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TESI DI DOTTORATO DI RICERCA

THE INTERPRETATION OF A BREAST MILK MONITORING OF DIOXIN-LIKE AND NON-DIOXIN LIKE POLLUTANTS IN ITALY BY A PBPK MODEL INTEGRATING A NOVEL APPROACH TO DESCRIBE PRE- AND POSTNATAL MOTHER/CHILD TRANSFER

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1. ABSTRACT

Polychlorinated dibenzo-p-dioxins, dibenzofurans (PCDD/Fs) and polychlorinated biphenyls (PCBs) are widespread environmental contaminants. They enter the human body mainly with food and because of lipophilic character they accumulate in fatty tissues. Knowledge about the intake and body burden of PCDD/Fs and PCBs in a population helps to focus efforts to diminish population exposure to these compounds.

This study aimed to interpret the results from a recent biomonitoring study of PCDD/Fs and PCBs in breast milk of Italian non-occupationally exposed mothers by a PBPK model of mother and infant. Measurements included detection of 2,3,7,8 substituted polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and polychlorinated biphenyls and estimation of the dietary intake of these pollutants by lactating infants. The modelling part was focused on one PCB congener – PCB 153. Here, reverse dosimetry was performed to estimate mother external dose, and forward dosimetry to predict tissue concentrations in mother and infant daily intake.

The modeling outcomes provided an historical perspective on the changing exposure of the Italian population to PCB-153. They realistically reflect the evolution of daily intake during the mother's life, and evolution of blood contamination. At the same time it was proved that breast milk is good biomarker of historical exposure and in combination with PBPK modeling can be used to characterize prospectively and retrospectively, internal and external exposures. So far, this is the first study which reconstructed the dynamic exposure for specific population. Results of this work can dramatically change the way to perform modeling for substances such us POPs, similar trends to PCBs is observed for PCDD/Fs , DDT, HCB etc.

2. INTRODUCTION

During life humans are exposed to the mixtures of different toxic chemicals like persistent organic pollutants such as: PCDDsFs PCBs, PBDE, PBBs etc. They enter the human body mainly with food and because of lipophilic character accumulate in fatty tissues. Dioxins and PCBs are than eliminated from the body by metabolic degradation, which is minimal for most congeners, and by excretion which is slow and primarily in faeces. However they may also concentrate in human milk, which is considered an important route of excretion and a primary source of intake for newborns. Pregnant and nursing woman transfer PCBs and dioxins to their babies trans-placentally and by breast-feeding (respectively prenatal and postnatal exposure), the latter being considered the most important source (Jacobson et al., 1984). They are in fact very efficient absorbed in the digestive tract of infants to the extent of about 95% of the amount ingested (Dahl et al., 1995; Abraham et al., 1994).

Breast milk therefore is widely used in human biomonitorig studies to assess exposure of mothers and infants to environmental chemicals (WHO 1989 and 1996). Biomonitoring of human tissues provides the evidence of human internal exposure, however to evaluate or bound potentially related health risks additional information about external exposure are needed. Therefore human biomonitoring is frequently and effectively combined with physiologically based pharmacokinetic (PBPK) models which describe the relationship between external and internal exposures.

The aim of PBPK modelling is to describe the physical and physiological processes that are involved in the uptake, distribution, and elimination of a compound in an organism in great detail. The combination of PBPK modelling and human biomonitoring results in a powerful tool for the health risk assessment at individual or population level. Application of PBPK models are needed to build hypothesis about possible relationships between exposure, dose and disease, and to monitor trends in environmental quality. Studies like this one show that PBPK models are broadly applicable as a tool for relating dose biomarker to measures of individual and population exposure and health risk. Moreover application of PBPK model gives the possibility to better relate biomarkers to specific source and scenario of exposure.

This study aimed to interpret results from a recent biomonitoring study of PCDD/Fs and PCBs in breast milk of Italian non-occupationally exposed population by a PBPk model of mother and infant. The modelling part focuses on one PCB congener – PCB 153. There are several reasons why PCB-153 has been selected to carry out computer simulations. First of all PCB-153 occurs in human milk at the highest concentration, therefore its amount is usually measured precisely. This congener is suggested as a valuable indicator for total amount of PCBs in the food of animal origin (i.e. fish) (Atuma et al 1996). Moreover concentration in human serum seems good marker of exposure to PCBs via food intake (Axmon et al 2006, Jonsson et al 2005), occupational exposure to PCBs during sealant removal operations (Wingfors et al 2006), or exposure of the population living in urban zones (Covaci et al 2002). Its presence in breast milk was found to be a biomarker of the total sum of TEQ PCDDs/Fs and TEQ DL-PCB (Glynn et al 2001). Moreover serum level of PB153 was inversely correlated with free testosterone levels, and high level of PCBs – with reduced sperm mobility (Rich Hoff et al 2003, Bush et al 1986). In woman positive association between PCBs and increasing menstrual cycle length was observed by Copper (Cooper et al 2005). PCBs 138, 153 and 180 were associated with increasing risk of asthma (Van Den Heuvel et al 2002). Some authors found a strong correlation between maternal blood level of PCB-153, 180 and 138 and endometriosis occurrence in Italian woman (Porpora et al 2006, De Filip et al 2004)

In this study we applied PBPK model for forward and reverse dosimetry in mother and infant based on results from biomonitoring of breast milk. Reverse dosimetry was performed in order to reconstruct the dietary intake over the mother's life, taking into consideration decreasing tendency in PCBs occurrence in environment. Forward dosimetry focused on prediction of mother blood concentration, and infant daily intake.

3. CHARACTERISTIC OF PCDD/Fs AND PCBs

3.1 PCDD/Fs. Physical and chemical properties

Term "*Dioxins*" is a common name of family of organic compounds which consists of 210 congeners. This group can be divided into two subgroups: PCDDs (polychlorinated dibenzo-p-dioxins) and PCDFs (polychlorinated dibenzofurans). PCDDs and PCDFs are almost planar tricyclic aromatic compounds formed by two benzene rings connected by two oxygen atoms in PCDDs and one oxygen atom in PCDFs (See Figure 1). The hydrogen atoms may be replaced by up to eight chlorine atoms what gives 75 PCDDs and 135 PCDFs congeners. Above mentioned compounds showed similar chemical properties.

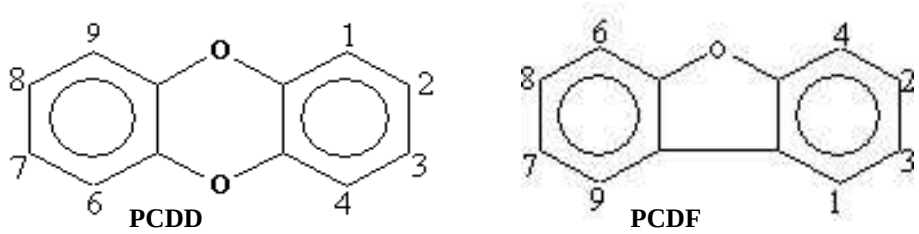


Figure 1. Structures of PCDDs and PCDFs (1-9 indicate the possible positions of the chlorine atoms)

These compounds are highly fat soluble, they have high melting points and low vapor pressures and are stable to acids and bases; these characteristics make them very persistent in the environment. They bioaccumulate through the food chain. Table below shows several chemical properties of PCDDs and PCDDFs

	Aberg et al 2008	Kadowaki et al 2000			Aberg 2008	
	S_W,	P^o_L	P^o_L	K_p	Log10 K_{OA}	Log10 K_{AW}
2378-TCDD	1.59E−5	-3.93	-3.28	-3.32	9.89	-2.91
12378-PeCDD	8.62E−5	-5.37	-3.83	-2.17	10.32	-3.24
123478-HeCDD	1.55E−5	-5.84	-4.43	-1.23	10.88	-3.28
123678-HeCDD						
123789-HeCDD						
1234678-HpCDD	1.93E−5	-6.75	-5.05	-0.595	11.42	-3.55
OCDD	2.93E−6	-6.02	-5.64	-0.567	12.05	4.37
2378-TCDF	1.35E−4	-3.7	-3.14	-2.4	10.28	-3.17
12378-PeCDF						
23478-PeCDF	5.25E−5	-4.76	-3.76	-2.26		-3.28
123478-HeCDF	3.16E−6	-5.51	-4.22	-1.91	10.77	-3.29
123678-HeCDF		-5.44	-4.24	-1.91		
123789-HeCDF						
234678-HeCDF						
1234678-HpCDF	1.74E−6	-6.24	-4.77	-0.888	10.63	-3.24
1234789-HpCDF	6.31E−7	6.27	-5.03	0.58	11.43	-3.19
OCDF	2.29E−7	-7	-5.5	0.444		-3.05

Table 1.
Several physicochemical properties of PCDDs and PCDFs

S_W - Liquid phase water solubility at 20°C [mol/m^3]

P^o_L - Subcooled liquid vapor pressures at 25°C [Pa]

K_p - particle–gas partition coefficient at 25°C [m^3/µg]

K_{OA} - Octanol-air partition coefficients

K_{AW} - Dimensionless Henry´s law constants

It is important to highlight that dioxins are unwanted compounds and often unavoidable by-products of industrial processes: production of pesticides, bleaching of paper pulp. They appear during incomplete combustion processes: industrial and natural.

3.2 PCBs. Physical and chemical properties

Polychlorinated biphenyls (PCBs) are aromatic compounds formed by substitution of hydrogen atoms on the biphenyl molecule with chlorine atoms. The chemical formula can be presented as $C_{12}H_{10-n}Cl_n$, where n is a number of chlorine atoms (See Figure below). There are 209 congeners, where 130 occur in commercial products. All congeners of PCBs are lipophilic, accumulate in fatty tissues and entrance the food chain. PCBs do not crystallize, they turn into solid resins. Polychlorinated biphenyls form vapors heavier than air but do not form any explosives mixtures with air. They are chemically very stable under normal conditions and non-flammable; they have a very low electrical conductivity and high dielectric constant. Because of above mentioned properties they have been widely use in industry (hydraulic and heat transfer systems as cooling and insulating fluids. Other applications included pigments, dyes, repellents, plasticizers etc. Since 1970 production of PCBs in USA is prohibited, since 1980 also in Europe.

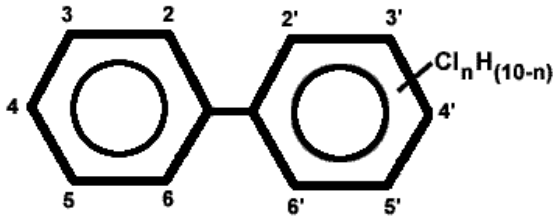


Figure 2. Structural formula of PCBs

Several chemical and physical properties such as liquid phase water solubility, octanol–water partition coefficient, vapour pressures, Henry´s law constants, octanol-air partition coefficient and octanol solubility for selected PCBs congeners are presented in the Table 2 below (Li et al 2002)

congener	S_W,	Log_{10} K_{ow}	P_L	Log 10 K_{aw}	Log K_{OA}	Log 10 S_{oL}
PCB-28	8.85-04	5.66	2.70E-02	1.48	7.85	2.89
PCB-52	4.78E-04	5.91	1.20E-02	1.39	8.22	2.90
PCB-101	1.02E-04	6.33	2.46E-03	1.38	8.73	2.72
PCB-118	6.83E-05	6.69	9.91E-04	1.16	9.36	2.96
PCB-138	1.87E-05	7.21	5.63E-04	1.47	9.66	3.00
PCB-153	3.07E-05	6.87	6.06E-04	1.29	9.44	2.83
PCB-180	1.32E+05	7.16	1.08E-04	0.91	10.16	2.80

Table 2. Several physicochemical properties of PCDDs and PCDFs [Li et al 2002] S_W - Liquid phase water solubility at 20^{o}C [mol/m^3] K_{ow} - Octanol–Water Partition Coefficient, P_L - vapor pressures [Pa], K_{AW} - Dimensionless Henry´s law constants, K_{OA} - octanol-air partition coefficients, S_{OL} Octanol Solubility in [mol/m^3]

PCBs congeners can be grouped according to their degree of chlorination, endocrine disrupting activity or pattern of enzyme induction. From the toxicological point of view PCBs are divided into 3 groups: non-ortho, mono-ortho and di-ortho substituted PCBs. Between them congeners non-ortho and mono-ortho bound to the Ah receptor, show toxicological activity comparable to that of dioxins, and that's why they are called "dioxin-like PCBs".

Dioxin like PCBs		**Non dioxin like PCBs**
Non orto PCBs	***Mono orto PCBs***	**Di-orto PCBs so called PCBs Indicators**
3,3',4,4'-TeCB (77)	2,3,3',4,4'-PeCB (105)	2,4,4' TriCB (28)
3,4,4',5-TeCB (81)	2,3,4,4',5 -PeCB (114)	2,2',5,5'-TeCB (52)
3,3',4,4',5 PeCB (126)	2,3',4,4',5-PeCB (118)	2,2',4,5,5'-PeCB (101)
3,3',4,4',5,5' HxCB (169)	2,3,4,4',5- PeCB (123)	2,3',4,4',5-PeCB (118)
	2,3,3',4,4',5 -HxCB (156)	2,2',3,4,4',5'-HxCB (138)
	2,3,3',4,4',5'-HxCB (157)	2,2',4,4',5,5'-HxCB (153)
	2,3',4,4',5,5'-HxCB (167)	2,2',3,3',4,4',5-HpCB (170)
	2,3,3',4,4',5,5'-HpCB (189)	2,2',3,4,4',5,5'-HpCB (180)

Table 3. PCBs nomenclature : IUPAC names and Ballschmiter & Zell (1980) name system in parenthesis

Non dioxin like PCBs create a group of congeners called PCBs indicators due to the fact that occur at higher concentration in environment than other congeners and account for 50-80% of total amount of PCBs (Noren et al 2000). Instead of laborious and expensive analysis of all 2,3,7,8 substituted PCBs congeners it is possible to estimate the total amount of PCBs based on the measurement of only the PCBs indicators.

3.3 Toxicological characteristic of PCDD/Fs and PCBs

It is very difficult to assess a human health effects separately for PCBs, PCDFs, PCDDs because those compound usually occur as a mixtures of many congeners. 17 dioxins and dioxin like PCB congeners have been shown to be toxic at low levels, they bind to the same dioxin-receptor but with the different intensities and with the different toxicity. This different toxicity is expressed by a TEF (toxicological equivalency factor), which express the toxicity of the various congeners. Congeners substituted in the 2,3,7,8 position are of special importance and for the most toxic 2,3,7,8 tetrachlorodibenzeno-p-dioxin (2,3,7,8 TCDD) TEF is of 1 (See Figure 3 below)

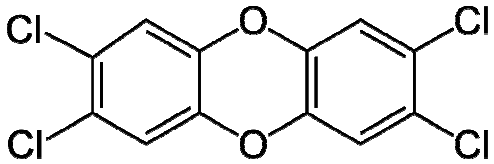


Figure 3. Chemical formula of 2,3,7,8 tetrachlorodibenzeno-p-dioxin (2,3,7,8 TCDD)

Usually the toxicity of a mixtures of dioxins is expressed as a TEQ value (toxic equivalents). To determine the TEQ of a mixture, the amount of each of the toxic members of the family is multiplied by its TEF, and the TEQs are summered (See Table 4)

$$\mathbf{TEQ = \Sigma\ (m_i\ x\ TEF_i)}$$

Congener	**WHO-TEF 1998**	**WHO – TEF 2005**	**Congener**	**WHO – TEF 1998**	**WHO-TEF 2005**
2,3,7,8-TCDD	**1**	**1**	***Mono orto PCBs***		
1.2.3.7.8-PeCDD	**1**	**1**	2,3,3',4,4'-PeCB (105)	0.0001	0.00003
1,2,3,4,7,8- HxCDD	0.1	0.1	2,3,4,4',5 -PeCB (114)	0.0005	0.00003
1,2,3,7,8,9- HxCDD			2,3',4,4',5-PeCB (118)	0.0001	0.00003
1,2,3,6,7,8- HxCDD			2,3,4,4',5- PeCB (123)	0.0001	0.00003
1,2,3,4,6,7,8- HpCDD	0.01	0.01	2,3,3',4,4',5 -HxCB (156)	0.0005	0.00003
OCDD	0.0001	0.0003	2,3,3',4,4',5'-HxCB (157)	0.0005	0.00003
2,3,7,8- TCDF	0.1	0.1	2,3',4,4',5,5'-HxCB (167)	0.00001	0.00003
2,3,4,7,8- PeCDF	**0.5**	**0.3**	2,3,3',4,4',5,5'-HpCB (189)	0.0001	0.00003
1,2,3,7,8- PeCDF	0.05	0.03	***Non orto PCBs***		
1,2,3,4,7,8- HxCDF	0.1	0.1	3,3',4,4'-TCB (77)	0.0001	0.0001
1,2,3,6,7,8- HxCDF			3,4,4',5-TCB (81)	0.0001	0.0003
1,2,3,7,8,9- HxCDF			3,3',4,4',5 PeCB (126)	**0.1**	**0.1**
2,3,4,6,7,8- HxCDF			3,3',4,4',5,5' HxCB (169)	0.01	0.03
1,2,3,4,6,7,8- HpCDF	0.01	0.01			
1,2,3,4,7,8,9- HpCDF					
OCDF	0.0001	0.0003			

Table 4. Comparison of TEF factor. Van den Berg et al 2006

A number of human epidemiologic and animal experimental studies have established an association between dioxins and effects on endocrine systems and

immune system (Pluim HJ et al 1993, Safe S et al 1990). Dioxins can block the action of estrogens under certain conditions, can lower the levels of androgens and affect the amount of thyroid hormones in the body (Safe S et al 1991). They increase serum triglycerides and cholesterol, well-established risk factors for cardiovascular disease (Martin JV et al 1984, Pazderova-Vejlupkova J et al 1981). TCDD is the most toxic among all dioxins congeners and classified as a group 1 carcinogen (IARC 1997), during infancy and prepuberty has permanent effect on semen quality and causes reduction of estradiol and growth of follicle-stimulating hormone (Mocarelli et al 2008). Dioxins showed also a significant negative relationship to the frequency of delta SCEs (sister chromatid exchanges), seemed to elicit some genotoxic / clastrogenic effects on infants postnatal of around 10 months (Nagayama et al 2003) and was associated with higher prevalence of coughing, chest congestion, and phlegm . (Weisglas – Kuperus et al 2000)

Mothers poisoned with rice oil (Yu-Cheng disease) reported lower birth weight, hyperpigmentation, conjunctivitis, nail changes, and natal teeth in their offspring and delay in developmental milestones (Rogan et al 1988). Exposed Yu- Cheng children at school age showed lower IQs (Chen et al 1992), behavioral effects (Chen et al 2994) and physically and mentally delayed (Guo et al 1994, Chen et al 1992) .Moreover effects on the external genitalia of boys at puberty who were born to exposed mothers had been also observed Guo YL, et al 1993; Jacobson and Jacobson 1990). Increased maternal consumption of fish contaminated with dioxins and furans in Sweden was associated with lower infant birth weights (Rylander et al. 2000) and higher proportion of spontanius abortion was observed in mothers living ion contaminated zone next to chemical plant in Chapayevsk, Russia (Revich et al. 2001)

PCBs are carcinogens and cancer promoter and chronic exposure results in chromosomal aberrations (Silberhorn et al 1990). A number of occupational studies have reported increased numbers of various PCB associated cancers—liver, gall bladder, biliary tract, leukaemia, gastrointestinal, skin (especially malignant melanoma), lymphoma, lung, pancreatic (ATSDR,2000; Longnecker et al 2003; Sinks et al 1992; Moysich et al 2002; Demers et al 2002; Yassi et al 1994). An exposure to PCBs can suppress the antibody and cellular immune response (Lu et al 1985, Weisglas-Kuperus et al 1995) and what is the consequence increased susceptibility to infections and to cancer.

A wide variety of endocrine systems are affected by PCBs i.e estrogens and androgen system (Golden et al 1998), retinoid system corticosteroid system, thyroid hormone system (Koopman-Esseboom et al 1994, Wang et al 2005) also at background levels (Schell et al 2005). Serum level of PB153 was inversely correlated with free testosterone levels, and high level of PCBs – with reduced sperm mobility (Richthoff et al 2003, Bush et al 1986). In woman positive association between PCBs and increasing menstrual cycle length was observed by Copper (Cooper et al 2005). PCBs 138, 153 and 180 were associated with increasing risk of asthma (Van Den Heuvel et al 2002). Many authors found a strong correlations between exposure to PCBs (mostly level in serum) and: plasma triglyceride levels and /or lipids level (Mochizuki 1998, 2004; Boll et al 1998; Bell et al 1994; Chase et al 1982; Moysich et al 2002; Tokunaga et al 2003), higher serum cholesterol and blood pressure (Kreiss et al 1981). Notable serum lipid levels and hypertensions are risk factors for cardiovascular diseases. (Wilsgaard et al 2001).

In Dutch cohort study conducted by Weisglas-Kuperus et al.1995, 2000; 2004, higher PCB levels were associated with a higher incidence of recurrent middle-ear infections (elevated 3-fold) and of chicken pox (elevated 7.6-fold) in 42 months old children, but a lower prevalence of asthma. The children with higher PCB levels had more coughing, chest congestion, and phlegm. The same children several years later were found to have a persistently higher prevalence of ear infections (Weisglas-Kuperus et al 2004). In utero exposure to PCBs has been linked to adverse effects on intellectual function in infants and young children like irreversible decrement of IQ. Results of pre- and postnatal exposure study of Jacobson and Jacobson 1996, in children born to women who during pregnancy had eaten fish contaminated with PCBs showed a significant reduction in full scale IQ and poorer performance on reading mastery. Investigations on background levels of PCBs in breast milk (Rogan et al 1986) and cord blood (Walkowiak et al 2001) showed negative effect on both mental and motor development in children at all ages and alteration of the nervous system performance of children. Also exposure to PCBs during the adulthood results with negative effects on health. Study on memory function between 49- to 86-year-old adults (Schantz et al 2001) exposed to contaminated Great Lakes fish clearly showed decrement of IQ and reduction of memory.

In utero exposure to environmental levels of PCBs in negatively associated with birth weight and postnatal growth until 3 mo of age (Koopman-Esseboom et al 1996).

Prenatal PCBs exposure showed correlation with lower growth rate (Patandin et al 1998), lower psychomotor scores at 3 months age (Koopman-Esseboom et al 1996) 6 and 12 months (Gladen et al 1988), poorer neurological conditions at birth (Huisman et al 1995), hypotonicity and hyporeflexia in newborns (Rogan et al 1986), poorer short-term memory functions at 4 years (Jacobson et al 1990). Infants exposed to higher toxic equivalence levels (PCBs, PCDDs and PCDFs) showed lower plasma free thyroxine and total thyroxine levels in the 2nd week after birth (Koopman-Esseboom et al 1994).

4. HUMAN EXPOSURE TO PCDD/Fs AND PCBs

Human environmental exposure to dioxins and PCBs occurs on several ways: via inhalation, food consumption, dermal absorption and ingestion of contaminated soil. Taking into consideration the chemical and physical properties of dioxins and PCBs is easy to deduce that food will be the most important path. Indeed many scientific studies focused on dioxins and PCBs exposure assessment via food (Llobet et al 2003, Fattore et al 2006, Fattore et al 2008) and proved this hypothesis. Figure 4 below presents schematic human exposure to persistent organic pollutants.

