A TEST OF THE HYPOTHESIS THAT ENVIRONMENTAL CHEMICALS INTERFERE WITH THYROID HORMONE ACTION IN HUMAN PLACENTA

A Thesis Presented

by

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ABSTRACT

ENVIRONMENTAL CHEMICALS POTENTIALLY INTERFERE WITH THYROID HORMONE ACTION IN HUMAN TERM PLACENTA

FEBRUARY 2012

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Thyroid hormone is essential for normal brain development and recognition of this has led to universal screening of newborns for thyroid function to ensure that circulating levels of thyroid hormone are within a range known to be supportive of normal growth and mental development. Environmental chemicals that interfere with thyroid function are known to inhibit normal growth and mental development. Work from our lab and from labs internationally demonstrates in animal systems that some industrial chemicals such as PCBs, PBDEs, and others may interact with the thyroid hormone receptor(s) in ways that are not predicted by changes in serum thyroid hormone levels. Our work demonstrates that the enzyme CYP1A1 must metabolize some individual PCB congeners before they can interact with the thyroid receptor. In animals, this requirement appears to be manifested in part by a strong correlation between *CYP1A1* and TH target gene expression. Here we present that this pattern extends to humans by demonstrating a correlation between increased *CYP1A1* mRNA and an abundance of thyroid hormone responsive gene mRNA.

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CHAPTER 1

INTRODUCTION

Thyroid Hormone Action and Disruption

Thyroid hormone (TH) is necessary for the proper functioning of the human body, but temporally and spatially specific actions of thyroid hormones play important roles in development. It is widely recognized that TH is necessary for proper fetal neurodevelopment, with TH deficiencies being linked to lower cognitive function. When hypothyroidism is untreated at birth, serious mental deficiencies quickly develop, and recognition of this has led to a screening of all newborns for thyroid function.[1]

The effects of thyroid hormone within the adult human body and the developing fetus are controlled by a complex and multifaceted system. Serum levels regulate the production of the thyroid hormones triiodothyronine (T_3) and thyroxine (T_4). Transporters control the movement of these hormones into specific tissues, where they interact with the thyroid receptor (TR), which binds to the thyroid response element (TRE) and induces or represses gene transcription. Thyroxine-binding globulin binds T_4 in serum, creating a reservoir of hormone in circulation. The amount of deiodinases (e.g. D2, D3) also plays a role in the level of thyroid hormones in different tissues, activating TH by converting T_4 to the thyroid receptor activator T_3, or deactivating TH by converting it to an unusable form. The complexity of thyroid hormone signaling, as well as the mechanisms of chemical disruption are fully reviewed by Zoeller (2010)[2]. Because thyroid hormone

signaling is so complex, it should be expected then that any chemical disrupting some point in this system would have a complex pattern of effects.

One such group of chemicals that have been linked to altered levels of TH is polychlorinated biphenyls (PCBs). PCBs could affect TH signaling by reducing serum TH levels, interfering with the TR, or both.

PCBs Disrupt Thyroid Hormone Action in a Way That Is Inconsistent with Hypothyroidism

PCBs are persistent environmental pollutants. Once used in industry for a variety of purposes, production of these chemicals was banned in the 1970s. Despite this, they remain ubiquitous, unavoidable contaminants in the environment and in the human population[3]. PCBs and their hydroxylated metabolites (OH-PCBs) are present in cord blood in concentrations that are predicted by maternal exposure[4], indicating the fetus is also exposed to these chemicals during development.

PCBs are composed of two linked benzene rings with varying degrees and placement of chlorination. Because of the differences in structures among these individual congeners, they can have different biological effects[5]. Study of these effects is complicated because commercial mixtures of PCBs are composed of different congeners at varying ratios of concentration. Further, the ratios found in the environment and in the human body are different from those of commercial mixtures. Little research has focused on how mixtures of these chemicals are interacting in the human body[6, 7].

In different mixtures, PCB congeners could interact to have different effects. Congeners with similar actions could have additive effects while congeners with

different actions may together form new patterns of disruption. Gauger et al. (2007) have shown that mixtures of PCBs can cause an increase in TH responsive genes, but when individual congeners (shown in Figure 1) are tested alone this effect is not seen[8]. What chemical properties then of different PCB congeners could interact to create this?

