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CHARACTERIZING LIFE-LONG HUMAN EXPOSURE TO PERSISTENT ENVIRONMENTAL POLLUTANTS

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To my family

POPULÄRVETENSKAPLIG SAMMANFATTNING

I denna avhandling redovisas olika metoder för att mäta exponeringen för miljögifterna polyklorerade bifenyler (PCB), polyklorerade dibenzo-*p*-dioxiner och polyklorerade dibenzofuraner (PCDD/F) i olika grupper av befolkningen.

PCBer är industrikemikalier som framställts främst för användning i elektrisk apparatur. PCDD/F bildas framförallt oavsiktligt vid förbränning. Under 1970-talet förbjöds användningen av PCB i många länder på grund av risken för negativa hälsoeffekter hos djur och människor vid exponering för PCB och PCDD/F.

Våra undersökningar visade att ammade spädbarn har 11 gånger högre exponering för PCB och PCDD/F, beräknat utifrån halter uppmätta bröstmjölk, än barn 1-3 år gamla beräknat med hjälp av kostenkäter och uppmätta halter i livsmedel. Exponeringen uttryckt per kg kroppsvikt sjönk med stigande ålder, upp till vuxen ålder (ca 70% minskning). Fisk och fiskprodukter var den största källan till exponering hos både barn och vuxna, följt av mejeri- och köttprodukter. De två exponeringsmodeller som vi använt, så kallad deterministisk och probabilistisk, gav liknande resultat med avseende på den genomsnittliga exponeringen. Den deterministiska modellen gav dock ett högre exponeringsmått (överskattar exponeringen) hos högexponerade. Vi testade även hur väl en kostenkät kan spegla individuell exponering från kosten. Resultaten visade att enkäten var användbar för att beräkna exponeringen för PCB som vi kunde använda för att skatta risken för hjärtinfarkt hos kvinnor (48 – 83 år). Kvinnor med en genomsnittlige exponering på 280 ng PCB/dag hade 70% större risk att drabbas av hjärtinfarkt än kvinnor med en exponering på 98 ng PCB/dag. Hjärtinfarkt med dödlig utgång var ca två gånger högre hos de högst exponerade kvinnorna jämfört med kvinnor med låg exponering.
Sedan förbudet mot PCB infördes, för ca 30 år sedan, har halterna av PCB och PCDD/F sjunkit både i miljön och hos människor men det finns indikationer på att halterna inte minskar längre. Resultaten från denna avhandling tyder på att exponeringen för PCB och PCDD/F fortfarande kan utgöra ett hot mot människans hälsa. Därför är det viktigt att karakterisera exponeringen i känsliga grupper av befolkningen med hjälp av olika exponeringsmodeller, och att åtgärder för eliminering eller reducering av exponeringskällor fortsätter.

LIST OF PUBLICATIONS

This thesis is based on the following articles:

I. **Bergkvist C**, Öberg M, Appelgren M, Becker W, Aune M, Halldin-Ankarberg E, Berglund M, and Håkansson H. Exposure to dioxin-like pollutants via different food commodities in Swedish children and young adults. *Food and Chemical Toxicology* (2008), 46 (11): 3360 - 67

II. **Bergkvist C**, Lignell S, Sand S, Aune M, Persson M, Slob W, Håkansson H, and Berglund M. A probabilistic approach for estimating infant exposure to environmental pollutants in human breast milk. *Journal of Environmental Monitoring* (2009), 12 (5): 1029 - 36

III. **Bergkvist C**, Åkesson A, Glynn A, Kiviranta H, Rantakokko P, Wolk A, Berglund M. Validity of food frequency questionnaires for estimating past and current dietary exposure to polychlorinated biphenyls. (2011) *Manuscript*

IV. **Bergkvist C**, Berglund M, Glynn A, Wolk A, Åkesson A. Dietary exposure to polychlorinated biphenyls and risk of myocardial infarction in women: a population-based prospective cohort study. (2011) *Manuscript*

The articles will be referred to in the text as **Paper I – IV** and are reproduced in full as appendices.

OTHER PUBLICATIONS

The following articles are not discussed in this thesis

V. **Bergkvist C**, Kippler M, Hamadani JD, Grandér M, Tofail F, Berglund M, Vahter M. Assessment of early-life lead exposure in rural Bangladesh. *Environmental Research* (2010), 110 (7): 718 – 24

VI. **Bergkvist C**, Aune M, Nilsson I, Sandanger TM, Hamadani JD, Tofail F, Oyvind-Odland J, Kabir I, Vahter M. Exposure in early infancy to organochlorine compounds in Bangladesh. (2011) *Submitted*

ABSTRACT

Humans are exposed to a variety of potentially harmful contaminants on a daily basis. Characterizing the exposure in different subgroups of the general population is important in order to protect public health sufficiently.

The overall aim of this thesis was to refine human exposure assessment by applying different methodological approaches for characterizing the exposure to harmful contaminants such as polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) in different subgroups of the Swedish general population.

We assessed the exposure to PCBs and PCDD/Fs in breastfed infants (up to 6 months of age), children and adolescents (1 – 24 year of age) and first-time mothers based on food consumption data (1989) and contaminant concentrations in food (1998 - 2004) and breast milk (2000 - 2006). A food concentration database was developed for the assessment of food frequency questionnaire (FFQ)-based PCB153 exposure in middle-aged and elderly women (born 1914 - 1948).

Median exposure to PCBs and PCDD/Fs declined from 44 pg/kg body weight in 1 month old breastfed infants to less than 1.4 pg/kg body weight in adults 15 - 40 years of age. The proportion of individuals exceeding the current TDI of 2 pg TEQ/kg body weight decreased from 100% in infants and young children to less than 26% in adults. Mean exposure to PCBs and PCDD/Fs was comparable when using either the probabilistic or the deterministic approach, whereas the deterministic worst-case scenario estimate was up to 1.7 times higher than the probabilistic estimated 95th percentile. We obtained a reasonable validity of the FFQ for estimating concurrent (Spearman correlation r_s 0.37; $p<0.001$) and long-term (r_s 0.32; $p<0.05$) dietary PCB153 exposure assessment against serum PCB153 concentrations. Based on the validated FFQ we found a significantly increased risk (hazard ratio 1.67; 95% CI 1.17 – 2.40) of myocardial infarction in middle-aged and elderly women of the highest exposure group (median 280 ng/day) compared to the lowest (median 98 ng/day) using a Cox regression model. Highly exposed individuals were characterized by a high fish consumption independent of age. A high dietary PCB153 exposure and a low intake of fish fatty acids (<0.20 g/day of EPA - DHA) were associated with a greater risk of myocardial infarction (hazard ratio 2.22; 95% CI 1.25 to 3.94) compared to a low exposure and a high intake of EPA and DHA (> 0.29 g/day). Thus, continued actions are needed to reduce environmental levels and at the same time conduct risk-benefit analysis for efficient dietary recommendations.

This thesis provides new and detailed knowledge of exposure to environmental contaminants in different subgroups of the population by using different methodological approaches, necessary to increase the precision in the exposure estimates. Refining the exposure assessment is a prerequisite for sustaining public health.

LIST OF ABBREVIATIONS

AhR	Aryl hydrocarbon receptor
BMD	Benchmark dose
BMI	Body mass index
Bw	Body weight
CI	Confidence interval
CVD	Cardiovascular diseases
DDT/DDE	Dichlorodiphenyltrichloroethane/Dichlorodiphenyldichloroethylene
DHA	Docosahexaenoic acid
ECEH	European Centre for Environment and Health
EFSA	European Food Safety Authority
EPA	Eicosapentaenoic acid
EU-SCF	European Union - Scientific Committee on Food
FFQ	Food frequency questionnaire
HCB	Hexachlorobenzene
HR	Hazard ratio
IARC	International Agency for Research on Cancer
ICPS	International Programme of Chemical Safety
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kg	Kilogram
LOAEL	Lowest observed adverse effect level
LOQ	Limit of quantification
MeHg	Methylmercury
MI	Myocardial infarction
MLE	Maximum likelihood estimation
NFA	Swedish National Food Agency
NOAEL	No observed adverse effect level
ng	Nanogram
PBDE	Polybrominateddiphenyl ethers
PCBs	Polychlorinated biphenyls
PCDDs	Polychlorinated dibenzo-*p*-dioxin
PCDFs	Polychlorinated dibenzofurans
pg	Picogram
POPs	Persistent organic pollutants
PTMI	Provisional tolerable monthly intake
SMC	Swedish Mammography Cohort
TCDD	2,3,7,8-tetrachlordibenzo-*p*-dioxin
TEF	Toxic equivalency factors
TEQ	Toxic equivalence
TDI	Tolerable daily intake
TWI	Tolerable weekly intake
UK	United Kingdom
US	United States of America
WHO	World Health Organization

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1 INTRODUCTION

Humans are exposed to contaminants on a daily basis via food, air, water and consumer products. Exposure can lead to deleterious health effects depending on the inherent properties of the chemical and the degree of contact, the frequency and the duration of exposure, as well as the individual characteristics such as behavior and physiological factors. Hence, the exposure distribution in a population may vary between different population subgroups including children, pregnant women and elderly. Characterizing the exposure in different subgroups of the population is important in order to be able to sufficiently protect them from harmful contaminants, thereby maintaining public health and well-being in the community. Hence, knowledge of the exposure is the basic prerequisite for risk reduction.

Human exposure assessments are performed for different purposes, including human health risk assessment, epidemiology, status and trends analysis, and risk management, all of which rely on reliable exposure data (Figure 1).

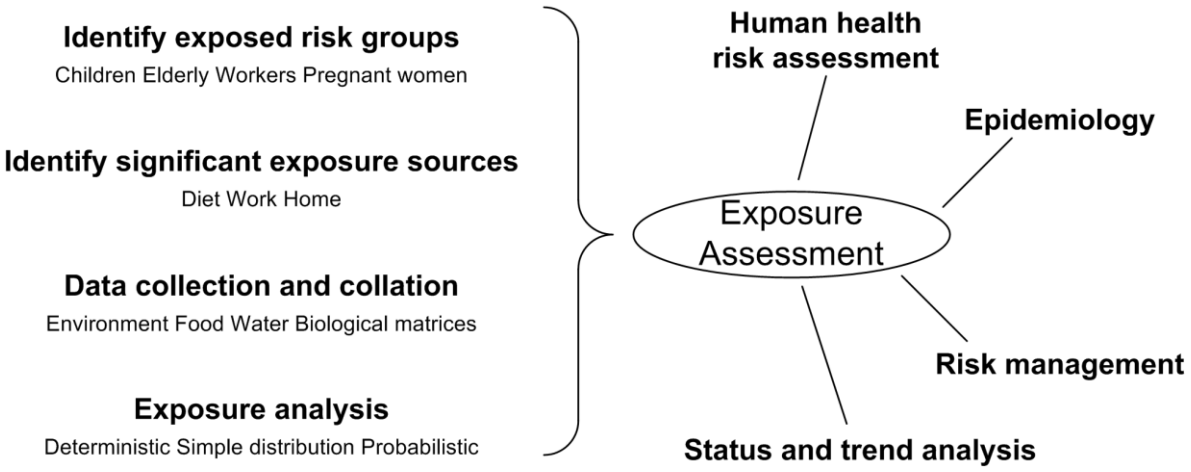

Figure 1. Components of human exposure assessment and the role of exposure assessment in different fields.

2 BACKGROUND

2.1 Human exposure assessment

Exposure can be defined as the contact over time between contaminants and a human, whereas *exposure assessment* is the process of estimating or measuring the magnitude, frequency, duration, and route of exposure to the contaminants along with the number and characteristics of the population of interest.[1]

Human exposure assessment can be approached in two ways; directly and indirectly. A direct approach involves personal exposure monitoring and biological monitoring, also called biomonitoring, which includes measurements of a contaminant or its metabolites in suitable human media such as blood, urine or breast milk.[1] An indirect approach involves questionnaires, data modeling and environmental monitoring, which includes measurements of a contaminant in food, water, air, soil and plants.[1] The choice of approach depends on the purpose of the study, the data availability and data quality that is needed to address the questions of interest. It is often useful to combine two or more approaches to gain as much information as possible.

