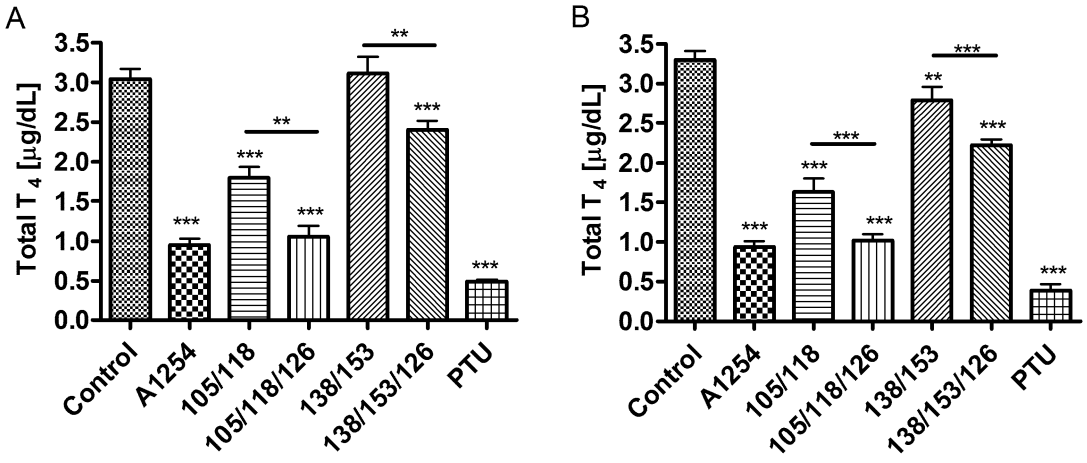
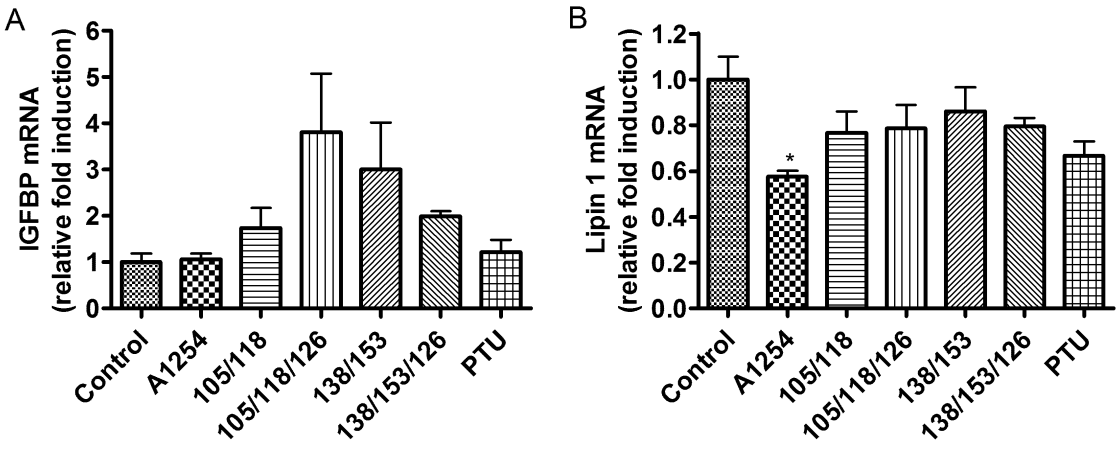
Supplemental Table 2.1. *Sequence of primer sets used for quantitive (real-time) PCR as described in the text.*

Gene	Forward Primer	Reverse Primer
CYP1A1	GAAGAAGCTAATCAAAGAGCACTACAGG	CAATGCTCAATGAGGCTGTCTG
Spot 14	AAACGGACACGGAATCAAAGGCAC	TGCTCAGCCTCATCAGATCCAACA
ME	AGGCCTCTTTATCAGTATCCAC	CCATCCCGTACAACCAA
D1	TCCGTGGACAGATCACTGAG	AGTCGTCTACGAGTCTCTTG
TSHβ	AATGCTGTCGTTCTCTTTTCC	AGAATGTCTGTGGCTTGGTGC
GH	TCCGTGGACAGATCACTGAG	AGCAGGCTGAAGGTCAGG
D2	AACAGCTTTCTCCTAGACGC	TGCTTCAGGATTGGACACG
MHCα	AACGTTTAAGCCCACTTGAA	CATTGGCACGGACTGCGTCA
MHCβ	GAGCCTCCAGAGTTTGCTGAAGGA	TTGGCACGGACTGCGTCATC
Glut4	GCCAGCCTACGCCACCATAG	AGCAGAGCCACCGTCATCAAG
Myh2	ACTGCTGAAGCAGAGGCAAGTAGT	TCCTGAGGTTGGTCATCAGCTTGT
Lipin1	ACCCCAACCTCGTGGTCAA	TGCATCGCCAGAAGTAGAGGA
IGFBP	CCTGTGTACTAGAACCTGC	TGTTCCTCTGTCATCTCTGG
β-actin	TGAACCCTAAGGCCAACCGTGAAA	ATACAGGGACAACACAGCCTGGAT

Supplemental Figure 2.1. *Serum total T_4 levels in P14 (A) and P16 (B) Pups.* One-way ANOVA revealed significant differences among the treatment groups for pups on both P14 [$F_{6,31} = 55.38$; $p < 0.0001$] and pups on P16 [$F_{6,32} = 88.44$; $p < 0.0001$]. Individual differences among means were nearly identical to those obtained on P15, and pooling data from all three days did not alter our interpretation of the effects of PCB mixtures on serum T_4.