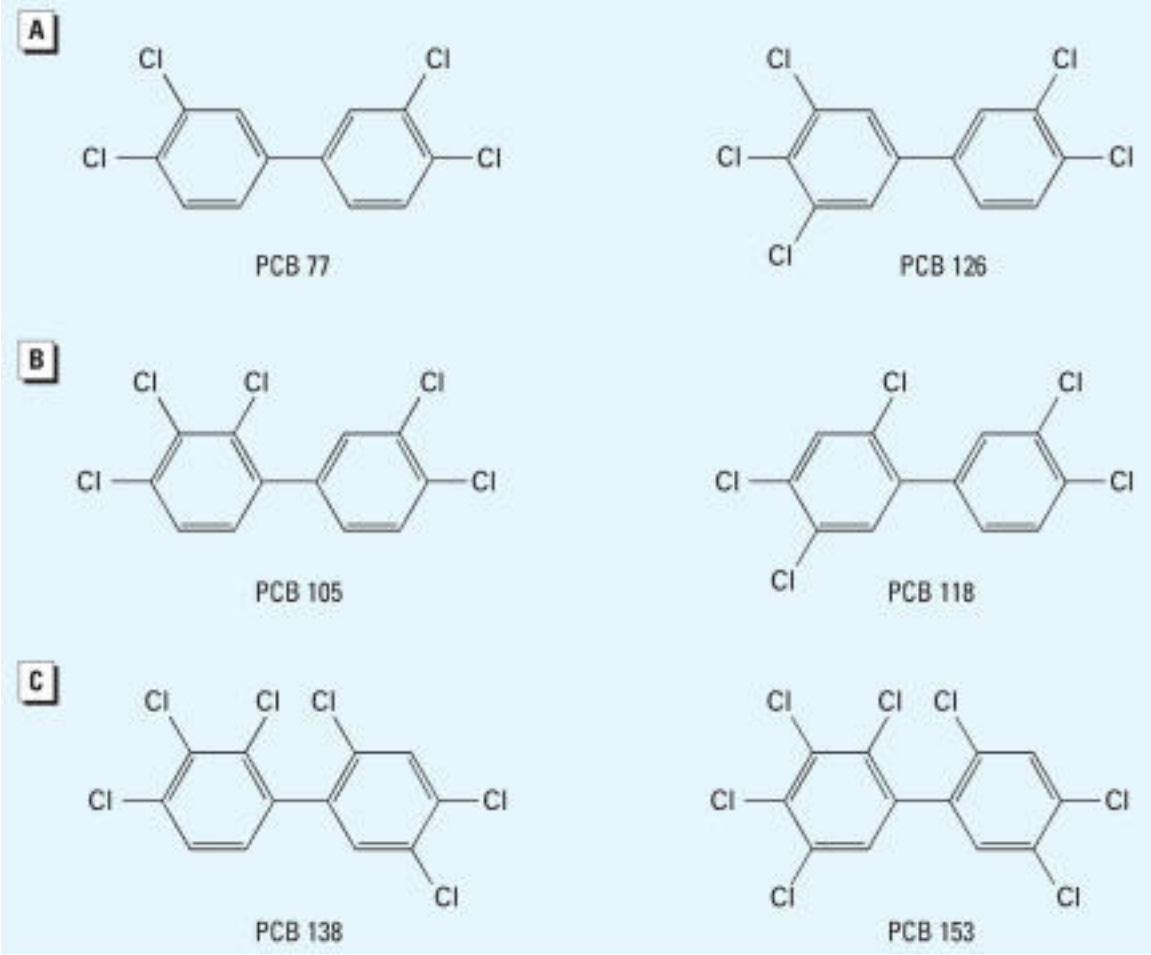

Figure 1. Representative examples of PCB congeners used in the Gauger et al. 2007 experiment. (A) Non-*ortho* PCB congeners, "dioxin-like": 3,3',4,4'-tetrachlorobiphenyl (PCB 77) and 3,3',4,4',5-pentachlorobiphenyl (PCB 126). (B) Mono-*ortho* PCB congeners: 2,3,3',4,4'-pentachlorobiphenyl (PCB 105) and 2,3',4,4',5-pentachlorobiphenyl (PCB 118). (C) Di-*ortho* PCB congeners: 2,2',3,4,4',5'-hexachlorobiphenyl (PCB 138) and 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153).[8]

Congeners can be coplanar or non-coplanar, depending upon the placement of chlorine. The coplanar PCBs, such as PCB 126, have dioxin-like effects: that is, they activate the arylhydrocarbon receptor (AhR) and induce transcription of the gene encoding the phase-I metabolic enzyme cytochrome P4501A1 (CYP1A1)[6].

The AhR is expressed in many fetal tissues as well as being highly expressed in the human placenta. Areas of higher expression in the fetus include the lung and liver, while the brain shows lower expression levels.[9]

Non-coplanar PCBs are not dioxin-like, but have other biological actions[6]. Results of dual-luciferase assays by Gauger et al. (2007) show that CYP1A1 induction and activity is necessary for specific non-coplanar, mono-*ortho*-substituted, PCBs 105 and 118 to drive thyroid receptor (TR) activation[8].

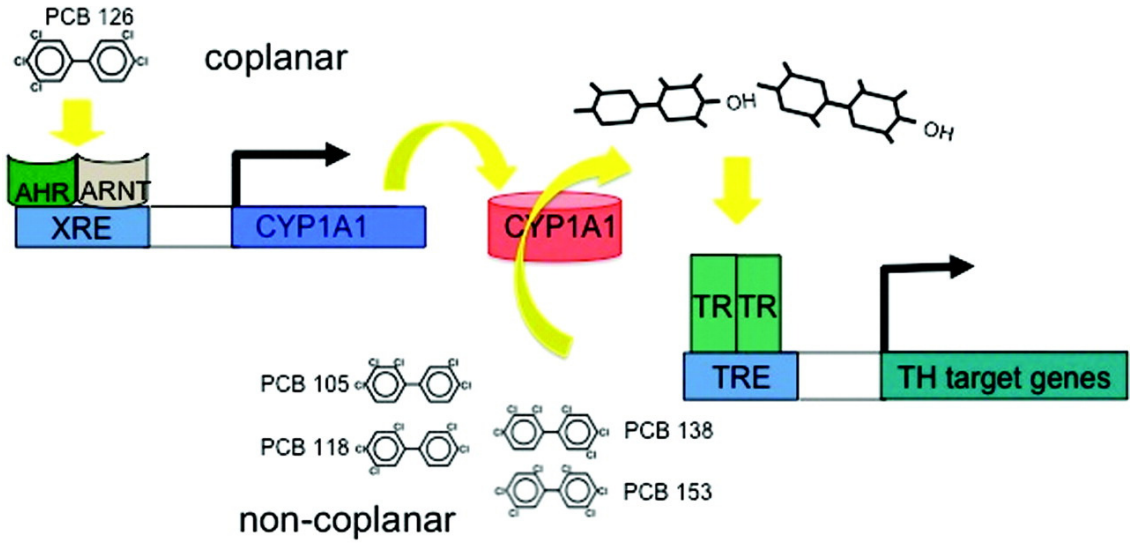

Figure 2. Proposed model for thyroid receptor activation by CYP1A1 metabolism of non-coplanar PCBs. The AhR is activated by coplanar PCBs or other chemicals, inducing the expression of CYP1A1. The CYP1A1 enzyme metabolizes non-coplanar, mono-*ortho*-substituted PCBs into their 4-hydroxy metabolites. These hydroxylated forms interact with the thyroid receptor and cause the transcription or regulation of thyroid hormone responsive genes.[10]

The following model has been proposed: that CYP1A1 metabolizes non-coplanar, mono-*ortho*-substituted PCBs, such as 105 and 118, into their 4-hydroxy metabolite forms [e.g. 4-hydroxy-2,3,3′,4′,5-pentachlorobiphenyl (4-OH-PCB107)[11]] and that these metabolites, which have similar chemical structure to thyroid hormone, interact with the TR[12](Figure 2). This two-step process, in which *CYP1A1* mRNA expression is positively correlated with the expression of genes regulated by thyroid hormone, provides us with a prediction to test this action in other systems.

