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Cohort Comparison of Halogenated Hydrocarbons and Chiral Persistent Bioaccumulating Endocrine Disrupting Chemicals in Mother Samples After Delivery

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List of Abbreviation:

***c,t*-CHL**: *cis-, trans*-chlordane; **OXC**: oxychlordane;
HC: heptachlor; ***c, t*-HE**: *cis-, trans*-heptachloroepoxide;
END-1: endosulfan-I (endosulfan-α); **END-2**: endosulfan-II (endosulfan-β);
***p, p´*-DDT**: 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane;
***o, p´*-DDT**: 1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane;
***p, p´*-DDD**: 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane;
***o, p´*-DDD**: 1,1-dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane;
***p, p´*-DDE**: 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane;
***o, p´*-DDE**: 1,1-dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane);
3-MeSO$_2$-DDE: 3-methyl sulfonyl-*p, p'*-DDE;
2-MeSO$_2$-DDE: 2-methyl sulfonyl-*p, p'*-DDE;
MOC: methoxychlor (1,1,1-trichloro-2, 2-bis(4-methoxyphenyl)ethane);
mono-OH-MOC: 2-(p-hydroxyphenyl)-2-(p-methoxyphenyl)-1,1,1-trichloroethane;
HPTE: 2,2-bis(p-hydroxyphenyl)-1,1,1-trichloroethane;
tris-OH-MOC: 1,1,1-trichloro-2-(4-hydroxyphenyl)-2-(3, 4-dihydroxyphenyl)ethane;
HCH: 1,2,3,4,5,6-Hexachlorocyclohexane;
PeCB: pentachlorobenzene;
HeCB: hexachlorobenzene;
OCS: octachlorostyrene;
PCA: pentachloroanisole;
PCP: pentachlorophenol;
PCN: pentachloronitrobenzene;
2,3,7,8- TCDD: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin;
PCB: polychloronated biphenyl

PBT: persistent bio-accumulated toxicant;
EDC: Endocrine disrupting chemical;
DHT: dihydrotestosterone;
E2: estradiol;
DHT: dihydrotestosterone;

ERα, ERβ: estrogen receptor α, β;
ER: enantiomeric ratio(in the disscussion of enantiomeric biotransformation);

FP: Finland placenta; **DP**: Denmark placenta; **DM**: Denmark milk; **FM**: Finland milk;
PC: principal component.

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Summary

Persistent organochlorine pesticides are accumulated over time in human fat tissue and can be transferred to the fetus during pregnancy by crossing the placenta. In the present study, levels of PeCB, HeCB, HCHs, DDT and the metabolites, OCS, PCA, aldrin, dieldrin, *c-, t*-CHL, HC, OXC, *c-, t*-HE, MOC, END-1, –2 and mirex were determined in the lipid phase of homogenized placenta and breast milk samples (placenta 112 and milk 65 from Turku, Finland, placenta 168 and milk 65 from Copenhagen, Denmark). Anti-androgenic *p, p'*-DDE was a dominant pollutant in all samples; other major pollutants were β-HCH, HeCB, END-1, dieldrin, OXC, *c*-HE and *p, p'*-DDT, which were all weak EDCs. No sample was negative for the 8 abounding compounds and they counted 89%, 95%, 98%, and 98% of the all investigated compounds in FP, DP, FM, DM, respectively. The total amount of the 8 compounds changed from 9.76 to 293.9ng/g in placenta and from 42.93 to 524.49ng/g in milk samples. PC analysis showed a general simultaneously increase for the 8 compounds. Comparing the two cohorts, Denmark samples, which have lower levels of lipid in placenta and milk, contained higher level of the investigated pollutants in placenta (1.73 times) and milk (1.82 times) than the corresponding Finland samples, with emphasizing the more higher contaminated samples in Denmark cohort than in Finland ones. The concentration correlations for the 10 compounds (*p, p'*-DDE, β-HCH, HeCB, dieldrin, END-1, OXC, *c*-HE, *p, p'*-DDT, mirex and OCS) between 42 paired Finland milk and placenta samples confirmed the transplancental contamination. The contents of these lipophilic pollutants in milk were 1.5 to 3.0 times higher than in placenta. PC analysis also suggested that there might be common exposure patterns for the two cohorts, respectively. The two common patterns, which might imply common exposure sources or paths for the two cohorts, are similar but with small differences in the content of END-1 and OXC. Interestingly, the END-1 and OXC contents were linearly correlated in DM, DP, FM, FP and pooled milk and placenta samples, respectively, which might reflect a source correlation of the two pollutants for most investigated samples. Two Finland samples have abnormally high levels of HCH with isomers ratios near to the technical mixture (one sample combined high levels of PeCB and δ-HCH) but the other investigated compounds were in the normal ranges, which might suggest a strong HCH contact of the mothers. The chiral exposure information expressed by *c*-HE

and α-HCH might suggest a different recent uptake level for the related pollutants. Additionally, the less estrogenic active (+)-*o, p'*-DDT were the dominant residual compared to its counterpart. The residual levels for most investigated compounds were much lower than the *in vitro* test levels (usually from several to hundreds nano-mole per liter, which might be equal to the contaminated levels of 10, 20 or even 30 years ago in these two countries) to introduce endocrine disrupting function. However, the data will be used in the following case-control studies as a whole. Considering the combined effects even at low levels, the metabolisms and the other unpredictable factors *in vivo*, these pollutants should raise concern for the reproductive organogenesis and function, especially for the contaminated heavily mothers.

1-1: Introduction

1-1-1: Research background

Environmental factors such as smoking, diet, and pollutants play a role in most human diseases. New molecular evidence indicates that specific people, who are characterized by predisposing genetic traits, children, or females, may have elevated risk from certain exposures (Perera, 1997). Therefore, there has been an increasing focus on environmental risk assessment of children (defined to include humans from conception through organ maturation, i.e. in adolescence) as a potentially susceptible population (Daston et al., 2004) in recent years. The possibility of these environmental factors expressed as diseases for boys and girls, such as testicular dysgenesis syndrome (Skakkebaek et al., 2001) and polycystic ovarian syndrome (Battaglia et al., 2002), have being concerned. The particular concern of environmental factors is human exposure to the persistent organic pollutants (POPs), the synthetic chemicals with unique and harmful characteristics. These long-lived compounds, because of their affinity for body fat, can build up to very high levels in human body along the food chains. In summary, they are PBTs, the "worst of the worst" in toxic substances. PBTs are ubiquitous toxicants and everyone has a body burden that their ancestors never had. Some of them have (had) been widely and extensively used as pesticides in worldwide with little or no regulatory control, while others are industrial chemicals or unintended by-products of industrial processes and incineration (Smith et al., 2002).

The exposure to PBTs is a global problem. Firstly, when released into the environment, PBTs can be transported on air currents to places far from the points of their origin by three stages: evaporation, transport in the atmosphere and condensation at lower temperatures (Blais et al., 1998). This phenomenon is so called the "grasshopper effect." Because evaporation is minimal in colder regions, they tend to build up in arctic and mountain ecosystems. Secondly, ocean currents and rivers which funnel agricultural runoff and industrial discharges to the ocean are also important transport pathways. Thirdly, long distance transfer is also possible through animals, which act as bulk-transfer vectors when they migrate. One of the examples is spawning salmon

dump toxic industrial compounds in Alaskan lakes on their return from the ocean and the amount of PCBs transported by sockeye salmon to these lakes is greater than the traditional assignment from atmospheric pathways (Krümmel et al., 2003).

The levels of PBTs tend to be the highest in species at the top of the food chain, such as eagles, polar bears and killer whales. This is so called biomagnification (Beek, 2000). For example, in marine ecosystem, contaminant can move from phytoplankton (single-cell micro-organism) to zooplankton (multicellular organisms), to small fish, to crustaceans and shellfish, and to larger fish and sea mammals (Smith et al., 2002). Also PBTs can bioaccumulate in human body by agricultural food chain represented by grass, milk cows, and beef cattle and aquatic food chain represented by zooplankton, planktivorous fish, and piscivorous fish (Czub et al., 2004) because of their lipophlic prosperities. The accumulated level of persistent trace chemicals in fish or other animals can pose a significant ecological risk, also to human health.

Many PBTs are confirmed as EDCs, chemicals that can lead to an increase in birth defects, sexual abnormalities and reproductive failure, which are suspected to associate with a wide range of biological effects in the environment on wildlife and human including carcinogenic effects, reproductive effects, neurological effects and immunological effects (Kavlock et al., 1996). Sometimes, they are called xenooestrogens because of the oestrogenic activity. Endocrine disruption refers to the interference of endocrine system function, which transporting information through the circulation from on tissue to another via chemical messengers or hormones (Witorsch, 2002b). Epidemiological data suggested an increase of disorder of the male reproductive tract in the last decades and clinical and experimental studies show the exposure of excess of oestrogens may cause such disorders (Hosie et al., 2000). Although they express only weak oestrogenic effects, these EDCs can accumulate in human body up to a high level and then pose a risk to normal hormones' function.

1-1-2: EXPORED project and the present study

The linking of specific exposures to specific effects in environment would often be difficult

because of the complexities of exposure, the latency of the effects, and the subtle nature of the outcomes. The confirmation of endocrine disrupting hypothesis will heavily depend on application of Hill criteria (strength of the association, presence of a dose-response relationship, specificity of the association, consistency across studies, biological plausibility, and coherence of the evidence) (Kavlock et al, 1996). Additionally, the potential adverse effects at low-dose or inverted U effects, the combination effects of mixture EDCs (Payne et al., 2001; Witorsch, 2002ab, Silva et al., 2002) and the extrapolation of dose in animals to exposure in human ask us pay attention to treat the exposure outcome relationships in human exposure risk assessment.

As one part of the research project EXPORED, which is concerning of the exposure-outcome relationships in male urogenital malformation with special reference of EDCs, we investigated some important organochlorine hydrocarbons and pesticides in mother samples after the delivery used for the further case-control study. The earlier cohort studies in Denmark and Finland showed the two countries with different incidences of male urogenital disorders, such as cryptorchidism and hypospadias (Toppari et al., 1996). The boys with malformations had been diagnosed at birth and followed up to 18 months together with matched controls. Biological samples had been systematically collected during these studies for exposure assessment. Comprehensive endocrine evaluation of the children had also been performed and a large database had been created on the basis of questionnaires. The aim is to combine these databases with the exposure data of endocrine disrupters to assess their roles in the formation of male urogenital malformations. The mother exposure data to PBTs, such as PCDD/Fs, PBDEs, PCBs, phthalates, alkylphenols, bisphenol A and halogenated hydrocarbons and selected pesticides, will be used to make a risk analysis combining the other database. It is expected to answer the crucial question: whether any of the exposures to endocrine disrupters is associated with altered incidence of urogenital malformations. Our work focus on halogenated hydrocarbons and selected pesticides, some of them have been identified as potential or might be EDCs (Lyons, 2000), for examples, *p, p'*-DDE is a potent antiandrogen and HeCB induce enzyme that hydroxylate testosterone (Toppari et al., 1996). DDT, CHL, HC, aldrin, dieldrin, mirex, and HeCB (it is both a pesticide and an industrial byproduct) are the 7 of the 'dirty dozen', which are the first time to seek the worldwide elimination in July 1998 by a negotiating committee of the United Nations Environmental Programme. β-HCH, CHL, DDT,

dieldrin, END, HeCB, lindane, MOC have been identified as potential endocrine disruptors in the list produced under the auspices of the Oslo and Paris Commissions. Similarly, aldrin, CHL, DDT (DDE, DDD), MOC, dieldrin, α-HCH, γ-HCH, OXC, HC, HE and mirex are also part of insecticides listed by World Wide Fund for Nature in the environment reported to have reproductive an /or endocrine disrupting effects. Some of the pesticides are chiral compounds. Their paired enantiomers have the same physical and chemical properties. When released, the transport processes (leaching, volatilization, and atmospheric deposition) and abiotic reactions (hydrolysis and photolysis) do not affect enantiomeric ratios (ERs). However, their bioaccumulating, biodegradation processes are usually chiral related processes. Especially, biotic reactions lead to nonracemic residues and an alteration of the original ERs. Our interest also refers to enantioselective degradation and accumulation processes of these chiral PBTs in human body by the detection of their ERs. α-HCH, *o, p'*-DDT, *c*-HE are some examples (Bidleman et al. 1999). This technique could provide a sensitive indicator for biodegradation, bioconcentration, and transport cross cell membrane, and also might be the clues about the origins of pesticides found in the samples.

1-2: Prenatal and postnatal exposure to PBTs from mother

1-2-1: General trends and some factors affect the levels of PBTs in mother samples

One of the widely used biomarkers for evaluation of human body burden to PBTs is breast milk since it's easy collecting, enriching of lipophilic compounds and exposure to suckling infant (Smith, 1999). The first evolution of human milk for PBTs contamination occur in 1951 when 32 non-occupationally exposed black women were surveyed in Washington, DC, 30 of whom had detectable levels of DDT with a mean value of 0.13 ppm (Laug et al., 1951). After more than a decade, many reports have been published on the levels of PBTs in breast milk. DDT concentrations in human milk have declined in most area of the world, from 5000-10 000 μg DDT/kg milk fat to around 1000 in 1999 in many areas. Nevertheless, levels can be high in areas still using DDT, ever higher than the WHO's recommended limit for infants (Smith, 1999). Also there is a declined trend for other PBTs such as HCH, dieldrin, CHL and relative compounds, and PCBs because of the ban or regulating throughout many global areas (Smith, 1999; Waliszewski et al., 1998; Noren et al., 2000; Solomon et al., 2002). Comparing the change of *p, p'*-DDE by reduced consumption of local fish and elevations of mirex from 1986-1992, HeCB showed no difference at any point due to the ban in Mohawk women milk (Fitzgerald et al. 2001), which might confirm its continuous release as industry by-products (van Birgelen, 1998). Although a decline of the time trend of PBTs in human samples, the exposure levels still can be detected easily because of their persistence.

Two processes, bioaccumulation in adipose tissue and excretion to breast milk, are the result of PBT levels in milk (Czaja et al. 1997). However, many factors like age of mother, parity, length of previous lactation and sampling method might affect the investigated levels (Harris et al. 2001). For example, the predictors of plasma concentrations of DDE and PCBs investigated in a group of 240 USA women showed the most reliable predictors of DDE were age and serum cholesterol, and the most important predictors of PCBs were age, serum cholesterol, and residence in the Midwest

or Northeast (Laden et al., 1999). For general people, diet should be one of the major factors that influence the exposure levels of PBTs, with patterns in fish consumption playing a particularly significant role (Solomon et al., 2002). Despite of some negative results (Rauhamaa-Mussalo et al., 1988), mothers with contaminated fish consumption exhibited a higher level of PBTs (Kostyniak et al., 1999; Stewart et al., 1999; Laden et al., 1999; Harris et al., 2001). For example, Mohawk mothers (Fitzgerald et al., 2001), with the greatest estimated local fish consumption, had a significantly higher geometric mean of *p, p'*-DDE level in milk than that of the control women, but no differences in mirex or HCB concentrations. Following the recommendation against the consumption of local fish by pregnant and nursing Mohawk women, the reduction of *p, p'*-DDE levels from 1986 to 1990 in Mohawk women breast milk was found. Great Lakes fish consumption was also associated with increased blood plasma PCB levels in men and mirex levels in both men and women. Waterfowl consumption was associated with higher plasma PCB (men and women), DDE (men only), and mirex levels (men and Cornwall women) (Kearney et al., 1999). The age of the mothers positively correlated with the PBTs levels (Covaci et al., 2002; Rauhamaa-Mussalo et al., 1988; Brunetto et al., 1996; Czaja et al., 1997) because of the longer exposure time and the possible higher daily uptake for the older mothers. Some reports showed that PCBs and DDE were significantly negatively related to both parity (Nair et al., 1996) and duration of lactation (Nasir et al., 1998; Kostyniak et al., 1999) and some showed no correlations (Czaja et al., 1997). It might be due to the correlation studies attributed to the simultaneous operation of many factors, such as age, number of deliveries, body weight, dietary habits, and sample collection time (Czaja et al., 1997; Harris et al., 2001). For example, *p, p'*-DDE in maternal adipose tissue was positively correlated with *p, p'*-DDE in cord blood ($P = 0.0001$) and breast milk ($P<0.0001$) and marginally correlated with change in body mass index (BMI) ($r = -0.03088$; $P = 0.06$) (Dorea et al., 2001). Historical and current local use patterns might be another reason for of the differences.

1-2-2: PBTs incorporate with fetus (infants) from mother via placenta and milk

The very high fasting triglyceride levels (Haggarty, 2002) suggest that maternal body fat has higher turnover during pregnancy than in the non-pregnant state, with the total lipid increasing in

maternal blood from 6.17 to 9.0 g/l (DeKoning et al., 2000). Except the instant nutrition supply to fetus, the relatively high ratio of milk energy preparation also demands a cycle of energy storage during pregnancy. In situations of low-energy supply, the stored lipid can be critical for the establishment of lactation and maintenance of maternal health (McNamara, 1999). All these may lead to the release of lipophilic contaminants from the adipose tissues into maternal blood in similar situation with their release into plasma in response to subcutaneous adipocyte basal lipolysis (Chevrier et al., 2000; Imbeault et al., 2001; Charlier et al., 2002). The release of β-HCH in response lipolysis of fasting can be in quantities sufficient to stimulate estrogen target uteri of ovariectomized mice (Bigsby, 1997). Although placenta is a predominantly non-fatty tissue, the difference of total lipids between cord blood (3.47 g/l serum) and maternal blood (9.0 g/l serum) (DeKoning et al., 2000) forms a gradient between the two sides of the placenta membrane resulting in PBT diffusion. For the production of milk, the human body employs approximately 75% of endogenous fats during pregnancy (Waliszewski et al., 1999), which might also result in the release of PBTs from the adipose tissue stores.

The common materials used to investigate the prenatal exposure to PBTs are maternal blood, cord blood, placenta, and even amniotic fluid (Foster et al., 2001; 2002). Although not quite clear for the mechanism, all researches confirmed the transplacental transfer of PBTs. It was suggested these xenobiotics travel with the lipid components of blood, such as very low density lipoprotein (VLDL), low density lipoprotein (LDL), high density lipoprotein (HDL), and chylomicron remnants, and likely gains access the many tissue compartments of the human body by utilizing the lipoprotein lipase mechanism (Dekoning et al., 2000). For examples, Aroclor 1254 increased hepatic lipid synthesis, but decreased hepatic production of albumin and apolipoproteins in pigeon (Borlakoglu et al., 1990a). Mice or Sprague-Dawley rat's administration and *Ah* receptor and 4S protein *in vitro* incubation suggested 2,3,7,8-TCDD was bound to lipoproteins, which subsequently underwent rapid and pronounced degradation processing, possibly catalyzed by lipoprotein lipase, to heavier entities. At the highest doses of xenobiotic administered, an almost complete disappearance of lipoprotein particles was observed (Souès et al., 1989).

It used to be thought that the placenta created a perfect bulwark against toxic exposure and thus

came the term 'placental barrier'. In fact, the placenta sorts out chemicals primarily on the basis of molecular weight, electrical charge, and lipid solubility. Small, neutrally charged molecules that readily dissolve in fat are afforded free passage regardless of their capacity for harm. Although the early suggestion that placenta restricts the transformation of PBTs to some extent (Eckenhausen et al., 1981), the followed researches (Saxena et al., 1981; Rosival et al., 1983; Cariati et al., 1983; Ando et al., 1985) all suggested the transfer of PBTs to fetus through placenta. Most of the results come from the comparison of PBT levels between maternal substance and placenta or cord blood, some data involved fetal tissues (Bosse et al., 1996; Waliszewski et al., 2000).

Paired sample analysis, especially using maternal blood *vs.* cord blood, is in principal a direct method to test potential transfer of persistent pollutants through the placenta. Many investigations found the significant correlation of PBTs, such as PCBs, PBBs, HeCB, HCHs and DDT with related compounds, between paired blood samples (Jacobson et al., 1984; Waliszewski et al., 2000; Covaci et al., 2002; Dallaire et al., 2004). For example, *p, p'*-DDE concentrations of the mean venous blood (7.12 μg /g) and cord blood (6.39 μg /g) were not significantly different, but were significantly correlated (Dorea et al., 2001). A more recent work showed not only lipophilic PCBs, but also their more polar metabolites OH-PCBs, can be transfered across the placenta to the fetus (Soechitram et al., 2004). A more broad list of compounds, from β-HCH, *p, p'*-DDE, *p, p'*-DDT, HeCB, mirex, OXC, trans-nonachlor, PCB 99, 118, 138, 153, 156, 170, 180, 187 and Aroclor 1260 to Parlar 26, 50 and total toxaphene, had been shown to correlate between maternal blood *vs.* cord blood samples (Walker et al., 2003). Generally, maternal serum levels were higher than cord serum levels for PBTs mightily because of higher lipid content (DeKoning et al., 2000) in maternal than in cord serum.

Apart from maternal blood *vs.* cord blood, a broad type of maternal-fetus paired samples, such as placenta *vs.* serum (Schlebusch et al., 1994), placenta *vs.* cord blood (Ando et al., 1985) and cord blood *vs.* milk (Jacobson et al., 1984; Stewart et al., 1999) had been used to test the PBTs incorporation to fetus. For example, both cord serum and maternal milk levels of PCBs and PBBs were examined in relation to maternal serum levels (Jacobson et al., 1984). A significant linear correlation had been found between the HeCB concentration in placenta and that in cord blood

(Ando et al., 1985). However, the linear correlations did not always exist for all investigated compounds. Cord blood *vs.* milk analysis show that blood PCB homologues of light (Cl 1-3) or moderate (Cl 4-6) chlorination did not correlate with their breast milk homologues, the most persistent and heavily chlorinated PCB homologues (Cl 7-9) were significantly and positively correlated with breast milk levels (Stewart et al., 1999). Although no significant correlation between maternal serum and placental concentrations was observed, the placenta / serum ratios of HCH isomers, HeCB, total DDT, total PCB with 0.48, 0.99, 0.45, 0.32 show the incorporation of these PBTs into fetus (Schlebusch et al., 1994). 6 types of tissues (adipose tissue, predelivery blood, cord blood, placenta, postpartum blood and breast milk of 1 or 2 months after delivery) had been analyzed to investigate the partitioning of dioxins, dibenzofurans and the dioxin-like coplanar PCBs. No correlation was found probably because only 5 samples had been included. However, the mean measured values, PCDD, PCDF and coplanar PCB with 352, 526, 182, 165, 352, 220 pg/g lipid for adipose, pre-blood, placenta, cord blood, post-blood and breast milk, show after the delivery, the burden levels of blood for all pollutants (or TEQs) are descending (Schecter et al., 1998). All these findings suggest the existence of a dynamic equilibrium of PBT among human tissues through blood circuit (Fig.1-2-2 (1)).

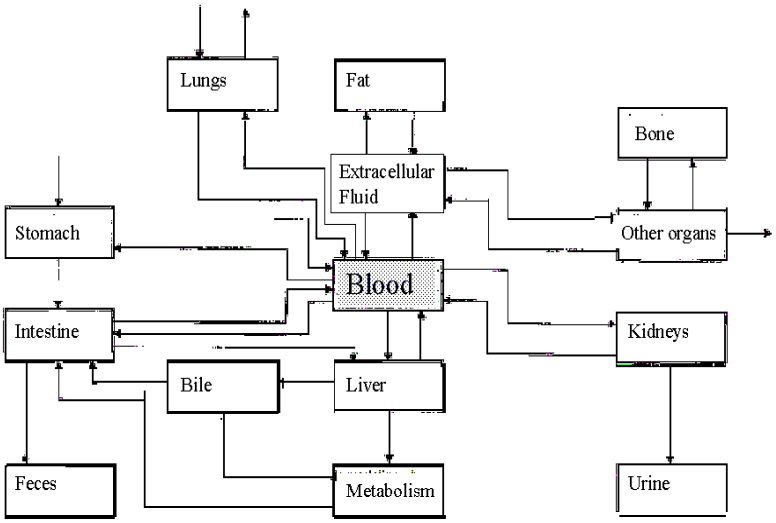


Fig. 1-2-2 (1): Distribution of PBTs among body through blood circuit (*Toxicology: principles and applications*, *CRC Press*)

Despite of the dynamic equilibrium potential, there might be many factors that affect the correlation of PBTs between the paired samples. Firstly, PBT distribution might be lipid type related. Comparing the lean charr (the most tissues and organs had a substantially lower

triacylglycerols but only a slightly lower phospholipids and cholesterol) with the fat charr (Jorgensen, et al., 1997), the tissue concentration of OCS was positively correlated with the concentration of triacylglycerols and negatively correlated with phospholipids and cholesterol. The proportion of the total body burden of OCS deposited in extra-adipose tissues was higher in the lean charr (28%) than in fat charr (4 times higher in brain of the lean charr than in fat charr). Also the partitioning of PBT pesticides between adipose tissues and serum might relate to the variation of lipid content in serum due to the association of PBTs with the lipid (lipoproteins) (Waliszewski et al. 1999). The congener specific distribution of PCBs in plasma fractions is more complex than can be explained solely by their solubility in the lipid components of plasma fractions (LDL, HDL and the lipoprotein-poor (predominantly albumin) fractions), and may suggest a complex association with apolipoproteins and plasma proteins that are important in transporting PCB to tissues (Borlakoglu et al. 1990b). Also DDT and HCH showed a positive correlation between paired breast milk and maternal serum while no correlation could be established either between breast milk and cord serum or maternal serum and cord serum (Nair et al., 1996). The fasting effect suggested there was different mobility for β-HCH and *o, p'*-DDT from fat depots by lipolysis in animals (Bigsby et al., 1997). An altered hormonal status, a different degree of metabolic activity and an increased deposition of fat in the breast during pregnancy perhaps favored degradation and selective partitioning of a few metabolites from the blood serum to the breast. It might explain that the light (Cl 1-3) or moderate (Cl 4-6) chlorinated PCBs in cord blood did not correlate with their breast milk homologues, the most persistent and heavily chlorinated PCB homologues (Cl 7-9) were significantly and positively correlated with breast milk levels (Stewart et al., 1999). The detected α-HCH in the similar level with *p, p'*-DDE but could not detected β-HCH in the non-fatty amniotic fluid (Foster et al., 2001; 2002) suggested the sample status responded to the variation of PBT patterns. The variation of reliability in the measurement of compound levels (Jacobson et al., 1984) and some other unpredictable factors might also cause the deviation from the dynamic equilibrium hypothesis.

The children serum levels of PBB, PCB and DDE increasing with months of breast-feeding (Anderson et al. 2000) indicated breast milk as the main source of these pollutants after the delivery. The paired analyses of adipose tissue *vs.* mature milk (colostrums *vs.* mature milk)

indicate a high degree of coherence, principally of total DDT; suggest lactation as a more effective decontamination means than through placenta (Waliszewski et al., 2001), especially, for primigravidae mothers (Nair et al., 1996). Other study also reported that breast milk was the main source of pesticide contamination to the newborn because the levels of maternal serum and cord serum are nearly equal but much lower than breast milk (Nair et al., 1996). Factually, the lipid content of the fetal tissue was 0.65% (8-14 week) much less than the amount of usually present in adults, which is generally from 15 to 30% of the body weight. That means the fetus has low potential to store PBTs. The estimated body burden of mother (from milk), placenta (lipid content 0.85%) to fetal tissue are 16.7, 10.1 to 5.3 TEQ (ng/kg lipid) for each (Schecter et al., 1996). Although a lower burden, transplacental exposure might be more relevant with regard to physical development and cognitive functioning of the child than postnatal exposure via breast milk (Przyrembel, et al., 2000).

1-3: PBT exposure, EDCs and human reproductive health

1-3-1: PBT exposure and human reproductive health

Human health and human disease result from three interactive elements: environmental factors, individual susceptibility, and age (Perera, 1997). Children's responses to environmental toxicants will be affected by toxicokinetic factors (such as their systems absorb, distribute, metabolize, and excrete chemicals) and toxicodynamic factors (inferred toxic mechanisms and mode of action). These vary during development, in utero where maternal and placental processes play a major role, to the neonate in which emerging metabolism and clearance pathways are key determinants (Ginsberg et al., 2003; Daston et al., 2004).

Scientifically sound documents underpin that environmental chemicals are capable of acting as endocrine disrupters in laboratory. However, clinical data linking the environmental exposure levels and the present human adverse reproductive outcomes, such as early pregnancy loss, birth defects, reduced birth weight, and altered functional development is limited (Feldman, 1997; Johnson et al., 2000). The scientific community awaits further epidemiological assessment. However, one of the major limitations in environmental studies is the use of crude exposure quantification. Surrogate exposure measures, such as time living in a contaminated area (Dolk et al., 1998), do not adequately reflect the true nature and / or degree of real exposure to the chemical substance(s) of interest. Also, as they are no quantitative measures of absorbed dose, the data cannot supply numerical measures of a dose response relationship, limiting their use in quantitative risk assessment (Sim, 2002). On the other hand, people exposed to many pollutants, which have been documented to be EDCs, and surrogated measure might not reflect the real xenoentrogenic exposure personally. Additional testing systems are therefore required to screen for estrogenicity and to identify appropriate biomarkers of human exposure (Rivas et al., 2001). The time of sample collection is also important because of the possible transient chemical exposure and the sensitive period of reproduction organ development (Kelce et al., 1997; Sultan et al., 2001). For example, by evaluating the incidence rates of acute infections with prenatal

exposure to PCBs and DDE, the researchers suggested that there is a possible association between prenatal exposure and not postnatal exposure to these compounds and the acute infections in early life in the investigated Inuit population (Dallaire et al., 2004). The association between PBTs and the higher levels of total IgE in newborns (with higher allergic sensitization) supported the higher incidence rate of atopic eczema cases in the industrial region (Reichrtová et al., 1999).

Two large outbreaks of PCB poisoning occurred in Asia showed that women pregnant at or after the exposures had children who were developmentally impaired (Tilson et al., 1990). Women's occupational exposure can affect hormone level and might suggest the direct reproductive endocrine disrupting action to fetus during pregnancy, for instance exposure to aliphatic hydrocarbon correlated with lower preovulatory luteinizing (Reutman et al., 2002). The lifetime exposure to PCBs of maternal but not paternal by consumption of contaminated fish may reduce fecundability among couples attempting pregnancy (Buck et al., 2000). However, TCDD is unlikely to increase the risk of low birth weight or preterm delivery through a paternal mechanism (Lawson et al., 2004). These might suggest maternal exposure is more crucial to children health. But the recent report referred paternal exposures as potential factor of cryptorchidism (Pierik et al., 2004). Also based on self-reported parental pesticide application, an increased risk of cancer was supported among children whose fathers did not use chemically resistant gloves compared with children whose fathers used gloves (Flower et al., 2004).

Many paediatric diseases have been suspected with the environmental factors. Children exposed transplacentally to PCBs' levels considered to be background have hypotonia and hyporeflexia at birth, delay in psychomotor development at 6 and 12 months, and poorer visual recognition memory at 7 months in the USA (Tilson et al., 1990). The general PCB exposure, measured by both contaminated fish consumption and cord serum PCB levels examined during the immediate postpartum period, had been shown to predicate lower birth weight and smaller head circumference (Fein et al., 1984). However, another report (Gladen et al., 2004) showed that the exposure to *p, p′*-DDE is correlated with the increase of height and weight in adolescent boys. The recent epidemiological risks based on biological measures (chemical analysis combined with bioassay) showed that the moderate exposure of polychlorinated aromatic hydrocarbons (PCB 138,

153, and 180 level in serum and dioxin-like compounds by chemically activated luciferase expression assay) might interfere with sexual maturation and in the long run adversely affect human reproduction (Den Hond et al., 2002). Case-control investigation of 117 male schoolchildren (10–19 years of age) lived in a more than 20 years END aerially sprayed region found that sexual maturity rating (scoring for development of pubic hair, testes, penis, and serum testosterone level) was negatively related to aerial exposure to END (using serum END levels) and serum luteinizing hormone levels were significantly positively related to serum END levels after controlling for age. Further they suggested that the prevalence of congenital abnormalities related to testicular descent among study and controls subjects was 5.1% and 1.1%, respectively, might (not significantly) correlate the long-term END exposure (Saiyed et al., 2003). Although only the concentration on lipid basis of *cis*-nonachlordane was significantly increased in testicular cancer cases, the case mothers showed significantly increased concentrations of the sum of 38 PCB congeners, HeCB, *trans*- and *cis*-nonachlordane, and the sum of CHLs (61 cases 58 age-matched controls) (Hardell et al., 2003). Recent case-control study based on the interview information (pregnancy aspects and personal characteristics, lifestyle, occupation, and dietary phyto-oestrogen intake of both parents) showed, apart from small-for-gestational age for hypospadias, and preterm birth for cryptorchidism, paternal pesticide exposure was significantly associated with cryptorchidism and paternal smoking was associated with hypospadias in male offspring (Pierik et al., 2004). However, to reach the dose-outcome relation of EDCs exposure with adverse effects like in lab (Ulrich et al., 2000) might need more detailed information including multiple routes of exposure; the timing, frequency, and duration of exposure; need for qualitative and quantitative data; sample collection and storage protocols; and the selection and documentation of analytic methods (Rice et al., 2003).

1-3-2: Debate on risk assessment of PBTs as EDCs:

PBTs acting as endocrine disruptors is harmful to human health is still hypothetical since no data yet proving a causal relationship. The critical argument is that humans are exposed to relatively higher levels of natural EDCs than xeno-EDCs (Safe 1995). Species-specific estrogenic action is another concern (Spearow et al., 1999; Witorsch, 2002b) of the extrapolation of animal data to

human. However, research need progress and deepen because the environmental mixed EDCs might interact additively, antagonistically, or synergistically; very little information of the effects of their metabolism, serum and intracellular protein binding and uptake by target cell is known (Safe et al., 1997); there are more than one agonists/antagonists endocrine response pathway (McLachlan, 2001; Cooper et al., 1997; Bulayeva et al., 2004, Fig. 1-3-2 (1), (2)); the other gene toxicity of PBTs might produce 'cross-talk' to the estrogenic receptor activity (for example, Ah-AhR-ARNT complex (aryl hydrocarbons, Ah receptor and AhR nuclear locator) bound to dioxin-response element, which locate close to EREs) (Witorsch, 2002b); small molecular structural difference, such as different PCB congeners (Tabb et al., 2004), even enantiomeric difference can appear to be the factors in hormonal activity.

Feldman (1997) have colleted the further attentions to PBTs: species-specific effects (Spearow et al., 1999) have raised the concern to the genetic susceptibility of subpopulations and tissue-specific effects (Ishihara et al., 2003) resulted the question of calculation of estrogenic potency based on breast or uterus models, which may not be quantitatively predictive of effects in other tissues such as the liver, bone, or brain or in the developing fetus that may be more sensitive to hormonal influences; pharmacokinetic or other *in vivo* factors, such as the inadequate metabolism of PBTs to more active analogs and their accumulation and storage in fat causing an increased or prolonged exposure, may cause the estrogenic effect to be greater than expected based solely on extrapolations from *in vitro* data. Additionally, compared to E2 with a higher affinity with serum protein, certain xeno-EDCs, namely diethylstilbestrol, *o, p'*-DDT, and octylphenol, may be more potent estrogen *in vivo* because of their bioavailability (Chen et al., 1997).

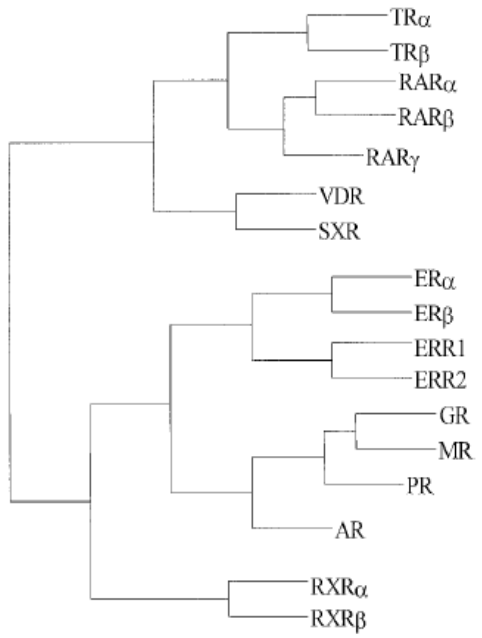


Fig. 1-3-2(1): Most of the known environmental chemicals with hormonal activity derive that activity through interaction with one or more of steroid / thyroid / retinoid gene family of nuclear receptors. Abbreviations used are: TR, thyroid hormone receptor; RAR, retinoid receptor; VDR, vitamin D receptor; SXR, steroid xenobiotic receptor; ER, estrogen receptor; ERR, estrogen-related receptor; GR, glucocorticoid receptor; MR, mineralocorticoid receptor; PR, progesterone receptor; AR, androgen receptor; RXR, retinoid orphan receptor; ERγ (recently been reported) (McLachlan JA, 2001).