Supplemental Figure 2.2. *PCB Effects on Estrogen-Regulated Genes in Liver.* To monitor the estrogenic activity of the various PCB mixtures employed in this study, we measured mRNA levels of Insulin-like Growth Factor-1 Binding Protein (IGFBP) and Lipin1 in the liver of P15 pups. IGFBP mRNA levels in liver are increased (1) and Lipin1 mRNA levels are decreased by estrogen (2). We explored this possibility because some PCBs can act on the estrogen receptor (ER) (3), and because estrogenic agents can increase malic enzyme expression (4). Pups exposed to the different treatments exhibited no significant difference in the expression levels of IGFBP compared to controls, although mean levels were quite variable (Supplemental Fig. 2.3A). In contrast, Lipin1 mRNA levels in the liver were significantly reduced in P15 pups treated with A1254. Pups of the other treatment groups did not display a difference in Lipin1 mRNA expression compared to the untreated pups (Supplemental Fig. 2.3B). Thus, it would appear that some PCB congeners present in the A1254 mixture may influence estrogen signaling in the liver, but these congeners are not PCBs 105, 118, 138, 153 or their metabolites. Note: Numbers of animals in each group are identical to those shown for manuscript Fig. 2.2.


A
IGFBP mRNA
(relative fold induction)
0
1
2
3
4
5
6
Control
A1254
105/118
105/118/126
138/153
138/153/126
PTU
B
Lipin 1 mRNA
(relative fold induction)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
*
Control
A1254
105/118
105/118/126
138/153
138/153/126
PTU

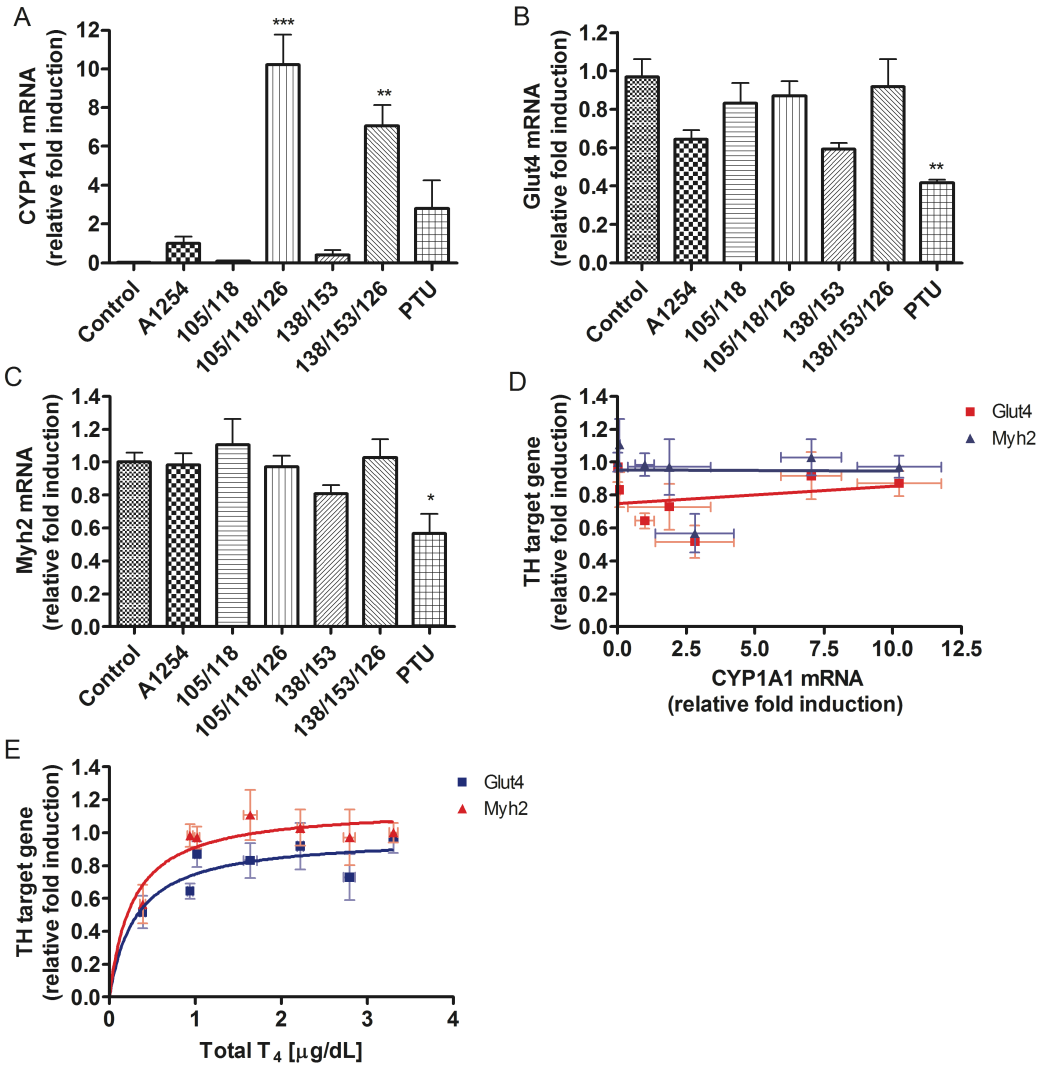
Supplemental Text for Figure 2.3. *Treatment Effects on TH signaling in Skeletal Muscle of P16 Pups.* In the skeletal muscle on P16, CYP1A1 expression was not detected in animals of the control group (Supplemental Fig. 3A). CYP1A1 mRNA in animals treated with A1254 was therefore used to assess the relative induction of CYP1A1 in groups where CYP1A1 was detected. The skeletal muscle of P16 pups exposed to 105/118/126 or 138/153/126 had significantly higher CYP1A1 mRNA levels than the pups treated with A1254; about 10-fold and 7-fold higher, respectively. It is not clear why A1254 and PCB126-containing mixtures induced CYP1A1 mRNA levels in the liver to the same extent, but that A1254 was less potent in skeletal muscle. CYP1A1 levels in animals treated with PTU or with 105/118 or 138/153 were not different from animals in the A1254 group (Supplemental Fig. 3A).