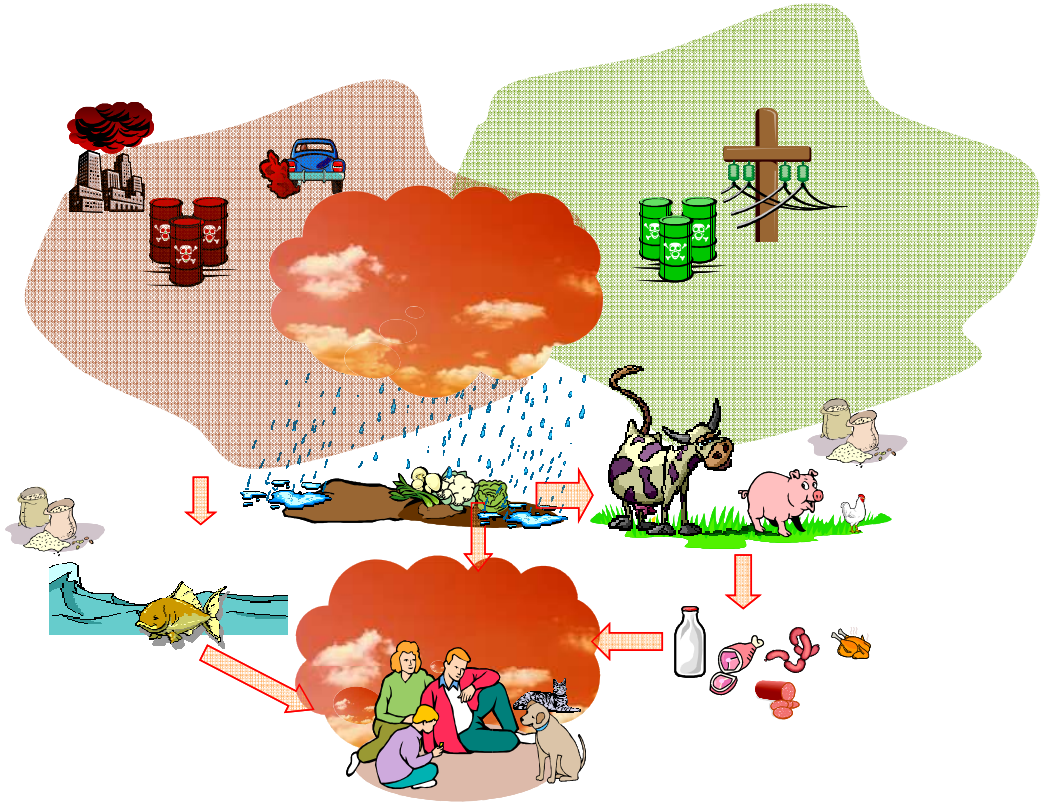


Figure 4 Human exposure to persistent organic pollutants

Additional exposure may occur in accidents or because of occupational contact with these pollutants. Several incidents in which populations were exposed to high levels of PCDDs PCBs are: an industrial explosion that occurred during the production of 2,4,5-TCP at the ICMESA plant in Seveso, Italy in 1976; and an incident with 2,3,7,8-TCDD-contaminated waste oil in Missouri in 1971/72. High exposure may be caused by accidentally contaminated food like contaminated rice oil in Yusho in Japan (1968) and Yu-Cheng in Taiwan.

However for general population the accidental and occupational exposure is minimal, much more important is impact from consumed food. 90% of daily dioxins and PCBs intake arrives from food consumption, mainly fish, egg, oil, meat, milk and its products (De Filip et al 2004, Parzefall 2002). Therefore a number of authorities have assessed or re-assessed risks of PCDD/Fs and DL-PCBs. WHO re-evaluated the risk assessment of PCDD/Fs and related compounds in 1998. Based on investigations such as: with laboratory animal results on decreased sperm count, immune suppression, increased genital malformations, neurobehavioral effects, and endometriosis, the consensus meeting ultimately suggested a range of TDI intakes for humans (1-4 pg TEq/kg body weight (bw)) (van Leeuwen and Younes 2002). Than The Scientific Committee on Food (EU SCF) of the European Commission assessed a TWI of 14 pg WHO-TEq/kg bw for PCDD/Fs and for DL- PCBs (European Commission 2001) based on the rodent studies. This guideline is in line with the tolerable monthly intake (70 pg WHO-TEq/kg bw) established by the Joint FAO/WHO Expert Committee on Food Additives, JECFA (WHO/FAO 2001).

In November 2000 the Scientific Committee on Food of the European Commission published an ‘Opinion of the SCF on the Risk Assessment of Dioxins and Dioxin-like PCBs in Food’ (SCF, 2000). On the basis of this extensive review the committee recommended a temporary tolerable intake on a weekly basis (t-TWI), because of the long half-lives of dioxins in humans (7 years or more) with a value 7 pg WHO-TEQ/kg bw per week. Few months later on the basis of further findings this limit was changed to 14 pg/kg bw per week.

Recent guidelines on tolerable intakes (as pg WHO-TEq / kg bw) of PCDD/Fs and PCBs according to risk assessments by different organizations are presented below:

- **WHO (2000):** TDI 1–4 pg WHO TEQ/kg bw per day

- **SCF (2000):** t-TWI 7 pg WHO TEQ/kg bw per week
- **SCF (2001):** TWI 14 pg WHO TEQ/kg bw per week
- **JECFA (2001):** PTMI 70 pg WHO TEQ/kg bw per month

4.1 CONTAMINATION OF FOOD.

As mentioned above, more than 90% of daily dioxins and PCBs intake comes from food consumption. Between different kinds of foodstuffs the most contaminated are fishes, meats and animal origin products. Contamination of food is primarily caused by deposition of emissions of various sources (like waste incineration, production of chemicals etc.) and subsequent accumulation in the food chain.

Table 5 below shows concentrations of PCDDs, PCDFs, and PCBs in foods in various European countries. Data for most of European conuntries are available in European Commission Report from 7th June 2000 “Assessment of dietary intake of dioxins and related PCBs by the population of EU Member States. However official EU Commission data are not available for countries: Austria, Bulgaria, Cyprus, Czech Republic, Estonia, Greece, Hungary, Latvia, Lithuania, Luxembourg, Malta, Poland, Portugal, Romania, Slovakia, Slovenia, Spain.

Food category	Country	1990-1994	1995-1999	1990-1994	1995-1999	Food category	Country	1990-1994	1995-1999	1990-1994	1995-1999
Eggs	DE	1.08	1.16			Beef	IT		0.7		
	FI	1.55					NL	1.77	0.72		
	FR		0.46				SV		0.98		1.08
	IT		2.67			Liver caw					
	NL	2	1.08				DE		2.6		
	NO	1.97		8.91			FR		3.29		
	SV		1.03		1.45		IT		2.3		
	UK	8.27	1.77	2.36	0.97		NL	5.7			
Fat and Oil							SV		0.95		1.65
	NL	1.51				Poultry					
	SV		0.77		0.42		DE	0.62	0.58		
	UK	1.26	0.26	1.29	0.39		IT		0.92		
Milk and milk products	BE	2.6	2.06				NL	1.63	0.89		
	DE	0.86	0.57				SV		0.77		0.7
	DK		0.49			Herring					
	FI	0.99	0.34		0.23		DE		0.79		
	FR		0.67				NO	0.91		2.64	
	IT		0.57				SV		0.73		1.11
	NL	1.56	0.94				UK		2.1		6.24
	NO	0.32		1.28		Mackerel					
	SV	0.92	0.75		0.43		DE		0.29		
	UK	1.41	1.01	0.91	1.8		IT		0.86		
Cereals and it products							NO	0.52		1.4	
	FI		0.02				UK		0.61		2.5
	FR		0.02				DE		0.05		
	NL	0.01				Cod					
	SV		0.17		0.11		NO	0.2			
	UK	0.11		0.02			SV		0.13		0.23
Fruits and vegetables							UK		0.03		0.07
	DE	0.01	0.01								
	FI		0.02		0.01						
	FR		0.06								
	DE	0.71	0.66								
	FI	0.02									

Table 5. National average concentration of dioxins and PCBs in foods. (SCOOP 2000)

Data confirm that fish, egg, and milk are the main (Domingo et al 1999, SCOOP 2000). source of dioxins and PCBs Concentration of dioxins in eggs are in the range 0.5-2.7 pg I-TEQ/ g fat. This level depends on type of breeding. In oils and fats dioxins and PCBs were ca 1 pg I-TEQ/ g fat. Concentration was relatively higher in oils of animal origin, professionally at fish oil. In fact high-trophic-level marine species are known to contain high concentration of POPs (e.g PCBs, dioxins, PBDEs) (Fangstrom et al 2005,

Dam et al 2000, Lindstrom et al 1999). Average concentration in milk and milk products were in the range 0.3-2.1 pg I-TEQ/ g fat for dioxins and 0.2-1.8 pg I-TEQ/ g fat for PCBs. Cereals, fruit and vegetables had lower concentration near to the limit of determination (0.05-0.1 pg I-TEQ/ g of product). Concentration in meet and meets products were in range 0.6-1.0 pg I-TEQ/ g fat. Salmon, eel, herring were the fish with the highest level of contamination. Comparing data between farmed fishes, and wild fishes, the first one showed lower contamination. Fishes from Baltic Sea presented relatively higher level of dioxins and PCBs in comparison with the same species from other regions.

More recent data (2003-2004) are available for PCBs in salmon, butter and cabbage samples from Italy, Belgium, Portugal and Spain. Data with average TEQ pg/g fat are summarized in Table below. Data are not available for dioxins. The comparison with concentrations reported by European Commission 2000 showed that levels are slightly lower.

	Italy	**Belgium**	**Portugal**	**Spain**
Salmon [TEQ pg/g fat]	3.2	0.9	1.2	2.7
Butter [TEQ pg/g fat]	0.16	0.13	0.04	0.12
Cabbage [TEQ pg/g ww]	0.0006	0.00004	0.00006	0.0009

Table 6. WHO-TEQs equivalents for dioxin like PCBs in salmon, butter and cabbage (Zuccato et al 2008)

There is no comprehensive report at the international level about dioxins and PCBs levels between new members of European Union neither complex report at European level from 2004-2008. There are individual information’s about level of those contaminants in foodstuff coming from single country.

Research from between 1996-2008 determined the concentrations of PCDD/PCDFs in a foodstuffs samples consumed in northern regions of Spain (Llobet et al 2003, Bocio et al 2005, Domingo et al 1999, Martin-Cid et al 2008). With respect to the PCDD/Fs WHO-TEQ values, practically all food groups showed very notable reductions in comparison with the WHO-TEQ values from 1996. Data for different food stuffs are presented in Table 7 are presented below.

pg WHO-TEQ PCDD/Fs	Vegetables	Pulses cereals	Fruits & Vegetables	Fish and shelfish	Meat	Eggs	Milk and products	Fats & oils
1996	1.61	0.75	0.49	6.23	1.27	1.25	1.35	0.79
2000	0.11	0.07	0.06	5.57	0.47	0.58	0.8	0.18
2002	0.006	0.011	0.008	0.27	0.03	0.037	0.019	0.238
2006	0.004	0.015	0.003	0.086	0.012	0.134	0.003	0.147

Table 7. PCDD/PCDF concentrations in food samples collected in different locations from Tarragona Region (Catalonia, Spain*)* Llobet et al 2003, Bocio et al 2005, Domingo et al 1999, Martin-Cid et al 2008

Monitoring of PCDD/Fs and DL-PCBs (PCB-77, PCB-88, PCB-126, PCB-169) in Greece showed similar effects (Papadopoulos et al 2004), samples from 2002 had dioxin content far below the EC Regulation (2375/2001/EC) limits. It can be notice also that food of animal origin contain higher levels of contaminants than food of plan origin. (See Table 8 below)

Mean (range)	**Butter (*n* = 5)**	**Yoghurt (*n* = 2)**	**Cow milk (*n* = 3)**	**Beef (*n* = 3)**	**Pork (*n* = 4)**	**Poultry (*n* = 3)**	**Fish—aqua culture (*n* = 7)**	**Eggs (*n* = 6)**	**Olive oil (*n* = 5)**	**Fruit ** (n=5)**	**Vegetable** (n=4)**
ΣPCDD/F [pg/g fat]**	9.93 (7.10–14.64)	5.56 (3.90–7.23)	3.43 (3.08–3.72)	10.94 (7.64–14.34)	23.29 (4.37-37.08)	5.55 (2.91–9.38)	13.19 (8.76-17.9)	4.06 (3.65–4.82)	10.27 (1.90-38.49)	3.3 (2.64-4.43)	3.55 (2.95-4.33)
ΣTEQ PCDD/F	0.79 (0.29–1.33)	0.44 (0.38–0.51)	0.39 (0.34–0.47)	0.55 (0.52–0.59)	0.39 (0.32–0.42)	0.30 (0.18–0.40)	0.47 (0.22–1.12)	0.37 (0.26–0.45)	0.30 (0.23–0.36)	0.47 (0.37-0.54)	0.43 (0.37-0.48)
Σ PCB * [pg/g fat]**	5.16 (1.23–9.30)	6.07 (3.25–8.90)	3.04 (1.64–5.30)	16.37 (13.06-20.46)	169.4 (67.35-347.7)	8.22 (5.78–13.1)	394.03 (222–749)	12.24 (7.10–24.32)	2.40 (2.02–4.83)	0.36 (0.27-0.40)	0.36 (0.36-0.37)
ΣTEQ-PCB*	0.32 (0.01–0.66)	0.41 (0.14–0.67)	0.18 (0.07–0.34)	0.41 (0.01–0.69)	0.69 (0.27–1.22)	0.06 (0.04–0.11)	1.19 (0.56–2.90)	0.13 (0.06–0.33)	0.04 (0.01–0.09)	0.01 (0.01-0.01)	0.01 (0.01-0.01)

Table 8. Average results of PCDD/Fs and PCBs (Papadopoulos et al 2004) *ΣPCB=PCB-77+PCB-88+PCB-126+PCB-169,
**For Fruits and Vegetables ΣPCDD/F and Σ PCB expressed in pg/g wet weight

Cited data demonstrated significant time reduction of dioxins and PCBs contamination in foodstuff. Less contaminated food has an influence on human exposure to dioxins and PCBs. In Netherlands a decrease of 50 percent of the intake of I-TEQ/kg bw/day was observed, in United Kingdom research data showed the exposure has also fallen around 45%. (SCOOP 2000). Investigations of German food have shown exposure approximately 50% lower than 10 years ago (SCOOP 2000). In general every year the average dietary exposure to dioxins within EU Members is decreasing around 9%-12%.

(SCOOP 2000). It is a result of changing model of food consumption and decreasing concentration of dioxins in food and feed.

4.2 Reconstruction of historical food contamination and historical dietary intake of PCB-153.

Consumption of food is considered as the major source of non-occupational human exposure to PCBs. More than 90% of total daily intake of these contaminants derives from the food. Here is presented an assessment of dietary exposure to PCB-153, one of the PCB indicator which occur at higher concentrations respect to other contaminant of interest. Estimation is obtained by combining data from National Italian Food Consumption Survey (INRAN-SCAI) (Leclerq et al 2008) with literature data about concentrations and profiles of PCB in Italian foodstuffs. (Fattore et al 2008)

Details about foods consumption were obtained from cross sectional study of Leclerq et al 2008 conducted in Italian territory between 2005-2006 which is the key reference for Italian food consumption and is utilized for risk analysis or assessment of nutrients intakes. In this study subjects were divided into several groups: infants, children, teenagers, adults and elderly. Last three groups were divided into male and female subgroups. Classification of food was based on classification developed by European Food Safety Authority (EFSA). Fat content of each food item was consulted with Italian Tables of Food Composition developed by INRAN and available on line: *http://www.inran.it/646/tabelle_di_composizione_degli_alimenti.html.* All data are presented in Table 9 below:

Consumption g/day	Fat content (%)	Infant (0-3)	child (3-10)	teenage (10-18)	adult (18-65)	Elderly (65-86)
Cereals and its products	-	125	238	265.3	232.3	228.3
fruit and vegetables	-	242.4	350	447.6	511.1	560.2
eggs	8.7	13.4	25.7	26.1	25.7	26.5
fatt and oils	91.825	15.5	33.2	39.1	38.8	34.4
fish and fishery products	10.51	47.9	58.9	68	65.4	56.2
meat and meat products	9.24	60	100	107.4	98.4	90.3
milk and milk products	17.15	397	259	218	194.5	201.5

Table 9 Food consumption in Italy and fat content (Leclerq et al 2008, INRAN on-line Data base)

Concentrations of PCB-153 in foods were obtained from Italian study of Fattore et al 2008, where values were expressed in ng/g of fat or ng/g of fresh weight for following food groups: cereals and its products, fruit and vegetables, fats and oils, fish and fishery products, meat and meat products, milk and dairy products.

Daily intake of PCB-153 was calculated as multiplication of food consumption rate, fat concentration in each food item and contamination with PCB-153 (see equation below).

$$Intake = I_{meat} + I_{fish} + I_{cereal} + I_{vegetables} + I_{milk} + I_{oil} + I_{eggs}$$

$$I_{meat} = M_{meat}\ [g/day] * FC\ [\%] * C_{PCB\text{-}153}\ [ng/f\ of\ fat]$$

Where:

I – Intake

M – Consumption of meat and meat products in [g/day]

FC – fraction of fat in meat (%)

$C_{PCB\text{-}153}$ – concentration of PCB-153 in meat and meat products [ng/g of fat]

The aim of this exposure assessment was to reconstruct the most realistic history of PCB intake for woman born in around 1970-1980, putting special attention on changing level of contaminants since the mid seventies up to 2009/2010.

To have clear idea on distribution of PCBs in environment in the past several studies and documents were used: “Italian emission Inventory 1990-2007” (Ispra 2009), “Towards a global historical emission inventory for selected PCB congeners – a mass balance approach. Parts: I, II, III” (Breivik et al 2002a, 2002b, 2007), and other single studies: Breivik et al 2004, 2006, 2010, Van Der Gon et al 2007, Wania et al 1999, Norstrom et al 2009, Pacyna et al 2003.

Data on PCB-153 emission for Italy (1943-2000) were obtained from the study of Breivik et al 2002a, 2002b, 2007, and were downloaded from the website as the excel files *http://www.nilu.no/projects/globalpcb/globalpcb2.htm*. Data since 2000 to 2007 were implemented with “Italian emission Inventory 1990-2007” (Ispra 2009).

Results of Breivik’s study are presented as three type of emission scenarios High, Mid and Low. High and Low scenarios were created in order to adjust the default (mid) estimates. This adjustment avoids the over- or underestimation which may appear due to uncertainty which affects several model parameters and due to limited or lack of empirical evidence

(Breivik et al 2002a, b). According to Breivik's methodology, three scenarios of air contamination with PCB-153 were created, and than three scenarios of food contamination. Food contamination data were used to calculate daily intake for each year from 1940 to 2009. Information about PCB153 concentration in the air in Italy were obtained from analytical measurement in 2008 (data not published). Concentration in the food in the past was calculated as the simple ratio based on literature data of Fattore et al 2008 and Zuccato et al 1999. The results of estimation for food contamination and dietary intake are presents in Figures 5 A, B below. Figure 5B shows an example of historical daily intake for woman born in 1980. As presented, the difference between three intake scenarios is of 10^2. All three estimations keep decreasing tendency, arriving to plateau around the mid 90ties. This is a very general estimation, based on simple assumption that certain concentration of PCB-153 results in certain concentration of PCB in the food. Even if is simple, this estimation is sensible enough to reflect dynamic change in environmental pollution. The shape of intake curves show strong decreasing trend in early years of life, which is the result of drop in PCB emission. Observed plateau in adulthood is due to the constant dietary habits and lower food contamination. This estimation was introduced to the model in order to it compare with reverse dosimetry results.

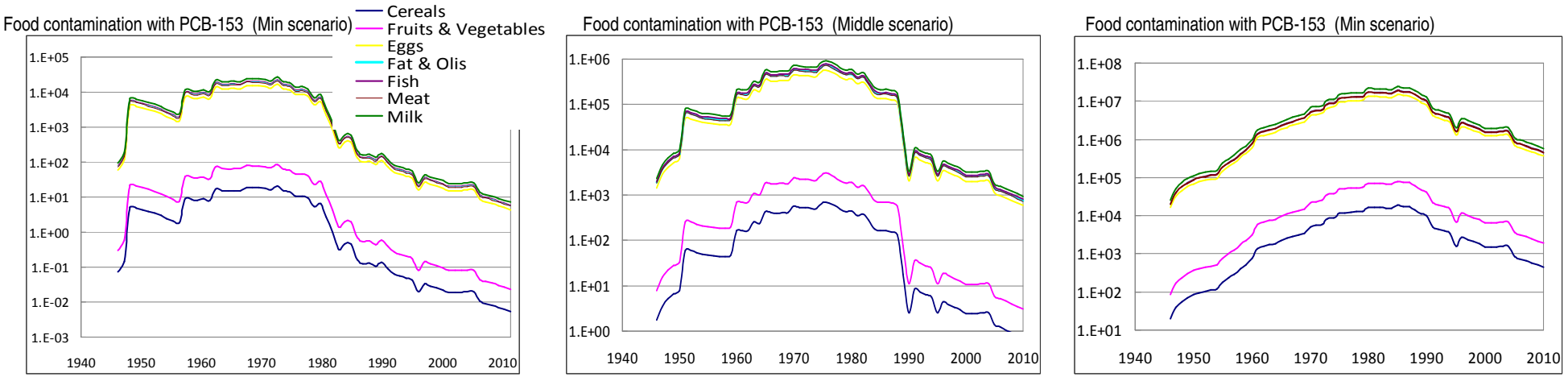


Figure 5A. Estimation of food contamination with PCB-153 in Italy since 1940-2010

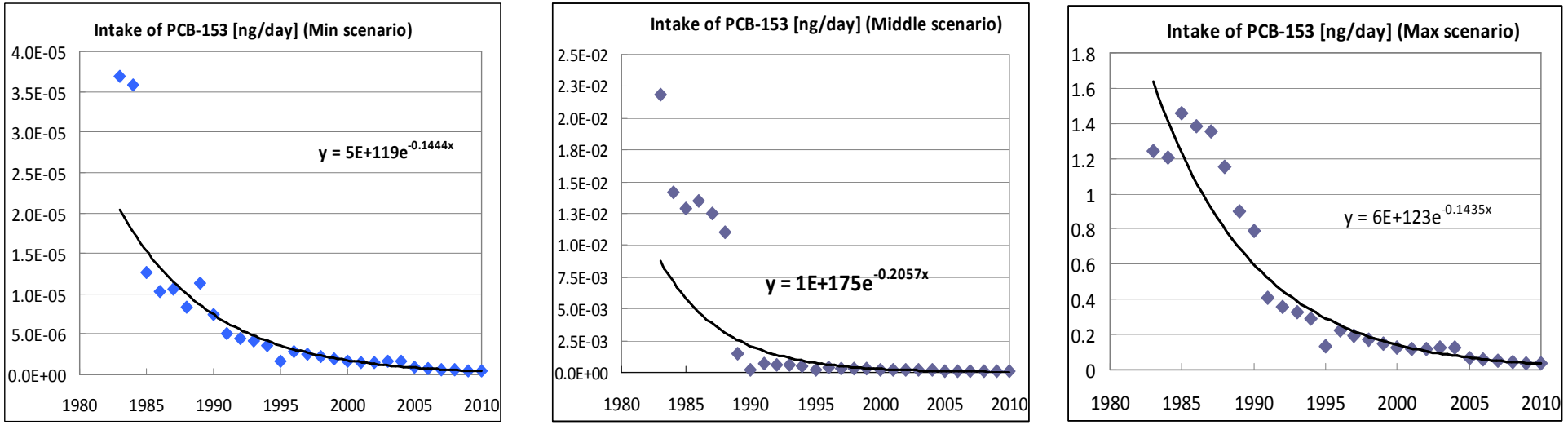


Figure 5B. Estimation of daily intake of PCB-153 in Italy since 1980-2010. An example for woman born in 1980.

5. CONTAMINATION OF HUMAN MILK. INFANT EXPOSURE

5.1 PCDD/F and DL-PCB LEVELS IN HUMAN MILK: HISTORICAL STUDIES

Several surveys on dioxins and PCBs in human milk have been made in the past. For instance the report prepared for European Commission DG Environment and the UK Department of the Environmental Transport and the Regions in 1999 (Buckley-Golder, 1999) reviews results from samplings carried out in 1988-1993. Several EU member states took part in this study, including Austria, Belgium, Denmark, Finland, France, Germany, Italy, Netherlands, Spain, Sweden and UK. During the eight year period, milk samples from mothers living in industrial, urban and rural zones of these countries were collected and analyzed for their dioxin content. Table 9 shows the average concentrations of PCDDs and PCDFs in human milk measured in this survey. Between 1988 and 1993, concentrations decreased by about 35% (8.3% per year) with lower mean concentrations and a slightly smaller decrease in rural than in industrial areas.

	Average concentration pg I-TEQ/g fat 1988	**Average concentration pg I-TEQ/g fat 1993**
Rural area	28.2	17.7
Urban area	29.5	19.2
Industrial area	35.9	24

Table 9. Mean PCCD and PCDF concentrations in human breast milk. (pg I-TEQ/g fat) (Buckley-Golder, 1999).

