Chapter 6

Estimating the levels of Polychlorinated Biphenyls and evaluating the correlation between PCBs toxicity and the cause of Subclinical hypothyroidism in Gujarat infertile female population

6.1 Introduction

Studies on the disruption of endocrine system by environmental chemicals termed as "Endocrine disrupting compounds" (EDCs) in the last few decades has revealed a marked increase in the deposition and accumulation of a large number of industrial chemicals in the environment. Air, soil and water can be contaminated by EDCs. Human body can be exposed to EDCs through inhalation, absorption, and ingestion. EDCs act by disrupting various pathways in the endocrine system. Fertility in both men and women is affected by EDCs due its direct effect or through effect on axes like the hypothalamic-pituitary-gonadal axis (HPG). The disruption of thyroid homeostasis by environmental chemicals known as thyroid disruptors (TDs) is one amongst the most prevailing etiological factors causing thyroid disorders with the data reporting hypothyroidism as the most common thyroid disorder, worldwide. TDs interfere with thyroid homeostasis at various stages mainly through their effects on hypothalamic–pituitary axis or via nuclear receptors, figure 6.1.

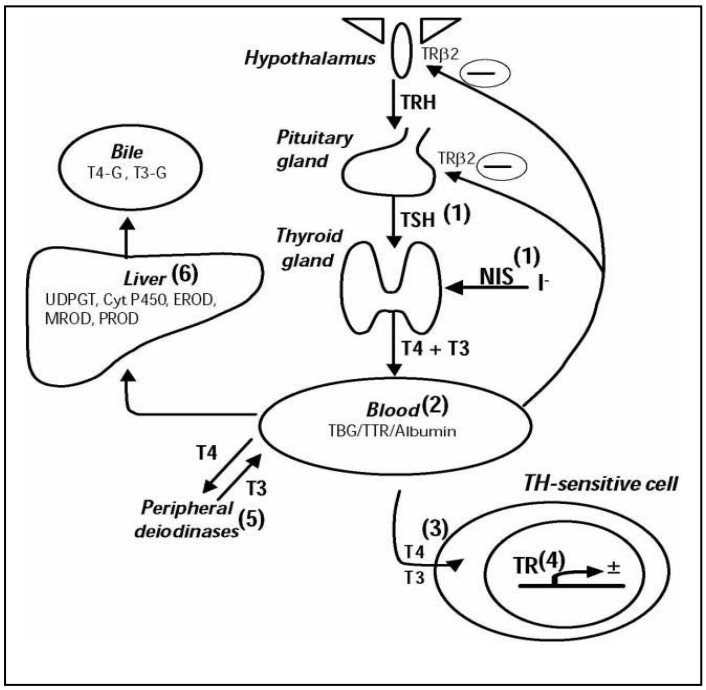


Figure 6.1 Possible steps where environmental chemicals disrupt the hypothalamic–pituitary–thyroid axis:
(1) Synthesis of THs: interference with NIS, TPO or TSHreceptor.
(2) Transport proteins.
(3) Cellular uptake mechanisms.
(4) The TH receptor.
(5) Iodothyronine deiodinases.
(6) Metabolism of THs in the liver.
(7)TRH, thyrotropin releasing hormone.

Most of the TDs have a high degree of structural resemblance to the thyroid hormones (THs) thyroxine (T4) and triiodothyronine (T3); figure 6.2 (Howdeshell *et al.,* 2002; Massart *et al.,* 2006). On account of similarities in their structures with that of THs the TDs interfere with binding of THs to transport proteins or receptors, thereby interfering in its action. Poly Chlorinated Biphenyls (PCBs) is a well-known example of TDs. PCBs are industrial chemicals consisting of paired phenyl rings with various degrees of chlorination (Chana *et al.,* 2002).

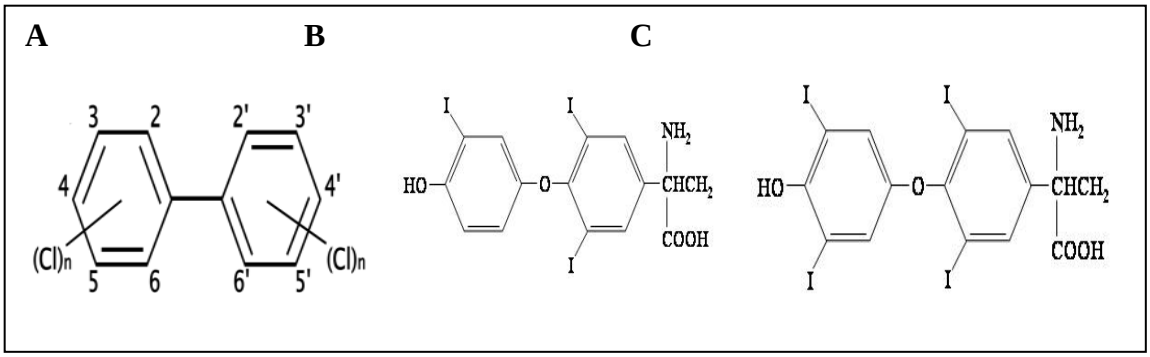


Figure6.2 A. Chemical structure of PCB showing structural resemblance with the thyroid hormones T3 and T_4. **B.** Chemical structure of Triiodothyronine / T3. **C.** Chemical structure of Thyroxine/ T4.

PCBs are one of the numerous "Persistant Organic Pollutants" (POPs), which are also termed as "Persistent, Bioaccumalative, and Toxic substances (PBTs)". The production of PCBs was banned in 1970s, but still these contaminants are characteristically detected in the surrounding environment (Breivik *et al.*, 2002) as well as in human tissues (Fisher *et al.*, 1999). PCBs are resistant to molecular degradation and 1.5 metric tons have accumulated on earth's surface. In the year 2001, more than 100 countries all around the world have signed "The Stockholm Convention", a commitment to restrict or discontinue use of POPs and PCBs are one among the total 12 chemicals in the list of these chemicals (Annex *et al.,* 2008; Patrick *et al.,* 2009; Kim *et al.,* 2013). PCBs are the members of the class of organochlorine compounds classified as persistent organo-halogenated pollutants that include dioxin (polychlorinated dibenzodioxin or PCDD), PCDFs, fire retardants (PBDEs), bisphenol A, and pesticides and herbicides hexachlorobenzene (HCB), DDT, and DDE. Their use was prohibited as they can disrupt the endocrine system and can also exert carcinogenic effects (Faroon *et al.,* 2001). A study on predator fish and herring gulls provided the confirmation for the link between PCBs and thyroid disruption in animals (Leatherland *et al.,* 2000).

PCBs are a group of compounds that exist in the forms of congeners which can be defined as the paired phenyl rings with various degrees of chlorination. The trade name for PCBs is "Aroclor". Out of total 209 probable congeners that have been synthesized only 50 are considered as an environmental threat, while less than 25 are considered a burden in animals and humans (Langer *et al.,* 1999). Owing to their lipophilic nature they are concentrated and thus measured in human adipose tissue and in blood as a fraction of blood lipids. The high persistence of PCBs in adipose tissues and their toxic potential for animals and humans resulted in an almost international ban in the 1970-80s (Fisher *et al.,* 1999; Safe *et al.,* 2000; Breivik *et al.,* 2002).