To test this hypothesis *in vivo* Giera et al. (2011) treated timed-pregnant rats with various PCB mixtures and observed the gene expression in tissues of the pups (i.e. liver, heart, pituitary). PCBs 105 and 118 induced the thyroid hormone responsive genes *Spot 14* and *malic enzyme* in liver, where the AhR is highly expressed, only in the presence of the dioxin-like PCB 126. This addition of PCB 126 also corresponds to an increase of *CYP1A1* mRNA, consistent with our hypothesis that CYP1A1 is necessary to create metabolites which in turn cause induction of TH responsive genes.

PCB exposure displayed different effects in different tissues. Mixtures containing PCB 126 were able to cause an increase in *CYP1A1* mRNA to a lesser extent in pituitary, and yet there was no corresponding decrease in the thyroid hormone inhibited gene, *TSHβ*, mRNA. Despite the pups given mixtures containing PCB 126 still showing the decrease in T_3 and T_4, the tissue mRNA response was not consistent with the positive control for hypothyroidism. It was concluded that, within these tissues, CYP1A1 was not highly expressed enough to metabolize PCB

congeners and cause a change in TH signaling. This clearly underlines that a mixture of chemicals, as well as having unanticipated interactions, can have paradoxical tissue and serum specific effects[10].

Maternal Thyroid Hormone and Role of the Placenta

The next question this raises is whether this metabolism observed *in vitro* and *in vivo* can create metabolites in humans that can interact with the TR and cause gene expression changes. Placenta was an obvious choice for examination; it is easily collected after birth from live healthy donors and it plays an important role in thyroid hormone delivery to the fetus[13].

Until relatively recently, it was believed that the placenta blocked the transfer of thyroid hormone from the mother to the fetus. Deiodinases D2 and D3, enzymes that inactivate thyroid hormone, are indeed present at high levels in the in the placenta[14]. However, the fact that permanent developmental effects of congenital hypothyroidism can be almost completely prevented by immediate T_4 treatment after birth indicated that the developing fetus is protected by the mothers T_4 production. By treating pregnant rats with the antithyroid drug methimazole and then rescuing with T_4 injections, Calvo et al. were able to demonstrate in 1990 the necessity of maternal T_4 to the protection of pup brains from hypothyroidism[15]. Vulsma et al. proved the same protective effect in humans by demonstrating that newborns completely lacking the genetic ability to synthesis T_4 have detectable T_4 in cord blood at delivery, and develop the profile of hypothyroidism only after birth [16].

Because the thyroid hormone axis of the mother is linked to thyroid hormone levels in the fetus and to its development, chemicals that disrupt TH signaling of the mother affect the fetus as well. Not only is placenta one of the few tissues readily available from healthy human donors, but endpoints observed in this tissue have implications for both mother and child.

The TH responsive genes measured in this study were *placental lactogen* (*PL*) and *placental growth hormone* (*GH-V*). *In vitro* studies have shown the TR to interact with a thyroid response element of these genes[17]. Addition of T_3, the active form of TH, is observed in cell line studies to increase the transcription of *PL*[18-20] and *GH-V*[18]. If the expression of these genes correlates with expression of *CYP1A1* mRNA, this is evidence that environmental chemical metabolites are affecting thyroid responsive gene expression.

By collaboration with Dr. Larissa Takser (Sherbrooke University Hospital, Quebec, Canada) we were provided with an opportunity to test this model in humans. Dr. Takser provided us with a large sample of human placentas in order to examine gene expression in this tissue.

These placentas were collected as part of an epidemiological study (named GESTE) that recruited women in 2007 living in the Eastern Townships region of Quebec, Canada during pregnancy. GESTE will follow these mother-infant pairs for at least four years to assess the effects and magnitude of endocrine disruption on maternal health and child development.

Summary

Through our interdisciplinary collaboration, this research has the potential to increase our understanding of the mechanisms of this example of xenobiotic metabolism and eventually combine it with developmental endpoints. Being able to assess the *CYP1A1* and TH responsive gene mRNA levels on a large sample of human placentas provides us with a unique and exciting opportunity to determine if the proposed model of metabolism extends to humans.

We hypothesized that expression of *CYP1A1* mRNA is positively correlated with the abundance of mRNAs coding for TR genes in the human term placenta. This would be consistent with AhR driven metabolism of certain chemicals to forms that interact with the TR.

CHAPTER 2

METHODS

Human Participation

Recruitment and sample collection took place in 2007 at Étienne-Lebel Clinical Research (CRC) Center of the Sherbrooke University Hospital (CHUS) (Quebec, Canada). The Human Research Ethics Committee of CHUS approved the study protocol. Four hundred pregnant women were recruited for this study at their first physician visit. Each participant gave informed consent. All women were at or over the age of 18, less than twenty weeks pregnant, and had no known thyroid disease.