2.1.1 Direct exposure assessment approaches

Biomonitoring is the measurement of environmental substances including contaminants in a biological media such as blood and breast milk, and has been used extensively to assess and monitor the exposure to contaminants in humans. Biomarkers of exposure take into account both intra- and inter-individual differences in exposure and may give a more precise measurement of the internal dose integrated over all exposure sources.[1] The disadvantage, however, is that biomonitoring is often expensive to perform due to the high costs of sampling and chemical analyses of biomarkers of exposure, resulting in restricted sample sizes, which in turn can lead to discrepancies in results between studies because of low statistical power and chance. Furthermore, to obtain reliable

biomarkers of exposure knowledge about the relationship between exposure and internal dose is necessary.

2.1.2 Indirect exposure assessment approaches

2.1.2.1 Dietary questionnaires

Well-designed dietary questionnaires are used for assessing individuals' food consumption. The dietary record method is based on the participants' records of the amount and the type of food and beverages that are consumed over one or more days.[2] The amounts consumed are reported in terms of household measures or estimated based on pictures or models of portion sizes.[3] Theoretically, dietary records give an accurate estimate of the amount of food consumed and minimize errors such as recall bias because the information is recorded at the time of the eating occasion. The disadvantage with dietary records is the high number of days required in order to capture the average consumption or seasonal variation in dietary habits, which increases respondent burden.[3] This relatively high burden requires that the respondent is motivated otherwise it can *i)* lead to a risk of incomplete records with increasing number of consecutive days of recording and/or *ii)* lead to altered dietary habits as the respondent may modify the diet in order to decrease the burden of recording, resulting in an under- or overestimation of intakes, and thus limiting the generalizability of the results. In dietary recording there is also a risk that the respondents report a lower consumption of unhealthy foods, such as desserts and alcohol and a higher consumption of healthy foods such as grains, salads and vegetables in order to report a socially desirable diet.[3]

Food frequency questionnaires (FFQs), on the other hand, record the average food consumption over a longer period of time.[2] The respondents are asked about their usual frequency of food consumption from a food list. The FFQs can also include portion sizes such as number of glasses or slices for some foods or beverages. Otherwise the consumption frequencies are multiplied with standard portion sizes to obtain an estimate of the amount of food consumed.

FFQs are relatively easy to use for obtaining information of an individual's usual dietary consumption and are cost-effective in comparison to other methods such as measurements of biomarkers of exposure. The disadvantages with FFQs are; *i)* the food list, that is supposed to cover the usual consumption of the respondents, may lack some important foods or beverages, *ii)* the number of questions asked can influence the reported consumption,[4] *iii)* misreporting by the participants due to recall bias or by overestimating "healthy" food and underestimating foods such as sweets and alcohol, and *iv)* the standard portion-sizes assigned to the consumption frequencies may not represent the respondent's usual portion sizes.[3]

2.1.2.2 Data modeling

Most exposure assessments rely, to some extent, on exposure models that combine measurements and assumptions to produce an estimate of the exposure to a contaminant or chemical.[5] An exposure model is a computational frame-work designed to reflect real-world human exposure scenarios.

Exposure to contaminants through the diet can be estimated by combining data on food consumption with concentration data of contaminants in the food and/or food groups.[6] The approaches for estimating such exposure include the deterministic, the simple distribution and the probabilistic approach.

The deterministic approach relies on point estimates of each input variable and refers to a method whereby fixed values, e.g., the average, of food consumption and of the concentration data on contaminants in food are combined in order to obtain a single estimated exposure value.[7] A worst case scenario approach is typically based on point estimates covering the upper-bounds of exposure, i.e., 95^{th} or 99^{th} percentile or the maximum value, in order to protect public health. Consequently, the deterministic approach assumes that all individuals consume the same amount of food and that the pollutant is always present at a constant level in the particular exposure media. Though, an advantage with the deterministic approach is that it is time-efficient and will easily give a rough estimate of the exposure.

The deterministic approach is conservative and does not take into account variability, which is the natural inherent difference in data. The simple distribution approach, also known as range estimates, often employs a distribution for the food consumption data, whereas the concentration data on contaminants in food is appointed a fixed value.[7] Simple distributions take into account the variability that is inherent in the pattern of the consumption data resulting in a distribution of the exposure, which is more informative than the exposure estimated by the deterministic approach. Nonetheless, conservative assumptions are still related to the presence of the contaminants in food.

The probabilistic approach, on the other hand, applies probability density functions to the food consumption and contaminant concentration data since it is often not practical to measure the exposure for every person in a population.[8] Within the probabilistic exposure model, simulations of exposure (e.g. Monte Carlo simulations) are carried out by randomly selecting values from the probability distributions where every possible value is weighed by the probability of its occurrence. The probabilistic exposure model is run several times generating new exposure estimates (Figure 2). By considering the whole distribution of the exposure, the probabilistic approach ensures that the variability and/or uncertainty, which is the lack of knowledge about the correct value for a specific exposure measure or estimate, are reflected in the output of the exposure model.[8]

One way to quantify the uncertainty is to use the bootstrap method, a statistical technique based on multiple resampling with replacement of the sample values or of the sample distributions resulting in a confidence interval around the statistics. Sensitivity analysis is a useful technique to identify variables that have the greatest effect on the variance of the outcome in an exposure model. The results from a sensitivity analysis can be useful as an underlying basis for an uncertainty analysis by providing with information about the variables.[8]

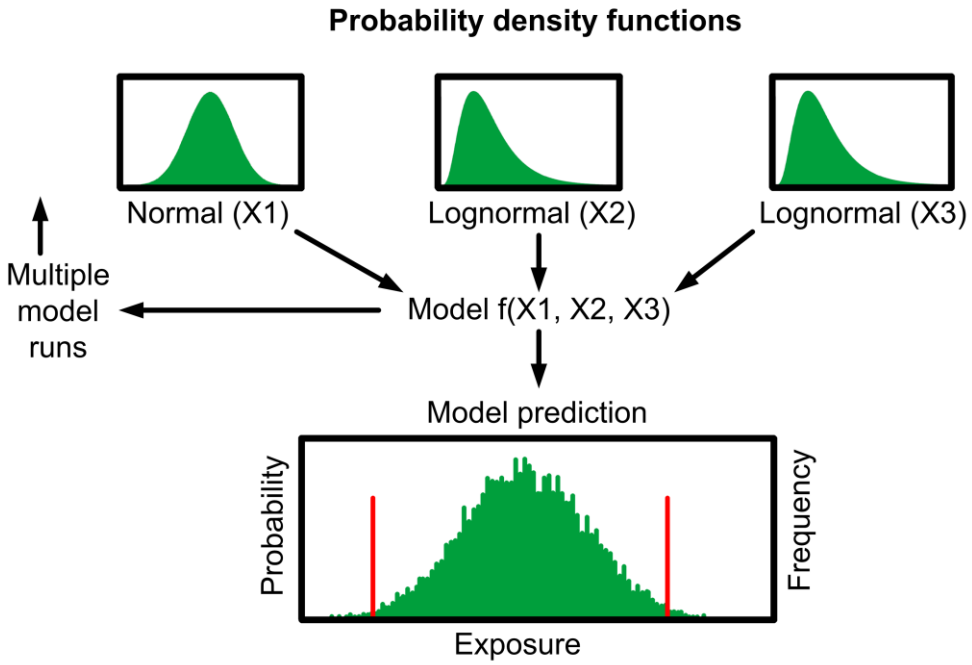

Figure 2. Schematic view of the probabilistic exposure assessment model.

2.1.3 Left censored data

A difficult step in dietary exposure assessment is the handling of analytical concentration data below the quantification limit, known as non-detects, resulting in a left-censored distribution of occurrence values. The level of censoring is based on the confidence with which the analytical signal can be discerned from the noise. Censored data are measurable but the quantity is uncertain, i.e., the quantification limit is the lowest level at which a chemical may be accurately and reproducibly quantified.

There are numerous ways of dealing with values below the quantification limit and, so far, there is no one procedure that outweighs all other procedures for various exposure assessment circumstances. Techniques for analyzing censored data sets include substitution methods, log-probit regression methods, maximum likelihood estimation methods (MLE) and non-parametric methods.[9] The substitution method is the most

commonly encountered technique and includes ignoring the censored data, replacing censored data with zero, replacing censored data with the quantification limit or replacing censored data with one-half of the quantification limit.[10] It has been widely recognized that the substitution method is biased; the bias being a function of true variability in the data, the percentage of censored observations and the sample size.[9] The MLE method makes use of the data above the quantification limit to extrapolate below it. Data below and above the quantification limit are assumed to follow a given statistical distribution. The parameters of the chosen distribution are estimated to best fit the distribution of the observed values above the quantification limit, compatibly with the percentage of data below the limit. The estimated parameters are the ones that maximize the likelihood function.[9,11]

2.2 Polychlorinated biphenyls, dibenzo-p-dioxins and dibenzofurans

In this thesis, exposure to manufactured chemicals and unintentionally formed by-products such as polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) was characterized. These contaminants are designated as persistent organic pollutants (POPs) and consist of 209, 75 and 135 congeners, respectively.[12] The congeners are determined by their chlorine pattern on the biphenyl ring (Figure 3). These contaminants can persist in the environment for long periods of time due to their resistance to bio-degradation, and can travel long distances in the atmosphere and have been found in remote areas such as the Arctic.[13] PCBs and PCDD/Fs also bioaccumulate and biomagnify up the food chain resulting in higher concentrations in species at the top of the chain. These contaminants may pose toxic threats to humans and wild-life resulting in endometrial, reproductive, immunological and carcinogenic health effects.[14] The term ‘dioxins’ includes 12 PCB congeners, often termed dioxinlike PCBs (DL-PCBs), and 17 PCDD/F congeners.[15] Dioxins are of special toxicological concern because they have similar properties as the most potent congener, 2,3,7,8-tetrachlordibenzo-*p*-dioxin (TCDD), which is classified as a known human carcinogen (Group 1) by the International Agency for Research on Cancer.[16]

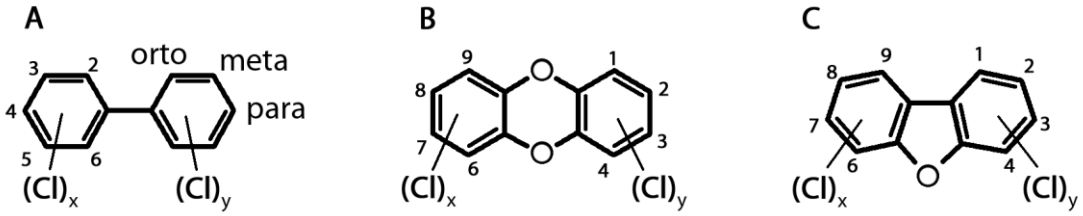

Figure 3. Chemical structure of polychlorinated biphenyls (A), polychlorinated dibenzo-*p*-dioxins (B), polychlorinated dibenzofurans (C).