PCBs are persistent in the environment and have travelled far away up to the Arctic Circle. The common sources of PCBs include coolants / lubricants in transformers, capacitors, hydraulic fluid, adhesives, fire retardants, pesticides, inks, carbonless reproducing papers, old fluorescent lighting, electrical devices, microscope and hydraulic oils (Patrick *et al.,* 2009; Diamanti *et al.,* 2009)**.** The humans and animals can get exposed to PCBs through contaminated water, Fish caught in contaminated waters, Dairy Products, Fats & Oils, Contaminated air around hazardous waste sites at work while preparing and maintaining PCB transformers, accidental fires or spills, improper disposal, Old fluorescent lighting, Old electrical appliances and devices such as TV's, refrigerators microwaves and microscopes may leak while heating up and cause skin exposure (Diamanti *et al.,* 2009), figure 6.3.

Figure 6.3 Sources of PCBs in the environment: A collection of pictures depicts some of the common sources of PCBs to which human and animal bodies are vulnerable to get exposed such as; paints and plasticizers, depositions of various industrial chemicals in the air, water and land, milk and dairy products, meat and sea foods, plastic bags and bottles.

A number of studies have been conducted in human populations, to address and analyze the serious concern of PCBs in altering thyroid homeostasis and reporting hypothyroidism with increased TSH levels in human subjects with presence of PCBs in their blood and body tissues (Hagmar *et al.,* 2003; Hagmar *et al.,* 2001; Persky *et al.,* 2001; Hsu *et al.,* 2005).

The present study aims to find out the possible etiological factors for subclinical hypothyroidism in the female subjects suffering from infertility. As we are mainly considering infertility as a consequence of subclinical hypothyroid condition in the infertile female population, henceforth we are looking for three different most alarming factors in the present scenario which can be the silent precipitator of subclinical hypothyroidism, one of which is EDCs and more specifically TDs as a cause of subclinical hypothyroidism (SCH) and among these PCBs are the main compounds of interest to be focused for the present

study. Thus the aim of the study is estimation of the levels of Polychlorinated Biphenyls (PCBs) and evaluation of the correlation between thyroid disruptors (PCBs) and SCH in Gujarat infertile female population.

6.2 Material and Methods

6.2.1 Ethical consideration

It was ensured that the study design complies with the ethical standards of the Institutional Ethical Committee for Human Research (IECHR), Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India (FS/IECHR/BC/PR/1) and with the 1964 Helinki declaration.

6.2.2 Study Population

The present retrospective study is a age matched, case-control study. The study population consists of parous control and IF-SCH female subjects with primary infertility as case subjects, (as was screened and reported in the first objective of the present study. Detail of the study population is as mentioned in chapter 2 [2.2.2.].

6.2.3 Blood collection and sample preparation

Blood (3mL) was collected from all the patients in a vacuum system tube, transported in a cooling pail, and centrifuged (2,500 × *g* for 15 minutes) within 24 hours after collection. The plasma (1.5 mL) was pooled and kept frozen at -20°C until PCBs was analyzed. All solvents used were of analytical grade purity (HPLC grade; Qualigens, Ltd., Mumbai, India). Eight standard PCB congeners mix (PCB Mix 525, Supelco, Bellefonte, PA) was selected at a concentration 500 g/mL in hexane of each PCB. Figure 5.4 depicts the overall strategy followed for the extraction and detection of PCBs from the blood plasma samples.

6.2.4 Gas chromatography

Gas chromatography for the extraction of PCBs was divided into five phases. Extraction PCBs was performed by the method described by Rozati et al (2002) which is a modification of the original method of Burce et al (Burce *et al.,* 1994), figure 6.5.

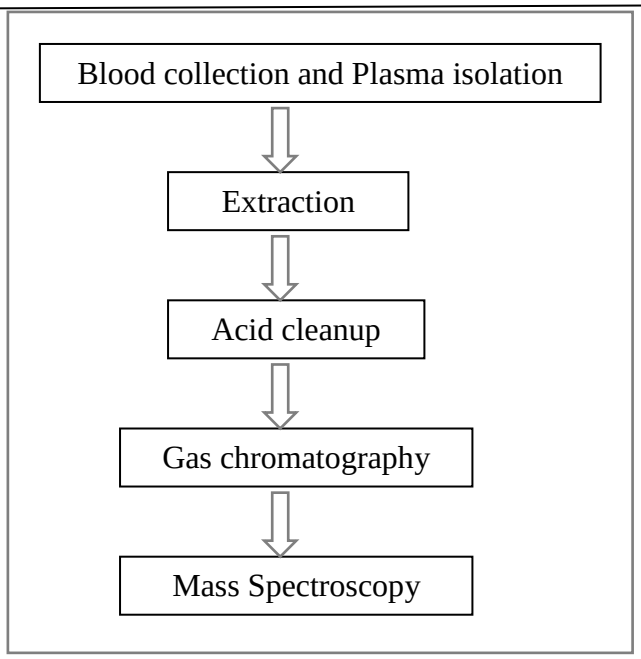
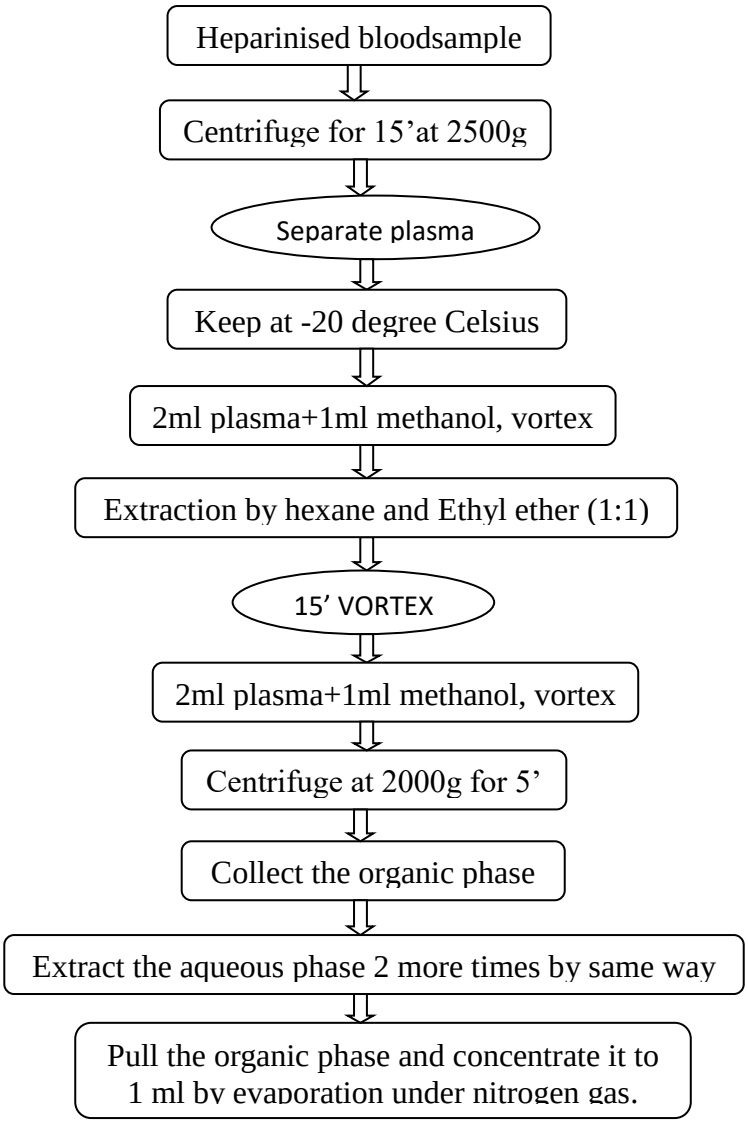


Figure 6.4 Strategy for PCB extraction and detection (Rozati *et al.*, 2002).