Thyroid Function Analysis

TH levels were measured by the Takser lab at Sherbrooke University. Serum free T_4 (fT_4) and total T_4 were assessed in the mothers at recruitment (<20 weeks of pregnancy) and at delivery, as well as in cord blood at delivery. fT_4 was determined by the electrochemiluminescent immunoassay system ADVIA Centaur (Bayer) and total T_4 by the Coat-A-Count radioimmunoassay (DPC Inc., Los Angeles, CA) at CHUS.

Placental Samples

Placentas were collected immediately upon delivery. Two placental lobes (i.e. cotyledons), morphological units of the placenta, were sampled from the center of the maternal side of each placenta (Figure 3). Samples were immediately stored at -80°C. Of these samples, one cotyledon was cut in half on ice and sent, stored in dry

ice, to our facility at the University of Massachusetts Amherst. 172 placental samples were received for use in this study.

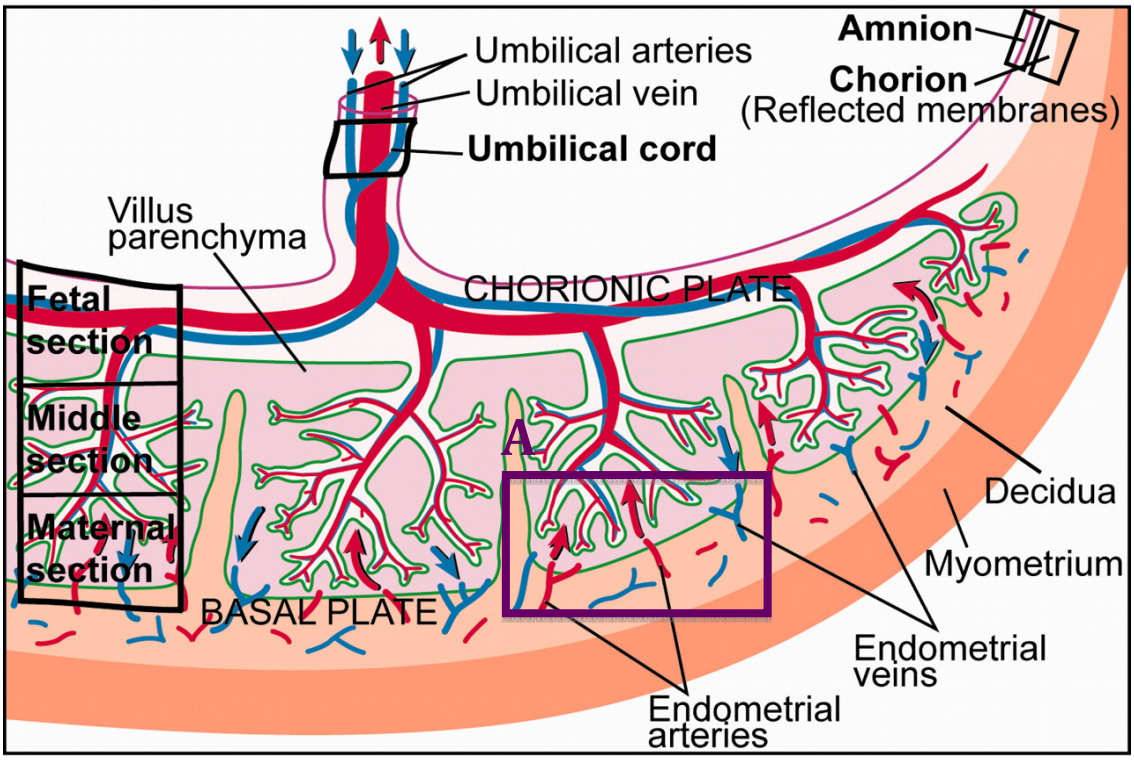

Figure 3. Cross-sectional diagram of the human term placenta. (A) Highlights a cotyledon. Two such units were sampled during the course of this experiment. This sample contains largely maternally derived tissue, however some fetal tissue is also present.[21]

RNA Isolation and DNase Treatment

For each sample received, a piece of frozen placental tissue approximately 300mg in weight was disrupted and homogenized with a Bullet Blender (Next Advance Inc., Averill Park, NY). Total RNA was isolated using TRIzol Reagent (Invitrogen Corporation, Carlsbad, CA), according to the manufacturer's instructions. RNA was dissolved in DEPC treated water. Concentration of RNA was determined with a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE).

A subset of samples was tested for the presence of genomic DNA. With contamination of genomic DNA in the samples confirmed, all RNA samples were treated with DNase using the Applied Biosystems TURBO DNA-free procedure (Applied Biosystems, Inc., Foster City, CA). Concentration of treated RNA was again determined with the NanoDrop 1000.

Quantitative RT-PCR

One microgram of RNA from each sample was reverse transcribed using the Applied Biosystems High Capacity cDNA Reverse Transcription Kit by the manufacturer's instructions.

Each quantitative PCR reaction was 10µL in volume and was comprised of 300nM forward and reverse primers each of the target gene (Table 1), nuclease free water, 5µL FastStart Universal SYBR Green Master (ROX) (Roche Diagnostics Corp., Indianapolis, IN) and cDNA. Because of the low abundance of *CYP1A1* transcripts in placenta, it was necessary to use 4µg cDNA to obtain a significant signal in *CYP1A1* PCR reactions. For all other reactions, 1µg cDNA was used.

Table 1. Quantitative RT-PCR Primers.

