EFFECTS OF ACUTE AND SUBCHRONIC ORAL DOSING ON IMMUNE FUNCTION AND EROD INDUCTION IN C57BL/6 FEMALE MICE: ASSESSMENT OF TEF VALUES

M. Steinberg, N. Harper, S. Safe
Department of Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M University, College Station, Texas, USA 77843

ABSTRACT
This study evaluates the effects of acute and subchronic oral dosing of TCDD and 3,3',4,4',5-pentachlorobiphenyl (3,3',4,4',5-PCB) in 26-28 week old C57BL/6 female mice and determines whether the toxic equivalency equivalency factors (TEFs) generated from these females are comparable to TEFs published in the literature. The experimental parameters were body weight loss, % liver:body weight, % spleen:body weight, suppression of the plaque-forming cell (PFC) response, suppression of anti-TNP-LPS IgM titer, and induction of EROD enzyme activity. There was no significant change in body weight and the % liver:body weight was significantly increased in mice treated at the highest acute dose of 14 μg/kg TCDD. The % spleen:body weight was significantly increased only in mice treated chronically with 20 μg/kg 3,3',4,4',5-PCB. After probit conversion, ED_{50} values were calculated for the PFC response, IgM titer, and EROD induction. The ED_{50} values from the orally dosed 24-28 week old female mice were compared to 7-10 week old male mice treated with TCDD by i.p. injection given that the immunotoxicity data used to generate TEFs was largely derived from young male mice treated by i.p. injection. Overall, the ED_{50} values from the female mice were greater than those from the male mice and the relative potency ranges were similar to those published. This study confirms that the published TEFs are valid for animals that differ in age, sex, and route of exposure.

INTRODUCTION
Commercial polychlorinated biphenyls (PCBs) are complex mixtures of congeners with various degrees of chlorination and are often contaminated with polychlorinated dibenzofurans. These halogenated aromatic hydrocarbons (HAHs) bind to the Ah receptor with different affinities and elicit a number of biological responses which differ with respect to age, sex, strain, species, and route of exposure. Two of the most sensitive biological indicators of exposure to PCBs and polychlorinated dibenzo-p-dioxins (PCDDs) are immunosuppression in mice and microsomal enzyme induction. Several studies have developed structure-activity relationships for the various PCB and PCDD congeners. Based on these studies, the relative toxicities of individual halogenated aromatics are determined relative to

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which is the most potent HAH. TEFs derived from this information can be used in the risk assessment of halogenated aromatic hydrocarbon mixtures. This study evaluated the effects of acute and subchronic oral dosing of TCDD and 3,3',4,4',5-PCB in 26-28 week old C57BL/6 female mice on immune function and hepatic microsomal enzyme (EROD) induction. Since 7-10 week old male mice treated i.p.with 2,3,7,8-TCDD were used in this laboratory for immunotoxicity studies that generated TEFs, the results from the female mice were compared to this model. Relative potency ranges and TEFs for the female mice relative to the male mice were then compared to relative potency ranges and TEFs published in the literature.[1]

MATERIALS AND METHODS

Chemicals Sheep red blood cells (SRBCs) in Alsevars' solution were obtained from M.A. Bioproducts, Maryland, U.S.A. Guinea pig complement was purchased from GIBCO Laboratories, New York, U.S.A. Trinitrophenyl lipopolysaccharride (TNP-LPS), picryl sulfonic acid, glycyl glycine, goat anti-mouse IgM and IgG conjugated to alkaline phosphatase, and nitrophenylphosphate were purchased from Sigma (St. Louis, MO, U.S.A). TCDD and 3,3',4,4',5-PCB were synthesized in this laboratory. All other chemicals used were the highest grade commercially available.

Animals Female C57BL/6 mice (26-28 weeks) were obtained from an in house breeding colony and male C57BL/6 mice (7-10 weeks) were obtained from Harlan Sprague-Dawley Houston, TX. All mice were maintained on a 12 hour light/dark schedule with free access to food and water.

Treatment Protocol 3,3',4,4',5-PCB and TCDD were dissolved in a corn oil vehicle. In the acute studies, the female mice were gavaged with vehicle, 3,3',4,4'5-PCB (6 or 20 μg/kg), or TCDD (4.2 or 14 μg/kg) in a total volume of 10 μL/g body weight. For the subchronic studies, female mice were gavaged with vehicle, 3,3',4,4',5-PCB (0.43 or 1.43 μg/kg/day), or TCDD (0.3 or 1.0 μg/kg/day) in a total volume of 10 μL/g body weight for 14 days to equal the dose given in the acute studies. The mice were immunized on day 11 with 50 μg TNP-LPS in 0.2 ml saline and sacrificed on day 15. The male C57BL/6 mice were injected i.p. with TCDD in a total volume of 10 μL/g body weight on day 0, immunized on day 4 with 50 μg TNP-LPS in 0.2 ml saline, and sacrificed on day 8. Each treatment group contained 5 mice and all animals were bled prior to sacrifice by cervical dislocation.

The Plaque-Forming Cell Assay The "Cunningham" modification of the Jerne plaque-forming cell (PFC) assay was used.[2,3] After removal of the liver and spleen, a single cell suspension of spleen cells was prepared, washed and resuspended in RPMI 1640 media. The spleen cells were incubated in a "Cunningham" chamber with guinea pig complement and SRBCs that were haptenated according to the method of Rittenberg and Pratt.[3] Viable spleen cells were counted by trypan blue staining.