A similar study was done by the World Health Organization (WHO). PCDDs, PCDFs and PCBs in human milk were measured in 1987-1988, 1992-1993, and 2001-2003 in Australia, Belgium, Brazil, Bulgaria, Croatia, Czech Republic, Egypt, Fiji, Finland, Germany, Hong Kong SAR, Hungary, Ireland, Italy, Luxembourg, New Zealand, The Netherlands, Norway, Philippines, Romania, Russia, Slovak Republic, Spain, Sweden, Ukraine, and United States (WHO 1989 and 1996; Van Leeuwen et al., 2002).

In the third study period (2001-2003) levels of contaminants in human milk were lower in the Southern hemisphere (Fiji, Brazil, Philippines, Australia, New Zealand), eastern Europe (Bulgaria, Croatia, Hungary), Ireland and USA, and higher in Western Europe (Italy, Spain, Germany, Luxembourg, Belgium, Netherlands) and in Ukraine, where dioxin-like PCBs were particularly high. In breast milk samples from Egypt PCDD/Fs (median

22.3 pg WHO-PCDD/F-TEQ/g fat) contributed a remarkably high proportion to the total toxic equivalent (TEQs). The survey identified a decrease in WHO-TEQ PCDD/Fs and PCBs in human milk from 1992-1993 to 2000-2003 (Figure 6). Results from this studies are summarized in Table 10

Country	PCDDs/PCDFs WHO-TEQ pg/g fat		PCBs WHO-TEQ pg/g fat		Sum indicator PCBs ng/g fat		Country	PCDDs/PCDFs WHO-TEQ pg/g fat		PCBs WHO-TEQ pg/g fat		Sum indicator PCBs ng/g fat	
	Median	Range	Median	Range	Median	Range		Median	Range	Median	Range	Median	Range
Italy	12.66	9.40 – 14.83	16.29	11.02 – 19.33	253	195 – 323	**Australia**	5.57	5.39 – 5.75	2.89	2.52 – 3.26	30	25 – 36
Luxembourg	14.97	13.68 – 16.25	13.67	12.98 – 14.36	217	196 – 237	**Belgium**	16.92	14.78 – 19.07	12.6	11.22 – 14	191	169 – 213
New Zealand	6.86	6.08 – 7.00	3.92	3.50 – 4.71	37	30 – 41	**Brazil**	3.92	2.73 – 5.34	1.77	1.30 – 12.28	16	10 – 97
Norway	7.3	7.16 – 7.43	8.08	6.56 – 9.61	119	106 – 132	**Bulgaria**	6.14	5.08 – 7.11	4.21	3.74 – 4.70	42	32 – 52
Philippines	3.94	3.64 – 4.24	2.38	2.22 – 2.54	26	26 – 26	**Croatia**	6.4	5.99 –6.80	7.17	6.82 – 7.52	135	121 – 150
Romania	8.86	8.37 – 12.00	8.06	8.05 – 8.11	173	165 – 198	**Czech Republic**	7.78	7.44 – 10.73	15.24	14.32 – 28.5	502	496 – 1009
Russia	9.36	7.16 – 12.93	13.45	12.92 – 22.95	126	84 – 311	**Egypt**	22.33	14.90 – 51.50	5.48	4.41 – 8.26	106	12 – 140
Slovak Republic	9.07	7.84 – 9.87	12.6	10.72 – 19.49	443	331 – 621	**Fiji**	3.34	3.17 – 3.51	1.75	1.70 – 1.80	17	16 – 19
Spain	11.56	10.24 – 18.68	9.42	6.93 – 17.94	241	162 – 467	**Finland**	9.44	9.35 – 9.52	5.85	5.66 – 6.03	91	84 – 98
Sweden	9.58	–	9.71	–	146	–	**Germany**	12.53	11.14 – 12.72	13.67	12.80 – 14.3	220	188 – 238
The Netherlands	18.27	17.09 – 21.29	11.57	10.90 – 13.08	192	178 – 210	**Hong Kong SAR**	8.69	5.80 – 10.09	4.73	2.80 – 6.58	45	16 - 80
Ukraine	10.04	8.38 – 10.16	19.95	14.10 – 22.00	136	103 – 148	**Hungary**	6.79	5.26 – 7.46	2.87	2.38 – 4.24	34	29 – 59
USA	7.18	6.22 – 8.14	4.61	3.69 – 5.52	54	43 – 64	**Ireland**	7.72	6.19 – 8.82	4.57	2.72 – 5.19	60	41 – 65

Table 10. PCDD/Fs, DL-PCBs and NDL-PCBs (the so called six indicator PCBs) in human milk (Van Leeuwen et al., 2002).

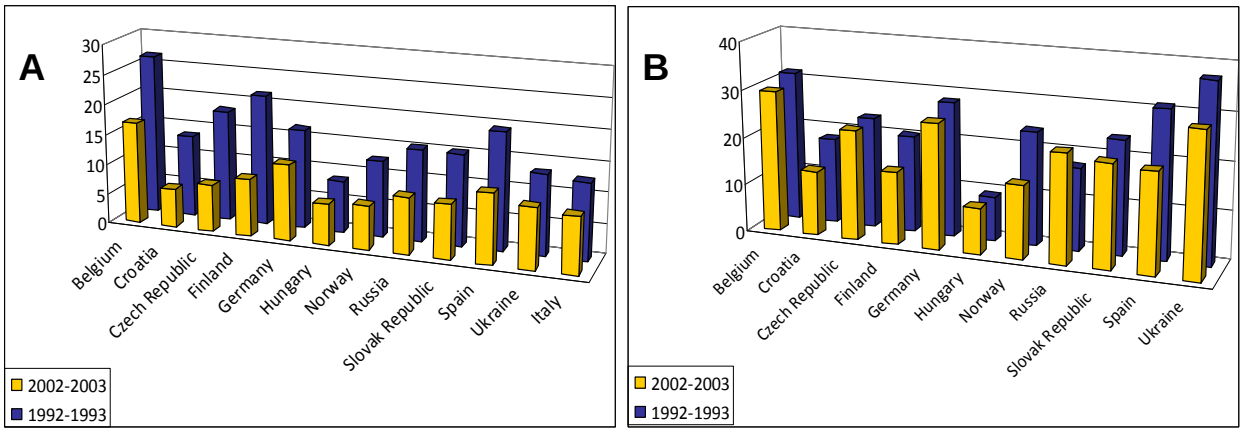


Figure 6 A-Levels of WHO-TEQ PCDD/Fs in breast milk, B-Levels of WHO-TEQ DL-PCBs in breast milk (Van Leeuwen and Malish 2004, WHO second round 1998)

These results will be used in this review as an “historical” reference for the time- and geography-related changes in these contaminants in human milk in more recent years. However the representativeness of the “national reference levels” reported in these surveys is sometimes questionable, particularly for Russia and Ukraine whose reference values were calculated from only two and five samples, respectively.

5.2 LEVELS IN ASIA

Thirty-three articles published between 2000 and 2008, reporting PCDD, PCDF and PCB concentrations in human milk samples collected in Asian countries, were identified and included in this review.

In China, most of the studies were conducted in the coastal provinces from northern Liaoning to southern Guangdong (Kunisue et al., 2004a; Sun et al., 2005 and 2006; Hedley et al., 2006 ; Hui et al., 2007 ; Zhao et al., 2007; Li et al., 2009). Concentrations of the contaminants generally varied widely. For instance Hedley et al. (2006) and Hui et al. (2007) independently recruited two comparable groups of primiparous mothers in Hong-Kong in 2001, but found substantially different PCDD/F and dioxin-like (DL) PCB levels in their milk (mean WHO-TEQ were respectively 8.25 and 4.67 pg/g fat). Moreover, these studies reported substantially lower TEQ PCDD/F levels than those measured in 2000-2003 in samples from the cities of Shijiazhuang and Tangshan (Hebei Province, northern China) (Sun et al., 2005 and 2006) where TEQ for PCDD/Fs were respectively 19 and 13 pg/g fat. In Korea, Yang et al. 2002 measured PCDD/Fs and DL-PCBs in human milk samples from primiparous to identify differences between an industrial (Inchon) and an urban (Seoul) area. PCDD/Fs in milk were 1197 pg/g fat in Seoul and 467 pg/g fat in Inchon. Values in this study were higher than those reported in northern China (Sun et al., 2005 and 2006; Li et al., 2009), and substantially higher than in Japan.

For Japan there were several papers dealing with PCDD/Fs and PCBs in human milk published after 2000 (Yoshida et al., 2000; Sampei, et al., 2002; Takekuma et al., 2004; Tajimi et al., 2005; Saito et al., 2005; Sun et al., 2006; Sasamoto et al., 2006a and 2006b; Kunise et al., 2006; Guan et al., 2006; Uehara et al., 2006; Tanabe et al., 2007; Uehara et al., 2007; Mato et al., 2007; Nakamura et al., 2008; Todaka et al., 2008). Overall, more than 1000 primiparous and multiparous mothers were recruited in Japan from 1997 to 2004. Detailed results are reported in Table 11A and 11B in the end of this chapter. In 1997-2002 Uehara et al. (2006) collected 839 milk samples from primiparous and multiparous mothers living in various Prefectures in Japan and investigated the relationships between mothers' ages and number of deliveries and PCDD/F concentrations in their milk. Mean levels of PCDD/Fs and DL-PCBs in the general population were respectively 14.2 and 8.8 pg TEQ/g fat and WHO-TEQ for PCDD/F were respectively 8.8 and 14.4 pg/g fat for multiparous and primiparous mothers. Findings were

similar for DL-PCBs, with WHO-TEQ of 8.6 and 5.7 pg/g fat for multiparous and primiparous mothers. Several studies were done in Taiwan (Hsu et al., 2007; Chao et al., 2004; Wang et al., 2004). In 2000-2001 Hsu et al. (2007) recruited young mothers with a mean age of 28 years, representative of the general population of southern Taiwan. Levels of PCDDs and PCDFs in their milk were respectively 8.37 and 6.33 pg WHO-TEQ/g fat.

In the Philippines, Vietnam, Cambodia, India, Malaysia and Indonesia studies were carried out by Tanabe et al. (2007), Sudaryanto et al. (2005 and 2006), Devanathan, et al. (2009), Subramanian et al. (2007), Kunisue et al. (2004a and 2004b), Minh et al. (2004). Only scant information is available on human exposure to POPs in these countries but the population is thought to be exposed to relatively high levels of contaminants, because of the fast industrial development. Sampling in these countries were therefore conducted mainly in the proximity of open dumping sites and concentrations of PCDD/Fs and DL-PCBs (pg TEQ/g fat) were 21 and 16.3 in India, 5.6 and 3.6 in Cambodia, 6.0 and 7.5 in Vietnam, 7.5 and 4.4 in Philippines (Kunisue et al. 2004b). Levels were thus quite high in India but not in the other countries investigated. Dwernychuk et al. (2002) reported higher levels of PCDDs by in the Aluoi Valley in Vietnam, where the US Air Force systematically spread Agent Orange in 1965-1966. Concentrations of TCDD in human milk were in the range 2.97-14.62 pg I-TEQ /g fat, contributing 51.7%-96.3% to total I-TEQs for dioxin and DL-compounds.

Extensive investigations were also conducted in Northern Russia, particularly in the Kola Peninsula and around the White Sea (Polder et al.1998; 2003; 2008), in Siberia (Schecter et al., 2002), in the Samara Region (Revich et al., 2001), in the Republic of Buryatia (Tsydenova et al., 2007) and Uzbekistan (Ataniyazova et al., 2001). Results are shown in Table 12 below.

	Murmansk		Arkhangelsk		Kargopol		Severodvinsk	Naryan-Mar	Angarsk	Usolye Sibirskoye	Chapaevsk
	1993	2000	1996	2002	1996	2002	1996-1997	1996-1997	1998	1998	1997-1998
ΣTEQs of PCDDs/Fs	18	10	15	5	11	12	N.A	N.A	(10.1-33.2)	(16.31-38.43)	42.26
ΣTEQs of non-ortho PCBs	12	6	10	3	7	7	N.A	N.A	(5.3- 23)	N.A	N.A
ΣTEQs of mono-ortho PCBs	18	13	15	7	11	15	16.59[a]	17.66[a]	N.A	N.A	N.A

Table 12 Mean TEQ for PCDDs/Fs, non-ortho and mono-ortho PCBs in breast milk samples from Northern Russia (pg/g fat) (Polder et al., 2008; Polder et al., 2003; Polder et al., 1998; Schecter et al., 2002; Revich et al., 2001; Tsydenova et al., 2007) [a] Sum of PCBs congeners 105, 114, 118, 156, 157, and 189. N.A = not analysed

Levels of PCDD/Fs were particularly high (42.26 pg WHO-TEQ/g fat) in milk of mothers living around the contaminated area of Chapaevsk, near a chemical plant producing crop protection chemicals, but the concentrations of PCBs were the highest in samples from the areas of Severodvinsk and Naryan-Mar, with maximum values of 16.59 and 17.66 pg TEQ/g fat, respectively. Such high level were probably related to the heavy industrialization of the White Sea area, resulting in considerable pollution of the environment. Particularly high levels of 2,3,7,8-TCDD (16.10 pg WHO-TEQ/g fat) were detected in milk samples from Uzbekistan, near the Aral Sea, in concomitance with several factors, including heavy use of DDT in that area (Ataniyazova et al., 2001). The results of all these studies are summarized in Table 11A and 11B in the end of this chapter.

5.3 LEVELS IN EUROPE

Among European countries relatively high levels of PCDDs, PCDFs and PCBs in human milk were reported in Sweden. A study published in 2000 (Noren et al 2000) summarized 30 years of investigations in Sweden on PCDDs, PCDFs, PCBs and other persistent organic pollutants in human milk. Mean concentrations in samples collected in 1996-1997 were 14.66 pg WHO-TEQ/g fat for PCDD/Fs and 14.76 pg TEQ/g fat for PCBs. These high values were believed to be caused by a diet rich in fish. Similar results were found by Glynn et al. (2001), who reported 18.9 pg WHO-TEQ/g fat for PCDD/Fs, and 8.95 pg TEQ/g fat for DL-PCBs. Data from Norway (Polder et al., 2008a) indicated quite low levels of mono ortho PCBs in Tromso and Oslo, respectively 0.7 and 0.9 pg WHO TEQ /g fat.

In Spain and Portugal some monitoring studies of PCDD/Fs and DL-PCBs in human milk were conducted in groups of mothers living in the proximity of hazardous wastes incinerators (HWI) (Schuhmacher et al., 2002, 2004a, 2004b, 2007 and 2009; Reis et al., 2007, Marti-Cid et al., 2008). These studies indicated that the mothers were not over-exposed to PCDD/Fs and DL-PCBs. Mean PCDD/F levels in the milk of these mothers and control mothers in Portugal were in fact 9.1 and 9.5 pg WHO-TEQ/g fat respectively (Reis et al., 2007). A recent study (Schumacher et al., 2009) compared levels in three different periods (1998, 2002 and 2007) in Tarragona, Spain. Samples collected in 2007 had levels of PCDD/Fs and DL-PCBs of respectively 7.6 and 4.8 pg WHO-TEQ/g fat. Comparison with concentrations measured in 2002 and 1998 by the same group and in the second

round of the WHO coordinated exposure study in Spain confirmed a time-related decrease of these contaminants in human milk. In Portugal Reis et al. (2007) monitored a group of mothers in Lisbon and Madeira. Levels of PCDD/Fs in their milk were 10.6 pg WHO-TEQ/g fat in Lisbon and 6.5 pg WHO-TEQ/g fat in Madeira, very similar to those measured in Tarragona by Schumacher et al.(2009).

A study in Greece (Costopoulou et al., 2006) showed PCDD, PCDF, and DL-PCB levels in human milk of respectively 3.66, 3.61, and 6.56 pg TEQ/g fat, suggesting that exposure to these compounds in some Southern European countries might sometimes be lower than in northern Europe. However this does not seem a general rule. In fact studies in Italy, particularly in Milan, Seveso (Weiss et al., 2003), Rome and Venice (Ingelido et al., 2007, Abballe et al., 2008) reported concentrations of PCDD/Fs and DL-PCBs in human milk much higher, with levels of PCDD/Fs and DL-PCBs respectively (pg TEQ/g fat) 9.40 and 11.01 in Rome, 13.7 and 19.21 in Venice, 10.67 and 6.02 in Seveso, and 11.74 and 8.03 in Milan.

In Central Europe studies have been conducted in Germany (Wittsiepe et al., 2007; Wilhelm et al., 2007; Cao et al., 2008, Raab et al., 2007 and 2008), Belgium (Focant et al, 2002), Latvia (Bake et al., 2007), and the Czech Republic (Bencko et al., 2004). A large study was recently done in Duisburg and Munich (Germany). Mean levels in human milk collected in these two cities were 13.30 and 8.17 WHO-TEQ pg/g fat for PCDD/Fs and 12.60 and 6.31 WHO-TEQ pg/g fat for DL-PCBs. concentrations were higher in Liege (Belgium), with mean values of 29.4 pg TEQ/g fat for PCDD/Fs and 11.5 TEQ pg/g fat for DL-PCBs. In another monitoring study in seven cities in the Czech Republic, PCDD/F and DL-PCB concentrations in human milk were quite high, in the range of 11.52-23.90 and 17.6-70.3 pg WHO-TEQ/g fat. Levels of DL-PCBs were particularly high in samples from inhabitants of the polluted area of Uherske Hradiste. In Latvia too, milk samples from the highly polluted area of Olaine (Bake M. A, et al, 2007) had extremely high levels of DL-PCBs (152.25 pg TEQ/g fat), but strangely enough, still higher levels (191.38 pg WHO-TEQ/g fat) were also given for a control group of mothers living 10 km or more from the polluted area. Detailed results from all these studies are shown in Table 11A and 11B in the end of this chapter

5.4 LEVELS IN AMERICA AND AUSTRALIA

Harden et al. (2007) measured PCDD/Fs and DL-PCBs in human milk sampes collected in 2002-2003 in 12 regions of Australia. Mean levels, expressed as WHO-1998 TEQs were 5.7 pg/g fat for PCDD/Fs, and 3.1 pg/g fat for DL-PCBs, thus confirming that the Southern hemisphere is less contaminated than the Northern, as suggested by the third WHO exposure study. A study by Bates et al. (2002) in New Zealand by also confirmed these findings. Moreover these authors reported that PCDD/F levels in breast milk collected in 1998 were about 75% lower than in samples collected ten years before, in 1988.

Concerning South America, recent data on concentrations of PCDD/Fs and DL-PCBs in human milk were found only for Brazil (Paumgarten et al, 2000). The mean PCDD/F level in human milk was 9.7 pg TEQ/g fat.

It was difficult to find recent data for North America either. Among the latest available, we identified only a study published in 2001, concerning samples collected in 1990, with PCDD/Fs of 18.8 pg WHO-TEQ/g fat (LaKind et al., 2001), another study reported levels measured in samples collected in 2001 in the USA, where PCDD/F and DL-PCBs levels were 7.18 and 4.61 pg WHO-TEQ/g fat (Van Leeuwen et al., 2002).

5.5 FACTORS AFFECTING PCDD/F AND DL-PCB LEVELS IN BREAST MILK

Several factor can potentially affect a population's exposure to PCDD/Fs and DL-PCBs and therefore the excretion of these contaminants in breast milk. The area where the mother lives seems particularly important, as previously discussed. Other variables potentially related to PCDD/Fs and DL-PCBs in breast milk are discussed below and include the mother's diet, her age, parity, and smoking habits. The time of the sampling also seems important. A time-trend toward a significant decrease in the exposure of the population to these contaminants and therefore of their levels in breast milk was documented until 2003, and its continuation seems confirmed by the papers cited in this review.

Effect of the diet. Several studies have shown that dietary habits have a strong effect on PCDD/F and DL-PCB intakes, and diets rich in fish and seafood have been

frequently associated with higher levels in human breast milk (Li et al 2009; Chao et al., 2003; Polder et al., 2008; Glynn et al., 2001; Noren et al., 2000). However these conclusions are not univocal, since a study in Venice and Rome, in Italy, failed to confirm the association of PCDD/F and DL-PCB levels with fish intake (Ingelido et al., 2007; Aballe et al., 2008) and a further study in Japan did not find any significant correlations between levels of the contaminants and the intake of meat and eggs (Takekuma et al, 2004)

Effect of age and parity. A correlation has been observed between mother's age and PCDD/F and DL-PCB levels in breast milk has been observed. Younger mothers were reported to have significantly lower levels of the contaminants in their milk than older mothers, probably because of their shorter lifetime exposure (Chao et al., 2004; Sasamoto et al., 2006; Kunisue et al., 2006; Guan et al., 2006; Nakamura et al 2008; Nakatani et al 2005; Uehara et al 2006; Todaka et al 2008; Takekuma et al 2004; Wittsiepe et al 2007; Glynn et al 2001). Significant differences were also reported for primiparous and multiparous mothers, with lower levels of the contaminants in breast milk of multiparous (Figures 7AB). (Minh et al., 2004; Subramanian et al., 2007; Uehara et al., 2006; Yang et al., 2002; Todaka et al., 2008; Guan et al., 2006).

