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# The Antiestrogenic Activity of 3,3',4,4'-Tetrachlorobiphenyl in the Immature Mouse Uterus

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## ABSTRACT

The estrogenic and antiestrogenic effects 3,3',4,4'-tetrachlorobiphenyl (tetraCB) were investigated in the female B6C3F1 mouse. Animals were cotreated with 50 or 75 mg/kg and 0.02 μg/kg17β-estradiol ($E_2$), $E_2$ alone, tetraCB alone or corn oil alone on three consecutive days. Results indicate that tetraCB alone is not estrogenic in the mouse uterus but in combination with $E_2$, tetraCB inhibited $E_2$-induced uterine wet weight and peroxidase activity.

## INTRODUCTION

The aryl hydrocarbon receptor (AhR) has been widely identified in mammalian tissues. An endogenous ligand for this receptor has not been reported however Poland and coworkers (1976) initially showed that the environmental toxin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) binds with high affinity to this receptor protein. Subsequent studies have identified a large number of structurally-related TCDD-like polychlorinated biphenyls (PCBs), dibenzofurans (PCDFs) and dibenzo-*p*-dioxins (PCDDs) which also bind to the AhR. AhR agonists induce a broad spectrum of biochemical and toxic effects in laboratory animals and mammalian cells in culture. AhR agonists have been extensively characterized as endocrine disruptors and recent studies have shown the estrogenic activity of some AhR agonists. Nesaretnam and coworkers (1996) recently reported the estrogenic activity of 3,3',4,4'-tetrachlorobiphenyl (tetraCB) in human breast cancer cells and in the immature mouse. However, previous studies have shown that tetraCB is an inhibitor of 17β-estradiol ($E_2$)-induced secretion of procathepsin D in MCF-7 human breast cancer cells (Krishnan and Safe, 1993). The estrogenic and antiestrogenic activity of tetraCB in the immature female mouse uterus have now been investgiated in this laboratory. Data from this study suggest that tetraCB is not an estrogen in the mouse uterine model and, in fact, some antiestrogenic activity was observed.

## METHODS

***Animals.*** B6C3F1 female mice were bred in an on site animal facility and housed 6-9 per cage with ad libitum access to food and water. TetraCB was dissolved in corn oil with slight warming and the total dose divided into 3 daily injections. Groups of mice (n=6-9) received 0.1 ml of tetraCB solution or vehicle control i.p. for 3 days beginning at 21 days of age.

Some groups also received 0.02 μg/day of $E_2$ (in corn oil) by i.p. injection on the same 3 treatment days (21-23). The doses of $E_2$ were the minimal effective dose which induced the 3 uterine responses of interest. Animals were killed by carbon dioxide asphyxiation 20 h after the last treatment and the uteri were quickly removed, cleaned of connected tissue, weighed, nicked, and blotted. The uteri were then bisected into right and left halves, each half containing an entire uterine horn.

***Estrogen and Progesterone Receptor Binding Assays (ER and PR)*.** The method used for these assays was carried out as previously described (Ramamoorthy *et al.*, 1997). Analysis was conducted on pooled uteri from each treatment group.

***Uterine Peroxidase Assay (UPO).*** This assay was conducted as previously described (Ramamoorthy *et al.*, 1997). Analysis was conducted on pooled uteri from each treatment group.

