Evaluation of DDTs Intake through Food Items and Serum from Reproductive Age Group Women in Bangladesh

バングラデシュ再生産年齢女性の食物摂取および血清からの DDTs 摂取評価

The United Graduate School of Agriculture Science, Kagoshima University Allied to Saga University, Japan

By

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Approval of Dissertation

This dissertation entitled **"Evaluation of DDTs intake through food items and serum from reproductive age group women in Bangladesh"**, submitted to the The United Graduate School of Agriculture Science, Kagoshima University Allied to Saga University, Japan by Rehnuma Haque, is hereby approved on the recommendation of

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Abstract

This research was conducted to identify and reduce human exposure to DDT, especially women of reproductive age in Bangladesh, and determine a relation between the DDT exposure levels in serum and major food items.

DDT is a persistent, lipophilic chemical that is known to accumulate in human tissues. Exposure to these chemical has been linked to reproductive health effects, cancer, and impaired growth and development of children. Primary routes exposure to DDT are through diet, breastfeeding, and placental transfer. DDT has significant potential to bio accumulate in the food chain and living organisms due to its persistence, and is a major public health concern, especially in areas prone to malaria.

In Bangladesh, the utilization of DDT as a pesticide in agriculture to increase crop production can be traced back to the mid-1950s. DDT products have been mostly used chemicals for public health, particularly for mosquito eradication program, started in 1965, as indoor residual spray (IRS) which was supplied by the World Health Organization. In early 1980s DDT was prohibited for agricultural purposes. Around 1992/93, all usages of DDT products were banned in every sector. Currently, they are used only if a detrimental outbreak occurs in certain focal areas. Although Bangladesh has signed Stockholm convention and restricted using toxic pesticides. Despite this, certain violations of the code can occur, e.g., the export to developing countries of pesticides which have been banned or whose use has been severely restricted.

In this study several POPs (DDTs, PCBs, Chlordanes, HCHs, HCBs, and PeCBs) were quantified. Among them DDTs showed the highest concentration. It was followed by PCB > PeCBs, > HxCBs. Furthermore, meat and fish exhibited higher concentrations of DDT and its metabolites (DDTs: *p, p'*-DDT, *p, p'*-DDD, and *p, p'*-DDE). However, only *p, p'*-DDE was detected in the serum samples. Statistical results suggested that consumption of meat such as beef and mutton may contribute to higher serum levels of *p, p'*-DDE.

要旨

本研究は、バングラデシュの特に再生産年齢女性の DDT 暴露源を特定し、総暴露量を減少させることを目的として、彼らの血清と主要摂取食物中の DDT レベルを測定し関連性を検討した。

DDT は、ヒト組織に持続的に蓄積する親油性化学物質であり、これに暴露されると、リプロダクティブ・ヘルス（生殖機能や生殖活動）、癌、子供の成長や発達障害に影響を与える。DDT は、食事、母乳、胎盤を通して体内に取り込まれ、長期間体内にとどまり続けるため、食物連鎖の過程でヒトに蓄積される可能性が高く、特にマラリア多発地域では、公衆衛生上の重大な懸案事項となっている。

バングラデシュでは、DDT は作物生産を増加させる農薬として 1950 年代半ばころから利用され始めた。 さらに、1965 年からはじまった蚊撲滅プログラムでは、世界保健機関（WHO）によって提供された DDT が屋内用噴霧として使用された。しかし、DDT の生態学的影響から、1980 年代初めには DDT を農薬として使うことが禁止された。1992・3 年頃には、国内ですべての DDT 製品を使用することが禁止され、現在では、特定の地域で緊急事態が発生した場合に使用されるのみである。

本研究では、主要食品中の POPs（DDTs、PCBs、Chlordanes、HCHs、HCBs、PeCBs）を測定したところ、DDT が最も高濃度で検出され、PCB、 PeCBs、HxCBs の順に高濃度を示した。肉および魚中の DDT およびその代謝産物(DDTs: *p, p'*–DDT, *p, p'*–DDD, and *p, p'*–DDE)の濃度が高かった。しかしながら、ヒトの血清中

では、*p, p´* –DDE のみが検出された。統計的には、牛肉および羊肉などの肉の消費が、これらヒト血清の *p*、*p*'–DDE レベルの上昇に寄与していることが示された。

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CHAPTER 1

Introduction

1.1 Pesticides contamination status in Bangladesh

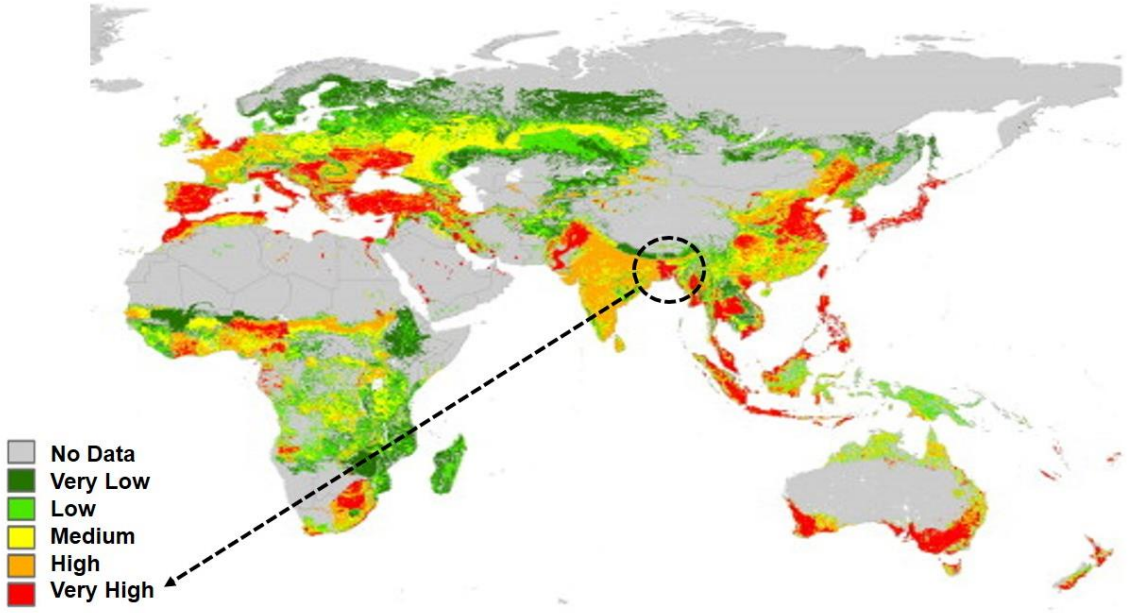
Bangladesh is an agrarian country, representing 30% of the gross domestic product (GDP) from the agro sector. Agriculture is the main occupation of the people employing 70% of the labor force and more than 90% of the rural development (ESDO 2005). However, flooding, drought, attacks from pests and diseases on crops and vegetables can dramatically reduce agricultural output. It has been reported that 20% of agricultural products in Bangladesh are destroyed per year during harvesting and storage period (Chowdhury et al. 2012). Therefore, like many other developing countries (Fig 1.1), pesticides are used extensively in Bangladesh.

Pesticide use in Bangladesh, negligible until the 1960s, has recorded a dramatic increase over the past four decades (Fig 1.2). This is partly due to government's preference to adopt chemical control measures to increase crop production as well as to prevent pre- and post-harvest crop losses (Matin et al. 1998). The growth in the number of brands has exploded in recent years. (Rahman 2003) reported only 48 brands of pesticides in 1983 increasing to 158 brands in 1989. Besides, many pesticides used in Bangladesh are in the banned such as DDT or restricted list under international agreements. Most farmers apply pesticide without knowing its actual requirements and/or effectiveness, and thus there are very high frequencies of pesticides application (Zamir et al. 2013). Pesticide suppliers in Bangladesh even continue to sell the 12 particularly controversial pesticides known by

activists campaigning worldwide as the “dirty dozen”. In addition, substantial anecdotal evidence suggests that users’ lack of information have led to widespread overuse or misuse of pesticides. As a result, pesticide poisonings and ecological damage have become common in Bangladesh several recent studies investigating this behavior found that inadequate product labeling and farmers’ lack of information often lead to widespread overuse or misuse of dangerous pesticides in developing countries (Gill S, Singh L, and R. 2012). FAO analysis of pesticide composition revealed high shares of toxic chemicals and the country is encountered with environmental health problems from the contamination by pesticides.

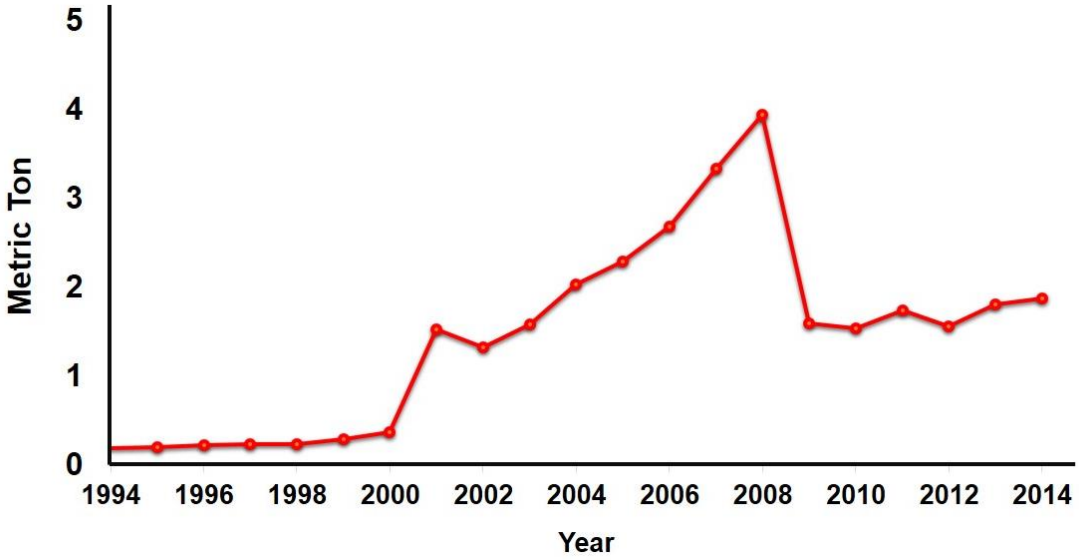
Agrochemicals, including pesticides, are considered a critical aid to improving agricultural production and the prevention of crop losses pre and post-harvest.

Very little effort has been made in Bangladesh to develop methods of pest management, other than the use of pesticides. Along with the increasing use of pesticides there is also an increasing awareness of the effect of pesticides on human health and the environment in general (Rahman, Malek, and Matin 1995). Although Bangladesh has signed Stockholm convention and restricted using toxic pesticides. Despite this, certain violations of the code can occur, e.g., the export to developing countries of pesticides which have been banned or whose use has been severely restricted. In addition, many of the developing countries have no specific pesticide legislation. A number of pesticides such as DDT, BHC, ethyl parathion, methyl parathion, etc. are banned in Bangladesh, but the evidence of using these banned pesticides are available (Fig 1.3).



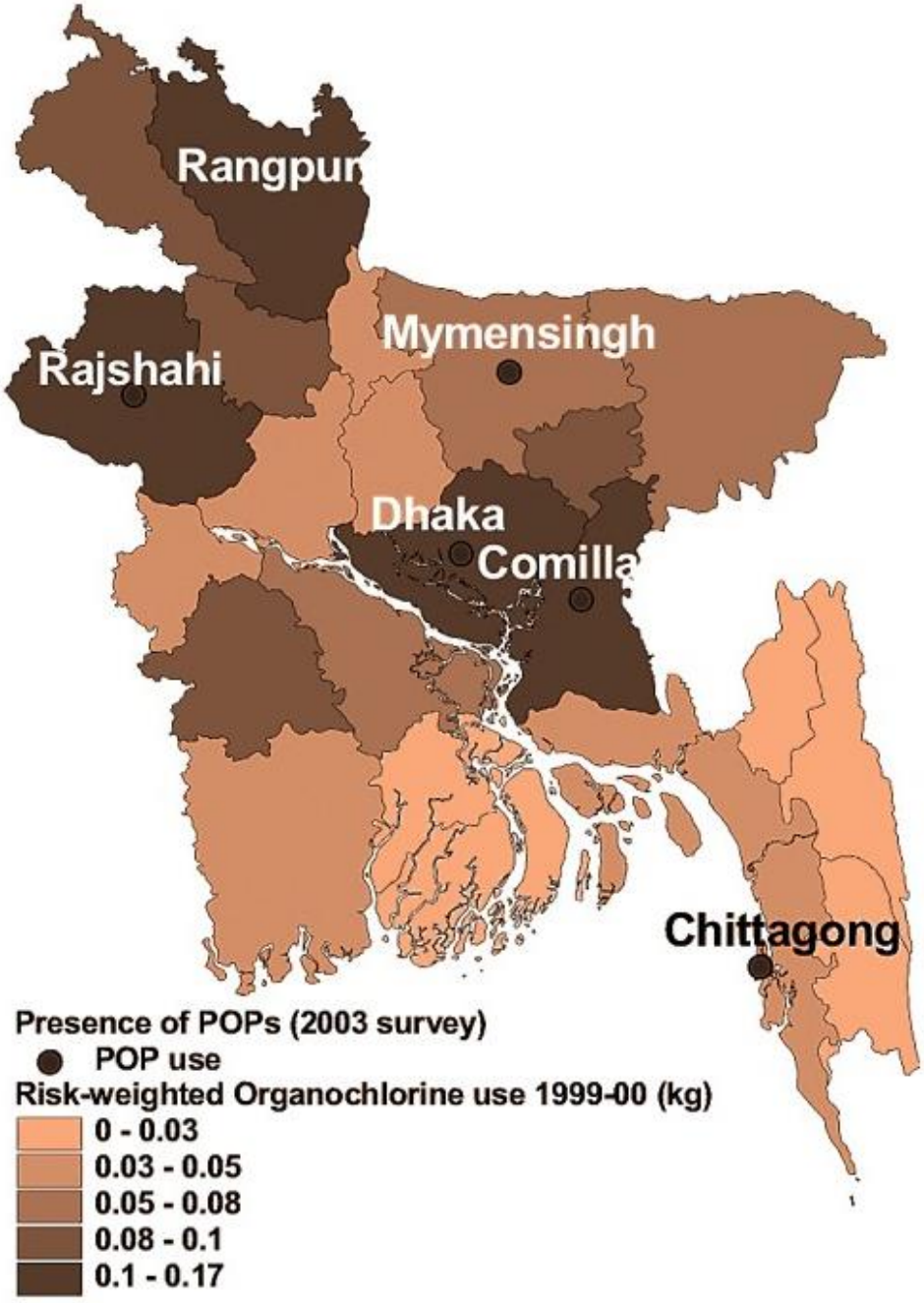
(Ippolito et al. 2015)

Fig: 1.1 Global pesticides runoff hazard map



(FAOSTAT 2018)

Fig 1.2 Pesticide use trend in Bangladesh.