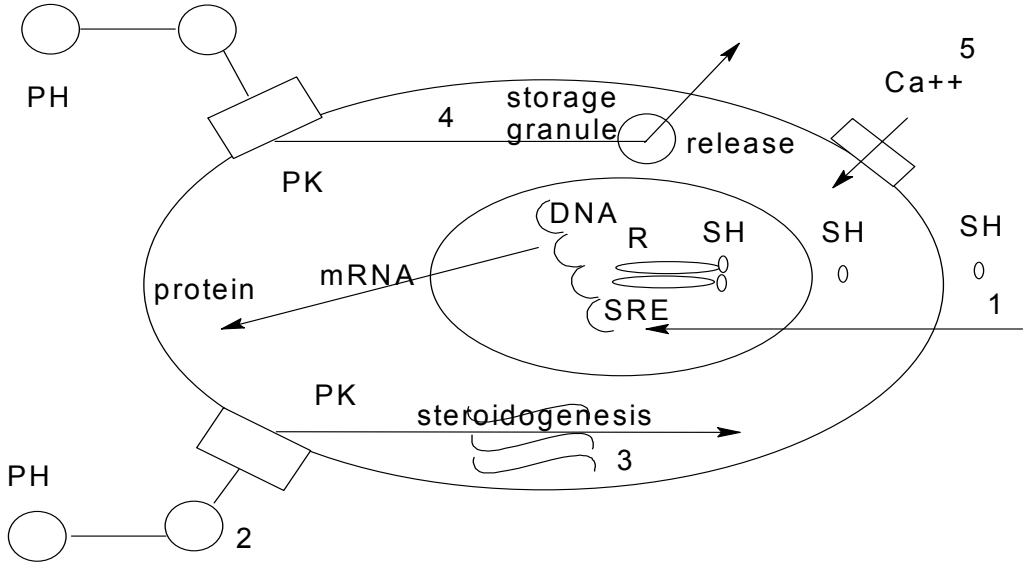


Fig. 1-3-2(2): Multiple pathways of EDCs: (1) The better-studied steroid hormone receptor-mediated gene pathway changes in protein synthesis and /or mitosis (e.g. weak estrogen methoxychlor or anti-androgen DDE). Some of the other include (2) compounds that interfere with membrane receptor binding (e.g. chlordimeform, an α-noradrenergic receptor blocker), (3) steroidogenesis (i.e. certain imidazole compounds) or (4) compounds that interfere with the synthesis of other hormones (e.g. dithiocarbamates disrupt adrenalin synthesis) and (5) compounds that alter the flux of ions across the membrane (e.g. pyrethroid insecticides alter sodium and chloride ion flux and metals compete for normal calcium ion flux) within certain types of hormone-secreting cells. SH = steroid hormone in blood or cytoplasm, PH = peptide hormone, R = steroid hormone receptor, SRE = steroid response element, PK = protein kinase (Cooper RL et al. 1997).

1-3-3: Human exposure characters to EDCs

Ambient endocrine disrupters: The endocrine disrupting action mode of PBTs has been

suspected to associate with the wide range of human health effects (Kavlock et al., 1996). Factually, EDCs range from natural plant oestrogens (e.g. genistein, coumesterol) and mycoestrogens (e.g. aflatoxins, zearalenone) to growth promoting pharmaceuticals (e.g. trenbolone acetate, melengastrol acetate) to chemicals spread in water, sewage sludge or the atmosphere such as detergents and surfactants (e.g. octylphenol, nonylphenol), plastics (e.g. bisphenol A (BPA), phthalates), pesticides (e.g. methoxychlor, dieldrin, DDT) and industrial chemicals (e.g. PCB, TCDD) (Torres, 2002). The various environmental chemicals are capable of acting as endocrine disrupters as either hormone agonists or antagonists, which can potentially alter the hormonal balance in animals and people. However, controversy of the adverse effects on human health still remains (Feldman, 1997).

Low-dose effects: Beyond the traditional threshold model (assumes the threshold dose as no observed adverse effect level) and linear non-threshold model (extrapolates risks to very low doses of adverse effect), especially the later, as the dose below the standard threshold, the response becomes more likely to exceed the control value, so called hormetic-like. Hormesis (Calabrese et al., 2003ab) has been defined as a dose-response relationship in which there is a stimulatory response at low doses, but an inhibitory response at high doses, resulting in a U- or inverted U-shaped dose response, which is also called low-dose effect (Kaiser, 2000). Sex differentiation period is sensitive to low-dose chemical effects and the exposure might be transient, the effects are irreversible and not easy discovered until after puberty (Kelce et al., 1997). For example, utero exposure of mice shown the low-dose stimulating responses to diethylstilbestrol and estradiol (vom Saal et al., 1997), and the effects are organ and strain specific, transient and not sustained into adulthood for natural estrogens (Putz et al., 2001). *In vitro* research has shown that EDCs, like coumestrol, endosulfan, dieldrin, DDE and *p*-nonylphenol produced rapid (3–30 min after application), concentration (10^{-14}–10^{-8} M)-dependent ERK-1/2 phosphorylation but with distinctly different activation patterns. But BPA does not exhibit phosphorylation. These actions may help to explain the distinct abilities of EDCs to disrupt reproductive functions at low concentrations via multiple membrane-initiated signaling pathways (Bulayeva et al., 2004). Although the low-dose effects was argued to appear unlikely during human pregnancy because of the species differences (particularly higher estrogen levels attained in human pregnancy compared to the mouse)

(Witorsch, 2002), low levels of POPs exposure have raise concerns for future generations. For example, levels of PCBs now shown to affect human brain development are nearly one million-fold lower than levels previously believed safe (Solomon et al., 2002).

Combined effects: As Daughton (EPA in Las Vegas) said: no organism is ever exposed to a single chemical in isolation; the individual compounds are just part of the story and the biggest unknown right now is interactions. It is necessary to consider the impact of combined effects, which are commonly assessed in terms of synergism, additivity, or antagonism by comparing of the observed response with the usually expected one (Payne, 2001). Many reports have focus on the mixture EDC activities. For examples, pig immature cumulus-oocyte complex exposed to EDC mixture, which mimic contaminants of the Arctic marine food chain and the highly exposed populations of women, supported the concerns that such pollutants harm reproductive health in human and other mammalian species (Campagna et al., 2001). The extracts of air, subsurface soil, and superficial dust from a landfill, was used to examine multiple biological responses by a 2-day prepubertal female rat bioassay, where soil, dust, and air extracts effectively reduced serum total thyroxine (T4) with similar dose-response relationships, despite the significantly different TCDD toxic equivalent (TEQ) values of these three extracts (Li et al., 1996).

Additivity: Many reports support that mixtures of EDCs at doses that are individually inactive can give active response. For example, the mixtures has been reported at doses that are individually inactive in the immature rat uterotrophic assay, can give an uterotrophic response (Tinwell et al., 2004). The additive effect of *o, p'*-DDT, *p, p'*-DDE, β-HCH, and *p, p'*-DDT acted together on MCF-7 cells could be predicted on the basis of data about single agent concentration–response relationships (Payne et al., 2001). Similar results have been reported on hydroxylated PCBs, benzophenones, parabenes, BPA, and genistein mixture using a recombinant yeast estrogen screen (Silva et al., 2002) and on estradiol-17β and ethynylestradiol-17α mixture (equi-potent fixed-ratio) using vitellogenin induction in a 14-day *in vivo* juvenile rainbow trout screening assay (Thorpe et al., 2003). Based on the pharmacologically well-founded models of concentration addition and independent action, the contribution of BPA or *o, p'*-DDT to the overall mixture effect combined with 17ß-estradiol (E2) was tested using yeast estrogen screen (Rajapakse et al., 2001). At molar

mixture ratios proportional to the levels normally found in human tissues (i.e., below 1:5000 of E2: BPA or *o, p′*-DDT), the effects of individual xenoestrogens are too weak to create an impact on the actions of steroidal hormones; however, at mixture ratios more in favor of the xenoestrogens (1:20,000 and 1:100,000 for E2: BPA or *o, p′*-DDT), a significant contribution to the overall mixture effect was predicted and the predictions were tested experimentally. The researchers suggest that the assumption that weak xenoestrogens are generally unable to create an impact upon the already strong effects of endogenous steroidal estrogens (Safe, 1995) is not supported.

Synergism: When screened in a simple yeast estrogen system (YES) containing human estrogen receptor (hER), combinations of two weak environmental estrogens, such as dieldrin, endosulfan, or toxaphene, were 1000 times as potent in hER-mediated transactivation as any chemical alone. Hydroxylated PCBs shown previously to synergistically alter sexual development in turtles also synergized in the YES (Arnold et al., 1996). After this report, at least five teams using the same chemicals in 10 standard endocrine test systems to look for the synergy and only additive in every case (Kaiser, 1997), for instances, estrogenic activity of a dieldrin / toxaphene mixture in the mouse uterus, MCF-7 human breast cancer cells, and yeast-based estrogen receptor assays (Ramamoorthy et al., 1997). Other kinds of EDCs, from the representative alkylphenols and phthalates, the pesticides dieldrin and toxaphene, to the mycoestrogen zearalenone and the phytoestrogen genistein, interacted with three major teleost steroid-binding sites (estradiol receptor (ER), testosterone receptor (TR) and cortisol receptor (CR)) was evaluated. These compounds are exclusively estrogenic in rainbow trout, albeit weakly so, and do not display any synergistic effects (Knudsen et al., 1999). However, curcumin and genistein, which inhibit the growth of estrogen-positive human breast MCF-7 cells induced individually or by a mixture of the pesticides END, DDT and chlorease or 17-beta estradiol when present at micromolar concentrations, can synergistically inhibit the induction was noted (Verma et al., 1997). Although most of the negative results in endocrine disruption toxicity, synergism can be found in other toxic tests or exposure reports, such as induced EROD activities (Borlakoglu et al., 1992; Basu et al., 2001). Further reports might refer the synergistic effects by testing manufactured gas plant-PAHs mixture *in vitro* test (Chaloupka et al., 1993) and by epidemiological investigating of combined exposure of high environmental tobacco smoke (plasma cotinine) and PAHs (using BaP-DNA

adducts as a molecular dosimeter) (Perera et al., 2004).

Antagonism: A recent report (Rajapakse et al., 2004) on combined effect of 17 β-estradiol, 17α-ethinylestradiol, genistein, BPA, 4-nonylphenol, and 4-*tert*-octylphenol using MCF-7 human breast cancer cells by E-SCREEN fell short of the additivity expectations because of the weak antagonism when the presence of 4-nonylphenol and 4- tertoctylphenol in mixture. It implies that, in sometimes, some interactions might compromise the predictability of estrogenic combination effects. Due to the inappropriate of the simple addition activities, methodology, it is suggested that isobole analysis is only suitable for 2- or 3-component mixtures, and concentration addition requires access to dose response data and EC_{50} values for the individual components of the mixture (Tinwell et al. 2004).

1-4: Enantiomeric ratio information of *c*-PBT residuals in biota

1-4-1: Chiral PBTs in biota samples

When racemic chiral PBTs (*c*-PBTs) entered the environment, enantioselective biodegradation might occur in water, soil (sediment) (Falconer et al., 1995; Pakdeesusuk et al., 2003; Robson et al., 2004) and their enantiomeric ratios (ERs), which have been widely used as tracers of air-soil transportation process (Bidleman et al., 1998; Hühnerfuss, 2000; Robson et al., 2004), could tell the processes information. Following review would focus on chiral transformation in biota, especially, in animals.

Enantioselective Residuals: The enantioselective biotransformations of *c*-PBTs have been well documented in aquatic biota. Early studies showed the ER and the correlation between the ERs and the concentrations of α-HCH and γ-HCH, respectively, could characterize the different microbiological degradation pathways in the North Sea (Faller et al., 1991). Different enzymatic characters were also revealed in different marine animals from the liver of Common eider ducks, the liver of flounders, blue mussels, and even the North Sea water in German Bight by ERs (Pfaffenberger et al., 1992). Additionally, enzyme activity might be affected by health status (Mössner et al., 1992). The other *c*-PBTs, such as OXC, *c*-HE in Baltic herring, Baltic salmon, Baltic seal even in human adipose tissue (Buser et al., 1992), *c, t*-CHL, *o, p'*-DDT, *o, p'*-DDD in cod liver oils and fish oils (Koske et al., 1999) had been observed. Except 2 brain samples, all organs showed that (-)-α-HCH was enantioenriched in pork (Covaci et al., 2004). In human samples (Chu et al., 2003), the racemic α-HCH was found in three liver samples, while chiral PCB95, 149, and 132 showed racemic or nearly racemic in muscle, kidney, and brain and the higher ERs for the three chiral PCBs were found in liver samples. Recent researches have paid more attention on the metabolites of PBTs, including some chiral metabolites, which formed from chiral of prochiral parental compounds. For example, the methyl sulfonyl ($MeSO_2$-) substituted of PCBs and DDE, they are also persistent and bioaccumulation like their parents and might be more

toxic. The preferential formation one atropisomer of certain chiral $MeSO_2$-PCB have been observed in animals, such as arctic ringed seal and polar bear (Wiberg et al., 1998), harbor porpoises (Chu et al., 2003) and grey seal (Larsson et al., 2004). In grey seals, all of the investigated metabolites found in significantly higher concentrations in the liver than in lung and blubber and the equal enantiomeric specificity for chiral metabolites in all tissues (Larsson et al., 2004) might suggest their formation in liver and redistribution among the other tissues.

ER as tracer of biotransformation in biota: The investigations in the polar bear food chain (arctic cod-ringed seal-polar bear) (Wiberg et al., 2000) and Lake Superior aquatic food web (Wong et al., 2004) suggested ERs might be magnified through predator-prey relationships with the increasing enzyme activity along the trophic levels. In the Lake Superior aquatic food web, chiral PCBs (91, 95, 136, 149, 174, 176, and 183) showed no biotransformation potential in phytoplankton and zooplankton (low trophic level organisms); macrozooplankton (diporeia and mysids) might stereoselectively metabolize them or, alternatively, obtained their nonracemic residues from feeding on organic-rich suspended particles and sediments; forage fish (lake herring, rainbow smelt, and slimy sculpin) and lake trout suggested a combination of both *in vivo* biotransformation and uptake of nonracemic residues from prey because of the widely nonracemic PCB residues (Wong et al., 2004). In polar bear food chain, cod showed near-racemic mixtures for α-HCH and CHL related compounds and, in contrast, ringed seal and polar bear were frequently nonracemic. Along the food chain, (+)-α-HCH became more abundant relative to (-)-α-HCH (Wiberg et al., 2000). The first order kinetic depuration rate models have been used to calculate the relative half-life time (Walter et al., 2001) and minimum biotransformation rates (Wong et al., 2002; 2004). The metabolic elimination rates calculation suggested that at least 58% of the *t*-CHL and the entire PCB-136 depuration rate could be attributed to metabolism in Rainbow Trout (Wong et al., 2002) and minimum biotransformation rates (calculated from enantiomer mass balances between predators and prey) suggested that significant biotransformation might occur for PCB congeners over the lifespan of trout and sculpins (Wong et al., 2004). Chiral biomagnification factor (relative to CB-153) analysis in polar bear food chain indicated that OXC might be formed by ringed seal and metabolised by polar bears and the linear relationships of ERs of some highly recalcitrant CHLs in polar bear adipose with the bears' age (Wiberg et al., 2000)

might suggest a continuous feeding and enantioselective depuration mode. Relationships between ER of PCB95 with 1/ER of PCB132 and ER of PCB149 with of 1/ER 132 suggest that the bioselective metabolism of chiral PCBs has the same trend in human, although the ratio is different (Chu et al., 2003). Additionally, multivariate statistical methods revealed the ERs were good indexes of sample groupings in response of the enzyme activity (Wiberg et al., 2000).

1-4-2: Chiral and prochiral PBTs and endocrine disruption activity

A pair of enantiomer molecules, with the same chemical composition, is asymmetrical in the arrangement of their carbon atoms or in energy barrier to ring rotation like in some PCBs or metabolite (Nezel et al., 1997) and would be different to them in terms of the orientation of elements in space. It has been shown that this kind of difference can definitely cause different endocrine activity. Binding and transactivation tests on the mutational ligand-binding domain of ER (estrogen receptor) (Kohno et al., 1996) and on the different receptors such as ERα, ERβ or AR (Gaido et al., 2000) showed the ligand-receptor binding and transactivation were stereochemically specific. For example, yeast-based assay assess the isomer-specific transcriptional activity of *o, p′*-DDT with the human estrogen receptor (hER) showed (similar in rat), the (-)-isomer was the active estrogen mimic whereas hER activity of *(+)-o, p′*-DDT was negligible (Hoekstra et al., 2001). The other aspect of the bioaccumulation EDCs is that most of them exhibit very week EDC but their metabolites, sometimes, have stronger transactivation, such as the in vivo estrogenic activity of methoxychlor is mainly caused by metabolism to phenolic estrogenic metabolites (Gaido et al., 2000). Some of the metabolites are chiral compounds, such as mono-OH-MOC. (S)-mono-OH-MOC showed 3-fold higher binding activity than that of the (R)-isomer. The result also pointed out the estrogenic activity of MOC after metabolic activation *in vivo*, which predominantly produced the (S)-mono-OH-MOC, might be higher than estimated from the in vitro activity of racemic mixtures (Miyashita et al., 2004).

Compounds like MOC are referred to prochiral compounds because they can be usually metabolized enantiomeric selectivity in biota (Hu et al., 2002). Dieldrin can also be mainly (86%) metabolized to one of the two 6,7-trans-hydroxy-dihydro-aldrin enantiomers in rabbits (Korte et

al., 1965). Also styrene, an industrial solvent, is mainly oxidized by cytochrome P450 to an electrophilic, chiral epoxide metabolite styrene-7,8-oxide and the R- and S-isomers with different cytotoxic and genotoxic properties (Wenker et al., 2000). Then, the additional endocrine disrupting effect is complex for the PBTs because they store in fat tissue and can be metabolized slowly and continuously *in vivo* to produce more or less bioactive compounds usually with apparent different effects than the parent compounds tested *in vitro*. Enantioselective residual or formation analysis for *c*-PBTs could be helpful in exposure-health risk assessment. However, it is little knowledge about the level and toxicity of the PBT metabolites *in vivo*.

2-1: Sampling and analysis

2-1-1: Sample collection information:

The study was approved by the local ethics committee and conducted according to the Helsinki II declaration. Mothers gave a written informed consent and allowed placentas and milk to be used for chemical analyses. 112 placenta samples were collected at Turku University Central Hospital in Turku, Finland, during the years 1997-01 and 168 placenta samples at University Department of Growth and Reproduction, Rigshospitalet, Copenhagen, Denmark. Only male children of Finnish families (both parents had to be born and raised in Finland, with a maximum stay abroad of three years for the mother and 10 years for the father and grandparents) were included in the study. Case-control information was listed in Table 2-1-1 (1).

Table 2-1-1 (1): Methodological information of breast milk and placenta used for case-control study

		Index cases		Matched controls		Additional reference
Denmark	Placenta	43	87	79	165	265
	Milk	57		117		117
Finland	Placenta	104	128	169	215	
	Milk	54		90		

Midwives collected placentas at birth and froze them in LD-polythene bags in –20 ^{o}C. The protocol of placenta and breast milk preparation was from Dr. Hannu, Kuopio, Finland. Homogenization and samples analysis for controls and cases from both countries were run in random order and during approximately the same time periods to avoid systematic errors from technical changes. When defrosted, every placenta was mechanically homogenized, shared in small glass bottles (*Neolab scintillation tubes 20 ml, code: 9-0621*) and stored in –20 ^{o}C until analyses.

Collected milk samples (FM weeks 4-6 and DM 4-12 weeks post partum) with more than 150 mL were primarily to select (65 samples from Denmark and 65 from Finland). But in order to get enough case samples it can be necessary to choose few samples around 125 mL. The protocol of

milk preparation was following: 1. Open the cork/cap/stopper of the frozen bottle. 2. Place the bottle in a "minigrip" pack or suchlike (other glass) in order to avoid possible loss of sample if bottle will break during defrosting. 3. Defrost the bottle in a fridge. 4. Temperate the bottle to room temperature after thawing. 5. Shake/mix the sample at 38-40 ^{o}C for 1 hour 6. Close the cork/cap/stopper of the bottle and shake vigorously. 7. Aliquot the sample 8. Freeze the aliquots as soon as possible and send them to other laboratories on dry ice.

2 × 10 g placenta and 2 × 10 mL milk sub-samples, sealed in scintillation glass with aluminium padded cap, was accepted by IÖC, GSF in München for analysis. When starting analysis, a pooled control sample of placenta and milk (1-2 L) have been provided by Copenhagen to establish the sample preparation procedure.

2-1-2: Sample preparation method:

The standard operation procedure was modified to suit the placenta and breast milk. The modified procedure is based on a cold-extraction method including gel permeation chromatography (GPC) and small sandwich column or cartridge cleanup to remove the lipids from the wet placenta samples for HRGC-HRMS analysis of selected halogenated hydrocarbons by isotope dilution method (Körner, 1996).

Materials: A whole deep frozen placenta (laboratory code 0202027) was frosted in a refrigerator and homogenized using automatic mill. The homogenized sub-sample are packed in sealed glass bottles and stored under –190 ^{o}C. These are the samples used to modify SOP for sample preparation. Solid materials are anhydrous sodium sulfate (*Water corporation*), sea sand (*Riedel-de Haen*), basic alumina (*ICN Biomedical GmbH*), silica gel and florisil (*Promochem*). Anhydrous sodium sulfate, sea sand, silica gel and florisil are heated at 650°C at least 6 hours for removing the possible pesticide residues and / or activation. Heating alumina B at high temperature is also important for the recovery of endosulfan and dieldrin at our experimental conditions. Solvents are acetone, n-hexane, toluene and benzene, which are all from *Promochem* with picograde quality. The standard compounds, including isotope labeled standard and native

compounds, are listed in Table 2-1-2 (1), special chiral standard compounds listed in Table 2-1-2 (2).

Table 2-1-2 (1): The listed isotope internal standards and native standards with method recovery

No.	Native Compounds	^{13}C Internal Standard	Recovery (%)	Other Information
1	α-HCH	α-HCH	69	Cambridge isotope lab, Inc.
2	γ-HCH	γ-HCH	71	
3	β-HCH	β-HCH	66	
4	δ-HCH	δ-HCH	81	Cambridge isotope lab, Inc.
5	ε-HCH	-	-	No label standard
6	Endosulfan-I	Endosulfan-I	68	Cambridge isotope lab, Inc.
7	Endosulfan-II	Endosulfan-II	75	Cambridge isotope lab, Inc.
8	Methoxychlor	Methoxychlor	119	Cambridge isotope lab, Inc.
9	o, p'-DDE	o, p'-DDE	68	Cambridge isotope lab, Inc.
10	o, p'-DDT	o, p'-DDT	93	Dr. Ehrenstorfer GmbH
11	o, p'-DDD	-		
12	p, p'-DDD	p, p'-DDD	70	Cambridge isotope lab, Inc.
13	p, p'-DDE	p, p'-DDE	66	
14	p, p'-DDT	p ,p'-DDT	73	
15	Pentachlorbenzene	Pentachlorbenzene	51	
16	Hexachlorbenzene	Hexachlorbenzene	61	
17	Pentachloroanisole	Pentachloroanisole	63	Dr. Ehrenstorfer GmbH
18	Aldrin	Aldrin	64	Cambridge isotope lab, Inc.
19	Dieldrin	Dieldrin	71	Cambridge isotope lab, Inc.
20	Oxychlordane	Oxychlordane	65	Cambridge isotope lab, Inc.
21	trans- Chlordane	trans- Chlordane	56	Cambridge isotope lab, Inc.
22	cis-Chlordane	-	-	No label standard
23	cis-Heptachloroepoxide	cis-Heptachloroepoxide	72	Cambridge isotope lab, Inc.
24	trans-Heptachloroepoxide		-	
25	Heptachlor	Heptachlor	79	Cambridge isotope lab, Inc.
26	Mirex	Mirex	71	Cambridge isotope lab, Inc.
27	Octachlorstyrene	Octachlorstyrene		Cambridge isotope lab, Inc.
28	PBB-1	^{13}C-PCB Mixture*		No label standard
29	PBB-3	^{13}C-PCB Mixture*		No label standard
30	PBB-4	^{13}C-PCB Mixture*		No label standard
31	PBB-15	^{13}C-PCB Mixture*		No label standard
32	PBB-18	^{13}C-PCB Mixture*		No label standard
33	PBB-31	^{13}C-PCB Mixture*	88 of ^{13}C-PCB-28	No label standard
34	PBB-37	^{13}C-PCB Mixture*		No label standard
35	PBB-49	^{13}C-PCB Mixture*		No label standard
36	PBB-52	^{13}C-PCB Mixture*		No label standard

37	PBB-77	^{13}C-PCB Mixture*		No label standard
38	PBB-80	^{13}C-PCB Mixture*		No label standard
39	PBB-101	^{13}C-PCB Mixture*		No label standard
40	PBB-103	^{13}C-PCB Mixture*		No label standard
41	PBB-153	^{13}C-PCB Mixture*	78 of ^{13}C-PCB-153	No label standard
42	PBB-155	^{13}C-PCB Mixture*		No label standard
43	PBB-169	^{13}C-PCB Mixture*		No label standard
44	PBB-209	^{13}C-PCB Mixture*		No label standard
45	Hexabromobenzene	hexa-Chlorbenzene*		No label standard

Table 2-1-2 (2): Native standards of purity enantiomers (from Dr. Ehrenstorfer GmbH)

Compound	Purity	(+)-Impurity	Org. Con. (mg/l)
(-)-α-HCH	99%	1%	1
(-)-oxychlordane	71.4%	28.6%	1
(-)-cis-heptachlorepoxide	99.4%	0.6%	1
(-)-trans-heptachlorepoxide	91.6%	8.4%	1
(-)-cis-chlordane	85.8%	15.3%	1
(-)-trans-chlordane	99.2%	0.99%	1
(-)-heptachlor	82.6%	17.4%	1
Compound	Purity	(-)-Impurity	Org. Con. (mg/l)
(+)-α-HCH	99%	1%	1
(+)-oxychlordane	71.4%	28.6%	1
(+)-cis-heptachlorepoxide	97.3%	2.7%	1
(+)-trans-heptachlorepoxide	80.5%	19.5%	1
(+)-cis-chlordane	75.2%	24.8%	1
(+)-trans-chlordane	92.6%		1
(+)-heptachlor	75.6%	24.4%	1
(±)-o, p'-DDT(^{13}C)	91.0%		100

Extraction: The extract glass column (25 × 2 cm) is packed with 10 g placenta, which is homogenized with about 30 g anhydrous sodium sulfate and 15 g sea sand. The packed column is spiked on its top with 10 µl internal standard ^{13}C-CKW (*Cambridge isotope lab, Inc.* and *Dr. Ehrenstorfer GmbH,* there are no labeled standards for ε-HCH, *o, p'*-DDD, *cis*-chlordane, *trans*-heptachlor epoxide and octachlorostyrene). The extracting solvent is a mixture acetone and n-hexane (2:1 v/v) of 250 ml. The elute flow was controlled in mediate drops but not in flow (~2 drops/min) and collected in a carefully weighted (using analysis-grade balance) 250 mL round bottom flask. This extraction process may be arranged overnight.

Lipid determination: Evaporate the solvent by rotary vacuum evaporator with a water-bath temperature up to 45ºC and a mediate rotary rate; the vacuum was controlled around 600 mbar. When the extract was condensed to about 0.5 ml, removed the round bottom flask from the evaporator and then evaporated it to hemi-dry with very mediate stream of nitrogen. After that, the flask was placed for 6 hours into a desiccator until stable weight was achieved (i.e. error of two separate weight measurements over at least two hours ±0.0005g). The lipid content was calculated on the base of wet weight sample. Like the organic solvent, dry lipids also can keep the chlorinated organic compounds. So the lipid residue could be used for further compound analysis and the results show that the recovery is sound (Table 2-1-2 (1)). The use of lipid residue after lipid determination can save the extraction step for analysis of the chlorinated organic compounds. After recording of the lipid data, the lipid residue was redissolved using 2 mL toluene and sealed and kept at –28 ^{o}C for cleanup.

Cleanup procedure: The first cleanup step was done on a GPC column. It is a 1.27 × 95 cm column packed with *Bio-Beads S-8*, spherical porous styrene-divinylbenzene copolymer with 8% crosslinkage, a constant-volume pump, an autosampler and an automatic fraction collector (*Gilson*) worked together with the column to perform the automatic chromatographic processes. The collected fraction is from around 32 min to 42 min for toluene eluent at a flow rate of 2ml/min (the result was checked and adjusted in the whole procedure of the two cohorts sample preparation).

Most part of lipids can be removed after GPC. However, the additional cleanup was needed to reach good analytical conditions for HRGC-HRMS. Further cleanup was performed on a sandwich glass cartridge (75×12 mm, *Merck*), which was packed with alumina B 0.8g, anhydrous sodium sulfate 0.3g, florisil 0.5g, silica gel 1g, and anhydrous sodium sulfate 0.5g from bottom to top. Here, 25 ml toluene was used to elute the pesticide residues from the cartridge.

Condense the cleaned elute by *Turbo Vap 500* (with the operation parameters: 5 min after sensor detection, temperature of water-bath 45 °C and fan speed level C). The eluent was evaporated to about 200 µl, and then, the sample was transferred to a 200µl micro-vial and was condensed by a gentle stream of nitrogen to a final volume about 10 µl in a fume hood. Add 10ul 1,2,3,4-TCDD

standard as the recovery standard to the final volume. Finally, the about 20 µl samples was sealed and stored at -28 °C for analysis.

2-1-3: HRGC-HRMS analysis and data collection:

HRGC is a *HP5890 (series II)* device equipped with a capillary column *DB-XLB* (length 60m, internal diameter 0.25mm and thin film 0.25 µm). A 30m the same column was used to analyze PBBs including HeBB. A capillary chiral column BGB-172 (two columns with 30m length and 0.25 and 0.18µm film thickness) was used for the enantiomers separation (Fig. 2-1-3 (1), (2), (3), (4), (5), (6)). (+) and (-)-isomers for *o, p'*-DDT were determined according literature (Falconer et al., 1997). Because the stationary phases were bleeding slowly in the processes, the GC programs were changed to suit the enantiomeric separation. Carrier gas was helium. HRMS is a *MAT95 (Finnigan)* engine using electron impact ion source mode. Multiple ion detection modes, combined with retention control, were used to identify the investigated compounds. The selected masses for compound identification and calibration and listed in Table 2-1-3 (1). The reference compound for mass detection was *FC43* (*MasCom GmbH*).

Limit of detection (LOD) was set as signal/noise (S/N) = 3. The deviation of the ion ratios of the calibration mass to the reference mass for the ^{12}C-investigation compounds from the counterpart ratios of ^{13}C-internal standard compounds were controlled in range ±15%; the retention time differences were controlled ±3s. To some compounds without the labeled standard, the native standards were run at the same chromatographic condition of the real samples, and the mass information and retention time were used to screening these compound in the samples. Only the data supported the upper conditions were considered for collection. Two blank samples and one reference (placenta or milk) sample were applied in each 30 real samples treating procedure. The data higher that 3 time mean blank values are used as quantified data and, on the contrary, the lower ones were taken as unquantifiable data.

Table 2-1-3 (1): The masses used to identify and calculate the investigated compounds

No.	Native Compounds	Mass	^{13}C Internal Standard	Mass
1	α-HCH	216.9145, 220.9087	α-HCH	222.9347, 226.9288
2	γ-HCH	216.9145, 220.9087	γ-HCH	222.9347, 226.9288

3	β-HCH	216.9145, 220.9087	β-HCH	222.9347, 226.9288
4	δ-HCH	216.9145, 220.9087	δ-HCH	222.9347, 226.9288
5	ε-HCH	216.9145, 220.9087	-	
6	Endosulfan-I	236.8413, 238.8384 338.8553	Endosulfan-I	241.8581, 243.8551 347.8854
7	Endosulfan-II	236.8413, 238.8384 338.8553	Endosulfan-II	241.8581, 243.8551 347.8854
8	Methoxychlor	227.1072	Methoxychlor	239.1475
9	o, p'-DDE	246.0003, 247.9975	o, p'-DDE	258.0406, 260.0376
10	o, p'-DDT	235.0081, 237.0053	o, p'-DDT	247.0484, 249.0454
11	o, p'-DDD	235.0081, 237.0053	-	243.0583, 245.0555
12	p, p'-DDD	235.0081, 237.0053	p, p'-DDD	243.0583, 245.0555
13	p, p'-DDE	246.0003, 247.9975	p, p'-DDE	258.0406, 260.0376
14	p, p'-DDT	235.0081, 237.0053	p, p'-DDT	247.0484, 249.0454
15	Pentachlorbenzene	249.8492, 251.8462	Pentachlorbenzene	255.8693, 257.8663
16	Hexachlorbenzene	283.8102, 285.8072	Hexachlorbenzene	289.8303, 291.8273
17	Pentachloroanisole	264.8363, 266.8333	Pentachloroanisole	270.8564, 272.8534
18	Aldrin	262.8570, 264.8541	Aldrin	269.8805, 271.8775
19	Dieldrin	262.8570, 264.8541	Dieldrin	269.8805, 271.8775
20	Oxychlordane	386.8053, 388.8024	Oxychlordane	396.8388, 398.8359
21	trans- Chlordane	236.8413, 238.8384 372.8260	trans- Chlordane	241.8581, 243.8551 382.8595
22	cis-Chlordane	236.8413, 238.8384	-	241.8581, 243.8551
23	cis-Heptachloroepoxide	352.8442, 354.8413	cis-Heptachloroepoxide	362.8778, 364.8748
24	trans-Heptachloroepoxide	352.8442, 354.8413		362.8778, 364.8748
25	Heptachlor	336.8493, 338.8464 271.8102, 273.8072	Heptachlor	346.8828, 348.8799 276.8269, 278.8240
26	Mirex	271.8102, 273.8072	Mirex	276.8269, 278.8240
27	Octachlorstyrene	342.7790, 344.7761	Octachlorstyrene	350.8059, 352.8029
28	PBB-1	231.9887, 233.9867	^{13}C-PCB Mixture*	
29	PBB-3		^{13}C-PCB Mixture*	
30	PBB-4	311.8972, 309.8992	^{13}C-PCB Mixture*	
31	PBB-15		^{13}C-PCB Mixture*	
32	PBB-18	389.8077, 391.8057	^{13}C-PCB Mixture*	
33	PBB-31		^{13}C-PCB Mixture*	
34	PBB-37		^{13}C-PCB Mixture*	
35	PBB-49	469.7162, 467.7182	^{13}C-PCB Mixture*	
36	PBB-52		^{13}C-PCB Mixture*	
37	PBB-77		^{13}C-PCB Mixture*	
38	PBB-80		^{13}C-PCB Mixture*	
39	PBB-101	547.6266, 549.6246	^{13}C-PCB Mixture*	
40	PBB-103		^{13}C-PCB Mixture*	

41	PBB-153	627.5351, 625.5371	^{13}C-PCB Mixture*	
42	PBB-155		^{13}C-PCB Mixture*	
43	PBB-169		^{13}C-PCB Mixture*	
44	PBB-209	-	^{13}C-PCB Mixture*	
45	Hexabromobenzene	551.5039	hexa-Chlorbenzene*	

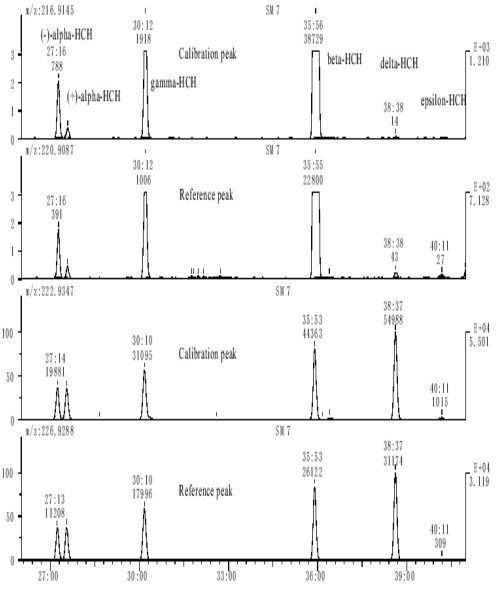


Fig 2-1-3 (1): Chromatography of enantiomeric separated α-HCH and the other HCH isomers

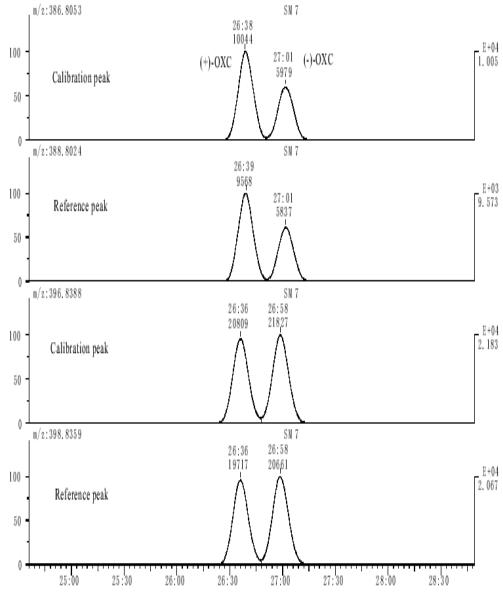


Fig 2-1-3 (2): Chromatography of enantiomeric separated OXC isomers

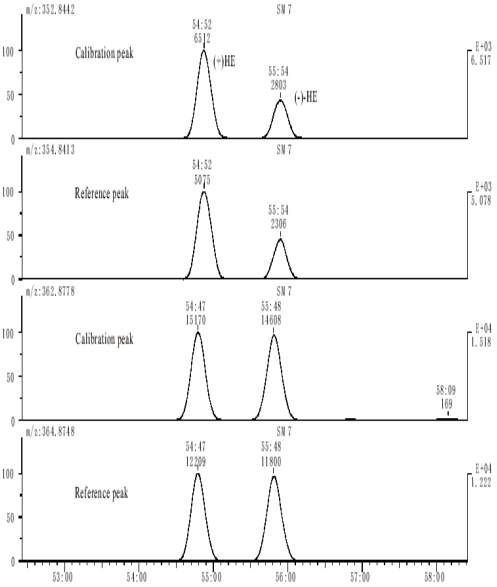


Fig 2-1-3 (3): Chromatography of enantiomeric separated c-HE

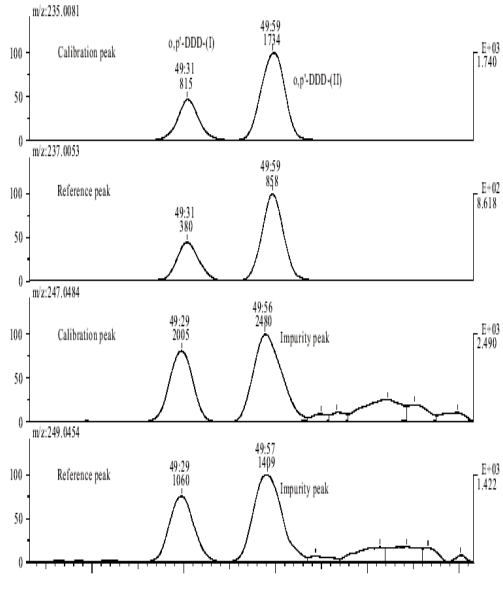


Fig 2-1-3 (4): Chromatography of enantiomeric separated *o, p'*-DDD

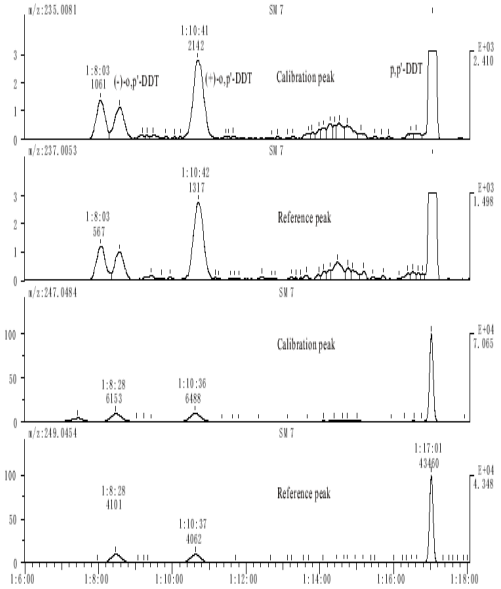


Fig 2-1-3 (5): Chromatography of enantiomeric separated *o, p'*-DDT and *p, p'*-DDT

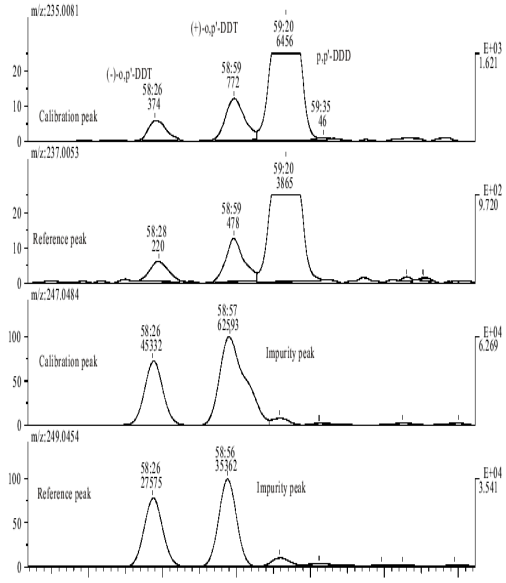


Fig 2-1-3 (6): Chromatography of enantiomeric separated *o, p'*-DDT (other temperature program)

2-2: Statistical analysis

Excel program was used to do the simple statistic, such as calculation of average value, geometric mean, standard deviation (STD) and a few linear regressions. Most of the linear fittings are applied with Origin 6.0 software. Further statistic analysis, such as data distribution testing, principal component analysis were done by the programmable Matlab 6.5 (*MathWorks Inc.*) using its statistic tools.

