($p<0.01$). T800-animals responded at lower rates than T200-animals, being comparable to Control-animals. The dose-response pattern of response rates after TCDD exposure showed an inverted U shape, suggesting that the neurobehavioral effects of TCDD were dose-specific rather than dose-dependent.

As well as the dose-specific pattern in the response rate, the reinforcement rate of T50 group during the FR component was significantly lower, and that of T200 group were higher than that of Control ($p<0.01$, Figure 1b). Reinforcement rate of the T800 group was comparable to that of Control.

During the DRL component, animals in T50 group tend to respond at lower rate although there was no significance. Animals in T200 group responded at significantly higher rate than Control ($p<0.01$, Figure 2a). The inverted U shape dose-response pattern was also found in the

response rate during DRL component. In the reinforcement rate, all TCDD-exposed groups, T50, T200 and T800, showed significantly lower rates than the Control group ($p<0.01$, Figure 2b).

Thus, the present study clearly demonstrated that a single oral administration of TCDD at 50 ng/kg to dams, caused a reduction of learning performance of their offspring in the SCOB. On the other hand, the T200 group acquired more rewards than the Control group during the FR component. However, it can be said that TCDD in this level did not facilitate the learning performance, because the T200 group acquired lower rewards than Control group during the DRL component. It is suggested that the T200 group did not discriminate between the FR and DRL components, but just responded at highly rates in both components. T800-animals also acquired less rewards than Control during the DRL components. Taken together, maternal exposure to 50, 200 or 800 ng TCDD/kg, were found to affect the SCOB performance of offspring in a dose-specific manner.

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***PCB126 exposed groups*:** Exposure to PCB126 (shown as shaded bars in figures) also altered the learning and behavioral performance of offspring in SCOB. Both response and reinforcement rates of P2000 group during the FR component were significantly higher than those of the Control group ($p<0.01$, Figure 1). In P8000 group, those were significantly lower than those of the Control group ($p<0.01$). P500 group tends to show lower rates but without a statistical significance.

During the DRL component, there were no significant differences in the response rates between Control and PCB126-exposed groups. In the reinforcement rate, only P2000 group reduced it significantly. The reinforcement rates of P500 and P8000 were comparable to that of Control ($p<0.01$, Figure 2).

***TCDD vs. PCB126 exposed groups*:** Based on the TEF of PCB126 as 0.1, the doses of PCB126 in the present study, 500, 2000 or 8000 ng/kg are equivalent to 50, 200 or 800 ng TEQ/kg, respectively. When effects of PCB126 are compared with those of TCDD, similarities and differences in toxicities on SCOB performance were found.

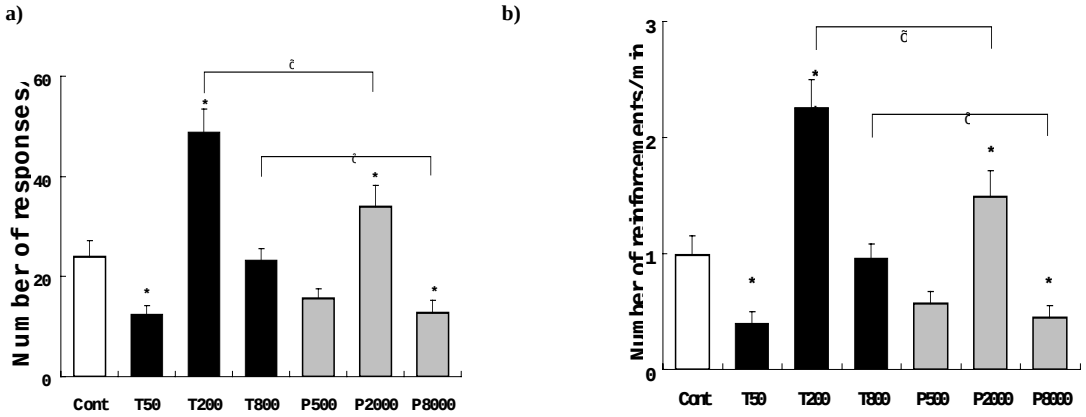
Dose-response pattern of PCB126-adminitered groups showed an inverted U shape in response and reinforcement rates during FR component, as well as that of TCDD. Significant reductions in response rates in FR component were found in T50 group only, but with an apparent reduction in P500 group as well. Both T200 and of P2000 increased response and reinforcement rates, whereas those of T200 were significantly higher than those of P2000 ($p<0.01$). These results suggest that PCB126 shows a dose response pattern similar to TCDD, based on the TEQ, but its toxicities are relatively lower than TCDD in the neurobehavioral performance.