Gene	Forward Primer (5'➔3')	Reverse Primer (5'➔3')
CYP1A1	GCTCAGCTCAGTACCTCAGCCAC	GGTCTTGAGGCCCTGATTACCCAG
PL[22]	CATGACTCCCAGACCTCCTTC	TGCGGAGCAGCTCTAGATTCT
GH-V[23]	AGAACCCCCAGACCTCCCT	TGCGGAGCAGCTCTAGGTTAG
B-actin[24]	CCTGGCACCCAGCACAAT	GGGCCGGACTCGTCATAC
18S[25]	ACACGGACAGGATTGACAGA	GGACATCTAAGGGCATCACAG

Quantitative PCR was performed using the Strategene Mx3000P real-time PCR machine (Agilent Technologies, Inc., Santa Clara, CA). Samples were run in duplicate for each gene. A calibrator pool was run in triplicate for each gene on each

plate. The following thermal profile was used for all runs: one cycle of 10 min at 95°C; 40 cycles of 15 s at 95°C, 30 s at 60°C, and 15 s at 72°C; one cycle of 1 min at 95°C, 30 s 55°C, and 30 s at 95°C. Melting-curve analysis was preformed to identify the creation of non-specific products, in which case all genes for the sample were rerun on a new plate.

Relative mRNA levels were determined by the Pfaffl method[26]: the difference between cycle threshold number, as calculated by the Mx3000P software, for a target gene and the reference gene, *β-actin*, was compared between the sample and a calibrator pool to obtain a relative fold change value.

Statistical Analysis

Statistical analysis was carried out using Prism 4 (GraphPad Software, La Jolla, CA). Relative gene expression level data sets were tested for normality using the D'Agostino-Pearson omnibus K2 normality test and log transformed if necessary to be consistent with a Gaussian distribution. Pearson's correlation test was used to compare the relative levels of *CYP1A1* transcripts and thyroid hormone gene target transcripts. If a correlation was observed, the linear regression line was calculated.

CHAPTER 3

RESULTS

CYP1A1 mRNA Expression Is Correlated with the Expression of mRNAs of TH Responsive Genes

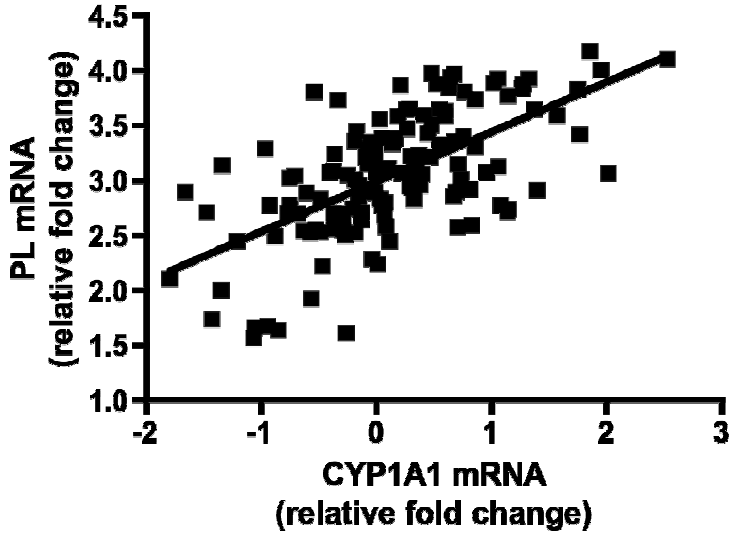

Figure 4. Correlation between *CYP1A1* and *PL* relative mRNA levels. *PL* and *CYP1A1* show a weak but significant positive log-linear correlation [r2 = 0.3924(df = 136), P < 0.0001]. Data were log transformed for statistical analysis.

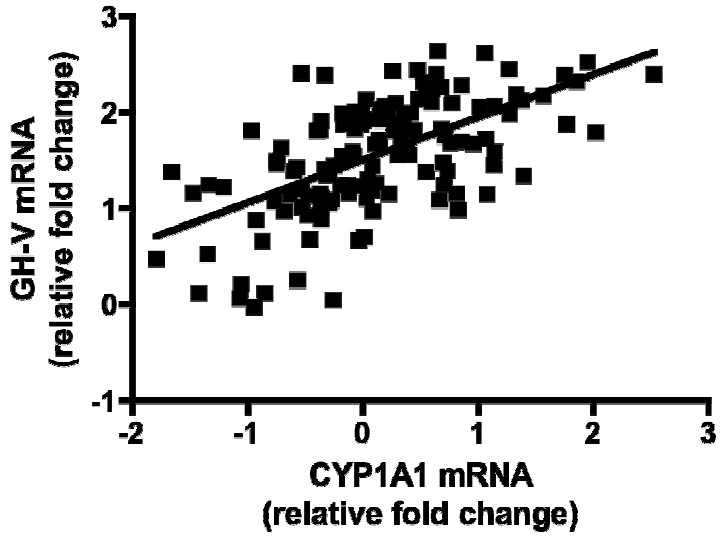

Figure 5. Correlation between *CYP1A1* and *GH-V* relative mRNA levels. *GH-V* and *CYP1A1* show a weak but statistically significant positive log-linear correlation (r2 = 0.3565(df = 136), P < 0.0001). Data were log transformed for statistical analysis.

Expression of *CYP1A1* mRNA showed a correlation with the TH responsive genes *PL* and *GH-V*. *PL* mRNA was correlated with *CYP1A1* mRNA (Figure 4), r^2 = 0.3924(df = 136), P < 0.0001. *GH-V* mRNA was correlated with *CYP1A1* mRNA (Figure 5), r^2 = 0.3565(df = 136), P < 0.0001. mRNA expression data was log transformed to create a Gaussian distribution for statistical analysis.

As a control, the fold change for the reference gene *18S* was also compared to *CYP1A1* (not shown). There was a significant but small correlation between *CYP1A1* and *18S* relative fold change, r^2 = 0.09425(df = 136), P = 0.0002 (log transformed data).

