# The Effects of Hydroxylated Polychlorinated Biphenyls (PCBs) on the Immature Rat and Mouse Uterus

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## ABSTRACT

The antiestrogenic effects of several hydroxylated polychlorinated biphenyls (PCBs) were investigated in female B6C3F1 mice and Sprague-Dawley rats. Animals were cotreated with 25, 50, 100 mg/kg and 0.02μg/kg17β-estradiol ($E_2$), $E_2$ alone, PCB alone or corn oil alone on three consecutive days. Antiestrogencity was observed in two of the compounds, 2,2',4',6' tetrachloro-4-biphenylol and 2,2',3',5',6' pentachloro-4-biphenylol.

## INTRODUCTION

Hydroxylated polychlorinated biphenyls are metabolites which have been identified in wildlife tissue and serum and in human serum (Jansson, *et al.*, 1975; Bergman *et al.*, 1994). Hydroxy PCBs competitively bind to transthyretin (Lans, *et al.*, 1993) and these compounds also bind to estrogen receptor (ER) and exhibit ER agonist activity in the female rat uterus (Korach, *et al.*, 1988b). Limited structure-activity studies with hydroxy-PCBs suggest that the most active compounds, typified by 2',3',4',5'-tetrachloro-4-biphenylol and 2',4',6'-trichloro-4-biphenylol, were substituted with a hydroxyl group in the 4-position in one phenyl (Korach, *et al.*, 1988b). Additional chloro substituents *ortho* to the hydroxyl group decreased the estrogenic activity of the resulting compounds. This study investigates the estrogenic and antiestrogenic activity of a series of hydroxy-PCBs which contain a 2-chloro-4-hydroxy and 3-chloro-4-hydroxy substitution pattern on one ring and 2,4,6-trichloro- and 2,3,5,6-tetrachloro- substituents on the second phenyl ring in both the immature rat and mouse uterus. These compounds were relatively weak ER agonists but exhibited response-specific antiestrogenic activity in the female rat and mouse uterus. Studies on the 2,3,4,5-tetrachloro- and 2,3,4,6-tetrachloro- substituted analogs were also investigated.

## METHODS

***Chemicals and Biochemicals.*** Eight synthetic hydroxy PCB congeners (Figure 1) were prepared via Cadogan coupling procedures as previously described (Safe, *et al.*,1995). The compounds were >95% pure. All remaining chemicals and biochemicals were of the highest purity available from commercial sources.

***Animals.*** Nineteen day-old female Sprague Dawley rats were obtained from Harlan Sprague-Dawley (Houston, TX) and housed 4 to a cage with ad libitum access to food and water. 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. Each hydroxy PCB was dissolved in corn oil with slight warming and the total dose divided into 3 daily injections. Groups of animals (rats n=4-5, mice n=6-9) received 0.2 ml or 0.1 ml (rat or mouse) of a hydroxy-PCB solution or vehicle control i.p. for 3 days beginning at 21 days of age. Some groups also received 0.1 mg/day (rat) or 0.02 μg/day (mouse) 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.

***Progesterone Receptor Binding Assays .*** Method was as previously described in Connor *et al.*, (1996). Analysis was conducted on pooled uteri from each treatment group.

***Uterine Peroxidase Assay.*** Method was as previously described in Connor *et al.*, (1996). Analysis was conducted on pooled uteri from each treatment group.