Principal component (PC) analysis: The basic idea of PC analysis is to find the components 1st PC, 2nd PC, 3rd PC, … nth PC, which are the linearly combined component of the original variables (Jolliffe, 1986; Kendall, 1975). These transformed components can explain the total amount of variance using the fewer variables. In other words, the few combined variables just refer to the common factors in factor analysis, which focus on the finding of common factors that affect the variance of set of data among multi-variables. All the PCs are orthogonalized and then are independent. 1st PC, interpreting the maximum percent of the total variance, is applied firstly. And then the 2nd PC, interpreting the second maximum percent variance, is determined in orthogonality with 1st PC. The other PCs are searched one by one in the same way. Each PC would refer a common factor, which is described separately by the original variables at different extent. To this point, there is no essential difference between PC analysis and factor analysis method. Before PC analysis treatment, the original data usually need undergo transformation, such as normalization; the most used transformation is so called standardization by formulae 1, 2, 3.

Methodologically, standardization results in the fact that every variable looks like similar in their variance ranges. However, the relative position for each observation in each variable is unchangeable. And then, it is unchangeable of the relationship of each observation to the remaining ones in the whole set of population. Additionally, the exact value of compound PC score and sample PC score are not as important as it's sign (i.e. positive and negative) when comparing the compounds (variables) and samples (observations) by their PC scores; and also it is important for the relative value of these PC scores.

$$\bar{x}_{.,j} = \frac{1}{m}\sum_{i=1}^{m} x_{i,j} \qquad (1)$$

$$s_j = \left(\frac{1}{m-1}\sum_{i=1}^{m} (x_{i,.} - \bar{x}_{.,j})^2 \right)^{\frac{1}{2}} \qquad (2)$$

$$stdx_{i,j} = \frac{x_{i,j} - \bar{x}_{.,j}}{s_j} \qquad (3)$$

Data ($x_{i,j}$) are expressed as a matrix ($m \times n$) with m rows and n columns. m is the number of observation (sample) and n is variable (compound). Formula (1) shows the mean of j^{th} variable along the m times observation; (2) shows the standard deviation (s_j) of j^{th} variable; (3) the final result of $x_{i,j}$ after the transformation.

2-3: Repeatability of the analytical procedure

The relative deviation (R-SDT: STD / Mean ×100%) of all abounding components like β-HCH, HeCB, OXC, *c*-HE, *p, p'*-DDE, END-1 and dieldrin can be controlled below 20-25% in the whole placenta sample analysis procedure (Table 2-3 (1)). Also in breast milk sub-samples coded 0202029 and 0202030, the repeatability was well controlled (Table 2-3 (2), (4)). However, for sub-samples coded 0202026 (Table 2-3 (3)), the relative standard deviations of OXC, *c*-HE, dieldrin reached from 30-40% might because of the not homogenized well of these sub-samples. Additionally, because the STD changed with the level of compound approximately by formula STD = 0.2~0.3 $Mean^{0.7\sim0.8}$ (Fig. 2-3 (1)), therefore, it is difficult to control the relative deviation at lower concentrations. That might the reason that the uncertain was bigger for the low level compounds, especially, when content less than 1 ng/g. The final factor of error induction is the blank. Because of pollutant emission every, such as *p, p'*-DDE, it should affect the repeatability more or less. It was not important for the higher concentration compounds, but it is a problem to compound like PeCB, α- HCH and γ-HCH in some situation, their contents are around 1~2 ng/g lipid. The lipids repeat detection showed R-STD was 6.20% for lipid reference samples (Table 2-3 (1)) and 8.75 and 12.3% for milk reference samples (Table 2-3 (2), (3)).

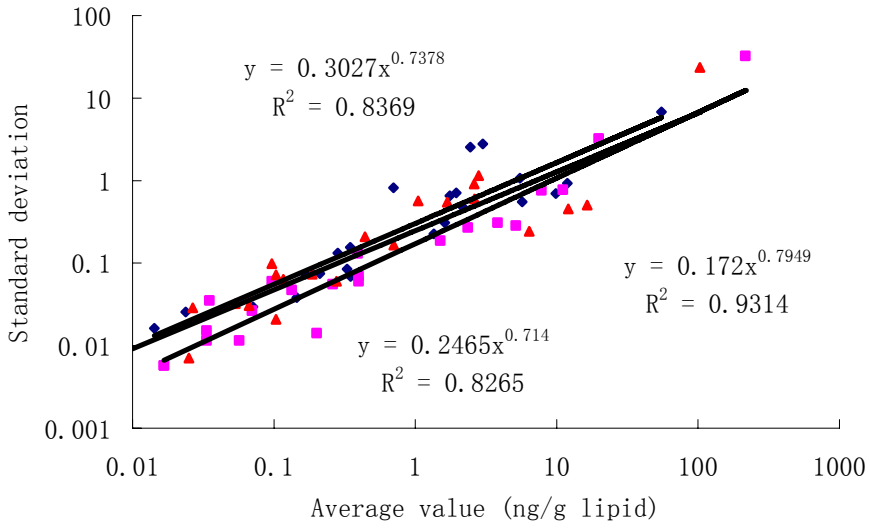


Fig. 2-3 (1): The trend of STD versus the average value (Mean), data come from Table 2-3 (1), (2), (3)

Table 2-3 (1): Repeated 7 sub-samples of the homogenized reference placenta

No.	PeCB	α-HCH	β-HCH	γ-HCH	δ-HCH	PCA	HeCB	OCS	OXC	c-HE	o,p'-DDE	p,p'-DDE
1	10.4*	6.2	11.5	1.8	2.8	0.6	9.1	0.1	2.2	1.4	0.1	45.8
2	5*	1.7	12.1	2	1.2	0.5	10.9	0.2	2.3	1.5	0.1	50.6
3	1.7	1.5	10.1	1.8	0.4	0.3	9.1	0.2	1.7	1.4	0.1	49.5
1	1.9	0.87	11.4	1.49	0.64	0.29	9.88	0.17	2.42	1.66	0.07	62.62
2	1.84	3.05	12.2	2.03	0.54	0.21	9.72	0.18	1.6	1.58	0.04	56.33
3	1.98	7.78	13.6	1	0.06	0.12	10.1	0.09	1.8	1.14	0.02	64.65
4	1.67	1.48	11.4	1.35	0.46	0.2	10.2	0.05	1.71	1.45	0.04	56.25
5	2.01	0.41	11.6	1.34	0.05	0.5	9.67	0.21	2.5	2.1	0.06	57.22
6	2.02	0.47	12.2	1.36	0.07	0.3	10.9	0.16	3.03	2.09	0.09	62.05
7	1.5	1	12.7	3.36	0.79	0.44	8.91	0.31	2.6	1.81	0.09	46.59
STD	2.79	2.54	0.93	0.65	0.82	0.16	0.7	0.07	0.47	0.31	0.03	6.78
Mean	3.00	2.45	11.9	1.75	0.7	0.35	9.85	0.17	2.19	1.61	0.07	55.16
R STD	92.9	104	7.82	37.3	117	45.1	7.11	44.1	21.6	19.2	41.7	12.3

*Because the high back ground at the early stage of the analysis

Table 2-3 (1): Repeated 7 sub-samples of the homogenized reference placenta (continue)

No.	t-CHL	c-CHL	END-1	END-2	p,p'-DDD	o,p'-DDD	Dieldrin	o,p'-DDT	p,p'-DDT	MOC	Mirex	lipid
1	0	0	5.6	0.5	1.4	0.3	5	0.4	1.6	0.2	0.2	0.83
2	0	0	4.7	0.3	1.2	0.3	5.4	0.3	1.4	0.1	0.3	0.78
3	0	0	3.9	0.1	1.3	0.2	6	0.4	3.9	0.2	0.2	0.71
1	0.05	0.03	6.82	0.24	1.89	0.46	6.43	0.36	2.14	0.16	0.18	0.73
2	0.02	0.02	4.69	0.17	1.12	0.34	5.19	0.34	1.78	0.16	0.13	0.72
3	n.d.	n.d.	4.58	0.24	1.3	0.22	5.39	0.19	1.83	0.1	0.12	0.68
4	n.d.	n.d.	5.38	0.21	1.32	0.29	5.41	0.31	1.72	0.11	0.18	0.76
5	0.02	0.04	5.96	n.d.	1.34	0.4	6.35	0.37	1.77	0.13	0.18	0.73
6	0.03	0.01	6.13	0.32	1.53	0.43	6.45	0.41	1.81	0.12	0.26	0.68
7	0.07	n.d.	7.31	0.47	1.12	0.35	5.35	0.41	1.56	0.17	0.36	0.75
STD	0.03	0.02	1.07	0.13	0.23	0.08	0.55	0.07	0.71	0.04	0.07	0.05
Mean	0.02	0.01	5.51	0.28	1.35	0.33	5.70	0.35	1.95	0.15	0.21	0.74
R STD	108	113	19.5	46.5	16.7	25.8	9.70	19.7	36.5	26.5	35.5	6.20

Table 2-3 (2): Repeated 3 times of the breast milk sub-sample coded 0202030

No.	PeCB	α-HCH	β-HCH	γ-HCH	δ-HCH	PCA	HeCB	OCS	OXC	c-HE	o,p'-DDE
1	0.19	0.1	16.5	0.37	0.06	0.16	11.1	0.17	5.07	1.46	0.05
2	*	0.05	20.1	0.27	0.01	0.04	10.3	0.08	4.89	1.33	0.05
3	0.21	0.06	22.9	0.53	n.d.	0.09	11.8	0.15	5.45	1.7	0.07
STD	0.01	0.03	3.23	0.13	0.04	0.06	0.78	0.05	0.29	0.19	0.01
Mean	0.2	0.07	19.8	0.39	0.04	0.1	11.1	0.13	5.14	1.50	0.06
R STD	7.07	37.8	16.3	33.6	101	62.4	7	35.4	5.57	12.5	20.4

Table 2-3 (2): Repeated 3 times of the breast milk sub-sample coded 0202030 (continue)

No.	p,p'-DDE	t-CHL	c-CHL	END-1	p,p'-DDD	o,p'-DDD	Dieldrin	o,p'-DDT	p,p'-DDT	MOC	Mirex	lipid
1	203.45	0.04	0.02	7.12	0.31	0.02	2.32	0.39	3.9	0.02	0.39	5.95
2	191.7	0.02	0.01	7.67	0.27	0.01	2.1	0.34	3.47	0.03	0.33	4.85
3	252.79	0.04	0.02	8.63	0.2	0.02	2.64	0.46	4.07	0.05	0.47	4.85
STD	32.42	0.01	0.01	0.76	0.06	0.01	0.27	0.06	0.31	0.02	0.07	0.64
Mean	215.98	0.03	0.02	7.81	0.26	0.02	2.35	0.40	3.81	0.03	0.40	5.22
R STD	15.0	34.6	34.6	9.79	21.4	34.6	11.5	15.2	8.11	45.8	17.7	12.2

Table 2-3 (3): Repeated 3 times of the breast milk sub-sample coded 0202026

No.	PeCB	α-HCH	β-HCH	γ-HCH	δ-HCH	PCA	HeCB	OCS	OXC	c-HE	o,p'-DDE
1	0.49	0.12	15.9	1.67	0.21	0.15	12.3	0.16	3.72	1.8	0.04
2	0.21	0.08	16.4	0.56	0.04	0.02	11.6	0.13	1.52	1.09	0.03
3	0.62	0.11	16.9	0.91	0.04	0.14	12.4	0.27	3.17	2.17	0.09
STD	0.21	0.02	0.51	0.57	0.1	0.07	0.45	0.07	1.15	0.55	0.03
Mean	0.44	0.10	16.4	1.05	0.1	0.1	12.1	0.19	2.80	1.69	0.05
R STD	47.6	20.1	3.09	54.2	102	70	3.76	39.5	40.8	32.5	60.3

Table 2-3 (3): Repeated 3 times of the breast milk sub-sample coded 0202026 (continue)

No.	p,p'-DDE	t-CHL	c-CHL	END-1	p,p'-DDD	o,p'-DDD	Dieldrin	o,p'-DDT	p,p'-DDT	MOC	Mirex	lipid
1	103.24	0.02	0.01	2.52	0.22	0.01	2.99	0.7	6.18	0.08	0.06	0.84
2	126.83	n.d.	n.d.	2.07	0.27	0.01	1.56	0.54	6.35	0.08	0.04	1
3	79.62	0.03	n.d.	3.26	0.34	0.06	3.26	0.87	6.66	0.19	0.1	0.91
STD	23.60	0.01		0.60	0.06	0.03	0.91	0.17	0.24	0.06	0.03	0.08
Mean	103.23	0.025	0.01	2.62	0.28	0.03	2.60	0.70	6.40	0.12	0.07	0.92
R STD	22.9	28.3		22.9	21.8	108	35.1	23.5	3.80	54.4	45.8	8.75

Table 2-3 (4): Repeated 3 times of the breast milk sub-sample coded 0202029

No.	PeCB	α-HCH	β-HCH	γ-HCH	δ-HCH	PCA	HeCB	OCS	OXC	c-HE	o,p'-DDE
1	0.21	0.15	9.84	0.51	0.02	0.08	6.92	0.15	2.98	1.85	0.05
2	0.22	0.22	10.6	0.33	n.d.	0.02	7.66	0.08	2.82	2.01	0.08
Mean	0.22	0.19	10.2	0.42	0.02	0.05	7.29	0.12	2.9	1.93	0.07

Table 2-3 (4): Repeated 3 times of the breast milk sub-sample coded 0202029 (continue)

No.	p,p'-DDE	t-CHL	c-CHL	END-1	p,p'-DDD	o,p'-DDD	Diel	o,p'-DDT	p,p'-DDT	MOC	Mir	lipid
1	69.91	0.03	0.03	3.66	0.19	0.02	3.11	0.31	2.37	0.05	0.35	3.08
2	61.95	0.02	0.03	2.19	0.58	0.21	3.19	0.42	3.5	0.37	0.52	3.63
Mean	65.93	0.03	0.03	2.93	0.39	0.12	3.15	0.37	2.94	0.21	0.44	3.36

All compounds are expressed in ng/g lipid and lipid content in g/g 100% in wet weight

2-4: Intra- and inter-laboratory comparison of the lipid data

Because the final data used to evaluate the exposure levels of the two cohorts are normalized using lipid data. Lipid determination has to be decided carefully. The robustness of the method for lipid determination was checked via intra-laboratory and inter-laboratory studies. The fat contents of paired placenta sub-samples (N = 2 × 15) and milk sample (N = 9) were applied for intra-laboratory evaluation. Assumed the paired sub-samples homogenized well, theoretically, their lipid contents should be having the same values (Y = X). Factually, both homogenized procedure for sample aliquot and the lipid determination at each time some uncertainties could have been introduced. When using the ideal model to fit the data, the inter-laboratory residuals of milk samples (N = 2 × 65) are 0.11 (±0.05) with the average of the relative errors (Y-residual / Y-prediction) are 12.92% (STD 10.53%), and the intra-laboratory residuals are 0.03 (±0.03) with the average of the relative errors 5.11% (STD 4.06%). This suggested that, generally, the detection results could be predicated between two laboratories of Kuopio and Munich (95% confidence), except some outlier data (Fig 2-4 (1), (2)).

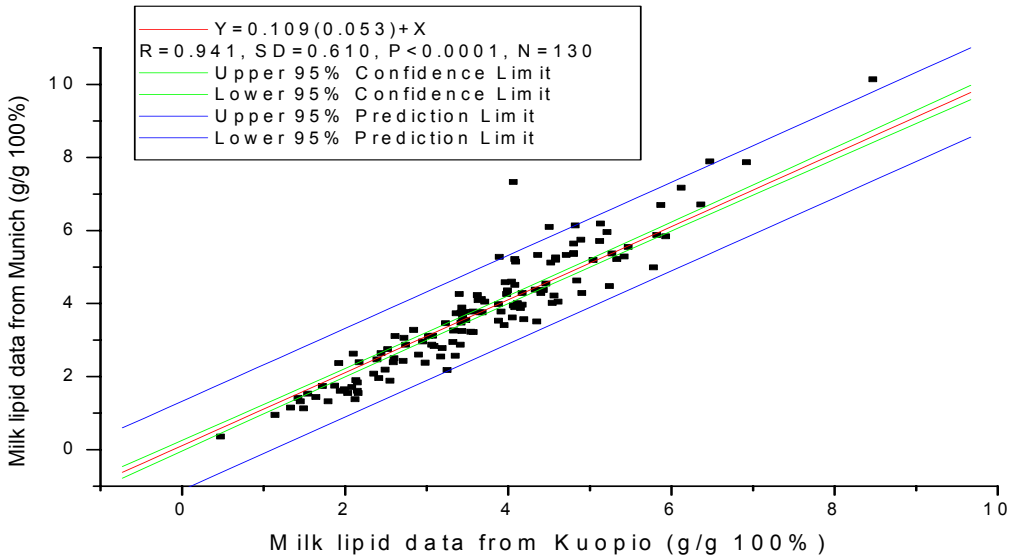


Fig. 2-4 (1): The inter-laboratory lipid data comparison between paired sub-samples of Finland and Denmark breast milk samples (65 samples for each cohort).

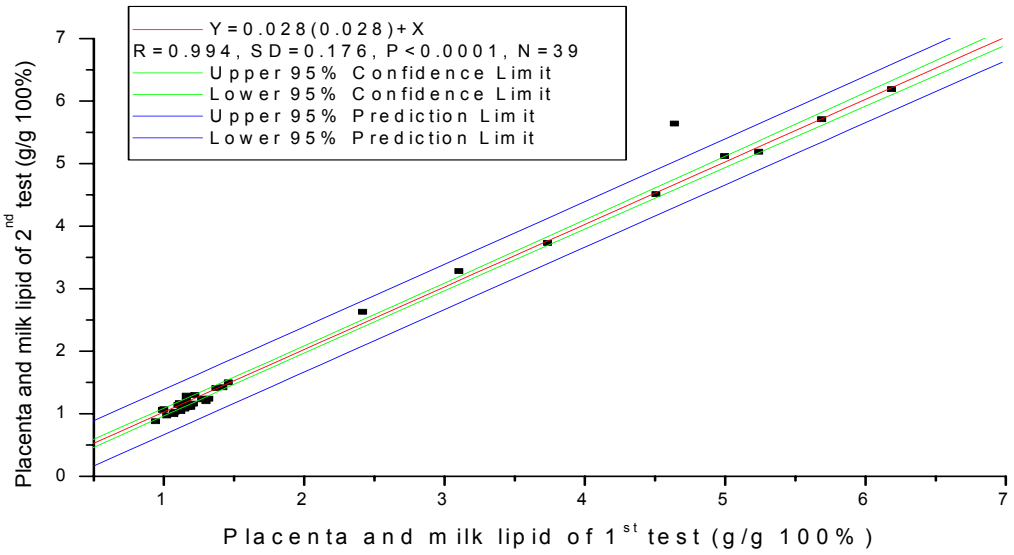


Fig. 2-4 (2): The intra-laboratory lipid data comparison between paired sub-samples of Finland and Denmark breast placenta samples (15 placenta samples for each cohort and 9 milk from Finland cohort).

3-1: Lipid comparison between Denmark and Finland cohorts

3-1-1: Lipid contents of placenta and milk samples in the two cohorts

Comparing the documented data of mother samples (DeKoning et al. 2000), it seems that the lipid data of Finland cohort for both placenta and breast milk samples are more close to the reference data (Table 3-1-1 (1)) than Denmark cohort. The calculated results (g/g 100% of lipid / wet weight) of Finland placenta samples are 1.21 for geometric mean, average value 1.22 with standard deviation (STD) = 0.13 and range from 0.93 to 1.52; Denmark placenta samples are geometric mean 1.07, average value 1.09 with STD = 0.17 and changed from 0.55 to 1.50; Finland breast milk samples are 4.24 of geometric mean, 4.52 average value with STD = 1.56 and range 0.95-10.14; Denmark breast milk samples are only 2.66 of geometric mean, 2.99 of average value with STD 1.38 and range 0.36-7.33. Fig. 3-1-1 (1) and Fig. 3-1-1 (2) give more detail information of the distributions of lipid data. It should be concluded that Danish cohort has lower lipid contents than Finnish cohort.

Table 3-1-1 (1): Some reference data of mother samples (DeKoning et al., 2000)

Specific density	Blood = 1.05	Plasma =1.027	Serum = 1.026
Total lipids in blood (g/l serum or plasma)	Cord blood = 3.47	Non-pregnant blood female = 6.17	Pregnant blood female = 9.0
Total lipids in breast milk	45.4 g/l (4.5%)		
Total lipids in placenta	1-1.5% by weight (wet)		
Fat content in foetus	12% by weight		
Composition of blood	45% cells	55% serum	

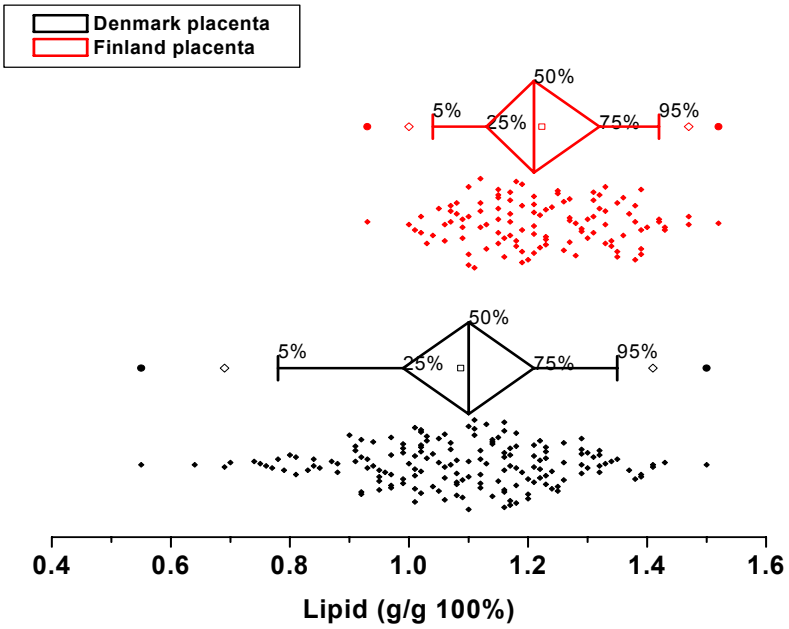


Fig. 3-1-1 (1): Comparing the distribution of placenta lipid data of the two cohorts.

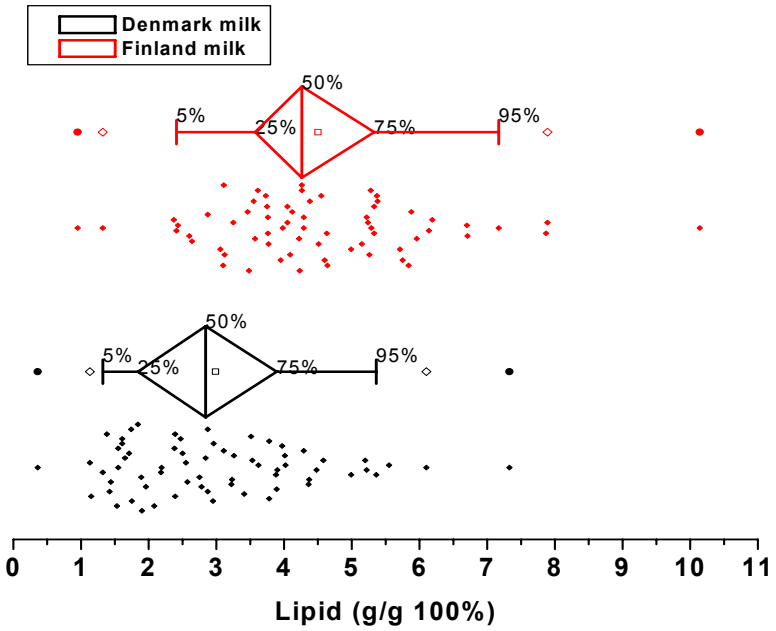
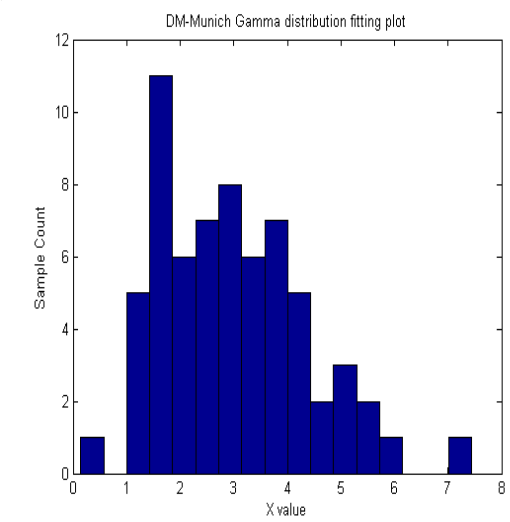


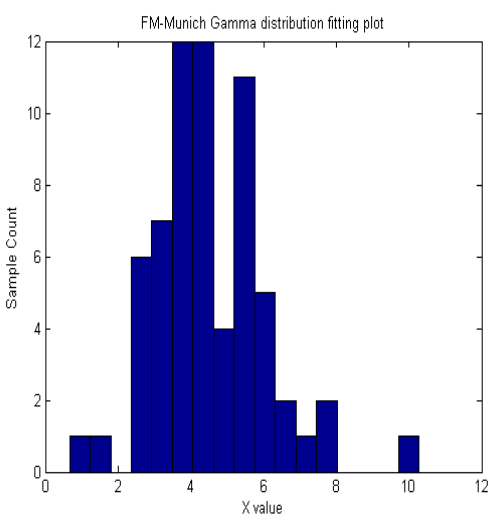
Fig. 3-1-1 (2): Comparing the distribution of breast milk lipid data of the two cohorts.

Note of figures: Diamond box represents percentile ranged from 25% to 75% and whisker from 5% to 95%; Maximum and minimum values are signed by ●, 99% and 1% values by ◇, and mean value by □.

3-1-2 Distributions of the lipid data

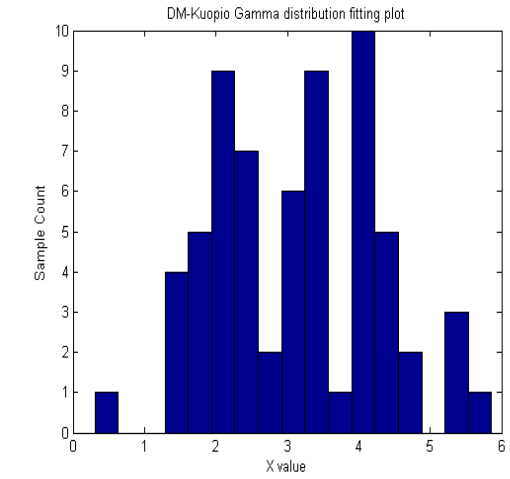
The best fitting distribution of DM-Kuopio and DP-Munich data were Weibull distribution; FM-Kuopio data were Normal distribution with higher probabilities than Gamma distribution. The best fitting of the pooled milk data M-Kuopio and placenta data P-Munich were Normal distribution and M-Munich data were Weibull distribution (Table 3-1-2 –(1)). Also all of them can be described as the normal distribution with fewer probabilities than the best fitting distributions (Table 3-2-2 (2)). In other words, all of them could be assumed as normal distributions and cannot be refused when tested by Kolmogorov-Smirnov method. This suggested the lipid data distributions are all near to normal ones and might suggested a representative sampling processes. For comparing the distribution analysis of compounds (next chapters), these lipid data were also assumed as the Gamma distribution and the fitting results suggested the distribution shape parameters' and scale parameters' changing trend between the two cohorts was similar in milk samples and placenta samples (Table 3-1-2 –(1)).

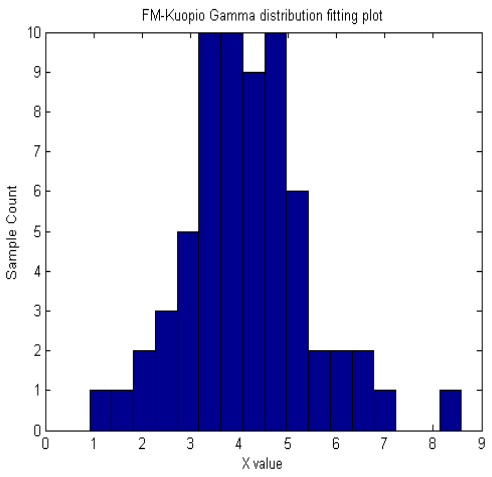




Denmark breast milk lipid investigated in Munich

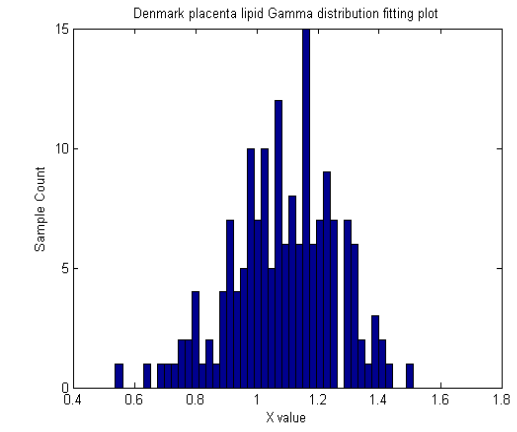
Finland breast milk lipid investigated in Munich

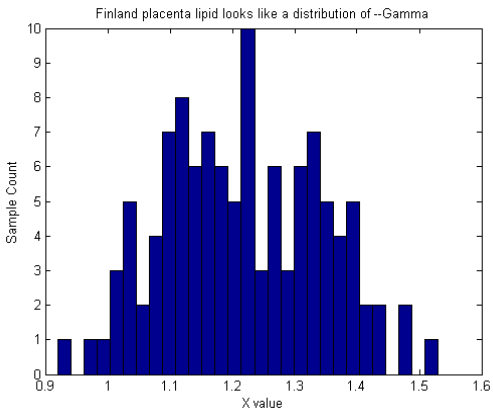




Denmark breast milk lipid investigated in Kuopio

Finland breast milk lipid investigated in Kuopio





Denmark placenta lipid investigated in Munich

Finland placenta lipid investigated in Munich

Fig. 3-1-2 (1): Histograms of the lipid data grouped by different laboratories, sample types and cohorts

Table 3-1-2 (1): The estimated distribution types and parameters of the fat content of breast milk and placenta samples of the two cohorts

Gamma Fit	DM-Kuopio		DM-Munich		FM-Kuopio		FM-Munich		DP-Munich		FP-Munich	
	γ (a)	γ (b)	γ (a)	γ (b)	γ (a)	γ (b)	γ (a)	γ (b)	γ (a)	γ (b)	γ (a)	γ (b)
Parameter	6.415	0.489	4.470	0.668	9.590	0.434	7.903	0.572	37.921	0.029	92.974	0.013
Interval	4.569	0.324	3.084	0.438	7.056	0.307	5.957	0.416	30.595	0.023	63.066	0.009
	8.260	0.654	5.856	0.898	12.124	0.561	9.849	0.729	45.247	0.035	122.882	0.017
Probability	0.601		0.976		0.532		0.853		0.498		0.815	
SK	0.093		0.058		0.099		0.074		0.063		0.059	
Best Fit	DM-Kuopio		FM-Kuopio		DP-Munich		M-Kuopio		M- Munich		P-Munich	
	Weib (a)	Weib (b)	N (a)	N (b)	Weib (a)	Weib (b)	N (a)	N (b)	Weib (a)	Weib (b)	N (a)	N (b)
Parameter	0.023	2.989	4.160	1.286	0.340	7.329	3.647	1.322	0.031	2.407	1.139	0.167
Interval	0.003	2.359	3.836	1.101	0.257	6.440	3.414	1.175	0.014	2.118	1.119	0.153
	0.044	3.619	4.483	1.470	0.424	8.219	3.881	1.469	0.048	2.696	1.160	0.180
Probability	0.761		0.915		0.960		0.948		0.940		0.584	
SK	0.082		0.068		0.039		0.045		0.046		0.046	

Parameters were resulted from Maximum likelihood estimation (Rayleigh, Exponential, Poisson, Gamma, Normal, Continuous uniform and Weibull are the candidates of the distribution) and the distributions are tested by Kolmogorov-Smirnov test (Probability and SK value); formula (1) describe the probability density of Gamma distribution (*a* is so called shape parameter and *b* is scale parameter, location parameter is 0), (2) for Weibull distribution (b is so called shape parameter and $a = scale^{-b}$, location parameter is 0) and (3) for normal distribution (a is location parameter and b is scale parameter). γ (a) and γ (b) in the tables are the parameters of *a* and *b* in formulae of Gamma distribution; weib (a) and weib (b) in the tables are the parameters of *a* and *b* in formulae of Weibull distribution, N (a) and N (b) in the tables are the parameters of *a* and *b* in formulae of Normal distribution. Milk and placenta lipids of the two cohorts were also pooled for distribution fitting and the results showed in columns named M-Kuopio, M-Munich and P-Munich (Matlab 6.5).

$$f(x|a,b)=\frac{1}{b^a\Gamma(a)}x^{a-1}e^{-\frac{x}{b}} \qquad (1)$$

$$f(x|a,b)=abx^{b-1}e^{-ax^b}\mathrm{I}_{(0,\infty)}(x) \qquad (2)$$

$$f(x|a,b)=\frac{1}{b\sqrt{2\pi}}e^{\frac{-(x-a)^2}{2b^2}} \qquad (3)$$

3-2: Exposure patterns and levels of the investigated compounds

3-2-1: Overview of the investigated compounds

Usually, geometric mean is less affected by the outlier values of a set of samples, which means it can show the central tendency better that arithmetic value when some data deviated far from the central point, for instant, log-normalized data (Walker et al., 2003). The general exposure patterns are compared using their geometric mean data. Aldrin, *t*-HE, ε-HCH, and END-2 cannot be determined in most of the investigated samples of the two cohorts. δ-HCH, *c*-CHL, *t*-CHL, *o, p'*-DDE, *o, p'*-DDD, MOC cannot be detected in many samples or usually at the levels too low to be quantified. These compounds are not being considered in the following comparative analysis. α-HCH, γ-HCH, δ-HCH and PeCB showed similar pattern with decreasing levels FP > DP > DM > FM; PCA and *p, p'*-DDD also showed apparently different patterns, with higher placenta levels than breast milk levels. These quite different patterns of these compounds might reflect their lower stability or pronounces different biodegradation among the samples. The other factor might be the higher but differently distributed background values of these lower determination level compounds, especially for α-HCH, γ-HCH and PeCB. All of these factors might additionally cause pattern differences. Mirex levels decreased in order of FM > DM > FP > DP. All other compounds, especially the 8 most abundant compounds *p, p'*-DDE, β-HCH, HeCB, dieldrin, END-1, OXC, c-HE, *p, p'*-DDT (Fig. 3-2-1 (1) and Table 3-2-1 (1)) and also the low level compounds such as *o, p'*-DDT and OCS showed similar pattern (DM > FM > DP > FP). The pattern should be accepted as the characteristic pattern of the investigated sample type and cohort because almost all of the abundance of the investigated contaminants appears in this pattern. Except for the placenta lowest *o, p'*-DDT, they are all linearly correlated for paired placenta and breast milk samples in Finland cohort (the same correlation for mirex). The 8 most abundant compounds count 89%, 95%, 98%, and 98% (the geometric mean of each compound dividing the summary of their geometric means) for FP, DP, FM, DM, respectively, in the total of the investigated compounds. Generally, *p, p'*-DDE occupies the around half of the total amount of investigated pesticides and the compound covers the largesse part of the difference of the two cohort's exposure to these PBTs. The next abundant ones are HCH and PeCB. As a result, the geometric means of the investigated

compounds are 40.77, 70.68, 105.89 and 192.30 (ng/g lipid) for FP, DP, FM and DM respectively. In other words, generally, the Denmark cohort exposure level is 1.73 or 1.82 times higher than the Finland level when calculated from placenta or breast milk. Correlation analysis and principal component analysis focus mainly on the 8 compounds and the Finland paired compounds. The final profile of PBT residuals depends on the exposure sources (esp. net uptake from food) and the resistance to enzyme biodegradation of these compounds in human bodies.