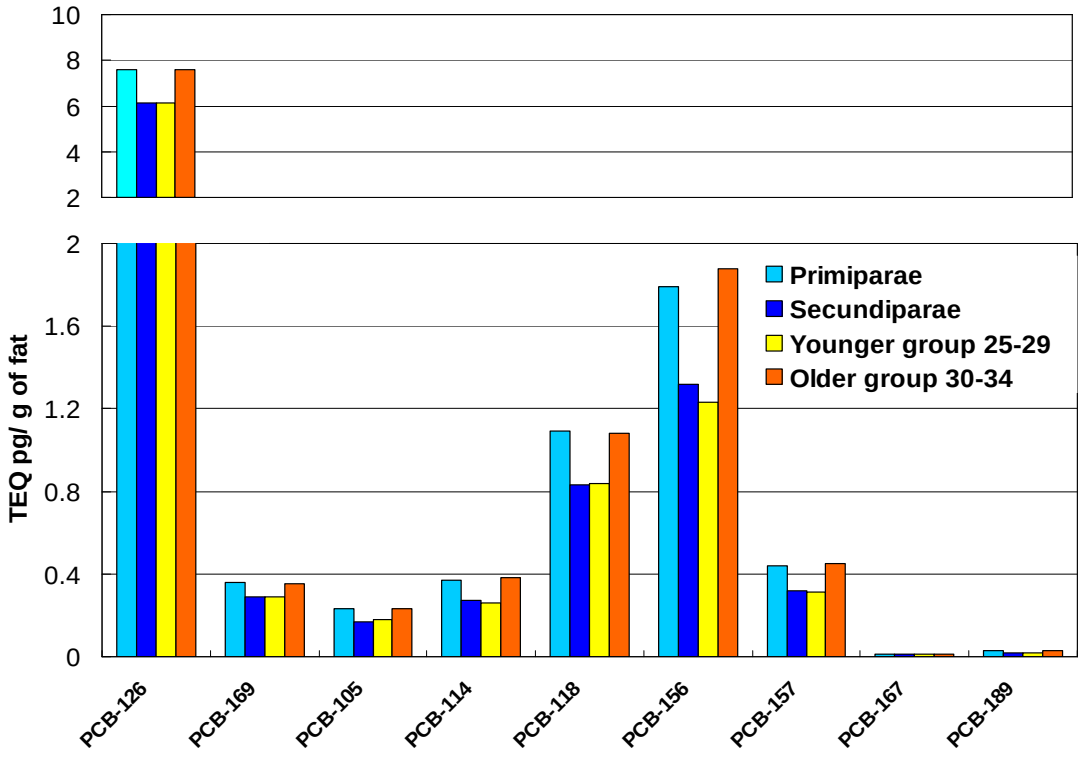


Figure 7A. Distribution of PCB congeners in breast milk samples. Comparison of levels and profile in primiparous and multiparous mothers and younger or older ones, in Tokyo (Guan et al., 2006)

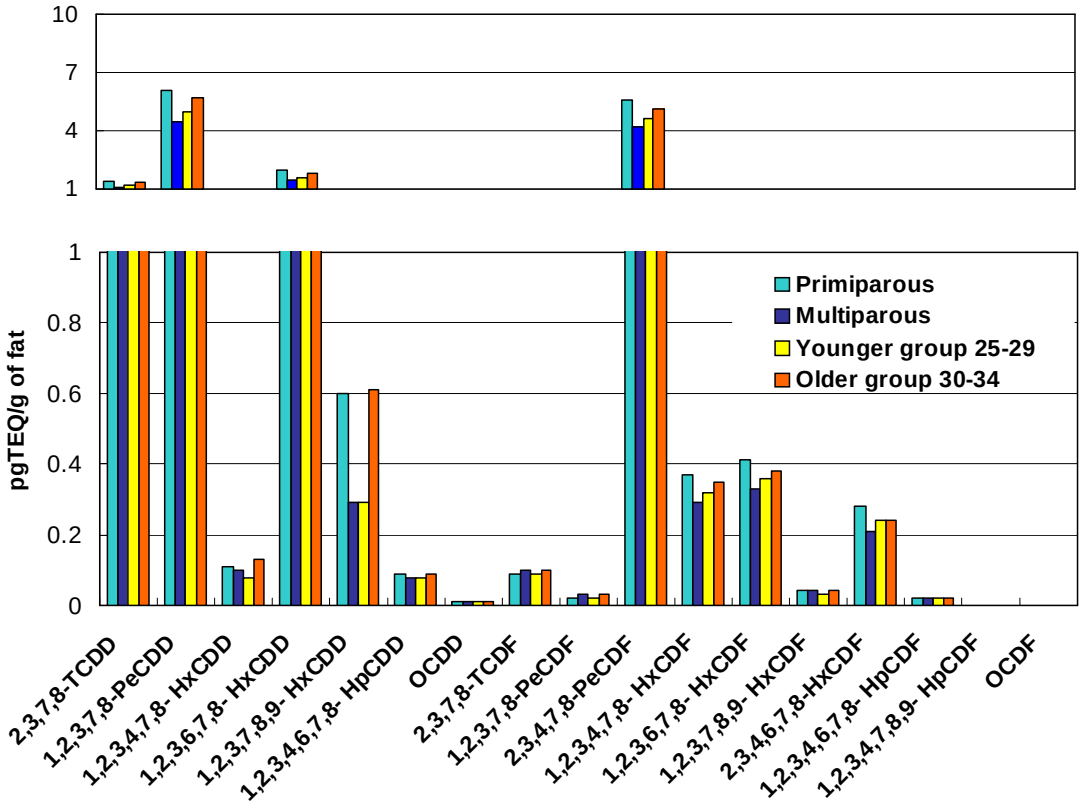


Figure 7B. PCDD/F in breast milk samples. Comparison of levels and distribution in primiparous and multiparous mothers and younger or older donors, in Tokyo (Guan et al., 2006)

Effect of nutrition of the mother during infancy. Nutrition during infancy also seemed to affect PCDD/F and DL-PCB levels in milk during lactation, and breast-fed mothers had higher levels of the contaminants in their milk than formula-fed mothers. In the study by Takekuma et al. (2004) PCDD/F mean levels in milk were respectively 16.31 and 14.69 pg TEQ/g fat in mothers who had been breast-fed or formula-fed during infancy. Similar results were observed for PCBs, with total TEQs for the sum of congeners 77, 126 and 169 of 6.78 and 5.96 pg/g fat in breast-fed and formula-fed mothers. Breast-fed mothers had significantly higher levels of total PCDD/F ($p=0.07$) and particularly higher levels of 2,3,4,7,8-PCDF in their milk ($p<0.02$) (Figure 8).

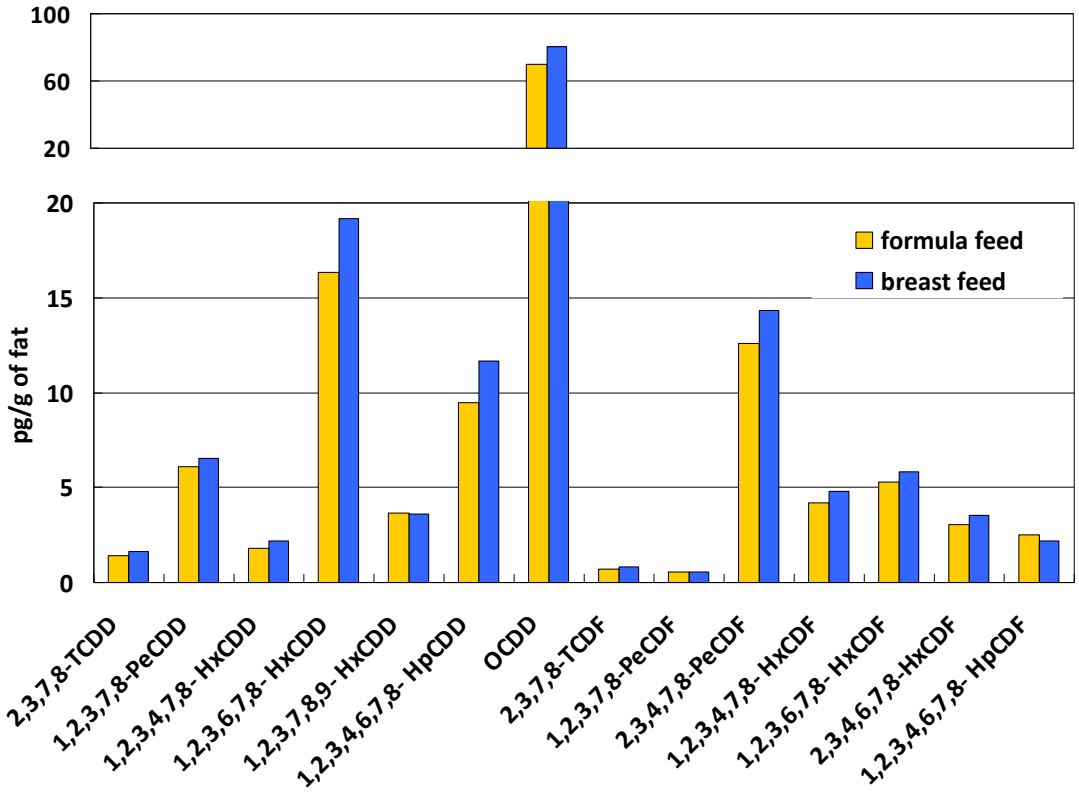


Figure 8. PCDD/F levels and distribution in milk samples from mothers who had been formula-fed or breast-fed during infancy (Takekuma et al., 2004).

Effect of smoking. The effect of smoking on PCDD/F and DL-PCB in breast milk is still debated (Uehara et al., 2007; Takekuma et al., 2004; Hedley et al., 2006). However, some studies reported significantly lower PCB levels in milk of active smokers than of never smokers, and results were similar for PCDD/Fs but with less significant differences (Uehara et al., 2007) (Figure 9). Takekuma et al. (2004) reported that total TEQ for the sum of PCDD/Fs and DL-PCBs in breast milk were 19.81 pg/g fat in active smokers and 23.07 pg/g fat in no- smokers ($p=0.001$), with particularly higher levels of the congeners 2,3,4,6,7,8-HxCDF and 1,2,3,4,6,7,8-HxCDD ($p<0.001$).

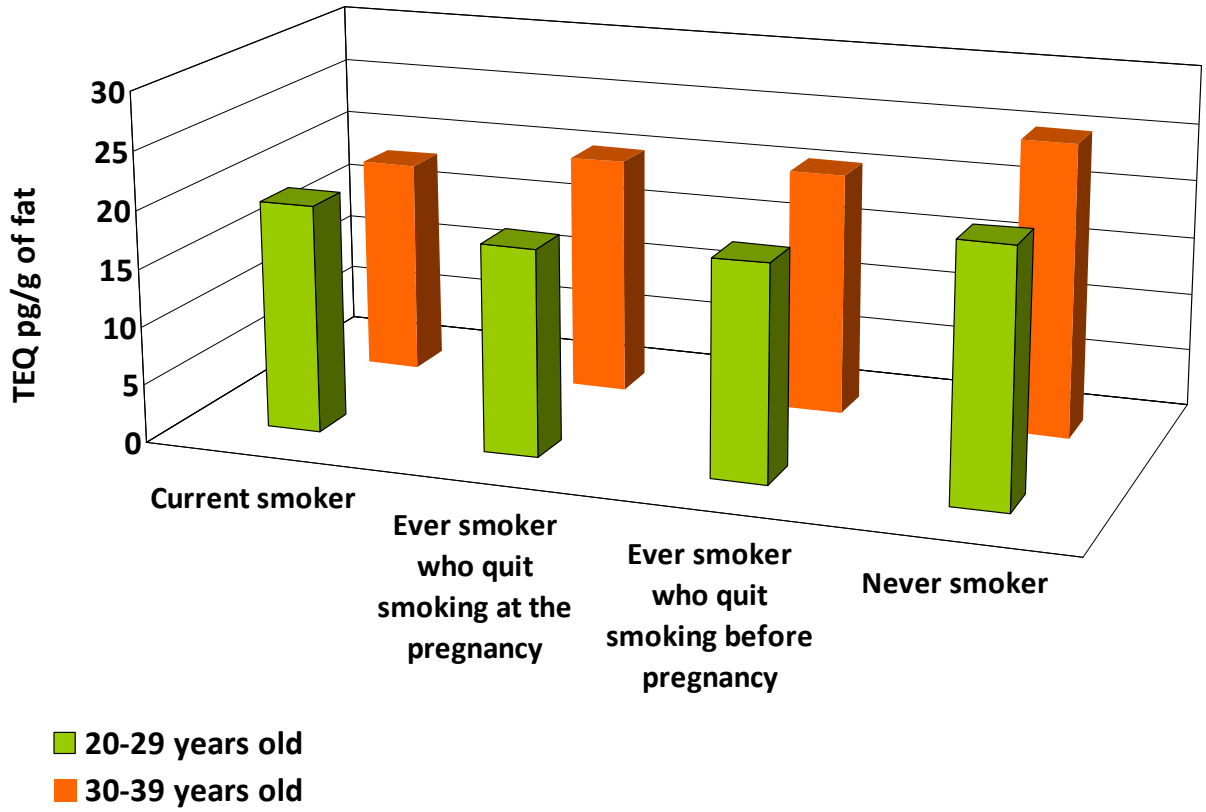


Figure 9. PCDDs/Fs in breast milk of mothers grouped according to age and smoking habits (Uehara et al., 2007)

Effect of living in urban or industrialized areas. According to Wittsiepe et al. (2007) mothers living in industrial areas had particularly high levels of PCDD/Fs and DL-PCBs in their milk. Li et al. (2009) also reported that inhabitants of urban areas had higher levels in their milk than people living in rural zones, with significant differences for PCDD/F ($p<0.05$ for WHO-TEQ). Another study in Spain (Schuhmacher et al., 2009), compared PCDD/F and DL-PCB levels in human milk of mothers living in an urban area with those living in an industrial. Samples from subjects in the urban area had a 38% excess of PCDD/F and 40% of DL-PCB over those from the industrial area. Yang et al. (2002) in Korea reported similar findings. However, still higher PCDD/Fs were found in milk samples collected in cities with heavy chemical industries, and manufacturing plants (Schecter et al., 2002), where urbanization and industrialization were therefore coexisting.

Effect of living near an incinerator or a dump. Several studies investigated the effect on PCDD/F and DL-PCB levels in breast milk of living close to municipal wastes and public waste incinerators (Nakatani et al., 2005; Sudaryanto et al., 2006; Tajimi et al.,

2005; Kunisue et al., 2004a, Subramanian et al., 2007; Tanabe et al., 2007; Zhao et al 2007; Wang et al., 2004; Reis et al., 2007; Schumacher et al., 2007 and 2009) (See Table 13). For instance, Tajimi et al. (2005) studied the correlation between the distance of the place of residence from waste incinerators and the concentrations of contaminants in milk. Samples from 240 volunteers from six Tokyo districts showed no correlations between TEQ for PCDDs, PCDFs and DL-PCBs in their milk, and the distance between their home and the nearest public or industrial waste incinerator. The only significant relationship was a weak correlation (r=0.25) between distances and concentration in milk of some PCDD congeners. PCDD/F congener profiles in these samples was similar to those reported in milk of mothers living in industrial areas, with a relative abundance of OCDD, 123678-HxCDD, 23478-PeCDF, and 1234678-HpCDD (Figure 10). In a group of mothers recruited in an area near a solid wastes incinerator in Taiwan, Wang et al. (2004) found levels of PCDD/Fs and DL-PCBs similar to those in the milk of mothers living in Athens (Costopoulou et al., 2006) or in Malaysia (Sudaryanto et al., 2005). The effect of living in the proximity of a hazardous wastes incinerator (HWI) was also investigated in Tarragona (Spain) by Schuhmacher et al. (2002; 2004a; 2004b; 2007; 2009). The most abundant PCDD/F congeners in milk samples collected in 2002 were OCDD (50.9 pg/g fat) followed by 1,2,3,6,7,8-HxCDD (27.7 pg/g fat) and OCDF (27.4 pg/g fat) (Figure 10). The total TEQ-WHO of 11.9 pg/g fat suggests that living near a hazardous wastes incinerator (HWI) does not imply any additional exposure to PCDD/PCDFs. However, comparing the levels in milk samples collected in 1998 and 2002 confirmed a strong decrease of PCDD/F and DL-PCB concentrations in this region (Schuhmacher et al., 2004a). Similar results were obtained by Reis et al. (2007) in a study carried out in the proximity of incinerators in Lisbon and Madeira (Portugal).

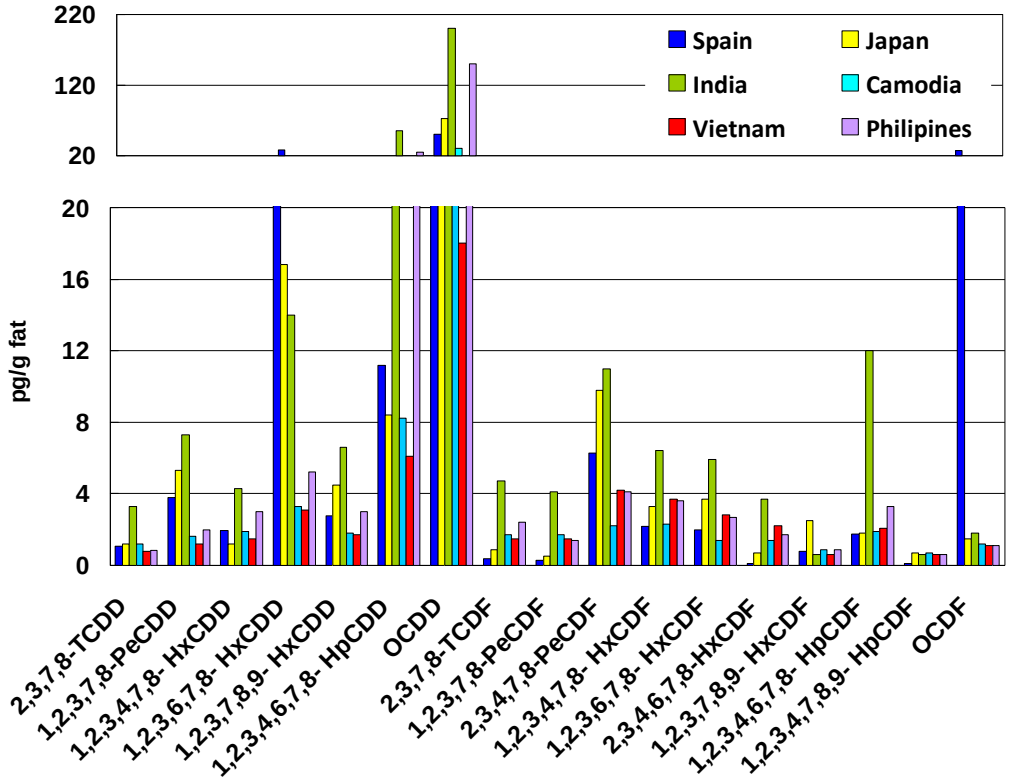


Figure 10 PCDD/F levels and distribution in breast milk samples from mothers living near a hazardous wastes incinerator in various countries

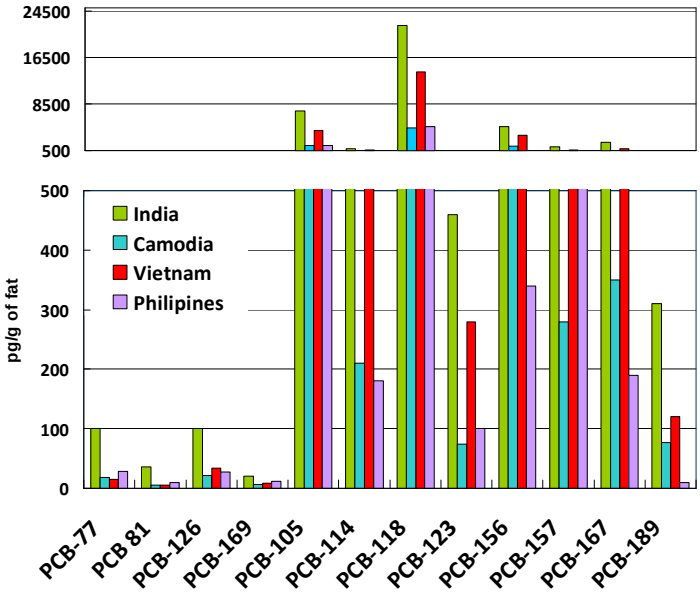


Figure 11 DL-PCBs levels and congener distribution in breast milk samples from mothers living near a hazardous wastes incinerator in various countries (Kunisue et al., 2004).

Reference/Location	PCDDs/Fs	DL- PCBs
Tajimi et al., 2005 /Tokyo	14.9	10.6
Wang et al., 2004 /Taiwan	7.62	5.19
Schuhmacher et al., 2002/Spain	11.9	-
Schuhmacher et al., 2009/Spain	7.6	4.8(for Co-PCBs)
Faitma Reis et al., 2007 / Portugal	9.2	-
Kunisue et al., 2004 / Cambodia	5.6	3.6
Kunisue et al., 2004 / Vietnam	6.0	7
Kunisue et al., 2004 / Philipines	7.5	4.5
Kunisue et al., 2004 / India	21	17

Table 13. PCDDs/Fs and DL-PCBs (pg TEQ/g fat) in breast milk samples of mothers living near an incinerator.

Studies conducted in some Asian developing countries (India, Vietnam, Philippines and Cambodia) in the proximity of large open dumping sites (Kunisue et al., 2004c), showed that PCDD/F and DL-PCB levels in breast milk collected in this area were not significantly higher than in samples collected in Europe, and comparable to those collected in countries like Sweden and Germany (Noren et al., 2000; Wittsiepe et al., 2007). In these samples, PCDD/F and DL-PCB profiles were similar to those from industrialised countries, with a relative abundance of OCDD, 1234678-HpCDD, 23478-PeCDF, and 123678-HxCDD (Figures 10 and Figure 11); among DL-PCBs the most abundant congeners were CB-118, 156, and 105. Milk samples from donors living near an open dumping site were also compared with samples from control sites far from the polluted area. In a study carried out in India, PCDD/F and DL-PCB levels were significantly different, with total WHO-TEQ levels of 38 pg/g fat at the dump site and 12 pg/g fat at the control site (Kunisue et al. 2004a; 2004c).

Effect of time (time-trend). A time-related decrease in the concentrations of contaminants in breast milk was reported in several of the studies reviewed (Raab et al., 2008; Noren et al., 2000; Schuhmacher et al 2009; Kunisue et al 2004; Nakamura et al 2008; Bates et al 2002; Polder et al 2008). For instance a marked decrease of PCDD/Fs and DL-PCBs between 1993 and 2002 was described in Murmansk and Arhangelsk, in Northern Russia, with 40% and 61% drops in of WHO TEQs for PCDD/Fs and DL-PCBs (Figures 12).

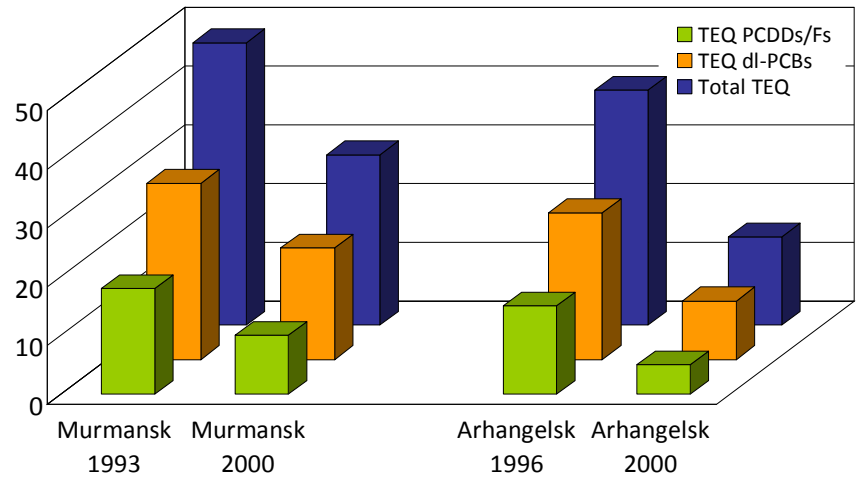


Figure 12 Time-related changes of PCDD/F and DL-PCB levels in breast milk samples from Murmansk (1993 and 2000), and Arhangelsk and Kargopol (1996 and 2000) in Russia (Polder et al., 2008)

Similar findings were reported in Sweden (Noren et al., 2000) and Japan (Uehara et al., 2006), where this was probably the result of strict regulations and restrictions on the use and production of these compounds. Schuhmacher et al. (2009), in a study in Spain, provided strong evidence of this decrease showing an overall reduction of 58% for PCDD/Fs and 33% for PCBs over the past 18 years. However, some studies confirmed it only in primiparous and not multiparous mothers (Uehara et al 2006), while others (Schuhmacher et al., 2009) suggested that the tendency is now slowing, and reported smaller decrease between 2002 and 2007 than between 1998 and 2002, for PCDD/Fs and DL-PCBs. However, overall comparison of PCDD/F and PCB concentrations in breast milk between 1992, 2002 and 2007 as reported in the studies reviewed (Figure 13 and 14), seems to confirm that a time-related decrease is still in progress.

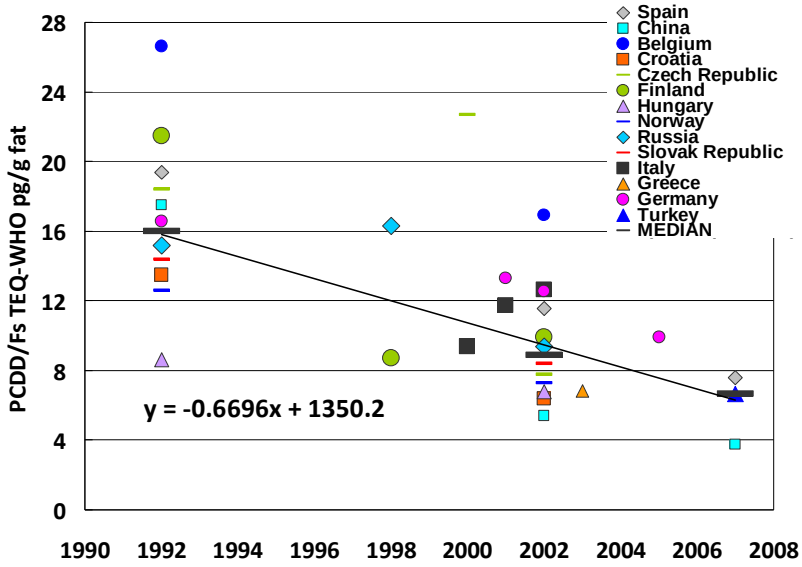


Figure 13
Mean PCDD/F levels (pg WHO-TEQ /g fat) in breast milk samples collected in different years (from 1992 to 2008) and locations.