(ESDO 2005)

Figure: 1.3 POPs use trend in Bangladesh.

1.2 DDTs

1.2.1 Overview

DDT (1, 1, 1-trichloro-2, 2-bis (p-chlorophenyl ethane) is an organochlorine compound which have been extensively used in agriculture as pesticides and for malaria prevention worldwide since the 1940s. DDT was first synthesized in 1874, and its insecticidal properties were described by Paul Müller in the late 1930s. During that period, it was first used to protect military areas and personnel against malaria, typhus, and other vector-borne diseases. Commercial sales began in 1945, and DDT became widely used in agriculture to control insects. Initially, twelve POPs have been recognized as causing adverse effects on humans and the ecosystem and these can be placed in 3 categories: Pesticides: aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, toxaphene; Industrial chemicals: hexachlorobenzene, polychlorinated biphenyls (PCBs); and by-products: hexachlorobenzene; polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/PCDF), and PCBs (Fig. 1.4).

In 1972, DDT use was banned in many parts of the world, except for use in controlling emergency public health problems. DDT is still used in certain parts of the world to control malaria. Technical-grade DDT is a mixture of three forms, *p, p'*-DDT (85%), *o,p'*-DDT (15%), and *p, p′* -DDT (trace amounts). All of these are white, crystalline, tasteless, and almost odorless solids (ATSDR 2002). *p,p*'-DDE (1,1-dichloro-2,2-bis(p-chlorophenyl) ethene; also referred to as dichlorodiphenyl dichloroethylene, DDE) and 1-chloro-4- [2,2-dichloro-1-(4- chlorophenyl) ethyl] benzene (DDD) are the metabolites and breakdown products of DDT in the environment. DDE is the main

metabolite of *p, p′*-DDT. It has a longer-half-life, is more toxic, and usually occurs at higher levels than *p, p′*-DDT, the term "total DDT" is often used to refer to the sum of all DDT related compounds (*p, p′*-DDT, *o, p′*-DDT, DDE, and DDD) in a sample. In humans, *p, p′*-DDT is metabolized to *p, p′*-DDE within about six months (ATSDR 2002). The ratio *p, p′*-DDE/p, p'- DDT provides information about how recently exposure took place. *p, p′*-DDE is the most abundant organochlorine pesticide both in the environment and the human body.

The global distribution for DDTs is almost exclusively associated with countries where malaria is still a significant health problem.

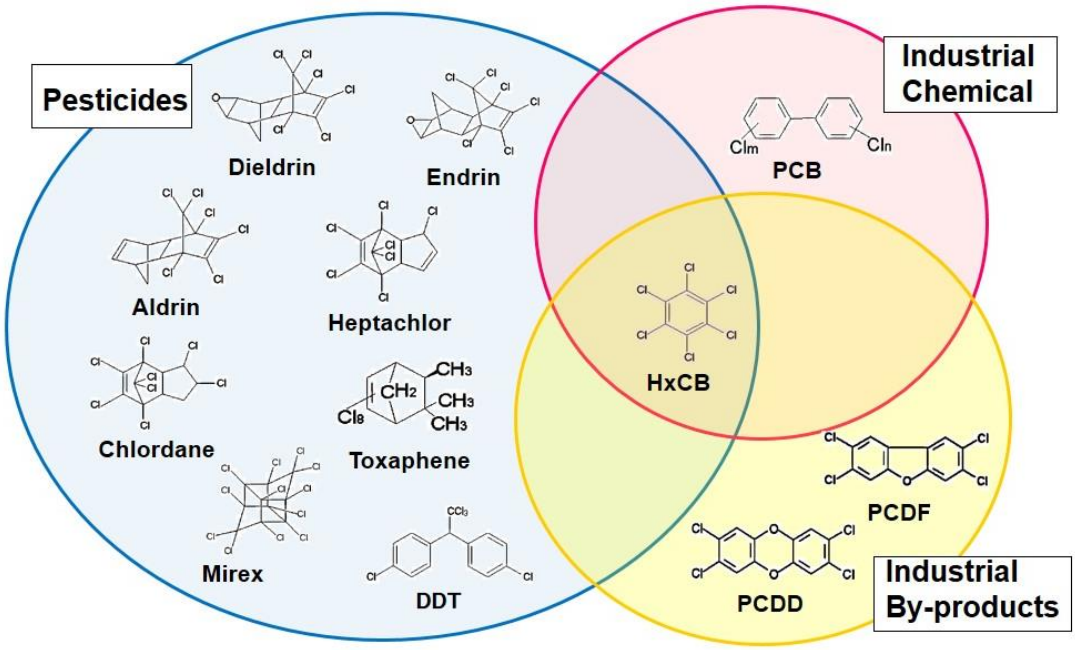


Figure: 1.4 The 12 initial POPs under the Stockholm Convention including DDT.

1.2.2 Route of exposure

The predominant route of exposure of the general public to DDT and its metabolites is through the diet. From the standpoint of dietary exposures, the main exposure route is through the consumption of foods either obtained from areas of the world where DDT is still used or that have the potential to contain bio accumulated residues of DDT and its metabolites (e.g., meat, fish, poultry, dairy products). Exposure to DDT in drinking water is considered negligible because of the extremely low water solubility of DDT and the efficiency of standard drinking water methods. With the ban on the use of DDT, occupational exposures that result Although DDT and its metabolites are ubiquitous in the atmosphere, they are present in such low concentrations that exposures through inhalation or dermal contact are considered to be negligible. DDT and its metabolites are essentially immobile in soil, becoming strongly absorbed onto the surface layer of soils. (ATSDR 2002). Results of previous studies indicated that over 90% of the body burden of DDTs in the general population are derived from food, particularly fish and other fatty foods of animal origin (Wang et al. 2013). Although, the major exposure pathways in the general population are diet breastfeeding and placental transfer also has been reported (Siddiqui et al. 1981; Thomas et al. 2017; Dewana et al. 2013). DDT from the mother can enter her unborn baby through the placenta. DDT has been found in amniotic fluid, human placentas, fetuses, and umbilical cord blood. DDT has been measured in human milk; therefore, nursing infants are also exposed to DDT. In most cases, however, the benefits of breast-feeding outweigh any risks from exposure to DDT in mother's milk. Nevertheless, women with unusually high amounts of DDT or metabolites in their bodies (compared to background amounts measured in the general

population) should be informed of the potential exposure of the fetus if they become pregnant and the potential risks of breast-feeding.

1.2.3 Toxic kinetics

The most frequently proposed reactions during DDT degradation are: reductive dechlorination, dehydrohalogenation, dioxygenation, hydroxylation, hydrogenation and meta-ring cleavage (attack Aerobic Degradation between the 2, 3 carbons on the ring structure). The first primary intermediate of DDT from the aforementioned reactions is either DDD or DDE; the occurrence will depend on the respiration mode and microbe being used (Figure 1.5). Most studies cite that DDD is the common anaerobic metabolite while DDE is associated with aerobic conditions (Kirman, Aylward, Hays,Krishnan, 2011).

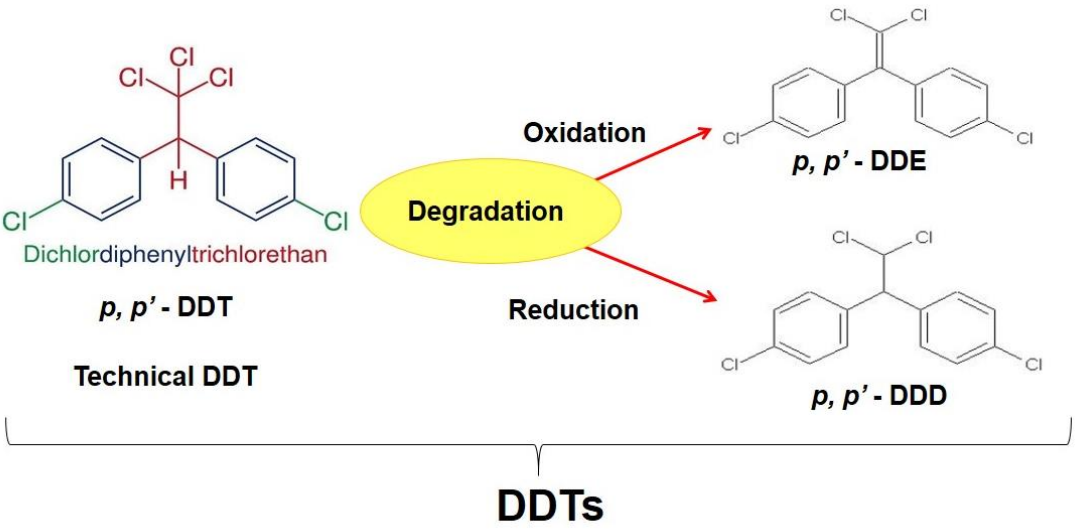


Figure 1.5 Metabolic pathway of DDTs.

1.2.4 Health effects of DDT and its metabolites

Due to the ability of DDT to accumulate in body tissues, their long half-life of elimination from the body, and emerging evidence of potential toxicity to human health, many countries throughout the world went on to ban many of the agents within the OCP family. Additionally, there is limited research on the full long-term impact of DDT and other accrued toxicants within the human body. As a result of the unfolding health problems associated with bioaccumulation of adverse agents and the likelihood of vertical transmission and potential trans generational impact, however, there is emerging attention to the development of effective interventions to facilitate elimination of toxic compounds (Wang et al., 2013). Numerous studies have been conducted on the effects of DDT and related compounds in a variety of animal species, but the human data are somewhat limited.

Reproductive Health Effects

Various reproductive and hormonal endpoints have been examined in both men and women, and although associations have been recorded, causal links have not been confirmed. DDE correlated with the risk of spontaneous abortion and preterm delivery. DDE has also shown an association with small-for-gestational-age in data from the US Collaborative Perinatal Project 100 times high DDE concentration in breast milk has shown an association with a shortened duration of lactation. An increase of 15 µg/L of DDE in maternal serum was associated with a 1-year advance of the age at menarche in daughters (Rogan and Chen 2005).

A study in humans showed that increasing concentrations of *p, p'*-DDE in human breast milk were associated with reductions in the duration of lactation. An additional

study in humans found that as the DDE levels in the blood of pregnant women increased, the chances of having a pre-term baby also increased. A plausible explanation for this decrease in the longevity of lactation is the anti-androgenic/weak estrogenic effect of DDE, as estrogens inhibit milk secretion (ATSDR 2002).

Children Health Effects

Neurological

DDT is an organochlorine pesticide whose best known effect is impairment of nerve impulse conduction. Effects of DDT on the nervous system have been observed in both humans and animals. Eating food with large amounts (grams) of DDT over a short time would most likely affect the nervous system (Katrina et al. 2013).

DDT poisoning usually results in paresthesia, dizziness, headache, tremor, confusion, and fatigue (Rogan and Chen 2005). As many basic functions such respiratory and cardiovascular functions are controlled by the nervous system, exposure to high amounts of DDT is expected to produce a wide array of symptoms and central and peripheral signs of toxicity.

Cancer

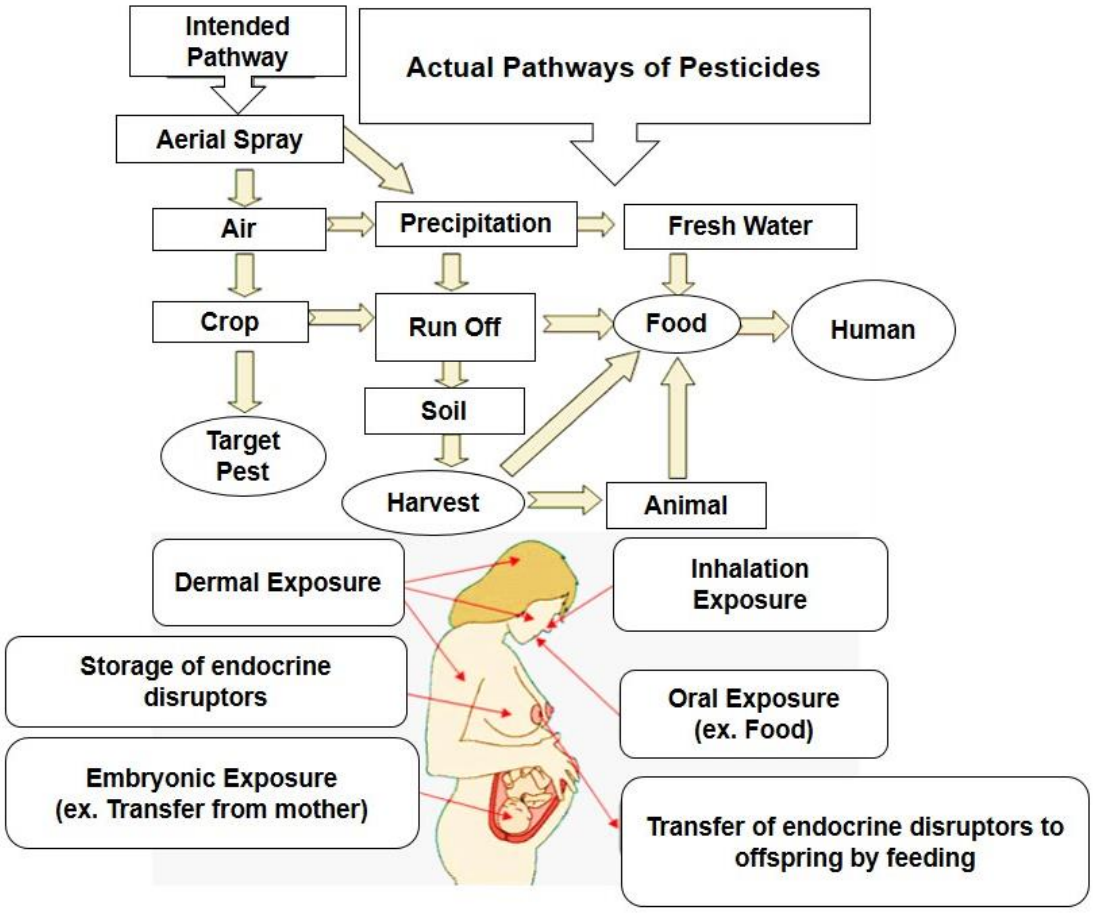
DDT is classified as a possible carcinogen (class 2B) by the International Agency for Research on Cancer (IARC). Breast cancer has been examined most closely for an association with *p, p'*-DDE. In a study in 1993 (Cohn, Cirillo, and Christianson 2010; Barbara et al. 2015; Cohn et al. 2007; Landrigan 2017; Wolff et al. 2000). Breast cancer patients had higher serum DDE concentrations (11·8 µg/L) than controls (7·7 µg/L), and results from several subsequent studies supported such an association. Studies in animals have shown that DDT, DDE, and DDD can cause cancer, primarily in the liver. The

possible association between exposure to DDT and various types of cancers in humans has been studied extensively, particularly breast cancer. Also, possible associations with other cancers that have been investigated include pancreatic cancer, lymphoma, multiple myeloma, prostate and testicular cancer, endometrial cancer, and liver cancer.