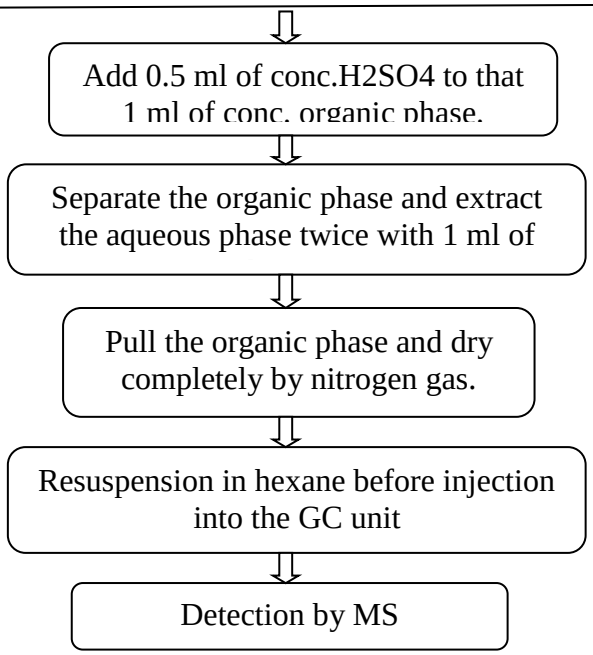


Figure 6.5 Protocol for PCBs extraction and detection (Burce *et al.,* 1994; Rozati *et al.,* 2002).

6.2.5 Body mass index (BMI)

The body mass index (BMI) was measured according to the following equation: dividing the weight in kilograms by the height in squared meters (kg/m2) (Flegal *et al*., 2005).

6.2.6 Lipid profile

The lipid profile results were ready to use as it was procured from the results of the control and IF-SCH subjects in the chapter 4.

6.2.7 Sampling method

The sampling method for selecting the participants was purposive (also called convenience method).

6.3 Statistical analysis

The statistical analysis was done by using Prism 5 software (GraphPad Software Inc.; 2007).The tests done were Non-parametric unpaired t-test. Pearson's correlation coefficient was calculated to determine the relationship.A two–tailed, at minimum 95% confidence intervals and a p-value <0.05 was considered statistically significant.

6.4 Results

The PCB standard (Sigma; PCB Mix 525, Supelco, Bellefonte, PA) was used for the present study. A mixture of total eight PCBs congeners was used for the identification of possible

congeners present in the study population which are more commonly found in the surrounding environment. The PCBs standard shows total eight peaks at different time intervals for eight different PCB congeners as shown in the figure 6.6.

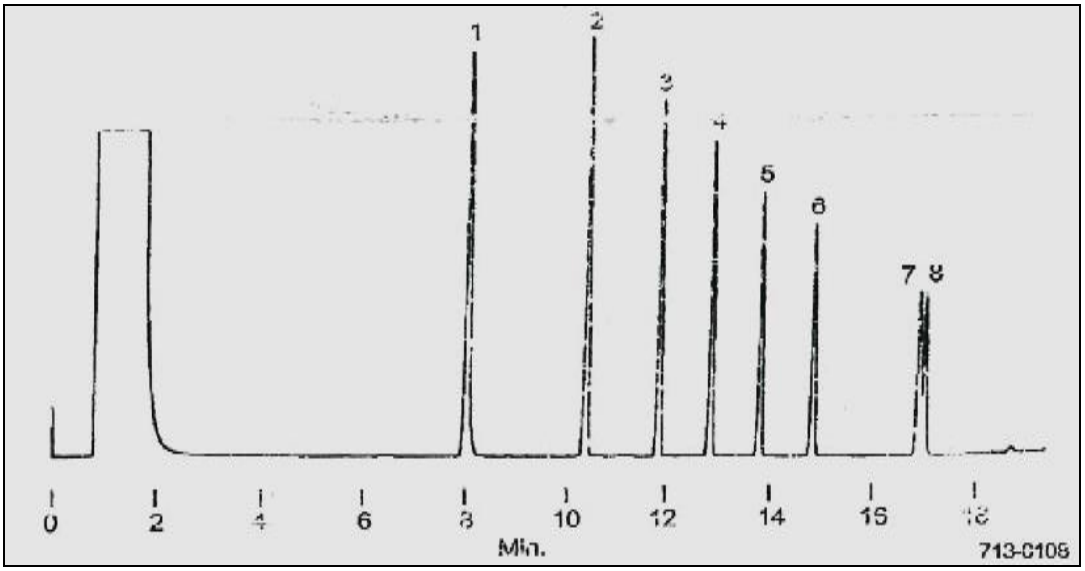


Figure 6.6 PCBs standard consisting of PCBs congeners: The PCBs standard used for the present study was a mixture of total eight most common PCBs congeners, which are **(1)** 2-chlorobiphenyl**(2)** 2,3-dichlorobiphenyl**(3)** 2,4,5-trichlorobiphenyl**(4)** 2,2',4,4' tetrachlorobiphenyl**(5)** 2,2',3',4,6-pentachlorobiphenyl**(6)** 2,2',4,4',5,6'-hexachlorobiphenyl **(7)** 2,2',3,3',4,4',6'-heptachlorobiphenyl **(8)** 2,2',3,3',4,4',6,6'-octachlorobiphenyl(PCB Mix 525, Supelco, Bellefonte, PA)**.**

Figure 6.7 shows the presence of different congeners along with probably the metabolites and/ or other impurities in a sample of IF-SCH female subject. The Samples processed for the extraction of PCBs from Control (n=3) and IF-SCH (n=3) subjects GC were subjected for the detection by MS. Out of three samples of the Control subjects we could not obtained any corresponding peaks as that of the PCBs standard. While, as shown in the figure 6.7, out of three samples of the IF-SCH females in one of the sample we obtained various peaks at different time intervals corresponding different PCBs congeners; which were compared with the PCBs standard results and are enlisted in table 6.1.