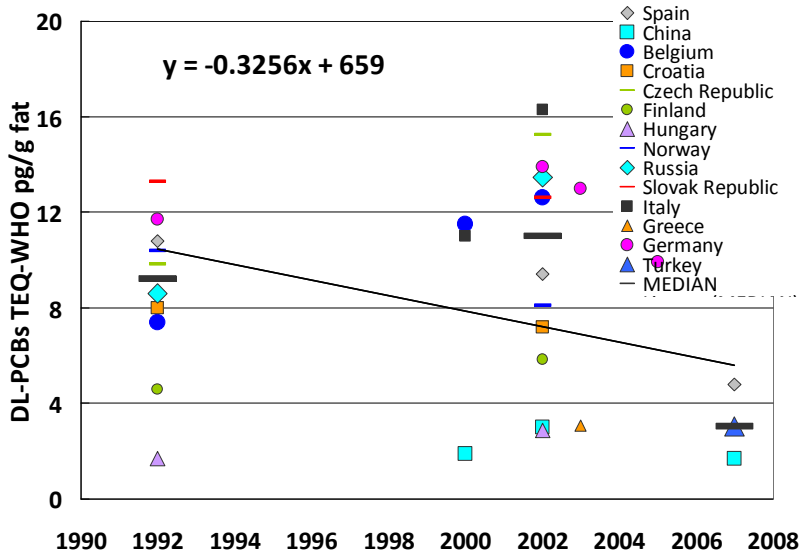


Figure 14
Mean PCB levels (pg WHO-TEQ /g fat) in breast milk samples collected in different years (from 1992 to 2008) and locations

5.6 HISTORY OF BREAST MILK CONTAMINATION IN ITALY SINCE 1970-2010

For this study of particular interest is the detailed information on the contamination of breast milk in Italy. Based on literature past studies conducted in Italy , the WHO second round of breast milk monitoring (WHO 1998) and implementing the lack of data with large Norwegian study of Noren et al 2000 was possible to reconstruct precisely the concentrations of contaminants since 1970 up to nowadays. It is clear that the concentration of persistent organic pollutants in Italian breast milk diminished and this trend is continuing for PCB and PCDD/Fs. Still between all contaminants the most abundant is PCB-153. In Tables 14ABC below are presented respectively the concentrations of PCDD/Fs in pg/g fat, DL-PCB in pg/g fat, and PCB Indicators in ng/g fat.

Year / concentration pg/g fat	1972	1976	1980	1984	1987	1992	1993	1998	1999	2000	2001	2009	Tendency line
2,3,7,8-TCDD	5	5	3		4.50	2.00	2.00	1.54	1.25	1.11	1.55	0.11	y = 9E+66e-0.0771x
1,2,3,7,8-PeCDD	12	7	6	5	7.50	6.00	6.00	3.74	3.31	2.67	3.61	1.54	y = 1E+38e-0.0432x
1,2,3,4,7,8- HxCDD					3.30	3.10	3.10	2.55	2.47	1.67	1.76	0.72	y = 2E+60e-0.0692x
1,2,3,6,7,8- HxCDD	42				28.00	33.40	33.40	11.10	9.24	7.08	9.52	4.57	y = 3E+58e-0.0661x
1,2,3,7,8,9- HxCDD	10	40	31	30	6.40	5.80	5.80	2.58	2.22	1.64	0.37	0.72	y = 4E+99e-0.1144x
1,2,3,4,6,7,8- HpCDD	160	96	70	69	55.00	32.60	32.60	15.30	12.80	11.40	11.46	4.23	y = 1E+83e-0.0944x
OCDD	600	400	300	200	231.00	157.80	157.80	65.30	57.40	52.10	49.45	28.29	y = 2E+74e-0.0836x
2,3,7,8-TCDF	3	3	3	2	4.90	0.90	0.90	0.76	0.67	0.46	0.76	0.20	y = 9E+66e-0.0771x
1,2,3,7,8-PeCDF	2				0.90	0.40	0.40	0.45	0.40	0.21	0.20	1.45	y = 3E+30e-0.0355x
2,3,4,7,8-PeCDF	43	29	17	14	21.00	16.90	16.90	11.50	9.39	7.78	8.87	4.81	y = 9E+43e-0.0495x
1,2,3,4,7,8- HxCDF	13				4.30	5.00	5.00	3.43	2.66	2.21	3.27	2.94	y = 1E+39e-0.0444x
1,2,3,6,7,8- HxCDF	11				3.20	4.00	4.00	3.04	2.40	1.84	2.40	1.40	y = 3E+46e-0.0531x
1,2,3,7,8,9- HxCDF	5					0.10	0.10	0.20	0.30	0.05	0.07	0.52	y = 2E+72e-0.0842x
2,3,4,6,7,8-HxCDF		14	8	8	1.50	1.50	1.50	1.62	1.34	0.88	0.84	0.16	y = 9E+101e-0.1175x
1,2,3,4,6,7,8- HpCDF	50	20	7	8	7.90	3.00	3.00	1.69	4.43	1.09	1.19	1.37	y = 2E+84e-0.0968x
1,2,3,4,7,8,9- HpCDF	2					0.20	0.20	0.09	0.13	0.06	0.12	0.08	y= 6E+81e-0.0953x
OCDF	4	4	5	5		0.50	0.50	1.03	5.16	0.56	1.42	0.82	y = 2E+45e-0.0521x
WHO TEQ-PCDD/Fs	29.59	19.85	14.57	10.25	18.76	14.41	14.41	8.48	7.28	5.80	7.53	3.42	y = 6E+42e-0.0482x

Table 14A Concentrations of PCDD/Fs in pg/g fat in Italy since 1972-2009

Year / ng/g fat	1972	1976	1980	1987	1990	1992	1993	1998	1999	2000	2009	Tendence line concentration of comtaminants in time
PCB 28	34	22	29	12		3.9	3.9	6.2	2.1	3.5	1.58	y = 3E+77e-0.0886x
PCB-52	4			3		1.7	1.7	0.33	0.26	0.26	0.28	y = 5E+80e-0.0933x
PCB-101	11			1.1		1.3	1.3	1.1	0.91	0.59	0.45	y = 3E+71e-0.0824x
PCB-118	60	46	31		40	31	31	23	16.30	14.10	8.34	y = 2E+41e-0.0462x
PCB-153	215	197	152	170	150	146	146	130	87	77	21.85	y = 9E+42e-0.0473x
PCB-138	190	177	134	130	107	116	116	92	66	58	10.72	y = 3E+53e-0.0596x
PCB-180	80	84	65	90	77	78	78	76	48	56	14.77	y = 2E+28e-0.0307x

Table 14B Concentrations of DL-PCB in pg/g fat in Italy since 1972-2009

Year / pg/g fat	1987	1992	1993	1998	1999	2000	2001	2009	Tendency line
PCB-81				0.0047	0.0042	0.002	0.005	0.08	-
PCB-77		0.0015	0.0015	0.00713	0.00988	0.00433	0.026	0.07	-
PCB-123				0.01	0.01	0.02		0.26	-
PCB-118				22.2	23	14.1		8.34	-
PCB-114				1.37	1.40	0.891		0.64	-
PCB-105		8	8	4.470	5.39	2.87		1.55	y = 1E+88e-0.1008x
PCB-126		0.0636	0.0636	0.078	0.0829	0.0309	0.076	0.03	y = 1E+38e-0.0453x
PCB 167	6.3			4.970	4.34	2.9		1.00	y = 1E+72e-0.0824x
PCB 156	20.4			12.50	10.90	0.765		3.51	y = 5E+81e-0.0933x
PCB 157	4.2			2.41	2.57	14.8		0.85	y = 6E+52e-0.0602x
PCB 169		0.0371	0.0371	0.054	0.051	0.0309	0.049	0.08	-
PCB-170	0							7.48	-
PCB-189	2.4			1.19	0.98	0.671		0.32	y = 2E+80e-0.0925x

Table 14C Concentrations of PCB Indicators in ng/g fat in Italy since 1987-2009

This strong decreasing trend might be a result of well functioning legislation, for example European Directives on of the reduction of the emission limits of PCDDs/Fs and PCBs from waste incineration set by EU Directive 2000/76/EC implemented in 2000, or Commission Regulations such as (EC) No 466/2001 and 1881/2006 setting maximum levels for certain contaminants in foodstuffs.

The following equation was proposed by Patandin et al. (1999) to estimate PCDD/F and DL-PCB intake from breast feeding in newborns and infants:

$$I = \left(0.95 \times V \times [BMF] \times [TEQ]_{breastmilk} \times \int_0^T e^{-0.017t}\, dt \right) \div 7$$

Where **0.95** = fraction of intestinal absorption of PCDD/Fs and DL-PCBs in breast-milk, **V** = weekly consumption of milk, in mL. [The newborn consumes 800 mL milk per day in the first 0-24 weeks (*Period 1*), 500 mL per day in the next 24-36 weeks (*Period 2*), and 400 mL per day in the 36-48 weeks (*Period 3*)]. **BMF** = milk fat concentration (3%). **T** = duration of breast feeding in weeks. **TEQ** = toxic equivalents of PCDD/Fs and DL-PCBs expressed as pg/g fat. **7** = average infant's body weight (BW), in kg. **0.017** = percentage used to take to account of a 1.7% weekly decrease in the PCDD/F and DL-PCB concentrations

in the milk of the breast-feeding mother (Patandin et al., 1999; Chao et al., 2004; Paul et al., 1988; US Department of Health, 2001).

An estimate of PCDD/F and indicator PCBs intake by newborns (0-24 weeks) and infants (24-36 and 36-48 weeks) and for the first year of breast feeding was calculated with this equation and is presented in Table 15 below, and in Figure 16.

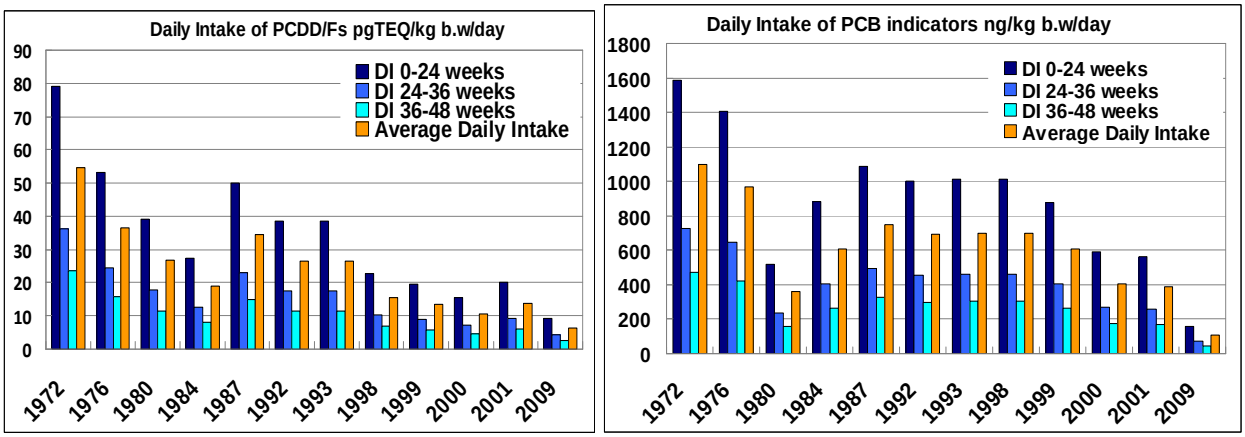


Figure 16 Estimation of daily intake for Italian infants since 1972-2009

Year	PCDD/F pg WHO-TEQ	DI 0-24 weeks	DI 24-36 weeks	DI 36-48 weeks	Average DI	Indicator PCBs [ng/g fat]	DI 0-24 weeks	DI 24-36 weeks	DI 36-48 weeks	Average DI
1972	29.59	79.14	36.24	23.64	54.54	594.00	1588.68	727.39	474.53	1094.82
1976	19.85	53.09	24.31	15.86	36.59	526.00	1406.81	644.12	420.20	969.48
1980	14.57	38.97	17.84	11.64	26.86	194.00	518.86	237.56	154.98	357.57
1984	10.25	27.42	12.55	8.19	18.89	331.00	885.27	405.33	264.42	610.07
1987	18.76	50.17	22.97	14.99	34.57	406.10	1086.13	497.29	324.42	748.49
1992	14.41	38.55	17.65	11.51	26.56	374.00	1000.28	457.98	298.78	689.33
1993	14.41	38.55	17.65	11.51	26.56	377.90	1010.71	462.76	301.89	696.52
1998	8.48	22.68	10.38	6.77	15.63	350.96	998.51	421.43	301.89	696.52
1999	7.28	19.48	8.92	5.82	13.43	328.63	878.93	402.43	262.53	605.71
2000	5.80	15.51	7.10	4.63	10.69	220.57	589.92	270.10	176.21	406.54
2001	7.53	20.13	9.22	6.01	13.88	209.45	560.18	256.48	167.32	386.04
2009	3.42	9.14	4.19	2.73	6.30	57.98	155.08	71.01	46.32	106.87

Table 15. Estimation of daily intake for Italian infants since 1972-2009 for PCDD/Fs and PCB Indicartors

The first 24 weeks of breast feeding are considered the period of highest daily intake, the average for this period for PCB Indicators was in the range of 155.08-1588.68 ng/day/kg b.w and for PCDD/Fs in the range 9.14-36.24 pgTEQ/day/kg b.w. For the B however no tolerable daily intake TDI or reference dose has been established specifically.

The main reason for that is the difficulty to distinguish between effects due to DL- NDL PCB congeners as several sensitive effects occur at the same target organs (liver, thyroid). Results for PCDD/Fs are much higher than the tolerable daily intake (TDI) proposed by WHO, 4 pg TEQ/kg BW/day (Van Leeuwen et al., 2000), and by the European Commission, 2 pg TEQ/kg BW/day (EC, 2001). The risks of this exposure, however, should not be overestimated because the TDI refers to life-span exposure while lactation is restricted to a limited period. However, this is a further indication that exposure of the population to these contaminants might exceed reference limits and that measures are needed to reduce it.

Table 11A Summary of PCDD/Fs levels in human milk in cited studies

Reference	City / country	2,3,7,8-TCDD	1,2,3,7,8-PeCDD	1,2,3,4,7,8-HxCDD	1,2,3,6,7,8-HxCDD	1,2,3,7,8,9-HxCDD	1,2,3,4,6,7,8-HpCDD	OCDD	2,3,7,8-TCDF	1,2,3,7,8-PeCDF	2,3,4,7,8-PeCDF	1,2,3,4,7,8-HxCDF	1,2,3,6,7,8-HxCDF	2,3,4,6,7,8-HxCDF	1,2,3,7,8,9-HxCDF	1,2,3,4,6,7,8-HpCDF	1,2,3,4,7,8,9-HpCDF	OCDF	WHO 2006 TEQ PCDDs/Fs	WHO TEQ PCDD/Fs+DL-PCBs
Abballe, et al., 2007[B]	Venice / Italy Low Fish Consumption	1.79	4.17	2.42	12.2	3.26	16.5	68.4	0.62	0.48	12.1	3.3	2.9	1.33	<0.1	1.75	0.06	0.19	14.8	34.14
	Venice / Italy Medium Fish Consumption	1.54	3.74	2.55	11.1	2.58	15.3	65.3	0.76	0.45	11.5	3.43	3.04	1.62	<0.2	1.69	0.0937	1.03	13.70	32.91
	Venice / Italy High Fish Consumption	1.25	3.31	2.47	9.24	2.22	12.8	57.4	0.67	0.4	9.39	2.66	2.4	1.34	<0.3	4.43	0.13	5.16	11.6	24.93
	Roma/ Italy	1.11	2.67	1.67	7.08	1.64	11.40	52.10	0.47	0.21	7.78	2.21	1.84	0.89	<0.05	1.09	0.06	0.56	9.40	20.41
Ataniyazova, et al., 2001[B]	Republic of Uzbekistan	16.10	4.84	1.00	2.98	0.79	4.71	20.70	0.48	0.32	3.90	1.84	1.57	0.92	<0.05	1.26	0.06	0.18	23.15	23.15
Bates, et al., 2002[B]	New Zeland	1.22	2.53	NA	NA	2.28	13.02	67.87	0.20	0.19	2.16	NA	NA	NA	0.16	1.20	0.20	0.68	4.83	4.83
Bake, et al., 2007[B]	Olaine/ Latvia	1.41	1.61	0.53	2.14	0.58	3.1	21.7	0.54	0.4	6.49	1.48	1.22	0.43	<0.04	1.04	<0.035	0.33	5.72	5.92
Bencko, et al., 2004[B]	Uherske Hradiste/ Czech Rep.	1.84	3.73	1.36	6.63	1.77	8.87	41.5	2.29	1.19	31.03	9.66	5.46	2.16	0.6	6.38	0.47	2.72	18.08	47.09
	Prague/ Czech Rep.	5.83	3.86	1.85	9.07	2.75	16.03	82.1	2.31	0.89	20.5	6.84	5.15	2.01	0.41	6.13	0.74	4.6	19.16	34.83
	Usti n.L/ Czech Rep.	1.94	3.86	2.19	8.87	3.26	12.3	54.1	5.95	2.97	22	12.6	6.88	4.42	0.25	8.38	0.86	2.48	17.16	36.74
	Kolin/ Czech Rep.	1.72	3.19	1.52	7.59	2.23	14.3	75.5	1.95	0.87	17.8	5.84	4.14	1.66	ND	3.34	0.16	2.3	12.97	26.91
	Liberec/ Czech Rep.	1.62	2.21	0.89	4.45	1.26	6.3	28.5	0.97	0.36	12	3.4	2.49	0.85	ND	1.84	0.13	0.96	8.96	16.83
	Kladno/ Czech Rep.	1.43	2.3	1.31	6.56	1.99	9.51	49.2	2.36	0.84	11.1	4.1	2.7	1.34	0.28	2.9	0.46	3.01	9.29	18.25
	Telc/ Czech Rep.	1.19	1.94	0.89	3.95	1.32	7.37	31.4	3.46	2.16	15.3	6.89	3.46	2.31	0.15	5.14	0.25	0.98	10.17	23.15
Chao, et al., 2004[B] (≥29 years old)	Taichung/ Taiwan	0.9	3.05	1.59	10.4	1.78	14.9	166	0.52	0.48	4.69	3.05	1.97	0.82	0.02	2.9	0.13	1.14	7.62	7.62
Chao, et al., 2004[B] (<29 years old)	Taichung/ Taiwan	0.6	2.33	1.07	5.61	1.11	9.3	87.6	0.39	0.41	3.96	2.49	1.63	0.67	<LOD	2.99	0.07	3.92	5.58	5.58
Cok, et al., 2009[B]	Istanbul / Turkey	0.29	0.95	2.21	3.2	1.08	22.8	61.2	0.32	0.29	4.9	6.1	4.7	6.1	0.0	31.6	5.10	25.9	5.71	8.24
	Afyon / Turkey	0.41	0.69	1.10	3.2	1.08	7.8	21.9	0.43	0.25	4.9	2.9	2.9	2.3	0.0	10.9	1.49	7.5	4.18	5.67
	K.Maras / Turkey	0.23	0.64	1.80	1.41	0.54	107.8	180.1	0.34	0.60	5.9	6.1	4.4	5.3	0.0	25.0	6.3	20.2	6.10	9.27
	Antalya / Turkey	1.1	0.93	5.8	1.67	0.23	38.81	92.8	0.42	0.72	8.2	12.1	8.2	11.9	0.0	57.1	9.80	47.8	9.64	12.80
	Ankara / Turkey	0.46	0.88	4.2	5.5	0.85	12.15	45.3	0.30	0.55	6.0	3.9	4.0	4.6	0.0	17.2	3.3	14.8	5.84	8.74
Costopoulou, et al., 2006[B]	Athens/ Greece	0.73	2.14	1.1	5.3	1	5	ND	0.5	0.2	6.26	1.6	1.6	0.7	0.2	1.0	ND	ND	6.01	10.08
Focant, et al., 2002[B]	Liege/ Belgium	2.3	8.3	8	28.8	4.7	26.6	223.2	1.3	0.8	25.2	5.4	6.5	3.2	0.1	5.3	No data	No data	24.37	37.20
Glynn, et al., 2001[B]	Uppsala/ Sweden	0.95	2.49	1.29	10.54	2.38	20.58	108.5	0.49	0.23	6.95	1.59	1.31	0.67	0.05	2.42	0.07	0.31	7.63	14.27
Guan, et al., 2006 [B](primiparous)	Tokyo/ Japan	1.4	6.05	1.1	19.4	6.0	9.0	100	0.9	0.4	11.16	3.7	4.1	2.8	0.4	2.0	ND	ND	14.79	24.05
Guan, et al., 2006[B] (multiparous)	Tokyo/ Japan	1.09	4.45	1.0	14.2	2.9	8.0	100	1.0	0.6	8.34	2.9	3.3	2.1	0.4	2.0	ND	ND	10.97	18.42
Guan, et al., 2006[B] (age 25-29)	Tokyo/ Japan	1.17	4.91	0.8	15.7	2.9	8	100	0.9	0.4	9.2	3.2	3.6	2.4	0.3	2.0	ND	ND	11.96	21.22
Guan, et al., 2006[B] (age 30-34)	Tokyo/ Japan	1.32	5.69	1.3	17.9	6.1	9	100	1	0.6	10.3	3.5	3.8	2.4	0.4	2.0	ND	ND	13.90	21.33
Harden, et al., 2007[B]	Australia	0.8	2.3	1.5	8.6	1.5	10.6	61.2	0.5	0.4	2.5	0.8	0.9	0.4	0.2	1.8	0.1	0.8	5.45	7.57
Hedley, et al., 2006[B]	Hong-Kong	1.23	2.76	2.06	5.69	1.69	11	59.5	1.06	0.78	5.03	2.46	1.89	0.1	0.82	1.56	0.07	0.3	7.24	10.31
Hsu, et al., 2007[B]	Taiwan	1.91	4.17	4.46	12.3	4.51	15.1	133	2.7	1.4	9.5	4.77	3.3	2.22	1.41	5.53	1.18	5.59	12.80	12.80