Overall, in spite of some positive correlations, there is no clear evidence that exposure to DDT/DDE causes cancer in humans (Ippolito et al. 2015).

Endocrine Disruptor and immunosuppression

Hormones influence the growth, differentiation, and functioning of many target tissues, including male and female reproductive organs and ducts such as mammary gland, uterus, vagina, ovary, testes, epididymis, and prostate (Cohn, Cirillo, and Christianson 2010). Developing organisms respond to endocrine-disrupting chemicals very differently than adults. Fetuses lack feedback regulatory mechanisms and active metabolism of steroid hormones that regulate maintenance of secondary sex tissues, estrous cycling, and pregnancy. Consequently, low levels of exogenous hormones or xenobiotic may induce severe effects in the development of the reproductive organs.



(Sharpe and Irvine 2004)

Figure 1.6 Routes of human exposure to some common environmental chemicals.

1.3 Aim of the Study

In recent lancet pollution commission states that Pollution is the largest environmental cause of disease and premature death in the world today. Diseases caused by pollution were responsible for an estimated 9 million premature deaths in 2015—16% of all deaths worldwide and 3 times more deaths than from AIDS, tuberculosis, and malaria combined (Landrigan 2017).

The result is that chemicals and pesticides whose effects on human health and the environment were never examined have repeatedly been responsible for episodes of disease, death, and environmental degradation.

Reproductive health is an area of priority research worldwide. Children can be exposed to DDT, DDE, or DDD by eating food or drinking breast milk contaminated with these compounds from mothers. DDT is a pesticide, and even though it has not been used in this country since 1992, soil has small amounts and, under certain conditions, contaminated soil transfers DDT to crops. Children can be exposed also by eating food imported from countries where DDT is still being used. Because of their smaller weight, intake of an equivalent amount of DDT by children and adults would result in a higher dose (amount of DDT ingested per kilogram of body weight) in children than in adults. (ATSDR 2002).

Reproductive age group women and children are at high risk of pollution-related disease and even extremely low-dose exposures to pollutants during windows of vulnerability in utero and in early infancy can result in disease, disability, and death in childhood and across their lifespan (Landrigan 2017).

In order to elucidate the contamination status of DDT in reproductive age group women and also to limit the exposure of Bangladeshi people, the following primary objectives were identified:

1. Quantify DDTs concentration in different foods and biological samples.
2. Estimate DDTs oral intake and risk.
3. Propose the way to reduce DDTs exposure, especially reproductive age group women in Bangladesh.

CHAPTER 2

Intakes of DDT and its Metabolites through Food Items among Reproductive Age Women in Bangladesh

2.1 Materials and Methods

2.1.1 Study area

Dhaka is the capital and largest city of Bangladesh. It lies along the east bank of the Buriganga River in the heart of the Bengal delta and located in an eponymous district and division. Dhaka is the economic, cultural and political center of Bangladesh. It is one of the world's most populated cities, with a population of 18.89 million people. It is a major financial center of the country.

The continuing growth reflects ongoing migration from rural areas to the Dhaka urban region, which accounted for 60% of the city's growth.

Chittagong is located towards south-east of the Capital city of Dhaka which is around 280 Km. from the capital. Total area: 168.07 km^2 (64.89 sq mi) and total population is about 2.5 million. Marine fish samples were collected from part of the Chittagong area.

Chittagong city is situated on the bank of Karnaphully River and the city is surrounded by rich natural resources like the green hilly terrain and the Bay of Bengal on the west. Chittagong is the second largest city, prime sea port and the heart of all commercial and business activities in Bangladesh.

Chittagong has been contributing the national economy since the independence of the country in 1971. The major economic establishments/resources are (1) Chittagong Port. (2) Lots of garments industries. (3) Huge numbers of medium and heavy Industries.

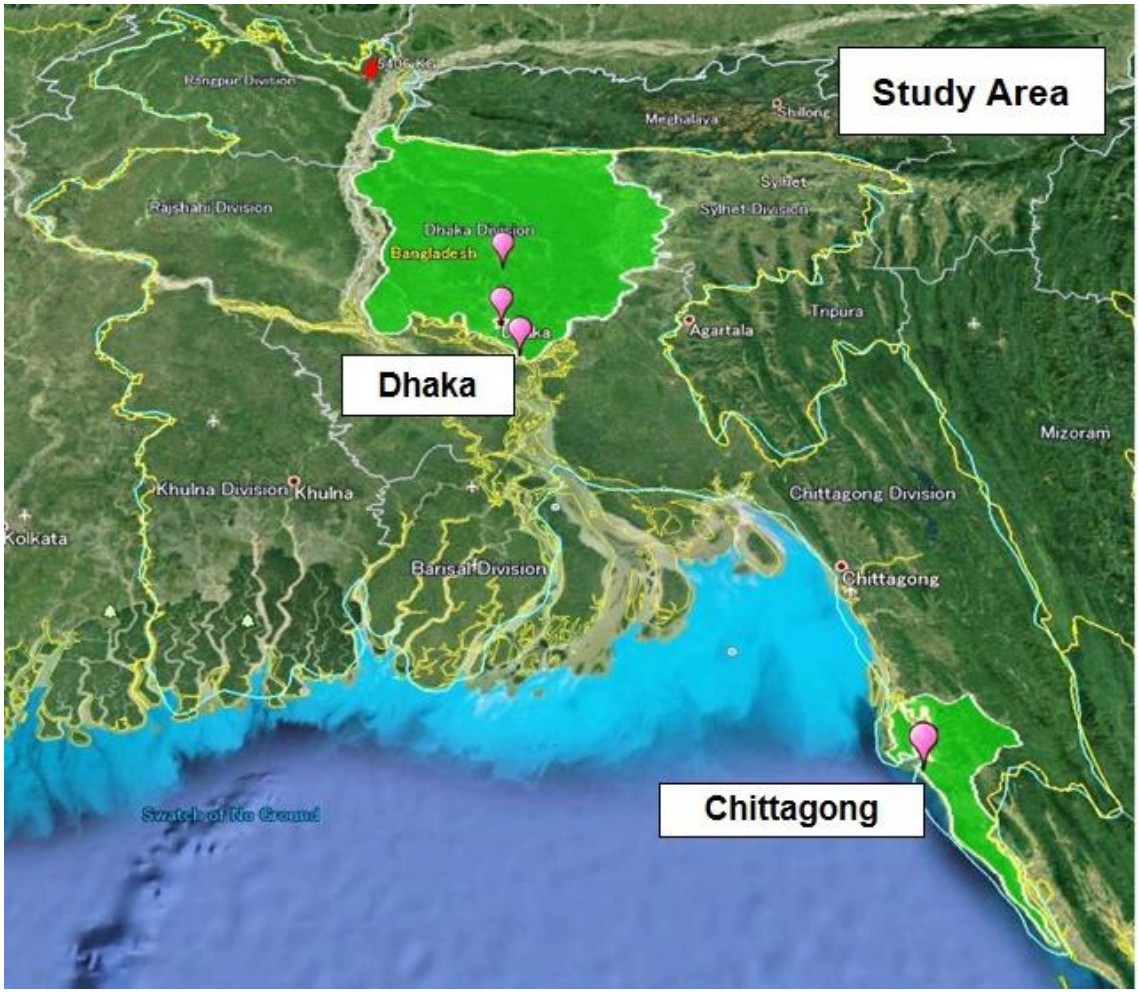


Figure 2.1 Study area map.

2.1.2 Study sample

Food and drinking water sample collection

Collected food items, human breast milk, and house dusts were summarized in (Table 2.1). Those food samples were collected from Dhaka and Chittagong in Bangladesh from 2011 to 2012. The food items were divided into 7 food groups.

Fish were selected from three different sources (fresh, brackish, and sea water). The meat was comprised of beef, chicken and mutton. Dairy products were milk, milk made butter, and special types of local butter called ghee. Cereals were rice and bean which were commonly eaten foods in Bangladesh. Oil was vegetable oils made from soybean. Drinking waters were collected from tap water and tube well.

Human breast milk sample collection

Human breast milk samples were collected from the primipara mother who was between 18 to 22 years old and lived in Dhaka more than 10 years. Those samples were homogenized and stored at -20°C until chemical analysis.

Table 2.1 Description of food items, human breast milk, and drinking water and house dusts collected in Bangladesh.

Food Group	Description	Number	Consumption (Average) (g day-1)
Fish [1)]	Freshwater [a)]	5	41.64
	Seawater	2	6.85
	Brackish	2	3.29
	Beef	5	3.56
Meat[1)]	Chicken	3	3.56
	Mutton	3	3.56
Cereals[1)]	Beans	1	18.63
	Rice	1	474.79
Oil[1)]	Soybean oil	1	4.66
Dairy products [1)]	Milk	2	54.79
	Butter, Ghee [b)]	2	0.55
Breast milk[2)]		8	700
House dust [3)]	Adult	9	0.004
	Child and infant	0	0.06
Drinking Water[4)]	Tube well	8	3000
	Tap	4	3000

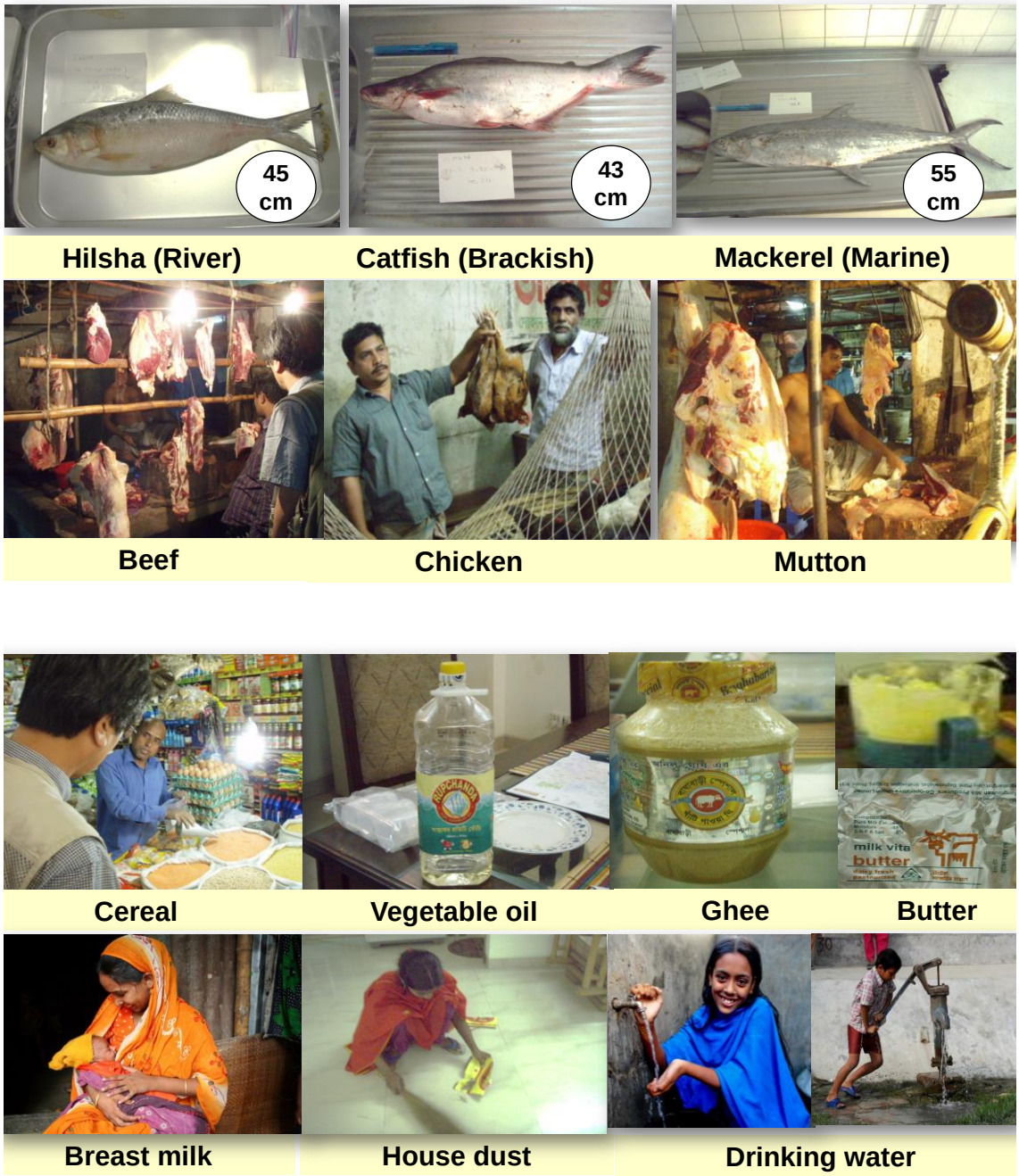


Fig 2.2 Some pictorial view of collected samples.

2.1.3 Chemical analysis and quality control

POPs compounds, polychlorinated biphenyls (PCBs: non-dioxin like tri- to deca-isomers), DDT and its metabolites (DDTs: *p, p'*-DDT, *p, p'*-DDD, and *p, p'*-DDE), chlordane and related compounds (CHLs: trans-chlordane, cis-chlordane, trans-nonachlor, cis-nonachlor, and oxychlordane), and hexachlorobenzene (HCB), were analyzed following the methods described previously (Kajiwara et al. 2003) with slight modifications. Briefly, a portion of the sample (5 g of breast milk or food homogenate) was mixed with surrogate standards of 13C12-PCB mix (from mono- to deca-CB) and grounded with sodium sulfate. It was extracted using a Soxhlet apparatus, and the extract was concentrated using rotary evaporator. An aliquot of the extract was used for determination of crud fat by measuring total non-volatile extract. Another aliquot of it was subjected to gel permeation chromatography (GPC) for lipid removal. The GPC fraction containing target compounds was concentrated and passed through an activated florisil packed glass column for further cleanup. The fraction was micro-concentrated and injected into a gas chromatograph coupled with a mass spectrometer (GC-MS) using selected ion monitoring (SIM) mode. HP-5ms (30 m length, 0.25 mm i.d., 0.25 μm film thickness) were used for analysis. Recoveries of 13C12-PCB ranged between 70% and 120%. Method detection limit (MDL) was calculated as a three-times standard deviation of background peak (or lowest concentration S/N 3 of authentic standard peak, in the case of no background peak) in the procedural blanks (n=5). MDL of each compound were 0.002 to 0.01 ng g-1 wet and 0.1 to 0.7 ng g-1 lipid weight basis, respectively. Standard Reference Materials (fish oil, EDF-2525 by CIL and sediment, SRM 1944 by NIST) were analyzed using this analytical procedure and the data from our laboratory were in good agreement with reference values.