2.2.1 History of PCBs and PCDD/Fs

Since the early 1930s, PCBs have been commercially manufactured as chemical mixtures under different trade names in the U.S., Japan, Germany, France, Spain, U.K., Italy, Czechoslovakia, Poland, Austria, Russia and China.[17] PCBs have been used for a number of applications including transformers and capacitors where they were used as dielectric fluids, but also in building material (sealants), carbonless copy paper, lubricants, surface coatings, adhesives, plasticizers, and inks among other things.[18,19] Of the estimated 1.5 million metric tons of PCBs produced globally since the 1930s, one third is thought to have found its way into the environment, mainly in industrialized countries (Figure 4).[17,20]

PCBs have never been produced in Sweden but the Swedish industry has imported PCBs from U.S., Germany, France and U.K.[21] In 1972, Sweden took regulatory actions to restrict the use of PCBs to closed systems such as capacitors and transformers.[21] In 1985, a new law was passed stating that all use of PCBs should be phased out by 1995. In 2001, a global action (the Stockholm Convention Treaty initiated by the United Nations Environmental Programme) was taken to eliminate or restrict the production and use of POPs around the world.[22]

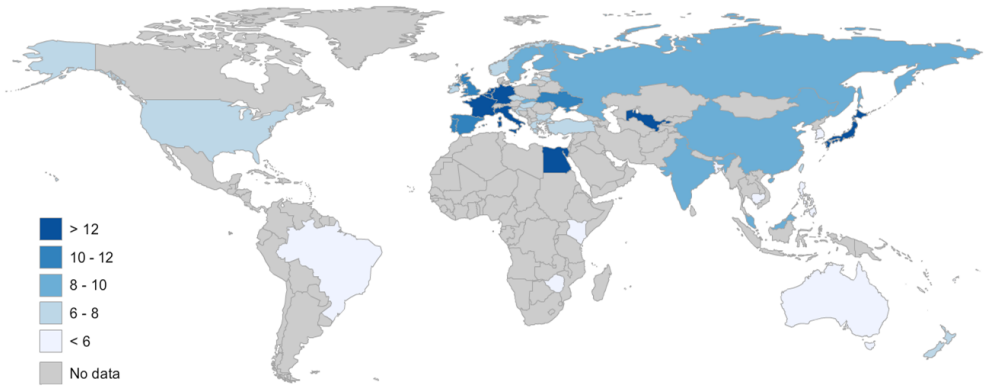

Figure 4. Concentrations of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans in breast milk expressed as toxic equivalence (TEQ) values (pg/g lipid weight). World map constructed with STATPLANET based on data from LaKind et al., 2007.[23]

PCDD/Fs are formed as unintentional by-products during incomplete combustion processes such as in waste incinerators or as unwanted by-products of industrial processes. In Sweden, iron and steel production, as well as pulp and paper mills that use chlorine have been important sources of environmental PCDD/F contamination.[24] Waste and uncontrolled waste burning such as backyard burning, as well as secondary emissions from chlorophenol (PCP)-treated wood are ongoing sources of environmental contamination.

Throughout the 1900s, several large incidents have been reported involving excessive exposure to PCBs and PCDD/Fs and their association with health effects in humans and animals.[25] Among these incidents are the use of the contaminated herbicide Agent Orange as a defoliant during the Vietnam War in the 1960s,[26] the explosion of the 2,4,5-trichlorophenol factory in Seveso (Italy 1976),[27] the poisoning from edible rice oils in Japan (Yusho, 1968)[28] and Taiwan (Yu-Cheng, 1978),[29] and the poisoning incidences of food and animal feed (Ireland 2008; India 2007; Belgium 1999; Brazil 1998; U.S. 1997).[25,30] Studies from these incidents revealed and supported previous theories about the association between exposure to PCBs and/or PCDD/Fs and deleterious effects in humans such as carcinogenic, developmental, reproductive, dermal and cardiovascular effects.[27-29] Monitoring concentrations of PCBs and

PCDD/Fs in food is an important step in detecting impurities at an early stage and to prevent the contaminants from reaching humans.

2.2.2 PCBs and PCDD/Fs in the environment and food

Over the subsequent decades, almost 30 years, since the use of PCBs was restricted in Sweden, the concentrations of PCBs and PCDD/Fs in the Swedish environment (swine, bovine, guillemot egg) have decreased by roughly 80%, but since the 1990s the rate of decline has been lower.[31,32]

In the general population, diet constitutes more than 95% of the total exposure to PCBs and PCDD/Fs when taking into account other exposure routes such as dermal absorption, air inhalation, and soil and dust ingestion.[33] At least 75% of the dietary exposure to PCBs is of animal origin,[34] particularly fish and fish products which are the major food sources in Sweden [35] as well as in other countries such as Finland, Spain, Japan, and Belgium.[36-39]

Because of the high concentrations of PCBs and PCDD/Fs in the diet and the fact that diet is the major source of exposure in the general population, the European Commission stipulates that levels of dioxins in foodstuffs should not, when placed on the market, exceed the maximum levels specified in the European regulations.[40] However, certain fish species originating from the Baltic region (herring and salmon) that contains high levels of dioxins often exceed these maximum levels. Excluding fish from the Baltic region from the Swedish and Finnish diet may have a negative health impact on the population because of the many nutrients in fish (including omega-3 fatty acids, selenium and vitamin D)[41] and a negative effect on the fishing industry. In previous years, a temporary exemption has been granted for Sweden and Finland regarding the sale of Baltic-sourced fish but from 2012 this exemption will be permanent. At the same time, the Swedish National Food Agency (NFA) is obliged to inform consumers of the dietary recommendations concerning restrictions on consumption of fish from the Baltic region in order to avoid potential health effects in

the general population as well as in sensitive sub-groups such as girls and women of child-bearing age and pregnant women.[42]

2.2.3 PCBs and PCDD/Fs in humans

Once ingested through the diet, up to 100% of PCBs and PCDD/Fs are absorbed through the intestines of infants[43] and between 48 and 99% is absorbed in adults.[44] The absorption, by passive diffusion, is congener specific and depends on the molecular size, lipophilicity, composition of the food that is ingested together with the contaminants, and concentrations already present in blood.[44] As with fats and other fat-soluble chemicals, PCBs are most likely absorbed from the gut via the lymphatic circulation and finally deposited mainly in the adipose tissue.[45]

The metabolism of dioxins is through the affinity to the aryl hydrocarbon receptor (AhR), whereas non-dioxinlike PCBs bind to several different receptors such as the constitutive androstane receptor (CAR)/pregnane X receptor (PXR), both inducing the transcription of cytochrome P450 (CYP) enzymes.[46,47] Congeners of PCBs and PCDD/Fs accumulate in the human body with different persistence, reflected by the congener's half-life that can vary from a couple of months to several decades partly attributable to personal characteristics such as age, body fat, and lactation.[48]

During pregnancy, concentrations of PCBs and PCDD/Fs are transferred from maternal blood to the blood of the fetus. During breastfeeding, PCBs and PCDD/Fs are eliminated from maternal stores to breast milk. The ratio of PCBs concentrations in cord blood, cord tissue and placenta compared to maternal blood is 0.6, 0.4 and 0.4, respectively, whereas the ratio for breast milk is 1.5.[49] Several studies have found strong correlations between concentrations of PCBs and PCDD/Fs in mothers' blood, cord blood, placenta as well as between blood and adipose tissue.[50,51] Suggested predictors of high concentrations of PCBs and PCDD/Fs in blood and breast milk are attributable to personal characteristics and life-style factors such as age, serum lipids, parity, duration of breastfeeding, fish consumption, body fat mass, early life exposure

to PCBs and PCDD/Fs, physical activity, place of residence, education and social class.[52]

Since 1972, the concentrations of PCBs and PCDD/Fs in breast milk have decreased by at least 70% [53] and mirror the decrease in concentrations of PCBs and PCDD/Fs in food and in the environment. The amount of PCBs in the breast milk of Swedish mothers is estimated to represent two thirds of the total amount of chemicals present, a total which includes DDT, DDE, HCB and PBDE.[53]

2.3 Human health risk assessment of PCBs and PCDD/Fs

Human exposure assessment is one of four activities in health risk assessments of PCBs and PCDD/Fs the others being *hazard identification*, *hazard characterization* and *risk characterization*.[54] Human health risk assessment, on the other hand, is a component in *risk analysis* together with *risk communication* and *risk management*. In the first activity, *hazard identification*, the type and nature of the health effect that a contaminant is capable of causing are typically identified from *in vivo* and *in vitro* studies. *Hazard characterization* aims to characterize a dose-effect relationship between the contaminant of interest and the biological effect identified in the hazard identification, in order for a human health-based guideline value to be derived. The guideline value is often defined as the NOAEL (no-observed adverse effect level) or the BMDL (the lower confidence interval of the benchmark dose).[55] Assessment factors, also known as uncertainty factors or safety factors are applied to the NOAEL or BMDL, to account for uncertainties in the data such as differences in toxicokinetics and dynamics between experimental animals and humans.[56] The aim of *risk characterization* is generally to compare the human health-based guideline values with exposure levels, estimated from the exposure assessment, in the population of interest to give an estimate of the occurrence and of the potential risks to the population.

2.3.1 Toxic equivalency factors

Mixtures of PCBs and PCDD/Fs are assumed to have an additive effect. To facilitate risk assessment and regulatory control, the concept of toxic equivalency factors (TEFs) has been developed for those congeners whose chemical characteristics and toxic responses are similar to those of the most toxic dioxin, TCDD (Table 1). To be included in the TEF scheme, a congener must be; *i*) structurally related to TCDD, *ii*) bind to the AhR, *iii*) elicit AhR mediated biochemical and toxic responses, and *iv*) be persistent and accumulate in the food chain. By multiplying the concentration of each congener by its TEF value the toxic equivalent (TEQ) of a mixture can be calculated and the analytical results can be expressed as TEQ concentrations.[15] TEF schemes from 1998[15] and 2005[47] initiated by the World Health Organization (WHO) have been used in the present thesis.

2.3.2 Risk characterization of PCBs and PCDD/Fs

2.3.2.1 Human health-based guidance values

Several international expert groups within the WHO and the European Union have established human health-based guideline values for dioxins on the basis of toxicological studies on *in utero* exposure to TCDD in experimental animals.

In 1998, the European Centre for Environment and Health (ECEH) and the International Programme of Chemical Safety (ICPS) reassessed the previous established tolerable daily intake (TDI) in humans of 10 pg TEQ/kg body weight. Based on new data on hormonal, reproductive, immunological and behavioral effects in offspring of rats and monkeys orally exposed to single and chronic doses of TCDD, the TDI was set to 1 - 4 pg TEQ/kg body weight using an uncertainty factor of 10, which was applied to the estimated body burden of the animals.[57] The upper range was considered to be a maximal tolerable intake on a provisional basis and the ultimate goal was to reduce human intake levels below 1 pg/kg body weight.[57]

Table 1. Summary of WHO 1998 and WHO 2005 TEF values.

Compound	WHO 1998 TEF	WHO 2005 TEF
chlorinated dibenzo-p-dioxins		
2,3,7,8-TCDD	1	1
1,2,3,7,8-PeCDD	1	1
1,2,3,4,7,8-HxCDD	0,1	0,1
1,2,3,6,7,8-HxCDD	0,1	0,1
1,2,3,7,8,9-HxCDD	0,1	0,1
1,2,3,4,6,7,8-HpCDD	0,01	0,01
OCDD	0,0001	**0,0003**
chlorinated dibenzofurans		
2,3,7,8-TCDF	0,1	0,1
1,2,3,7,8-PeCDF	0,05	**0,03**
2,3,4,7,8-PeCDF	0,5	**0,3**
1,2,3,4,7,8-HxCDF	0,1	0,1
1,2,3,6,7,8-HxCDF	0,1	0,1
1,2,3,7,8,9-HxCDF	0,1	0,1
2,3,4,6,7,8-HxCDF	0,1	0,1
1,2,3,4,6,7,8-HpCDF	0,01	0,01
1,2,3,6,7,8,9-HpCDF	0,01	0,01
OCDF	0,0001	**0,0003**
non-ortho substituted PCBs		
3,3',4,4'-tetraCB (PCB 77)	0,0001	0,0001
3,4,4',5-tetraCB (PCB 81)	0,0001	**0,0003**
3,3',4,4',5-pentaCB (PCB 126)	0,1	0,1
3,3',4,4',5,5'-hexaCB (PCB 169)	0,01	**0,03**
mono-ortho substituted PCBs		
2,3,3',4,4'-pentaCB (PCB 105)	0,0001	**0,00003**
2,3,4,4',5-pentaCB (PCB 114)	0,0005	**0,00003**
2,3',4,4',5-pentaCB (PCB 118)	0,0001	**0,00003**
2',3,4,4',5-pentaCB (PCB 123)	0,0001	**0,00003**
2,3,3',4,4',5-hexaCB (PCB 156)	0,0005	**0,00003**
2,3,3',4,4',5'-hexaCB (PCB 157)	0,0005	**0,00003**
2,3',4,4',5,5'-hexaCB (PCB 167)	0,00001	**0,00003**
2,3,3',4,4',5,5'-heptaCB (PCB 189)	0,0001	**0,00003**

Bold values indicate a change in TEF value between 1998 and 2005

In 2001, JECFA (Joint FAO/WHO Expert Committee on Food Additives) established a provisional tolerable monthly intake (PTMI) in humans ranging from 40 - 100 pg TEQ/kg body weight. The PTMI was based on different modeling approaches using data on developmental effects in offspring of rats exposed to long-term subcutaneous dosing of TCDD, applying a safety factor of 9.6.[58] The mid-point of 70 pg TEQ/kg body weight was chosen as the PTMI value (corresponding to a daily intake of 2.3 pg TEQ/kg body weight).