Table 3-2-1 (1): Relative contents (fractions) of the 8 main components in the two cohorts

	Total *	p,p'-DDE	β -HCH	HeCB	OXC	c-HE	END-1	p,p'-DDT	Dieldrin
FP	40.77	0.44	0.12	0.11	0.02	0.02	0.04	0.01	0.03
FM	105.9	0.61	0.1	0.08	0.03	0.02	0.05	0.04	0.02
DP	70.68	0.58	0.12	0.11	0.01	0.01	0.03	0.01	0.03
DM	192.3	0.66	0.09	0.06	0.03	0.01	0.04	0.03	0.03

*Total exposure level (ng/g lipid) was the summary of geometric means of all investigated compounds, which were detectable in the two cohorts.

The level of total DDT had decreased much from 1570 (N = 49) or 2320 (no sample size) in 1974 (Smith 1999) to 699 (N = 50, recalculated using lipid data 4.5% g/g wet weight) in 1982 (Nasir et al., 1998), further to 570 (N = 165) in 1985 (Mussalo-Rauhamaa et al, 1988), and to the present data 84.06 ng/g lipid (N =65). In Denmark milk samples (N = 57), total DDT was 1150, total HCH 80, and dieldrin 40 ng/g lipid in 1982 (Nasir et al., 1998) (Table 3-2-1 (2)). The reported β-HCH, HeCB, dieldrin, *p, p'*-DDT, *p, p'*-DDE and total DDT were 116.43, 282.76, 33.10, 1123.69, 143.02, 1373.78, 1526.30 ng/g (recalculated the mean values of two time measurements) in serum of samples from 1976-1978 (Hoyer et al. 2000). Considering the possible sample specific difference (Table 3-2-1 (3)), the body burden of the DDT of Finland people could be lower than Denmark people at the beginning of the ban and the following clearance periods. Apparently, all the other investigated PBTs could also decrease with the year because of their ban (Table 3-2-1 (2)). The general levels of these PBTs have decreased in first order rate in human samples (Smith, 1999; Noren et al., 2000; Solomon et al., 2002) after the ban.

Table 3-2-1 (2): Comparison of PBT contents of the present data of the two cohorts with reported Finland milk samples data:

Mean (ng/g lipid)	*p, p'*-DDE	Total DDT	HCHs	HeCB	*t*-CHL[a]	*c*-CHL[a]	HC[a]	OXC	*c*-HE
Finland 1997-2001	79.04 (±57.81)	84.06 (±60.09)	12.46 (±5.38)	8.51 (±3.17)	0.04 (±0.03)	0.02 (±0.02)	0.23 (±0.34)	3.93 (±1.9)	2.37 (±2.06)
Denmark 1997-2001	148.78 (±84.93)	157.12 (±88.31)	21.86 (±13.06)	12.82 (±4.13)	0.06 (±0.05)	0.04 (±0.02)	-	3.08 (±1.59)	5.2 (±1.89)
Finland 1984-1985[b]	600 (±600)	660 (±640)	200 (±250)	80 (±60)	200 (±190)	100 (±60)	70 (±60)	230 (±210)	100 (±400)
Decline[c]	32.6	36	11.7	4.5	12.5	6.2	4.4	14.1	6.1

[a] Detection positive samples of *t*-CHL, *c*-CHL and HC for the present Finland cohort are 82, 46 and 5% and for the present Denmark cohort are 85, 40 and 0%; [b] Mussalo-Rauhamaa et al, 1988; The mean data for samples above the detection level 10 ng/g lipid; [c] Simply estimated decline rate per year (from 1985 to 2001).

Table 3-2-1 (3): Ratio of PBT distribution between tissue compartments on lipid basis

	Milk/ Adipose	[e]Placenta/ Milk	Placenta/ Ma serum	Cord plas/ Ma plas	Cord serum/ Milk	Ma serum/ Milk
β-HCH	0.43[d]	0.51	0.48[a]	0.70 [b]	0.061[c]	0.157[c]
HeCB	0.43 (0.50)[d]	0.63	0.99[a]	0.91 [b]		
p,p'-DDT	0.53 [b]	0.09	t0.45[a]	0.41 [b]	0.165[c]	0.525[c]
p,p'-DDE	0.91[d]	0.33		0.94 [b]	0.016[c]	0.042[c]
c-HE		0.36				
END-1		0.46				
OXC		0.36		0.74 [b]		
Mirex		0.81		0.19 [b]		

[a]Schlebusch et al., 1994; [b]Walker et al., 2003; [c]Nair et al., 1996; [d]Waliszewski et al., 1999; 2001; [e]Paresent data (slope of regression line); Maternal: Ma; Plasma: Plas

3-2-2: Distribution types of the investigated compounds

The distribution profiles of the 8 compounds are well characterized respectively in placenta samples and breast milk samples, and interestingly, for all compounds, the profile was quite similar for placenta and breast milk (Fig. 3-2-2 (2)). This information should also be a reflection of the similar life habits of the investigated cohort members. Comparing the lipid data, which could be accepted as normal distributed at higher probabilities, the probabilities of the 8 compounds distribution as normal types were lower, except END-1 and HeCB in DM samples (Table 3-2-2 (1) and (2)). The best fitting distributions for the investigated compounds were listed (Table 3-2-2 (3)) and most of them were Gamma distributed. The deviation from the normal distribution depicts

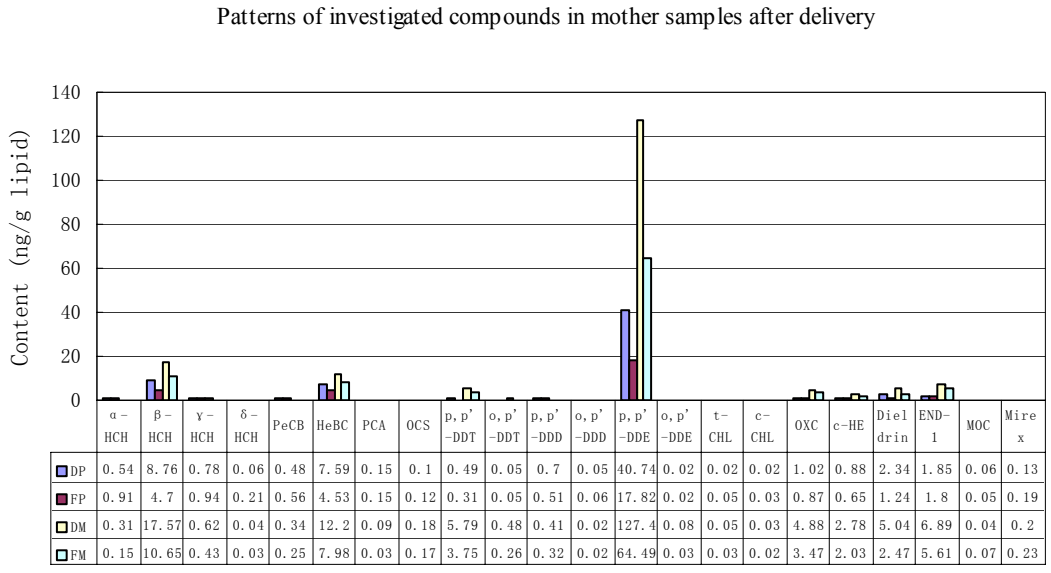
coactions of more than one independent random factor on the PBT bioaccumulation in human bodies.

Table 3-2-2 (1): Normal-fitting probability of the 8 compounds in the two cohorts samples

Normal fitting	β-HCH	HeCB	OXC	c-HE	p,p'-DDE	p,p'-DDT	END-1	Dieldrin
Probability -DM	-	0.66	0.46	0.11	0.29	-	0.83	-
SK value-DM	-	0.09	0.10	0.15	0.12	-	0.08	-
Probability -FM	0.17	-	0.32	-	0.05	-	0.18	-
SK value-FM	0.14	-	0.12	-	0.16	-	0.13	-
Probability -DP	-	-	0.13	0.06	-	-	0.12	-
SK value-DP	-	-	0.09	0.10	-	-	0.09	-
Probability -FP	-	0.15	0.07	0.10	-	-	-	0.09
SK value-FP	-	0.11	0.12	0.11	-	-	-	0.12

Table 3-2-2 (2): Normal-fitting probability of the lipid data of the two cohorts

Normal fitting	DM-Kuopio	DM-Munich	FM-Kuopio	FM-Munich	DP-Munich	FP-Munich
Probability	0.58	0.77	0.92	0.53	0.73	0.81
SK value	0.09	0.08	0.07	0.10	0.05	0.06



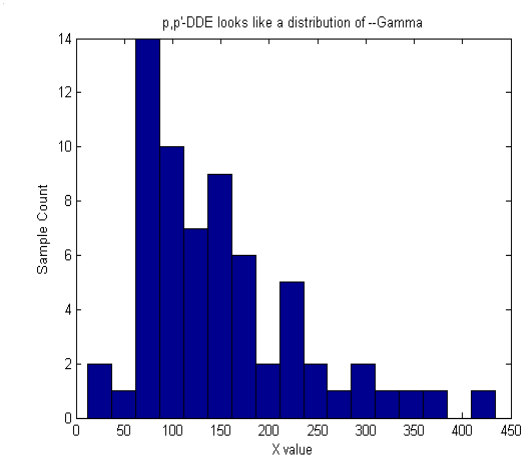
	α-HCH	β-HCH	γ-HCH	δ-HCH	PeCB	HeBC	PCA	OCS	p,p'-DDT	o,p'-DDT	p,p'-DDD	o,p'-DDD	p,p'-DDE	o,p'-DDE	t-CHL	c-CHL	OXC	c-HE	Dieldrin	END-1	MOC	Mirex
DP	0.54	8.76	0.78	0.06	0.48	7.59	0.15	0.1	0.49	0.05	0.7	0.05	40.74	0.02	0.02	0.02	1.02	0.88	2.34	1.85	0.06	0.13
FP	0.91	4.7	0.94	0.21	0.56	4.53	0.15	0.12	0.31	0.05	0.51	0.06	17.82	0.02	0.05	0.03	0.87	0.65	1.24	1.8	0.05	0.19
DM	0.31	17.57	0.62	0.04	0.34	12.2	0.09	0.18	5.79	0.48	0.41	0.02	127.4	0.08	0.05	0.03	4.88	2.78	5.04	6.89	0.04	0.2
FM	0.15	10.65	0.43	0.03	0.25	7.98	0.03	0.17	3.75	0.26	0.32	0.02	64.49	0.03	0.03	0.02	3.47	2.03	2.47	5.61	0.07	0.23

Fig 3-2-1 (1): The profile of the investigated compounds in placenta and breast milk samples (DP, Denmark placenta; FP, Finland placenta; DM, Denmark milk and FM, Finland milk)

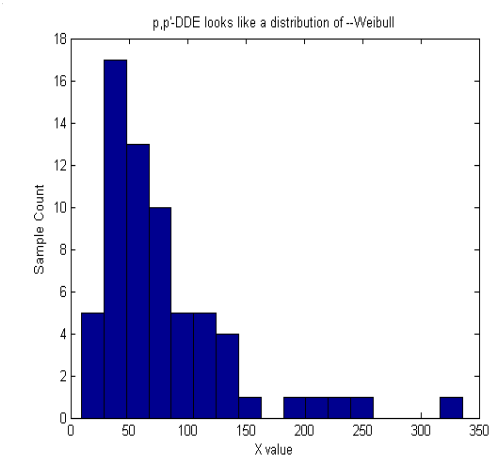
Table 3-2-2 (3): The Gamma distribution fitting parameters of the 8 abundant compounds in breast milk and placenta samples of the two cohorts

	β -HCH		HeCB		OXC		*c*-HE		*p, p'*-DDE		*p, p'*-DDT		END-1		Dieldrin	
	γ (a)	γ (b)	γ (a)	γ (b)	γ (a)	γ (b)	γ (a)	γ (b)	γ (a)	γ (b)	γ (a)	γ (b)	γ (a)	γ (b)	γ (a)	γ (b)
DM Parameter	3.353	6.129	10.204	1.256	7.976	0.652	4.982	0.619	3.391	43.862	2.461	2.921	4.703	1.636	3.542	1.649
Interval	2.004	4.005	6.140	0.762	5.096	0.417	3.435	0.452	2.178	28.097	1.584	2.082	2.963	0.992	2.575	1.351
	4.701	8.254	14.268	1.750	10.855	0.887	6.529	0.786	4.604	59.626	3.339	3.761	6.443	2.281	4.509	1.947
Probability	0.505		0.963		0.957		0.637		0.794		0.239		0.980		0.267	
SK value	0.101		0.061		0.062		0.091		0.079		0.126		0.057		0.122	
FM Parameter	5.605	2.083	7.952	1.070	4.182	0.939	3.326	0.714	2.613	30.246	4.082	1.044	2.400	2.921	4.323	0.644
Interval	3.963	1.485	5.616	0.764	2.790	0.599	2.455	0.605	1.586	20.102	2.540	0.701	1.406	1.675	2.628	0.412
	7.248	2.682	10.287	1.377	5.574	1.280	4.196	0.823	3.641	40.391	5.623	1.388	3.393	4.167	6.018	0.876
Probability	0.797		0.101		0.749		0.065		0.494		0.196		0.720		0.426	
SK value	0.079		0.149		0.083		0.160		0.101		0.131		0.085		0.107	
DP Parameter	4.234	2.340	9.705	0.824	3.400	0.350	4.359	0.228	3.474	13.638			3.227	0.675	3.343	0.819
Interval	3.468	2.013	8.265	0.710	2.662	0.271	3.549	0.182	2.844	11.475			2.460	0.495	2.784	0.715
	5.001	2.667	11.146	0.939	4.137	0.429	5.170	0.274	4.104	15.801			3.994	0.854	3.902	0.922
Probability	0.142		0.419		0.836		0.964		0.570				0.521		0.221	
SK value	0.088		0.067		0.047		0.038		0.060				0.062		0.052	
FP Parameter			7.911	0.611	3.443	0.294	5.938	0.119	2.558	8.581			2.375	0.951	4.666	0.298
Interval			6.086	0.479	2.355	0.199	4.453	0.090	1.785	6.169			1.691	0.690	3.449	0.224
			9.735	0.743	4.531	0.388	7.422	0.148	3.331	10.992			3.059	1.212	5.883	0.371
Probability			0.767		0.880		0.653		0.769				0.441		0.899	
SK value			0.062		0.055		0.069		0.062				0.081		0.053	

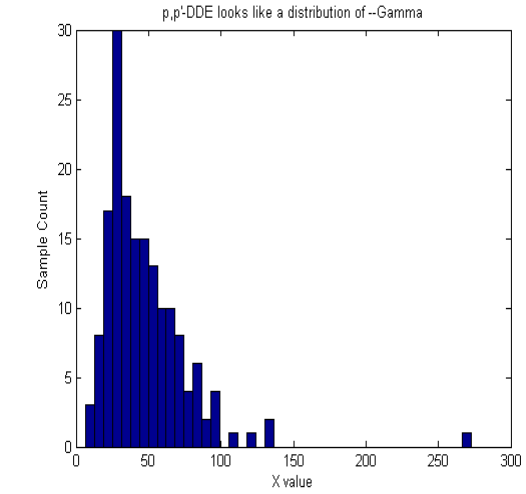
Note: the best fitting distributions for FM *p, p'*-DDE, END-1 and DP END-1 are weibull distribution with parameters Weib (a) 0.001, 0.037 and 0.170; Weib (b) 1.544, 1.600 and 1.967; probability 0.552, 0.737 and 0.738 and SK value 0.097, 0.084 and 0.052



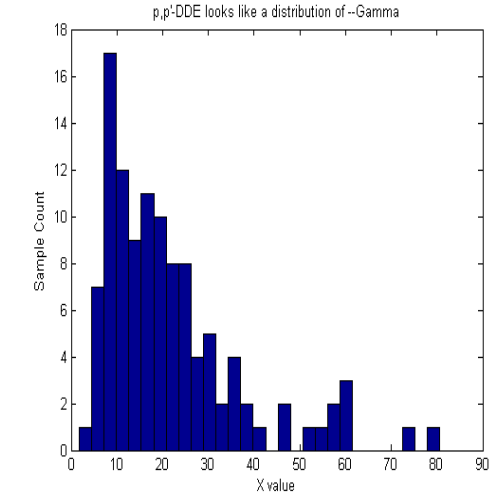
Distribution of *p, p'*-DDE in DM samples



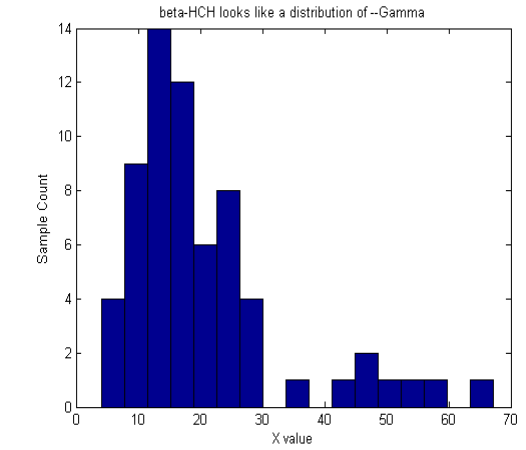
Distribution of *p, p'*-DDE in FM samples



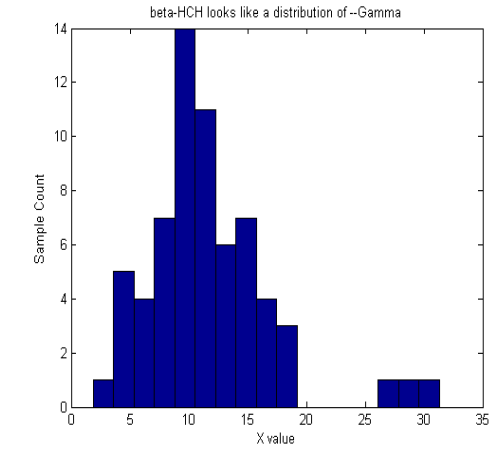
Distribution of *p, p'*-DDE in DP samples



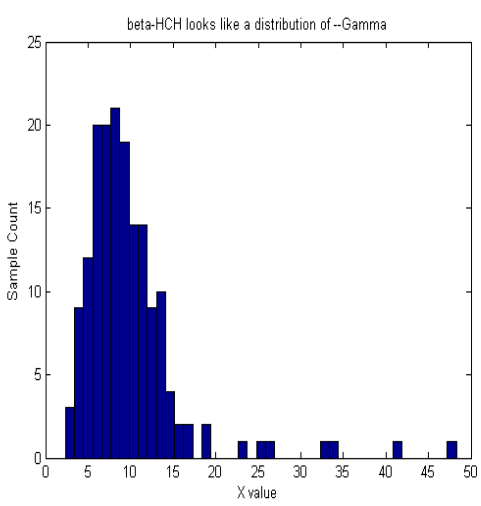
Distribution of *p, p'*-DDE in FP samples



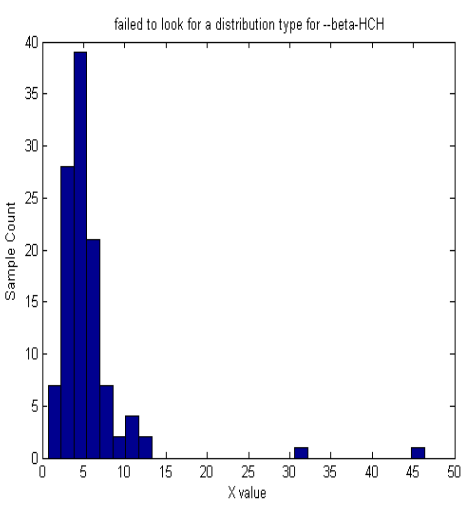
Distribution of β-HCH in DM samples



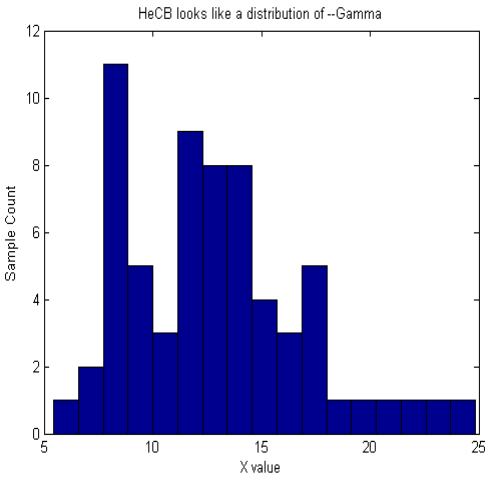
Distribution of β-HCH in FM samples



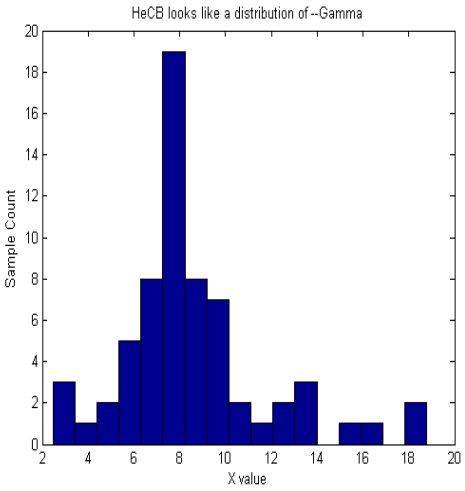
Distribution of β-HCH in DP samples



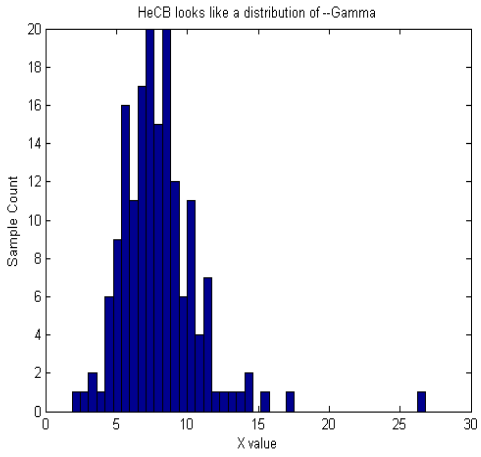
Distribution of β-HCH in FP samples



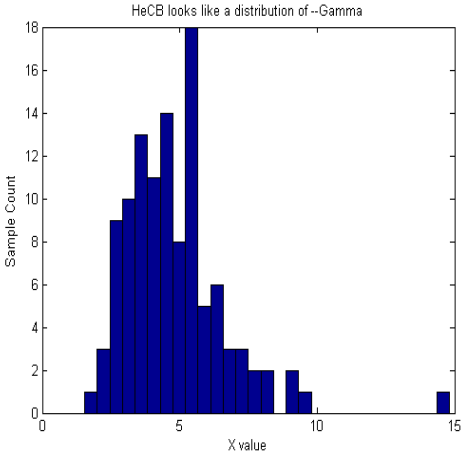
Distribution of HeCB in DM samples



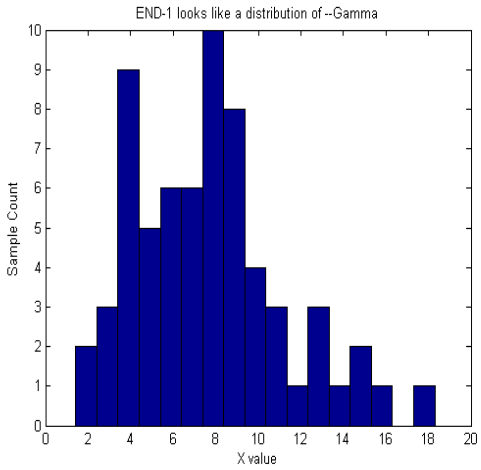
Distribution of HeCB in FM samples



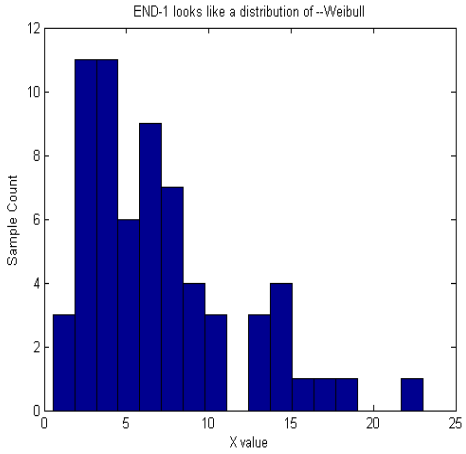
Distribution of HeCB in DP samples



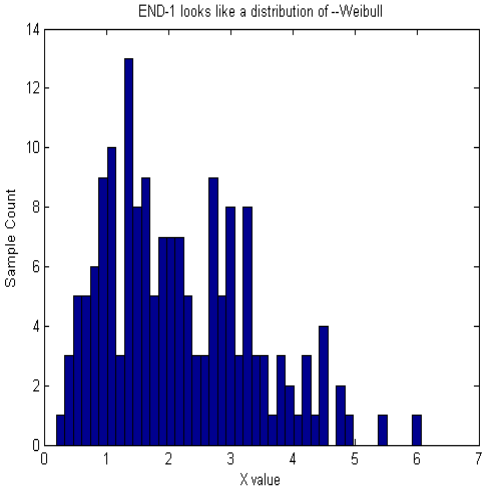
Distribution of HeCB in FP samples



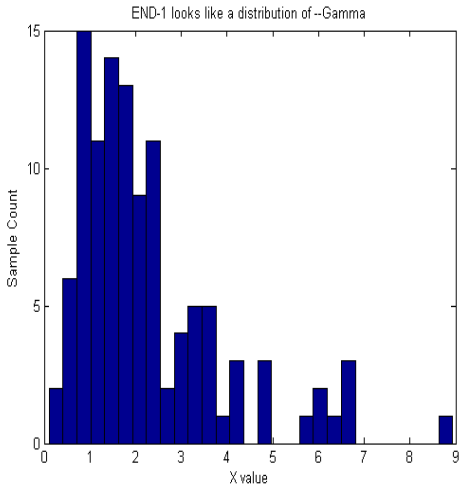
Distribution of END-1 in DM samples



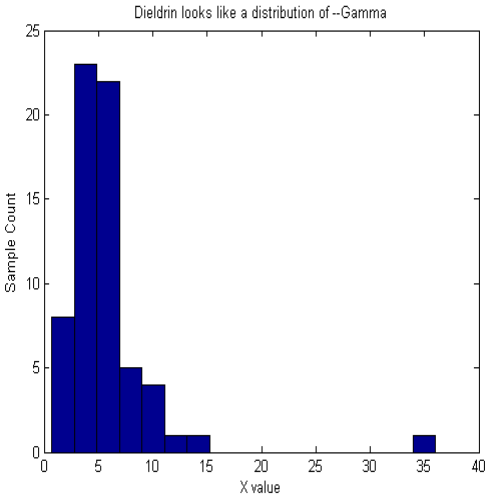
Distribution of END-1 in FM samples



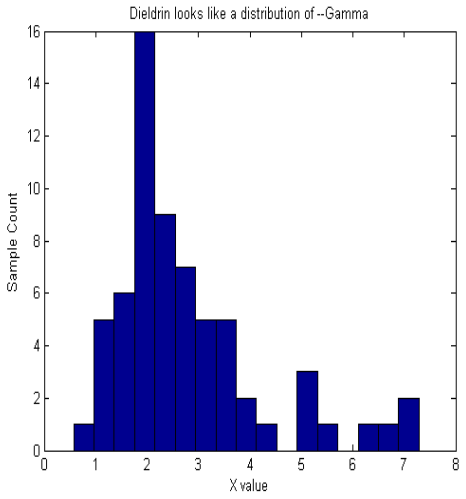
Distribution of END-1 in DP samples



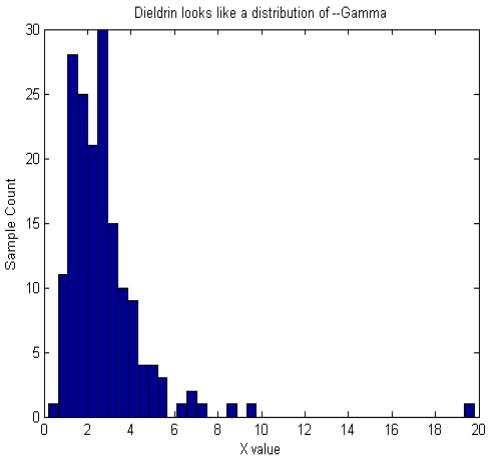
Distribution of END-1 in FP samples



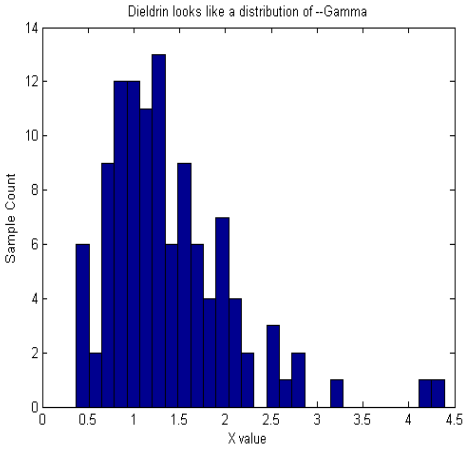
Distribution of dieldrin in DM samples



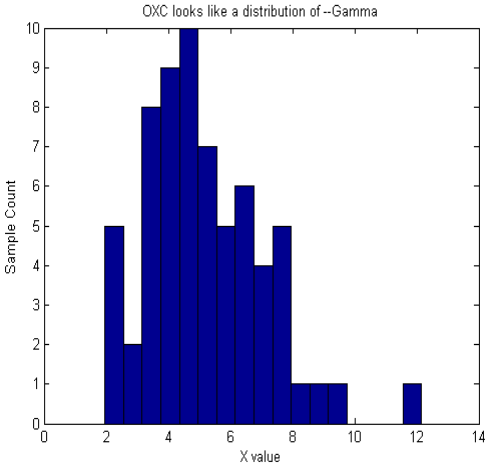
Distribution of dieldrin in FM samples



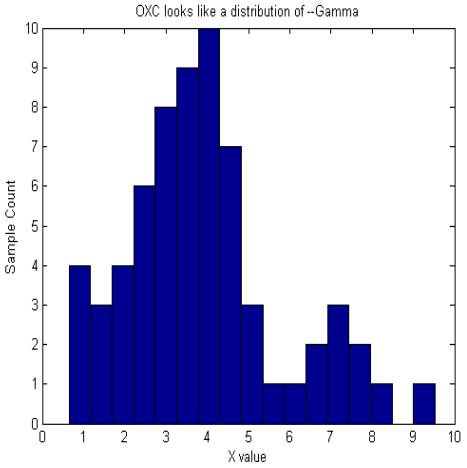
Distribution of dieldrin in DP samples



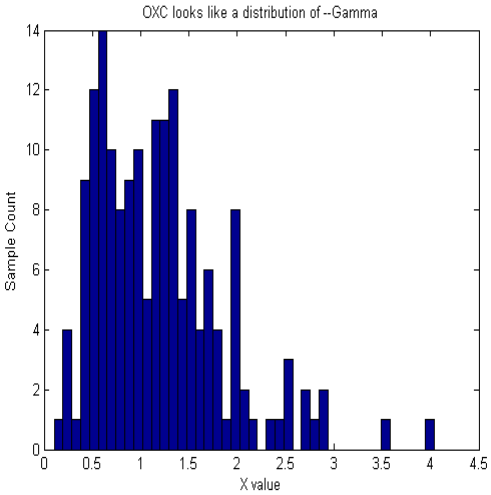
Distribution of dieldrin in FP samples



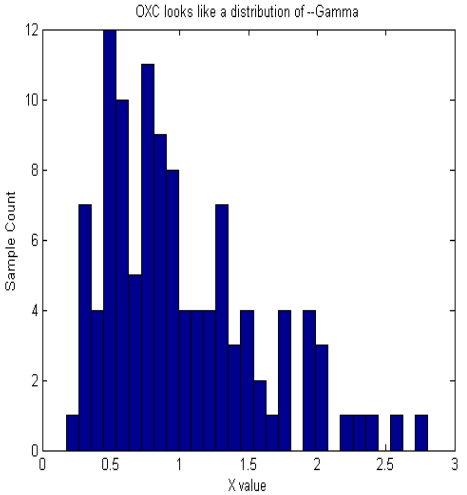
Distribution of OXC in DM samples



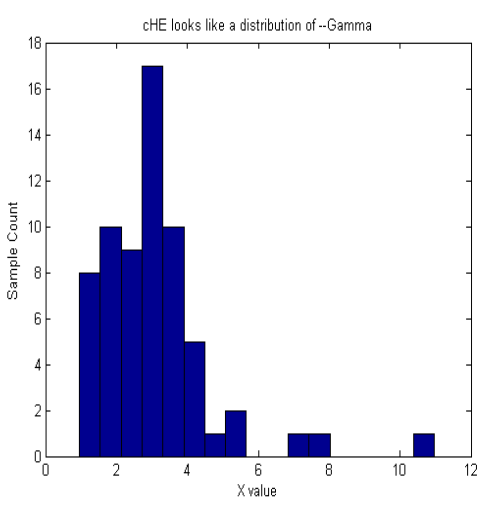
Distribution of OXC in FM samples



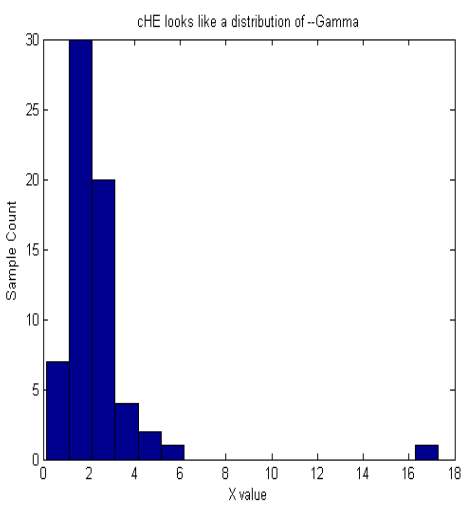
Distribution of OXC in DP samples



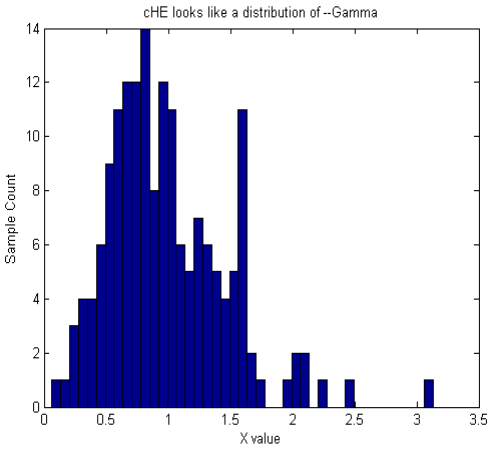
Distribution of OXC in FP samples



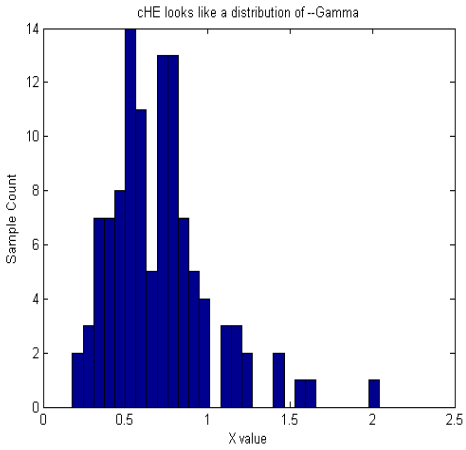
Distribution of *c*-HE in DM samples



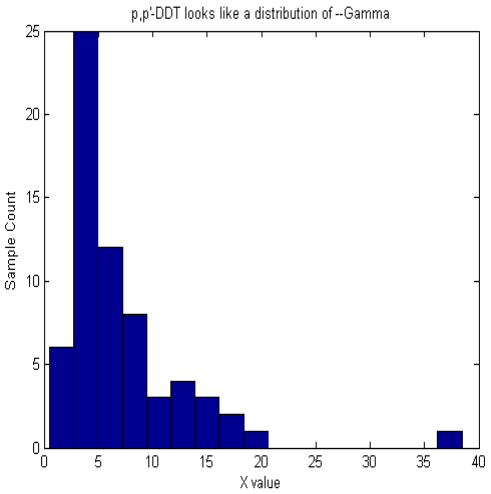
Distribution of *c*-HE in FM samples



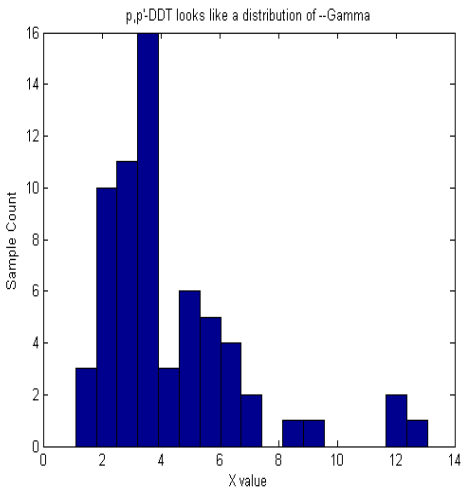
Distribution of *c*-HE in DP samples



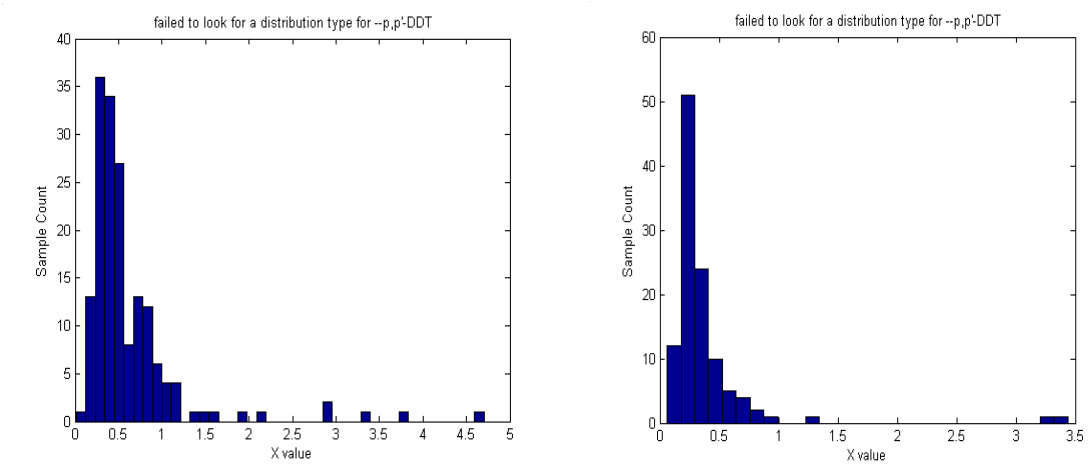
Distribution of *c*-HE in FP samples



Distribution of *p, p'*-DDT in DM samples



Distribution of *p, p'*-DDT in FM samples



Distribution of *p, p'*-DDT in DP samples Distribution of *p, p'*-DDT in FP samples