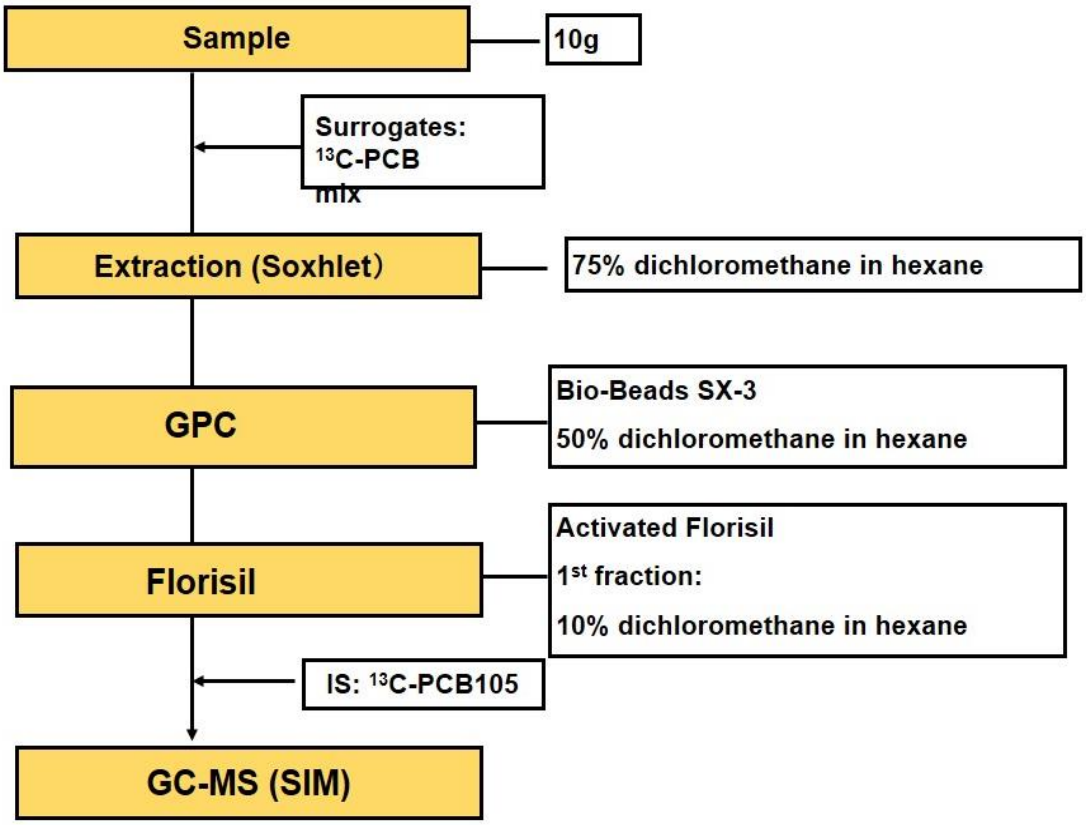


Figure 2.3 Chemical analytical procedure in this study.

2.2 Results and Discussions

2.2.1 Contamination status of POPs in food items

The highest detection frequencies of POPs analyzed in this study was PCBs (95%), and it was followed by DDTs and PeCBs (75%), CHLs (73%), HxCBs (71%) and HCHs (34%). DDTs, HxCBs, PeCBs, PCBs and CHLs were detected from almost all the food samples (n= 56) analyzed in this study (Fig 2.3). Concentrations of POPs in food items including breast milk and house dust from Bangladesh are summarized in (Fig 2.4). These results indicate ubiquitous contamination of these POPs in food items in Bangladesh.

Among POPs analyzed in this study, DDTs showed the highest concentration (maximum 1100 and median 21 ng^{-1} gwet weight basis), and it was followed by PCB (maximum 28 and median 0.56 ng^{-1} gwet weight) > PeCBs, > HxCBs. Concentration ranges of DDTs detected in this study showed 50 times higher than those of PCB, and concentration ranges of other POPs showed much lower than those of DDTs. According to this result, it will be discussed about DDTs contamination in this study because considerable level of POPs in Bangladesh seems to be only DDTs.

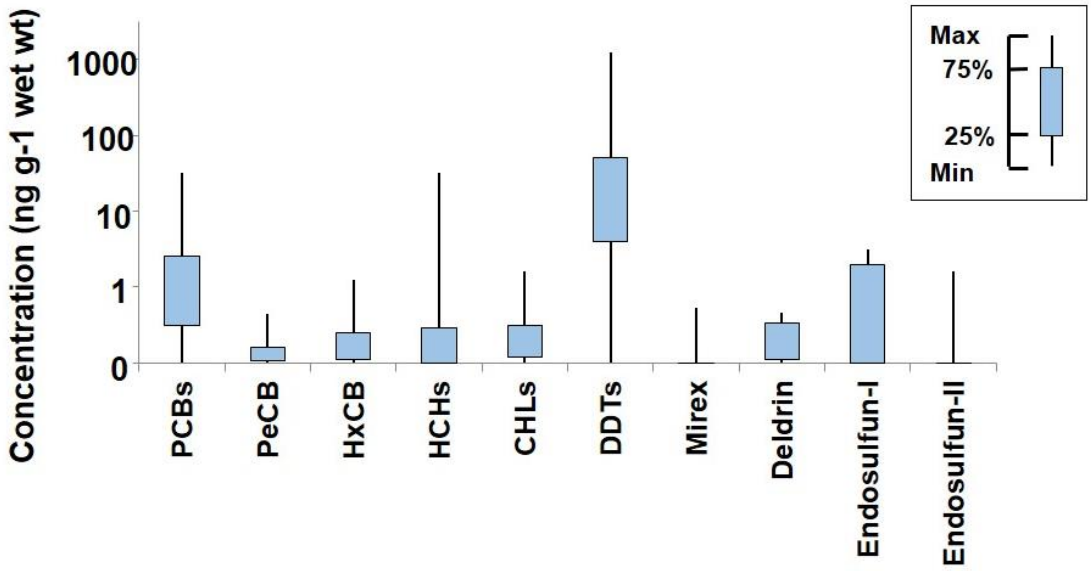


Figure 2.4 POPs concentrations in food items, human breast milk, house dust and drinking water quantified in this study.

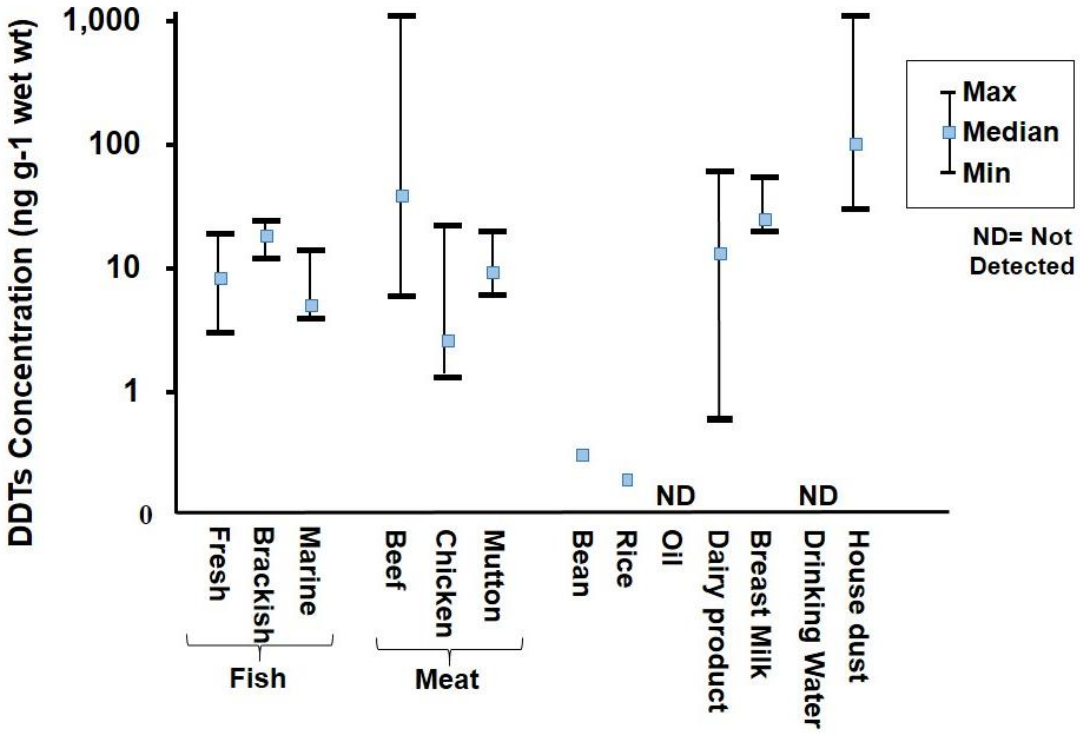


Figure 2.5 Contamination status of DDTs in food items, human breast milk, house dust and drinking water quantified in this study.

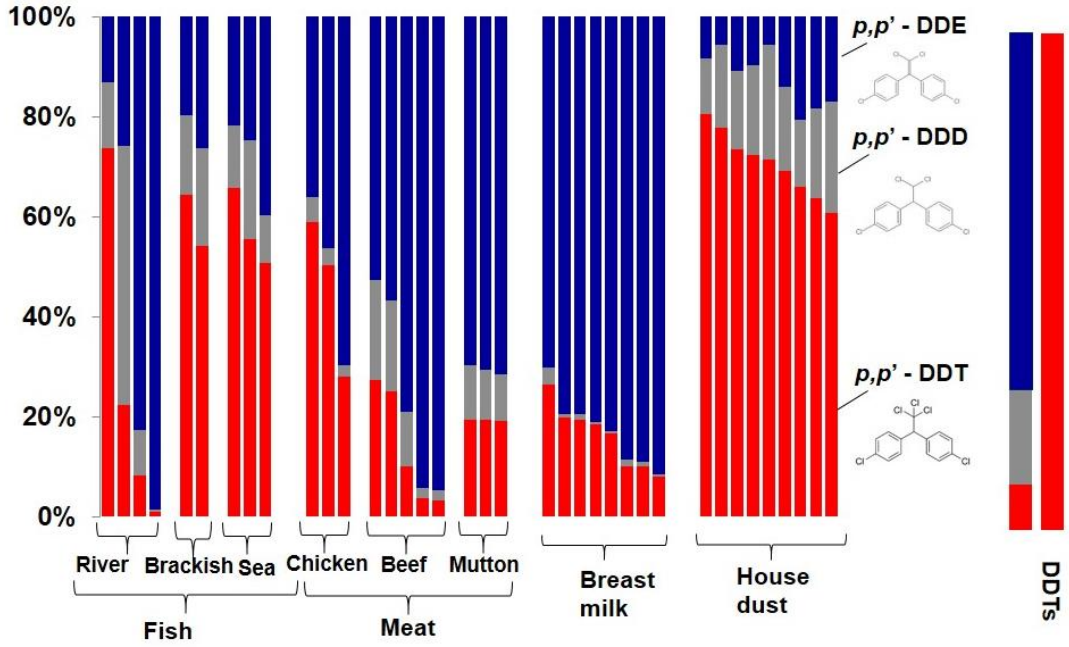


Figure 2.6 DDTs composition in food items, human breast milk, house dust and drinking water quantified in this study.

Table 2.2 DDTs concentrations in food items, human breast milk, house dust and drinking water quantified in this study.

	p,p'-DDE			*p,p'*-DDD			*p,p'*-DDT			Total-DDT		
Items	Med	Max	Min	Med	Max	Min	Med	Max	Min	Med	Max	Min
Fish												
Fresh water	2.5	5.3	1.0	0.78	5.6	0.029	2.2	14	0.045	5.4	19	3.03
Brackish	4.1	4.9	3.2	3.2	4.04	2.4	11	16	6.6	18	24	12
Sea	3.3	5.4	1.1	0.99	1.3	0.63	5.1	6.9	3.3	9.3	14	4.99
Meat												
Beef	36	600	4.7	0.81	230	0.35	1.4	310	0.52	38	1100	5.9
Mutton	6.7	14	4.4	0.87	2.2	0.62	1.8	3.9	1.2	9.3	20	6.1
Chicken	2.5	8.0	0.6	0.080	1.1	0.041	1.0	13	0.64	3.6	22	1.3
Vegetable oil	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cereal	0.054	0.060	0.048	0.069	0.12	0.022	0.088	0.15	0.031	0.21	0.32	0.100
Dairy Products	9.5	19	0.38	2.2	22	0.070	1.2	20	0.037	13	61	0.54
Breast Milk	21	50	16	0.26	0.84	0.12	4.3	6.4	2.6	25	55	20
Drinking water	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
House dust	12	62	4.9	16	250	5.4	75	780	19	100	1100	30

Fish

In this study, DDTs were detected from fish samples in 100%, and the concentration range of DDTs in fish was 3.0-24 ng g^{-1} wet weight (Table 2.2). The concentration range of DDTs in fish in previous studies was summarized in (Table 2.3). Concentration ranges of DDTs in the previous report showed 23-83 ng g^{-1} wet weight basis (converted from dry basis). Those were comparable or higher than the result in this study. The reason of higher concentrations of DDTs in dry fish was that fisherman spraying technical DDT for dry fish to protect it from insects (Bhuiyan et al. 2009; Chowdhury et al. 2010; Jabber, Khan, and Rahman 2001). Actually, higher percentage of *p, p'*-DDT (maximum ratio of *p, p'*-DDE/ *p, p'*-DDT was 0.17, which was 74 %) were found in fish from fresh, brackish, and sea water (Fig 2.4 and Table 2.3). This result also suggests that recent usage of technical DDT even amount of usage can be smaller.

Table 2.3 POPs concentrations detected in fish samples.

POPs		Fresh water fish			Brackish water fish			Sea water fish		
	Concentration (ng/g)	Med	Min	Max	Med	Min	Max	Med	Min	Max
	Lipid content %	5.1	3.1	7.7	19	16	21	1.7	1.0	2.4
Total-PCBs	Lipid weight	11	5.2	19	4.8	3.6	5.99	30	27	33
	Wet weight	0.55	0.40	0.57	0.86	0.75	0.96	0.55	0.28	0.81
PeCB	Lipid weight	0.25	0.08	2.00	0.34	0.021	0.65	0.42	0.12	0.72
	Wet weight	0.0092	0.0034	0.15	0.10	0.10	0.10	0.01	0.00	0.02
HxCB	Lipid weight	0.20	0.14	0.54	1.49	0.06	2.92	1.71	0.81	2.61
	Wet weight	0.010	0.0045	0.042	0.38	0.30	0.47	0.04	0.01	0.06
Total-HCHs	Lipid weight	11.10	2.98	19	ND	ND	ND	8.90	3.31	14
	Wet weight	0.78	0.091	1.47	ND	ND	ND	0.19	0.03	0.35
Total-CHLs	Lipid weight	1.23	0.083	2.98	1.96	0.33	3.59	3.45	3.31	3.59
	Wet weight	0.061	0.0029	0.17	1.10	0.58	1.62	0.05	0.01	0.09
Total-DDTs	Lipid weight	150	60	260	105	60	150	520	480	560
	Wet weight	5	3	19	18	12	24	9	5	14

Table 2.4 Domestic comparison of DDTs concentrations detected from fish samples.