## RESULTS AND DISCUSSION

1. Competitive ER binding assays were performed on mouse uterine cytosol. Unlabelled $E_2$ competitively bound to the ER and the $IC_{50}$ values was $6.31 \times 10^{-14}$ M (Figure 1). The results also showed that tetraCB did not competitively bind the ER (Figure 1) and this contrasted to a previous study which reported binding to ER isolated from human breast cancer cells (Nesaretnam *et al.*, 1996).
2. In female B6C3F1 mice treated with different doses of tetraCB, the compound did not significantly increase uterine wet weight, PR binding and uterine peroxidase activity at dose levles of 50 and 75 mg/kg X 3 (Figure 2). Moreover, at the higher dose of tetraCB there was a significant decrease in uterine peroxidase activity compared to control.
3. In female B6C3F1 mice cotreated with 0.02 μg $E_2$ and different doses of tetraCB, there was a significant ($p < 0.05$) inhibition of $E_2$-induced uterine wet weight gain and uterine peroxidase levels but no inhibitory effect on PR levels was observed. The lower dose of tetraCB (50 mg/kg) inhibited uterine wet weight and peroxidase activity by 7% and 33%, respectively. The higher dose of tetraCB (75 mg/kg) inhibited both parameters by 32% and 55%, respectively. (Figure 3).
4. This study has shown that 3,3',4,4'-tetrachlorobiphenyl is not an estrogen in the mouse uterine model and, in fact, exhibits some antiestrogenic activity. The antiestrogenic activity of tetraCB in MCF-7 cells has previously been reported (Krishnan et al, 1993) and this is consistent with the well characterized antiestrogenic activity of a diverse spectrum of AhR agonists (Safe, 1995). The reasons for the differences observed in this research compared to results of a previous study (Nesaretnam et al, 1996) are unknown and are currently being investigated.

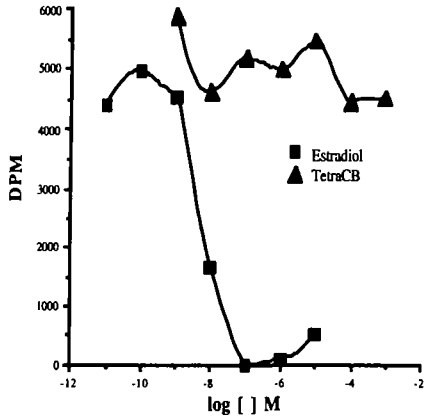


Figure 1. Competitive binding of tetraCB to cytosolic mouse uterine ER. Uterine ER was incubated with 10 nM [3H]Estradiol.

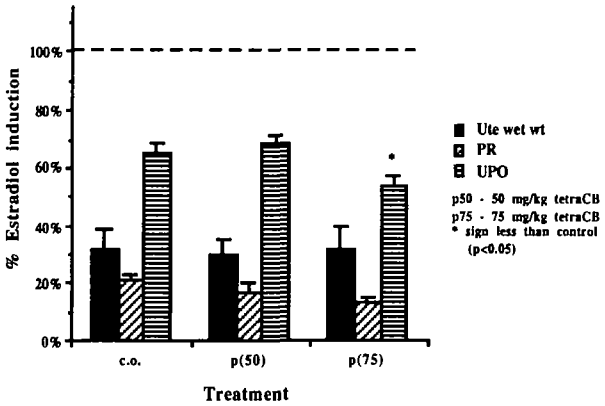


Figure 2. Effect of tetraCB on ute wet weight, prog rec and ute perox activity in B6C3F1 female mouse uterus.

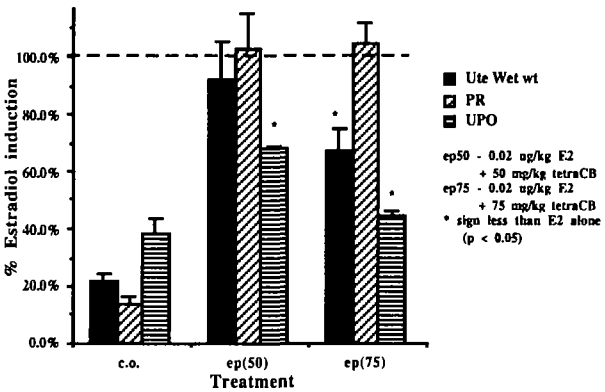


Figure 3. Effect of cotreatment of tetraCB + estradiol on ute wet wt, prog rec and ute perox activity in the B6C3F1 female mouse

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