Fig. 3-2-2 (1): Histograms of the 8 most abounding compound data grouped by different sample types and cohorts

3-3: Compounds correlations of the abundant pollutants

Not only showing apparent correlation in paired samples (chapter 3-5), the most abundant pollutants of *p, p'*-DDE, β-HCH, HeCB, dieldrin, END-1, OXC, *c*-HE and *p, p'*-DDT also exhibited some pronounced concentration correlations in DM, FM, DP and FP samples, respectively. These correlations supported the similar patterns of the 8 compounds in placenta and milk of the two cohorts. Additionally, it implies that most people face the similar types of sources of the pollutants in different levels. An interesting example is the content of END-1 and OXC, which are highly correlated in both types of samples in the two cohorts (Table 3-3 (1)). This might underpin the co-exposure pattern for these two compounds. In milk samples, there are more complete sets of correlated compounds than in placenta sample, because of higher concentrations of these compounds in milk (easy to quantify). By excluding some outlier data, the correlation range is broadened to the other compounds for the most of these samples (Fig. 3-3 (1), (2), (3), (4) and Fig. 3-4-1 (1)). The correlations for OXC with END-1 also occurred when milk and placenta samples are pooled. Additionally, the slope values for Denmark samples (1.69 and 1.54) were different from the ones for Finland samples (2.25 and 2.53), which might reflect some cohort specific character of OXC *vs.* END-1 exposure. This will be discussed deeply in the further PC analysis. The regression results for the pooled samples are compromised by the separated regressions (Table 3-3 (1)).

Table 3-3 (1): Correlations of OXC *vs.* END-1 in the two cohort samples

Sample	END-1 = Intercept (standard error) + Slope (standard error) * OXC	Correlation coefficient	SD	p-Value	N
DM	Y=-1.09(0.55)+1.69(0.10)*X	0.90	1.52	<0.0001	65
DP	Y=0.35(0.09)+1.54(0.07)*X	0.87	0.59	<0.0001	168
FM	Y=-1.83(0.57)+2.25(0.13)*X	0.91	1.99	<0.0001	65
FP	Y=-0.30(0.15)+2.53(0.13)*X	0.88	0.77	<0.0001	112
Pooled milk	Y=-0.98(0.45)+1.83(0.09)*X	0.87	2.03	<0.0001	130
Pooled Placenta	Y=0.18(0.09)+1.81(0.07)*X	0.83	0.75	<0.0001	280

SD: Standard deviation of fitting, here $SD = \sqrt{\sum_{i=1}^{N}(y_i - (a + bx_i))^2 \Big/ (N-2)}$

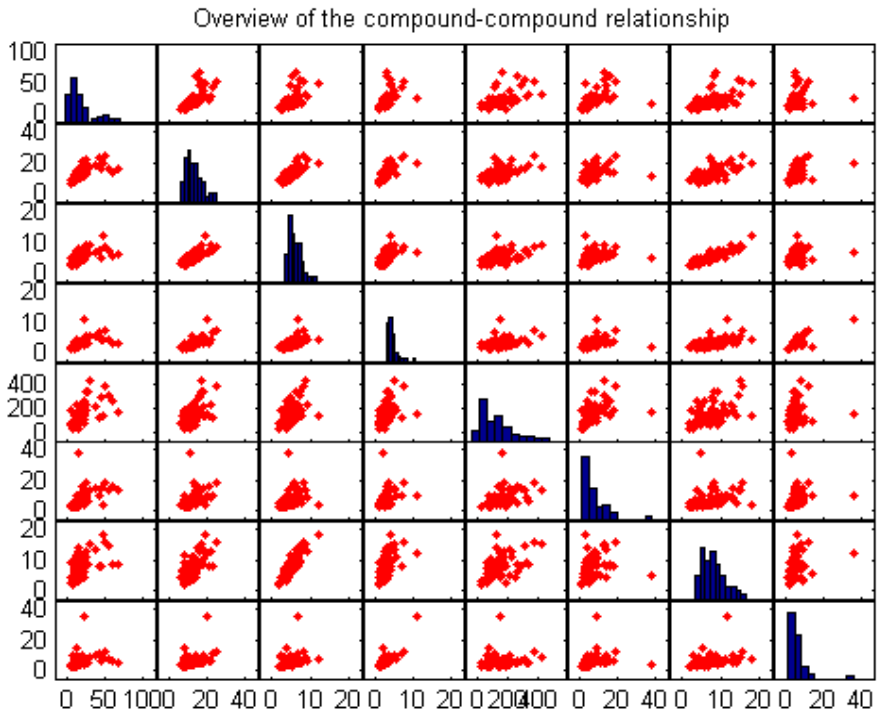


Fig. 3-3 (1): β-HCH HeCB OXC c-HE p,p'-DDE p,p'-DDT END-1 Dieldrin

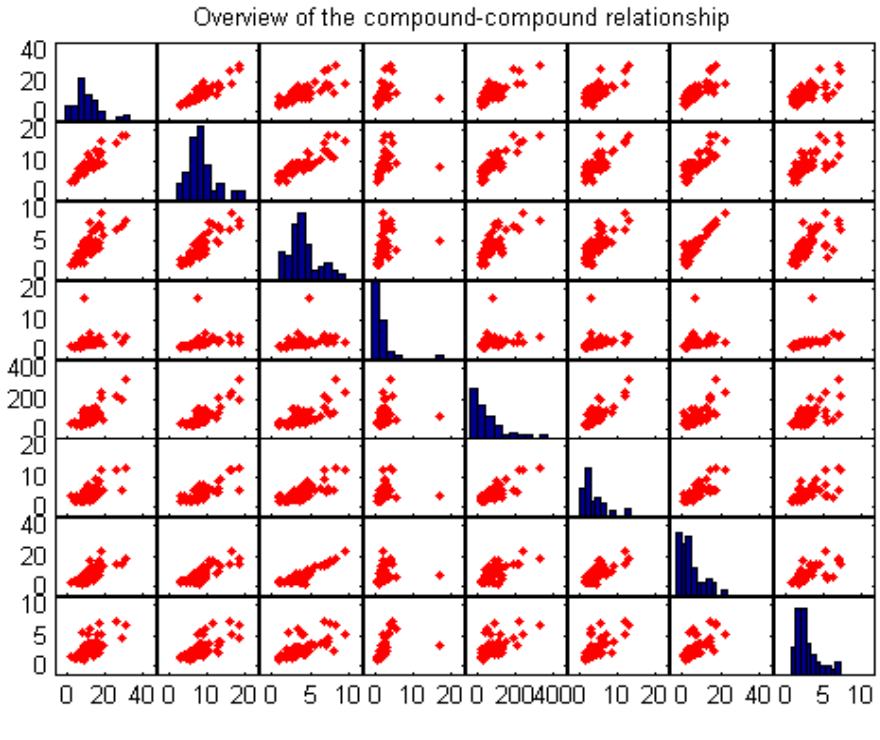


Fig. 3-3 (2): β-HCH HeCB OXC c-HE p,p'-DDE p,p'-DDT END-1 Dieldrin

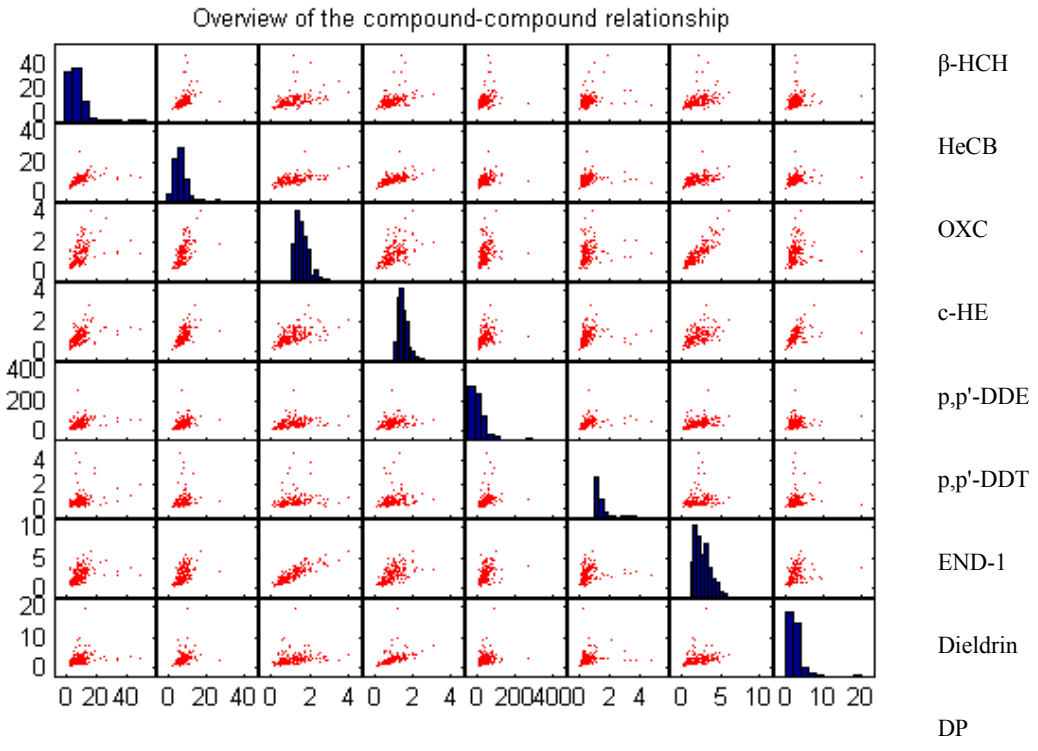


Fig. 3-3 (3): β-HCH HeCB OXC c-HE p,p'-DDE p,p'-DDT END-1 Dieldrin

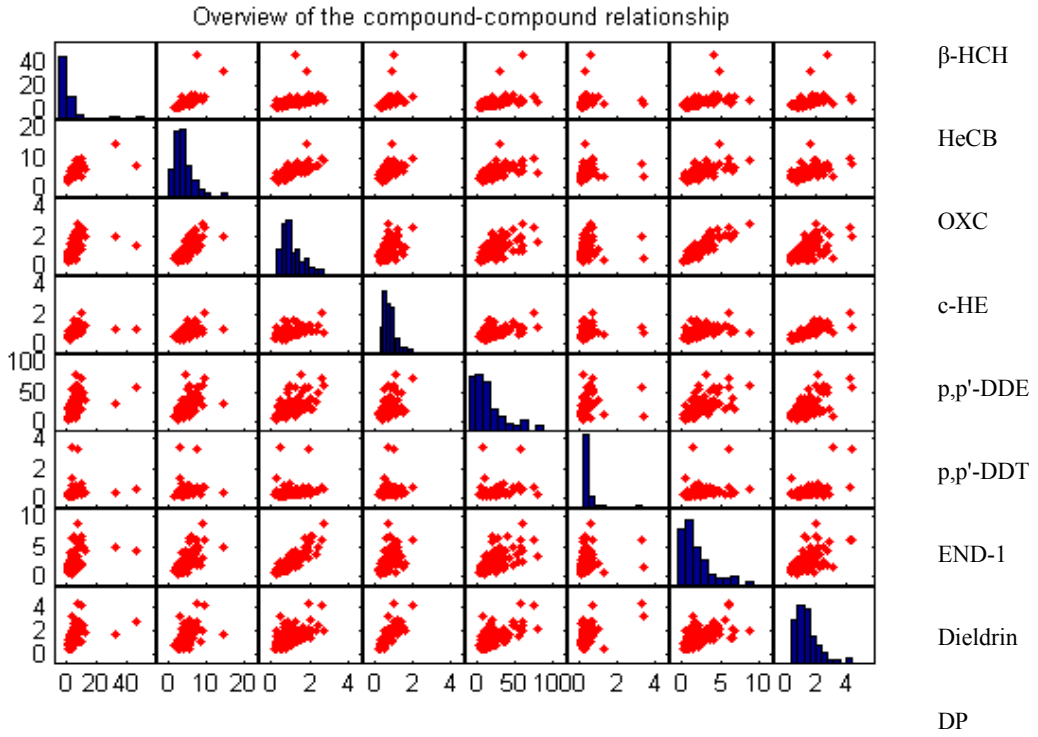


Fig. 3-3 (4): β-HCH HeCB OXC c-HE p,p'-DDE p,p'-DDT END-1 Dieldrin

3-4:What the PC analysis tell

3-4-1: PC analysis for DM, DP, FM and FP

The compound scores characterize the investigated compounds in how far they similarly or differently contributed to the certain factors behind the variance of the set of samples. For example, the correlation of OXC with END-1 in DM, DP, FM and FP have been discussed and this relationship can also be reflected in compound scores for each PC. The apparent character was OXC and END-1 always occurred closely on the compound score figures, which showed that they have approximately the same values for 2nd, 3rd PC compound scores. Fig. 3-4-1 (4)-1 to 18 show the compound score plots and sample score plots of DM, DP, FM and FP samples.

The exposure pattern of every sample might be affected by one or more factors. The 1st PC interpreted 55.1%, 44.6%, 70.6% and 57.6% of the total variance of DM, DP, FM and FP samples, respectively. The 1st PC compound scores suggest that all of the 8 abundant compounds positively contribute to the variance gradient, which were recorded by 1st PC sample scores. That means, many samples, with the less variance 2nd, 3rd and 4th sample scores (i.e., approximately equal to 0), could be considered as similarly exposed to pollutant sources. For these samples, the relative contents for the 8 compounds were similar but at different concentration levels. These mothers could be expected to have contacted to similar pollutant sources at different extents, such as similar food feeding habits. These samples could be grouped dictatorially according to their 2nd, 3rd and 4th sample scores and their pollutant source pattern was the most common one, here we called the most common pollutant pattern. The concentrations of the 8 compounds are nearly correlated in linear ways, for instance, the 48 DP samples with the rounded 2nd, 3rd and 4th sample scores of 0 (round the data to integer) (Fig. 3-4-1 (1)). The proposed common exposure patterns were calculated as the absolute values of 1st PC compound scores multiplied the standard deviation of the related compound respectively (Fig 3-4-1(2)).

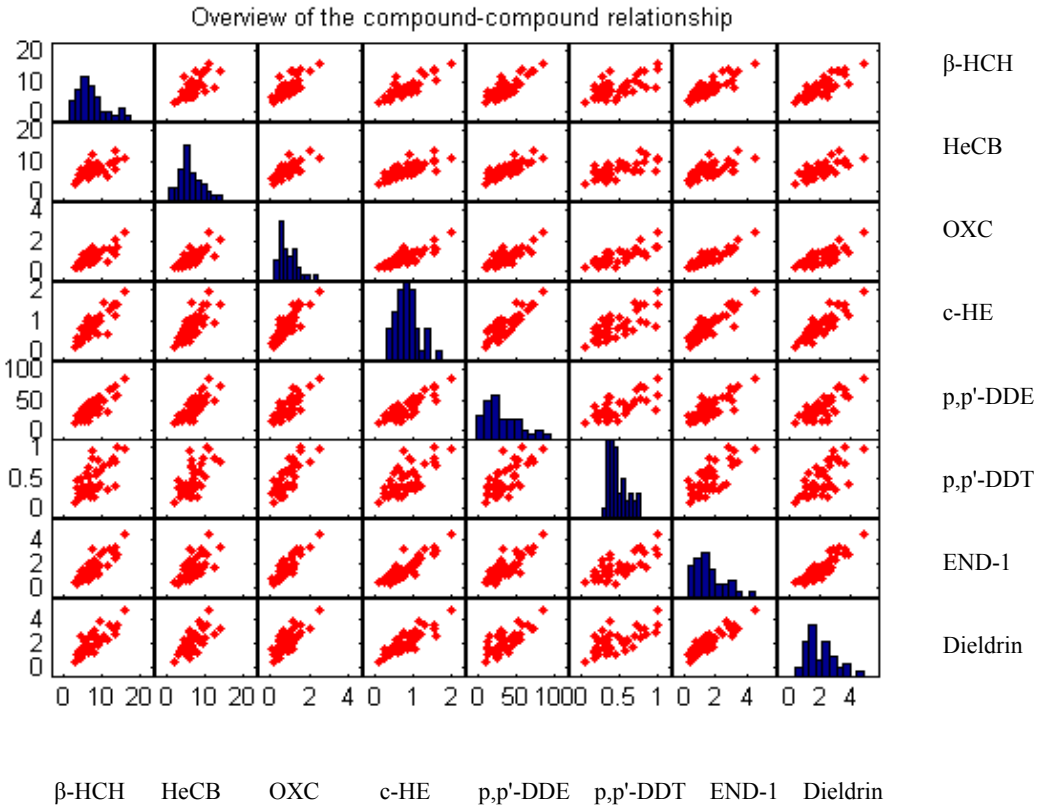
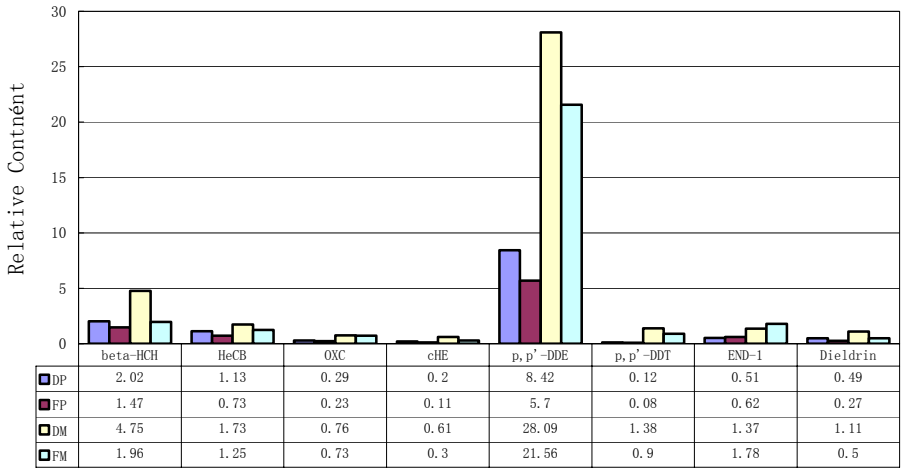


Fig. 3-4-1 (1): 48 DP samples with less variance of 2nd, 3rd and 4th PC sample scores (rounded to 0) showed linear correlation for each two the 8 compounds (ng/g lipid).

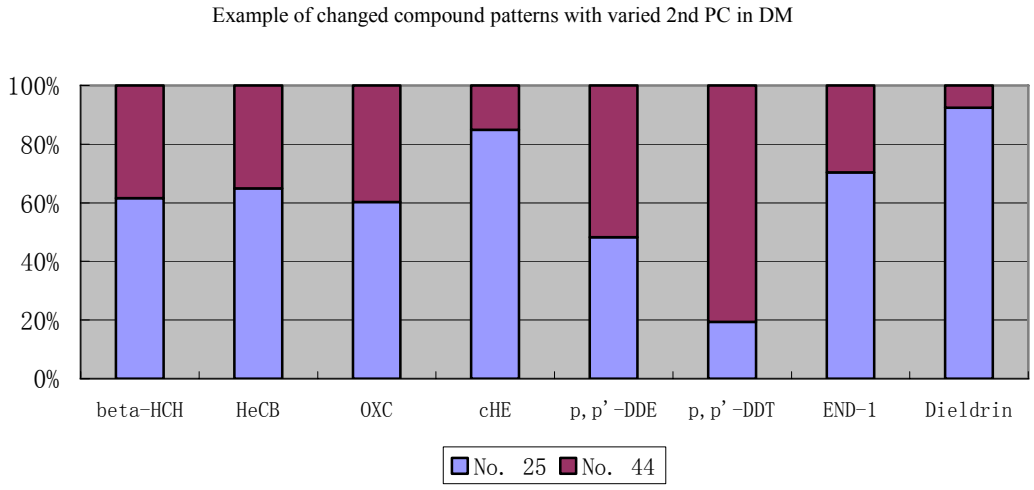


	beta-HCH	HeCB	OXC	cHE	p,p'-DDE	p,p'-DDT	END-1	Dieldrin
DP	2.02	1.13	0.29	0.2	8.42	0.12	0.51	0.49
FP	1.47	0.73	0.23	0.11	5.7	0.08	0.62	0.27
DM	4.75	1.73	0.76	0.61	28.09	1.38	1.37	1.11
FM	1.96	1.25	0.73	0.3	21.56	0.9	1.78	0.5

Fig. 3-4-1 (2): The proposed common exposure patterns (multiply the absolute value of 1st PC compound score by the standard deviation of certain compound level to special sample).

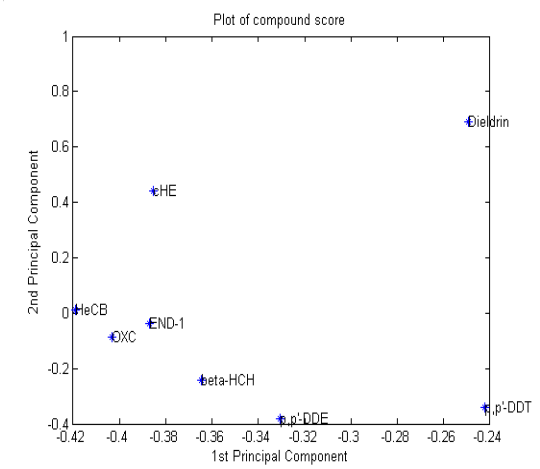
A different character, comparing the patterns described in geometric means (see the last chapter), is the relative content of END-1 in the common exposure patterns of Finland cohort was little higher than in Denmark cohort, and the differences of the relative contents of OXC between the

two cohort samples were less than geometric mean patterns. This character did not result from the lighter exposure of Denmark cohort to END-1 and OXC, but from their different exposure sources. The further interpreting will be shown in the next chapter by applying PC analysis to the pooled milk and placenta samples. The other samples might be contaminated by the different pollutant sources or the sources combined with the most common pollutant pattern. For example, the relative content of dieldrin and c-HE in sample 25 of DM were apparently higher than that in sample 44; *vice versa*, *p, p'*-DDT in sample 25 was apparently lower (Fig 3-4-1 (3)). The larger variances for 2nd, 3rd or 4th PC sample scores usually occurred when the samples have higher total content of the investigated compounds. However, for the samples with the smaller variance of 2nd, 3rd or 4th PC sample scores, their loading of these pollutants covered the whole concentration changes. It shows that the heavy contamination for these compound might come from various pollutant sources including the emphasized most common one.

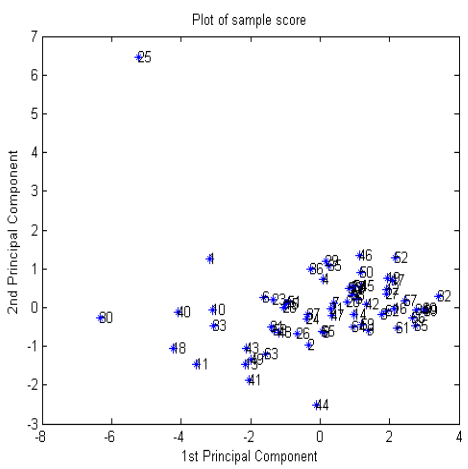


Score	β-HCH	HeCB	OXC	c-HE	p,p'-DDE	p,p'-DDT	END-1	Dieldrin
1st PC	-0.36	-0.42	-0.40	-0.39	-0.33	-0.24	-0.39	-0.25
2nd PC	-0.24	0.01	-0.09	0.44	-0.38	-0.34	-0.04	0.69

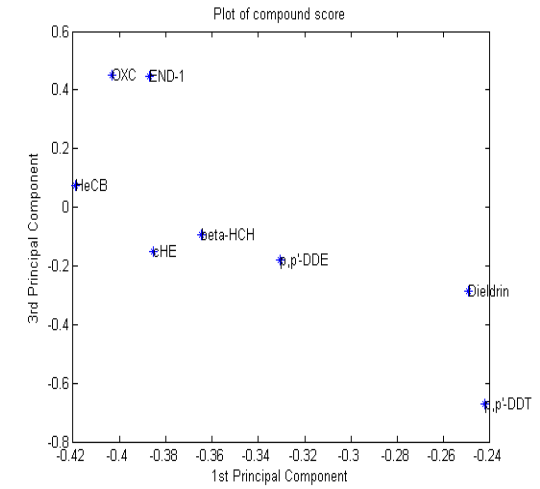
Fig. 3-4-1 (3): Compound patterns changing with 2nd PC, especially for c-HE and dieldrin contrary to the rest.



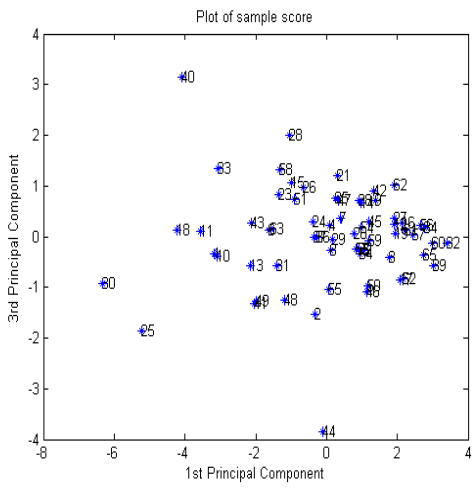
1: 1st PC vs.2nd PC compound score in DM



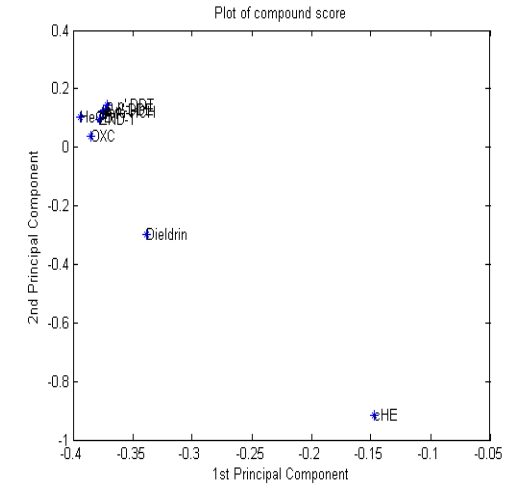
2: 1st PC vs.2nd PC sample score in DM



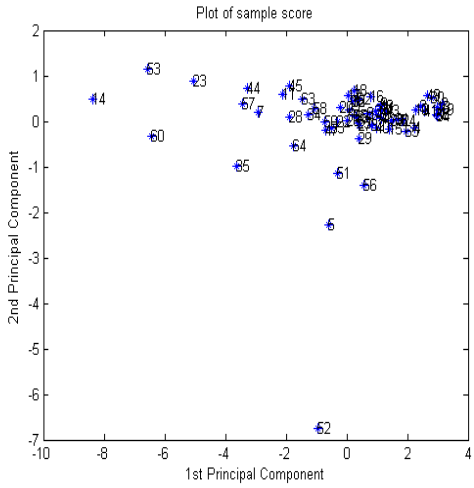
3: 1st PC vs.3rd PC compound score in DM



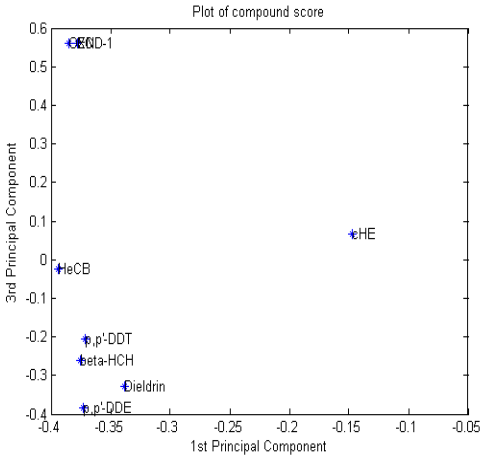
4: 1st PC vs.3rd PC sample score in DM



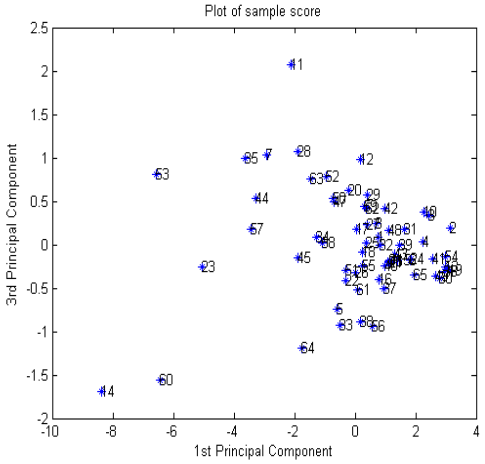
5: 1st PC vs.2nd PC compound score in FM



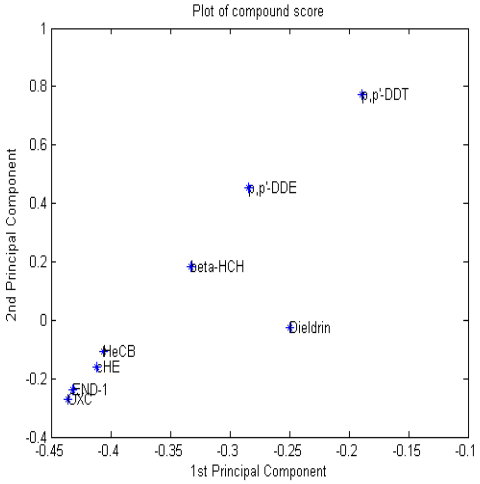
6: 1st PC vs.2nd PC sample score in FM



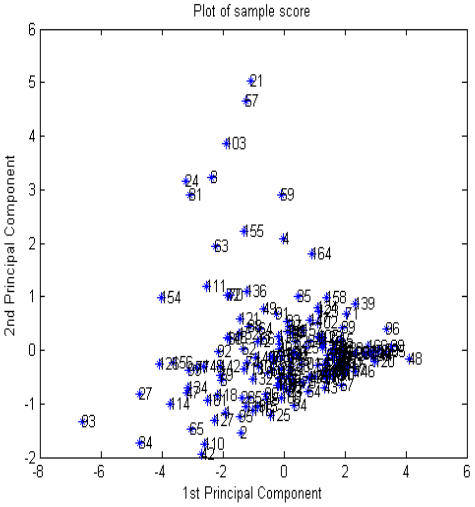
7: 1st PC vs.3rd PC compound score in FM



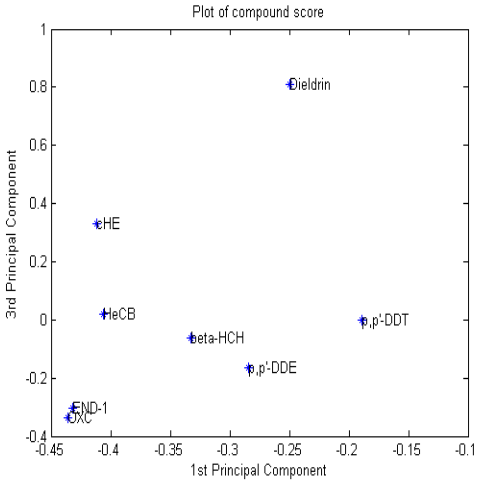
8: 1st PC vs.3rd PC sample score in FM



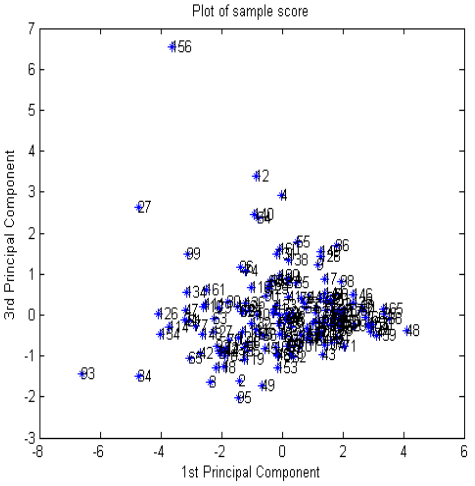
9: 1st PC vs.2nd PC compound score in DP



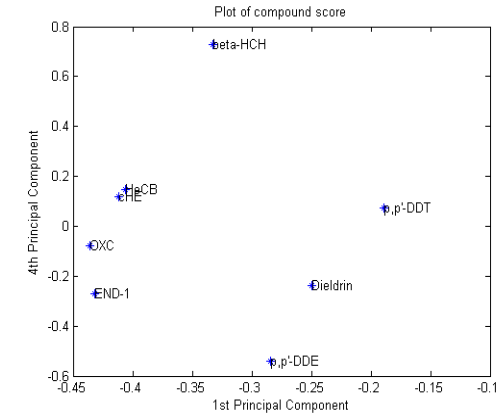
10: 1st PC vs.2nd PC sample score in DP



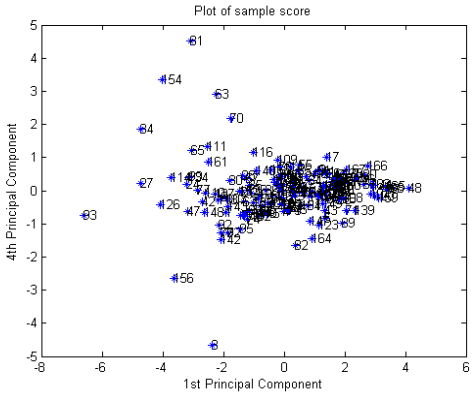
11: 1st PC vs.3rd PC compound score in DP



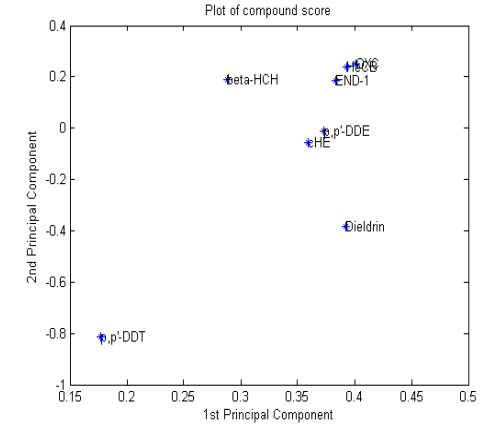
12: 1st PC vs. 3rd PC sample score in DP



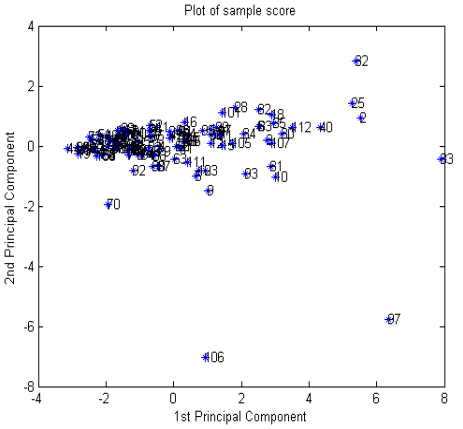
13: 1st PC vs. 4th PC compound score in DP



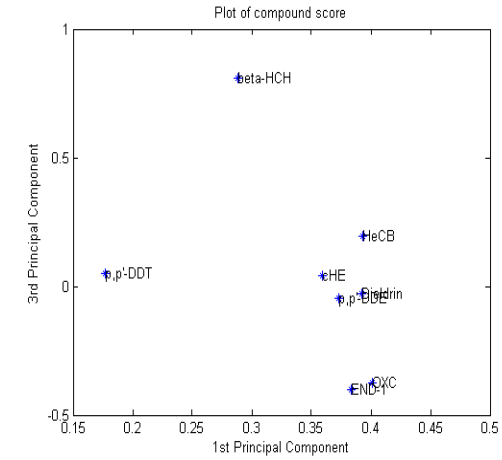
14: 1st PC vs. 4th PC sample score in DP



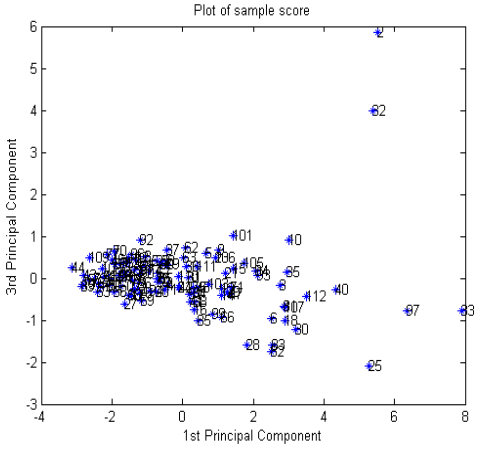
15: 1st PC vs.2nd PC compound score in FP



16: 1st PC vs.2nd PC sample score in FP



17: 1st PC vs.3rd PC compound score in FP



18: 1st PC vs.3rd PC sample score in FP

Fig. 3-4-1 (4): Compound and sample score plots of DM, DF, FM and FP in 1 to 18; larger variance for 2nd and 3rd and 4th PC sample score usually occur in the high concentration side (1st PC samples score interpreted the total concentration variance of the 8 pollutants, *also see* Fig. 3-4-2 (1)).