Fish	Year	Area	ng/g-1 wet weight	References
Catfish-1	2011	Chittagong	19	This Study
Carp-2	2012	Dhaka	3.03	This Study
Eel-1	2012	Dhaka	11	This Study
Shrimp-2	2012	Dhaka	5.4	This Study
Hilsha-1	2011	Dhaka	24	This Study
Hilsha-2	2012	Dhaka	12	This Study
Tilapia-1	2012	Chittagong	3.9	This Study
Mackerel	2011	Chittagong	14	This Study
Mackerel	2012	Chittagong	5.0	This Study
Ribbon fish	2012	Chittagong	20	(Siddique and Aktar 2012)
Bombay duck	2012	Chittagong	25	(Siddique and Aktar 2012)
Shrimp	2012	Chittagong	7.9	(Siddique and Aktar 2011)
Ribbon fish	2009	Chittagong	0.54 - 83	(Bhuiyan et al. 2009)
Shrimp	2009	Chittagong	3.2 - 55	(Bhuiyan et al. 2009)
Hilsa	2009	Chittagong	23 - 42	(Bhuiyan et al. 2009)
Rui	2013	Dhaka	10 - 279	(Zamir et al. 2013)
Pangus	2013	Dhaka	6 - 669	(Zamir et al. 2013)
Katla	2013	Dhaka	8 - 113	(Zamir et al. 2013)
Catfish	2016	Mohangonj	54	(Hossain, Mohammad, and Nilufar 2016)
Shrimp	2016	Brahmanbaria	13	(Hossain, Mohammad, and Nilufar 2016)
Eel	2016	Brahmanbaria	79	(Hossain, Mohammad, and Nilufar 2016)
Telapia	2016	Brahmanbaria	19	(Hossain, Mohammad, and Nilufar 2016)
Carp	2016	Brahmanbaria	11	(Hossain, Mohammad, and Nilufar 2016)

* calc moisture by 80% of total component

DDTs: *p, p′*-DDE+*p, p′*-DDD+*p, p′*-DDT

Meat

In this study, the highest concentration of DDTs was detected from meat (Table 2.4). Among meat samples, the highest concentrations of total DDTs were found in beef (median: 38 and range 6-1100 ng g^{-1} wet weight), and it was followed by chicken (median: 3.6 and range 1.3-22 ng g^{-1} wet weight) and mutton (median: 9.3 and range 6.1-20 ng g^{-1} wet weight). Regarding to percentages of p, p'-DDT in meat, chicken showed a relatively higher percentage of p, p'-DDT, (maximum ratio of p, p'-DDE/ p, p'-DDT was 0.64, which was 58%) and it was followed by beef (maximum ratio was 2.6, which was 28 %) and mutton (Fig 2.3 and Table 2.4). In this study, beef purchased from markets in Dhaka showed the highest concentration of DDTs, and it was the first report showing higher levels of DDTs in beef from Bangladesh market. The reason of higher concentrations of DDTs in these beef might be due to that either through indoor residue spray (IRS) of cattle house or direct application on the animal (John, Bakore, and Bhatnagar 2001). IRS was the primary malaria control method, and DDT has long been the cheapest insecticide and the one with the longest residual efficacy against malaria vectors until stop to used it in 1993 (6–12 months depending on dosage and substrate) (WHO 2015). After IRS on the wall of cattle house, soil and fodder could be contaminated by DDT. The livestock in the cattle house reared on the soil and eat fodders. Animal can accumulate these substances from contaminated soil, feed and water. Also due to lipophilic nature of DDT, milk and dairy products can be accumulating those chemicals (John, Bakore, and Bhatnagar 2001) . However, dairy products analyzed in this study showed relatively lower concentrations of DDTs (median: 13 and range 0.54-61 ng g^{-1} wet weight). The higher DDTs concentrations in beef and lower those in milk may be due to that the farms producing

beef and milk could be different. It was reported that almost all of milk has been domestic produced in Bangladesh (M.M. Uddin et al. 2011).

On the other hand, almost 60 % of beef has been imported from India (Khatun et al. 2016). It was also reported that DDT had been used for IRS on cattle house in India (John, Bakore, and Bhatnagar 2001; Mishra, Sharma, and Kumar 2011) and higher concentrations of DDTs in milk and dairy products made in Northeastern India. These results suggest that the milk showed lower DDTs concentrations were domestic produced in Bangladesh, and the beef which showed higher concentrations of DDT might be imported from India where is the regions using DDTs in Northeastern India.

Only one report showed DDTs concentrations in chicken from Bangladesh (Table 2.5 and Fig 2.6). DDTs concentrations in this report showed comparable or higher (Shoeb et al. 2016) than those in this study. Chicken analyzed in this study showed the relatively higher percentage of p, p'-DDT (maximum ratio of p, p'-DDE/ p, p'-DDT was 0.64, which was 58%) than beef even those concentrations were lower than beef (Fig 2.4). It was reported that 195 million chicken per year were domestically produced in Bangladesh (BBS 2007; Shoeb et al. 2016). The higher percentage of p, p'-DDT in chicken analyzed in this study suggests recent usage of DDT in Bangladesh even amount of it can be limited.

Table 2.5 POPs concentrations detected in meat samples.

POPs		Beef			Chicken			Mutton		
	Concentration (ng/g)	Med	Min	Max	Med	Min	Max	Med	Min	Max
	Lipid content %	15.9	8.5	26	3.1	2.0	7.7	11.0	7.0	21.0
Total-PCBs	Lipid weight	10.60	4.17	37.81	3.22	1.08	4.16	2.63	2.29	2.74
	Wet weight	2.68	0.55	7.03	0.06	0.03	0.32	0.28	0.19	0.47
PeCB	Lipid weight	0.02	0.01	0.05	0.10	0.07	1.04	0.04	0.04	0.04
	Wet weight	0.00	0.00	0.01	0.01	0.00	0.02	0.00	0.00	0.01
HxCB	Lipid weight	1.15	0.40	4.12	0.23	0.22	2.11	0.34	0.34	0.36
	Wet weight	0.16	0.05	0.66	0.02	0.01	0.04	0.04	0.02	0.07
Total-HCHs	Lipid weight	3.46	1.63	41.62	ND	ND	ND	0.80	ND	0.82
	Wet weight	0.45	0.21	6.61	ND	0.00	0.00	0.11	0.06	0.16
Total-CHLs	Lipid weight	0.22	0.14	0.31	0.18	0.10	0.25	0.15	0.12	0.20
	Wet weight	0.03	0.02	0.08	0.01	0.00	0.02	0.01	0.01	0.04
Total-DDTs	Lipid weight	200	45	7200	47	41	1000	88	88	100
	Wet weight	38	5.9	1100	3.6	1.3	22	9.3	6.1	20

Table 2.6 Domestic comparison of DDTs concentrations detected from chicken samples collected in Bangladesh.

Chicken	Year	Area	ng/g wet weight	References
Chicken-1	2011	Dhaka	22	This study
Chicken-2	2012	Dhaka	3.6	This study
Chicken-3	2012	Dhaka	1.3	This study
Chicken-Aftab	2016	Dhaka	344	(Shoeb et al. 2016)
Chicken-Babu	2016	Dhaka	14	(Shoeb et al. 2016)
Chicken-Tareq	2016	Dhaka	95	(Shoeb et al. 2016)
Chicken-Sarwar	2016	Dhaka	39	(Shoeb et al. 2016)

* DDTs: *p,p′*-DDE+*p,p′*-DDD+*p,p′*-DDT

Other food items

In this study, dairy products (butter and ghee and milk), cereals (bean and rice), vegetable oil and drinking water were also employed for DDTs analysis. Generally, DDTs concentrations in those samples were low and/or not detected (Table 2.7 and Table 2.8). The reason of lower concentrations of DDTs in these food items was that DDT has not used for an agricultural purpose since 1993 (ESDO 2005).

Table 2.7 POPs concentrations detected in dairy samples.

POPs	Concentration (ng/g)	Ghee & Butter			Milk		
		Med	Min	Max	Med	Min	Max
	Lipid content %	86	78	93	3.5	3.5	3.5
Total-PCBs	Lipid weight	1.6	1.4	1.8	2.1	1.9	2.4
	Wet weight	1.4	1.1	0.0	0.1	0.1	0.1
PeCB	Lipid weight	0.0	0.0	0.1	0.0	0.0	0.0
	Wet weight	0.0	0.0	1.2	0.0	0.0	0.0
HxCB	Lipid weight	1.3	1.2	1.5	0.2	0.2	0.2
	Wet weight	1.1	1.1	0.0	0.0	0.0	0.0
Total-HCHs	Lipid weight	29	29	30	0.84	0.84	0.84
	Wet weight	25	22	0.0	0.0	ND	0.0
Total-CHLs	Lipid weight	0.17	0.11	0.24	2.6	0.1	5.0
	Wet weight	0.15	0.08	0.00	0.089	0.005	0.17
Total-DDTs	Lipid weight	53	27	78.2	15.9	15.3	16.5
	Wet weight	43	25	7.7	0.6	0.5	0.6

Table 2.8 POPs concentrations detected from cereal and oil samples collected in Bangladesh.

POPs	Concentration (ng/g)	Cereal		Oil
		Rice	Beans	Soyabean oil
	Lipid content %	0.12	3.11	77.43
Total-PCBs	Lipid weight	18.70	1.67	0.11
	Wet weight	0.023	0.052	0.084
PeCB	Lipid weight	2.06	0.50	0.017
	Wet weight	0.00	0.016	0.013
HxCB	Lipid weight	ND	1.85	0.039
	Wet weight	ND	0.058	0.031
Total-HCHs	Lipid weight	ND	ND	ND
	Wet weight	ND	ND	ND
Total-CHLs	Lipid weight	0.17	0.46	0.13
	Wet weight	0.00	0.014	0.10
Total-DDTs	Lipid weight	81.58	10.37	ND
	Wet weight	0.10	0.32	ND

2.2.2 Contamination status of POPs in human breast milk

In this study, breast milk also showed higher concentrations (Table 2.9). The breast milk samples collected in this study were primipara mothers in Dhaka. The concentration range of DDTs in breast milk was 20-55 ng g^{-1} wet weight. Those samples were also shown a relatively higher percentage of *p, p'*-DDT (maximum ratio of *p, p'*-DDE/ *p, p'*-DDT was 2.6, which was 27 %) (Figure 2.3 and Table 2.9). The previous report of DDTs concentrations in breast milk collected in Bangladesh were summarized in (Table 2.8). Only one report has been available on the DDTs concentrations in breast milk samples collected from primipara mothers in rural villages of Bangladesh. The level of DDTs in a previous study (median: 69 and range 12-1430 ng g^{-1} lipid weight) was 3-4 times higher than those in this study (median: 69 and range 12-1430 ng g^{-1} lipid weight) (Bergkvist et al. 2012). The percentage of *p, p'*-DDT in it also showed higher value (5-50 %), and it was similar or a bit higher than those in this study. This research reported the reason of higher DDTs levels that the rural agricultural village have been using heavy burden of pesticides including DDT for agriculture purpose (Rahman 2003). It indicates that contamination levels of DDTs in Bangladesh could be higher in rural villages than those in urban regions such as Dhaka.

Table 2.9 POPs concentrations detected in human breast milk samples.

POPs	Concentration (ng/g)	Med	Min	Max
	Lipid content %	4.8	2.5	8.1
Total-PCBs	Lipid weight	12	8.1	37
	Wet weight	0.53	0.27	1.18
PeCB	Lipid weight	0.070	0.055	0.10
	Wet weight	0.00	0.00	0.01
HxCB	Lipid weight	0.69	0.45	1.23
	Wet weight	0.03	0.02	0.05
Total-HCHs	Lipid weight	5.0	2.8	11.4
	Wet weight	0.2	0.1	0.3
Total-CHLs	Lipid weight	3.1	1.3	47.5
	Wet weight	0.1	0.1	1.2
Total-DDTs	Lipid weight	720	390	1100
	Wet weight	25	20	55

Table 2.10 Domestic comparison of DDTs concentrations detected from human breast milk.

Human breast milk	Year	Area	ng/g-1 wet weight	References
Breast Milk	2015	Dhaka	25 (20-55)	This Study
Breast Milk	2012	Matlab	70 (12-1400)	(Bergkvist et al. 2012)

DDTs: *p,p'*-DDE+*p,p'*-DDD+*p,p'* DDT

Table 2.11 International comparison of DDTs mean concentrations in human breast milk from various countries.

Country	Year	($ng^{-1}g$ lipid weight)	References
Asian			
Bangladesh	2016	540 (390-1100)	This study
Bangladesh	2012	1900 c	(Bergkvist et al. 2012)
China	2002	2100	(Kunisue et al. 2004)
Vietnam	2000-2001	2200	(Minh et al. 2004)
India	2011	1914 c	(Bedi et al. 2013)
India	2004-2005	1100	(Devanathan, Subramanian, and Someya 2009)
Malaysia	2003	1600	(Sudaryanto et al. 2005)
Japan	2001-2004	340	(Kunisue et al. 2006)
Philippines	2004	170	(Malarvannan et al. 2009)
Non-Asian			
Ethiopia	2010	14460	(Gebremichael, Birhanu, and Tessema 2013)
South Africa	2006	4797	(Bouwman, Sereda, and Meinhardt 2006)
Tunisia	2002-2005	1931	(Ennaceur, Gandoura, and Driss 2008)
Poland	2002-2005	1621 c	(Hernik et al. 2013)
Russia	2003-2004	660	(Tsydenova et al. 2007)
Mexico	1997-1998	4700 bd	(Waliszewski et al. 2001)
Brazil	2001-2002	493 c	(Azeredo et al. 2008)

a: *p,p′*-DDE+*p,p′*-DDD+*p,p′*-DDT

b: *p,p′*-DDE+*o,p′*-DDE+*p,p′*-DDD+*p,p′*-DDT+*o,p′*-DDE

c: median

2.2.3 Contamination status of DDTs in house dust

In this study, house dust showed the higher concentrations even in dry weight basis (Table 2.8). The concentration range of DDTs in house dust was 30-1100 ng g^{-1} dry weight. The highest percentage of *p, p'*-DDT (maximum ratio of *p, p'*-DDE/ *p, p'*-DDT was 0.073, which was 81 %) was also found in these samples (Fig 2.3 and Fig 2.5). It has not had any report on DDTs concentrations in house dust in Bangladesh. Possible reason of higher concentrations of DDTs and higher percentage of *p, p'*-DDT in house dust would be due to recent activities of IRS using DDT in Bangladesh. IRS was the primary malaria control method, and DDT has long been the cheapest insecticide and the one with the longest residual efficacy against malaria vectors until the stop to used it in 1993 (6–12 months depending on dosage and substrate) (WHO 2015). The higher concentrations and percentage of *p, p'*-DDT in house dusts suggests recent usage of DDT for IRS even DDT usage for IRS was restricted in 1993.