Kunisue, et al., 2004 [B]	India	3.3	7.3	4.3	14	6.6	55	200	4.7	4.1	11	6.4	5.9	3.7	<0.6	12	<0.6	1.8	19.38	31.17
	Cambodia	1.2	1.6	1.9	3.3	1.8	8.2	31	1.7	1.7	2.2	2.3	1.4	1.4	0.88	1.9	0.71	1.2	5.10	7.64
	Vietnam	0.8	1.2	1.5	3.1	1.7	6.1	18	1.5	1.5	4.2	3.7	2.8	2.2	<0.6	2.1	0.6	1.1	5.11	9.46
	Philipines	0.81	2	3	5.2	3	25	150	2.4	1.4	4.1	3.6	2.7	1.7	0.87	3.3	0.6	1.1	6.66	9.95
Kunisue,, et al., 2004a [B] (age 26-27)	Dalian/ China	0.94	0.91	1.1	2.5	1.6	3.8	29	0.71	0.92	4.1	2.9	2.4	1.8	0.87	2.1	1.2	5.5	4.58	7.24
Kunisue, et al., 2004a [B] (age 28-29)	Dalian/ China	0.65	0.87	0.49	2.7	0.47	3.8	32	0.89	0.68	3.9	2.3	1.8	1.1	0.32	1.8	0.19	0.71	3.79	6.35
Kunisue, et al., 2004a [B] (age 30)	Dalian/ China	0.87	0.85	1.3	3.1	0.73	4	40	1	0.82	4.9	3.2	2.2	1.8	0.85	1.6	0.91	2.6	4.71	7.44
Kunisue, et al., 2004a [B] (age 25-28)	Shenyang/ China	0.8	0.94	1	1.8	0.79	2.7	29	1.1	0.64	4.4	2.4	2.1	1.1	0.4	1.8	0.69	0.79	4.21	6.29
Kunisue, et al., 2004a [B] (age 29-32)	Shenyang/ China	0.5	0.64	0.44	1.3	0.52	1.8	15	0.63	0.22	3.2	2.2	1.7	0.43	<0.3	1.1	<0.4	<.5	2.90	5.14
Kunisue, et al., 2006 [B] (multiparous)	Fukoka/ Japan	0.82	3.9	2	14	2.3	9.8	110	0.71	0.28	6.2	2.2	2.2	1.2	<0.6	2.4	0.15	0.53	9.27	14.57
Kunisue, et al., 2006 [B] (primiparous)	Fukoka/ Japan	1.4	5.7	2.6	19	3	12	100	0.84	0.19	11	3.3	3.2	1.8	0.6	3	0.23	1.1	14.02	21.62
Nakamura, et al., 2008, [B]	Tohoku/ Japan	0.93	4.18	1.6	12.8	2.3	6.8	43.6	0.73	0.16	6.91	2.1	2.6	1.6	0.04	1.5	0.06	1.6	9.66	15.58
Nakatani, et al., 2005 [B]	Osaka/ Japan	1.3	5.3	1.7	28.1	5.1	8.3	57.2	0.7	0.3	10.8	3	3.5	1.6	ND	1.5	ND	ND	14.33	21.85
Noren, et al., 2000 [B]	Stockholm / Sweden	2	4	No data	21	5	30	100	ND	No data	11	4	3	No data	ND	5	1	4	12.99	22.52
Paumgrtten, et al., 2000 [B]	Rio De Janeiro/ Brasil	0.6	3.9	0.6	21	4.4	117	420	0.3	0.6	1.8	1.8	1.6	NA	0.3	4.9	NA	0.3	9.40	9.40
Raab, et al., 2008 [B]	Munich/ Germany	0.86	2.84	1.68	7.95	1.44	8.62	39.95	0.26	0.11	8.73	1.91	1.95	0.9	0.01	2.52	0.04	0.27	8.06	13.74
Revich, et al., 2001 [B]	Chapaevsk / Russsia	23.2	7.88	2.78	26.5	4.27	10.3	426.4	1.87	9.08	6.73	16.97	11.35	3.02	7.88	1.87	1.87	2	41.10	41.10
Saito, et al., 2005 [B]	Not precisided	1.5	6.3	2	19.3	3.9	11	73.6	0.7	0.5	13.3	4.6	5.5	3.3	0.00	2.3	0.00	0.00	15.89	22.94
Sasamoto, et al., 2006 [B] (sampling 1999-2000)	Tokyo/ Japan	1.1	4.9	0.44	16	6.7	7.7	75	1.02	0.42	9.06	3.1	3.2	1.8	0	1.8	0.025	0.17	12.07	17.22
Sasamoto, et al., 2006 [B] (sampling 2001-2002)	Tokyo/ Japan	0.8	3.4	0.71	10	1.5	6.5	48	0.7	0.32	3.4	2	2.6	1.1	0	0.98	0	0	7.18	16.17
Schecter, et al., 2002 [B]	Anagarsk/ Russia	3.3	2.3	no data	0.5	0.1	0.1	ND	0.2	NA	7.4	no data	2.3	NA	0.1	ND	NA	NA	8.14	9.17
	Usolye-Sibirskoye/ Russia	3.6	2.7	no data	0.4	0.1	ND	ND	0.4	0.1	16.7	no data	4.4	ND	0.1	ND	ND	ND	11.85	11.85
Schuhmacher, et al., 2004a [B]	Terragona / Spain	1.04	3.79	1.96	27.7	2.75	11.2	50.9	0.37	0.3	6.27	2.17	2	0.07	0.8	1.74	0.07	27.4	10.66	10.66
Sudaryanto, et al., 2005 [B]	Penang Kedah/ Malaysia	1.1	3.6	1.7	6.4	2.2	15	100	1.2	<0.16	4.1	2	1.6	0.92	NA	2.3	NA	NA	7.74	10.84
Sun, et al., 2006 [B]	Heibei/ China	0.46	0.89	0.54	1.66	0.34	3.08	31.2	0.34	0.34	2.98	1.85	1.25	0.46	0.2	1.16	0.22	0.87	2.97	4.50
	Tokyo/ Japan	1.5	6.85	1.97	22.28	2.8	7.71	53.1	0.66	0.39	10.05	2.38	3.2	0.07	2.19	2.51	0.19	0	15.05	20.12
Tajimi, et al., 2005 [B]	Tokyo/ Japan	1.2	5.3	1.2	16.8	4.5	8.4	72.4	0.9	0.5	9.8	3.3	3.7	2.5	0.7	1.8	0.7	1.5	12.95	21.30
Takekuma, et al., 2004, [B] not smokers donors	Saitma/ Japan	1.59	6.49	2.24	19.37	3.79	11.97	75.13	0.83	0.56	13.94	4.71	5.81	3.78	NA	2.41	NA	NA	16.50	24.20
Takekuma, et al., 2004 [B] Active smokers donors	Saitma/ Japan	1.41	5.91	1.61	18.19	3.24	8.46	71.62	0.6	0.4	12.58	4.43	5.7	2.43	NA	2.16	NA	NA	14.85	20.84
Todaka, et al., 2008 [B] (multiparous)	Sapporo/ Japan	0.6	2.3	<LOD	6.9	<LD	5	36	<LOD	<LOD	4.1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	4.88	9.04
Todaka, et al., 2008, [B] (primiparous)	Sapporo/ Japan	0.7	3.2	<LOD	9.8	<LD	5.9	40	<LOD	<LOD	5.4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	6.57	11.50

Weiss, et al., 2003[B]	Seveso / Italy	4.45	2.76	1.39	7.15	1.37	9.13	72.95	<0.82	0.2	5.81	2.59	1.58	0.64	0.12	1.5	0.13	<1.54	10.65	16.89
	Milan/ Italy	1.63	4.06	2.21	12.8	1.78	20.45	169.12	<0.76	0.21	9.71	3.3	2.44	0.85	0.05	1.44	0.12	<1.42	11.30	20.49
	Lombardian Village/Italy	1.58	4.28	2.79	13.07	2.11	20.49	190.39	<0.82	0.19	9.00	3.44	2.47	0.88	0.04	1.41	0.13	<1.54	11.41	19.57
Wittsiepe, et al., 2007[B]	Duisburg/ Germany	1.5	4	2.7	11.6	2.3	12.6	70.4	0.28	0.19	10.1	2.7	2.4	1	0.05	1.9	0.08	0.34	11.01	19.30
Yang, et al., 2002[B] (multiparous, urban zone)	Inchon / Korea	0.20	2.46	2.07	13.19	3.17	11.60	167.17	2.42	0.62	7.25	4.99	3.69	2.21	0.00	4.71	0.33	0.90	8.24	8.24
Yang, et al., 2002[B] (primiparous, industrial zone)	Inchon/ Korea	ND	1.90	3.64	22.12	1.75	34.07	303.89	1.62	1.54	7.87	6.58	5.78	10.91	0.23	28.56	4.93	22.21	10.34	10.34
	Seoul/ Korea	0.16	1.83	4.45	29.88	7.41	60.95	836.16	18.35	1.72	14.93	15.82	20.08	24.68	8.37	90.17	14.58	47.71	21.35	21.35

Values expressed as pg/g fat; ND – not detected; NA - not analysed; N CAL – not calculated; <LOD - below limit of detectio

Table 11B. Summary of DL-PCBs levels in human milk in cited studies

References	City / country	PCB-77	PCB 81	PCB-126	PCB-169	PCB-105	PCB-114	PCB-118	PCB-123	PCB-156	PCB-157	PCB-167	PCB-189	TEQ-WHO PCBs	TEQ-WHO PCBs
Abballe, et al., 2007[B]	Venice LC/ Italy	7.13	4.73	78	54	4470	1370	22200	<0.08	12500	2410	4970	1190	19.34	19.34
	Venice MC/ Italy	9.88	4.22	82.9	51.9	5390	1400	23000	<0.09	10900	2570	4340	982	19.21	19.21
	Venice HC/ Italy	9.68	5.67	61	36.2	3770	953	16300	<0.1	7010	1570	3210	613	13.33	13.33
	Roma/ Italy	4.33	2.01	38.9	30.9	2870	891	14100	<0.2	7650	1480	2900	671	11.01	11.01
Bake, et al., 2007[C]	Olaine/ Latvia	3.59	10.22	1.27	2.33	4.33	12.57	9.57	1.87	5.46	7.02	4	1.33	166.52	0.20
Bencko, et al., 2004 [B]	Uherske Hradiste/ Czech Rep.	15.6	NA	256.6	111.5	6.47	NA	69.9	NA	65.6	5.15	21.3	7.38	29.01	29.01
	Prague/ Czech Rep.	18	NA	134	75.4	3.9	NA	34.4	NA	23.3	2.73	8.2	2.67	15.67	15.67
	Usti n.L/ Czech Rep.	8.38	NA	174	72.5	2.98	NA	29.1	NA	41.6	3.42	13.1	4.04	19.58	19.58
	Kolin/ Czech Rep.	10.5	NA	123	54.6	3.43	NA	22.6	NA	26.6	2.48	8.73	2.37	13.94	13.94
	Liberec/ Czech Rep.	12.9	NA	66.1	41.6	1.49	NA	14.9	NA	16.2	1.34	4.54	1.59	7.86	7.86
	Kladno/ Czech Rep.	21.9	NA	76.1	44.7	ND	NA	17.1	NA	19.9	2.36	ND	2.37	8.96	8.96
	Telc/ Czech Rep	27.7	NA	118	39.2	ND	NA	18.7	NA	15.2	2.01	no data	1.24	12.99	12.99
Cok, et al 2009[B]	Istanbul / Turkey	30.9	3.78	20.40	11.4	746.7	169.3	2264	49.3	810	177.4	253.3	83.2	3.05	2.53
	Afyon / Turkey	21.3	1.6	12.1	5.8	595.9	116.7	1742	46.8	499	110.6	164.7	43.8	1.88	1.49
	K.Maras / Turkey	30.4	5.5	27.2	12.4	443.5	84.6	1220	28.6	355	92.2	122.8	43.6	3.29	3.17
	Antalya / Turkey	43.3	4.7	25.0	15.4	974.9	221.2	3000	61.1	1185	251.2	313.1	103.2	3.91	3.16
	Ankara / Turkey	14.6	3.7	24.1	11.4	883	154.3	2600	55.7	725	172.5	269.3	68.5	3.4	2.90
Costopoulou, et al., 2006 [B]	Athens/ Greece	**ND**	ND	28.4	24	2500	340	6900	1100	3720	680	1000	500	4.06	4.06
Focant, et al., 2002[B]	Liege/ Belgium	45.9	no data	107.7	68.2	NA	NA	NA	NA	NA	NA	NA	NA	12.83	12.83
Glynn, et al., 2001[C]	Uppsala/ Sweden	no data	ND	53	21	1960	NA	14910	NA	5610	NA	1430	NA	6.65	6.65
Guan, et al., 2006[B] (primiparous)	Tokyo/ Japan	ND	ND	75.9	36	2300	740	10900	ND	3580	880	1000	300	9.26	9.26
Guan, et al., 2006 [B] (multiparous)	Tokyo/ Japan	ND	ND	61.3	29	1700	540	8300	ND	2640	640	1000	200	7.45	7.45
Guan, et al., 2006 [B] (age 30-34)	Tokyo/ Japan	ND	ND	76.1	35	2300	760	10800	ND	3760	900	1000	300	9.25	9.25
Guan, et al., 2006 [B] (age 25-29)	Tokyo/ Japan	ND	ND	61.1	29	1800	520	8400	ND	2460	620	1000	200	7.43	7.43
Harden, et al., 2007[B]	Australia	20.5	3.2	17	7.2	940	220	3400	67	1300	300	380	87	3.1	2.12
Hedley, et al., 2006	Hong-Kong/ China	3.91	3.27	26.8	12.7	1.24 [C]	0.25 [C]	4.59 [C]	<0.02 [C]	<1.88 [C]	0.38 [C]	0.73 [C]	0.14 [C]	4.67	3.06
Kunisue, et al., 2006 [B] (primiparous)	Fukoka/ Japan	18	4.3	56	47	2200	670	9600	190	4000	1000	1300	420	6.9	7.60
Kunisue, et al., 2006[B] (multiparous)	Fukoka/ Japan	13	3.1	40	31	1400	440	6200	120	2600	630	810	290	10	5.31
Kunisue, et al., 2004a[B] (age 26-27)	Dalian/ China	5.7	3.6	20	18	590	72	2400	35	540	160	210	49	2.9	2.66
Kunisue, et al., 2004a[B] (age 28-29)	Dalian/ China	8.00	3	19	17	690	130	2800	47	810	230	260	70	2.9	2.56
Kunisue, et al., 2004a[B] (age 25-28)	Shenyang/ China	2.8	2.3	17	8.8	570	93	2100	31	540	140	170	56	3.2	2.08
Kunisue, et al., 2004a[B] (age 29-32)	Shenyang/ China	6.6	2.5	19	6	830	94	3400	48	570	120	180	40	2.5	2.24
Kunisue, et al., 2004a[B] (age 30)	Dalian/ China	5.3	3.3	21	16	820	100	2700	45	700	180	270	71	2.8	2.73
Kunisue, et al., 2004[B]	India	100	36	100	20	7300	860	22000	460	4600	1100	1900	310	16.3	11.79
	Cambodia	18	5.4	21	6.5	1400	210	4400	74	1300	280	350	77	3.6	2.54
	Vietnam	15	5	34	8	4000	590	14000	280	3100	600	820	120	7	4.35
	Philipines	28	9.1	27	12	1400	180	4600	100	340	510	190	10	4.5	3.29
Nakamura, et al., 2008 [B]	Tohoku/ Japan	3.3	2.1	46.5	27.5	1782	504	8288	112	2460	591	942	184	7.8	5.92

Nakatani, et al., 2005 [B]	Osaka / Japan	38.3	6.5	59.4	34.6	1935.3	599.5	9268.1	202.3	3498.3	729	980	243.5	**9.9**	7.51
Noren, et al., 2000 [B]	Stockholm/ Sweden	16	N.A	76	39	4000	0	13000	NA	6000	2000	ND	NA	9.52	9.52
Polder, et al., 2008a[B]	Norway / Tromso	NA	NA	NA	NA	25000	1200	100000	ND	13400	3200	150000	3000	8.87	8.87
	Norway / Oslo Grunnerlokka	NA	NA	NA	NA	32000	2000	140000	ND	22000	5200	220000	7000	12.85	12.85
	Norway / Oslo Sondre Nordstrand	NA	NA	NA	NA	35000	1600	150000	ND	13600	3400	190000	4000	11.93	11.93
Raab, et al., 2008 [B]	Munich/ Germany	5.86	1.49	48.57	27.49	1.64	0.48	9.88	0.1	5.66	0.85	1.71	0.77	6.31	5.68
Saito, et al., 2005 [B]	Not precised	6.5	NA	59.5	36.6	NA	NA	NA	NA	NA	NA	NA	NA	N. CAL	7.05
Sasamoto, et al., 2006 [B] sampling 2001-2002,	Tokyo/ Japan	8.1	2.4	40	26	95	6600	1500	410	690	2200	520	180	11	5.15
Sasamoto, et al., 2006[B] sampling 1999-2000,	Tokyo/ Japan	30	25	75	32	36	9500	2000	650	1000	3200	780	230	6.6	8.99
Schecter, et al., 2002	Anagarsk/ Russia	nd	NA	10.2	0.4	NA	NA	NA	NA	NA	NA	NA	NA	10.6	1.03
Sudaryanto, et al., 2005[B]	Penang Kedah/ Malaysia	19	2.8	22	19	1700	450	5800	120	1800	390	490	140	4.5	3.10
Sun, et al., 2006[B]	Heibei/ China	9.97	3.29	12.34	6.07	746.7	92.28	1944	43.37	433.4	94.73	141	36.63	1.88	1.53
	Tokyo/ Japan	4.4	1.72	36.65	35.4	1490	307.3	5420	62.3	2312	807	812.5	227.2	6.48	5.07
Tajimi, et al., 2005[B]	Tokyo/ Japan	5.5	20.8	68.6	32.3	2018.5	639.6	9555.7	0.4	3111.5	759.4	984.4	230	10.6	8.35
Takekuma, et al., 2004[B] (not smokers donors)	Saitma/ Japan	5.99	NA	65.99	36.57	NA	NA	NA	NA	NA	NA	NA	NA	6.97	7.70
Takekuma, et al., 2004[B] (active smokers donors)	Saitma/ Japan	5.79	NA	49.96	32.83	NA	NA	NA	NA	NA	NA	NA	NA	5.33	5.98
Todaka, et al., 2008 [B] (multiparous)	Sapporo/ Japan	<LD	<LD	33	16	1707	396	7090	108	1953	447	711	186	4.2	4.16
Todaka, et al., 2008 [B] (primiparous)	Sapporo/ Japan	<LD	<LD	39	20	1939	480	8075	145	2209	515	801	198	4.9	4.93
Weiss, et al., 2003 [B]	Seveso/ Italy	ND	3.00	53	31	NA	NA	NA	NA	NA	NA	NA	NA	5.57	6.23
	Milan/ Italy	25	5.00	75	56	NA	NA	NA	NA	NA	NA	NA	NA	8.08	9.19
	Lombardian Village/Italy	27	3.00	66	52	NA	NA	NA	NA	NA	NA	NA	NA	7.13	8.17
Wittsiepe, et al., 2007 [B]	Duisburg/ Germany	no data	no data	67.3	28.9	1800	630	11000	230	6400	870	2000	380	8.29	8.30
Yang, et al., 2002[B] (industial zone, primiparous donor)	Inchon /Korea	75	NA	38	23	1333	294	5348	95	1211	NA	NA	NA	N CAL	4.76
Yang, et al., 2002, [B] (industrial zone, multiparous donor)	Inchon /Korea	39	NA	30	23	1354	264	5236	88	1631	NA	NA	NA	N CAL	3.96
Yang, et al., 2002[B] (urban zone multiparous donors)	Seoul/ Korea	470	NA	29	0	759	93	2757	45	359	NA	NA	NA	N CAL	3.16
Yang, et al., 2002[B] (urban zone, primiparous donors)	Seoul/ Korea	164	NA	51	28	1599	958	5228	156	1485	NA	NA	NA	N CAL	6.27
Zhao, et al., 2007[C]	Pingqiao/ China	15670	1600	NA	NA	4400	7380	5400	NA	NA	NA	NA	NA	5.38	5.38
	Luqiao/ China	5150	NA	NA	NA	15210	8660	17750	NA	9510	2680	NA	NA	3.16	3.16

Values expressed as pg/g fat; ND – not detected; NA - not analysed; N CAL – not calculated; <LOD - below limit of detection

6. HUMAN BIO-MONITORING OF PCDD/Fs and PCBs IN ITALIAN BREAST MILK

Polychlorinated dibenzo-*p*-dioxins (PCDDs), and dibenzofurans (PCDFs) are by-products of combustion or thermal processes involving organic matter and chlorine, such as those occurring during waste incineration, power/energy generation, metallurgical and other chemical–industrial processes as already mentioned in previous chapters (Konig et al., 1993; Cleverly et al., 2007), whereas polychlorinated biphenyls (PCBs) are produced by industry as technical mixtures for use as dielectric fluids, organic diluents, plasticizers, adhesives and flame retardants. As widespread in environment and easily fat soluble they bioaccumulate through the food chain and enter the human body mainly via food. They accumulate in fatty materials such as adipose tissue and breast milk. Several factors can potentially affect a population's exposure to PCDD/Fs and PCBs, and hence the occurrence of these contaminants in breast milk. The most important variables are the mother's diet (Li et al 2009; Chao et al., 2003), her age (Sasamoto et al., 2006; Kunisue et al., 2006), parity (Minh et al., 2004; Subramanian et al., 2007; Uehara et al., 2006), and smoking habits (Uehara et al., 2007; Takekuma et al., 2004).

Several studies have shown that concentrations of PCDDs/Fs and PCBs in breast milk of mothers living in urban areas are higher than those from rural zones (Yang et al., 2002; Schuhmacher et al., 2007 and 2009; Li et al., 2009). Particularly high levels of PCDDs/Fs were found in breast-milk samples collected near chemical industries and manufacturing plants (Schecter et al., 2002). Processes such as combustion of municipal/urban waste in incinerators, burning of domestic waste or backyard burning may produce optimal conditions for formation and emission of PCDDs/Fs (Gullett et al., 2001). However research conducted in the proximity of modern municipal solid-waste incinerators and hazardous-waste incinerators (Reis et al., 2007; Schuhmacher et al., 2004a; Tajimi et al., 2005) did not show significant increases of PCDDs, PCDFs and PCBs in the milk of the mothers potentially exposed. Moreover, according to Tajimi et al. (2005), there was no relationship between PCDD/F levels in breast milk and the distance of the mothers' home from the nearest waste incinerator. Indeed living near to a modern well controlled municipal solid-waste incinerator or hazardous-waste solid incinerator should not increase the risk of exposure to PCDDs/Fs and PCBs (Reis et al., 2007; Schuhmacher et al. 2002; 2004a; 2004b; 2007; 2009).

Recently the area of Naples, which includes the city of Giugliano, was reported to have serious problems regarding the disposal of domestic waste. For a long period, from the mid 1980's until recently, much waste has been disposed of illegally and burned on open-air fires; this additional source of PCDDs/Fs might have increased the exposure of residents either directly through the atmosphere or indirectly through the food-chain. Breast milk is known to accumulate these substances, and its monitoring can show whether such possible additional exposure resulted in increased intake.

6.1 Breast milk sampling

A total of 59 healthy mothers, non-occupationally exposed to PCDD/F and PCB, were recruited, comprising 21 mothers from Giugliano, 22 from Piacenza and 16 from Milan. Breast-milk samples were collected manually by each volunteer from April 2008 to November 2009 at one to three months after delivery. Mothers provided informed consent and filled in a "questionnaire for human milk donors" as proposed by WHO. The information included age, residence record, dietary habits (frequency of consumption of fish, meat, eggs, milk, vegetables and fruits), occupation before pregnancy, and smoking habits (Tables 16). Samples were collected in glass containers and stored at -20^{o}C until analysis.

%		Piacenza	Milano	Giugliano in Campania
Milk	Not at all	0	0	5
	Less than 1/week	0	0	5
	1/week	5	0	0
	2/week	5	0	10
	More than 2/week	36	19	25
	Every day	55	81	55
Meat	Not at all	0	6	0
	Less than 1/week	5	6	0
	1/week	5	6	0
	2/week	23	25	0
	More than 2/week	55	50	85
	Every day	14	0	15
Fish	Not at all	9	6	0
	Less than 1/week	18	13	5
	1/week	50	63	40
	2/week	23	6	45
	More than 2/week	0	6	10
	Every day	0	0	0
Vegetables	Not at all	0	0	5
	Less than 1/week	0	0	5
	1/week	0	0	10
	2/week	5	6	0
	More than 2/week	14	56	60
	Every day	82	38	20
Fruit	Not at all	0	0	0
	Less than 1/week	5	0	5
	1/week	0	0	10
	2/week	23	13	10
	More than 2/week	18	44	25
	Every day	55	38	50
Eggs	Not at all	0	0	25
	Less than 1/week	59	38	20
	1/week	18	31	25
	2/week	23	6	20
	More than 2/week	0	19	10
	Every day	0	0	0
Number of mothers		22	18	21
Primipara vs multipara		82/18	81/19	100/0
Younger (age 20-30) vs older (age 30-40)		32/68	18/82	85/15
primipara	20-30 years old	39	18	86
	30-40 years old	61	82	14
Smoking habits: Yes/No		23/77	31/69	10 /90
Occupational exposure: Yes/No		14/86	25 /75	25 /75

Table 16 Characteristics of milk donors from Piacenza, Milan and Giugliano in Campania,

6.2 Materials

PCB internal standards (19 $^{13}C_{12}$ congeners) were supplied by Cambridge Isotope Laboratories (Andover, Massachusetts, USA); PCDD and PCDF internal (16 $^{13}C_{12}$ congeners 2,3,7,8 chlorine-substituted) and external standards (2,3,7,8-TCDD ^{37}Cl substituted) were supplied by Wellington Laboratories (Shawnee Mission, USA).

Solvents and reagents such as n-hexane (purity >98%), acetone (>99.8%), dichloromethane (>99.8%) and carbon tetrachloride were obtained from Riedel, (Germany). Concentrated sulfuric acid (>98%), isooctane (>98%), methanol (99.9%) and, diethyl ether (> 99.5%) were obtained from Carlo Erba, Italy. Aluminum oxide for column chromatography, anhydrous sodium sulfate (99%), Extrelut NT cartridges and silica gel

were purchased from Merck (Darmstadt, Germany). Glass wool was obtained from Supelco (Bellefonte, PA) and sodium oxalate from Sigma Aldrich (Seelze, Germany)

6.3 Extraction

Breast milk was extracted using a liquid-liquid extraction method, samples (50 ml) being placed in a glass beaker with of sodium oxalate (1 g), to prevent coagulation. Then internal standards of PCDD/ F (200 pg) [$^{13}C_{12}$ – 16 isomers] and of PCB (1.00 ng) [$^{13}C_{12}$ WHO-PCB 12 dioxin-like PCBs isomers and seven PCB isomers called indicators] were added to the sample, and an external standard [2,3,7,8-TCDD Cl^{37}] was added after extraction to measure the instrumental recovery. A blank was also included in each series of samples. Lipids were liquid-liquid extracted from the aqueous sample with methanol (50 ml), followed by diethyl ether (3x50 ml) and n-hexane (3x50 ml) with the aid of an ultrasonic homogenizer. The extracts were dried by evaporation to constant weight for gravimetric fat determination.