3-4-2: Sample type and cohort characters

Pooling the two cohort samples of breast milk and placenta respectively and running the PC analysis, 1st PC, 2nd PC and 3rd PC interpreted 59.34%, 12.78% and 9.96% of the total variance for milk samples; and 1st PC, 2nd PC, 3rd PC and 4th PC interpreted 48.89%, 13.92%, 10.62% and 8.79% of the total variance for the placenta set. Fig. 3-4-2 (3) I-XII showed the compound and sample score plots of the pooled placenta (168 DP and 112 FP) and milk (56 for each cohort) samples.

The 1st PC represented the most part of the variance of the total amount of the 8 compounds. With the increase of the total amount, 1st PC sample score *vs.* the total amount (Fig. 3-4-2 (1), (2)) deviated a linear relationship more and suggested the larger change of exposure patterns. Although Finland cohort almost covered the whole variance range of the total amount, it was clear that Denmark samples dominated in the higher concentration range in both samples; also they represented the deviation from 1st PC. Additionally, the structures of 1st PC for milk sample and Finland sample were nearly equal, which might suggested that both milk and placenta samples can represent the investigated population to the same extent, although they have different sizes 130 and 280 respectively. 2nd PC sample scores for placenta sample and 3rd PC samples scores for milk samples might suggest a cohort sign of Finland exposure, the character was emphasized by the summary of OXC and END-1 (these two compounds were always correlated), which increase with 3rd PC and 2nd PC sample scores for milk samples and placenta samples (except 2 samples), respectively. Only part of the Denmark samples has similar relationship than the Finland cohort. The similar structure of 3rd PC of milk and 2nd PC of placenta (Table 3-4-2 (1)) suggest that they refer to the same factor behind the about 10% variance. This could be treated as a sign of difference of OXC and END-1 source of Finland cohort from Denmark cohort (might only the part of Denmark cohort). Had been discussed in the last chapter, they might represent the different common exposure patterns.

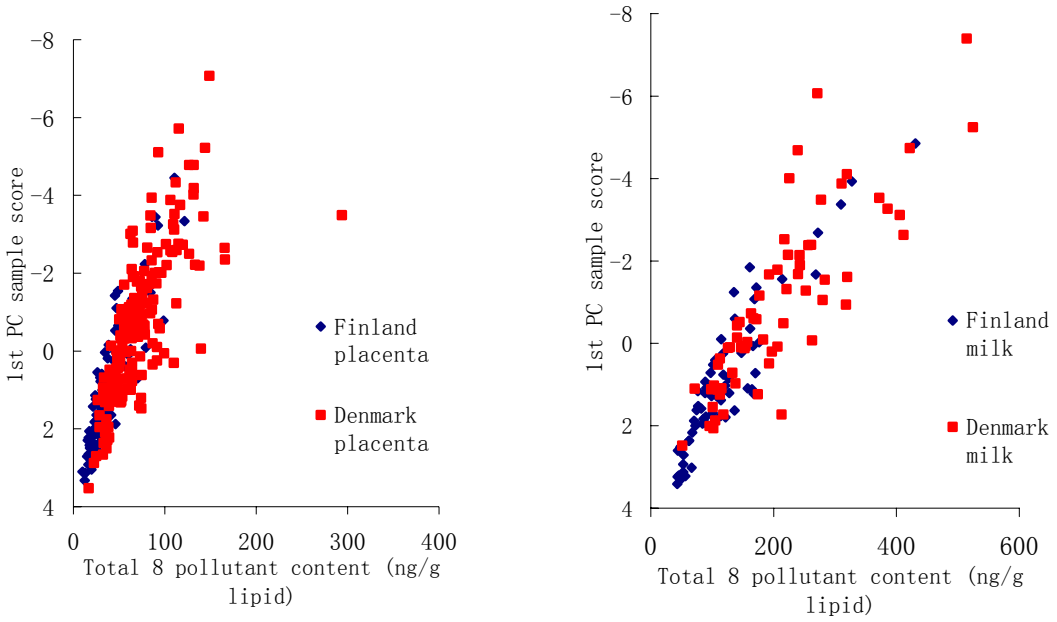


Fig 3-4-2 (1): 1st PC sample score interpreted the most part of the total variance amount of the 8 pollutants in placenta and milk; the other part variance mainly involved the higher contaminated Denmark samples because of the increasing deviation from a line with the increase of the total content.

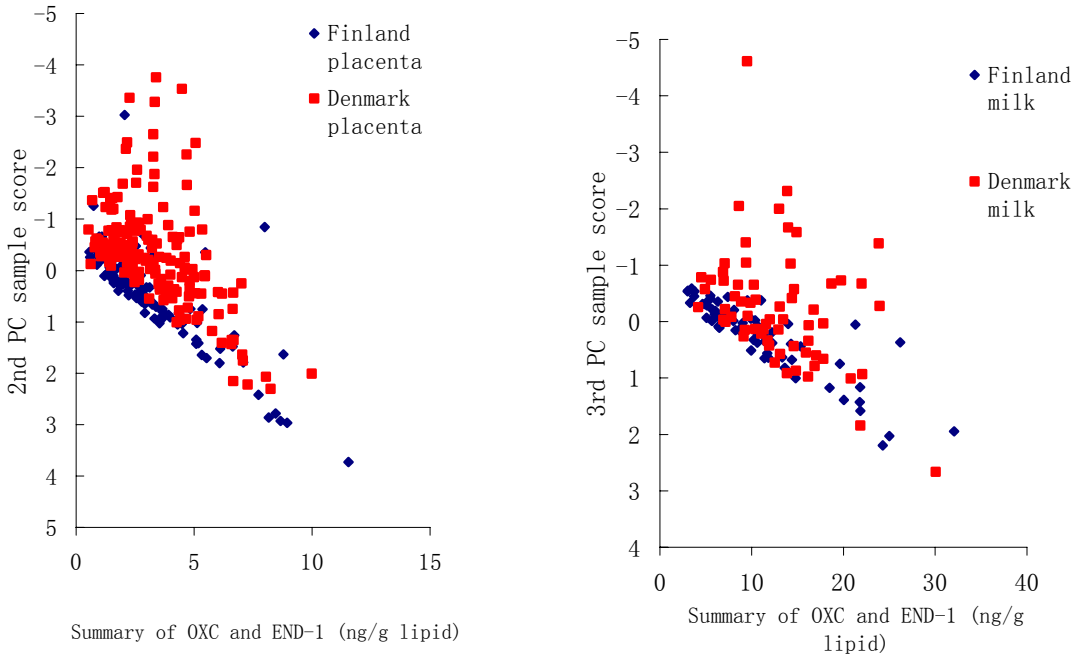
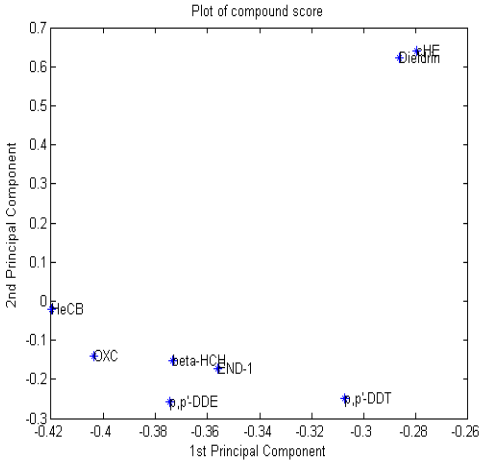
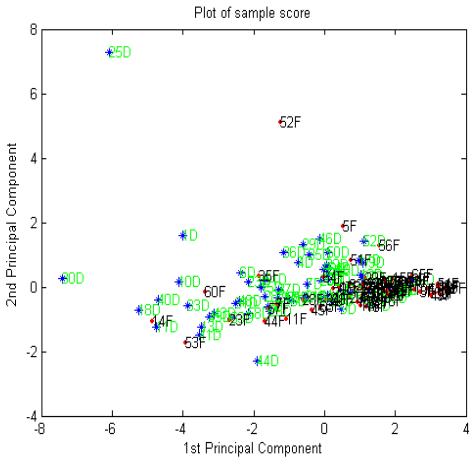


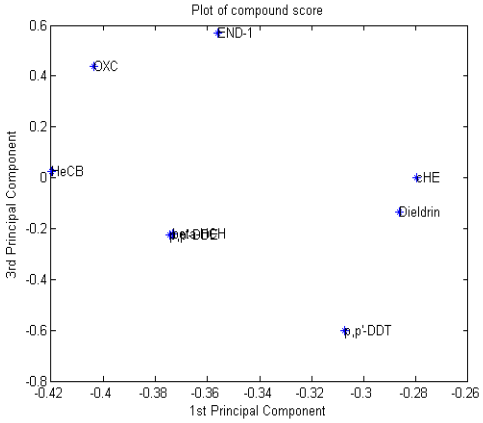
Fig 3-4-2 (2): the summary of OXC and END-1 increase with 3rd PC and 2nd PC sample scores for milk samples and placenta samples (except 2 samples) in Finland samples.



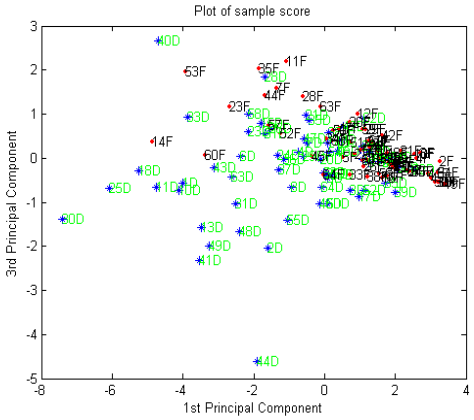
1: 1st PC vs. 2nd PC of the pooled milk



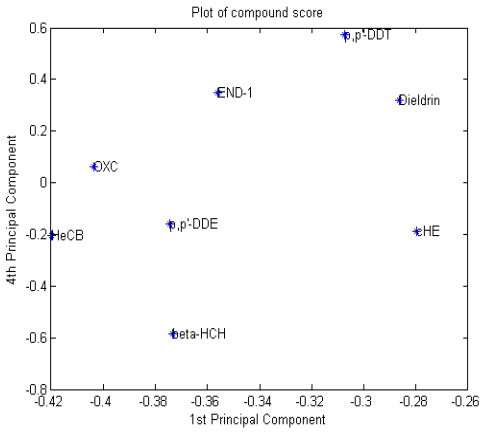
2: 25D and 52F showed different patterns of dieldrin and c-HE



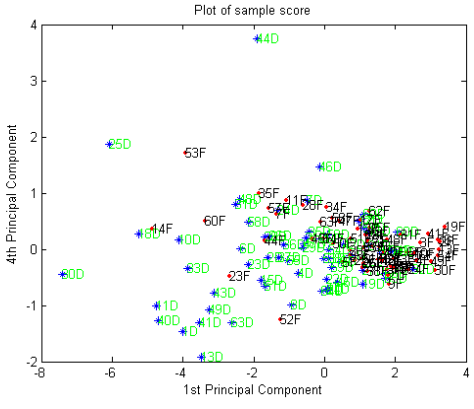
3: 1st PC vs. 3rd PC of the pooled milk



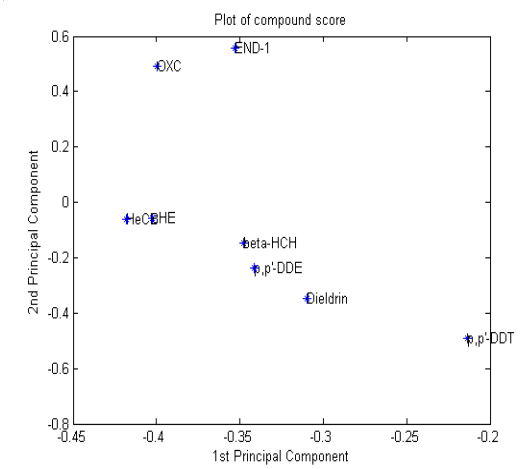
4: OXC and END-1 patterns might distinguish the two cohorts



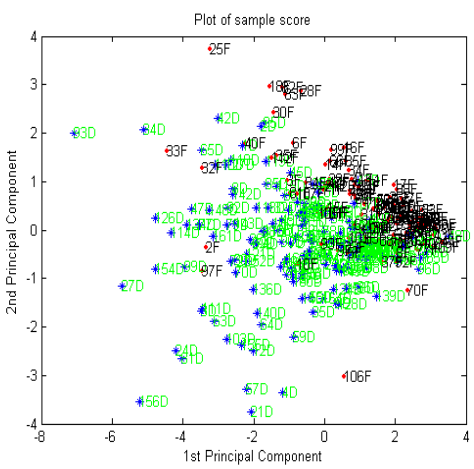
5: 1st PC vs. 4th PC of the pooled milk



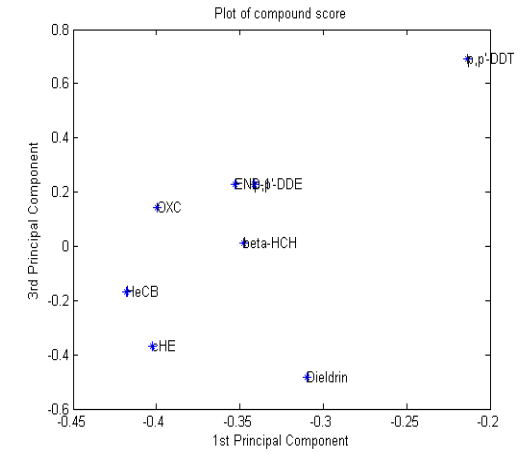
6: Interpreting only 5.56 % of the total variance



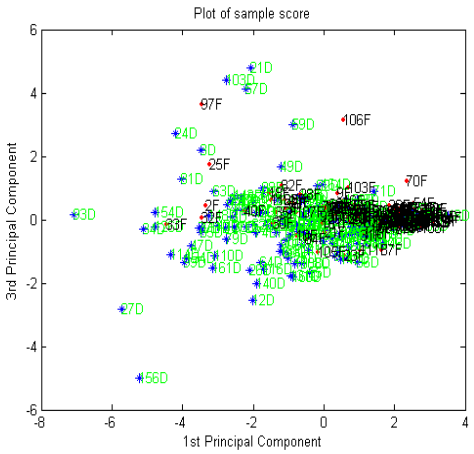
7: 1st PC vs. 2nd PC of the pooled placenta



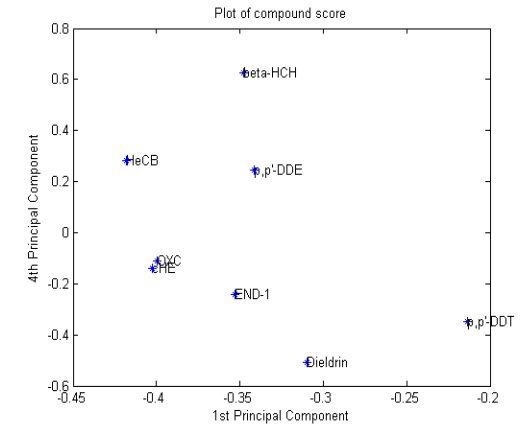
8: OXC and END-1 patterns might distinguish the two cohorts



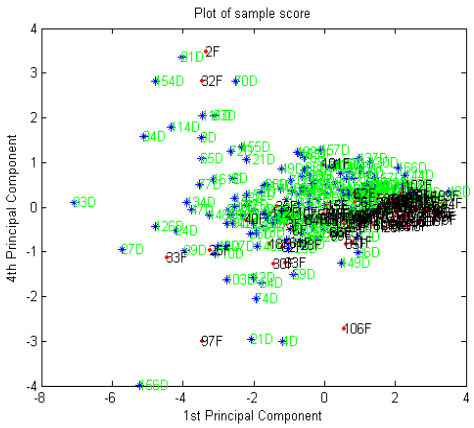
9: 1st PC vs. 3rd PC of pooled placenta



10: Interpreting only 10.62% of the total variance



11: 1st PC vs. 4th PC of the pooled placenta



12: Interpreting only 8.79% of the total variance

Fig. 3-4-2 (3): Compound and sample score plots (1 to 12) of the pooled placenta (168 DP and 112 FP) and milk (56 for each cohort) samples

Table 3-4-2 (1): Principal components interpreted more than 82% of the total variance of pooled milk (PM) and pooled placenta (PP) samples.

	β-HCH	HeCB	OXC	c-HE	p,p'-DDE	p,p'-DDT	END-1	Dieldrin
PM-1st PC	**-0.37**	**-0.42**	**-0.40**	**-0.28**	**-0.37**	**-0.31**	**-0.36**	**-0.29**
PM-2nd PC	-0.15	-0.02	-0.14	0.64	-0.26	-0.25	-0.17	0.62
PM-3rd PC	-0.22	0.03	0.44	0.00	-0.22	-0.60	0.57	-0.13
PM-4th PC	-0.58	-0.21	0.06	-0.19	-0.16	0.57	0.35	0.32
PP-1st PC	**-0.35**	**-0.42**	**-0.40**	**-0.40**	**-0.34**	**-0.21**	**-0.35**	**-0.31**
PP-2nd PC	-0.15	-0.06	0.49	-0.06	-0.24	-0.49	0.56	-0.35
PP-3rd PC	0.01	-0.17	0.14	-0.37	0.23	0.69	0.23	-0.48
PP-4th PC	0.63	0.28	-0.11	-0.14	0.25	-0.35	-0.24	-0.51

3-5: Paired milk and placenta samples from Finland cohort

Compound correlation of the paired samples: The most abundant pollutants of *p, p'*-DDE, β-HCH, HeCB, dieldrin, END-1, OXC, *c*-HE, *p, p'*-DDT, as well as the low level compounds such as OCS, not only showed similar patterns (DM > FM > DP > FP) in these two cohort samples, but also they were all linearly correlated for paired placenta and breast milk samples in Finland cohort. Moreover, the low level compound mirex also showed a similar correlation. Regression analysis (Table 3-5 (1)) shows that they all have the p-Value <0.0001. The correlation for γ-HCH might be not so confident because most of the data are close to low concentration range except two. The regression result was shown just for reference. These paired sample correlations were essentially different with compound specific slopes. The correlations reflected the dynamic distribution of these pollutants through blood circuit system and the slopes differentiated the capacities of these compounds to the two tissues. Additionally, these compounds increased with the lipid content in breast milk samples when expressed in wet weight calibration, suggesting a lipid dependent variance and will be discussed further.

PC analysis on Finland paired samples: In order to investigate how the extent of a factor affect the exposure level comparing the other factors, PC analysis was used for the 10 correlated compounds (p-Value less than 0.0001 except γ-HCH) with the pooled milk and placenta data on the both calibrations (Fig. 3-5 (1) 1-4). 1st PC can explain 66.1% and 67.1% of the total variance of the 42 samples on w. w. calibration and lipid calibration respectively, and 2nd PC explains the 11.4% and 10.2% of the variance on the relative calibrations.

The 1st PC structures for the both calibrations were similar and the compound scores for certain compound were also similar intra-1st PC and inter-1st PCs (Table 3-5 (2) and Fig. 3-5 (1) I and III). The absolute value of the 1st PC compounds score calculated on wet weight (w. w.) base are little lower than calculated on lipid base for the listed compounds (Table 3-5 (2)). These absolute values reflected the contribution of the investigated compounds to 1st PC, and also reflected how much of the variance could be interpreted by 1st PC. This inferred the lipid content as a factor to affect the

distribution of these compounds among tissues. 2nd PC compound scores for certain compound are also similar on the lipid calibration but with opposite signs on w. w. calibration. The similar structure of the two 1st PCs suggest that the both calibrations could reflect the variance controlled by the same factor at the same extent (Fig 3-5 (2)); this factor should be the environmental pollutant level faced by different subjects. In other words, in the two calibration methods were no difference on that. The 1st PC score similar (including 2nd PC compound scores on lipid calibration; Fig 3-5 (1) 1 and 3) for certain compounds reflected the correlations of these pollutants between the paired samples, which had been discussed (Table 3-5 (1)). The sign of the different compound scores for certain compounds on w. w. calibration (Table 3-5 (2) and Fig 3-5 (1) 1) depicts the variance introduced by lipid content difference, which mean the variance interpreted by 2nd PC sample scores was the result of sample specific lipid change (Fig. 3-5 (3)), and the variance were diminished by lipid calibration, so called lipid normalization. The gained additional information was that lipid differences introduce about 11.4% of the total variance.

The regression analysis of 2nd PC sample score versus lipid variance (Fig. 3-5 (3)) might characterize the more fatty, higher lipophilic contaminant level for milk samples, but at only about 1/6 contribution when comparing the personally faced environmental difference of levels. The lipid contribution to 2nd PC samples score for placenta sample was much lower because of the lower fat content of placenta tissues.

The 2nd PC sample scores on lipid calibration, referring to the compound pattern variance (Fig.3-5 (1) 3), might be interpreted as different pollutant sources for these compounds (have been discussed), especially emphasizing on compounds mirex *vs.* *c*-HE and dieldrin. An extreme example is sample No. 11 compared to No. 34. Few samples (No. 42, 15, 38, 14) could be clearly grouped with No. 11 and only No. 34 with No. 12 (Fig.3-5 (1) 4). Fig 3-5 (4) showed that the pollutant source of mirex might be different to the source of *c*-HE and dieldrin for these samples. However, 2nd PC counts only 10.2% of the total variance, which implies that these pollutants had been well 'homogenized' by the environmental physical or biological processes when they reached the human body after a long time-space transfer. The other PCs, which totally count the rest of 22.7% variance, should be considered as other compound patterns and each involved lower sample

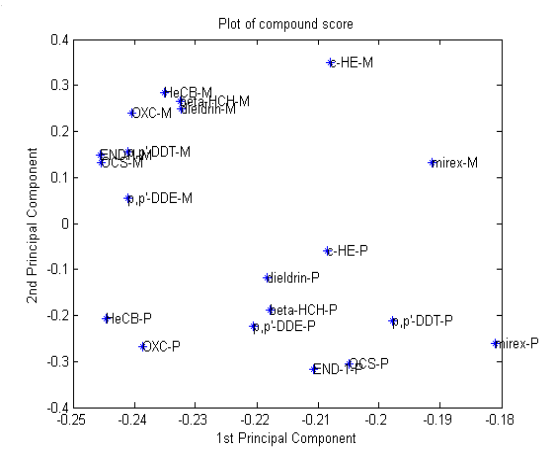
variance than 2nd PC.

Table 3-5 (1): Linear regression results of Finland breast milk samples versus placenta samples

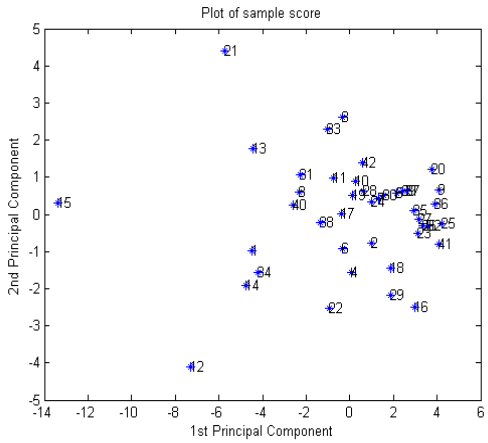
Compound	Milk = Intercept (standard error) + Slope (standard error) * Placenta	Correlation coefficient	Standard deviation	p-Value	N
β -HCH	y = 1.681 (±0.874) + 1.970 (±0.159) x	0.890	2.145	<0.0001	42
ɣ -HCH	y = -0.475 (±0.169) + 1.180 (±0.165) x	0.749	0.490	<0.0001	42
HeCB	y =0.663 (±0.535) + 1.592 (±0.103) x	0.926	1.152	<0.0001	42
OCS	y = 0.003 (±0.035) + 1.500 (±0.246) x	0.694	0.094	<0.0001	42
Mirex	y = 0.019 (±0.033) + 1.229 (±0.116) x	0.860	0.119	<0.0001	42
END-1	y = 1.729 (±0.690) + 2.188 (±0.221) x	0.843	2.593	<0.0001	42
Dieldrin	y = 0.216 (±0.222) + 1.931 (±0.144) x	0.904	0.671	<0.0001	42
c-HE	y = 0.209 (±0.143) + 2.741 (±0.181) x	0.923	0.381	<0.0001	42
OXC	y = 1.012 (±0.301) + 2.743 (±0.240) x	0.875	0.925	<0.0001	42
o, p'-DDE	y = 0.007 (±0.009) + 1.541 (±0.366) x	0.559	0.026	0.00014	41
p, p'-DDE	y = 12.397 (±6.346) + 3.043 (±0.246) x	0.890	24.078	<0.0001	42
p, p'-DDT	y = 0.984 (±0.657) + 11.20 (±1.948) x	0.673	1.876	<0.0001	42
p, p'-DDD	y = 0.241 (±0.071) + 0.277 (±0.097) x	0.410	0.265	0.0070	42

Table 3-5 (2): The structure of 1st PC and 2nd PC on wet weight and lipid calibrations

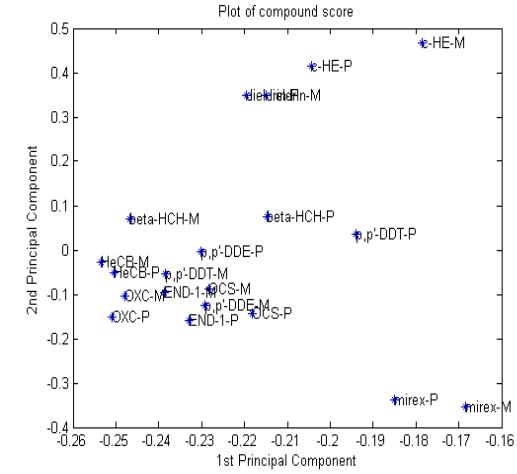
	β-HCH	HeCB	OCS	p,p'-DDT	p,p'-DDE	OXC	c-HE	dieldrin	END-1	mirex
1st PC-M (w.w.)	**-0.23**	**-0.24**	**-0.25**	**-0.24**	**-0.24**	**-0.24**	**-0.21**	**-0.23**	**-0.25**	**-0.19**
2nd PC-M (w.w.)	0.27	0.28	0.13	0.16	0.06	0.24	0.35	0.25	0.15	0.13
1st PC-P (w.w.)	**-0.22**	**-0.24**	**-0.20**	**-0.20**	**-0.22**	**-0.24**	**-0.21**	**-0.22**	**-0.21**	**-0.18**
2nd PC-P (w.w.)	-0.19	-0.21	-0.31	-0.21	-0.22	-0.27	-0.06	-0.12	-0.32	-0.26
1st PC-M (lipid)	**-0.25**	**-0.25**	**-0.23**	**-0.24**	**-0.23**	**-0.25**	**-0.18**	**-0.21**	**-0.24**	**-0.17**
2nd PC-M (lipid)	0.07	-0.03	-0.09	-0.05	-0.12	-0.10	0.47	0.35	-0.10	-0.35
1st PC-P (lipid)	**-0.21**	**-0.25**	**-0.22**	**-0.19**	**-0.23**	**-0.25**	**-0.20**	**-0.22**	**-0.23**	**-0.18**
2nd PC-P (lipid)	0.07	-0.05	-0.14	0.03	-0.00	-0.15	0.41	0.35	-0.16	-0.34



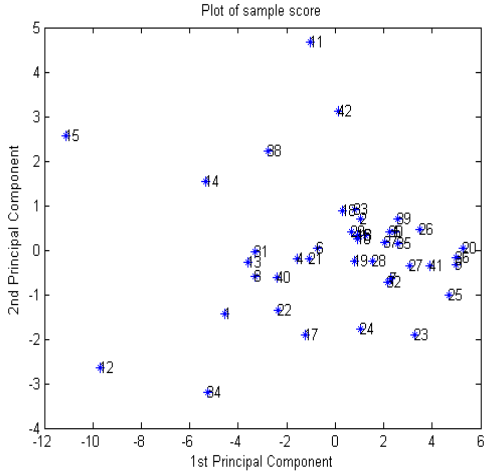
1: 1st PC-2nd PC compound score on w. w. base



2: 1st PC-2nd PC sample score on w. w. base



3: 1st PC-2nd PC compound score on lipid base



4: 1st PC-2nd PC sample score on lipid base

Fig. 3-5 (1): Compound score and sample score plots for paired samples

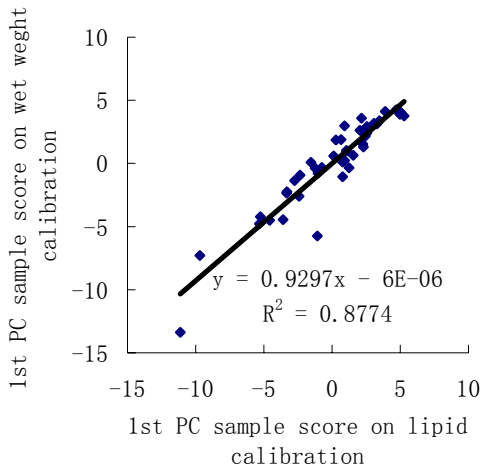


Fig. 3-5 (2): Sample score on lipid base & w. w. base referring the same factor behind the 1st PC.

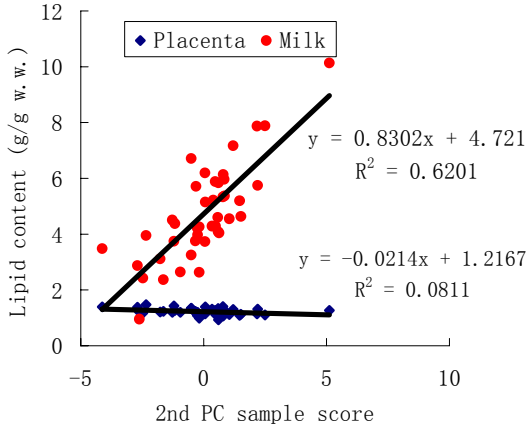


Fig. 3-5 (3): 2nd PC sample score on w. w. base referred variance introduced by lipid difference

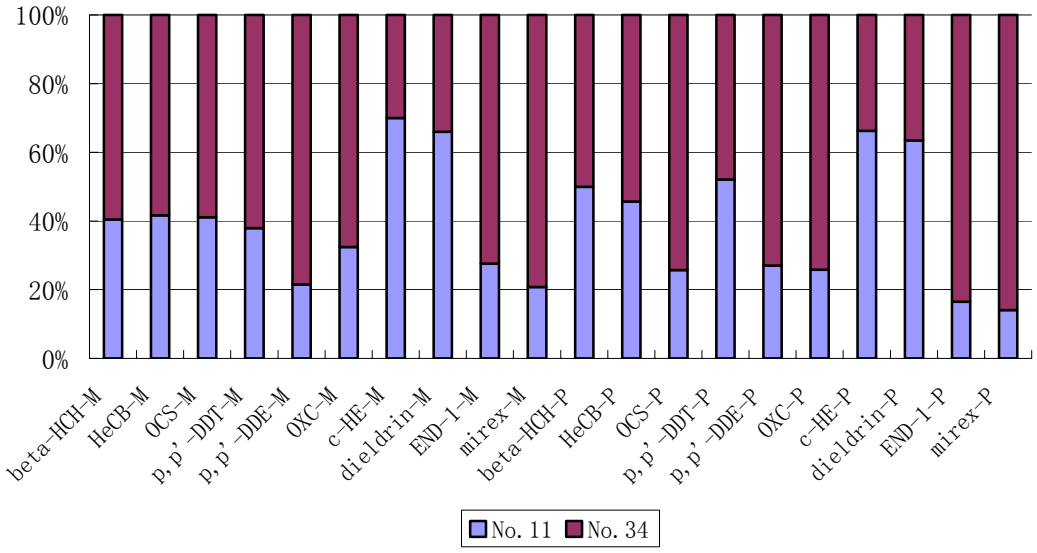


Fig. 3-5 (4): 2nd PC sample score calculated on lipid base referring the pattern variance, esp. to *c*-HE, dieldrin and mirex.

3-6: Enantioselective residuals of the *c*-PBTs in mother samples

3-6-1: Information from ERs

The physical processes (leaching, volatilization, and atmospheric deposition) and abiotic reactions (hydrolysis and photolysis) for chiral compounds are unaffected (Bidleman et al. 1999). ERs exhibit only the information of biotransformation or biodegradation of these pollutants. It is helpful because we usually not need to consider these factors when we focus on biotic processes of these compounds. However, ER values were usually difficult to determine at high precision because of the low concentrations. Although tissue specific ERs for certain *c*-PBTs had been well documented, these differences could be the result of different enzyme activities or blood brain barrier in different tissues, especially for tissue liver, kidney and brain (Kallenborn et al., 2000). Assuming dynamic equilibrium of *c*-PBTs among organs and tissues, the ERs of the low enzyme activity tissues, such as breast and adipose tissue, might mainly depended on the enzyme active tissues, with emphasizing the liver. However, the metabolic activity of the placenta is almost as large as that of the fetus itself (Berry et al., 1977; Chao et al., 1980; St-Pierre et al., 2002). ER analysis between paired milk and placenta samples (Fig. 3-6-1 (1)) showed placenta might contribute more metabolic activity to *c*-HE than breast tissues because of the generally larger ER values and the range in placenta than in milk samples. Enzyme activity for biotransformation might associate with the more effective detoxification function of placenta than breast tissue. No apparent correlation was found between the ERs of the invested *c*-PBTs in the paired samples. For α-HCH, *c*-HE (might include *o, p'*-DDD), ER *vs.* (+)-or (-)-enantiomer concentration profiles show the concentration tendency of the ERs (Fig. 3-6-1 (2), (3), (5)). Because of the probable continuous exposure mode and the possible variation of ER sources, the ER-concentration patterns could be more complex than the well-documented single dose model (Walter et al., 2001; Wong et al., 2002; 2004). No structural characters had been found for chiral OXC (Fig. 3-6-1 (4)). The possible reasons is that OXC is the metabolite of both *c*-CHL and *t*-CHL, which have been reported as apparent different OXC enantioselective sources in mammalians (Buser et al, 1992).