Table 2.12 POPs concentrations detected in house dust samples.

POPs	Concentration (ng/g dry)	Med	Min	Max
Total-PCBs	8.6	8.3	2.8	28
PeCB	0.089	0.1	0	0.4
HxCB	0.18	0.4	0	1.2
Total-HCHs	ND	ND	0	0
Total-CHLs	0.63	0.3	0	1.3
Total-DDTs	97	100	30	1100

2.3 Risk assessment of DDTs intake

To estimate the exposure to DDT of the population in Bangladesh, estimated daily intake (EDI) was calculated using DDT concentrations in food items, including breast milk and house dust, collected in this study. DDT intake was calculated based on the assumption that the body weights of adults, children and infants were 50 kg, 20 kg, and 5 kg, respective The food consumption data for Bangladesh are summarized in Table 1 based on the FAO Food Balance Sheet (FAO-STAT 2009; Milton et al. 2006). The ingestions of house dust by adults, children and infants were 0.1, 0.2, and 0.2, respectively. The milk ingestion of infants was assumed to be 700 ml per day (Bouwman, Sereda, and Meinhardt 2006; Oostdam et al. 1999; Wilford et al. 2005). The EDIs of total DDT and *p, p'*-DDT for Bangladesh are shown in (Fig. 2.7). The highest intake of total DDT was found in an infant (7800 ng day^{-1} body wt^{-1} in a high-exposure scenario) and it was was 99% due to breast milk feeding. The remaining 1% was due to house dust ingestion. The EDI for infants was greater than those for children and adults, which were 150 and 110 ng day^{-1} body wt^{-1}, respectively, in the high-exposure scenario. For children and adults, the EDIs of DDTs through meat were 71% and 75%, respectively (Fig. 2.6 and Table 2.13). In particular, the contributions of beef were 69% and 72% for children and adults, respectively (Table 2.13). This was due to the higher concentrations in beef than in other meats (chicken and mutton), even though consumption of meat was not so large (3.56 g day^{-1}) compared to rice (475 g day^{-1}) and fish (42 g day^{-1} for freshwater fish). Fish also accounted for relatively high contributions in adults and children (22% and 28%, respectively). This was due to large fish consumption (42 g day^{-1} of freshwater fish) and relatively high concentrations of DDTs in freshwater fish.

To evaluate the health risks of DDTs, the EDI was compared to the tolerable daily intake (TDI) proposed by the WHO (20,000 ng day^{-1} body wt^{-1}) (Oostdam et al. 1999). The highest EDI of DDTs, found in an infant, was 7800 ng day^{-1} body wt^{-1}, which did not exceed the TDI proposed by the WHO (Figure 2.6). On the other hand, a specific finding for Bangladesh was the relatively high percentage of *p, p'*-DDT detected in food items, breast milk and house dust. To evaluate the health risk of *p, p'*-DDT in this study, the EDI of *p, p'*-DDT was compared to the reference dose (RfD) proposed by the US EPA (500 ng day^{-1} body wt^{-1}) (US-EPA 1987). The highest EDI of *p, p*'-DDT, found in an infant, was 930 ng day^{-1} body wt^{-1}, which exceeded the RfD (Fig 2.7 and Table 2.14). In this study, the highest EDIs of SDDT and *p, p*'-DDT were found in the infant. Infancy is one of the most sensitive stages for environmental contaminants (Bouwman et al. 2012). In vitro studies using human oestrogen receptor showed that *p, p'*-DDT has an estrogenic effect (Rogan and Chen 2005). To reduce the exposure of fetuses and infants to DDT, the EDI of DDT for women of reproductive age should be reduced.

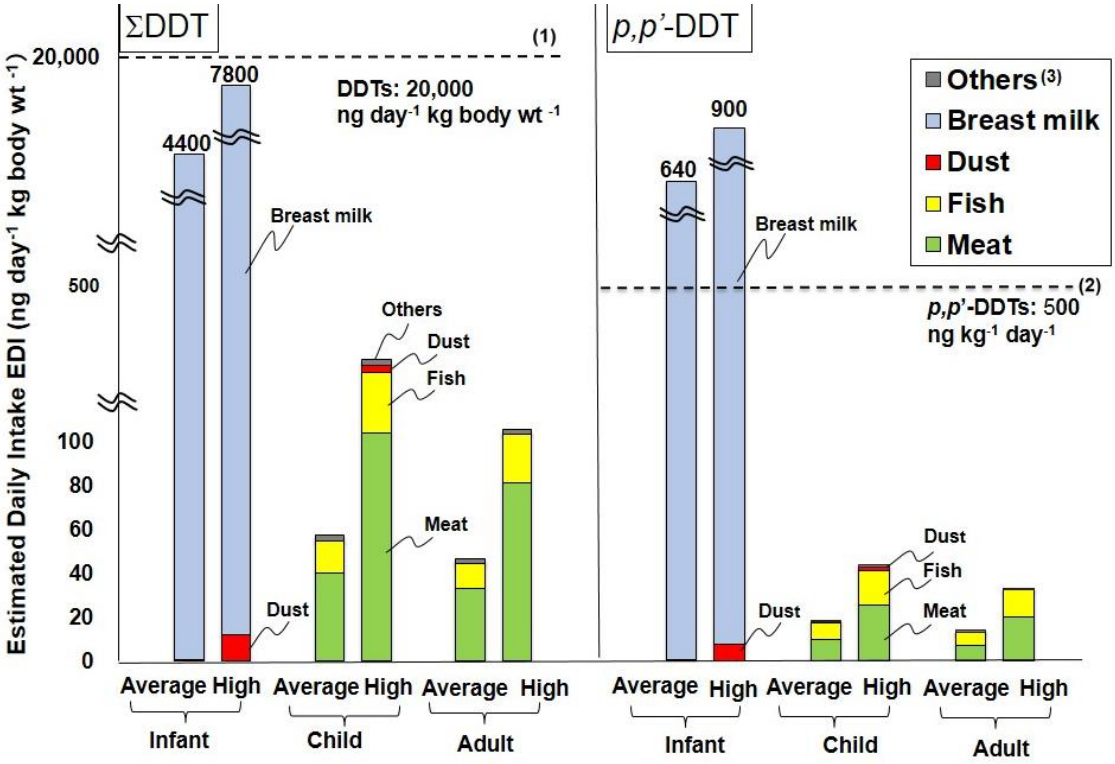


Figure 2.7 Risk assessment of DDTs and *p, p'* –DDT intake.

Table 2.13 DDTs intake on Bangladesh people through food items and house dust

	Adult						Child						Infant					
	Intake (ng day-1)		Intake (ng day-1 kg		Contribution (%)		Intake (ng day-1)		Intake (ng day-1 kg		Contribution (%)		Intake (ng day-1)		Intake (ng day-1 kg bw-		Contribution (%)	
Items	Average	High	Average	High	Average	High	Average	High	Average	High	Average	High	Average	High	Average	High	Average	High
Fish																		
Freshwater	520	1000	10	20	22	19	260	500	13.0	25.0	23	18	0	0	0.0	0.0	0	0
Seawater	52	96	1	2	2	2	26	48	1.3	2.4	2	2	0	0	0.0	0.0	0	0
Brackish	18	18	0	0	1	0	9	9	0.4	0.4	1	0	0	0	0.0	0.0	0	0
Meat																		
Beef	1500	3900	30	78	64	74	730	2000	37	100	63	73	0	0	0.0	0.0	0	0
Chicken	96	78	2	2	4	1	48	39	2.4	2.0	4	1	0	0	0.0	0.0	0	0
Mutton	42	71	1	1	2	1	21	36	1.1	1.8	2	1	0	0	0.0	0.0	0	0
Cereals																		
Bean	6	6	0	0	0	0	3	3	0.1	0.1	0	0	0	0	0.0	0.0	0	0
Rice	47	47	1	1	2	1	24	24	1.2	1.2	2	1	0	0	0.0	0.0	0	0
Vegetable oil	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0.0	0.0	0	0
Dairy products																		
Milk	31	32	1	1	1	1	15	16	0.8	0.8	1	1	0	0	0.0	0.0	0	0
Ghee	7	7	0	0	0	0	3	3	0.2	0.2	0	0	0	0	0.0	0.0	0	0
Butter	16	16	0	0	1	0	8	8	0.4	0.4	1	0	0	0	0.0	0.0	0	0
Breast milk	0	0	0	0	0	0	0	0	0.0	0.0	0	0	22000	39000	4400	7800	100	100
Drinking water	0	0	0	0	0	0	0	0	0.0	0.0	0	0	0	0	0.0	0.0	0	0
Dust	0	5	0	0	0	0	4	61	0.2	3.0	0	2	4	60.5	0.7	12.1	0	0
Total	2300	5400	47	106			1200	2900	58	137			22000	39000	4401	7812		

Table 2.14 *p, p′*-DDTs intake on Bangladesh people through food items and house dust.

	Adult						Child						Infant					
	Intake (ng day-1)		Intake (ng day-1 kg bw-1)		Contribution (%)		Intake (ng day-1)		Intake (ng day-1 kg bw-1)		Contribution (%)		Intake (ng day-1)		Intake (ng day-1 kg bw-1)		Contribution (%)	
Items	Average	High	Average	High	Average	High	Average	High	Average	High	Average	High	Average	High	Average	High	Average	High
Fish																		
Freshwater	330	670	6.6	13.4	41	35	160	330	8.0	16.5	40	33	0	0	0.0	0.0	0	0
Seawater	28	47	0.6	0.9	4	2	14	24	0.7	1.2	3	2	0	0	0.0	0.0	0	0
Brackish	0.15	0.15	0.0	0.0	0	0	0.07	0.07	0.0	0.0	0	0	0	0	0.0	0.0	0	0
Meat																		
Beef	390	1100	7.8	22.0	49	58	200	550	10.0	27.5	50	55	0	0	0.0	0.0	0	0
Chicken	17	46	0.3	0.9	2	2	8.7	23	0.4	1.2	2	2	0	0	0.0	0.0	0	0
Mutton	8.2	14	0.2	0.3	1	1	4.1	6.9	0.2	0.3	1	1	0	0	0.0	0.0	0	0
Cereals																		
Beans	2.8	2.8	0.1	0.1	0	0	1.4	1.4	0.1	0.1	0	0	0	0	0.0	0.0	0	0
Rice	15	15	0.3	0.3	2	1	7.4	7.4	0.4	0.4	2	1	0	0	0.0	0.0	0	0
Soyabean oil	0	0	0.0	0.0	0	0	0	0	0.0	0.0	0	0	0	0	0.0	0.0	0	0
Diary products																		
Milk	2.8	3.5	0.1	0.1	0	0	1.4	1.8	0.1	0.1	0	0	0	0	0.0	0.0	0	0
Ghee	0.67	0.67	0.0	0.0	0	0	0.34	0.34	0.0	0.0	0	0	0	0	0.0	0.0	0	0
Butter	5.6	5.6	0.1	0.1	1	0	2.8	2.8	0.1	0.1	1	0	0	0	0.0	0.0	0	0
Breast milk	0	0	0.0	0.0	0	0	0	0	0.0	0.0	0	0	3200	4500	640.0	900.0	100	99
Drinking water	0	0	0.0	0.0	0	0	0	0	0.0	0.0	0	0	0	0	0.0	0.0	0	0
Dust	0.03	3.245	0.0	0.1	0	0	3	44	0.1	2.2	1	4	3	44	0.6	8.8	0	1
Total	2300	5400	16	38			1200	2900	20	50			22000	39000	641	909		

2.4 Summary

In order to reduce the body burden of DDTs in reproductive age woman in Bangladesh, this study conduct to make clear the major intake route of DDTs on Bangladesh people. Several POPs (DDTs, PCBs, chlordanes, HCHs, HCB, and PeCB) concentrations were quantified in food items, human breast milks and house dust collected from Bangladesh in 2011-2012. Among POPs analyzed in this study, DDTs showed the highest concentration, and it was followed by PCB > PeCBs, > HxCBs. The higher median of DDTs concentrations was found in house dust (30-1100 ng g^{-1} wet weight), and it was followed by human breast milk (20-55 ng g^{-1} wet weight) and meat (1.3- 1100 ng g^{-1} wet weight). Estimated daily intake (EDI) was calculated using DDTs concentrations in food items showed the highest intake of DDTs was found on an infant and it was occupied 99 % by breast milk feeding. In case of child and adult, DDTs intake through beef showed 69 % and 72 % for child and adult, individually. Health risk evaluation was conducted using the highest EDI found in infant. EDI on DDTs was not exceeded TDI proposed by WHO, but EDI on *p, p*'-DDT was exceeded RfD proposed by US EPA.

CHAPTER 3

Dietary Patterns and Serum of DDTs Concentrations among Reproductive Age Group Women in Bangladesh

3.1 Materials and Methods

3.1.1 Study population

This research was conducted in between August 2015 to March 2017 on a randomly selected sample of 40 shop floor employees at a local garment factory in Kashimpur area of Dhaka city as reproductive age women group. The respondents were selected according to an inclusion criterion comprised of three conditions: 1) being an adult woman of reproductive age group, 2) being a resident of the study area for more than 2 years, and 3) absence of any diagnosed chronic disease.

The study protocol was approved by the ethics committees of the Faculty of Agriculture, Saga University in Japan and the Bangladesh University of Health Science (BUHS). The participants were required to sign a consent form before the questionnaire survey. The study purpose, methods, and treatment of personal information were elaborately explained to all the participants.

3.1.2 Questionnaire survey

The first part of the questionnaire was inquired about basic socio-demographic information (age, marital status, parity, education, number of family members, monthly income, duration of residence, drinking water source). The height and weight of participant were measured and BMI was calculated.

The second part of the questionnaire was inquired about the long term dietary habits on a predefined set of 10 food items. A structured food frequency questionnaire (FFQ) was used to collect data on participant's food habits. FFQ has been developed as a valid and reproducible tool to measure long-term dietary intake (Rockett, Wolf, and Acolditz 1995;

Sudo et al. 2004) and this method is more suitable than 24-h recall or 1-day food record for dietary assessment in developing countries because the former is relatively free from underestimation or overestimation (Sudo et al. 2004). Every food items in this FFQ were available at local markets in Bangladesh and it included staple foods (rice and legumes), red meat (beef and mutton), poultry (chicken), fish and dry fish, eggs, vegetables and milk. This FFQ required the participants to recall how frequently they had consumed the 10 food items concerned over the last year with six alternatives: never consumed = 0; 1-3 times/month= 1; 1 time/week = 2; 2-4 times/week= 3; 5-6 times/week= 4; 1 time/day= 5 and 2-3 times/day= 6. All the participants were interviewed by the first author.

3.1.3 Blood sample collection

Approximately 10 mL of peripheral blood was collected into two 10 mL vacutainer tubes containing EDTA, and sent it to a local laboratory for serum extraction by centrifugation. The collected serum samples were frozen and transported to Saga University in Japan to determine DDT concentration in them. Samples were stored at temperature of -30° C until chemical analysis.