In 2000, the European Scientific Committee on Food (SCF) decided on a tolerable weekly intake (TWI) of 7 pg TEQ/ kg body weight corresponding to a TDI of 1 pg TEQ/kg body weight.[59] After a re-evaluation of the pivotal studies one year later, the SCF decided upon a TWI of 14 pg TEQ/kg body weight (corresponding to a TDI of 2 pg TEQ/kg body weight) based on developmental effects observed in offspring of rats orally exposed to a single dose of TCDD, and applying an uncertainty factor of 9.6.[60] The TWI is considered to be an estimate of a safe level of intake.

In all aforementioned risk assessments, it is stated that the guideline values are not applicable to breastfed infants and the concept of the guideline values relates to a dietary intake throughout a lifetime whereby breastfeeding constitutes only a short period of that time. It is further stated that breast milk is thought to have beneficial effects on the child.

In 2005, the European Food Safety Authority (EFSA) conducted a human health risk assessment for the sum of six non-dioxinlike PCBs (28, 52, 101, 138, 153, 180).[61] No health-based guidance value was established because of limited toxicological data and difficulties in distinguishing the non-dioxinlike PCBs from the dioxinlike PCBs as well as other compounds in toxicological and epidemiological studies. EFSA concludes that there are indications that subtle developmental effects, caused by non-dioxinlike PCBs, dioxinlike PCBs, or PCDD/Fs alone, or in combination, may occur at maternal body burdens that are only slightly higher than those expected from the average daily intake in European countries.[61] Because some individuals and some European (sub)-populations may be exposed to considerably higher average intakes, a continued effort to lower the levels of non-dioxinlike PCBs in food was warranted.[61]

A risk assessment of dioxins based on non-developmental effects has been performed by the Institute of Environmental Medicine (Karolinska Institutet, Sweden) and the Swedish NFA and relates to the general population.[62] It was concluded that, based on carcinogenic effects in rodents, a TDI of 2-10 pg TEQ/kg body weight represents exposure levels where the human cancer risks are very low or non-existent.

2.3.2.2 Myocardial infarction and PCBs

Besides from the developmental and carcinogenic health effects on which the risk assessments are based, PCBs and PCDD/Fs are also associated with cardiovascular effects.

Myocardial infarction, often known as heart attack, is a state of change in the heart muscle. The myocardial tissue becomes subject to necrosis (cell death) caused by a blockage in circulating blood and thereby reduced oxygen supply. The reduced blood flow is most often the result of a blood clot surrounding a plaque rupture with thrombus formation in the vessel. The plaque development is often initiated by inflammatory responses.[63]

There are several risk factors for cardiovascular diseases including diabetes, hypertension, hypercholesterolemia, dietary habits and life-style factors such as cigarette smoking and reduced physical activity.[64] Long-chain n-3 polyunsaturated fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have, on the other hand, a possible protective role against myocardial infarction.[65] Both EPA and DHA are present at higher concentrations in fatty fish (such as herring, mackerel and salmon) and in fish oil compared to other foods.[66] However, fish can also contain high concentrations of contaminants especially PCBs, PCDD/Fs and methylmercury (MeHg).

The role of PCB in the development of cardiovascular diseases is not known. The lining of the blood vessels consists of endothelial cells where an activation or dysfunction of the cells is a critical event in the initiation of cardiovascular diseases.[63] Experimental studies of PCB-exposed endothelial cells have shown increased cellular oxidative stress,[67] CYP1A1 activity,[68] poly (ADP-ribose) polymerase activity (PARP; DNA repair enzyme),[68] induction of inflammatory transcription genes (cytokines and adhesion molecules),[69] NADPH oxidase activity,[70] and a disruption of the endothelial barrier function.[67] The possible mechanisms of PCB cytotoxicity are illustrated in Figure 5.

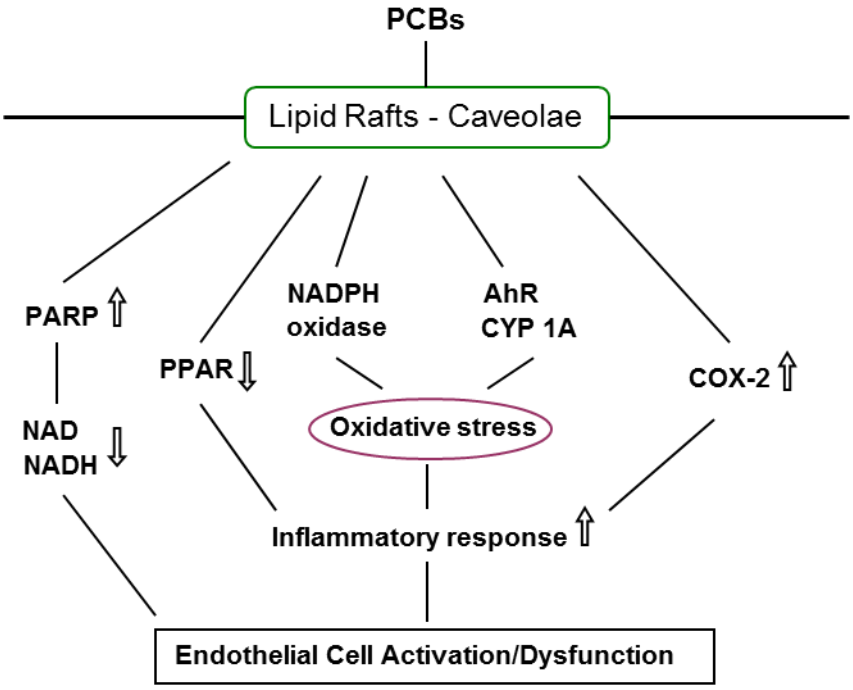

Figure 5. A schematic view of possible mechanisms of PCB endothelial cytotoxicity. Modified picture from Hennig et al., 2005.[71]

In animal studies of mice and rats, exposure to PCBs and/or PCDD/Fs was shown to cause increased blood pressure, serum cholesterol,[72] expression of inflammatory adhesion molecules,[73] markers of oxidative stress in aorta[74] and heart weight[75] as well as atherosclerosis[76] and degenerative cardiovascular lesions including cardiomyopathy and chronic active arteritis.[77]

Epidemiological studies investigating the association between PCB exposure and risk of cardiovascular diseases in the general population are few. A cross-sectional study on 463 American women (40 - 85 years of age) reported a statistically significant increased prevalence of self-reported CVD (coronary heart disease, angina, myocardial infarction and stroke) with increasing concentrations of several PCB congeners in serum.[78] The multivariable-adjusted odds ratio in the highest as compared to the lowest quartile was 10.4 (95 % CI 1.1 – 94) for PCB153, 5.0 (95 % CI 1.2 – 20) for dioxin-like PCBs and 3.8 (95 % CI 1.1 – 13) for non-dioxinlike PCBs.

Most studies on cardiovascular diseases have been conducted on PCDD/Fs but these mainly include ecological, accidental and occupational studies, including a follow-up

study on residents living close to the industrial accident in Seveso (1976), where an increased risk was found between proximity to the industrial site and cardiovascular diseases such as chronic rheumatic heart diseases (rate ratio 5.7; 95% CI 1.8 – 18) but not for myocardial infarction.[27] In a cross-sectional ecological study on residents living close to POP waste sites in New York, a 20% increase in rates of acute myocardial infarction was reported.[79] In a prospective cohort study of 242 occupationall- exposed Swedish men working at a manufacturing plant which used PCBs, a standardized mortality rate (SMR) of 1.44 (95 % CI 0.69 – 2.65) for circulatory diseases and a SMR of 1.49 (95 % CI 0.6 – 3.06) for ischemic heart disease was observed.[80] The PCB exposure was estimated based on the men's working tasks. In a follow-up study of PCB-exposed workers, defined through a job exposure matrix and employed at one of two chemical plants in the U.S. between 1939 and 1977, a slight but non-significant increased risk of ischemic heart diseases was observed (SMR 1.07).[81] In an extended study on PCB-exposed workers employed at any of the 12 U.S. plants between 1942 and 1984, a standard mortality risk of 1.8 (95% CI 1.1 – 2.9) was reported for ischemic heart diseases in the highest exposure category compared to the lowest.[82] In an occupational cohort of workers at a chemical plant in Hamburg (Germany), the exposure to TCDD and PCDD/F, estimated from a validated kinetic model, showed a significant relative risk of 2.4 (95% CI 1.3 – 4.7) and 2.06 (95% CI 1.2 – 3.5), respectively, for ischemic heart diseases.[83] Workers employed between 1969 and 1988 at a chemical plant in New Zeeland had an increased risk of ischemic heart diseases (SMR 1.10), however this was non-significant.[84] A international study by the International Agency for Research on Cancer comprising 36 occupational cohorts from 12 countries observed a rate ratio of 1.67 (95% CI 1.23 – 2.26) for ischemic heart disease in TCDD-exposed workers.[85]

Epidemiological studies of exposure to PCBs and PCDD/Fs in humans have also observed a statistically significant increased risk of established intermediate risk factors for cardiovascular diseases such as hypertension,[86,87] hyperlipidemia,[88-90] atherosclerosis,[91] metabolic syndrome[92] and type 2 diabetes.[93,94]

3 Aim

The overall aim of this thesis was to refine human exposure assessment by applying different methodological approaches for the purpose of characterizing the exposure to PCBs and PCDD/Fs in different subgroups of the general population.

The more specific objectives were;

- to characterize and compare the age and gender-specific, cumulative and long-term PCB and PCDD/F exposure in different subgroups and risk groups using biomonitoring and different dietary exposure assessment methods (**Paper I** - **IV**)

- to compare the deterministic and the probabilistic exposure assessment approach (**Paper II**)

- to develop food concentration databases for the assessment of food frequency questionnaire-based estimates of concurrent and long-term dietary PCB153 exposure (**Paper III** and **IV**)

- to validate and apply food frequency questionnaires in a large population-based prospective cohort study for assessment of the association between dietary PCB153 exposure and risk of myocardial infarction (**Paper III** and **IV**)

4 Subjects and Methods

4.1 Study populations and data sources

A short summary of the study design of the different papers is presented in Figure 6. Here follows a general description of the different data sources used in this thesis.

4.1.1 The nationwide dietary and household budget survey

For this thesis, data on children's and young adults' dietary consumption was obtained from a nationwide dietary and household budget survey that was carried out by the Swedish NFA in collaboration with the Statistics Sweden in 1989.[95] The study population was systematically selected from a registry of the total population in Sweden sorting residents into different districts, thereby, guaranteeing an even geographical distribution.[95] The inclusion criteria for the study population were individuals between 1 - 74 years of age (born 1915 - 1989), who lived in a household where every member shared a common household economy, and who were not institutionalized. An invitation to participate was sent out to 2,937 eligible subjects and 70% participated. Of these, 953 were boys/men and girls/women between 1 - 24 years of age. The response rate for this group was73% (Figure 6).[95]

One member of each household received a questionnaire on age, weight, dietary habits, major life-style factors and medical history. Each member also received a 7-day menu diary listing frequently consumed foods and pre-specified serving sizes, in terms of cups, pieces, slices, teaspoons, or by using portion guides of photographed meals. The participants reported their consumption of foods during breakfast, lunch and dinner by filling in the number of portions of the relevant food(s) listed. In-between meal eating occasions and foods not already listed were recorded as free text. The participants recorded their daily food consumption for one week starting at different time points during the year.[95] Young children filled in the menu diary with the help of their parents and by personnel at day care centers. The individuals were

divided into six age groups; 1 - 3, 4 - 6, 7 - 10, 11 - 14, 15 - 18 and 19 - 24 years.[95] Complete data were available for 670 boys/men and girls/women 1 to 24 years of age.

The most commonly consumed food items were collected from producers or purchased from two different stores in each of four major cities in Sweden (Uppsala, Göteborg, Malmö and Sundsvall) and analyzed during 1998–1999.[35,96] Each food item was weighed and homogenized.[96] Food items were collected again and analyzed between 2000 and 2004.[35,97] A combination of the 1998/1999 and 2000/2004 data sets were used in the present thesis.