Glucose transporter 4 (Glut4). Glut4 is a TH-responsive gene in skeletal muscle (5). In our experiments, the expression of Glut4 was significantly reduced by about 60% in the skeletal muscle of PTU treated animals compared to controls (Supplemental Fig. 3B). However, PCB treated pups did not exhibit a difference in Glut4 expression from untreated animals (Supplemental Fig. 3B). TH target gene expression was regressed against CYP1A1 induction and circulating levels of serum T_4. CYP1A1 expression and TH target gene Glut4 (r^2=0.0206; Supplemental Fig. 3D) displayed no association in the skeletal muscle. In contrast to CYP1A1 expression, serum T_4 levels was correlated with the expression of Glut4 (r^2=0.1734; Supplemental Fig. 3E), although the correlation was weak.

Myosin heavy chain 2 (Myh2). PTU treated pups displayed reduced mRNA levels of skeletal Myh2 of about 60% compared to that of the control levels (Supplemental Fig. 3C). Animals treated with the PCB mixtures did not exhibit altered Myh2 mRNA expression compared to controls. Expression of CYP1A1 and TH target gene Myh2 (r^2=0.00006; Supplemental Fig. 3D) did not correlate in the skeletal muscle. Contrary to CYP1A1 expression, serum T_4 levels correlated with the Myh2 expression in a non-linear manner (r^2=0.2103; Supplemental Fig. 3E), but again the correlation was weak.

Supplemental Figure 2.3: mRNA expression levels in P16 pup skeletal muscles using qRT-PCR.

Effects of experimental treatments on (A) CYP1A1 mRNA [$F_{6,27}$ = 15.59; p < 0.0001], (B) Glut4 mRNA [$F_{6,29}$ = 4.394; P = 0.0028], (C) Mhy2 mRNA [$F_{6,30}$ = 3.420; P = 0.0108] in P15 pups. Linear regressions of mRNA levels of TH responsive genes in skeletal muscle (D) against mRNA levels of CYP1A1 (Glut4 (r^2=0.0206) and Mhy2 (r^2=0.00006)) or (E) serum total T_4 levels (Glut4 (r^2=0.1734) and Mhy2 (r^2=0.2103)) in P16 pups treated with either PTU or PCB mixtures.


A
CYP1A1 mRNA
(relative fold induction)
12
10
8
6
4
2
0
***
**
Control
A1254
105/118
105/118/126
138/153
138/153/126
PTU
B
Glut4 mRNA
(relative fold induction)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
**
Control
A1254
105/118
105/118/126
138/153
138/153/126
PTU
C
Myh2 mRNA
(relative fold induction)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Control
A1254
105/118
105/118/126
138/153
138/153/126
PTU
D
TH target gene
(relative fold induction)
Glut4
Myh2
0.0
2.5
5.0
7.5
10.0
12.5
CYP1A1 mRNA
(relative fold induction)
E
TH target gene
(relative fold induction)
Glut4
Myh2
0
1
2
3
4
Total T4 [µg/dL]

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