3.1.4. Chemical analysis

DDT and its metabolites (*p, p'*-DDT, *p, p'*-DDD, and *p, p'*-DDE) were quantified in this study using method previous reported with slight modification (Haque et al. 2017). A portion of serum sample (3 to 5 g) was grounded with sodium sulfate. The surrogate standard of $^{13}C_{12}$-PCB mix comprised of mono- to deca-CB (Wellington Laboratories Inc.) was mixed with it since $^{13}C_{12}$-hexa-CB shows similar behavior to DDT and its

metabolites in this method. It was extracted using a Soxhlet apparatus with dichloromethane, and the extract was concentrated using a rotary evaporator. The extract was used for determination of crude fat by measuring the total nonvolatile extract. The extract was dissolved in hexane and subjected to pre-conditioned Supelclean™ Sulfoxide SPE 3g (Merck Inc.). The SPE was washed with 6 ml of hexane elution, and it was eluted with 20 ml of acetone. Acetone fraction was dried under nitrogen gas flow and dissolved to hexane. It was subjected to pre-conditioned SPE of Supelclean™ EZ-POP NP (Merck Inc.) and it was eluted with 20 ml of acetonitrile to separate target compounds and lipid. Eluate was micro concentrated after adding internal standard comprised of a $^{13}C_{12}$-PCB-105 and it was employed for quantification by gas chromatograph (Agilent 6890N) coupled with a mass spectrometer (Agilent 5973 inert) using the selected ion monitoring (SIM) mode. HP-5 ms (30 m length, 0.25 mm i.d., and 0.25 μm film thickness) was used for analysis.

Recoveries of $^{13}C_{12}$-PCBs as surrogate standards ranged from 70 to 120%. Actual recovery of spiked experiment also showed ranged from 70 to 120% in p, p'-DDT, p, p'-DDD, and p, p'-DDE and those recoveries were not adjusted for quantification. Quantification of the chemical was conducted by isotope dilution method. DDT and its metabolites were quantified using relative response factor (RRF) of $^{13}C_{12}$-PCB118 to target compounds since their retention time and recoveries have shown the same trend in the results of spiked experiment. The method detection limit (MDL) was calculated as a threefold standard deviation of the background peak (or a threefold standard deviation of the lowest concentration S/N 3 of the authentic standard peak in the case of no background peak) in the procedural blanks (n= 5). The average MDLs of these chemicals on wet weight and lipid weight were 0.5 ng g^{-1} and 50 ng g^{-1}, respectively. Standard

Reference Materials (SRM: fish oil, EDF-2525 provided by CIL) were analyzed (n= 5) using this analytical procedure and the data obtained in this laboratory which was the relative concentrations to certified value and their SD showed acceptable agreement (*p, p'*-DDE: 92±4.7%, *p, p'*-DDD: 105±12%, *p, p'*-DDT: not detected).

3.1.5. Statistical analysis

The statistical analyses were carried out using SPSS (version 22.0), SPSS Chicago, IL, USA. The stepwise multiple linear regression analysis was performed to identify the relationship between serum DDT concentrations and the result of questionable survey. This analysis employed forward selection with backward elimination to select the best subset of predictor variables. The principal component analysis (PCA) was performed to extract the composites of the FFQ items. PCA reduces the data, constructing new variables (principal components/factors) based on linear combinations of the original data. The factors explain as much of the variation in the original variables as possible. In this study, a Varimax Rotation with Kaiser Normalization was applied to enhance the interpretability of the results. Individuals were given factor scores for each dietary pattern.

3.2 Results and Discussion

3.2.1 Characteristics of socio-demographic and dietary pattern

The result of the survey on socio-demographic and food frequency questionnaire (FFQ) from the reproductive age women in the study was summarized (Table 3.1). The characteristics of socio-demographic in the participant in this study was summarized as below. The median age was 26 years (range 19–31 years). The majority of religion was Muslim (74%) and the rest were Hindu. Most of the women were married (82%). The majority of participants were finished their junior high school education level. Their mean monthly income was around 8192 Bangladeshi Taka. The parity was consisted as 54 % of nullipara (a woman who has never borne a child), 18 % of primipara (a woman who had a first child) and 28 % of multipara (a woman who had more than one child). The majority of family member number were four. The mean duration of residency in the factory premises was 4 years.

Table 3.1 Socio-demographic characteristics of the reproductive aged group in Bangladesh.

Socio-demographic factors	*N*	Min	Max	Mean	Median	Std. Deviation
Age	39	19	31	26	26	2.9
Religion*	39	0	1	1	1	0
Marital Status**	39	0	1	1	1	0
Parity	39	0	4	1	0	1.1
Education***	39	1	4	2	2	0.92
Living Duration	39	2	12	4	4	2.7
Family member	37	0	6	4	4	1.4
Monthly Income (Taka)	39	6000	10000	8192	8000	1235
BMI	37	14	27	22	22	2

The results of FFQ survey was summarized as below (Table 3.2). Every participant reported consuming rice 2-3 times a day. There was no variation in rice consumption among the respondents, and hence rice was excluded from the analysis. It was also revealed that Hindu participants had never consumed beef. On the contrary, Muslim participants had consumed beef frequently: 1-3 times/month and maximum 2-4 times/week. This research could not find any other variations in our participant's food consumption pattern on grounds of demographic differences. This observation is supported by the previously reported (Sudo et al. 2004), which found that the food menus of its study communities were confined to a small number of ingredients (except spices) and scarcely changed from day to day

Table 3.2 Characteristics of food frequency questionnaire (FFQ) on the reproductive aged group in Bangladesh.

FFQ	*N*	Min	Max	Mean	Median	Std. Deviation
Rice	39	2-3 times/day	2-4 times/day	2-5 times/day	2-6 times/day	
Beef	39	Never consumed	2-4 times/week	1-3 times/month	1-3 times/month	1.1
Mutton	39	Never consumed	1 time/week	1-3 times/month	1-3 times/month	0.52
Chicken	39	Never consumed	5-6 times/week	2-4 times/week	2-4 times/week	0.94
Fish	39	Never consumed	5-6 times/week	2-4 times/week	2-4 times/week	0.92
Dry fish	39	Never consumed	2-4 times/week	1-3 times/month	Never consumed	0.76
Egg	39	Never consumed	1 time/day	2-4 times/week	1 time/week	1.5
Beans	39	2-4 times/week	2-3 times/day	1 time/day	1 time/day	0.96
Vegetables	39	1 time/week	2-3 times/day	5-6 times/week	5-6 times/week	1.05
Milk	39	Never consumed	1 time/day	1 time/week	1 time/week	1.7

3.2.2 Contamination status of DDT in serum sample

Blood, breast milk and adipose can be good biomarkers to assess human exposure to environmental contaminants (Mueller et al. 2008; Wang et al. 2013). Among those tissues, blood is a feasible biomarker to cover a wide range of ages of both genders compared to breast milk which can only be obtained from the female population and only during lactation. Therefore, blood is considered to be a good matrix in which to assess concentrations of pollutants in the general population (Wang et al. 2013).

DDT and its metabolite concentrations detected in serum samples collected in this study were summarized (Table 3.3). Among the serum samples in this study, only *p, p′*-DDE were detected and the highest concentration was 5300 ng g^{-1} lipid weight basis (median was 330 ng g^{-1} lipid weight basis). Concentrations of *p, p′*-DDT and *p, p′*-DDD in all samples were lower than method detection limit even the result of recovery test of *p, p′*-DDT was good. The reason for it was due to small amount of serum samples and/or lower sensitivity of *p, p′*-DDT on low-resolution GC-MS. Also, several reports have indicated that *p*, *p′*-DDT is mainly metabolized to *p*, *p′*-DDE by dehydrochlorination in mammalian species, insects, and microorganisms, and *p*, *p′*-DDD is an intermediate of the reaction, which may proceed via α-hydroxyl-DDD. *p*, *p′*-DDT and*p*,*p′*-DDE are further oxidized to 2,2-bis(4-chlorophenyl) acetic acid (*p*, *p′*-DDA), the major excreted metabolite in animal (ATSDR 2002). Human body can be Improve of sensitivity is required for farther exposure assessment *of p, p′*-DDT.

To compare the concentrations of DDE in blood samples in this study to those collected in various countries, previously reported were summarized for international comparison (Table 3.4). Median concentrations of *p, p′*-DDE in serum samples of our study were relatively higher than that of serum and plasma samples collected in Mexico (Waliszewski

et al. 2001) Japan (Fukata et al. 2005), Portugal (Cruz, Celeste, and Irene 2003) and Hong Kong (Wang et al. 2013). On the other hand, *p, p′*-DDE levels in this study were lower than in that in previous report of Bangladesh (Zamir et al. 2008), India (Mishra 2011), Romania (Dirtu et al. 2006), China (Qu et al. 2010) and Sweden (Glynn et al. 2000).

Table 3.3 DDT concentrations in serum samples from the reproductive aged group women.

		Wet weight			Lipid weight		
	Lipid%	*p,p′-DDT*	*p,p′*-DDD	*p,p′*-DDE	*p,p′*-DDT	*p,p′*-DDD	*p,p′*-DDE
Detection frequecy%		0	0	100	0	0	100
Mean	1.0	<MDL	<MDL	2.7	<MDL	<MDL	700
Minimum	0.068	<MDL	<MDL	0.28	<MDL	<MDL	18
Maximum	3.2	<MDL	<MDL	10	<MDL	<MDL	5300
Percentile							
25th	0.25	<MDL	<MDL	0.97	<MDL	<MDL	120
50th	0.63	<MDL	<MDL	2.0	<MDL	<MDL	330
75th	2.2	<MDL	<MDL	3.4	<MDL	<MDL	950
90th	2.4	<MDL	<MDL	7.6	<MDL	<MDL	2600
SD	0.95	<MDL	<MDL	2.4	<MDL	<MDL	990

Table 3.4 Comparison of p, p`-DDE levels in human blood samples from different countries.

Country	Samples	Concentration (ng g^{-1} lipid weight)		References
		Mean	Median	
Asian				
Bangladesh	Serum	700	330	This study
Bangladesh	Plasma	1400	1500	(Zamir et al. 2008)
Bangladesh	Serum	-	3600	(Mamun et al. 2007)
India	Whole blood	14000	3900	(Mishra, Sharma, and Kumar 2011)
Hong Kong	Plasma	450	220	(Wang et al. 2013)
China	Whole blood	1900	1600	(Qu et al. 2010)
Japan	Serum	90	93	(Fukata et al. 2005)
Non-Asian				
Sweden	Serum	810	590	(Glynn et al. 2000)
United Kingdom	Serum	-	100	(Thomas et al. 2017)
Portugal	Serum	94	-	(Cruz, Celeste, and Irene 2003)
Romania	Serum	-	2400	(Dirtu et al. 2006)
Mexico	Serum	15	'-	(Waliszewski et al. 2001)

-: no data available

3.2.3 Temporal trend of DDT level in blood from Bangladesh

Concentration of *p, p′*-DDE in serum and plasma samples collected from Bangladesh were summarized (Table 3.4). Two previous studies regarding serum and plasma from adult male and female conducted in 2005 and 2006 (approximately 10 years ago) had reported that *p, p′*-DDE concentrations were 4 to10 times higher in these reports than that in this study (Zamir et al. 2008). Another report which has been conducted in 2008 among children also reported a higher concentration of *p, p′*-DDE than that in this study (Mamun et al. 2007). These results suggested that DDT exposure levels in Bangladesh have been being decreasing over the last decade after use of DDT for agriculture and public health purposes was banned in 1993.

The decreasing trend of DDT concentrations in biomarkers has been also reported in various countries. In Germany, neonatal serum *p, p′*-DDE concentrations have been decreased over time that from a mean value of 1.5 ng ml^{-1} in 1984 to that of 0.18 ng ml^{-1} in 2002 (Lackmann 2005). In Canada, the mean cord blood concentrations of DDTs considerably declined from 1993 to 2000 (Dallaire et al. 2002). In Sweden, (Hardell et al. 2010) observed approximately 10% annual reductions in the concentrations of DDE in their analysis of 392 blood samples collected from 1993 to 2007. In Japan, the concentrations (Konishi, Kento, and Hori 2001) of DDE in human breast milk have been decreased from 1972 to 1998 . In Northern Germany, the concentrations of DDT in human breast milk declined by 10–20% over the decade from 1986 to 1997 (Schade and Heinzow 1998). Recent reductions in the body burdens of DDT have appeared to be a global trend (Kanazawa et al. 2012). It is plausible that decreasing trend of DDE concentration was found in serum samples in this study.

Table 3.5 Comparison of DDE concentration (ng g-1 lipid weight) in blood samples from previous study in Bangladesh.

Location	Year	*N*	Subject	Median	References
Kashimpur	2017	21	Female	400	This study
Savar	2008	8	Teen agers	1000	Linderholm et al. (2011)
Dhaka city	2006	10	Female	1500	Zamir et al. (2008)
Dhaka city	2006	9	Male	2900	Zamir et al. (2008)
Dhaka city	2005	10	Male	3600	Mamun et al. (2007)
Dhaka city	2005	8	Children	3900	Mamun et al. (2007)
Dhaka city	2005	6	Teen agers	2900	Mamun et al. (2007)

3.2.4. Relationship between socio-demographic information and DDE concentrations in serum

To assess the effect of socio-demographic information variables on DDE level in serum, a stepwise multiple linear regression analysis was performed. The values of socio-demographic characteristics and concentrations of *p, p`*-DDE (ng g^{-1} wet weight) were defined as independent and dependent values, respectively. The result of multiple regression analysis was summarized (Table 3.6). Significant correlations were found in parity which was negatively correlated (β= -.979) to the concentration of *p, p′*-DDE in serum. Whereas education level (β= .930) and BMI (β= .347) were significantly and positively correlated. Detail of each result will be discussed below.

Education: This research found that the education level (β= .930) of the reproductive age women was correlated to *p, p′*-DDE concentrations (ng g^{-1} wet weight) in serum. Significant positive correlation was also found between education levels and income in this study ($p < 0.05$, Spearman's rank correlation). This result suggests that the person who has higher education and income in Bangladesh would pay more attention to have rich food which contains higher fat such as meat and/or big fish. Actually, higher DDT concentrations were detected in beef and fatty fish purchased from local market in Bangladesh (Haque et al. 2017). A similar trend was reported in previous studies and it suggested that people who had a higher monthly household income and education level were more likely to consume foodstuff in high nutrients and paid more attention to the quality of meat (Luo et al. 2016).