Seventeen PCDD/F congeners together with nine PCB congeners (77, 105, 114, 118, 126, 156, 157, 167 and 169) were analyzed in all food items. The analyses were performed at Umeå University, Dr. Wessling laboratorien in Germany, and at the Swedish NFA. The chemical analyses of food items during 2000–2004 were performed on all the aforementioned contaminants and an additional PCB congener (81). The TEQ values were based on the TEF-scheme from 1998 and values below the limit of quantification (LOQ) have been substituted with a value that is half of this limit at the Swedish NFA.[96]

4.1.2 The time-trend investigation on pollutants in breast milk

Breast milk concentration data as well as personal characteristics of Swedish mothers were obtained in a time-trend investigation on pollutants in breast milk performed within the National Health Related Environmental Monitoring Program run by the Swedish Environmental Protection Agency and carried out by the Swedish NFA.[98] First-time mothers who were Swedish by birth and delivered at Uppsala University Hospital between 2000 and 2006 were recruited.[98] First-time mothers were recruited in order to minimize the variation in contaminant levels due to parity. Women, who delivered during the first week in every month and on randomly selected days during this week, were asked to participate in the time-trend investigation. Every month, 2 - 3 mothers were recruited (Figure 6). The mothers sampled milk at home during the third week after delivery (approximately 14 - 21 days postpartum) using a manual breast

milk pump and/or a passive breast milk sampler.[98] The women were instructed to sample milk both at the beginning and at the end of the breastfeeding session. The goal was to sample 500 ml milk from each mother during 7 days of sampling. The breast milk was stored in the home freezer, in acetone-washed bottles. Newly sampled milk was poured on top of the frozen milk. At the end of the sampling week, a mid-wife visited the mothers to collect the bottles. Data on age and weight were obtained via questionnaires completed approximately 6 weeks after delivery.

Individual breast milk concentration data for each congener of both dioxinlike and non-dioxinlike PCBs (mono-ortho substituted congeners 28, 105, 118, 156 and 167, di-ortho substituted congeners 52, 101, 138, 153 and 180, and non-ortho congeners 77, 126 and 169) as well as for PCDD/Fs (sixteen congeners) were obtained from the Swedish NFA.[98] Di-ortho, mono-ortho and non-ortho PCBs, with the exception of non-ortho PCBs in samples from 2006, were analyzed at the Swedish NFA, whereas PCDD/Fs (2000 to 2004) were analyzed at the National Institute of Public Health and the Environment (the Netherlands; RIVM) and PCDD/Fs and non-ortho PCBs from 2006 were analyzed at the Department of Chemistry at Umeå University in Sweden.[98] The LOQ for mono- and di-ortho PCBs, non-ortho PCBs and PCDD/Fs was 0.23 - 1.3 ng/g, 11 - 39 pg/g and 0.04 - 4.0 pg/g milk fat, respectively. The percentage of values below LOQ varied from 3 to 71% with fewer values below LOQ in 2006. Concentrations of PCBs and PCDD/Fs in breast milk had been adjusted for the lipid concentrations in the biological media at the Swedish NFA.[98]

4.1.3 The Swedish Mammography Cohort

Data on food consumption in middle-aged and elderly women were obtained from the Swedish Mammography Cohort (SMC), a population-based prospective cohort study of women established between 1987 and 1990.[99] The source population consisted of 90,303 women who were born between 1914 and 1948 and who lived in central Sweden (Uppsala County and Västmanland County; response rate 74%; Figure 6). The women received a mailed invitation, together with a 67-item food frequency questionnaire (FFQ) and personal characteristics (e.g., age, weight, parity and

education), to participate in a free mammography screening program.[99] To update and expand exposure data, a second questionnaire was sent out in 1997 to all 56,030 eligible cohort members still living in the study area (response rate 70%).[100] The questionnaire from 1997 gathered additional information on diet (a 96-item FFQ), medical history and major life-style factors (including smoking status, physical activity and use of dietary supplements).[100] From November 2003 to August 2009, 5,022 women from the cohort (60 % response rate), still living in the town of Uppsala, participated in a health examination during which they donated blood and completed a questionnaire containing a 123-item FFQ.[101] Of these women, we included the first 201 consecutive women that completed the FFQ in 1997 and again in 2004 - 2006 at the time of the health examination for the validation of the FFQ. The 201 women were non-users of fish oil supplements to avoid influence of additional sources of PCB exposure.[102] Of those women, 165 filled in the same questionnaire again one year later, 2005-2007 (hereafter referred to as the reproducibility study).[103]

The FFQs from 1997 and 2004-2006 primarily reflected the women's average consumption of different foods and beverages during the previous year and were based on open-ended questions with pre-specified serving sizes for frequently consumed foods (i.e., dairy products) and eight predefined frequency categories (never, 1 - 3 times/month, 1 - 2, 3 - 4 and 5 - 6 times/week, and 1, 2 or 3 or more times/day).[100] Questions on seafood comprised of grouped fish, i.e., *high fat fish* (Atlantic herring, Baltic Sea herring, and mackerel), *medium fat fish* (Arctic char, salmon and whitefish) and *low fat fish* (cod, saithe and pollock), as well as one question each regarding consumption of *caviar* and *shellfish*. Compared to the 1997-FFQ, the later FFQ also contained questions on *other fish* and *tuna*, and three additional frequency questions on dairy and meat products. Age-specific portion sizes had been estimated from 5,922 weighed food records kept by 213 randomly selected women from the study area.

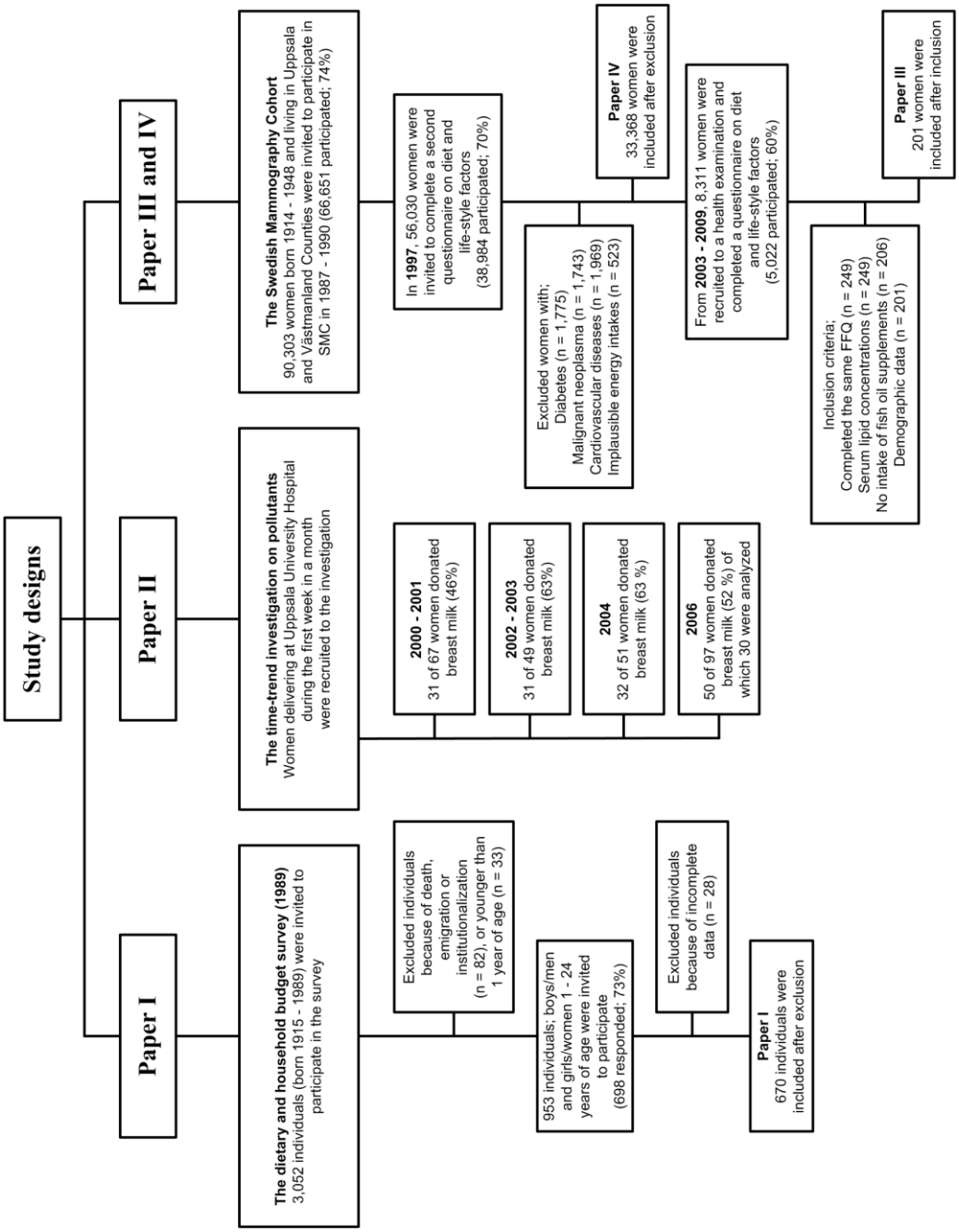

Figure 6. Study design of Papers I – IV.

For a construction of a food database, concentration data of PCB153 in food available on the Swedish market was obtained from the regular control and monitoring program run by the Swedish NFA. Concentrations of triglycerides and cholesterol were analyzed at Uppsala University Hospital, whereas phospholipids were analyzed at Karolinska University Hospital.

PCB congeners (118, 138, 153, 156, 170 and 180) in serum from 201 women were analyzed at the National Institute for Health and Welfare in Finland.[104] None of the analytical results were below the LOQ which varied from 2 to 5 pg/ml serum.

4.2 Mathematical exposure modeling and statistical analysis

In **Paper I**, a simple distribution approach was used to estimate the exposure to PCBs and PCDD/Fs in different age groups. The dietary exposure expressed as TEQ values was calculated by multiplying individual food consumption level with mean concentration data of the contaminants in various foods. Food consumption data were available for all 670 individuals expressed both in fresh (ng/day) and lipid (g fat/day) weight. The food items were assigned to food groups; *dairy products*, *meat* and *meat products*, *fish* and *fish products*, *egg* and *other fat containing food products*. Analysis of covariance (ANCOVA) using post-hoc test Scheffe ($p < 0.05$) was used for comparing mean estimates of TEQ exposure in different food groups and over all food groups between different age-groups and sex.

In **Paper II**, the deterministic and the probabilistic approach were compared for the estimation of exposure to PCBs and PCDD/Fs in breastfed infants. Values below LOQ were substituted with half of this limit for the deterministic calculations, whereas the MLE method was used for the probabilistic exposure calculations. Total TEQ as well as PCB-TEQ and PCDD/F-TEQ were estimated by multiplying the concentrations of each congener with their respective TEF value using the TEF-scheme from 2005.[47] Probabilistic exposure calculations were performed using a Monte Carlo simulation where each simulation contained 25 000 iterations after which

variations in parameters were assumed to be negligible. Non-parametric bootstrap sampling was used to estimate the uncertainty of the calculated probabilistic exposure estimations expressed as confidence intervals using 100 trials and 2000 iterations. The maximum likelihood method was used for fitting a Hill model to the daily milk consumption data, which was assumed to be normally distributed. Parameters for residue concentration data were also estimated with the maximum likelihood approach, where a log-normal distribution was fitted to the concentration data and the likelihood of values above and below the detection limit was optimized so that under the detection limit the predicted fraction of the total likelihood was approximately the same as the observed fraction of values below detection limit. Generalized mixed effect models using population-average mixed effect spline models were used for temporal trend analysis avoiding multicollinearity between the continuous variables.

For the purpose of calculating exposure to PCBs in a prospective population-based cohort, a large recipe-based database was created from concentrations of PCB153 in different food items retrieved from the time period between 1992 and 2009 (**Paper III** and **IV)**. The concentration data was extrapolated to reflect the levels of PCB153 in food between the years 1997 and 2004, assuming an 8% yearly decline in PCB153 concentrations. This figure is based on samples of breast milk in Sweden[98] as well as in swine and bovine adipose tissue sampled at slaughter houses in Sweden.[32] If sufficient concentration data for a specific food item was available for the analytical years 1997 or 2004 then these values were preferred over the extrapolated values. In total, over 1,200 samples were used for the concentration data of which 12% of the samples had a value below LOQ. Values below LOQ were substituted with half of this limit. A large recipe-based database was also created for methylmercury (MeHg) included 1,353 fish samples analyzed between 1975 and 2007 with no obvious observed temporal trend.