Surprisingly, P8000 severely reduced response rate during the FR component, whereas those of T800 were comparable to that of Control group. The response rate of P8000 were significantly lower than not only that of Control, but also T800 group ($p<0.01$). These results indicate the possibility that P8000 possess a different toxic effect from TCDD. The existence of different toxicity of PCB126 was also suggested by the results of the reinforcement rates; P8000 also inhibited the reinforcement rate although the TEQ-based equivalent dose of TCDD had no effect. Thus, maternal exposure to PCB126 at 8000 ng/kg, was clearly found to reduce learning performance of offspring, being different from TCDD.

Taken together, the present results demonstrated that TCDD exposure reduces learning performance of rats even at a low dose (50 ng/kg) and exerts its neurobehavioral toxicities in a dose-specific manner. The dose-specific pattern in effects of PCB126 were similar but a little lower than those of TCDD on a TEQ basis. In addition, the existence of 'non-dioxin-like toxicity'

of PCB126 was suggested to be present at the high level of exposure. In conclusion, the coplanar PCB is suggested to possess both 'dioxin-like' and 'non-dioxin-like' toxicities in learning behavior.

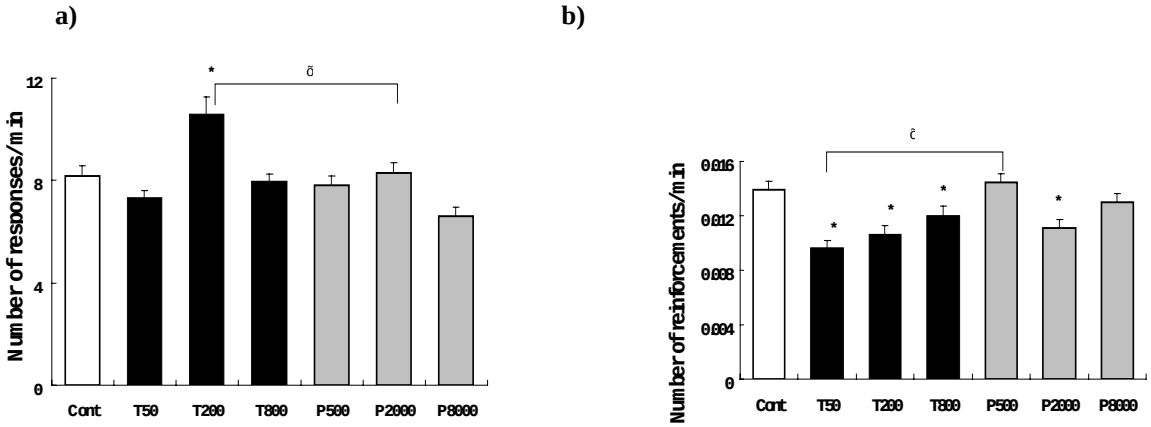
Figure 1.



Response rate in FR component Reinforcement rate in FR component

Figure 1. Means ± S.E.M. of response rate (number of responses/minute) and reinforcement rate (number of reinforcements/minute) during the FR component in the last 5 sessions in MultFRDRL after perinatal exposure to TCDD and PCB126. Control; vehicle-exposed controls (open bar), T50, T200 and T800; 50, 200 and 800 ng/kg of TCDD-exposed groups, respectively (solid bars), P500, P2000 and P8000; 500, 2000 and 8000ng/kg of PCB126-exposed groups, respectively (shaded bars). * indicates $p < 0.05$, vs. Control, indicates $p<0.05$, TCDD vs. PCB.

Figure 2.



Response rate in DRL component

Reinforcement rate in DRL component

Figure 2. Means ± S.E.M. of response rate (number of responses/minute) and reinforcement rate (number of reinforcements/minute) during the DRL component in the last 5 session in MultFRDRL after perinatally exposed TCDD and PCB126. Control; vehicle-exposed controls (open bar), T50, T200 and T800; 50, 200 and 800 ng/kg of TCDD-exposed groups, respectively (solid bars), P500, P2000 and P8000; 500, 2000 and 8000ng/kg of PCB126-exposed groups, respectively (shaded bars). * indicates $p < 0.05$, vs. Control, indicates $p<0.05$, TCDD vs. PCB.

Acknowledgements

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References

1 Hojo R., Stern S., Zareba G., Markowski VP., Cox C., Kost JT. and Weiss B. (2002) Environ. Health Perspect. 110, 247-54.

2 Kakeyama M., Sine H., Miyabara Y. and Tohyama C. (2003) Neurotoxicol. 24, 207-17.

3 Van den Berg, M., Birnbaum, L., Bosveld, A.T.C., et al. (1998) Toxic Equivalency Factors (TEFs) for PCBs, PCDDs, PCDFs for Humans and Wildlife. Environ. Health Perspect., 106 106, 775-792

4 Holene E., Nafstad I., Skaare JU. and Sagvolden T. (1998) Behav. Brain Res. 94(1), 213-24.

5 Schantz SL., Seo BW., Moshtaghian J., Peterson RE. and Moore RW. (1996) Neurotoxicol. Teratol. 18(3), 305-13.