BMI: Positive relationship between serum *p, p`*-DDE concentration (ng g^{-1} wet weight) and BMI (β= .347) was observed in this study. The people had higher BMI

usually tend to have larger consumption of fatty food such as meat and big fish in Bangladesh. As discussed above in the paragraph of education, fatty food in Bangladesh such as beef and big fish showed higher concentrations of DDT (Haque et al. 2017). Similar result was found in previous studies (Valvi et al. 2012). The influence of BMI on POPs concentration in blood remains controversial that researchers have reported non-significant, positive, or even negative associations between (Arrebola et al. 2012) BMI was positively associated with the adipose tissue concentrations of DDE, which is comparable with the results of some studies (Glynn et al. 2000). A positive association could be expected in relation to prolonged high consumption of fatty food, which is often associated with being overweight and possibly also with a high intake of lipophilic compounds accumulated through the food chain (Vaclavika et al. 2006). Those results in "education" and" BMI" suggest that the controlling of fatty food such as meat (beef) and big fish might reduce the body burden of DDT.

Parity: The stepwise multiple linear regression model showed a negative association between *p, p`*-DDE concentrations (ng g^{-1} wet weight) in serum and parity (β= -.979). The relationship between number of children and *p, p′*-DDE concentration was shown in (Fig. 3.1). Nullipara mothers had significantly higher concentrations of *p, p′*-DDE in serum than multipara mothers. The excretion of lipophilic DDT through breast feeding may decrease the body burden of these chemicals in lactating women (Wolff et al. 2000). The negative association between parity and serum DDT concentration will be ascribed to breastfeeding history (Barraza-Vázquez et al. 2008; Kanazawa et al. 2012; Lee et al. 2007; Luo et al. 2016).

Table 3.6 Result of multiple regression analysis between serum *p, p`*-DDE concentration (ng g-1 wet weight) and socio-demographic characteristics in this study.

	Unstandardized Coefficients		Standardized Coefficients		
	B	Std. Error	*Beta*	*t*	Sig.
(Constant)	-6.1	3.5		-1.7	.090
Parity	-0.98	0.35	-.40	-2.8	.008
Education	0.93	0.37	.36	2.5	.016
BMI	0.35	0.15	.33	2.3	.029

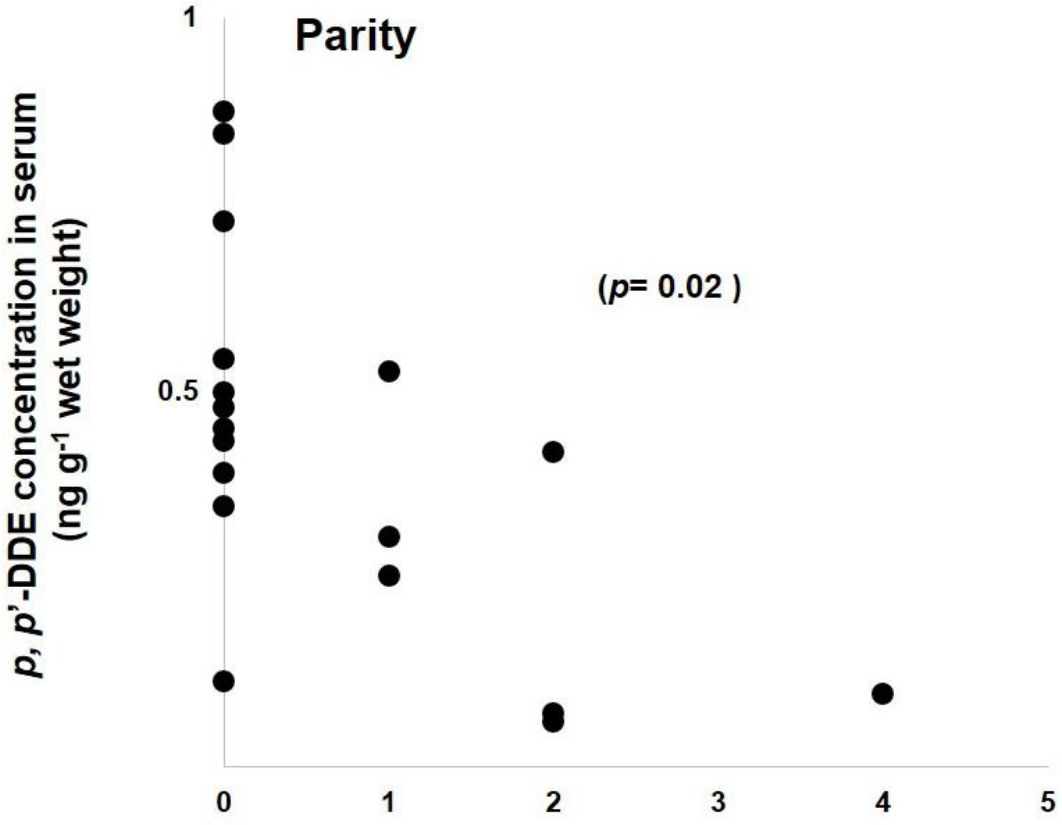


Figure 3.1 Relationship between number of children and serum DDE concentration in the reproductive age group women in Bangladesh (Spearman's rank correlation).

3.2.5 Relationship between FFQ and DDE concentrations in serum

To assess the effect of dietary pattern on *p, p′*-DDE level in serum, a stepwise multiple linear regression analysis was performed. Prior to the regression, the ten food items in the FFQ were subjected to PCA to group the items into dimensions. The PCA identified four factors that best represented the data, given the scree plot of eigenvalues, the interpretability of the factor loadings, and an eigenvalue above 1.2. Extracted factors were defined based on knowledge on local Bangladeshi diet as follows: Factor 1; "frequent consumption", Factor 2; "Muslim predominant", Factor 3;" Hindu predominant" and" less frequent consumption". The constituent food items of each factor with their factor loadings are listed in (Fig 3.2 and Table 3.7).

The result of regression substituting factors obtained by PCA was re-analyzed by multiple regression analysis to find correlation with individual food items and serum DDE concentration. However, none of the food items showed a significant correlation to serum DDE concentration.

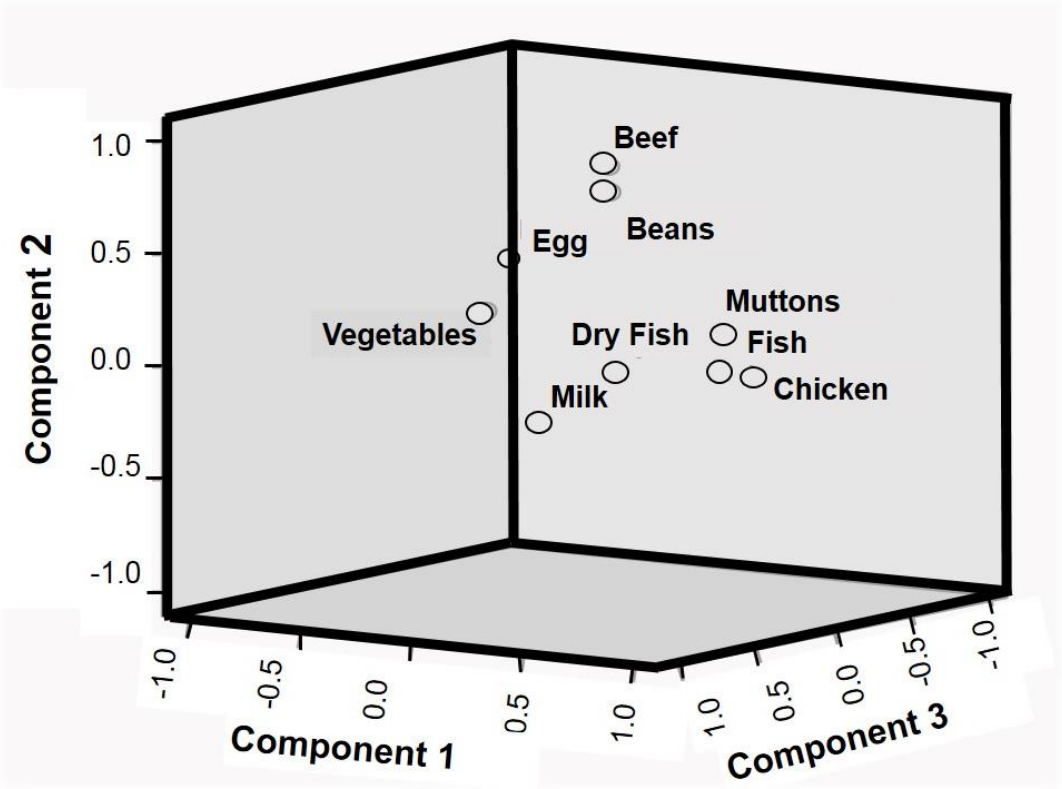


Figure 3.2 Principal component analysis showing typical food eating pattern in Bangladesh among low economic group.

Table 3.7 Factor loading matrix for dietary pattern derived from principal component analysis.

	Component			
Food items	Frequent	Muslim predominant	Hindu predominant	Less frequent
Beef	.032	.83	-.10	-.15
Mutton	.15	.060	-.73	-.14
Chicken	.90	.070	-.08	-.020
Fish	.89	.089	.16	-.0039
Dry fish	.06	-.085	-.12	.91
Egg	.11	.54	.66	-.024
Daal	.10	.74	-.014	.22
Vegetables	-.15	.25	.43	.69
Milk	.25	-.18	.65	-.085

Extraction Method: Principal Component Analysis.
Rotation converged in 6 iterations.

Although no statistical significance was found, a weak positive correlation between *p, p'*-DDE concentration (ng g-1 wet weight) and frequency of beef consumption was observed among the nullipara women sub-group (Fig. 3.3). It seems plausible because higher concentrations of DDT were detected from beef samples among various food collected from the market in Bangladesh in our previous research (Haque et al. 2017), and it was hypothesized that beef could be one of the major intake route of DDT to Bangladesh population. To make clear the effects of beef on DDE concentration in serum, participants in this study was divided into two group as "beef consumer (Muslim)" and "non-beef consumer (Hindu)". "Non-beef consumer" which was 0 frequency showed relatively lower concentrations than "beef consumer" even no statistical significance (Fig. 3.3). It suggests that the control of beef consumption might reduce the body burden of DDT in reproductive age women in Bangladesh. This result can be supported by previous studies that animal origin foods are the main DDTs exposure route to human body (Cao et al. 2011; Nakata et al. 2002). Further research is required on the effects of beef consumption. Regarding fish consumption, no clear trend was found between fish frequency and DDE (Fig 3.3), even it has been reported that fish consumption will be the major exposure route (Zamir et al. 2013). It might be caused that no clear variation in fish consumption frequency (there is no participant of "non-fish consumer") in Bangladesh as same as rice (Table 3.2). The discussion of "education" and" BMI" in this study suggested that fatty food such as big fish can be major intake route of DDT for Bangladesh population. Further research which including the information of fish species, fish length and amount of consumption is required.

It has been suggested that the dry fish was major route of DDTs intake to Bangladesh population because higher concentrations of DDT were detected from it and its consumption frequency reported was almost every day (Mamun et al. 2007). On the other hand, median frequency of dry fish consumption was 0 time/ month in this study (Table 3.2). This result indicates that dry fish might not have much contribution for DDT intake route for Bangladesh population even it has higher concentration of DDT.

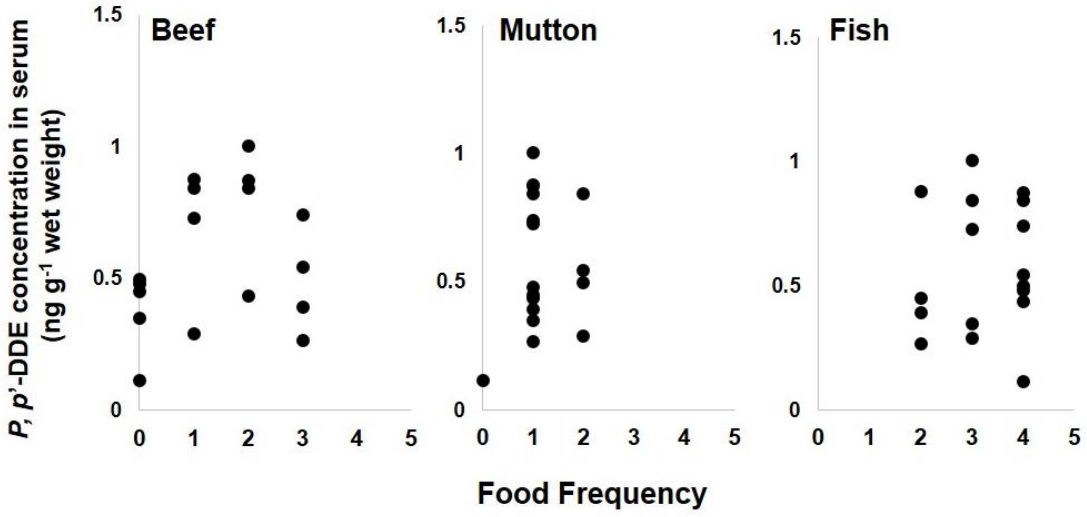


Figure 3.3 Relationship between food consumption frequency and serum DDE concentrations among nulliparous women sub-group.

3.3 Summary

This research was conducted to propose ways to reduce human exposure to DDT, especially women of reproductive age in Bangladesh, and identify a relation between the DDT exposure levels in serum and socio-demographic and food frequency questionnaire (FFQ) information. This study found a significant relationship between the education level and BMI and the serum *p, p′*-DDE concentration. This result suggests that people with higher education (which is related to higher income) and BMI in Bangladesh actively purchase expensive foodstuff, such as meat and/or fatty fish, which is related to a higher fat intake. A weak positive relationship between the serum *p, p′*-DDE concentration and the frequency of beef consumption was observed in the nullipara women sub-group. In a previous study, beef and fish contributed largely to DDT intake in the Bangladesh population. These results suggest that controlling fatty food consumption, such as meat (beef) and marine fish, may regulate the DDT serum levels.

CHAPTER 4

Conclusion and Recommendations

This study proposes the ways to reduce the human exposure to DDT, especially for women of reproductive age in Bangladesh, this research conducted to find the relationship between DDT exposure possible pathways which includes major food items, house dust, drinking water and breast milk for the infant and questionnaire information including socio-demographic and food frequency (FFQ).

The results of this study showed that the EDI of *p, p'*-DDT of infants exceeded the RfD, and the major intake routes of DDT for adults in Bangladesh were beef and fish.

Education level and BMI showed a significant relationship to serum *p, p`*-DDE concentration in this study. This result suggests that person who has higher education (relating to higher income) and higher BMI in Bangladesh would pay more attention to have "rich food" which contain higher fat such as meat and/or big fish. In addition, a weak positive relationship between *p, p'*-DDE concentration in serum and frequency of beef consumption was observed among the nullipara women sub-group.

In this study, beef and fish showed larger contribution on DDT intake of Bangladesh population. Those results suggest that control of fatty food consumption such as meat (beef) and big fish might help to reduce the body burden of DDT.

To reduce the exposure of fetuses and infants to DDT, its intake in women of reproductive age should be reduced. Further research is required to clarify the reasons for the higher levels of DDT in beef and fish in order to take counteractions to reduce the levels of these chemicals in them because beef and fish have nutritional benefits.

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