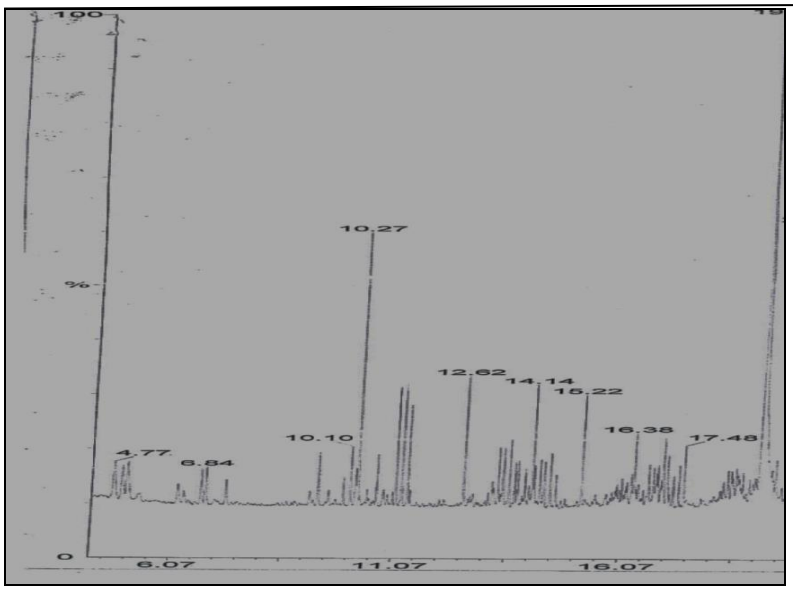


Figure 6.7 Peaks of PCBs Congeners obtained in a sample of IF-SCH subject: A total five peaks corresponding to peaks of PCBs standard were obtained at six different time intervals which are enlisted in the table 5.1. We also obtained a number of small peaks along with the peaks of PCBs which could be of PCBs metabolites and/or other impurities in the sample.

Table 6.1 PCB congeners reported in the sample of IF-SCH female

Sr. no.	**Name of the PCB congener reported**	**Time at which peak obtained after sample run for the identification of PCBs**	**Corresponding peak of PCBs standard**
1	2, 3-dichlorobiphenyl	At 10:27 minutes	2nd
2	2, 4, 5- trichlorobiphenyl	At 12:02 minutes	3rd
3	2, 2',3',4,6-pentachlorobiphenyl	At 14:14 minutes	5th
4	2,2',4,4',5,6'-hexachlorobiphenyl	At 15:22 minutes	6th
5	2,2',3,3',4,4',6'-heptachlorobiphenyl	At 17:48 minutes	7th

6.5 Discussion

There is growing evidence on the relationship between environmental exposure to a specific class of chemicals and its effects on the endocrine system. "The Endocrine Society" in 2009 coined the term "Endocrine-Disrupting Chemicals" (EDCs) [available online at http://www.endo-society.org/advocacy/policy/index.cfm.1] and defined them as "compounds natural or synthetic which through environmental or inappropriate developmental exposures alter the hormonal and homeostatic systems that enable the organism to communicate with and respond to its environment." Among these EDCs, Thyroid disruptors (TDs) are the chemicals which exert their effects on thyroid action in multiple ways. Effects of around 150 industrial chemicals have been discussed in a review summarizing the reports of various animal studies which concludes that TDs can cause reduction in thyroxine levels (Howdeshell *et al.,* 2002). Brucker-Davis et al. (1998) in his extensive review listed about 381 wild-lives,

experimental animal and human studies analyzing the effects of specific drugs and chemicals on thyroid metabolism. TDs have also been shown to cause disturbances in the female reproductive system including infertility (Howdeshell *et al.,* 2002; Nishimura *et al.,* 2002; Hardell *et al.,* 2003). TDs include both naturally occurring and industrial chemicals. The examples of naturally occurring TDs are soy isoflavones, genestien, coumesterol, thiocynate (in Cruciferous vegetables) etc. The common industrial chemicals are Polychlorinated Biphenyls-PCBs, Hexachlorobenzene-HCB, Bisphenol–A(Plastics), Perchlorates, Pentachlorophenol, Polybrominated diphenyl ethers, Dioxins, Phalates (Plasticizers), Pharmacutical Agents (DES), Pesticides (DDT), Fungicides (Viclozolin) (Guo *et al.,* 2000; Santini *et al.,* 2003; Gauger *et al.,* 2004 and Kiliç *et al.,* 2005). Several groups of chemicals have potential for thyroid disruption. There is substantial evidence that polychlorinated biphenyls (PCBs), Hexachlorobenzene (HCB), dioxins and furans cause hypothyroidism in exposed animals and that environmentally occurring levels affect human thyroid homeostasis. Thyroid disruption may be caused by a variety of mechanisms, as different chemicals interfere with the HPT axis at different levels. Even small changes in thyroid homeostasis may adversely affect human health especially fetal neurological development is the most vulnerable. Hence there is an urgent need to clarify whether the animal data showing effects of chemicals on thyroid function can be extended to humans in different populations.

We in our laboratory have initiated a study on animals (rat model) treated with HCB (which is also a member of TDs group) to find out its effects on the female reproductive system in rats. HCB which is a global environmental pollutant causes hypothyroidism as a consequence of endocrine disruption. We performed generation studies through F1 and F2 generations and investigated the effect of chronic exposure of HCB to explore the inter connection between female infertility and hypothyroidism. Thyroidectomy was also performed to evaluate the contribution of the thyroid gland in affecting ovarian dysfunction and reproductive aberrations. We confirmed that the preconception exposure of HCB leads to hypothyroidism which was reflected by an increase in the body weight, alteration in the thyroid hormones, and alteration of the lipid profile. Hypothyroid female rats exhibited a poor reproductive profile with altered steroidogenic pathways, altered estrus cyclicity, reduced litter size, and stunted growth. The external supplementation of thyroxine in thyroidectomized animals rescues the reproductive aberrations confirming the protective role of the thyroid gland in reproductive biology. All results highlight the jeopardizing functional connection of the

thyroid and ovary due to HCB, leading to serious consequences on future generations. These results are published in Dhaibar *et al.* (2021).

Data on the deleterious effects of HCB on female reproductive system as a consequence of hypothyroidism in animal model encouraged us to explore the effects of TDs on female fertility in human population. It has been reported that Polychlorinated Biphenyls (PCBs) apart from HCB also is a very potent thyroid disruptor amongst TDs. A considerable number of animal and human studies report the effects of PCBs on thyroid homeostasis resulting into hypothyroidism. Even though PCBs production was banned long back in 1970s, these contaminants are characteristically detected in the surrounding environment (Breivik *et al.*, 2002) and also in human tissues (Fisher *et al.*, 1999). There are aplenty of sources of contact in our day-to-day life through which the animal and human body gets exposed to PCBs. Contaminated water, soil and marine food chain, dairy products, fats & oils, contaminated air around hazardous waste sites, occupational exposure while at work, preparing and maintaining PCB transformers, accidental fires or spills, improper disposal are the most common sources of PCBs for their exposure. So, it is said that no one is PCB-free. PCBs easily get stored in body fat but are eliminated very slowly from the body (Patrick *et al.*, 2009; Diamanti *et al.*, 2009). High degree of structural resemblance of PCBs to THs, thyroxine (T4) and triiodothyronine (T3), interferes with binding of THs to receptors or transport proteins, which in turn may lead to subclinical hypothyroidism, which in adults is often diagnosed only by chance because of subtle symptoms. However, growth and development in fetal life and childhood is highly dependent on normal levels of THs. Particularly during gestation, normal levels of THs are crucial for the development of the central nervous system. This critical phase may be vulnerable to even subtle effects of synthetic chemicals on fetal and maternal TH levels. Such developmental deficiencies may not be identifiable until later in life (Van den Berg *et al.*, 1988). Another fall out of such subtle changes on TH levels is infertility as result of thyroid homeostasis.