Concentration data for serum PCB153 in 201 women was used for validating the FFQ (**Paper III**) which was subsequently used in **Paper IV**. An intra-class correlation coefficient was calculated to measure the strength of agreement between dietary PCB153 exposures estimated from two identical FFQs in the reproducibility study by assessing the proportion of the between-person variance to the total variance, i.e., the sum of both the within- and between-variation. Serum PCB concentrations were

divided by total serum lipid concentrations and expressed as ng/g lipid. Total serum lipid was calculated according to Grimvall and colleagues (1997) based on assumed molecular weights for triglycerides, phospholipids and cholesterol of 807, 571 and 714 u, respectively, and a proportion of free and esterified cholesterol in plasma 1:2.[105] Spearman's rank correlation coefficient (r_s) and 95% confidence intervals (CI) were used to measure the strength of the relationship between FFQ-based dietary PCB153 exposure and serum concentrations of PCB congeners. De-attenuated Spearman's correlation coefficient was used to account for the reproducibility by adjusting the Spearman's correlation coefficient with the intraclass correlation coefficient. Agreement of cross-classification was used to measure the proportion of subjects classified into the same or adjacent category of exposure.

In **Paper IV**, the association between validated FFQ-based dietary PCB153 exposure and risk of myocardial infarction was studied in 33,638 women. The women were followed from mid-September (1997) until the date of first myocardial infarction, death or end of follow-up (31 December 2008), whichever came first. We categorized women into quartiles of dietary PCB153 exposure at baseline. Hazard ratios (HR) and 95% CI were estimated using Cox proportional hazards regression models with attained age (in years) as the underlying timescale. In the multivariable-adjusted analysis, we adjusted for level of education (<10, 10–12, or >12 y), family history of myocardial infarction before the age of 60 years (yes/no), high cholesterol (yes/no), hypertension (yes/no), ever use of postmenopausal hormones (yes/no), use of aspirin (yes/no), smoking status (never, past or current), waist circumference (≤ 80/> 80 cm), weight loss of ≥5 kg within a year (yes/no), parity, ever use of fish oil supplements (yes/no), total physical activity (MET-h; quartiles), history of atrial fibrillation before 1997 (yes/no), alcohol intake (0; > 0 - 4.9; 5.0 - 14.9; >15.0 g/day), energy intake (continuous, kcal/day), dietary MeHg exposure (quartiles; μg/day) and consumption of fruit and vegetables (quartiles; g/day), meat (quartiles; g/day) and sum of EPA and DHA (quartiles; g/day). The Schoenfeld's residual test showed no indications of violation of the proportional hazard assumption. A linear trend across categories was tested using median dietary PCB153 values within categories as a continuous variable. To test for possible interactions, the likelihood ratio test was used to compare models with and without an interaction term of dietary PCB153 (quartiles) and waist circumference (≤ 80/> 80 cm) and parity (nulliparous/≥1 child).

All statistical analyses were either performed in PASW/SPSS for Windows (SPSS Inc., version 18.0, California, USA) or STATA (STATA Corp. Inc., version 11, Texas, USA). Probabilistic exposure calculations were performed in Crystal Ball® (Decisioneering Inc. Release 11.1.1.3.0) and the MLE method was performed using the statistical software R (version R 2.6.2, Wirtshaftsuniversität, Wien, Austria). Reported p values were from two-sided statistical tests where a p value of ≤ 0.05 was considered statistically significant.

5 Results and Discussion

This section summarizes the main results of the present thesis along with a discussion regarding different exposure assessment approaches and methodological considerations. For a detailed description of the results, the reader is referred to the separate papers (**Paper I** - **IV**).

5.1 Estimating variability in exposure using different approaches

There is a general lack of knowledge in the variability of exposure to PCBs and PCDD/Fs in different age and sex subgroups of the general population. Through the use of the simple distribution approach and the probabilistic approach, detailed estimates of the exposure to dioxinlike PCBs and PCDD/Fs (expressed as TEQ values) in different subgroups of the Swedish population was obtained based on a menu diary and contaminant concentrations in food (**Paper I**) as well as biomonitoring data (**Paper II**; Figure 7).

The estimated exposure to dioxinlike PCBs and PCDD/Fs per kilo body weight decreased from early infancy (median 44 pg/kg body weight in 1 month old infants) to adulthood (median 1.4 pg/kg body weight; Figure 7). The variability in exposure between individuals was large and resulted in 22 – 26% of young adults and 3% of first-time mothers exceeding the recommended TDI of 2 pg TEQ/kg bw (**Paper I** and **II**). Because of the high exposure during infancy and early childhood, infants and 1-10 year old children had a median dietary exposure that exceeded, by far, the TDI (**Paper I**). There was a statistically significant difference ($p < 0.05$) between children of younger and older age groups in their exposure to PCBs and PCDD/Fs. There was no difference between boys and girls within the same age group, except for girls in the 19 – 24 year age group who had a significantly lower exposure from meat products compared to boys of the same age group (**Paper I**).

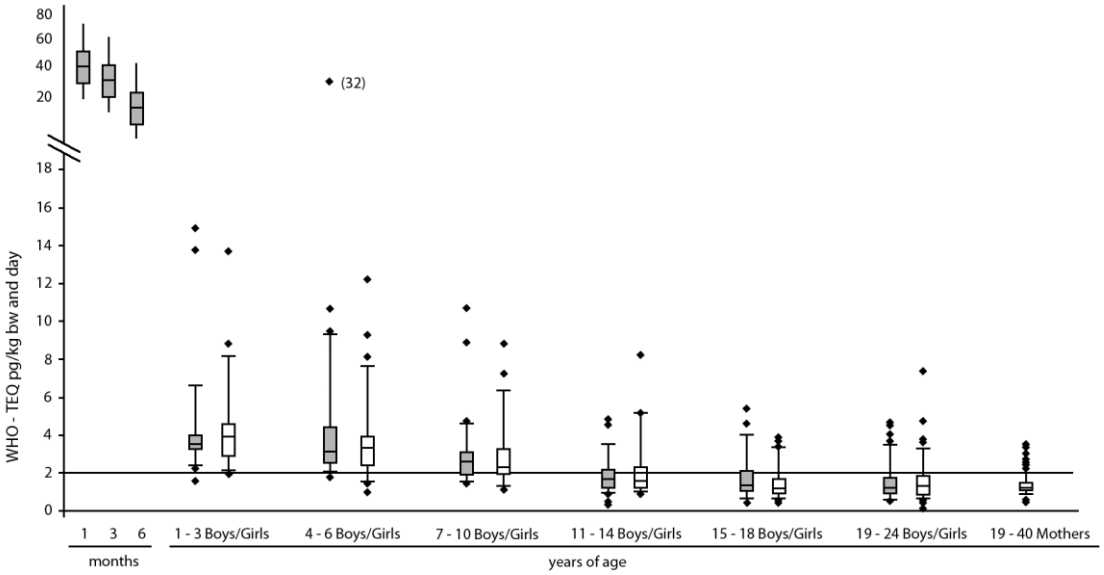

Figure 7. Dietary exposure to dioxinlike PCBs and PCDD/Fs in different subgroups of the Swedish population. Each box represents the 25th percentile (bottom), the median value (middle) and the 75th percentile (top). The line represents a TDI value of 2 pg TEQ/kg body weight.

The cumulative body burden in infants was 4 times higher (4.8 to 20 ng TEQ) at the end of 6 months of breastfeeding for dioxinlike PCBs and PCDD/Fs and 7 times higher (46 to 352 mg) for the non-dioxinlike PCBs compared to the initial exposure at the start of the breastfeeding period (**Paper II**).

General discussion about the levels of PCBs and PCDD/Fs

An age-dependent decrease in exposure levels has also been observed in populations from the Netherlands,[34] Spain,[106] Italy,[107] and the U.S.,[108] and is partly explained by the higher consumption of food by children relative to their body weight compared to adults. Children between 1 to 6 years of age eat about 3 times more food on a body weight basis than the average adult (**Paper I**). Even though the dietary exposure to PCBs and PCDD/Fs decreases by age due to an increase in body weight, the cumulative body burden of these contaminants increases with age.[51,109] In the present thesis, there was a 4 to 7 fold increase in mean cumulative

exposure to dioxinlike PCBs and PCDD/Fs during 6 months of breastfeeding (**Paper II**) as also observed in Dutch infants.[109]

The number of individuals exceeding the current TDI of 2 pg TEQ/kg body weight[60] decreased from almost 100% of infants and young children to less than 4% of first-time mothers (Figure 7). The current TDIs are not applicable to breastfed infants because of the short duration of this life period and the assumed overall beneficial effects of breastfeeding on child development.[57,58,60] However, results from studies on the association between exposure to PCBs and PCDD/Fs and the health effects of breastfeeding are contradictory.[110-113] Furthermore, risks appearing in adulthood due to high exposure early in life are unknown, although it seems that the exposure during early infancy has an impact on body burden later in adulthood.[52] Swedish women who were themselves breastfed had higher concentrations of PCBs and PCDD/Fs in breast milk compared to women who had not been breastfed.[52] Early life is a vulnerable stage of development which means that children are a special risk group and, thus, more studies are needed to disentangle the potential effects of relatively high exposure during a sensitive period in life to improve risk assessments.[114]

The current TDIs are based on prenatal effects and thus mainly concerns pregnant women and women of childbearing age. Of these women, 3 – 4% had dietary exposure levels of dioxinlike PCBs and PCDD/Fs that were close to the upper range of the TDI value (4 pg TEQ/kg body weight). The upper TDI value is only 3.5 times lower than the most sensitive end point in animals (14 pg TEQ/kg body weight). It may be discussed whether or not the safety margin (uncertainty factor) of ten is large enough to compensate for this narrow range. The potential health risks of such an exposure level is difficult to predict, however, due to the narrow safety margin efforts to continue reducing the exposure is essential to avoid potential adverse health effects in children.

The TDI of 2 – 8 pg TEQ/kg body weight based on carcinogenic effects is applicable for consumers of the general population and was also exceeded by many of the individuals in the present thesis.[62]

Methodological considerations

The dietary exposure to PCBs and PCDD/Fs was based on consumption data from a menu diary (covering 7 consecutive days) and contaminant concentrations in food. Menu diaries are similar to dietary records in the sense that the respondents have to fill in how much of their food is consumed on a daily basis.[3] In comparison to dietary records, the menu diary contains a food list that the respondent can choose from.

Variability in the exposure levels to PCBs and PCDD/Fs (**Paper I**) is probably, to some extent, reflected by the choice of dietary assessment method (menu diary) since the consumption of foods was recorded on a daily basis rather than as an average consumption covering a longer period as estimated from FFQs.[3] For this reason, seasonal differences in exposure will also have an effect on the exposure estimated from menu diaries.[115] The menu diary in the present study, did not always note the origin of the fish or what type of fish that was consumed by the individuals and, thus, Baltic Sea fish including salmon and herring containing high concentrations of the contaminants could have had a stronger, or less marked, impact on the exposure estimate.[116] Furthermore, the menu diary covered a relatively short period and does not represent the entire lifetime exposure of a child. In the present thesis, the estimated daily exposure to PCBs and PCDD/Fs in mother, based on breast milk concentration data, was comparable to those estimated from dietary questionnaires,[97] indicating that exposure estimations based on biomonitoring data could be a good alternative when food consumption data are not available or are of low quality.

Concentrations of PCBs and PCDD/Fs in breast milk were adjusted for the lipid content in milk to avoid influence due to temporal variability[117] and inter-individual differences (where two women with similar body burden of PCBs and PCDD/Fs but different breast milk lipid contents would otherwise be misclassified).