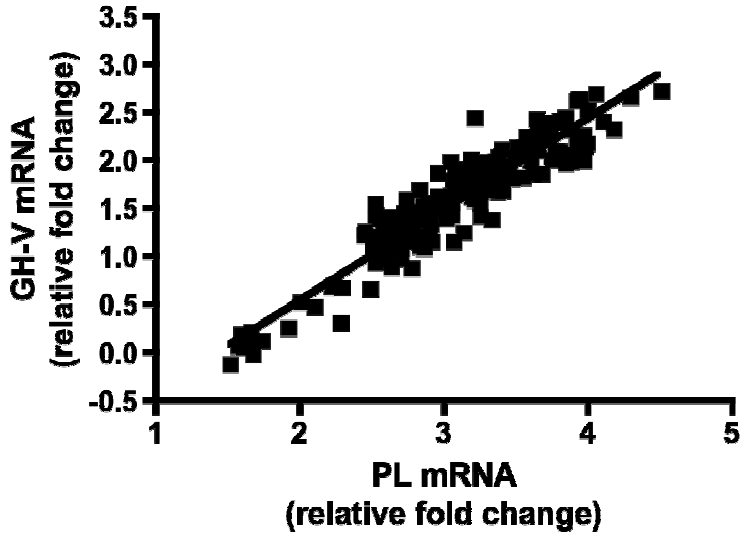

Figure 6. Correlation between *PL* and *GH-V* relative mRNA levels. *PL* and *GH-V* relative mRNA expression shows a high positive linear correlation (r2 = 0.8827(df = 170), P < 0.0001). Data were log transformed for statistical analysis.

PL and *GH-V* showed a very strong correlation with each other (Figure 6), r^2 = 0.8827(df = 170), P < 0.0001. This is expected, and consistent with other observations of parallel increase in transcription of these genes[27].

CYP1A1 mRNA Expression Is Not Correlated with Measures of Maternal T_4 or Cord Blood T_4 Levels

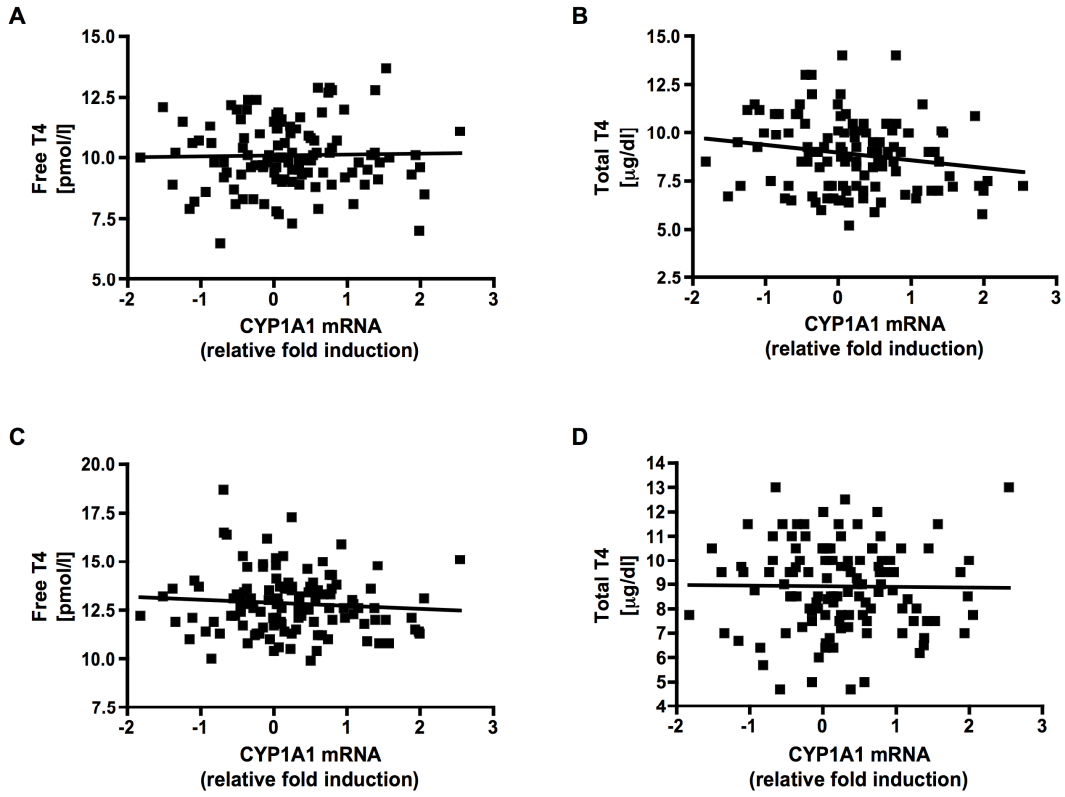

Figure 7. *CYP1A1* relative mRNA levels compared to measures of T_4. There was no correlation between CYP1A1 mRNA levels and maternal serum fT_4 (A) [r^2 = 0.0005026(df = 110), P = 0.8145] or total T_4 (B) [r^2 = 0.03176(df = 112), P = 0.0578] at delivery. There was no correlation between CYP1A1 mRNA levels and cord blood fT_4 (C) [r^2 = 0.006778(df = 118), P = 0.3714] or total T_4 (D) [r^2 = 0.0001525(df = 118), P = 0.8935]. CYP1A1 data is log transformed for statistical analysis.

The expression of *CYP1A1* mRNA showed no correlation with measures of circulating free and total T_4 levels, when data sets were examined as a group (Figure 7). There was no correlation between *CYP1A1* mRNA expression and maternal serum levels of fT_4 [r^2 = 0.0005026(df = 110), P = 0.8145] or total T_4 [r^2 = 0.03176(df = 112), P = 0.0578]. Though the low P value for the correlation of total

maternal T_4 and CYP1A1 mRNA could be interpreted as statistically significant, the r^2 of nearly zero indicates that this is not a correlation of biological interest.

To further analyze these findings, the data was broken into quintiles by T_4 levels (not shown). When examining maternal fT_4 at delivery in this way, the fifth quintile, representing the group with the highest levels of fT_4, shows a distinct positive correlation between relative levels of *CYP1A1* mRNA and fT_4 [$r^2 = 0.3527$(df = 20), P = 0.0036]. This effect was not observed in any of the lower quintiles of fT_4.