PCBs have been shown to affect thyroid function through several different mechanisms previously reviewed: (1) reducing the ability of thyroid hormones to bind to transport proteins in the bloodstream; (2) enhancing hepatic metabolism by up-regulating the glucuronosyltransferases or sulfotransferases that break down thyroid hormones in the liver; (3) inhibiting or up-regulating the production of deiodinases that allow T4 to be converted to T3 ; and (4) acting as either an agonist or antagonist at the site of the cellular thyroid receptor, figure 6.8 (Safe et al; 1990, Brouwer et al; 1995 Petrick *et al.*, 2009). Exposure to

these persistent chemicals reportedly results in a variety of toxic effects in experimental animals, including immunologic, neurochemical, neurotoxic, carcinogenic, and endocrine changes (Hsu *et al.*, 2005; Hagmer *et al.*, 2001 and Hagmer *et al.*, 2003). Multiple studies regarding PCB exposure have been carried out human populations, with the concern of environmental PCB levels altering thyroid homeostasis as summarized in Table 6.2.

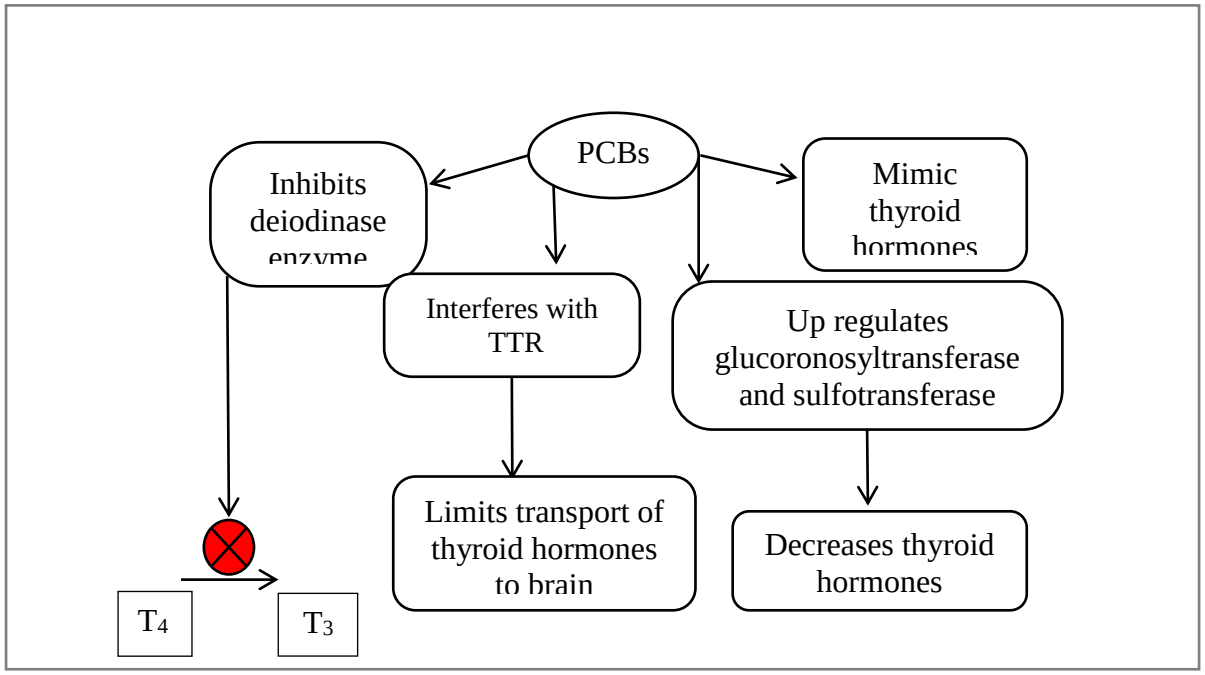


Figure 6.8 Effects of PCBs at various levels of thyroid metabolism: 1. PCBs mimic thyroid hormones thus interfere and compete with binding of thyroid hormones. 2. Decreases thyroid hormone levels by up regulating glucoronosyltransferase and sulfotransferase. 3. PCBs also interfere with TTR thus limit transport of thyroid hormone to brain. 4. Inhibits deiodinase enzyme as a result of which T4 is not getting converted to T3 (Petrick *et al.*, 2009).

Table 6.2: Effects of PCBs on Thyroid Hormones

Author/Year	**No. of Subjects**	**Effect**
Hsu et.al; 2005	60 boys	No effects
Hagmer et al; 2001	182 women	↑T T3
Persky et al; 2001	229 adults	↑ T4, FTI (females); ↓T3 -uptake (men)
Osius et al; 1999	320 children	↓FT3 , ↑ TSH
Tasker et al; 2005	101 mothers	TSH
Ribas-Fito et al; 2003	66 adults	Trends towards ↑TSH
Steuerwald et al; 2000		
Longnecker et al; 2000	160 cord blood	No effects
Koopman et al;1994	105 mothers and infants	Mothers: ↓ TT3 Infants: ↑ ↓ TSH
Matsuura et al; 2001	Maternal seafood exposure	Noeffects in infants
Curtis et al; 2019	744 Adult exposed to PBB and PCBs during childhood	Altered thyroid function

Various studies demonstrated a positive correlation between PCB exposure and TSH (Osius *et al.*, 1999; Persky *et al.,* 2001; Schell *et al.,* 2004; Bloom *et al.,* 2003; Ribas-Fito *et al.,* 2003; Tasker *et al.,* 2005), while other studies in contrast found no associations between PCBs and THs (Steuerwald *et al.,* 2000; Longnecker *et al.,* 2000). Measurements of PCBs in cord blood were not associated with infant THs. However, measurements of PCBs in maternal blood during pregnancy showed negative correlations to peripheral maternal THs and positive correlations to TSH as reported by some studies (Longnecker *et al.,* 2000; Koopman *et al.,* 1994; Matsuura *et al.,* 2001). Curtis et al has also reported altered thyroid function in adults who were exposed to PCBs during their childhood or prenatally (Curtis *et al.,* 2019). Similarly, most studies of PCB content in breast milk did not demonstrate significant associations with infant peripheral TH levels, although one study found significant positive correlation to TSH in the infants as well as significant negative correlations to maternal TT3 and TT4 (Boas *et al.,* 2006; Chavrier *et al.,* 2008 and Boas *et al.,* 2021). Overall studies pin point towards subtle, but significant, effects of low-dose PCB exposure on human thyroid function. For PCBs and their metabolites, relatively firm evidences on thyroid hormone disruption are present in humans. PCBs and their hydroxylated metabolites are structurally close to thyroxine (T_4), hence may disturb thyroid hormone balance by competing for thyroid binding proteins. Even at environmentally occurring concentrations, PCBs may decrease blood triiodothyronine (T_3) or T4 concentrations or elevate thyroid stimulating hormone (TSH) in humans. Significant inverse associations between PCBs and thyroidhormones in pregnant women clearly support the thyroid disrupting effects at the concentration occurring among general population. PCBs might be competing for common receptors due to their structural resemblance resulting in decrease levels of T_3 or T_4. PCBs also enhance hepatic metabolism that leads to elimination of thyroid hormones (Patrick *et al.,* 2009). Alvarez et al. (2009) reported PCBs measured at first trimester of pregnancy showed negative associations with T3 but positive associations with T4 in two pregnant women cohort of Spain. Most epidemiological studies on general population indeed showed negative associations between PCBs and thyroid hormones like T3 or T4, and positive associations with TSH (Takser *et al.,*2005; Chevrier *et al.,* 2008and Kim *et al.,* 2013). Effects of PCB exposure apart from thyroid homeostasis have also been studied for various reproductive destructions but very few for female infertility. Reddy *et al.* (2006) in their study reported PCBs and PEs as the cause of endometriosis in infertile female population Rier *et al.* (1996). has also reported exposure of organochlorine as a cause of endometriosis and thus infertility.