The exposure to dioxinlike PCBs and PCDD/Fs were estimated based on two different TEF schemes; one from 1998 and one from 2005 (**Paper I** and **II**). In the revised version of the TEF scheme, the authors conclude that the TEQ value (derived from

applying a TEF value to the concentrations in food or biota) decreased with 10 – 45% when using the revised TEF scheme from 2005 compared to the TEF scheme from 1997.[47] The largest decrease in TEQ was observed for chicken (- 45%) followed by Baltic herring (- 25%), and may lead to the erroneous impression that the dietary exposure to PCBs and PCDD/Fs (expressed as TEQ) has decreased.[118,119]

5.2 Comparing exposure assessment approaches

Reliable human exposure data is needed for effective risk assessment and management. By applying a probabilistic exposure assessment approach to the exposure data, true individual variations in exposure are taken into account, and thus, overly conservative exposure estimates based on deterministic exposure assessment approaches can be avoided. For this reason, the probabilistic exposure assessment approach was used for estimating infant exposure at different time points during breastfeeding as well as the daily exposure in nursing mothers based on biomonitoring data, and compared to the deterministic approach.

The estimated mean exposure to PCBs and PCDD/Fs was comparable when using the deterministic and the probabilistic exposure assessment approach; a mean exposure of 44 pg TEQ/kg body weight in 1 month old infants when using either approach was seen (**Paper II**). The estimated worst case scenario, on the other hand, was 1.25 times higher for dioxinlike PCBs and PCDD/Fs and 1.67 times higher for non-dioxinlike PCBs when using the deterministic worst case scenario approach compared to the 95th percentile from the probabilistic exposure distribution (Figure 8; **Paper II**). The sensitivity analysis showed that fat content and breast milk consumption were the variables with the greatest influence on the variation of exposure in the probabilistic model contributing to 63% and 19%, respectively. In total, 9 of the 25 input variables (9 PCBs and 16 PCDD/Fs) contributed to the variance of the exposure (0.1 – 11%) whereas the rest of the 16 input variables had very little effect on the variability of the exposure distribution.

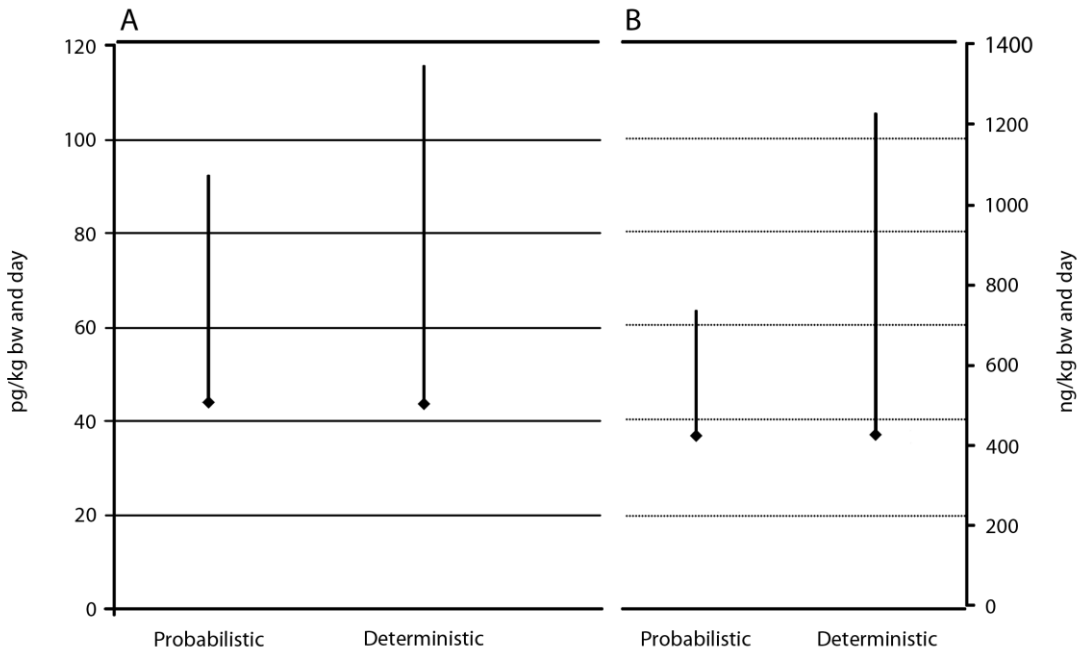

Figure 8. Comparing mean values (♦) and 95th percentiles (|) of exposure to dioxinlike PCBs and PCDD/Fs expressed as TEQ (A) and non-dioxinlike PCBs (B) in 1 month old breastfed infants using the probabilistic and the deterministic exposure assessment approach.

General discussion and methodological considerations

So far, the deterministic exposure assessment approach including the worst case exposure scenario has been the dominating methodology within exposure assessments. However, probabilistic modeling, including benchmark dose modeling and physiologically based toxicokinetic modeling, is increasingly being used to improve exposure and health risk assessments.[120,121] The point estimates that are used within the worst case scenario calculations are by definition conservative, which may tend to result in an overestimation of the exposure.[122] The probabilistic approach, on the other hand, is more practical because several upper-bound percentiles can be drawn from the same exposure distribution where a range of possible exposure scenarios are presented, and the likelihood of these exposures to occur is given.[8]

An accurate exposure distribution from the probabilistic model is derived by using probability density functions that give a good representation of the different input

variables. However, finding a distribution that best represents the input data can be difficult since the distributions are often based on empirical data to which several distributions may fit, especially when the collected sample size is small.[123] For example, the skewness of a distribution based on collected empirical data can affect the central tendency of the output.[8] It should be noted, however, that the choice of distribution is less important than the precision of the mean and variance estimates. Hence, based on results from sensitivity analyses, special attention should be put on the variables that have the greatest impact on the variance of the exposure outcome. For instance, increasing the mean of the input variable of fat content in a probabilistic model will equally increase the mean of the exposure.[124]

In conclusion, the deterministic approach should be considered as a first tier in human exposure assessments using the worst case approach to protect public health. The probabilistic approach, on the other hand, can be used to increase the accuracy of the exposure and to gain more information such as probability of risk levels, sensitivity analysis, uncertainty and variability analysis.

In **Paper II**, values below the quantification limit (censored data) were replaced by one-half of this limit or by using the MLE method. Assigning censored data with a value can result in an overestimation, whereas the opposite can occur if censored data are assigned with the value zero or neglected.[9] To minimize the bias in over- and underestimation of the exposure, the one-half of the quantification limit can be used assuming, however, that all values between the quantification limit are equally likely. The degree to which the results are biased will depend on the values below the quantification limit in relation to those above the limit.

5.3 Use of food contaminant databases for exposure assessment

In **Paper I**, concentration data for the different food items was considered constant between the analytical years 1998 - 2004 and no time-trend adjustments were therefore made to the concentrations in food. When calculating the total TEQ exposure, the difference between in TEQ exposure by using concentration data in food analyzed from

1998 to 1999 rather than food concentration data analyzed from 2000 to 2004, was only 0.18%, which was based on the sum of TEQ values in food items analyzed during the two periods (1998–1999 and 2000–2004).

For **Paper III** and **IV**, two large recipe-based databases for PCB153 were constructed for the FFQs completed in 1997 and 2004-2006. The concentration data for PCB153 was extrapolated by 8% per year[98] to reflect the concentrations in food during the years 1997 and 2004-2006. The number is based on time-trend studies of PCB concentrations in breast milk.[98] In **Paper II**, the temporal decrease in concentrations of PCBs and PCDD/Fs in breast milk between 2004 and 2006 were minor (41 versus 40.5 pg TEQ/kg body weight, respectively) and, thus, the food concentration data for year 2004 to 2006 was assumed to be the same. There is of course an amount of uncertainty in the two databases, including the standardized recipes and the extrapolation factor for the concentration data. The percentage of decrease in PCB153 concentrations may not be the same for all foods.[125] Food analyzed in the years 1997 or 2004 were used as far as possible to avoid this problem.

The dietary databases were created for PCB153 as an indicator for PCB and PCDD/F exposure.[126] In the present thesis, PCB153 was the most abundant congener in breast milk and serum, accounting for 42 - 46% of the total concentrations of PCBs and PCDD/Fs in breast milk (**Paper II and III**). Serum concentrations of PCB153 correlated well with five other PCB congeners (118, 138, 156, 170 and 180; r_s 0.72 – 0.94; p value <0.001) as well as with the total sum of the PCB congeners (r_s 0.93; **Paper III**). Similar correlations were observed in breast milk between PCB153 and eight other PCB congeners (118, 138, 156, 157, 167, 169, 170 and 180; r_s 0.69 – 8.89; p value <0.001) as well as with 10 out of 15 of the PCDD/Fs congeners (r_s 0.53 – 0.78; p value <0.05).

For the two food contaminant databases, the concentrations of PCB153 below the quantification limit were replaced with one-half of this limit (12% of the total number of samples). When comparing different approaches, that is, replacing censored data with one-half of the quantification limit, replacing censored data with zero and replacing censored data with square root of the quantification limit, in samples of dairy

products, the mean concentration of PCB153 was the same between the three approaches. The influence of the various methods for dealing with the censored data was minor because of the low number of data below the quantification limit.[9]

5.4 Validating food frequency questionnaires

Validated FFQs for estimating dietary exposure to PCBs can be used in large prospective epidemiological studies for studying the association between exposure and risk of health effects, thereby overcoming the disadvantages of high costs of sample collection and chemical analyses of biomarkers as well as cross-sectional study designs.

In **Paper III**, the validation of FFQs for estimating concurrent (median 152 ng/day ~ 2.2 ng/kg bw) as well as long-term (7 to 9 years prior to blood sampling; 169 ng/day ~ 2.4 ng/kg body weight) exposure assessment of PCB153 was based on concentrations in serum PCBs (118, 138, 153, 156, 170 and 180) in middle-aged and elderly women (n = 201; 48 – 83 years of age). The intra-class correlation coefficient was 0.51 based on FFQ-estimated exposure to PCB153 in the reproducibility study (median 153 ng/day in 2004 –2006 and 160 ng/day in 2005 – 2007; n = 165).

A reasonable validity was observed between FFQ-based dietary estimates of PCB153 exposure (expressed as ng/kg body weight and day) and age- and lipid-adjusted concentrations in serum PCB153. The deattenuated Spearman correlation coefficient was 0.37 (p value <0.001) for the concurrent exposure assessment and 0.32 (p value <0.05) for the long-term exposure assessment (**Paper III**). The correlation for the five other PCB congeners (118, 138, 156, 170 and 180) in serum ranged from 0.26 to 0.45 (95% CI; 0.06-0.62) and 0.23 to 0.42 (95% CI 0.00-0.62) for the respective exposure assessments.

The degree of cross-classification of tertiles of serum PCB153 levels was evaluated against tertiles of the 2004-06 FFQ-estimated PCB153 exposures. In the lowest tertile of serum PCB153, 32 of 67 women (48 %) had low levels of both serum PCB153 and

concurrent dietary PCB153 exposure (corresponding results were 51 % for serum sumPCB). In the highest tertile of serum PCB153, 39% of the women were categorized into the same dietary category (the same results were obtained for serum sumPCB). Very similar results were obtained for the 1997 FFQ-estimated PCB153 exposure. The percentage of women classified into the same or adjacent tertile was 85 % for both the 1997 and the 2004-2006 FFQ.

General discussion and methodological considerations

We obtained a reasonable validity of the FFQ justifying the use of the FFQ for estimating dietary PCB exposure. The dietary PCB153 exposure compares well to the estimated exposure in middle-aged and elderly women in Sweden.[127]

The FFQ-estimated PCB153 exposure was compared to the concentrations of PCB congeners in serum, presumed to represent long-term exposure to PCBs. Previous studies have found a strong correlation between concentrations of lipid-adjusted PCBs in serum and PCBs in adipose tissue,[50,128] the main storage site for life-long accumulation of polychlorinated pollutants.[129] The correlation between FFQ-based estimates of PCB exposure and serum PCB concentrations was stronger when adjusting the dietary exposure for body weight and the serum concentrations for age. The reason for this could be that the amount of PCB in serum is not equivalent to the amount of PCB ingested when expressed as g/day as would be observed in individuals with a larger body size. Instead, the larger the body weight, the more PCB is concentrated in the fatty tissues.[130] The mean PCB153 concentrations in blood of Swedish women 50 – 74 years of age increased by 1.8% per year of age[131] and was a strong predictor of body burden of PCBs.[105,132] Adjusting serum PCB concentrations for age may compensate for the cumulative serum concentrations.