When examining maternal serum total T_4 at delivery by quintile, no correlations were observed between *CYP1A1* relative mRNA levels and total T_4. Neither were correlations observed between *CYP1A1* relative mRNA levels and free or total T_4 of cord blood, when divided into quintiles.

TH Responsive Gene Expression in the Placenta Is Not Predicted by Maternal Serum T_4 Levels

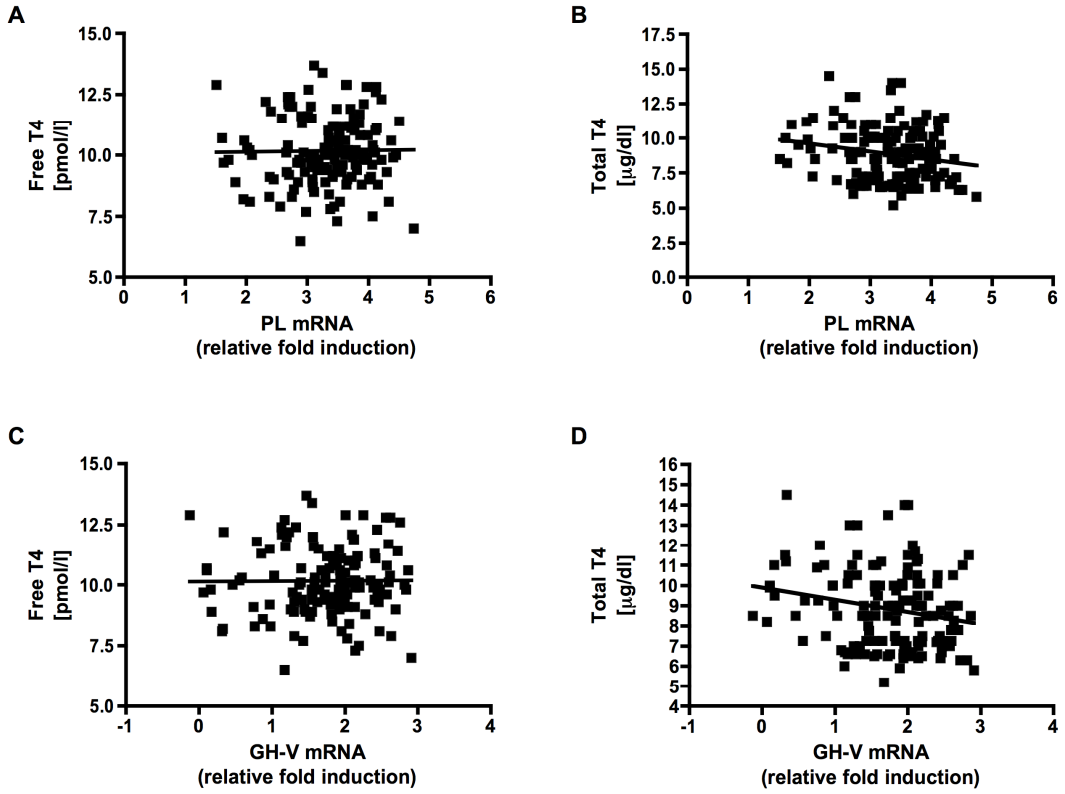

Figure 8. *PL* and *GH-V* relative mRNA levels compared to measures of maternal T_4. *PL* shows no correlation with maternal fT_4 at delivery (A) [r^2 = 0.0002523(df = 139), P = 0.8517] but a slight correlation with total T_4 (B) [r^2 = 0.03927(df = 141) P = 0.0177]. Likewise, *GH-V* shows no correlation with fT_4 at delivery (C) [r^2 = 0.00007266(df = 139), P = 0.9201] but a slight correlation with total T_4 (D) [r^2 = 0.04484(df = 141), P = 0.0111]. Relative fold induction data is log transformed for statistical analysis.

The relative mRNA levels of the thyroid hormone responsive genes were compared to measures of maternal T_4 (Figure 8). The expression of *PL* and *GH-V* mRNAs showed no correlation with the maternal level of fT_4 at delivery. However, both genes (log transformed) showed a weak correlation with maternal serum total T_4 at delivery. PL: r^2 = 0.03927(df = 141), P = 0.0177 and GH-V: r^2 = 0.04484(df =

142), P = 0.0111. Such a low r^2, however, indicates this effect is unlikely to be biologically significant.

No correlations were found between thyroid hormone responsive gene expression and measures of T_4 in cord blood (not shown).

CYP1A1 mRNA Expression Is Not Affected by the Smoking of Mothers

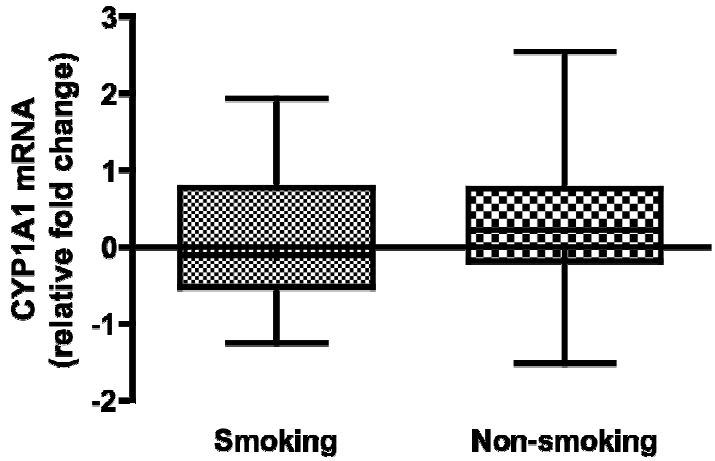

Figure 9. *CYP1A1* mRNA in smoking versus and nonsmoking mothers. *CYP1A1* data (log transformed for statistical analysis) was grouped based on mothers' reporting of smoking or not during pregnancy. The two groups were compared using the student's t-test. There was no significant difference between the means of the two groups [t = 0.5995(df = 107), P = 0.5501].