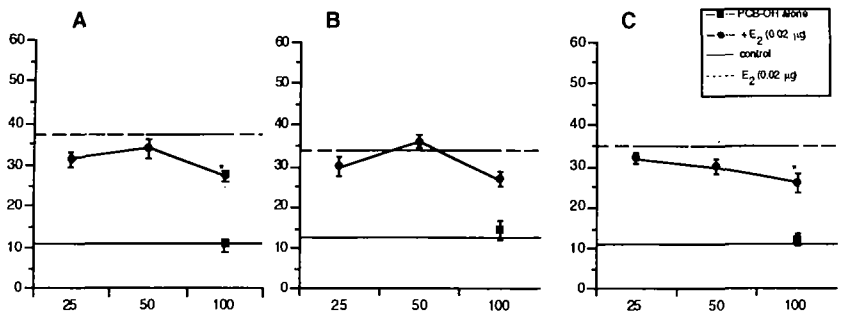
## RESULTS AND DISCUSSION

1. In female B6C3F1 mice cotreated with 0.02 μg $E_2$ and different doses of hydroxy-PCB congeners, the compounds with the highest antiestrogenic activity were 2,2',4',6' tetrachloro-4-biphenylol and 2,2',3',5',6' pentachloro-4-biphenylol, which inhibited $E_2$ induced uterine wet weight gain, progesterone receptor levels and uterine peroxidase levels by 27%, 28%, 1% (2,2',4',6') and 26%, 28%, 47% (2,2',3',5',6') respectively at the highest cotreatment dose (Figure 1, Table 1).

2. In female Sprague Dawley rats cotreated with 0.1 mg $E_2$ and different doses of the hydroxy-PCB congeners, the compounds with the highest antiestrogenic activity were also 2,2',4',6' tetrachloro-4-biphenylol and 2,2',3',5',6' pentachloro-4-biphenylol, which inhibited $E_2$ induced uterine wet weight gain, progesterone receptor levels and uterine peroxidase levels by 10%, 27%, 13% (2,2',4',6') and 7%, 22%, 43% (2,2',3',5',6') respectively at the second highest cotreatment dose (data not shown).

3. Of the eight congeners studied, 2,2',4',6' tetrachloro-4-biphenylol and 2,2',3',5',6' pentachloro-4-biphenylol were the most active antiestrogens in the in vivo assays in the mouse and rat uterus. Hydroxy-PCBs represent a new class of antiestrogens and current studies are focused on further characterizing the antiestrogenic and antitumorigenic activites of these compounds and related analogs as potential new drugs for treatment of breast cancer.

Figure 1. Uterine wet weight (mg) vs. dose of hydroxy PCB (μg/kg) in the immature female mouse.




A. 2,2',4',6' tetrachloro-4-biphenylol B. 3,2',4',6' tetrachloro-4-biphenylol
C. 2,2',3',5',6' pentachloro-4-biphenylol *statistically significant using Dunnett;s one tailed test $p<0.05$

Table 1. Effect of hydroxy PCBs on progesterone receptor (PR) and uterine peroxidase (UPO) levels in immature female mouse.

| Compound | Treatment | PR (fmol/uterus) | UPO (abs/mg protein) |
|---|---|---|---|
| A | E+PCB(25) | 125% ± 15 | 92% ± 11 |
| | E+PCB(50) | 135% ± 6 | 92% ± 6 |
| | E+PCB(100) | 72% ± 8 | 99% ± 6 |
| | PCB(100) | n/a | n/a |
| B | E+PCB(25) | 105% ± 18 | 156% ± 6 |
| | E+PCB(50) | 154% ± 4 | 141% ± 4 |
| | E+PCB(100) | 117% ± 2 | 112% ± 5 |
| | PCB(100) | 31% ± 3 | 41% ± 8 |
| C | E+PCB(25) | 88% ± 15 | 87% ± 10 |
| | E+PCB(50) | 99% ± 15 | 82% ± 10 |
| | E+PCB(100) | 72% ± 4 | 53% ± 4 |
| | PCB(100) | 11% ± 4 | n/a |

A. 2,2',4',6' tetrachloro-4-biphenylol B. 3,2',4',6' tetrachloro-4-biphenylol
C. 2,2',3',5',6' pentachloro-4-biphenylol

E=$E_2$ (0.02μg/kg mouse or 0.1μg/kg rat) PCB=compound at 25, 50 or 100 μg/kg

values expressed as % of $E_2$ induced response (mean ± s.e.)

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