Rozati *et al.* (2009) found the role of PCBs in deterioration of male factor infertility Various reproductive health and birth outcomes in women from exposure to PCBs have been evaluated (Gore *et al.,* 2015). It is reported that women with greater PCBs levels were found to altered menstrual cycles (Yang *et al.,* 2011), while Chavrier *et al.* (2013) has mentioned a decrease in fecundability, possibly due to PCBs causing an endocrine disruption in the oocyte and thus can be considered as a possible factor for a decrease in fecundability and also a delay in conception. Associations of increased PCB exposure and spontaneous abortions have also been reported (Leoni *et al.,* 1989), while no association was found in the study by Small *et al*. (2007). PCBs exposure is suggested to be associated with increased risk of uterine fibroids in several population studies (Lambertino *et al.,* 2011, Trabert *et al.,* 2015), endometriosis (Yao *et al.,* 2017), and polycystic ovarian syndrome (PCOS) (Yang *et al.,* 2015). PCB exposure has been correlated to Apgar scores in newborn (Terrell *et al*; 2015), birth weight (Lignell *et al.,* 2013; Gebbink *et al.,* 2015), preterm birth (Berkowitz *et al.,* 1996; Givens *et al.,* 2007), as well as birth defects studies (Ma J *et al.,* 2012). Studies have indicated there is transfer of PCBs across the human placenta causing in utero exposure and through high amounts in breast milk to neonatal exposure (Jacobson *et al.,* 1984). However, studies evaluating the correlation of female infertility with PCBs levels are very few and inconsistent for different populations (Buck *et al.,* 2009; Cohn et al; 2011; Chavrier *et al.,* 2013; Han *et al.,* 2016). Neblett *et al*. (2020) and other researchers in their studies reported the association of higher serum PCB levels with fewer numbers of lifetime pregnancies but not for female infertility (Small *et al.,* 2011).

The present study found the presence of PCBs in 33% of infertile females with subclinical hypothyroidism. The present study aimed to find out the presence of POPs specifically PCBs in female population of Gujarat and correlate the effects of non-occupational exposure of PCBs with subclinical hypothyroidism and female infertility. There are continuous and growing evidences of worldwide contamination of PCBs and other chemicals which have banned long back to the same extent as that of the chemicals which have not been phased out. In midst of paucity on toxicity and exposure data for the number of chemicals to which people are exposed at national level and specifically for western India, the study was designed to assess the safe level of exposure to hazardous man-made chemicals that remain persistent in the body for longer periods and casing infertility via disturbing the endocrine system. As PCBs have long biologic half-life and easy accumulation in the food chain as well as the continuous production of structurally similar compounds their exposure remains

widespread even though PCBs are no longer manufactured. Thus, the study of these older chemicals is still relevant and important. Further research and scrutiny are needed to determine if and how PCBs may cause subclinical hypothyroidism and female infertility. Because female infertility is a multi-factorial disease it requires a multi-dimensional approach to find out the interrelated causes and consequences, thus the present study hypothesized the cumulative effects of PCBs (environmental chemical disturbing endocrine system) along with autoimmune as well as genetic factor that play roles in its etiology.

The present study reports the presence of PCB congeners in infertile females with subclinical hypothyroidism, while the blood sample of control (fertile) female did not report the presence of PCBs. 33% of IF-SCH females were positive for the presence of PCBs, while none of the Control female samples reported the presence of PCBs. For the validation of pilot results thirty samples of both IF-SCH and control females were processed and prepared and stored for the detection by GC-MS, but we could not proceed further for the detection as all the prepared samples got denatured due to malfunctioning of -20 degree refrigerator during the lockdown period of Covid-19 pandemic.

6.6 Conclusion

The data on the exposure and accumulation of the environmental chemicals affecting female fertility mainly for the western part of India is not documented till date. The results of correlation of PCBs exposure and toxicity effects with female infertility can throw the light on the burden of the environmental chemicals such as PCBs and related compounds in our surroundings to which we are routinely exposed. And this finding thus can be used as a diagnostic approach for subclinical hypothyroidism and female infertility.

6.7 References

Alvarez-Pedrerol M, Guxens M, Ibarluzea J, Rebagliato M, Rodriguez A, Espada M, Goni F, Basterrechea M, Sunyer J. Organochlorine compounds, iodine intake, and thyroid hormone levels during pregnancy. Environmental science & technology. 2009 Oct 15; 43(20):7909-15.

Annex C. of the Stockholm Convention on Persistent Organic Pollutants. United Nations Environmental Programme, Geneva. 2008 Aug 27;29.

Berkowitz GS, Lapinski RH, Wolff MS. The role of DDE and polychlorinated biphenyl levels in preterm birth. Archives of environmental contamination and toxicology. 1996 Jan;30(1):139-41.

Bloom MS, Weiner JM, Vena JE, Beehler GP. Exploring associations between serum levels of select organochlorines and thyroxine in a sample of New York state sportsmen:: the New York State Angler Cohort Study. Environmental research. 2003 Sep 1;93(1):52-66.

Boas M, Feldt-Rasmussen U, Main KM. Thyroid effects of endocrine disrupting chemicals. Molecular and cellular endocrinology. 2012 May 22; 355(2):240-8.

Boas M, Feldt-Rasmussen U, Skakkebæk NE, Main KM. Environmental chemicals and thyroid function. European Journal of Endocrinology. 2006 May 1;154(5):599-611.

Breivik K, Sweetman A, Pacyna JM, Jones KC. Towards a global historical emission inventory for selected PCB congeners—a mass balance approach: 2. Emissions. Science of the Total Environment. 2002 May 6;290(1-3):199-224.

Brouwer A, Ahlborg UG, Van den Berg M, Birnbaum LS, Boersma ER, Bosveld B, Denison MS, Gray LE, Hagmar L, Holene E, Huisman M. Functional aspects of developmental toxicity of polyhalogenated aromatic hydrocarbons in experimental animals and human infants. European Journal of Pharmacology: Environmental Toxicology and Pharmacology. 1995 May 26;293(1):1-40.

BRUCKER-DAVIS FR. Effects of environmental synthetic chemicals on thyroid function. Thyroid. 1998 Sep;8(9):827-56.

Buck Louis GM, Dmochowski J, Lynch C, Kostyniak P, McGuinness BM, Vena JE. Polychlorinated biphenyl serum concentrations, lifestyle and time-to-pregnancy. Human Reproduction. 2009 Feb 1;24(2):451-8.

Burse VW, Groce DF, Caudill SP, Korver MP, Phillips DL, McClure PC, Lapeza Jr CR, Head SL, Miller DT, Buckley DJ, Nassif J. Determination of polychlorinated biphenyl levels in the serum of residents and in the homogenates of seafood from the New Bedford, Massachusetts, area: a comparison of exposure sources through pattern recognition techniques. Science of the total environment. 1994 Apr 29;144(1-3):153-77.