Few samples also showed inverse ERs for α-HCH (Fig. 3-6-1 (7) and Table 3-6-1 (1)). Only one sample had the detected level of HC, which showed an enantioselective residual (Fig. 3-6-1 (8)). The abnormal data (ER for *c*-HE near to 1 and for α-HCH much larger than 1) might result from a special source or might reflect different enzyme activity of the special mother. The generally enantioselective enrichment of (-)-α-HCH (Fig. 3-6-1 (2)) might be associated with a lower risk factor than the accumulation of (+)-α-HCH (Kallenborn et al. 2000). According the *in vitro* results, the estrogenic activity of *o, p'*-DDT residuals might be overestimated because the enantioselective biodegradation of the (-)-*o, p'*-DDT (Fig. 3-6-1 (6)), which is the active estrogen whereas hER activity of (+)-*o, p'*-DDT was negligible (Hoekstra et al., 2001). Therefore, it could assume that human evolved removing the more toxic isomer firstly from body than the less toxic one in case of the possible adverse effect.

Table 3-6-1 (1): Data of ER and (+)-, (-)- α-HCH concentrations not included in Fig. 3-6-1 (2)

Different ER	19DP	7FM	12FM	62FM	High level	2FP	13FP	19FP	32FP	71FP	79FP
(-)-α-HCH	0.07	0.21	0.39	0.06	(-)-α-HCH	392.94	12.05	17.82	50.46	6.45	10.64
(+)-α-HCH	0.1	0.58	0.48	0.11	(+)-α-HCH	413.66	12.37	18.12	49.33	6.51	10.16
ER(10%)	1.28	2.8	1.26	1.79	ER(10%)	1.05	1.03	1.02	0.98	1.01	0.95

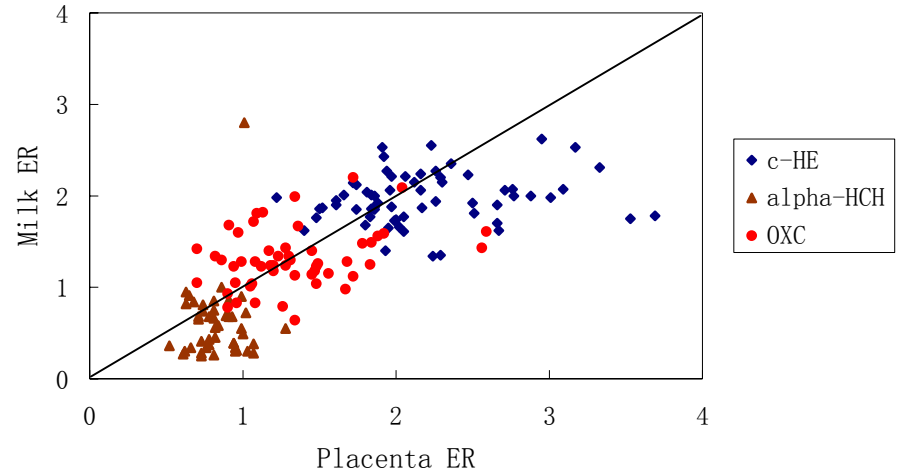


Fig. 3-6-1 (1): ER of the paired Finland and Denmark milk and placenta samples

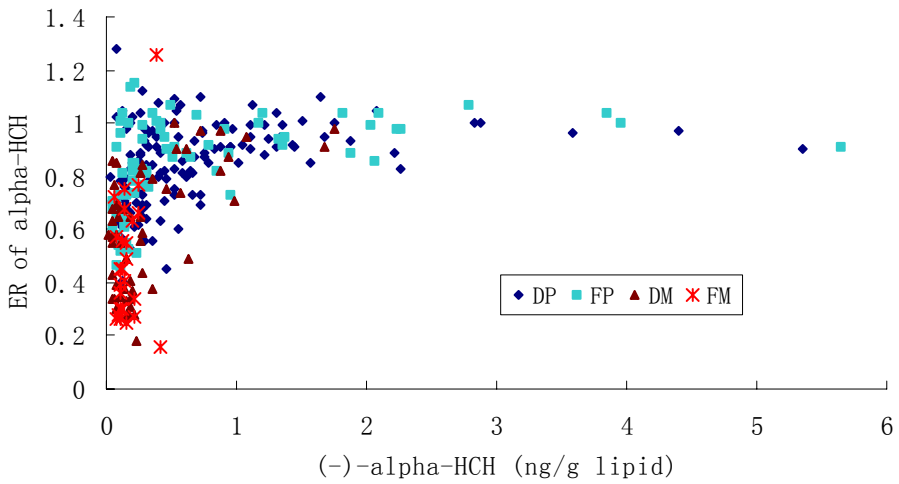


Fig. 3-6-1 (2): (-)-α-HCH is usually the isomer enantioselective enriched in the investigated mother samples.

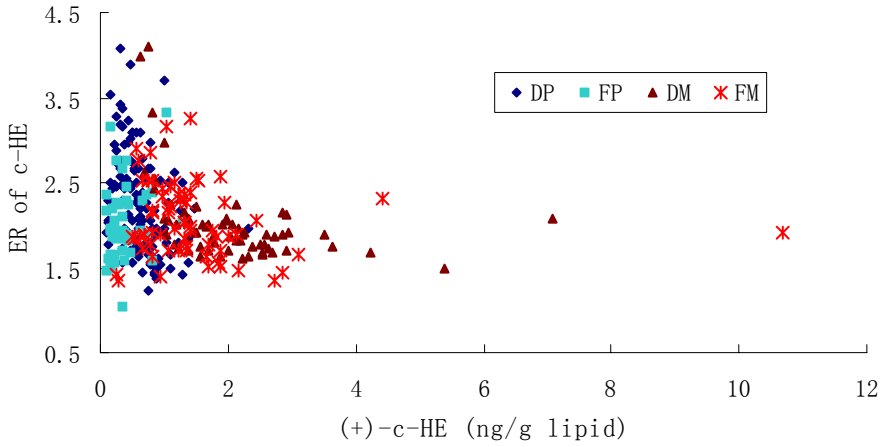


Fig. 3-6-1 (3): (+)-c-HE is the enantioselective enrichment isomer in the investigated mother samples

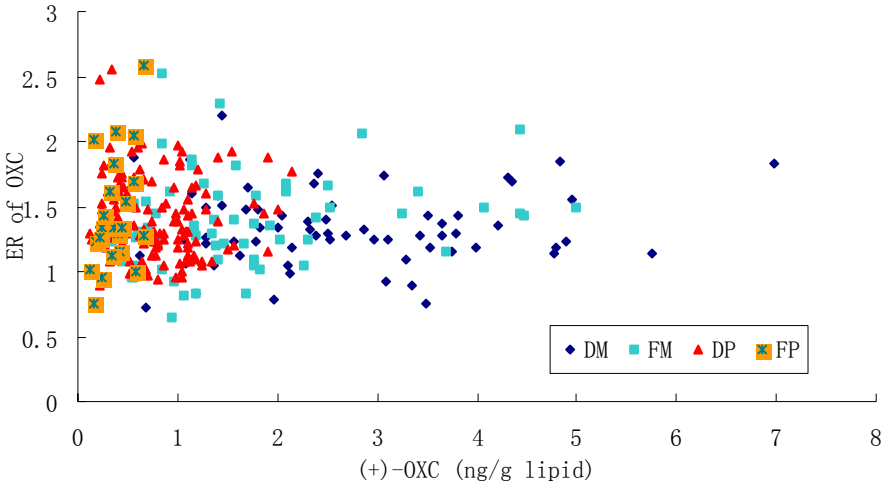


Fig. 3-6-1 (4): (+)-OXC, usually, is the enantioselective enrichment isomer in the investigated mother samples

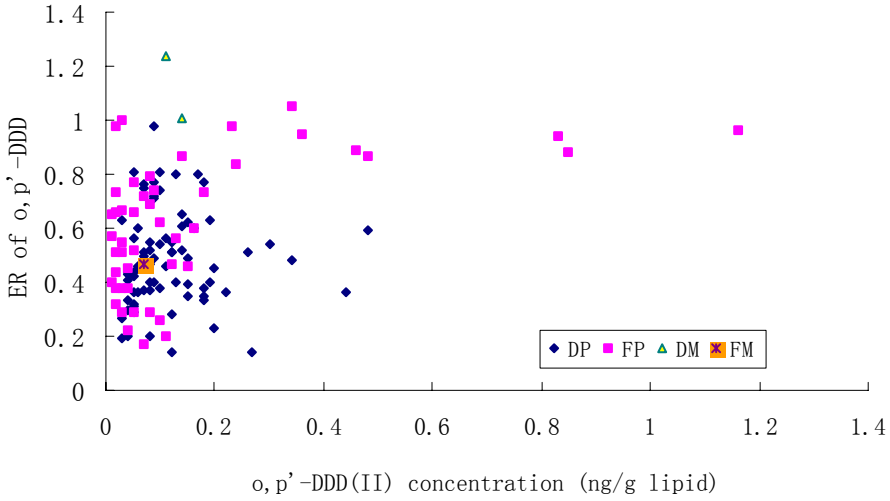


Fig. 3-6-1 (5): o,p'-DDD (II) is the enantioselective enrichment isomer in the investigated mother samples

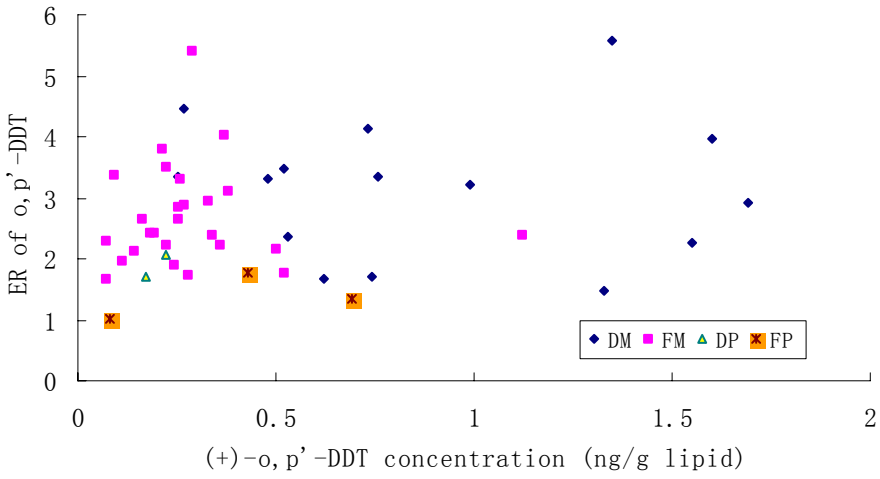
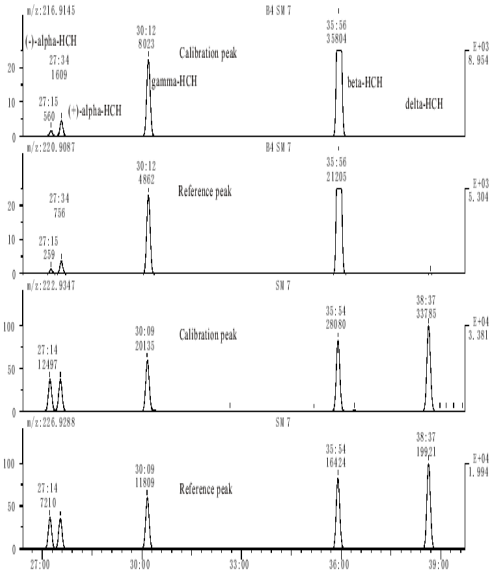


Fig. 3-6-1 (6): (+)-o,p'- DDT is the enantioselective enrichment isomer in the investigated mother samples



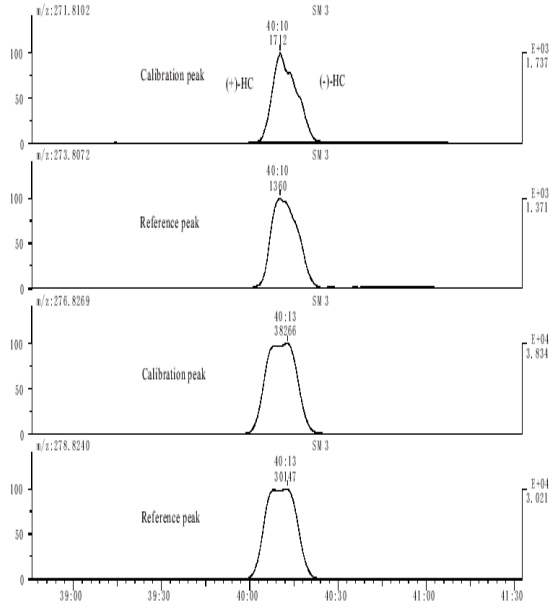


Fig. 3-6-1 (7): Chromatography of enantiomeric separated α-HCH (ER>1) and the other four isomers (upper left).

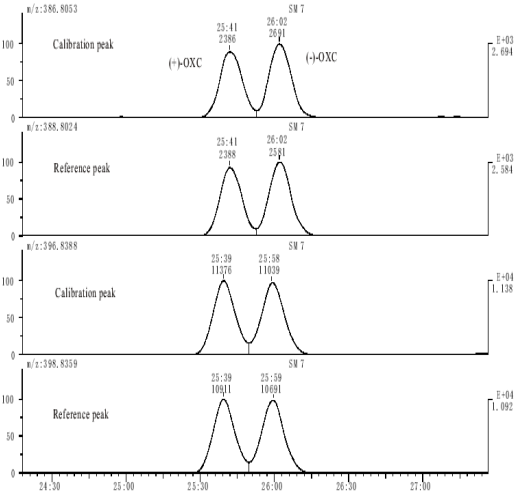


Fig. 3-6-1 (8): Chromatography of enantiomeric separated HC (only sample with detectable HC, upper)

Fig. 3-6-1 (9): Chromatography of enantiomeric separated OXC (ER<1, left)

3-6-2: Model interpreting human exposure to *c*-PBTs

First order clearance model: Generally, the PBTs could be decreased at first order in human samples (Smith, 1999; Noren et al., 2000; Solomon et al., 2002) after the ban (Table3-6-2 (1)). If the stored residuals are high enough and the dominant processes are clearance when compared to the recent uptake, it might be reasonable to assume the first order clearance model is, to some extent, suitable to interpret the time trend of *c*-PBT biodegradation difference for paired enantiomers.

Table3-6-2 (1): OCs in human milk (pool) adapted to a first order decrease in the past 20-30 years*

Compound	DDT	DDE	$MeSO_2$-DDE	Dieldrin	HeCB
Clearance rate (*k, year*$^{-1}$)	-0.162	-0.113	-0.121	-0.117	-0.118

*Noren et al, 2000, $C = C_0e^{-kt}$, C_0 is the initial concentration when clearance started.

In a cohort, the variance of each sample could be tentatively treated as the sample exited at a time-specific history point of a clearance processes from certain concentration. For paired enantiomers, time related enantiomeric residual of (+)-/(-)-isomer could be also simply described by first-order kinetics of clearance without considering the physical clearance like fecal excretion, which usually are non-enantioselective processes. Enantiomeric ratios of biota samples *ER* can be defined as:

$$ER = C_+ / C_- \quad (1)$$

In one-time single dose exposure to environmental chiral pollutants, the stored residual concentrations for (+)-, and (-)-isomers are C_{s+}, C_{s-}, and *ER* can be described as equation (2), (3) and (4) (Walter et al., 2001; Wong et al., 2002; 2004), where C_{0+} and C_{0-} are the initial respective concentrations:

$$C_{s+} = C_{0+}e^{-k_+t} \quad (2)$$

$$C_{s-} = C_{0-}e^{-k_-t} \quad (3)$$

$$ER_s = (C_{0+}/C_{0-})e^{-(k_+-k_-)t} \quad (4)$$

$$\alpha = C_{0+}/C_{0-} \quad (5)$$

$$\beta = k_+/k_- \quad (6)$$

Here we further define the parameters α, β (equation 5, 6), α describe the *ER* when exposure is terminated and β reflects the difference of enantioselective biodegradation. Transforming the time-function of ER (equation 4) to the concentration-functions and linearising to equation (7) and (8).

$$LnER = Ln\alpha + (1/\beta - 1)(LnC_{0+} - LnC_{s+}) \quad (7)$$

$$LnER = Ln\alpha + (1-\beta)(LnC_{0-} - LnC_{s-}) \quad (8)$$

New uptake changing stored ER: However, the observed ER *vs.* concentration patterns of *c*-HE and α-HCH suggested the partly true of the model. The upper border data of *c*-HE pattern can be well described by the linear relationship (7) and (8). However, ERs changed much at the low concentration range; this might be the result of the recent exposure, expressed as $C_{uptake+}$ or $C_{uptake-}$, respectively. That means at the higher concentration range, the clearance processes was dominated because of the relative higher-level of residuals. On the other hand, the uptake rate was dominated at the lower concentration range for some samples because the historical residuals were relative low. The ERs of these samples are determined by the recent uptake, which should reflect ER values of the environment pollutants. Assumption of all people facing a recent uptake, the model could be modified to equation (9).

$$ER = C_+ / C_- = (C_{uptake\ +} + C_{s+}) / (C_{uptake\ -} + C_{s-}) \qquad (9)$$

Observed data from mother samples: The patterns should basically reflect the combined single dose of enantioselective biodegradation and recent exposure. However, it is little knowledge about the environmental ER values (ER_{en}) of the chiral pollutants from person to person. Here it is just assumed that the recent uptake reflects ER_{en}, which should be equal to α. By the plotted patterns (Fig. 3-6-1 (2) and (3)), it could be suitable to assume ER_{en} is close to 1 for α-HCH and 1.4 for *c*-HE.

The milk data in 1985 showed HC was 70 (±60) and *c*-HE was 100 (±400) ng/g lipid (Mussalo-Rauhamaa et al, 1988) and the present determination showed HC are completely changed. Considering that HC is less stable than *c*-HE in the human body, the present ER changes might have resulted mainly from the slowly degradation of *c*-HE. Equation 2, 3 describe the first order clearance of the stored (+) or (-)-*c*-HE from C_{0+} or C_{0-} at time zero (t =0). When transforming the time-function of ER to the linearized C_+ or C_--function by assuming each observed C_+ or C_- reflecting the residuals of C_{0+} or C_{0-} at different time, equation 7 and 8 show the correlation between *LnER* and LnC_{s+} or LnC_{s-}. Assume that in case of upper border data (Fig. 3-6-2 (1)), the recent uptake for *c*-HE can be neglected (C_{s+} and C_{s-} is dominant). For this tentative evaluation the few border data could be used in a first order clearance process (Walter et al., 2001)

with the calculated β = 0.78. As lower border data values of ER = α = 1.4, which reflect recent uptake ($C_{+uptake}$ and $C_{-uptake}$) as dominating process, were used, the data between the upper border and lower border suggest a medium status of exposure, which suggest both the recent uptake and the stored residuals determine the ER values (equation 9). Generally, about 50 % of the (+)-*c*-HE and 66% of (-)-*c*-HE was estimated (from equation 10 and 11 and Fig. 3-6-2 (1)) as the average recent uptake and the other part should be counted as historical enantioselective degraded residuals in human body based on the upper assumptions (only calculated for the milk samples). Similar explanations could be used for α-HCH for most of the samples.

$$C_{+uptake} = \frac{C_+ - C\_ER_s}{1 - \frac{ER_s}{ER_{uptake}}} \qquad (10)$$

$$C_{s+} = \frac{C_+ - C\_ER_{uptake}}{1 - \frac{ER_{uptake}}{ER_s}} \qquad (11)$$

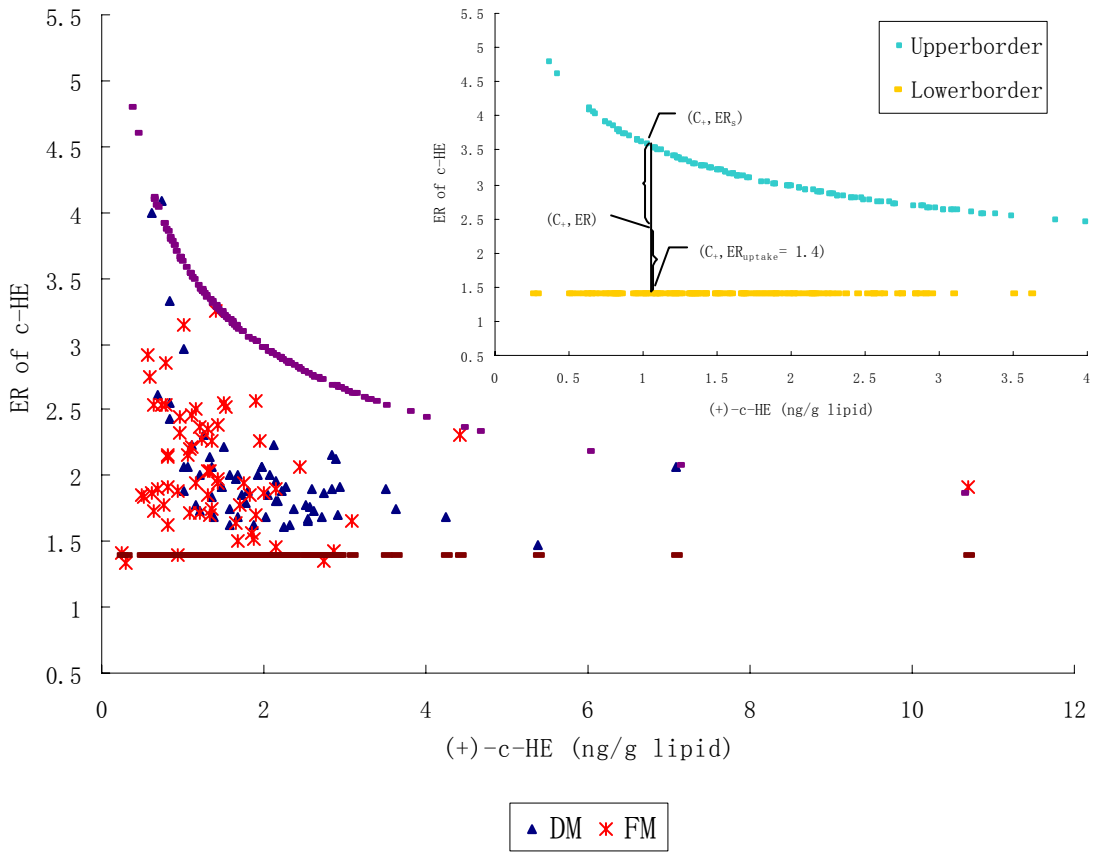
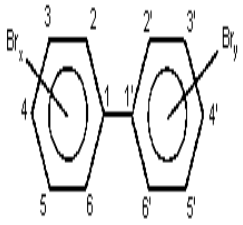


Fig. 3-6-2 (1): Upper border line ER=1.4×$(C_+/29.8)^{-0.28}$; β=0.78 and lower border line ER=1.4

3-7: PBBs in Finnish placenta samples

Only three commercial bromobiphenyl (BB) products were manufactured, which were hexabromobiphenyl (HxBB), octabromobiphenyl (OBB) and decabromobiphenyl (DBB) (Hardy, 2002). HxBB contained Br_5- through Br_7-congeners (or Br_4- through Br_{10}), which were 12-25% Br7, 60-80% Br6, 1-11% Br5; OBB contained Br7 through Br_{12} congeners (or B_6 through Br_{12}), which were 1-9% Br10, 28-65% Br9, 31-72% Br8 and 1-27% Br7; and DBB contained 70-90% Br10, 2-11% Br9 and less Br8, Br7 and even Br6 (EHC 152, 1994). The commercial production PBBs was started in the USA in 1970. OBB and DBB were produced in the USA until 1979. A mixture of highly brominated PBBs called Bromkal 80-9 D was produced in Germany until mid- 1985. Technical grade DBB (Adine 0102) was produced until 2000 in France (Hardy, 2002). Fish less accumulates more than 4-bromine atom substituted BBs than the corresponding PCB's from water. However, the accumulation of these BBs from food is higher or equal to that of Aroclor 1254 (Zitok, 1977). Additionally, HeBB was not accumulated either from water or from food by fish (Zitko et al. 1976). It has been suggested that the congener patterns found in fish could be the result of photochemical debromination of DBB (EHC 152, 1994). Although no congener information, the transfer of PBBs across the human placenta and into maternal milk had been reported (Jacobson et al., 1984) and the contents in placenta tissue were ND-370 ng/g (ww) for Michigan specimens (10 years later of the PBB accident) and the content correlated with maternal serum contents (Eyster et al., 1983). Because of the resistance of BB-15, BB-155 and BB-153 to biodegradation (Hakk et al, 2003), they can be detected frequently in Finland placenta samples. However, BB-37, BB-77 and BB-169 cannot be detected in almost all samples. Few samples contained more BB-155 than BB-153 and the congeners BB-4, BB-31, BB-80, BB-101 frequently occurred in the samples that exhibit a ratio BB-155/BB-153 more than 0.5 (expect sample 11377). The ratios could not be surprised when comparing the data from Europe, however, the BB155 were much lower than BB153 in USA data (Table 3). BB-153 (~60%) was the principal component of the widely used commercial HxBB (~87% of the total PBBs), but PBB-155 only occupied 0.5% in FM BP-6® (EHC 152, 1994; Hardy, 2002). The



exposure congener difference might reflect different pollutant sources when comparing Europe to USA, the continuing production of OBB and DBB might contribute to the different exposure patterns. DBB cannot be detected at the present analytical conditions.

Table 3-7 (1): PBB congeners in Finland placenta samples (lipid base pg/g)

Congener	BB4	BB15	BB31	BB80	BB101	BB155	BB153	PeBB	HeBB
Structure	2,2'-	4,4'-	2,4',5-	3,3',5,5'-	2,2',4,5,5'-	2,2',4,4',6,6'-	2,2',4,4',5,5'-		
STD	19	61	53	10	14	93	217	22	200
Mean	27	47	51	20	30	63	82	37	130
Maximum	60	360	150	30	40	370	1610	80	940
Minimum	10	10	10	10	20	10	10	10	10
GM	22	27	33	18	28	28	41	31	72
Frequency %	6.3	55	6.3	2.7	1.8	19.6	48.2	8.9	29

39 of 112 placenta samples have negative results to all PBBs and one of them was only positive for HeBB. In 64 samples only BB-15, BB-155 or BB-153 can be detected. STD: standard deviation; GM: geometric mean

Table 3-7 (2): Other congeners can be detected in 10 samples (lipid base pg/g)

	BB-4	BB-15	BB-31	BB-37	BB-80	BB-103	BB-101	BB-155	BB-153	PeBB	HeBB	BB-155/ BB-153
10006		170	10		30		40	40	40			1
10253		70		10	10	10	20	90	130	20	100	0.7
10428		10	30					50	110			0.5
10817	40	50	150		20			370	30		60	12.3
11262	10	50	100					270	40	50	120	6.8
11363	30	50						100		50	120	-
11377	10	30	10					30	140	30	110	0.2
11413	10	20	30					140				-
11518	30	30										-
14096	60	40	30					110	180			0.6

Table 7-3 (3): Reference data environmental samples from Europe and USA

Species		BB15	BB31	BB49	BB52	BB80	BB101	BB103	BB153	BB154	BB155	BB169
River fish[1]	Germany							0.02	0.6	0.22	0.66	<0.01
Baltic fish[1]								0.12	2.39	0.54	2.64	15.16
Fish[1]	North Sea							0.1	1.31	0.37	1.11	<0.01
SS[1]								< 0.02	0.81	<0.02	0.4	<0.01
Cow's milk[1]	Germany							< 0.02	0.04	<0.02	< 0.03	<0.01
Human milk[1]	Germany							-	1.03	0.01	0.05	0.05
SP1[2]	blubber	0.08		0.33	0.55		1.26		2.63			<0.14
SP2[2]	blubber	0.17		0.56	0.9		1.71		3.12			0.21
SP2[2]	liver	<0.03		<0.03	<0.03		1.99		56.78			<0.13
SP3[2]	blubber	3.04		8.7	15.65		30.43		47.83			<4.35
WD[2]	blubber	0.2		7.58	4.14		8.38		13.13			<0.2
WD[2]	liver	<0.37		2.22	1.11		27.41		703.7			<0.74
Minke whale[2]	blubber	0.79		1.93	1.71		3.86		5.86			<0.14
HS1[2]	blubber	<0.2		139.34	23.36		38.11		250			49.18
HS2[2]	blubber	<0.05		3.22	2.39		1.45		18.69			<0.21
HS3[2]	blubber	<0.07		4.16	0.72		1.52		18.01			<0.14
HS2[2]	liver	<0.29		2.86	1.43		17.71		42.86			<0.57
HS3[2]	liver	<0.2		1.96	0.59		0.78		16.08			<0.2
HS4[2]	liver	<0.33		30	4.67		14.67		433.33			<0.67
Mackerel[2]	muscle	0.07		0.07	0.07		<0.07		0.26			<0.2
Trout[3,*]	Superior		< 1.8	6.8	8.4	< 3.8	42	< 1.5	189		1.0	
Trout[3,*]	Huron		5.2	125	191	< 3.8	633	4.4	2083		5.8	
Trout[3,*]	Erie		< 1.8	20	24	< 3.8	71	< 1.5	220		< 0.98	
Trout[3,*]	Ontario		< 1.8	14	11	< 3.8	109	< 1.5	1008		1.1	

SS: spitzbergen seal SP: sperm whale; HS: harbour seal; WD: Whiteb. Dolphin

[1]EHC 152, 1994; [2]de Boer et al. 1998; [3]Luross et al., 2002; [*]Data from USA expressed as pg/g w. w. ±S.D. and the rest expressed as ng/g fat

3-8: The investigated compound perspectives

3-8-1: DDT and the metabolites with MOC

DDT and the metabolites: Typical technical DDT mixtures consist of 77.1% *p, p'*- DDT, and 14.9% chiral *o, p'*-DDT. Further compounds detected are the *p, p'*-DDE (4%), *o, p'*-DDE (0.1%), *p, p'*- DDD (0.3%) and chiral *o, p'*-DDD (0.1%), together with approximately 3.5% unidentified compounds (Vetter et al., 1997). *p, p'*-DDE, the major and persistent DDT metabolite, is the most abundant residue in the present study. Also *p, p'*-DDT have apparent residuals, especially in the investigated milk samples. The other residuals such as *p, p'*-DDD, *o, p'*-DDT, *o, p'*-DDD and *o, p'*-DDE can be detected in much lower level in most of the investigated samples. The maximum levels for *p, p'*-DDE in DM, FM, DP and FP are 427.55, 331.16, 269.83 and 79.21-ng/g lipids; for p, p'-DDT are 37.88, 12.9, 4.65, and 3.38-ng/g lipid; for *p, p'*-DDD are 2.2, 1.36, 6.35 and 2.4-ng/g lipids and for *o, p'*-DDT are 1.83, 1.21, 0.26 and 0.92-ng/g lipids.

DDT can result in a neurotoxic syndrome in both vertebrate and invertebrate species through the action on the axonal membrane and neonatal mice showed a more sensitive DDT response than adult (50 to 200 times) and the action is permanent (Evangelista de Duffard et al., 1996). *p, p'*-DDT and *o, p'*-DDT suppressed the neurite outgrowth dose dependently, and *p, p'*-DDE revealed a similar effect but a lesser extent. Apoptotic cell death was induced by *p, p'*-DDT and *o, p'*-DDT but also to lesser extent by *p, p'*-DDE (Shinomiya, et al. 2003). Several DDT analogs can mimic phenobarbitone in the mode of induction of hepatic drug-metabolizing enzymes in the immature male Wistar rat (Campbell et al., 1983). The transplacental toxicity of 3-$MeSO_2$-DDE in the earliest stage of developing adrenal cortex had been observed in mice (Jönsson et al. 1995). Following local CYP11B1-catalyzed metabolic activation and irreversible protein binding in the adrenal cortex, *o, p'*-DDD and 3-$MeSO_2$-DDE have been tested highly potent adrenal toxicant that induces mitochondrial degeneration and cell death in the murine and human; additionally, *o, p'*-DDD and 3-$MeSO_2$-DDE, have adrenocorticolytic effects in human, rodent, and fish adrenal tissue but 3-$MeSO_2$-DDE can impair hormone secretion only (Lindhe, et al. 2002). *p, p'*-DDT and *p, p'*-DDE associated with interleukin-4 (IL-4) plasma levels, which could contribute an

immunological abnormalities factors (Volker et al, 2002).

DDT and certain metabolites interact with estrogen, androgen and progesterone receptors by multiple hormone receptor signaling pathways. *p, p'*-DDE has little ability to bind the estrogen receptor (ER), however, it inhibits androgen binding to the androgen receptor (AR), androgen-induced transcriptional activity, and androgen action in developing, pubertal and adult male rats (Kelce et al., 1995). It was 36.9-fold less potent as antiagonist of AR than DHT (equilibrium dissociation constant K_B =36.9 $\times 10^{-8}$M when DHT was 1×10^{-8}M) (Maness et al., 1998; Gaido et al., 2000). Epidemiological study showed the overall concentrations of plasma DDE (median level was 1213 ng/g lipid) and androgens in 137 North Carolina black male farmers were unrelated but, among those whose DDE level was in the top tenth percentile, compared with all others, total testosterone and free androgen index were lower by 23% and 22% respectively (Martin Jr et al., 2002). However, developmental abnormalities cannot be predicted from exposure levels from adults with their higher tolerance (Kelce et al., 1997). *p, p'*-DDE can also produce rapid nongenomic signaling effects via second messenger systems (Bulayeva et al., 2004). Apart from *p, p'*-DDE, AR assay on the HepG2 human hepatoma cell line, which transiently transfected with the human AR and an androgen-responsive reporter, showed all the *o, p'*-DDT, *o, p'*-DDE, *o, p'*-DDD, *p, p'*-DDT, *p, p'*-DDE and *p, p'*-DDD behaved as antagonists at concentrations above 10^{-6} M and *p, p'*-DDE also showed some agonist activity at 10^{-5} M (Maness et al., 1998).

In contrast with *p, p'*-DDE, *o, p'*-DDT acted as EDC mainly by binding and activating the ER (Maruyama et al., 1999) and stimulated proliferation in a dose-dependent manner in the ER-positive cell lines MCF-7 and T47D (Steinmetz et al., 1996). The present data suggested the dominated enantioselective residuals are the less activated (+)-isomer whereas hER activity was negligible (Hoekstra et al., 2001). ER competitive binding assays showed *p, p'*-DDT, *o, p'*-DDD and *o, p'*-DDE were all able to bind specifically to the human estrogen receptor (hER) with approximately 1000-fold weaker affinities for the hER than estradiol (only *o, p'*-DDT but *p, p'*-DDT bound to the rat ER) (Chen et al., 1997). *o, p'*-DDE, *o, p'*-DDD can competitively bind to ER source in uteri from ovariectomized Sprague-Dawley rats (Blair et al., 2000). However, *p, p'*-DDT, *p, p'*-DDD and *p, p'*-DDE are virtually no evidence of estrogenicity to modulate

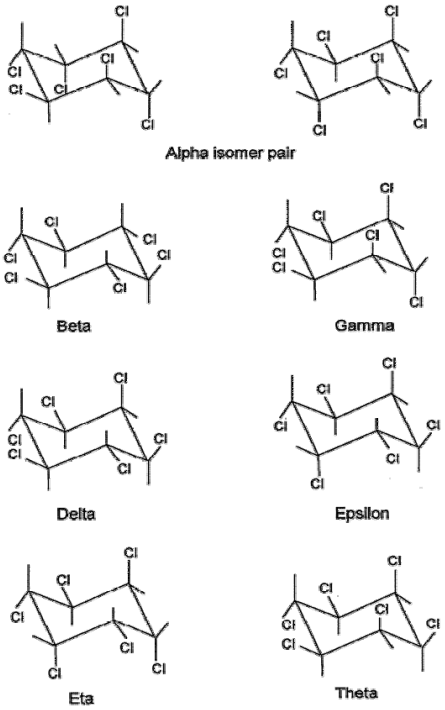
transcriptional activation of an estrogen-responsive reporter gene in transfected HeLa cells (Tullya et al., 2000).

Assays evaluated by yeast expressing human progesterone receptor (hPR) and T47D human breast cancer cells expressing endogenous hPR (Klotz et al., 1997) suggested *o, p'*-DDT, *p, p'*-DDT, *o, p'*-DDD, *p, p'*-DDD, *o, p'*-DDE, *p, p'*-DDE, *p, p'*-DDA and DDOH inhibited progesterone-induced reporter gene activity in a dose-dependent manner. None of them functioned as hPR agonists. Whole cell competition binding assays using T47D cells indicated that the progesterone-dependent inhibitory effects might occur through both hPR-dependent and hPR-independent pathways. *o, p'*-DDT and β-HCH as well were found to be potent activators of protein kinases, which activated c-Neu at extremely low (0.1-1nM) concentrations to elicit estrogen action by ligand-independent way (Enan et al., 1998). DDE can also produce rapid nongenomic signaling effects via second messenger systems (Bulayeva et al., 2004) and *o, p'*-DDT can modulate steroid hormone homeostasis through induction of heptatic enzymes (Gladen et al., 2004).