In the present thesis, serum had been sampled once after a 12-hour overnight fast and adjusted for the serum lipid content. Sampling after fasting reduces the influence of diet on the concentrations of PCBs in serum,[133] especially if the dietary concentration is higher than that in blood.[134] Thus, changes in serum PCB concentrations over time can

be compensated for if the PCB concentrations are reported on a lipid weight basis rather than on a fresh weight basis.[133] The serum lipid concentrations, in the present thesis, had been determined by the enzymatic method and, hence, the weight of the lipid content was calculated based on the method described by Grimvall and co-workers.[105] In the study by Grimvall, the correlation between the enzymatic method and the gravimetric method for determining serum lipids was 0.82.[105]

5.5 Exposure levels and associations with myocardial infarction

Through the use of validated FFQs for estimating PCB153 exposure, the risk of myocardial infarction was estimated in middle-aged and elderly women (Figure 9). An increased hazard ratio of 1.67 (95% CI 1.17 – 2.40) was observed for total myocardial infarction in the highest exposure quartile of dietary PCB153 exposure (median 280 ng/day) compared to those in the lowest (median 98 ng/day) in the multivariable-adjusted model (**Paper IV**). The hazard ratio was even more pronounced (2.62; 95% CI 1.15 – 5.98) for fatal myocardial infarction (Figure 9).

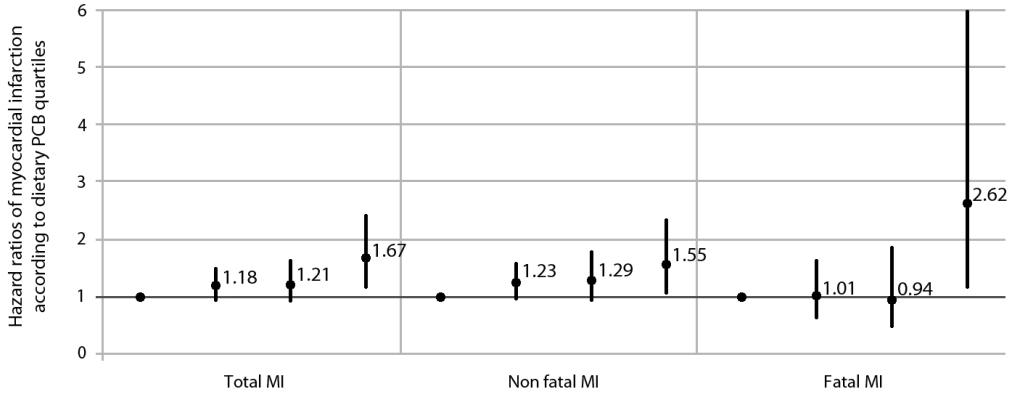

Figure 9. Multivariable-adjusted hazard ratios (95% confidence intervals) of any first myocardial infarction (MI) according to quartiles of dietary PCB153 exposure in 33,638 women.

General discussion and considerations

The strong correlation between PCB153 and other congeners of PCBs and PCDD/Fs hinders the possibility to make conclusions about a causal relationship. However, it has been suggested that since PCB congeners act differently in relation to predicting factors (e.g., BMI) they should not be considered to be assessed as a homogenous group in exposure assessments. Cell culture and animal studies support the theory of PCB exposure and associations with CVD as well as risk factors for CVD.[68,69,71,77] **Paper IV** is based on a prospective epidemiological study design in which exposure is estimated prior to the diagnosis of disease and, thus, reverse causality can be avoided.

Fish and seafood can also contain high concentrations of methylmercury (MeHg).[135] Adjusting for MeHg exposure was important to rule out the possibility of observing effects on myocardial infarction caused by MeHg exposure. Epidemiological findings behind the cardiovascular effects of MeHg are inconsistent. Of seven studies, [136] [137] [138] [139] [140] only two have shown an increased risk of coronary heart diseases associated with toenail mercury concentrations[141] and concentrations of mercury in hair.[142]

5.6 Dietary sources of PCBs and PCDD/Fs exposure and risk of myocardial infarction

One important aspect of exposure assessment is source identification. Once the pattern of exposure in highly exposed individuals or individuals at risk is characterized, public health polices can then be developed such as dietary recommendations and tolerable intakes. Through dietary questionnaires and databases on contaminants in food, the major dietary sources of exposure could be estimated in different age and risk groups in the present thesis.

Fish and dairy products were in general the main sources of exposure in children and young adults accounting for 29% and 30 %, respectively, of the total TEQ exposure (Figure 10; **Paper I**). On the other hand, fish and fish products were in general the main

sources of exposure in the highly exposed children and young adults contributing with 75% to the total dietary exposure. Fish and fish products were the main source of exposure to PCB153 in middle-aged and elderly women accounting for 66 – 78% of the total exposure (**Paper III**).

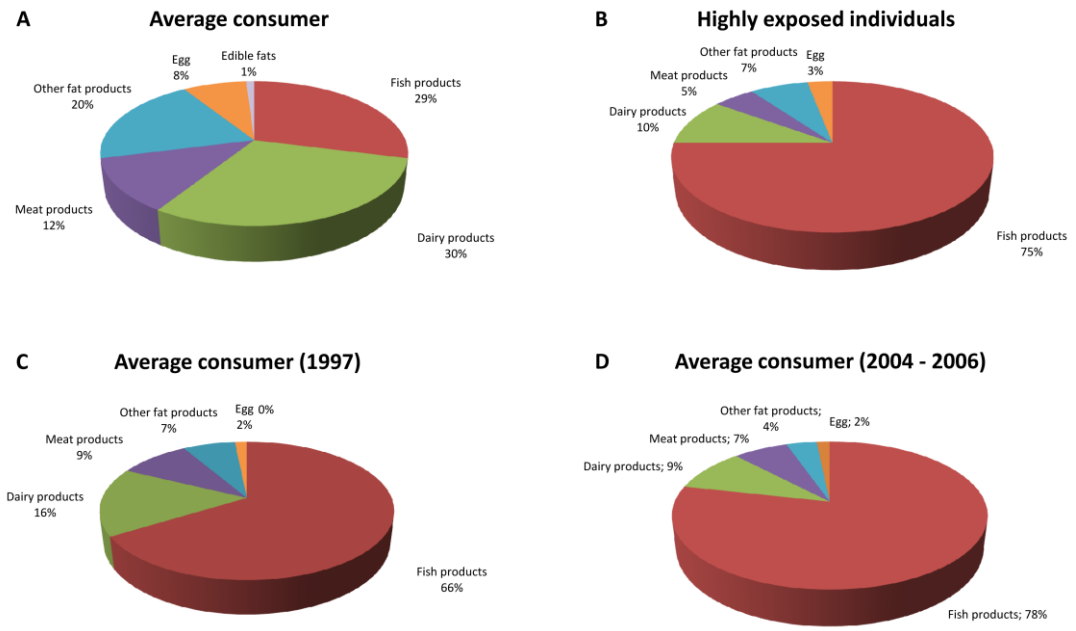

Figure 10. Contribution from food groups to the estimated exposure to dioxinlike PCBs and PCDD/Fs in Swedish children and young adults 1-24 years (A; average and B; highly exposed (95th percentile), and to PCB153 in middle-aged and elderly women (C; 1997 and D; 2004 - 06).

When taking into account the beneficial effects of long-chain polyunsaturated fish fatty acids that is eicosapentaenoic (EPA) and docosahexaenoic acids (DHA), the hazard ratio for myocardial infarction was 2 times higher (95% CI 1.25 to 3.94) in women in the highest quartile of dietary PCB153 exposure at the same time having a low intake of EPA and DHA (< 0.20 g/day), compared to those in the lowest quartile having a high intake of more than 0.29 g/day of EPA and DHA (Figure 11; **Paper IV**).

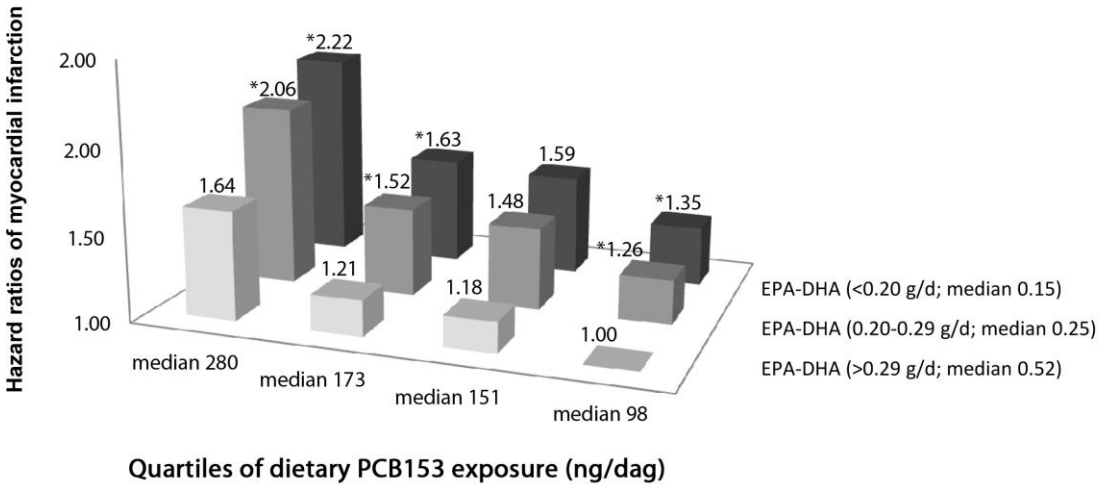

Figure 11. Multivariable-adjusted hazard ratios of myocardial infarction according to quartiles of dietary PCB153 exposure (ng/day) and intake of EPA and DHA (g/day).

General discussion

Even though fish in general contain higher concentrations of PCBs and PCDD/F than other food, dairy products were the main source of exposure in children and young adults from the present thesis. The reason for this is the relatively higher consumption of dairy products compared to fish and fish products. During recent years, the importance of different food exposure sources has changed, resulting in a higher contribution from fish and fish products to the total exposure in children.[143,144] In Sweden, [127] and in some other countries,[36-39] fish and fish products are the main dietary contributors to the exposure to PCBs and PCDD/Fs in adults. The higher contribution from fish in middle-aged women as compared to children and adolescents is probably due to a higher consumption of fish in this subpopulation.[41]

Fish is also the major source of long-chain n-3 polyunsaturated fatty acids (n-3 PUFAs) which is suggested to have a protective effect on CVD[145,146] and to improve mental development and cognition in infants and young children.[147]

Health risk analyses of PCBs in relation to the beneficial effects of n-3 PUFAs in fish, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are limited in number.[148] Hence, there remain difficulties in determining the appropriate dietary recommendations for consumption of fatty fish since these both pose a risk to the population from exposure to PCBs and PCDD/Fs and a potential health benefit derived from omega-3 fatty acids.[149,150] Only during recent years have the exposure to contaminants and the intake of nutrients from fish (and other foods) been considered together, i.e., risk-benefit analyses, when establishing dietary recommendations.[151]

6 CONCLUSIONS

I. Reliable exposure estimates of PCBs and PCDD/Fs in various age groups was achieved by the use of different exposure assessment approaches, taking into account variability and uncertainty in exposure data.

II. Age specific exposure assessments that consider sensitive and/or highly exposed individuals in the general population is a prerequisite for effective risk management.

III. The deterministic (worst case) exposure assessment approach should be considered as the first choice while the use of the more sophisticated approaches such as the probabilistic approach should be utilized when more detailed exposure information is needed.

IV. Highly exposed individuals were characterized by a high fish consumption independent of age. Thus, continued actions are needed to reduce environmental levels and at the same time conduct risk-benefit analysis for efficient dietary recommendations.

V. A reasonable validity of FFQ-based PCB153 exposure estimates in relation to PCB concentrations in serum was obtained, justifying the use of FFQs in large scale epidemiological studies.

VI. FFQ-estimated dietary PCB153 exposure was associated with increased risk of myocardial infarction among women. Further studies are needed to elucidate the observed association between the indicator biomarker PCB153 and risk of cardiovascular effects.

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/*Lotta*

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