Chana A, Concejero MA, de Frutos M, González MJ, Herradón B. Computational studies on biphenyl derivatives. Analysis of the conformational mobility, molecular electrostatic potential, and dipole moment of chlorinated biphenyl: searching for the rationalization of the selective toxicity of polychlorinated biphenyls (PCBs). Chemical research in toxicology. 2002 Dec 16;15(12):1514-26.

Chevrier C, Warembourg C, Gaudreau E, Monfort C, Le Blanc A, Guldner L, Cordier S. Organochlorine pesticides, polychlorinated biphenyls, seafood consumption, and time-to-pregnancy. Epidemiology. 2013 Mar 1:251-60.

Chevrier J, Eskenazi B, Holland N, Bradman A, Barr DB. Effects of exposure to polychlorinated biphenyls and organochlorine pesticides on thyroid function during pregnancy. American journal of epidemiology. 2008 Aug 1; 168(3):298-310.;

Cohn BA, Cirillo PM, Sholtz RI, Ferrara A, Park JS, Schwingl PJ. Polychlorinated biphenyl (PCB) exposure in mothers and time to pregnancy in daughters. Reproductive Toxicology. 2011 Apr 1;31(3):290-6..

Curtis SW, Terrell ML, Jacobson MH, Cobb DO, Jiang VS, Neblett MF, Gerkowicz SA, Spencer JB, Marder ME, Barr DB, Conneely KN. Thyroid hormone levels associate with exposure to polychlorinated biphenyls and polybrominated biphenyls in adults exposed as children. Environmental Health. 2019 Dec;18(1):1-2.

Dhaibar HA, Patadia H, Mansuri T, Shah R, Khatri L, Makwana H, Master S, Robin P. Hexachlorobenzene, a pollutant in hypothyroidism and reproductive aberrations: a perceptive transgenerational study. Environmental Science and Pollution Research. 2021 Mar;28(9):11077-89.

Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, Zoeller RT, Gore AC. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. Endocrine reviews. 2009 Jun 1;30(4):293-342.

Faroon OM, Keith S, Jones D, De Rosa C. Effects of polychlorinated biphenyls on development and reproduction. Toxicology and Industrial Health. 2001 Apr;17(3):63-93..

Fisher BE. Most unwanted. Environmental health perspectives. 1999 Jan;107(1):A18-23.

Gauger KJ, Kato Y, Haraguchi K, Lehmler HJ, Robertson LW, Bansal R, Zoeller RT. Polychlorinated biphenyls (PCBs) exert thyroid hormone-like effects in the fetal rat brain but do not bind to thyroid hormone receptors. Environmental health perspectives. 2004 Apr;112(5):516-23.

Gebbink WA, Berger U, Cousins IT. Estimating human exposure to PFOS isomers and PFCA homologues: the relative importance of direct and indirect (precursor) exposure. Environment international. 2015 Jan 1;74:160-9.

Givens ML, Small CM, Terrell ML, Cameron LL, Blanck HM, Tolbert PE, Rubin C, Henderson AK, Marcus M. Maternal exposure to polybrominated and polychlorinated biphenyls: infant birth weight and gestational age. Chemosphere. 2007 Oct 1;69(8):1295-304.

Gore AC, Chappell VA, Fenton SE, Flaws JA, Nadal A, Prins GS, Toppari J, Zoeller RT. Executive summary to EDC-2: the Endocrine Society's second scientific statement on endocrine-disrupting chemicals. Endocrine reviews. 2015 Dec 1; 36(6):593-602.

Guo YL, Hsu PC, Hsu CC, Lambert GH. Semen quality after prenatal exposure to polychlorinated biphenyls and dibenzofurans. The Lancet. 2000 Oct 7;356(9237):1240-1.

Hagmar L, Rylander L, Dyremark E, Klasson-Wehler E, Erfurth EM. Plasma concentrations of persistent organochlorines in relation to thyrotropin and thyroid hormone levels in women. International archives of occupational and environmental health. 2001 Apr;74(3):184-8.

Hagmar L. Polychlorinated biphenyls and thyroid status in humans: a review. Thyroid. 2003 Nov 1;13(11):1021-8.

Han L, Hsu WW, Todem D, Osuch J, Hungerink A, Karmaus W. In utero exposure to polychlorinated biphenyls is associated with decreased fecundability in daughters of Michigan female fisheaters: a cohort study. Environmental Health. 2016 Dec;15(1):1-3.

Hardell L, van Bavel B, Lindström G, Carlberg M, Dreifaldt AC, Wijkström H, Starkhammar H, Eriksson M, Hallquist A, Kolmert T. Increased concentrations of polychlorinated biphenyls, hexachlorobenzene, and chlordanes in mothers of men with testicular cancer. Environmental health perspectives. 2003 Jun;111(7):930-4.

Howdeshell KL. A model of the development of the brain as a construct of the thyroid system. Environmental health perspectives. 2002 Jun;110(suppl 3):337-48.

Hsu PC, Lai TJ, Guo NW, Lambert GH, Leon Guo Y. Serum hormones in boys prenatally exposed to polychlorinated biphenyls and dibenzofurans. Journal of Toxicology and Environmental Health, Part A. 2005 Sep 1;68(17-18):1447-56.

Jacobson JL, Fein GG, Jacobson SW, Schwartz PM, Dowler JK. The transfer of polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs) across the human placenta and into maternal milk. American journal of public health. 1984 Apr;74(4):378-9.

Kiliç N, Sandal S, Çolakoglu N, Kutlu S, Seyran A, Yilmaz B. Endocrine disruptive effects of polychlorinated biphenyls on the thyroid gland in female rats. The Tohoku journal of experimental medicine. 2005;206(4):327-32.

Kim S, Park J, Kim HJ, Lee JJ, Choi G, Choi S, Kim S, Kim SY, Moon HB, Kim S, Choi K. Association between several persistent organic pollutants and thyroid hormone levels in serum among the pregnant women of Korea. Environment international. 2013 Sep 1;59:442-8.

Koopman-Esseboom C, Morse DC, Weisglas-Kuperus N, Lutkeschipholt IJ, Van Der Paauw CG, Tuinstra LG, Brouwer A, Sauer PJ. Effects of dioxins and polychlorinated biphenyls on thyroid hormone status of pregnant women and their infants. Pediatric research. 1994 Oct; 36(4):468-73.

Lambertino A, Turyk M, Anderson H, Freels S, Persky V. Uterine leiomyomata in a cohort of Great Lakes sport fish consumers. Environmental research. 2011 May 1;111(4):565-72.

Langer P. Persistent organochlorinated pollutants (PCB, DDE, HCB, dioxins, furans) and the thyroid--review 2008. Endocrine regulations. 2008 Jun;42(2-3):79-104.

Leatherland JF. Contaminant-altered thyroid function in wildlife. Environmental endocrine disruptors: an evolutionary perspective. 2000 Aug 17:155-81.

Leoni V, Fabiani L, Marinelli G, Puccetti G, Tarsitani GF, De Carolis A, Vescia N, Morini A, Aleandri V, Pozzi V, Cappa F. PCB and other organochlorine compounds in blood of women

with or without miscarriage: a hypothesis of correlation. Ecotoxicology and environmental safety. 1989 Feb 1;17(1):1-1.