No difference in placental *CYP1A1* mRNA expression was found when mothers were grouped based on their reporting of smoking or not during pregnancy (Figure 9), t = 0.5995 (df = 107), P = 0.5501. This statistic, however, was reported for only a portion of participants, with only 14 participants reporting that they smoked during pregnancy.

CHAPTER 4

DISCUSSION

These findings lend support for the hypothesis that environmental chemicals can act as TH if they are metabolized by CYP1A1. This hypothesis has been tested *in vitro* and in an animal model, but the results reported here are the first indications that this mechanism extends into humans.

CYP1A1 and Thyroid Responsive Gene Induction

To see differences in TH action, we examined two genes known to be induced by T_3 in placenta, *PL* and *GH-V*. The increase in mRNA levels of these genes was predicted by an increase in *CYP1A1* mRNA. These data support the model that PCB metabolism by CYP1A1 can affect TH gene expression and capitulate observations *in vitro* and *in vivo*. This is consistent with the notion that CYP1A1 creates metabolites that act as TH agonists.

As a negative control, we also examined a characterized housekeeping gene in placenta, *18S*. This gene should not change expression as a response to thyroid hormone receptor activation. The correlation between *CYP1A1* and *18S* in placenta was comparatively smaller than the relationship between *CYP1A1* and the target genes mRNA, supporting the conclusion that this response is specific to thyroid hormone receptor activation.

In 20% of samples we were not able to obtain a qRT-PCR signal for *CYP1A1*. These samples were excluded from these comparisons; however, they represent an interesting subset of samples that should be investigated. It is likely the lack of

CYP1A1 signal represents no induction or a level of induction that is below our ability to detect. It would be interesting to examine if low exposure or genetic factors are the cause of the low *CYP1A1* induction in these individuals.

An additional interesting set of data to examine would be how *CYP1A1* single-nucleotide polymorphisms (SNPs) contribute to the observed correlation. *CYP1A1* SNPs have effects on gene expression[28] and enzyme activity[29, 30] and also are linked to an increased risk for a number of cancers[31-34].

T_4 Levels and Gene Induction

Studies have shown that T_3 increases transcription of *PL* and *GH-V* in *in vitro* cell line systems. Though T_3 levels account for much of the thyroid hormone action in tissue, T_4 levels provide a more accurate overview of signaling in the body. Since T_3 and T_4 levels are indirectly related, we expected that we could predict serum T_4 levels from the *PL* and *GH-V* mRNA levels.

We did not see any significant correlations between free or total T_4 levels in maternal serum, or in cord blood, and the induction of TH responsive genes. This indicates a disconnection between hormone levels in serum and hormone action in tissue. The next step then is to examine TH levels in the tissue samples themselves to better understand how TH levels and gene expression are linked in placenta.

We also observed that *CYP1A1* mRNA levels did not predict T_4 levels. We expected to see an effect on T_4 related to this marker of PCB metabolism. In animal models, PCB exposure and *CYP1A1* induction was linked to an increase in TR responsive genes mRNA[8, 10] that corresponded with a decrease in serum total T_4.

Previous human studies, however, by the Takser lab found only a correlation between PCB exposure and T_3 levels but not T_4 levels[35]. Some studies now indicate that OH-PCB levels, but not total PCB levels, are correlated with declining fT_4 levels[36].

These results indicate that T_4 levels cannot predict gene expression in human placenta. Combining this data with measures of chemical exposure, as well as examining tissue TH levels, could provide a more accurate picture of chemical metabolism and its effects on the thyroid hormone axis.

Smoking During Pregnancy

To examine how behavior can affect exposure we looked to see if individuals who reported smoking had higher *CYP1A1* mRNA levels, and therefore higher TH responsive gene mRNA levels. No difference in *CYP1A1* mRNA levels was found between self-reported smoking and non-smoking during pregnancy. It is well established that smoking increases levels of *CYP1A1* mRNA in human term-placenta[37], as well as increases related enzymatic activity[38].

A great amount of variability of this effect between individuals, however, has been reported and factors such as genetics and the extent and timing of smoking during pregnancy have been proposed to cause this[37]. Further, this statistic was reported for only 63% of participants, with only 14 individuals reporting to have smoked during their pregnancy. It is likely that for an enlightening analysis more data concerning this activity, such as examining chemical markers of smoking, is necessary than a self-reported binary.

CHAPTER 5

CONCLUSIONS

This test of the hypothesis that environmental chemicals can interfere with thyroid hormone signaling clearly supports the proposed model of PCB metabolism by CYP1A1. By observing the predicted effect of *CYP1A1* on TH responsive genes, we demonstrate that, within the human population, exposure to environmental chemicals can cause a change in TH signaling.

Further, we could not predict this effect by looking at serum hormone levels. This is not surprising, as this metabolism and disruption occurs on the level of the tissue, and previous studies indicate these tissue effects are not uniform. To what extent this affects the creation and circulation of TH remains to be seen.

This research also underlines that the study of chemicals in isolation cannot provide the whole picture of the risk a chemical poses. An endocrine disruptor may only have an effect after a series of other factors have occurred. In this model, people with elevated levels of CYP1A1 for any reason (e.g. exposure to other dioxin-like chemicals, smoking) could be at increased risk. Interaction with other chemicals must be considered.

Recognition of the fact that proper TH signaling is essential for healthy human development has led to nearly universal screening of thyroid function at birth. Yet this realization has not penetrated other areas of concern, such as how signaling can be disrupted by the chemicals we put into our environment. Clearly we need to discover new endpoints for assessing the power of chemicals to interfere with thyroid hormone action.

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