TOX IV

ASSESSING THE STRUCTURE ACTIVITY RELATIONSHIP OF POLYHALOGENATED AROMATIC HYDROCARBONS USING ENDOMETRIOSIS AS AN ENDPOINT

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1. INTRODUCTION

Endometriosis is a well-established disorder plaguing women of all ages throughout the world. This disease affects an estimated 10-15% of pre-menopausal women undergoing gynecological surgery. [1)] Endometriosis is primarily described as the growth of endometrial tissue outside the uterus, usually in the peritoneal cavity, which may cause infertility and a high degree of pain.[2)] Previous studies have shown an increased proliferation of endometriotic sites in rhesus monkeys,[3)] as well as in rats and mice,[4)] following subchronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Thus, exposure to environmental chemicals could potentially enhance the development of endometriotic lesions

All of the well-studied effects of 2,3,7,8-TCDD are mediated by the Ah receptor. [5)] Using 2,3,7,8-TCDD as a prototype, previous studies have derived toxic equivalency factors for polyhalogenated aromatic hydrocarbons which bind to the AhR. [6)7)] Thus, it maybe possible to predict the potencies of other PHAH's on the proliferation of endometriosis using the TEF methodologies. This work was an attempt to show the structure activity relationships between four chemicals and 2,3,7,8-TCDD and to determine if the mechanism for increased proliferation of endometriotic lesions by PHAH's is mediated by the Ah receptor. We assessed the structure activity relationships and mechanisms of endometriotic proliferation by using 2,3,7,8-TCDD and four additional PHAH's with varying degrees of affinity for the Ah receptor. In addition to 2,3,7,8-TCDD, two "dioxin-like" and two "non-dioxin-like" chemicals were used. The "dioxin-like" chemicals used were PCB 126 (3,4,5,3',4'-pentachlorobiphenyl) and 4-PeCDF (2,3,4,7,8-pentachlorodibenzofuran.) The two "non-dioxin-like" chemicals used were PCB 153 (2,4,5,2',4',5'-pentachlorobiphenyl) and 1,3,6,8-TCDD (1,3,6,8-tetrachlorodibenzo-*p*-dioxin.)

2. MATERIALS AND METHODS.

Dosing. Seventy day old B6C3F1 female mice were randomly assigned to treatment groups. Group I included four groups of ten animals per group dosed with 0,1,3, or 10 ug 2,3,7,8-TCDD per kg of body weight. Group II included six groups: one group of ten control animals and five groups of twelve animals per group dosed with 3 or 30 mg PCB 153 per kg body weight, or 100, 300, or 1000 ug PCB 126 per kg of body weight. Group III also included six groups: one group of ten control animals and five groups of twelve animals per group dosed with 10, 30, or 100 ug 4-PeCDF per kg of body weight, or 2 or 20 mg 1,3,6,8-TCDD per kg of body weight. The dosing volume used for all congeners was 10 mL per kg of body weight. All chemicals were dissolved and administered in corn oil. The animals were dosed by oral gavage a total of five times

with three weeks between each dosing and terminated three weeks after the last dose as described by Cummings, *et al.* [4] The dose ranges for 4-PeCDF and PCB 126 were based on their TEF values.[7] The doses of PCB 153 and 1,3,6,8-TCDD were based on their known toxicities and solubilities in the dosing vehicle.

Surgical Methods. Because mice have a closed reproductive tract with a bursa enclosed ovary and an estrous cycle instead of a menstrual cycle, they do not develop endometriosis naturally. Therefore, endometriosis must be induced in these animals through surgical methods performed during the week of the second dosing. The two major steps of the surgical process as described by Vernon and Wilson [8] in a rat model and extended to mice by Cummings and Metcalf [9] were ablation of the left uterine horn and implantation into the peritoneal cavity. Following anesthesia by ether, the mouse was placed in dorsal recumbancy and a 2-3 cm midline incision was made in the skin and underlying muscle. The left uterine horn was located, tied off with sutures, ablated, and cut longitudinally to expose the epithelial lining. After sectioning of the uterine horn into three segments, the uterine pieces were implanted into the peritoneal cavity by suturing to mesenteric blood vessels. After application of antibiotic media into the peritoneum, the abdomen muscles were sutured and the skin closed with wound clips.

Tissue Analysis. At the conclusion of sixteen weeks, the animals were randomly euthanized by carbon dioxide and necropsied. Endometriotic lesion diameters and weights were measured. Lesions, uterine horns, and ovaries were extracted and fixed for histopathology. Livers were also extracted for subsequent enzymatic analysis.