Lignell S, Aune M, Darnerud PO, Hanberg A, Larsson SC, Glynn A. Prenatal exposure to polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) may influence birth weight among infants in a Swedish cohort with background exposure: a cross-sectional study. Environmental Health. 2013 Dec;12(1):1-9.

Longnecker MP, Gladen BC, Patterson Jr DG, Rogan WJ. Polychlorinated biphenyl (PCB) exposure in relation to thyroid hormone levels in neonates. Epidemiology. 2000 May 1:249-54.

Ma J, Qiu X, Ren A, Jin L, Zhu T. Using placenta to evaluate the polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) exposure of fetus in a region with high prevalence of neural tube defects. Ecotoxicology and environmental safety. 2012 Dec 1;86:141-6.

Massart F, Massai G, Placidi G, Saggese G. Child thyroid disruption by environmental chemicals. Minerva pediatrica. 2006 Feb 1;58(1):47-53.

Matsuura N, Uchiyama T, Tada H, Nakamura Y, Kondo N, Morita M, Fukushi M. Effects of dioxins and polychlorinated biphenyls (PCBs) on thyroid function in infants born in Japan–the second report from research on environmental health. Chemosphere. 2001 Dec 1; 45(8):1167-71.

Neblett MF, Curtis SW, Gerkowicz SA, Spencer JB, Terrell ML, Jiang VS, Marder ME, Barr DB, Marcus M, Smith AK. Examining reproductive health outcomes in females exposed to polychlorinated biphenyl and polybrominated biphenyl. Scientific reports. 2020 Feb 24;10(1):1-9.

Nishimura N, Miyabara Y, Sato M, Yonemoto J, Tohyama C. Immunohistochemical localization of thyroid stimulating hormone induced by a low oral dose of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin in female Sprague–Dawley rats. Toxicology. 2002 Feb 28; 171(2-3):73-82.

Osius N, Karmaus W, Kruse H, Witten J. Exposure to polychlorinated biphenyls and levels of thyroid hormones in children. Environmental health perspectives. 1999 Oct; 107(10):843-9.

Patrick L. Thyroid Disruption: Mechanisms and Clinical Implications in Human Health. Alternative Medicine Review. 2009 Dec 1;14(4).

Persky V, Turyk M, Anderson HA, Hanrahan LP, Falk C, Steenport DN, Chatterton Jr R, Freels S, Great Lakes Consortium. The effects of PCB exposure and fish consumption on endogenous hormones. Environmental health perspectives. 2001 Dec;109(12):1275-83.

Reddy B.S., Rozati R., Reddy S., Kodampur S., Reddy P., Reddy R.: "High plasma concentrations of polychlorinated biphenyls and phthalate esters in women with endometriosis: a prospective case control study". *Fertil. Steril.*, 2006, *85*, 775.

Ribas-Fitó N, Sala M, Cardo E, Mazón C, De Muga ME, Verdú A, Marco E, Grimalt JO, Sunyer J. Organochlorine compounds and concentrations of thyroid stimulating hormone in newborns. Occupational and environmental medicine. 2003 Apr 1; 60(4):301-3.

Rier SE, Martin DC, Bowman RE, Dmowski WP, Becker JL. Endometriosis in rhesus monkeys (Macaca mulatta) following chronic exposure to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin. Fundamental and applied toxicology. 1993 Nov 1;21(4):433-41.

Rozati R, Bakshi HA, Baludu S, Sharma RS. Impact of high plasma concentrations of dioxin and polychlorinated biphenyls (PCBS) in South Indian women with endometriosis. Medical Journal of Islamic World Academy of Sciences. 2009; 17(1):37-44.

Rozati R, Reddy PP, Reddanna PA, Mujtaba R. Role of environmental estrogens in the deterioration of male factor fertility. Fertility and sterility. 2002 Dec 1;78(6):1187-94.

Safe S. Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). Critical reviews in toxicology. 1990 Jan 1; 21 (1):51-88.

Safe SH. Endocrine disruptors and human health--is there a problem? An update. Environmental Health Perspectives. 2000 Jun; 108(6):487-93.

Santini F, Vitti P, Ceccarini G, Mammoli C, Rosellini V, Pelosini C, Marsili A, Tonacchera M, Agretti P, Santoni T, Chiovato L. In vitro assay of thyroid disruptors affecting TSH-stimulated adenylate cyclase activity. Journal of endocrinological investigation. 2003 Oct;26(10):950-5.

Schell LM, Gallo MV, DeCaprio AP, Hubicki L, Denham M, Ravenscroft J, Akwesasne Task Force on the Environment. Thyroid function in relation to burden of PCBs, p, p′-DDE, HCB, mirex and lead among Akwesasne Mohawk youth: a preliminary study. Environmental toxicology and pharmacology. 2004 Nov 1; 18(2):91-9.

Small CM, Cheslack-Postava K, Terrell M, Blanck HM, Tolbert P, Rubin C, Henderson A, Marcus M. Risk of spontaneous abortion among women exposed to polybrominated biphenyls. Environmental research. 2007 Oct 1;105(2):247-55.

Small CM, Murray D, Terrell ML, Marcus M. Reproductive outcomes among women exposed to a brominated flame retardant in utero. Archives of environmental & occupational health. 2011 Oct 1;66(4):201-8.

Steuerwald U, Weihe P, Jørgensen PJ, Bjerve K, Brock J, Heinzow B, Budtz-Jørgensen E, Grandjean P. Maternal seafood diet, methylmercury exposure, and neonatal neurologic function. The Journal of pediatrics. 2000 May 1;136(5):599-605.

Takser L, Mergler D, Baldwin M, De Grosbois S, Smargiassi A, Lafond J. Thyroid hormones in pregnancy in relation to environmental exposure to organochlorine compounds and mercury. Environmental health perspectives. 2005 Aug;113(8):1039-45.

Terrell ML, Hartnett KP, Lim H, Wirth J, Marcus M. Maternal exposure to brominated flame retardants and infant Apgar scores. Chemosphere. 2015 Jan 1;118:178-86.

Trabert B, Chen Z, Kannan K, Peterson CM, Pollack AZ, Sun L, Louis GM. Persistent organic pollutants (POPs) and fibroids: results from the ENDO study. Journal of exposure science & environmental epidemiology. 2015 May;25(3):278-85.

Van den Berg KJ, Zurcher C, Brouwer A. Effects of 3, 4, 3′, 4′-tetrachlorobiphenyl on thyroid function and histology in marmoset monkeys. Toxicology letters. 1988 Apr 1;41(1):77-86.

Yang CY, Huang TS, Lin KC, Kuo P, Tsai PC, Guo YL. Menstrual effects among women exposed to polychlorinated biphenyls and dibenzofurans. Environmental research. 2011 Feb 1;111(2):288-94

Yang Q, Zhao Y, Qiu X, Zhang C, Li R, Qiao J. Association of serum levels of typical organic pollutants with polycystic ovary syndrome (PCOS): a case–control study. Human Reproduction. 2015 Aug 1;30(8):1964-73.

Yao M, Hu T, Wang Y, Du Y, Hu C, Wu R. Polychlorinated biphenyls and its potential role in endometriosis. Environmental Pollution. 2017 Oct 1;229:837-45.