ELISA Procedure Five μg of TNP-LPS in phosphate buffered saline (PBS) was incubated in each well of a 96 well microtiter plate overnight at 4°C. The plate was then washed with PBS/TWEEN (0.05 %) buffer. Diluted mouse serum added and incubated overnight at 4°C. The plate was washed with PBS/TWEEN and incubated with goat anti mouse IgM-AP for 2 hr at room temperature. The plate was

then washed and visualized with nitrophenylphosphate for 30-60 min at 20°C. The optical density was read at 410 nm on a Dynatech microtiter plate reader.
EROD Enzyme Activity Hepatic microsomes were prepared and EROD activity was measured as previously described.[4]

RESULTS AND DISCUSSION

No significant changes in body weight or % spleen:body weight occurred in 26-28 week old C57BL/6 female mice treated with acute or chronic doses of TCDD (Table 1). A dose-dependent increase in the % liver:body weight occur in mice receiving acute doses of TCDD which was significant at a dose of 14 μg/kg (Table 1). In mice treated with 3,3'4,4'5-PCB, the average body weght loss was greater than that seen in mice treated with TCDD even though the increased loss was not significant within the PCB treated groups. There were no differences in % liver:body weight for mice receiving either an acute or chronic dose of 3,3',4,4'5-PCB, however, % spleen:body weight ratio was significantly increased in mice dosed subchronically at 20 μg/kg.

TABLE 1

Treatment	Dose (μg/kg)	Ave. Body Weight Loss (g)	% Liver:Body Weight (g) ± S.E.	% Spleen:Body Weight (g) ± S.E.
TCDD				
Acute	0	0.00	6.90± 0.20	0.69 ± 0.06
	4.2	0.00	8.20 ± 0.50	0.60 ± 0.07
	14	0.00	8.80 ± 0.40*	0.56 ± 0.05
Subchronic	0	1.00	7.60 ± 0.70	0.56 ± 0.06
(total dose over	4.2	1.80	7.54 ± 0.29	0.58 ± 0.03
14 days)	14	0.79	7.99 ± 0.26	0.53 ± 0.04
3,3',4,4',5-PCB				
Acute	0	2.70	6.28 ± 0.24	0.62 ± 0.02
	6	2.00	7.38 ± 0.41	0.64 ± 0.06
	20	1.70	6.52 ± 0.42	0.70 ± 0.05
Subchronic	0	1.80	6.54 ± 0.54	0.54 ± 0.02
(total dose over	6	2.70	7.12 ± 0.68	0.46 ± 0.03
14 days)	20	2.60	6.47 ± 0.26	0.63 ± 0.03*

* $p < 0.05$ determined by ANOVA , Dunnett 2-tailed post hoc test.

Following probit conversion, ED_{50} values were calculated for the plaque-forming cell response, serum anti-TNP-LPS IgM titer, and EROD enzyme induction (Table 2). For the female mice treated acutely or subchronically with TCDD, the effective doses required to decrease 50 % of the PFC response and serum anti-TNP-LPS IgM titer were greater than those in the male mice receiving an acute dose of TCDD. The ED_{50} values for induction of EROD activity were similar for both female and male mice treated with TCDD. Relative potency ranges for female mice receiving acute or subchronic doses of TCDD were 0.2-1.2 and 0.2-1.0 respectively.

This data indicates that the female mice required doses up to five times greater than the male mice to produce the same effects.

Predictably, the ED50 values for females treated acutely and subchronically with 3,3',4,4',5-PCB were higher than the values for TCDD-treated females or males. The TEFs derived from this study for 3,3',4,4',5-PCB were similar to the published TEFs (supported by the National Institutes of Health P42-ES04917).

TABLE 2: Summary of Data

Treatment	ED_{50} PFC/1E6 Cells (µg/kg)	ED_{50} EROD (µg/kg)	ED_{50} IgM (µg/kg)	Comparative Relative Potency Range	Comparative TEF (Published TEF)
TCDD					
Acute	7.8	1.4	10.2	0.2 - 1.2	0.7 (1)
Chronic	8.2	1.7	11.9	0.2 - 1.0	0.7 (1)
3,3',4,4',5-PCB					
Acute	26.0	3.7	26 mg/kg	0.06 - 0.45	0.15 (0.1)
Chronic	19.0	3.1	25 mg/kg	0.08 - 0.56	0.15 (0.1)
TCDD Acute Male Mice	1.6	1.7	2.8	1	1 (1)

---

[1] Safe, S. Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: Environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). *Crit. Rev. Tox.*, 21, 51-88 (1990).

[2] Cunningham A, Szenberg A. Further inprovements in the plaque technique for detecting single antibody-forming cells. *Immunology* 14, 599-610 (1968).

[3] Rittenberg M, Pratt K. Antinitrophenyl (TNP) plaque assay. *Proc. Soc. Exp. Biol. Med.* 132, 575-581 (1969).

[4] Howie L, Dickerson R, Davis D and Safe S. Immunosupressive and monooxygenase induction activities of polychlorinated diphenyl ether congeners in C57BL/6N mice: quantitative structure activity relationships. *Toxicol. and Appl. Pharmacol.* 105 (2), 254-263 (1990).