Statistics. Primary statistical analysis of endometriotic lesion diameters from all the different chemical treatment groups was performed using the Dunnett's test.

3. RESULTS AND DISCUSSION

Figure 1 shows the dose-response effects of the administered chemicals on diameter size of endometriotic lesions. Analysis of lesion diameters using the Dunnett's test revealed statistically significant results for animals dosed with 2,3,7,8-TCDD and 4-PeCDF. Lesion diameters of animals dosed with 1 ug and 3 ug 2,3,7,8-TCDD per kg of body weight, and 100 ug 4-PeCDF per kg of body weight were statistically larger than lesion diameters of control animals. Animals dosed with 10 ug 2,3,7,8-TCDD per kg body weight did not have significantly larger lesion diameters than control animals possibly due to ovarian atrophy. Growth of endometrial lesions is estrogen dependent.[9] Since estrogen is produced in the ovaries, a decrease in ovarian size results in a decrease in estrogen production. Thus, a decrease in endometrial size results from ovarian atrophy, which is consistent with our data. Animals dosed with PCB 126 showed a trend of lesions with larger mean diameters than control animals, but due to variability the increase was not statistically significant. No effect on lesion diameter was apparent in animals dosed with PCB 153 or 1,3,6,8-TCDD.

Statistically significant increases in lesion diameters of animals dosed with 2,3,7,8-TCDD supports previous work stating exposure to 2,3,7,8-TCDD induces proliferation of endometriotic lesions. [4] Because neither of the "non-dioxin-like" chemicals induced increased proliferation and both "dioxin-like" chemicals caused increased lesion diameters, the data is consistent with the hypothesis that the mechanism of increased proliferation of endometriotic lesions is Ah receptor-mediated.

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4. REFERENCES

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Figure 1a. Endometriotic lesion diameter of 2,3,7,8-TCDD-treated mice

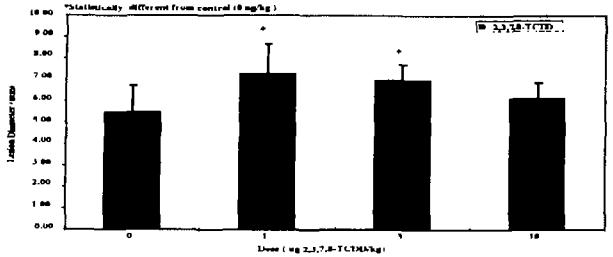

Figure 1b. Endometriotic lesion diameter of PCB 153-treated mice

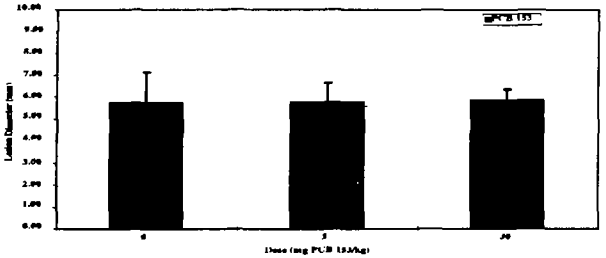

Figure 1c. Endometriotic lesion diameter of PCB 126-treated mice

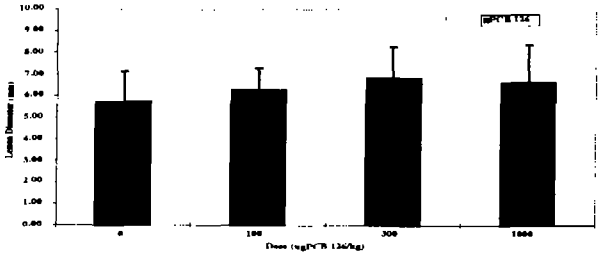

Figure 1d. Endometriotic lesion diameter of 1,3,6,8-TCDD-treated mice

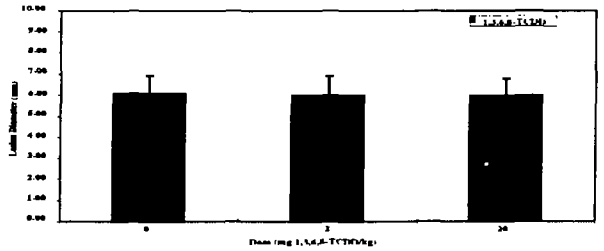

Figure 1e. Endometriotic lesion diameter of 4-PeCDF-treated mice