6.4 Clean-up

Dried samples were dissolved in 98% sulfuric acid (15 mL per 2.5 g fat), applied to an Extrelut column and eluted with n-hexane (120 mL). The solvent was concentrated by evaporation to 1 mL, and applied to an alumina column in order to separate PCDDs and PCDFs from PCBs. PCBs were first eluted with carbon tetrachloride (6 mL), and subsequently PCDDs/Fs and PCBs 77, 81, 126 and 169 were eluted with dichloromethane (7 mL). All extracts were dried with a gentle stream of nitrogen and redissolved in isooctane (50 µL) for instrumental analysis.

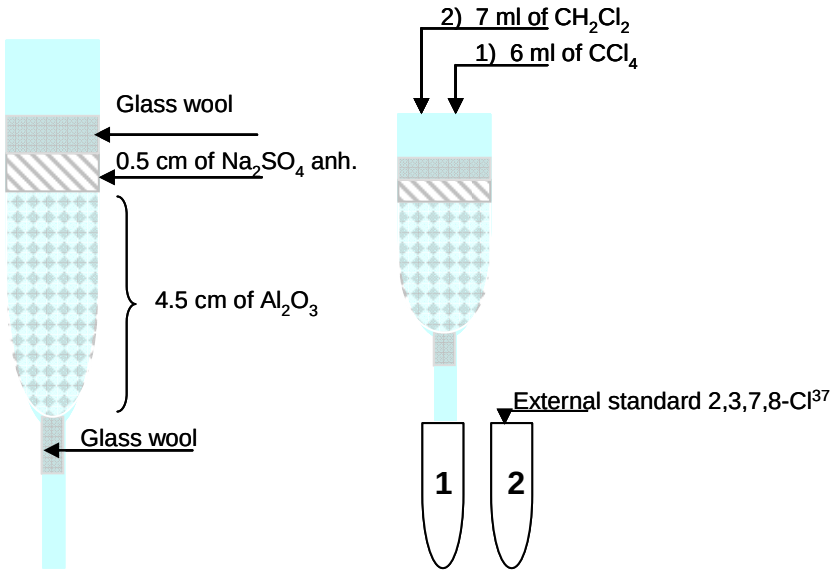


Figure 17. Alumina column preparation and elution step with using CH_2Cl_2 and CCl_4.

6.5 Instrumental analysis

PCBs (19 congeners, IUPAC No. 28, 52, 81, 77, 101, 123, 118, 114, 105, 126, 153, 138, 167, 156, 157, 169, 180, 170, 189) and PCDDs/Fs (17 congeners: 2,3,7,8-TetraCDD, 1,2,3,7,8-PentaCDD, 1,2,3,4,7,8-HexaCDD, 1,2,3,6,7,8-HexaCDD, 1,2,3,7,8,9-HexaCDD, 1,2,3,4,6,7,8-HeptaCDD, OctaCDD, 2,3,7,8-TetraCDF, 1,2,3,7,8-PentaCDF, 2,3,4,7,8-PentaCDF, 1,2,3,4,7,8-HexaCDF, 1,2,3,6,7,8-HexaCDF, 2,3,4,6,7,8-HexaCDF, 1,2,3,7,8,9-HexaCDF, 1,2,3,4,6,7,8-HeptaCDF, 1,2,3,4,7,8,9-HeptaCDF, OctaCDF) were measured by high-resolution gas chromatography-high-resolution mass spectrometry (HRGC-HRMS) using a TRACE GC 2000, Thermo Finnigan (Thermo Fisher Scientific), coupled with a Mat 95 XP Mass Spectrometer, operating in the electron impact ionization (EI^+) mode at resolution 10000.

For PCDD/F analysis, the following temperature program was used: 160 ℃ for 1 min, 2.5 ℃/ min increase to 300 ℃, 300 ℃ maintained for 6 min. Peaks were accepted if the isotopic ratio was within 15% of the corresponding ratio in the IS. To calculate the limit of detection (LOD), the minimum signal-to-noise ratio considered was 3:1. Recoveries were in the range 70-90%. Concentrations of most compounds were higher than the LODs, except for 2,3,7,8-TCDD and 2,3,7,8-TCDF in some samples. Concentrations below LOD were assigned a value of LOD/2 (middle bound). TEQ values were calculated using revision 2006 WHO-TEFs.

PCBs were analysed using a BPX-DXN (60 m x 0.20 mm x 0.25 µm) (SGE, Analytical Science, Melbourne, Australia) capillary column with splitless injection and the following temperature programme: 125 ℃ for 2 min, 8° C/min i ncrease to 190 ℃, 2 ℃/ min increase to 280 ℃, maintained for 7 min. The GC-MS was used in the selected-ion monitoring (SIM) mode and the monitored ions for PCBs and dioxins were M^- and $(M+2)^-$ for tetra- and penta-substituted compounds, and $(M+2)^-$ and $(M+4)^-$ for hexa-, hepta- and octa-substituted.

6.6 Results

PCDDs, PCDFs and PCBs in milk: comparison of samples from Giugliano vs. Milan and Piacenza.

The mean concentration of PCDDs/Fs in milk samples collected in Giugliano was 46.6 pg/g fat, this being slightly lower than that in samples from Milan (51.5, p=N.S.) and Piacenza (57.0, p=0.05) (Table 17). The mean total WHO-TEQ value was also lower in samples from Giugliano (3.78) than from Milan (5.03, p=N.S.) and Piacenza (4.67, p=0.05).

	Piacenza			Milan			Giugliano in Campania		
Summary	mean	range	S.D	mean	range	S.D	mean	range	S.D
PCDDs [pg/g of fat]	40.19	8.72-132.49	31.36	36.39	15.22-57.17	12.17	33.14	15.79-69.68	14.01
PCDFs [pg/g of fat]	16.76	4.41-37.95	7.89	15.16	9.34-31.28	7.38	13.49	5.24-46.33	9.82
PCDDs+PCDFs [pg/g of fat]	56.95	20.46-145.76	33.00	51.54	24.56-85.98	16.95	46.64	27.08-96.99	21.03
WHO-TEQ PCDDs+PCDFs	4.67	2.43-7.70	1.44	4.70	2.42-9.55	1.80	3.78	1.26-9.44	2.09
DL-PCBs [ng/g of fat]	48.65	14.87-116.46	30.68	31.48	9.20-76.13	21.69	37.31	16.92-86.83	16.34
PCBs Indicator [ng/g of fat]	113.69	26.95-267.37	70.00	65.47	19.85-192.47	55.13	72.42	23.59-160.49	29.20
PCBs (DL-PCBs+Indicators)	130.98	36.01-297.45	76.61	82.18	26.29-245.26	67.86	94.15	45.96-207.51	36.60
WHO-TEQ DL-PCBs	5.27	1.47-10.70	2.64	6.28	1.09-9.19	3.317	4.87	1.81-10.46	2.21
Total WHO-TEQ PCDDs/Fs+DL-PCBs	9.94	5.58-17.59	3.10	10.98	4.66-14.15	4.39	8.65	4.22-18.95	3.55

Table 17 Summary: mean and range of PCDD, PCDF and PCB concentrations in human milk collected in Piacenza Milano and Giugliano.

Analysis of the PCDD/F congener profiles showed that OctaDDs and 2,3,4,7,8-PentaCDF, followed by 1,2,3,6,7,8-HexaCDD and 1,2,3,4,6,7,8- HeptaCDD were the congeners with the highest concentrations in milk (Table 18- in the end of the chapter). Significant differences were observed for 1,2,3,7,8-PeCDF levels, which were much lower

in Giugliano (0.16 pg/g fat) than in Milan (0.89 pg/g fat, p=0.03) and Piacenza (4.46 pg/g fat, p=0.02). The congeners principally contributing to the total TEQ were always 1,2,3,7,8-PentaCDD and 2,3,4,7,8-PentaCDF (altogether 64-71% of the total), but only small differences in the PCDD/F profiles were observed in milk samples collected at the different locations. (see Figure 18 and 19). Whereas samples from Piacenza and Milan had comparable profiles with the highest contribution to total PCDDs/Fs given by PentaCDDs and PentaCDFs followed by HexaCDDs and HexaCDFs, in the Giugliano samples the highest contributions were given by PentaCDFs and PentaCDDs, followed by TetraCDD and HexaCDDs (See Table 18- in the end of this chapter, 18 and 19).

Table 18 shows also DL-PCB and the indicator PCB concentrations in milk. PCB congener profiles in the three groups were generally comparable. The most abundant congeners were PCB-170 and PCB-118 followed by PCB-156 among DL-PCBs, and PCB-153, PCB-180 and PCB-138 among the indicator PCBs.(See Figure 20) Total PCB concentrations were higher in samples from Piacenza (131 ng/g fat) than from Milan (82.2 ng/g fat, p=0.03), and Giugliano (67.9 ng/g fat p=0.05), but total WHO-TEQs for PCBs were higher in Milan (6.28 ng TEQ) than in the other two cities (Tables 17 and 18, Figure 18). The major contribution to total TEQs for PCBs was from PCB-126, followed by PCB-169. PCBs with 6- and 7-chlorines were the most abundant classes in all the three groups (Figure 20). Samples from Giugliano had the highest level of hexachloro PCBs but differences were not statistically significant.

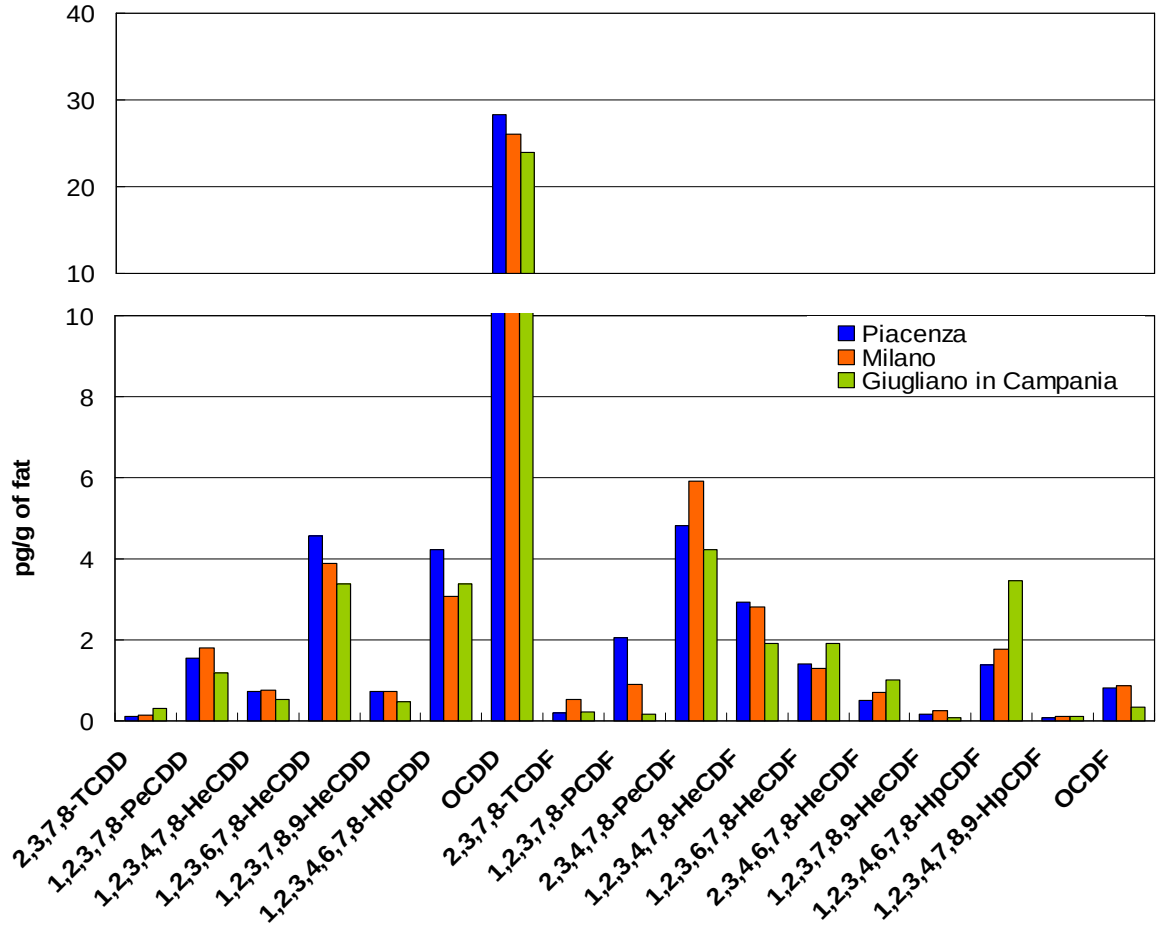


Figure 18. PCDD/Fs congener profiles in breast milk from Piacenza, Milano and Giugliano in Campania

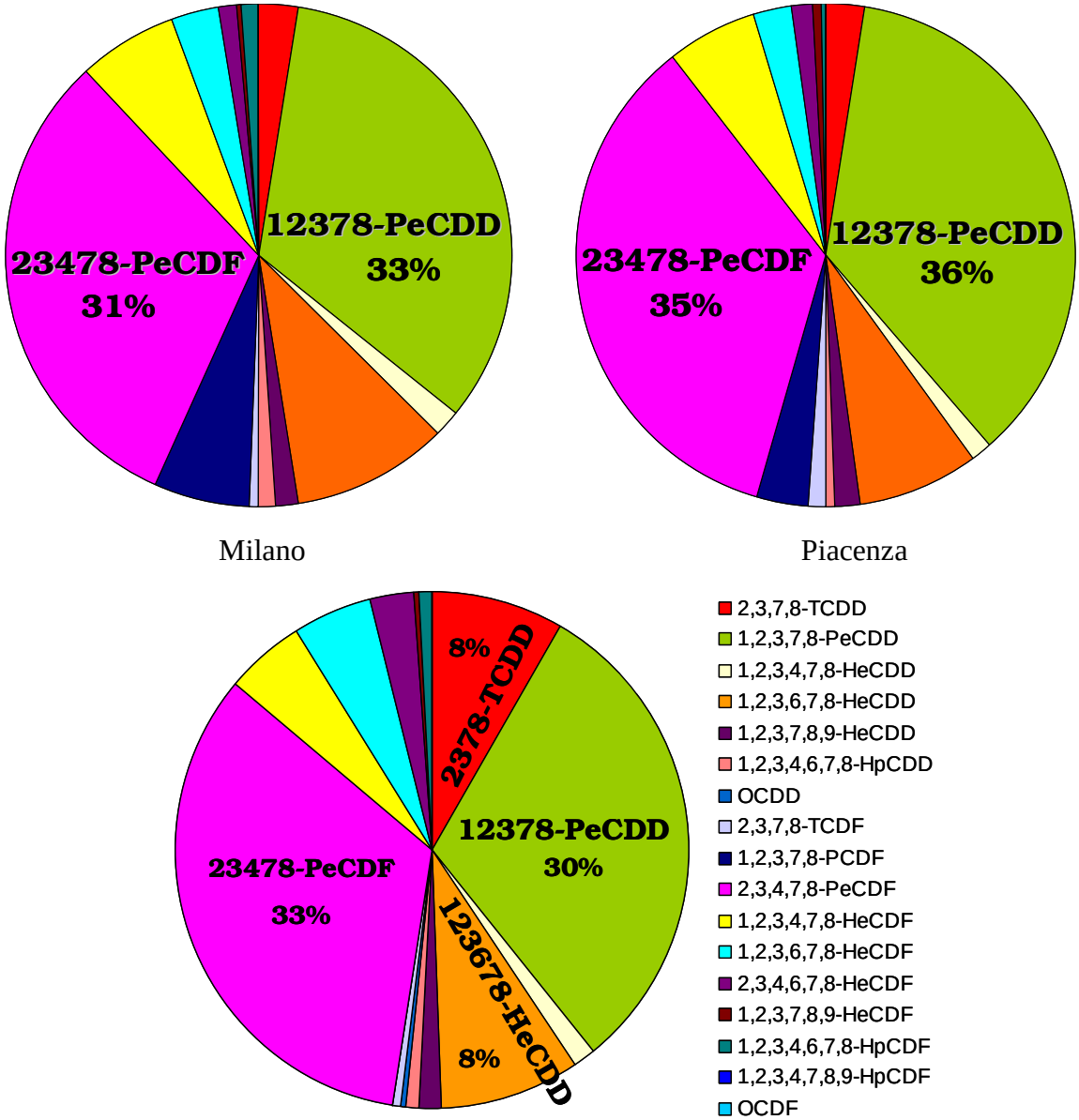


Figure 19 PCDDsFs contribution to total TEQ

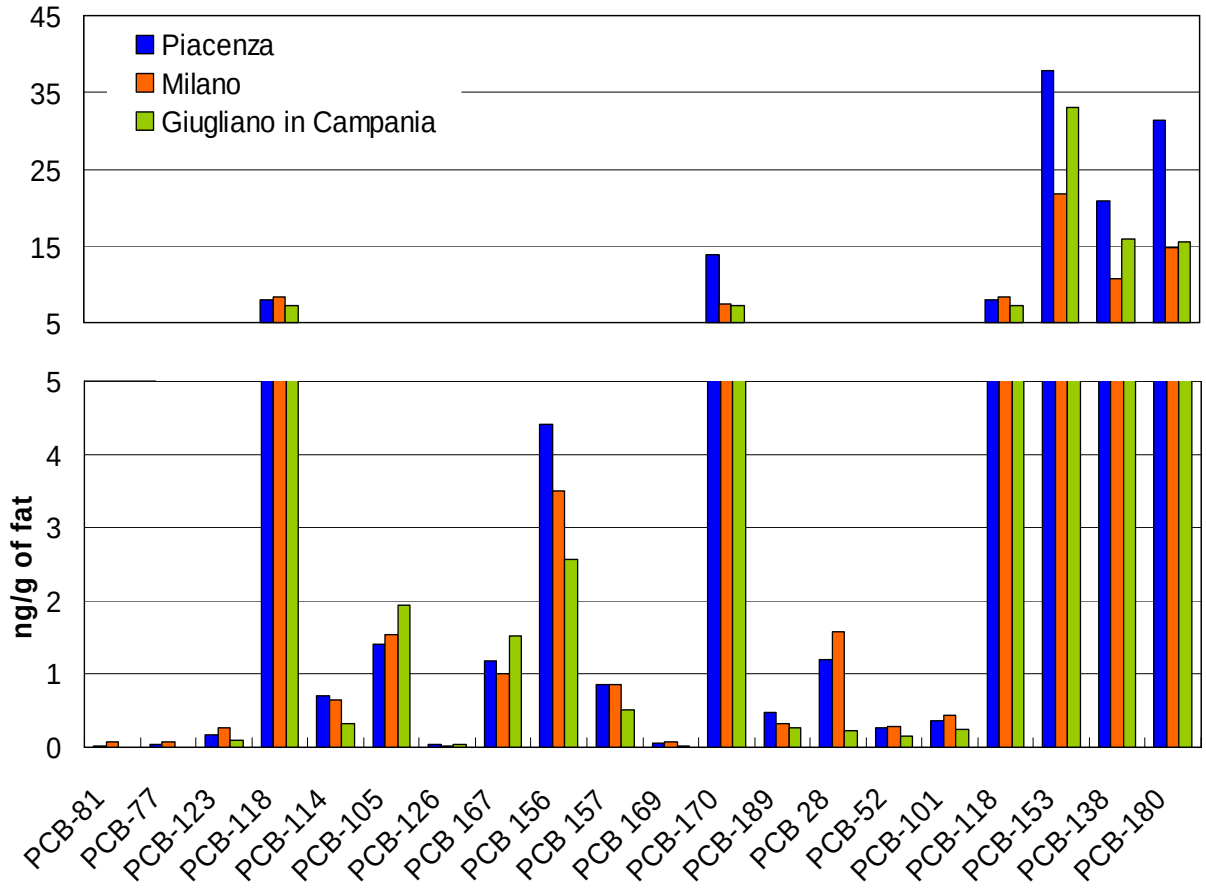


Figure 20 DL-PCB and PCB Indicators in milk samples from Piacenza, Milan, and Giugliano in Campania

Principal Component Analysis was performed to investigate the relationships among PCBs and PCDDs/Fs concentrations and profiles in milk and some variables, including location, diet, age, and smoking habits of donors. A significant correlation was found only between PCBs in milk and location. The score and loading plots are shown in Figure 21 A and B. The first, second and third principal components accounted for respectively 34%, 16% and 12.7% of the variability of the data set. The first component was positively influenced by PCB-123, PCB-157, PCB-77, PCB-81, and PCB-52, and negatively influenced by PCB-138. The second component was positively influenced mainly by PCB-118, whereas PCB-189 and PCB-153 were related to the third component. Score plots (Figure 21A) allowed the identification of at least two clusters. Samples marked “G” (from Giugliano) were all grouped in cluster 1 and were separated through the first and third component. Samples grouped in cluster 2 were of different origin, mostly from Piacenza but some also from Milan. The second component was responsible for the separation of only few samples collected in Milan and Piacenza from the others (Figure

21A, dotted line circle). When the first principal component is plotted against the third, three groups of samples can be better distinguished, and cluster 1 is clearly separated (Figure 21B). The PCB profile was similar in samples from Milan and Piacenza but differed in samples from Giugliano. This explains the grouping of all the samples from Giugliano in cluster 1 of the PCA score plots, and the presence of mixed samples from the other two cities in cluster 2 (Figures 21A,B). The major differences in the samples from Giugliano were given by PCB-123 and PCB-157 which occurred at very low concentrations and by PCB-81 and PCB-77 which were frequently at concentrations below the LOD. Congener PCB-189 was also detected at lower concentrations in milk samples from Giugliano and this might explain the cluster separation in the third principal component (Figure 21B). Overall the Principal Component Analysis showed that samples collected in Giugliano were homogenous for their PCB profiles, whereas samples collected in Piacenza and Milan had greater differences.