MOC: MOC is a currently used pesticide as the DDT substituted compound (lower toxicity and shorter half-life compared to DDT). It is metabolized fairly quickly to phenolic derivatives like mono-OH-MOC (77-87% of *S*-isomer in human liver microsomes) and di-OH-MOC (Hu et al., 2002). Because of a weak EDC (Nimrod et al., 1996; Guillette Jr et al., 2002; Maruyama et al., 1999), the *in vivo* estrogenic activity of MOC might be caused its phenolic estrogenic metabolites, which expressed as agonistic or antagonistic for ERα, ERβ or AR (Maness et al., 1998; Gaido et al., 1999; 2000; Blair RM et al. 2000; Cupp et al., 2001). MOC alter gonadotropin-releasing hormone (GnRH) biosynthesis by directly regulate GnRH gene expression in hypothalamic cell line but through ER by consistently mimic estrogen and might have endocrine disrupting effects on GnRH neurons in vivo (Gore AC et al. 2002). Present data showed MOC level was lower in all the investigated samples might because of its ready to degradation in environment. The maximum levels for MOC in DM, FM, DP and FP are 0.43, 1.12, 7.79 and 1.14-ng/g lipids. In conclusion, the endocrine disrupting action, if possible, might mainly refer the antiandrogenic function of p,p'-DDE in most samples.

3-8-2: HCHs with emphasis the β-isomer

Five of the eight possible isomers of HCHs are present in technical mixtures, typically containing 60-70% α, 5-12% β, 10-12% γ, 6-10% δ, and 3-4% ε. Technical HCH had been used widely as commercial pesticide before the use of pure highest pesticidal active Lindane (γ-HCH isomer to 99.9%). As a synthetic product, enantiomers of α-HCH are in the racemic ratio 1:1. Although a smaller component of technical HCH, β -isomer has much higher bioconcentration factor than α-, and γ- isomer in human fat (Willett, et al. 1998). HCHs were used in both human and veterinary medicine to treat ectoparasites, human scabies (skin disease caused by mites), pediculosis (infestation with lice), also had been used as a general insecticide to control structural pests such as termites. The present data suggest HCH, especially the β-isomer, are ranked the second high levels among the investigated compounds in most samples. The maximum levels for β-HCH in DM, FM, DP and FP are 66.23, 30.89, 47.76 and 45.54-ng/g lipids; for α-HCH are 3.45, 0.77, 8.92 and 692.79 (one of the two abnormal samples data)-ng/g lipid and for γ-HCH are 3.34, 4.05, 2.04 and 246.87 (one of the two abnormal samples data)-ng/g lipids. Lindane has been well reviewed as a potent convulsant agent in humans and other mammals. Other effects included intention tremors, memory impairment, irritability, and aggression. At the nonconvulsant doses, lindane can interfere with the ability to acquire and use new information (Evangelista de Duffard et al., 1996). The effects of four HCH isomers as inducers of hepatic drug-metabolizing enzymes in the immature male Wistar rat all resembled the mode of induction of phenobarbitone (Campbell, et al. 1983). HCHs might also affect the human immunological health combining with the other PBTs (Volker et al, 2002).



γ-HCH, α-HCH, β-HCH can cause greater than 50% displacement of estradiol-17β (0.0078 μM) from the alligator ERα, with IC_{50} of 37.2, 43.4, 48.3 μM, respectively (Guillette Jr et al., 2002).

However, as endocrine disruptor, β-HCH mainly stimulated cell proliferation and gene expression ER dependently, but its action is not through the classic ER pathway (Steinmetz et al., 1996). In fact, β-HCH acted estrogenic without being an agonist for ER in MCF-7 cells but by ligand-independent activation pathways (evoked by EGF through EGF receptor or β-HCH through c-Neu) (Hatakeyama et al., 2002ab). Long-term exposure of MCF-7 cells to β-HCH (100nM to 1μM) can not only enhance their transformation tendencies but also promoted their invasiveness (Zou et al., 2003). β-HCH has been reported as a significant risk factor of breast cancer (sampling from Oct. 1985 to Feb. 1986) at concentration 130 (±60) ng/g comparing the control 80 (±30) ng/g lipid in adipose breast tissue (Rauhamaa-Mussalo et al., 1990). The other isomers of HCH, α-, δ-, and γ-HCH inhibited steroidogenesis by reducing steroidogenic acute regulatory protein expression (assaying in mouse MA-10 leydig tumor cell line), an action that may contribute to the pathogenesis of lindane-induced reproductive dysfunction (Walsh et al., 2000). Considering the relative amounts, the endocrine functions of HCHs, if been expressed, should focus on β-HCH in the investigated samples.

Table 3-8-2 (1): β-HCH was the most persistent in HCH isomers in human body (Willett, et al. 1998)

Property	α-HCH	β-HCH	γ- HCH	δ- HCH	ε- HCH
Melting point (^{o}C)	159-160	309-310	112-113	138-139	219-220
Vapor pressure	$(1.6\pm0.9)\times10^{-2}$	$(4.2\pm0.3)\times10^{-5}$	$(5.3\pm1.4)\times10^{-3}$	2.1×10^{-3}	
Log Kow	3.9±0.2	3.9±0.1	3.7±0.5	4.1±0.02	
BCF in human fat	20±8	527±140	19±9		
BCF in aquatic	2.6±0.5	2.9±0.3	2.5±0.4		

3-8-3: HeCB, PeCB and PCA

HeCB and PeCB: It is one of the 12 banned POPs by WHO as a pesticide and also an industrial byproduct. HeCB as an antibacterial agent was widely used in soap and antiseptic solutions and has been found in blood or serum, urine, feces, adipose tissue, and breast milk. It also is suspected as the parent compound of the environmental PeCB (Smeds and Saukko 2001). The present data showed HeCB is one of the main residuals in all of the investigated samples, the concentration of the related compounds like PeCB and PCA are lower. The maximum levels for HeCB in DM, FM, DP and FP are 24.56, 18.55, 26.49 and 14.59-ng/g lipids; for PeCB are 1.41, 1.17, 3.45 and 171.71

(an abnormal data)-ng/g lipid and for PCA are 0.79, 0.11, 2.7 and 1.7-ng/g lipids. The ratios of PeCB/HeCB were 0.063, 0.124, 0.028 and 0.031 for DP, FP (one sample has abnormal high), DM and FM respectively; and PCA/HeCB were 0.02, 0.033, 0.007 and 0.004 for DP, FP, DM and FM respectively. However, no apparent correlation between these related compounds could be found.

The effects of HeCB as a microsomal enzyme inducer resembled those observed after coadministration of phenobarbitone plus 3-methylcholanthrene (Campbell et al., 1983). As a neurotoxicant, large doses fed rats suffered from the damage of white matter and peripheral neuropathy, motor dysfunction in adults, inhibited synthesis of myelin and suffered hyperactive upon subsequent behavioral evaluation when exposed *in utero* (Evangelista de Duffard et al., 1996). HeCB and its main metabolite PCP could induce hypothyroidism by involving of the different mechanisms in rat (Van Raaij et al., 1993). More general effects include stimulatory effects on the immune system and induction of enzymes involved in drug metabolism, porphyria, and the reproductive system in rat and human, such as porphyria turcica. As a result, the toxicant HeCB inflicts multisystemic damage through initiation of an inflammatory response (Ezendam et al., 2004; McGovern, 2004). HeCB can activate liver enzymes that hydroxylate androgens and might induce liver and testis dysfunction (Toppari et al. 1996). Lower level (0.5-5 nM) enhanced androgen action but higher level (>10 μM) of HeCB interfered in the prostate (Ralph et al., 2003). HeCB had been reported revealing a highly significant difference between patients from the control group and those from the undescended testes group (Hosie et al. 2000).

PCA: PCA is an environmental metabolite of structurally related, commercially important, ubiquitous chlorinated aromatic compounds such as pentachlorophenol (PCP) and pentachloronitrobenzene (PCN) (Mayring et al. 1984; Ikeda et al. 1995; Rüttimann-Johnson et al. 1997). PCN was introduced into agriculture from 1930 as powerful fungicide and its biotransformation into PCP, PCA had been investigated in soil, plants and animals (Torres et al., 1996). PCP has been used extensively as fungicide, insecticide and herbicide and the accidental spillage and inappropriate disposal at many wood treatment facilities (Chung et al. 1995). They are all potential teratogenic compounds (Welsh et al., 1987) and have been found in Finnish soil and earthworms of sawmill environments (Knuutinen et al., 1990; Palm et al., 1991). Animal

administration showed PCA could be metabolized to tetrachlorohydroquinone (TCH), free and conjugated-PCP in male Sprague-Dawley rats and New Zealand White rabbits (Ikeda et al., 1994) and was readily demethylated to PCP and conjugated PCP but TCH by beagle dog and miniature pig (Ikeda et al., 1995). PCP and PCA have been showed apparent reproductive toxicity to male rats (Welsh et al., 1987). PCP constituted 46% (geometric mean) of the total quantitated chlorinated phenolic compounds in Inuit blood and the concentrations ranged from 0.558 to 7.77 ng/g on a wet weight basis, which might also interfere thyroid hormone function (Sandau et al., 2000).

3-8-4: Dieldrin also reflected the exposure of aldrin

In many countries outside the United States, dieldrin is used as a broad-spectrum insecticide to protect food crops and control disease vectors, locusts, and termites. Levels of dieldrin in the environment reflect exposure to aldrin or dieldrin because aldrin can be degraded rapidly to dieldrin, which is more persistent and accumulates in living organisms (ATSDR 2002). The present investigation showed dieldrin was detectable for each samples and aldrin was almost nothing. The maximum levels in DM, FM, DP and FP are 35.5, 7.19, 19.5 and 4.32-ng/g lipids respectively.

Dieldrin-induced hepatocarcinogenesis in mice occurs through a nongenotoxic mode of action: the slow oxidative metabolism of dieldrin, accompanied by an increased production of reactive oxygen species, can deplete hepatic antioxidant defenses (particularly α-tocopherol), and peroxidize liver lipids. Dieldrin-induced oxidative stress or its sequelae apparently result in modulation of gene expression that favors expansion of initiated mouse liver cells (Klaunig, et al. 1998; Stevenson, et al, 1999). Antioxidant supplementation prevented the dieldrin-induced cellular changes. An early study (Vainio, et al. 1976) in rat liver shown dieldrin was a more potent inducer than the parent compound itself. Aldrin, dieldrin, mirex act as inducers of hepatic drug-metabolizing enzymes in the immature male Wistar rat resembled the induction mode of phenobarbitone (Campbell et al., 1983). After oral administration of dieldrin-^{14}C to rabbits (Korte et al. 1965), the main metabolite (about 86%) are one of enantiomorphic isomers of 6,7-trans-hydroxy-dihydro-aldrin with a specific rotation of α_{D20} = -13.7 with lower acute oral

toxicity to mammals than that of dieldrin, however, the metabolite was known as neurotoxicant by disruption in membrane Ca^{2+} transport mechanism (Mehrotra, et al. 1989; Khan, 1970; Van Den Bercken, et al. 1974; Akkermans, et al. 1975 a,b; Evangelista de Duffard et al., 1996). Dieldrin has estrogenic effects on human estrogen-sensitive cells (Soto et al. 1994). It has been suggested to affect the risk of developing breast cancer and the survival by assessment of the serum concentration with relative risk estimation (RR) 2.78 when >57.55ng/g in serum after adjusting for tumor characters (Hoyer et al., 2000). Consider the sample specific concentration difference, dieldrin level in DM might involve in the range of higher risk for breast cancer. Like E2, it can also produce rapid nongenomic signaling effects via second messenger systems (Bulayeva et al., 2004).

3-8-5: END

END in its pure form is a crystalline substance consisting of α-, β-isomers (isomer-I and -II) in the ratio of approximately 7:3. The environmental residuals are END-1, -2 and the major metabolites endosulfan ether, endosulfan sulfate, endosulfan lactone, and endosulfan diol (Kaur et al. 1997, Lentza-Rizos et al. 2001). All of the upper compoumds had been detected in human milk (Campoy et al. 2001). Like aldrin and dieldirn, it can induce increase in total glutathione peroxidase activity in hamster ovary cells and alternate the membrane permeability (Bayoumi, et al. 2001). In aqueous system the half-life of END-1 is much longer that END-2 (Guerin, 2001) and the isomerization can occur with the reaction favoring formation of END-1 (Schmidt et al, 1997), which is more toxic than END-1 in mammals (LD_{50} =9.4-40 mg/kg), a similar toxicity with their mainly biological oxidation products END sulfate, LD_{50} =8-76 mg/kg) (Guerin, 2001). In this study, END-1 was the dominated can be detected in all of the investigated and END-2 was almost nothing for most samples. Comparing the reported milk data (Campoy et al. 2001), it seems that the mother's main exposure result from environmental transformed residuals in seafood. Both isomer-I and II of END have the similar potential of estrogenic effects on human estrogen-sensitive cells with similar potency as *o, p'*-DDT (Soto et al. 1994). Like E2, END can also produce rapid nongenomic signaling effects via second messenger systems (Bulayeva et al., 2004). Serum luteinizing hormone levels were significantly positively related to serum END levels after controlling for age. The researchers (Saiyed et al., 2003) suggested that the prevalence of

congenital abnormalities related to testicular descent among study and controls subjects was 5.1% and 1.1%, respectively, might (not significantly) correlate to the long-term END exposure. In the present data, only END-1 should be considered for endocrine disrupting action in most samples. The maximum levels for END-1 in DM, FM, DP and FP are 18.05, 22.66, 6, and 8.78-ng/g lipids and for END-2 are 0.1, 0.12, 0.39 and 5.63-ng/g lipids respectively.

3-8-6: Biotransformed technical chlordane residuals

Technical CHL consists of more than 140 compounds, includes many chiral compounds. The major pesticides *c-, t*-CHL and HC, including the minor Miyazaki compounds and U82 (de Boer, 1999), are chiral compounds. In environmental samples, the major residuals are *c-, t*-CHL, *trans*-nonachlor, and two metabolites *c*-HE and OXC, sometimes together with *cis*-nonachlor and α-, β-chlordene (de Boer, 1999). HC is rapidly degraded in the environment and the main metabolic product *c*-HE is a persistent chiral toxicant (Vetter et al., 1997). *c*- and *t*-CHL were metabolized different and enantioselective in the environment (Ulrich et al., 1998). In biological samples the major residuals are achiral *trans*-nonachlor and chiral OXC. Technical CHL, *c-, t*-CHL, OXC, trans-nonachlor, HC and HE all resembled the mode of induction of phenobarbitone as inducers of hepatic drug-metabolizing enzymes in the immature male Wistar rat (Campbell et al., 1983). In the present data, *c*-HE and OXC are the dominant residual and the other residuals could be omitted for the most samples. The chiral residual information for *c*-HE could be useful to investigate the recent uptake and historical exposure level for this compound. As weak EDCs, *c*-HE, HC and

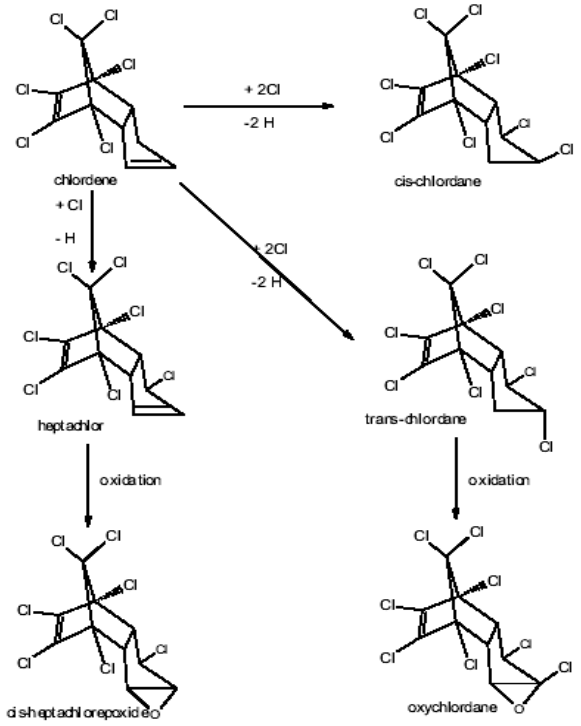


Fig 3-8-6 (1): Major chlordane-related compounds (Vetter et al., 1997)

OXC can cause greater than 50% displacement of estradiol-17β (0.0078 μM) from the alligator ERα, with IC_{50} of 19.4, 26.7, 34.7μM, respectively (Guillette et al., 2002). Hosie et al. (2000) had been reported *c*-HE revealing a highly significant difference between patients from the control group and those from the undescended testes group. The maximum levels for OXC in DM, FM, DP and FP are 12.01, 9.4, 3.99 and 2.76-ng/g lipids; for *c*-HE are 10.82, 17.02, 3.1 and 2.01-ng/g lipids; for *t*-CHL are 0.35, 0.11, 0.18 and 0.67-ng/g lipids and for *c*-CHL are 0.07, 0.05, 0.18 and 0.1-ng/g lipids. Therefore, only *c*-HE and OXC might need to be considered when assessing the endocrine disruption function of these compounds in the two cohorts.

3-8-7: Other detectable PBT residuals

OCS: Chlorostyrene congeners occur from hexa-, hepta- to octa- in biota, and OCS is the prevalent one ($BCF_w \approx 10^6$ for fish and Log $K_{ow} \approx 6.86$ - 7.68), which can be traced back to the by-products of electrolytic processes (Bester et al., 1998). OCS is the most permanent congener and had been found in livers of various fish from 20-3700 ng/g lipid in the North Sea in 1983 and no remarkable differences comparing the data of 1972 (Ernst et al., 1983). It was reported that OCS could be photo-degraded into penta- and tetra- congeners (Hustert et al., 1984) and metabolised into two isomeric $MeSO_2$-heptachlorostyrenes by blue mussel (*Mytilus edulis*) (Bauer et al., 1988) slowly. OCS is an inducer of the phenobarbital (PB)-type in mice; the induction of acetanilide 4-hydroxylase (AcA hydroxylase) is not regulated by the aryl hydrocarbon (Ah) hydroxylase locus (Holme et al., 1982). The maximum levels for OCS in DM, FM, DP and FP are 0.4, 0.7, 0.39 and 0.55-ng/g lipids in the present study.

Mirex: Although it has been used for control of insects e.g. ants, in Europe, mirex use as fire retardants in industrial products is thought to be the major source of mirex in the Baltic Sea environment and the biological samples are often contaminated with other compounds such as PCBs (Strandberg, et al., 1998). Photomirex has been measured in adipose by GC-MS (LeBel and Williams 1986). Elevations from 1986-1992 in the concentrations of mirex in the breast milk of the Mohawks are consistent with the fact that it is a common contaminant in the region and throughout the Lake Ontario-St. Lawrence River Basin (Fitzgerald, et al. 2001). Mirex can cause

greater than 50% displacement of estradiol-17β (0.0078 μM) from the alligator ERα, with IC_{50} of >50 μM, respectively (Guillette et al., 2002). Except the co-induction of cytochrome P450 2B1 and 2B2 mRNAs and immunoreactive proteins in rat hepatocytes (Kocarek et al. 1994), mirex is a potent non-phorbol ester tumor promoter in mouse skin and E2 is a major ovarian hormone regulator for mirex tumor promotion sensitivity in female mice (Porter et al. 2002). The maximum levels for mirex in DM, FM, DP and FP are 0.66, 1.54, 1.08 and 2.08-ng/g lipids in the present study.

4: Conclusions

Pollutant level and pattern characteristics for Denmark and Finland cohorts: The lipid data and the 8 most abundant pollutants have been evaluated of the distribution, the nearly normal distribution of the lipid data might suggest a representative sample collecting process in the case-control studies. The apparent deviation from normal distributions for most of the compounds suggested more than one random factor contributed the bioaccumulation of these pollutants. PC analysis showed a general simultaneously increase for the 8 compounds even had the much different levels. For both cohorts, there might be a common exposure pattern. The common patterns for Denmark cohort and Finland cohort are similar but with small difference in the content of END-1 and OXC. These patterns showed that most mothers faced nearly the same pollutant sources, which would suggest the types of food sources. All the general levels (cohort geometric means for milk and placenta respectively) and sample specific distributions (for the summary and for each compound) exhibit more samples in Denmark cohort were heavily contaminated than in Finland cohort. The Denmark cohort, with small fat content in milk and placenta samples, has a higher level of pollutants in general, with emphasis for some high contaminated samples, which might be a result of various pollutant sources because they had different compound patterns comparing the common patterns of the lower contaminated mothers. Interestingly, the END-1 and OXC contents were linearly correlated in DM, DP, FM, FP and pooled milk and placenta samples, respectively, which might reflect a source correlation of the two pollutants for almost all investigated samples. On the other hand, the correlations of END-1 and OXC confirmed that the whole analysis procedure was generally stable. Two Finland samples have abnormally high levels of HCH with isomers ratios near to technical mixture (one sample combined with high level of PeCB and δ-HCH) but the other investigated compounds were in normal ranges, which might suggest a heavy HCH contact of single mothers.

Prenatal and postnatal exposure: Paired analysis for the milk and placenta samples of Finland cohort suggested placental contamination by chemicals might act as a biological marker for the exposure of the mother or for the fetus via transplacental transfer. The concentration correlations

for the 10 compounds (*p, p'*-DDE, β-HCH, HeCB, dieldrin, END-1, OXC, c-HE, *p, p'*-DDT, OCS and mirex) confirmed the transplancental contamination. The slopes of regression lines were changed from 1.5 to 3.0 (except *p, p'*-DDT) suggesting that these compound had the similar transferring potentials from fatty tissue breast to non-fatty placentas. In other words, the contents of these lipophilic pollutants in milk were 1.5 to 3.0 times higher than in placenta, which might imply breast-feeding is a more attentive contaminated pathway for babies. However, considering the reproductive toxicity, placenta-transfer pathway might be more harmful. For the investigated cohorts, both sample types can be used as biomarker for the risk assessments.

Chiral information: Apart from the first order clearance process, recent uptake might also the important factor for the detected exposure to the chiral PBTs. The chiral exposure information expressed by c-HE and α-HCH might conclude about the different recent uptake level for the related pollutants, considering the general simultaneously exposure of the 8 main pollutants, which might also supply a general information of the recent total exposure to these pollutants. Additionally, the chiral residual information could be also used as index for the evolution of estrogenic activity of chiral pollutants like *o, p'*-DDT, the most strong xenoestrogen among DDT and its common metabolites.

Are EDCs harmful to human reproduction? For most of the investigated compounds, their concentrations in placenta samples are much lower than the active concentrations used *in vitro* tests. However, the maximum concentrations of *p, p'*-DDE in Denmark cohort and Finland cohort placenta are 0.8 nM and 0.24 nM and Kelce et al. (1997) suggested 0.22 nM in placenta might inhibit androgen action *in vitro*. Other information, such as bioavailability and protein binding in serum, needs to be known in order to evaluate *in vivo* function of *p, p'*-DDE and any other EDCs. Although all of the investigated compounds are weak EDCs, their phenolic metabolites, such as di-OH-DDE and HPTE, typically modulated multiple endocrine pathways, are only 25- and 17-fold less potent as ERα agonists than E2 (Gaido et al., 2000). The stored PBTs could be released from fatty tissue and biotransformed by a diverse enzyme system of the cytochrome P450 monooxygenases. Most of the known metabolic pathways involve the initial formation of phenolic metabolites, which can be excreted unchanged or further conjugated with glucuronic acid or

sulfate (Sandau et al., 2000). The significant correlation between the parent PCBs and resulting OH metabolites for all congeners in both maternal plasma and cord plasma samples (Soechitram et al., 2004) have been reported recently. 3-$MeSO_2$-DDE and 2-$MeSO_2$-DDE had been detected in human body and the ratios of the sum of $MeSO_2$-DDEs to *p*, *p´*-DDE ranged from 1/1000 to 1/142 (mean 1/455) and from 1/200 to 1/30 (mean 1/61) in adipose tissue and liver, respectively (Weistrand et al., 1997). In addition to the combined effect, the exposure of these PBTs as EDCs should be considered, especially to their possible metabolites.

In conclusion, PBTs can be transfered from mother to fetus during pregnancy and have potential harm to human reproductive health. There are definitely exposure differences between Denmark and Finland cohort, from levels to structure. These data will be employed in to the following case-control study to try to interpret the different case incidence of cryptorchidism and hypospadias in the two cohorts.

5: Outlook

Scientifically sound documents from laboratory studies underpin that environmental chemicals are capable of acting as endocrine disrupters. However, the association of PBT exposure to human interacting as endocrine disrupting chemicals with human reproductive health is still a complex problem. The reason is that, for each compound, both of the toxicokinetic factors and toxicodynamic factors need to be checked carefully and people are exposed to a complex mixture of compounds.

Considering the fact that prenatal and postnatal exposures take place to complex chemical matrix, combined effect analysis of complex mixed PBTs should be extended to the non-accumulating ones (such as plant sourced EDCs). Most PBTs are weak EDCs but their metabolites, such as phenolic biotransformation products, are usually more active than the parent compounds. PBTs stored in fatty tissues might just offer a sustainable pool opportunity of endocrine interrupting activities of their metabolites. Therefore, further studies should pay attention on the association of the enzyme activities for biotransformation, the metabolite concentrations and their possible correlations with these PBT-levels *in viv*o. The chiral information, expressed as ER values, is associated with the enantioselective biodegradation processes and is a promising tool to be used as indictor of the enzyme activities for biotransformation. Due to PBTs mimicry in terms of the functional important small molecules (hormones) in organisms and because the concentrations of these hormones are of defined homeostasis and programming controlled, it is also important to monitor the change of these hormones in mother's when investigating their PBTs exposure.

For an overview, experiments could be designed to evaluate systematically the endocrine disrupting activity of PBTs or their mixture within or without their metabolites by testing extracts (such as blood removal of natural hormones) from the administrated animal models, cell cultures or human samples in molecular assays. However, the compound specific PBT levels, their metabolite levels (reflected the enzyme activity) and the change of the interrupted hormone levels, if they are systemic and correlated factors, should be detected carefully and quantitatively for the

confirmed reply of their risk assessment. Therefore, comparable high quality compound specific data are still waiting for the work from trace analysis. Statistically, levels of PBTs, their metabolites and natural hormone level change should be considered as multivariate of the individual health outcome.

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Appended Table 1: Content of selected pesticides in Finnish human placentas (ng/g lipid)

Compound	Positive samples (%)	Geometric mean	Range	Mean (SD)	Limit of detection	Recovery (%)
lipid (% g/g)	100	1.21	0.93-1.52	1.22 (0.13)		
α-HCH	100	0.9	0.2-692.8	9.19 (66.05)	0.02-0.59	48
β-HCH	100	4.7	1.5-45.5	5.58 (5.08)	0.02-0.8	50
γ-HCH	100	0.9	0.4-246.9	3.51 (23.51)	0.03-0.68	48
δ-HCH	85.7	0.2	0.1-727	9.34 (78.1)	0.02-0.72	56
ε-HCH	51.8	0.2	0.1-40.2	1.87 (7.74)	0.03-0.85	-
Total HCH	100	7.6	2.4-985.2	18.28 (93.68)		
PeCB	100	0.6	0.2-171.7	2.55 (16.79)	0.02	50
HeCB	100	4.5	1.8-14.6	4.83 (1.85)	0.02	52
PCA	99.1	0.2	0.1-1.7	0.19 (0.18)	0.11	55
OCS	96.4	0.1	0.1-0.5	0.14 (0.07)	0.03	-
p, p'-DDT	100	0.3	0.1-3.4	0.39 (0.44)	0.07	70
o, p'-DDT	47.3	0.1	0.1-0.9	0.13 (0.12)	0.07	66
p, p'-DDD	100	0.5	0.2-2.3	0.55 (0.32)	0.02	64
o, p'-DDD	56.3	0.1	0.1-0.4	0.14 (0.08)	0.04	-
p, p'-DDE	100	17.8	3.1-79.2	21.94 (15.25)	0.01-0.17	57
o, p'-DDE	9.8	0.1	0.1-0.6	0.15 (0.15)	0.03	58
Total DDT	100	18.8	3.5-80	22.88 (15.6)		
DDE/DDT (p, p')	100	58.4	2.8-265	70.8 (45.0)		
t-CHL	8.9	0.2	0.1-0.7	0.21 (0.21)	0.11	47
c-CHL	6.3	0.1	-	-	0.13	-
OXC	100	0.9	0.2-2.8	1.01 (0.56)	0.1	53
HC	1.8	0.1	-	-	0.03	60
c-HE	100	0.6	0.2-2	0.7 (0.31)	0.05	57
t-HE	24.1	0.1	0.1-0.2	0.13 (0.05)	0.48	-
Aldrin	4.5	0.1	-	-	0.05	48
Dieldrin	100	1.2	0.4-4.3	1.39 (0.7)	0.1	53
END-1	100	1.8	0.3-8.8	2.26 (1.62)	0.01-0.15	52
END-2	50	0.1	0.1-5.6	0.22 (0.74)	0.01-0.25	54
MOC	42	0.1	0.1-1.1	0.14 (0.16)	0.08	114
Mirex	94	0.2	0.1-2.1	0.3 (0.2)	0.02	50

Appended Table 2: Content of selected pesticides in Denmark human placentas (ng/g lipid)

Compound	Positive samples (%)	Geometric mean	Range	Mean (SD)	Limit of detection	Recovery (%)
lipid (%g/g)	100	1.07	0.55-1.5	1.09 (0.17)		
α-HCH	51	1.34	0.46-8.92	1.76 (1.62)	0.00-0.04	70
β-HCH	100	8.77	2.87-47.76	9.92 (6.07)	0.00-0.08	72
γ-HCH	6	1.3	1.06-1.72	1.31 (0.19)	0.00-0.04	68
δ-HCH	20	0.31	0.15-0.7	0.33 (0.14)	0.00-0.04	79
ε-HCH	7	0.03	0.01-0.1	0.04 (0.03)	0.00-0.04	-
Total HCH	100	9.81	3.07-48.52	10.95 (6.61)		
PeCB	21	0.97	0.48-8.56	1.23 (1.39)	0.00-0.33	55
HeCB	100	7.63	2.17-26.49	8.08 (2.99)	0.00-0.03	59
PCA	37	0.32	0.13-2.7	0.4 (0.39)	0.00-0.03	67
OCS	48	0.15	0.08-0.39	0.16 (0.06)	0.00-0.04	65
p, p′-DDT	59	0.7	0.31-4.65	0.85 (0.72)	0.00-0.06	83
o, p′-DDT	8	0.18	0.14-0.26	0.19 (0.05)	0.00-0.04	80
p, p′-DDD	100	0.68	0.16-6.28	0.85 (0.8)	0.00-0.02	81
o, p′-DDD	89	0.06	0.02-0.35	0.08 (0.06)	0.00-0.02	-
p, p′-DDE	100	40.77	9.52-269.83	47.43 (29.69)	0.01-0.1	67
o, p′-DDE	49	0.04	0.02-0.2	0.04 (0.03)	0.00-0.02	68
Total DDT	100	42.04	9.86-273.57	48.88 (30.32)		
DDE/DDT (*p, p'*)	59	71.69	11.51-380.04	84.32 (51.51)		
t-CHL	5	0.05	0.03-0.18	0.06 (0.05)	0.00-0.07	67
c-CHL	5	0.05	0.03-0.19	0.06(0.05)	0.00-0.07	67
OXC	100	1.02	0.15-3.99	1.19 (0.66)	0.00-0.17	70
HC	-	-	-	-	0.02	75
c-HE	100	0.88	0.1-3.1	0.99 (0.47)	0.00-0.06	75
t-HE	-	-	-	-	0.01-0.34	-
Aldrin	11	0.04	0.01-0.73	0.08(0.17)	0.00-0.03	64
Dieldrin	100	2.34	0.42-19.57	2.73 (1.96)	0.00-0.12	64
END-1	83	2.21	0.72-6	2.45 (1.09)	0.01-0.22	65
END-2	8	0.21	0.13-0.39	0.22 (0.08)	0.01-0.16	66
MOC	1	0.59	0.59	0.59(-)	0.00-0.05	98
Mirex	99	0.14	0.02-1.08	0.19 (0.17)	0.00-0.02	61

Appended Table 3: Content of selected pesticides in Denmark human milk (ng/g lipid)

Compound	Positive samples (%)	Geometric mean	Range	Mean (SD)	Limit of detection	Recovery (%)
lipid (% g/g)	100	2.66	0.36-7.33	2.99 (1.38)		
α-HCH	38	0.63	0.1-3.45	0.93 (0.80)	0.00-0.05	49
β-HCH	100	17.6	5.97-66.23	20.55 (13.02)	0.00-0.09	51
γ-HCH	32	0.89	0.29-2.98	1.08 (0.72)	0.00-0.04	48
δ-HCH	5	0.18	0.14-0.27	0.19 (0.07)	0.00-0.03	57
ε-HCH					0.00-0.04	-
Total HCH	100	18.99	7.02-67.33	21.86 (13.06)		
PeCB	83	0.34	0.18-1.41	0.38 (0.23)	0.00-0.02	40
HeCB	100	12.20	6.01-24.56	12.82 (4.13)	0.00-0.04	49
PCA	43	0.17	0.05-0.79	0.24 (0.21)	0.00-0.03	53
OCS	86	0.20	0.07-0.39	0.21 (0.08)	0.00-0.03	58
p, p′-DDT	100	5.79	1.62-37.88	7.19 (5.70)	0.00-0.04	80
o, p′-DDT	100	0.48	0.13-1.83	0.58 (0.38)	0.00-0.04	69
p, p′-DDD	75	0.10	0.01-0.33	0.12 (0.07)	0.00-0.03	65
o, p′-DDD	46	0.03	0.01-1.07	0.08 (0.22)	0.00-0.01	-
p, p′-DDE	100	127.42	24.59-427.55	148.73 (84.93)	0.00-0.1	52
o, p′-DDE	92	0.09	0.03-0.28	0.09 (0.05)	0.00-0.02	54
Total DDT	100	135.07	26.63-442.7	157.12 (88.31)		
DDE/DDT (*p, p'*)	100	22.01	4.36-106.66	25.12 (15.14)		
t-CHL	66	0.05	0.01-0.35	0.07 (0.06)	0.00-0.04	51
c-CHL	37	0.03	0.01-0.07	0.04 (0.02)	0.00-0.04	51
OXC	100	4.88	2.26-12.01	5.20 (1.89)	0.00-0.25	56
HC					0.01	67
c-HE	100	2.78	1.25-10.82	3.08 (1.59)	0.00-0.14	64
t-HE					0.02-0.71	-
Aldrin					0.00-0.03	53
Dieldrin	100	5.04	1.74-35.5	5.84 (4.45)	0.00-0.17	54
END-1	100	6.89	1.92-18.05	7.70 (3.54)	0.01-0.31	48
END-2					0.01-0.34	52
MOC	8	0.16	0.07-0.26	0.18 (0.08)	0.00-0.04	94
Mirex	98	0.20	0.04-0.66	0.23 (0.12)	0.00-0.02	53

Appended Table 4: Content of selected pesticides in Finland human milk (ng/g lipid)

Compound	Positive samples (%)	Geometric mean	Range	Mean (SD)	Limit of detection	Recovery (%)
lipid(% g/g)	100	4.24	0.95-10.14	4.52(1.56)		
α-HCH	100	0.15	0.04-0.77	0.18(0.14)	0.00-0.02	47
β-HCH	100	10.65	2.74-30.89	11.68(5.23)	0.00-0.27	44
γ-HCH	100	0.43	0.08-4.05	0.59(0.64)	0.00-0.04	47
δ-HCH	38	0.03	0.01-0.16	0.05(0.04)	0.00-0.10	50
ε-HCH					0.00-0.12	-
Total HCH	100	11.45	3.64-31.74	12.46(5.38)		
PeCB	100	0.25	0.08-1.17	0.27(0.15)	0.00-0.01	39
HeCB	100	7.98	2.94-18.55	8.51(3.17)	0.00-0.08	45
PCA	98	0.03	0.01-0.11	0.04(0.02)	0.00-0.01	52
OCS	100	0.17	0.04-0.70	0.20(0.12)	0.00-0.02	69
p, p′-DDT	100	3.75	1.46-12.9	4.26(2.42)	0.00-1.35	51
o, p′-DDT	98	0.26	0.04-1.21	0.31(0.19)	0.00-0.33	43
p, p′-DDD	100	0.32	0.10-1.36	0.39(0.26)	0.00	44
o, p′-DDD	85	0.02	0.01-0.17	0.03(0.03)	0.00	-
p, p′-DDE	100	64.49	18.95-331.16	79.04(57.81)	0.01-0.11	29
o, p′-DDE	100	0.03	0.01-0.14	0.05(0.03)	0.00-0.05	44
Total DDT	100	69.24	20.74-346.12	84.06(60.09)		
DDE/DDT (*p, p'*)	100	17.19	8.10-42.51	18.49(7.69)		
t-CHL	82	0.03	0.01-0.11	0.04(0.03)	0.00-0.04	75
c-CHL	46	0.02	0.01-0.05	0.02(0.02)	0.00-0.03	-
OXC	100	3.47	0.91-9.40	3.93(1.9)	0.00-0.17	49
HC					0.01	67
c-HE	100	2.03	0.63-17.02	2.37(2.06)	0.00-0.05	57
t-HE					0.01-0.27	-
Aldrin					0.00-0.03	43
Dieldrin	100	2.47	0.77-7.19	2.78(1.48)	0.00-0.12	40
END-1	100	5.61	1.19-22.66	7.01(4.71)	0.01-0.38	27
END-2	11	0.05	0.02-0.12	0.06(0.04)	0.01-0.12	31
MOC	98	0.07	0.02-1.12	0.11(0.15)	0.00-0.17	81
Mirex	98	0.23	0.02-1.54	0.32(0.26)	0.00-0.02	36