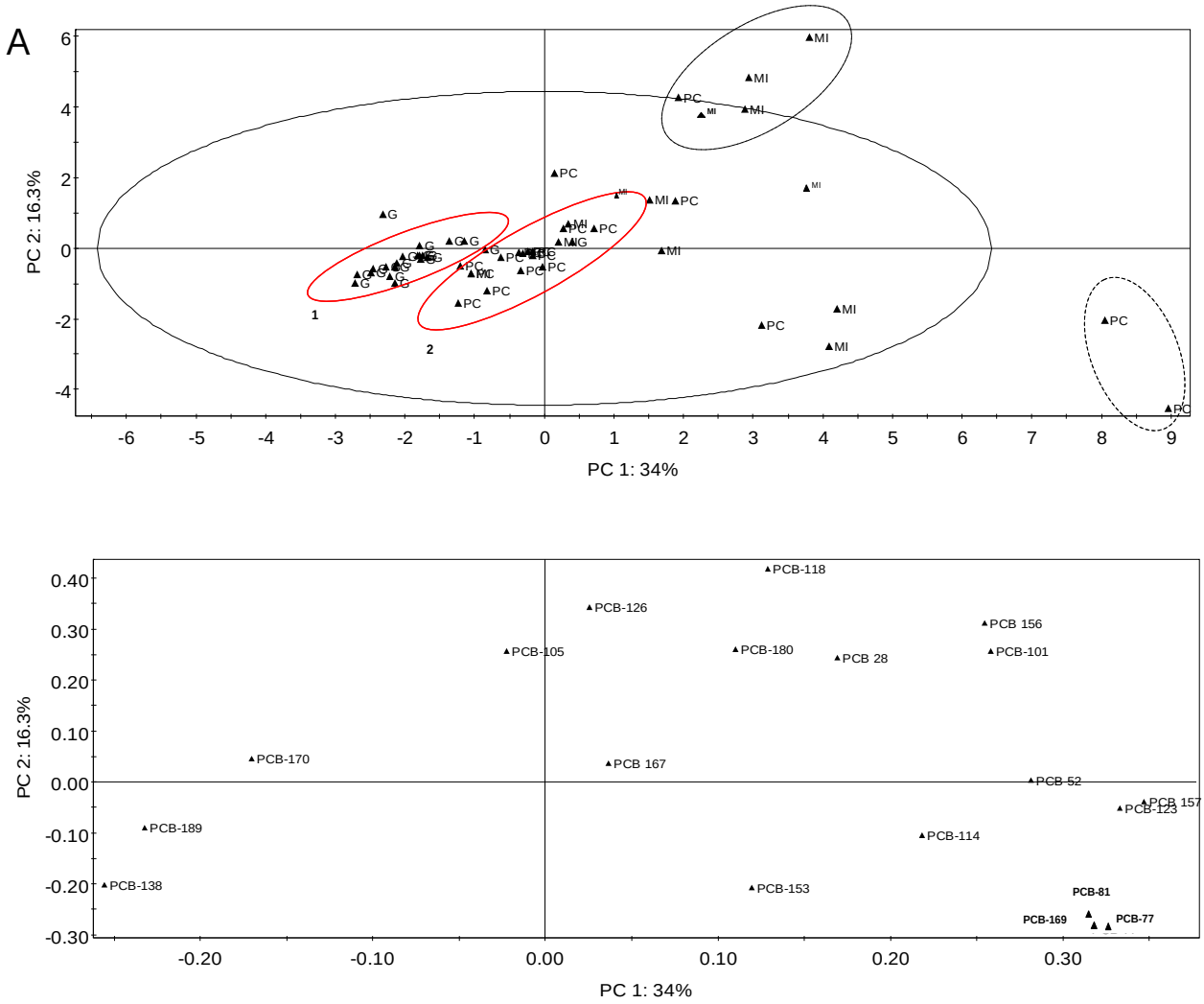


Figure 21 A PCA score and Loading plots of 59 samples and 19 viariables (19 PCB congeners). First and second PC;

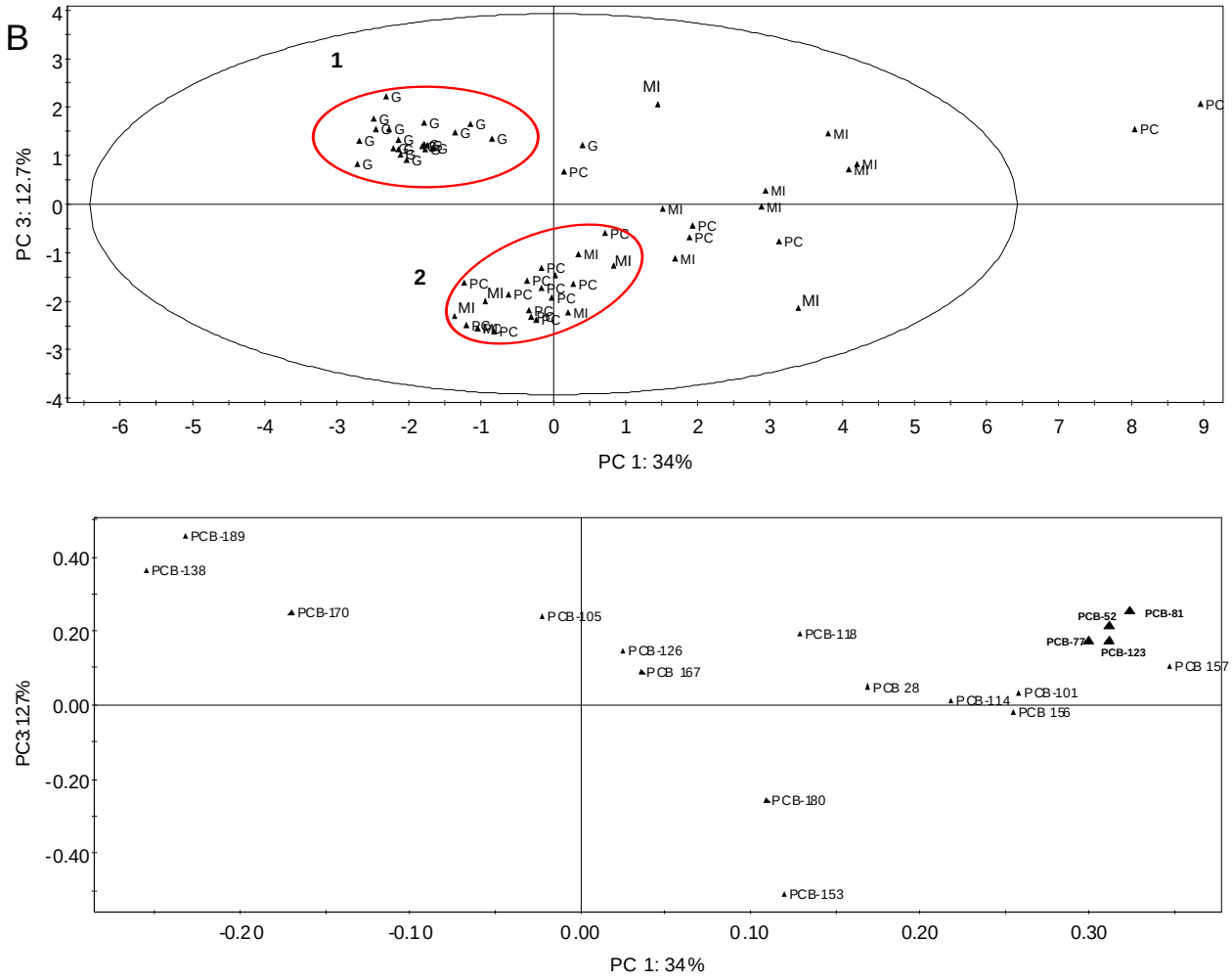


Figure 21B PCA score and Loading plots of 59 samples and 19 viariables (19 PCB congeners) First and third PC

Effect of diet and age on PCDD/F and PCB levels in breast milk

Samples collected from the different locations were grouped in order to analyse the effect of diet and age on contaminants level and profile. Based on questionnaire information, donors were divided into groups considering age (age groups 20-30 and 30-40 years) and dietary habits (high or low fish and meat consumption). PCDD, PCDF and PCB mean concentrations in milk samples from these subgroups are presented in Table 16. Age was significantly related to PCDD/F concentrations and older mothers had higher level of PCDDs/Fs in their milk than younger mothers (65.9 pg/g fat and 47.7 pg/g fat respectively, p=0.03). Statistically non-significant differences were observed for PCBs in

milk (4.98 and 5.36 ng WHO-TEQs respectively for young and old mothers; Table 19 and Figures 22A,B). No significant correlations were observed between consumption level of meat and fish and concentrations of these contaminants in breast milk.

Other dietary variables collected through the food-frequency questionnaire, such as consumption of milk and dairy products, vegetables, and fruits, and other potential variables such as the smoking habits and the delivery history of the donors, could not be investigated because of an insufficient number of subjects in some experimental groups.

PCDDs/Fs pg/g of fat	**20-30 year old**	**30-40 year old**	**Meat HC**	**Meat LC**	**Fish HC**	**Fish LC**
mean	47.67	65.8	56.19	37.24	46.50	52.39
St.Dev	21.85	35.16	29.91	0.62	21.65	22.66
Rel.St.Dev	0.45	0.53	0.53	0.02	0.47	0.43
Std.Err	4.37	7.67	5.29	0.36	5.41	9.25
p value	0.03		0.29		0.58	
PCBs ng/g of fat	**20-30 year old**	**30-40 year old**	**Meat HC**	**Meat LC**	**Fish HC**	**Fish LC**
mean	87.08	107.58	104.77	99.13	104.43	82.06
St.Dev	41.69	65.93	50.39	53.95	48.46	30.69
Rel.St.Dev	0.48	0.61	0.48	0.54	0.46	0.37
Std.Err	8.18	14.05	8.77	24.13	0.11	13.73
p value	0.19		0.82		0.34	
PCDDs/Fs pg TEQ-WHO	**20-30 year old**	**30-40 year old**	**Meat HC**	**Meat LC**	**Fish HC**	**Fish LC**
mean	3.94	4.90	4.22	4.29	4.28	4.08
St.Dev	1.94	1.72	2.02	0.94	2.30	0.70
Rel.St.Dev	0.49	0.35	0.48	0.22	0.54	0.17
Std.Err	0.38	0.38	0.30	0.55	0.57	0.31
p value	0.07		0.95		0.84	
PCBs pg WHO-TEQ Eq.	**20-30 year old**	**30-40 year old**	**Meat HC**	**Meat LC**	**Fish HC**	**Fish LC**
mean	4.99	5.37	4.94	5.33	5.09	4.48
St.Dev	2.61	2.62	2.51	3.84	2.34	2.14
Rel.St.Dev	0.52	0.49	0.51	0.72	0.46	0.48
Std.Err	0.51	0.57	0.46	1.72	0.59	0.96
p value	0.61		0.76		0.61	

Table 19. Correlations between age and dietary habits of donors and concentrations of dioxins and PCBs. Abbreviations: St.Dev – standard deviation; Rel.St.Dev – relative standard deviation; Std.Err – standard error; p-value – student t-test; HC indicate high consumption ,LC indicate low conssuption

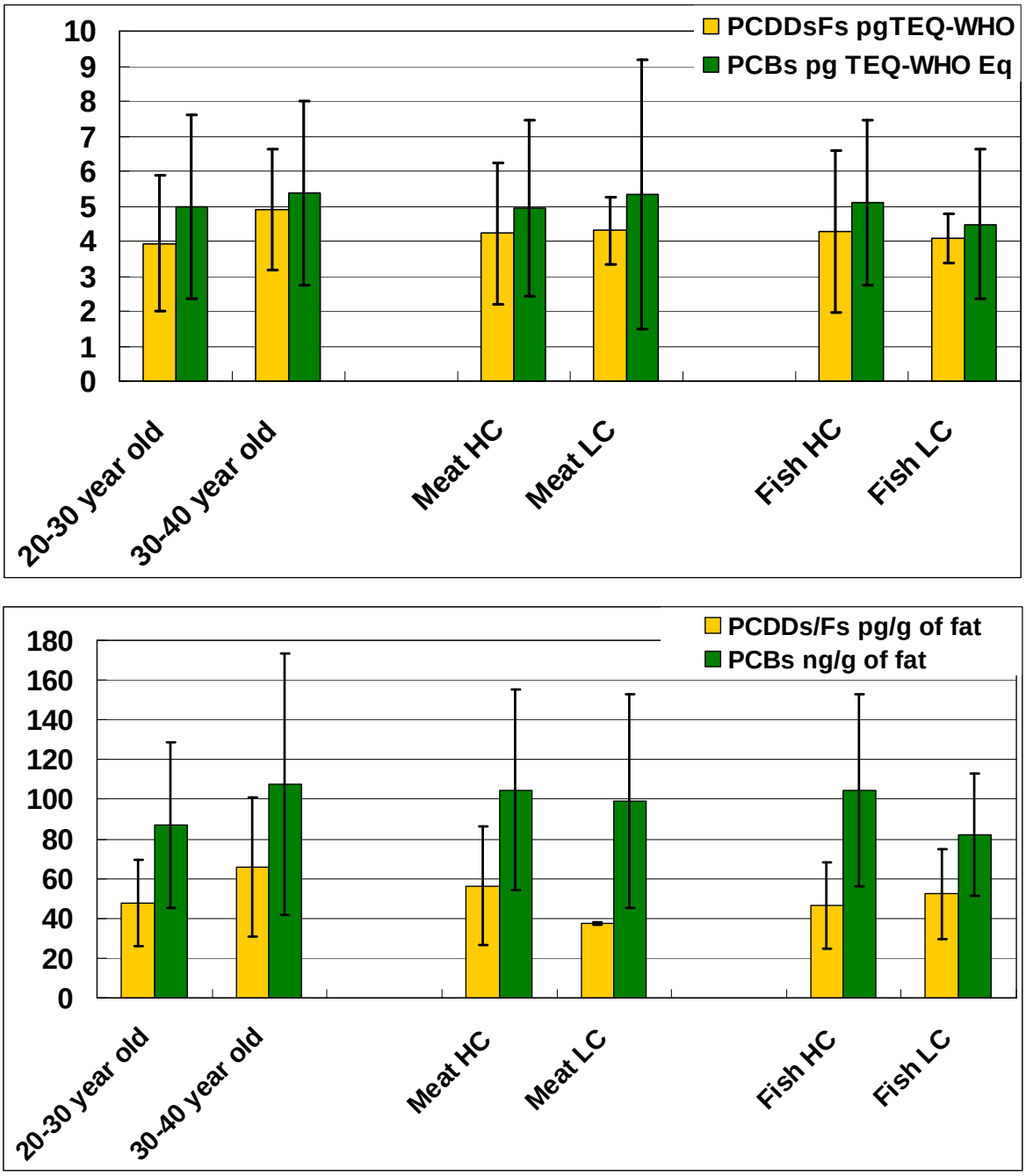


Figure 22 Correlations between age and dietary habits of donors and concentrations of dioxins and PCBs A expressed in pgTEQ-WHO , B in pg/g fat

6.7 DISCUSION

The main aim of this study was to investigate if the burning of wastes on open-air fires, as frequently occurs around the city of Giugliano, had resulted in an increase of PCDDs and PCDFs in the breast milk of local mothers. Milk samples of donors recruited in

Giugliano were compared with those of donors from two cities with different sources of pollution, Piacenza and Milan, for their PCDD and PCDF levels and profiles. PCBs were also measured as another group of polycyclic aromatic compounds entering the human body mainly via the food chain, as for PCDDs and PCDFs, but which are not produced by combustion. Other possible confounding variables such as diet, age and smoking habits of donors were also investigated.

Neither PCDD and PCDF levels nor WHO-TEQs in milk samples collected in Giugliano were significantly lower than those taken in Piacenza and Milan, indicating a lack of effect of open-air burning of wastes on the accumulation of these contaminants in breast milk. PCB levels and WHO-TEQ values of DL-PCBs were also slightly higher in samples from Piacenza and Milan than in those from Giugliano. However, apart from these slight differences, the findings were generally homogeneous and this allows us to conclude that these levels measured in breast milk resulted from a typical dietary intake of a general non-occupationally exposed population. The higher content of PCDDs/Fs and PCBs in milk samples collected in Milan and Piacenza might be a result of a greater contamination related to industrial activities or vehicular traffic in these cities but might be also related to the older age of the donors, at least in Milan (Table 17 and 18). Indeed our study confirmed the existence of a relationship between levels of these contaminants in breast milk and the age of the mothers, as reported by others (Sasamoto et al., 2006; Kunisue et al., 2006).

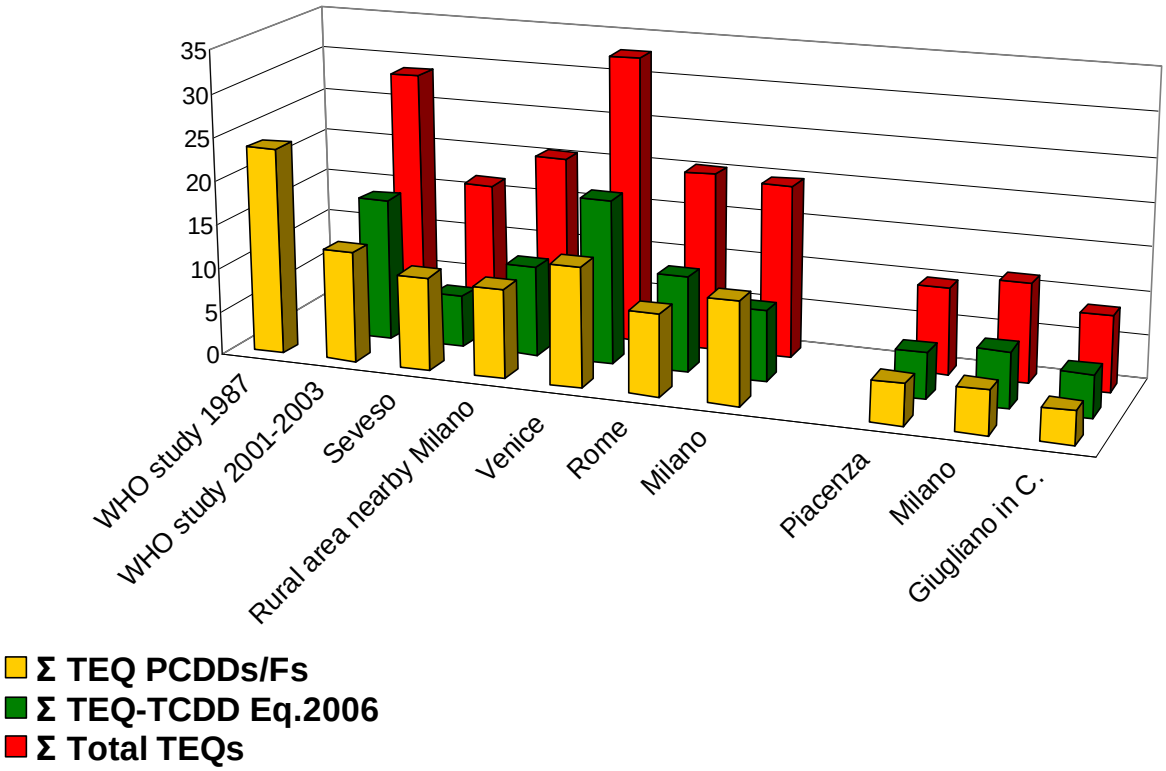


Figure 23 Comparison of PCDDs PCDFs and PCBs in breast milk from different Italian cities with recent study

Previous surveys of PCDDs/Fs and PCBs in human milk in Italy have been conducted in Rome and Venice in 1998-2001 (Ingelido et al., 2007; Abballe et al., 2008), in Milan and Seveso in 2000-2001 (Weiss et al., 2003) and in Milan in 2000-2001 during the second series of the international exposure study carried out by the WHO (Van Leeuwen et al., 2002). PCDDs/F and PCB levels in breast milk measured in this study were lower than those reported in these previous studies (Figure 23), and this study confirms the existence of a time-related trend of decrease of these contaminants in human milk, with a 57% and 20.5% decrease of the WHO-TEQ values for PCDDs/Fs and DL-PCBs respectively in the last 10 years (Weiss et al., 2003) and an 80% decrease in the last 20 years (WHO, 1987). In conclusion, mothers from Piacenza, Milan and Giugliano in this study seem to be exposed to lower doses of PCDDs/Fs and PCBs; and this might be a result of strict regulations and restrictions on the use and production of these compounds, as previously noted (Uehara et al., 2006; Noren et al., 2000).

Pg/g of fat	LOD (pg/g of fat)	Piacenza (22 samples)				Milan (16 samples)				Giugliano in Campania(21 samples)			
		mean	range	S.D	pg WHO-TEQ	mean	range	S.D	pg WHO-TEQ	mean	range	S.D	pg WHO-TEQ
2,3,7,8-TetraCDD	0.01	0.11 (22)	0.04-0.51	0.10	0.11	0.13 (12)	0.04-0.35	0.10	0.13	0.32 (14)	0.05-1.38	0.39	0.32
1,2,3,7,8-PentaCDD	0.02	1.54 (2)	0.14-2.89	0.70	1.54	1.81 (1)	0.35-4.31	1.15	1.81	1.17 (4)	0.10-3.32	0.83	1.17
1,2,3,4,7,8-HexaCDD	0.01	0.72 (3)	0.11-3.27	0.71	0.07	0.75 (3)	0.07-2.79	0.72	0.07	0.53 (6)	0.07-1.63	0.38	0.05
1,2,3,6,7,8-HexaCDD	0.01	4.57	0.75-14.10	2.98	0.46	3.90	1.76-10.12	2.19	0.39	3.37	1.50-9.23	1.68	0.33
1,2,3,7,8,9-HexaCDD	0.01	0.72 (3)	0.09-2.32	0.51	0.07	0.72 (3)	0.20-1.33	0.40	0.07	0.49 (6)	0.05-1.53	0.39	0.05
1,2,3,4,6,7,8-HeptaCDD	0.01	4.23	0.62-16.79	4.42	0.04	3.08 (2)	0.25-6.72	1.87	0.03	3.38 (2)	0.08-7.67	2.16	0.03
OctaCDD	0.01	28.29	5.18-110.83	17.70	0.01	25.99	8.90-41.30	10.09	0.01	23.89	10.46-58.31	11.25	0.01
2,3,7,8-TetraCDF	0.02	0.20 (16)	0.02-1.24	0.31	0.02	0.55 (8)	0.03-2.69	0.92	0.05	0.24 (12)	0.03-0.88	0.25	0.02
1,2,3,7,8-PentaCDF	0.02	4.46 (7)	0.03-32.00	2.03	0.13	0.89 (5)	0.05-3.24	1.01	0.03	0.16 (16)	0.03-0.84	0.20	0.12
2,3,4,7,8-PentaCDF	0.02	4.81	2.45-8.90	1.64	1.44	5.90	2.16-11.67	2.75	1.77	4.23	1.72-9.94	2.07	1.27
1,2,3,4,7,8-HexaCDF	0.01	2.94	0.04-8.59	2.30	0.29	2.81 (2)	0.15-7.37	2.43	0.28	1.92	0.73-5.31	1.22	0.19
1,2,3,6,7,8-HexaCDF	0.01	1.40	0.54-3.47	0.76	0.14	1.29 (2)	0.15-3.78	1.06	0.13	1.92	0.60-6.15	1.31	0.19
2,3,4,6,7,8-HexaCDF	0.01	0.52 (8)	0.05-1.85	0.55	0.05	0.70 (6)	0.03-3.51	0.99	0.07	1.01 (4)	0.04-4.86	1.03	0.10
1,2,3,7,8,9-HexaCDF	0.01	0.16 (19)	0.03-1.64	0.35	0.02	0.26 (11)	0.04-2.01	0.56	0.03	0.09 (21)	0.03-0.23	0.05	0.01
1,2,3,4,6,7,8-HeptaCDF	0.03	1.37	0.56-5.40	1.36	0.01	1.77 (2)	0.05-4.88	1.37	0.02	3.48	0.40-36.02	7.61	0.03
1,2,3,4,7,8,9-HeptaCDF	0.03	0.08	0.02-0.25	0.06	0.0008	0.12 (10)	0.03-0.25	0.08	0.0012	0.11 (21)	0.03-0.30	0.06	0.0011
OctaCDF	0.03	0.82 (2)	0.07-3.09	0.66	0.0002	0.87 (2)	0.15-2.01	0.64	0.0003	0.34 (18)	0.01-2.63	0.55	0.0001
Classes of dioxins													
Total TetraCDD	0.01	0.13 (17)	0.04-0.51	0.83		0.25 (9)	0.08-0.66	0.19		0.40 (7)	0.06-1.38	1.08	
Total PentaCDD	0.02	1.66	0.44-2.89	0.38		1.84	0.73-4.31	1.11		1.38 (1)	0.16-5.16	2.04	
Total HexaCDD	0.01	6.94	1.88-18.48	0.63		5.82	2.74-15.76	3.48		4.81	1.74-12.14	2.07	
Total HeptaCDD	0.01	4.46	0.62-16.79	0.99		3.28 (2)	0.50-6.72	1.84		3.69	0.15-7.67	7.78	
Total TetraCDF	0.02	6.33 (3)	0.07-9.23	1.36		7.84	2.16-28.45	5.79		1.02 (1)	0.07-6.49	0.44	
Total PentaCDF	0.02	16.15	4.46-36.10	0.82		16.01	4.13-39.73	11.45		4.32	1.73-10.00	2.26	
Total HexaCDF	0.01	18.71	2.73-85.14	1.14		13.82	0.31-49.27	14.71		4.82	0.07-14.66	1.37	
Total HeptaCDF	0.03	14.19	1.25-74.36	1.27		5.20 (1)	0.09-14.12	4.60		5.36	0.16-37.64	3.51	

[ng/g of fat]		Piacenza				Milan			Giugliano in Campania		
Dioxin like PCBs	LOD (pg/g of fat)	mean	range	S.D		mean	range	S.D	mean	range	S.D
PCB-81	0.5	0.02 (17)	0.0002-0.30	0.07		0.08 (4)	0.0005-0.34	0.12	0.003 (10)	0.001-0.007	0.001
PCB-77	0.5	0.03 (11)	0.0002-0.31	0.08		0.07 (4)	0.0005-0.28	0.09	0.003 (17)	0.0003-0.021	0.004
PCB-123	0.5	0.16	0.03-0.45	0.13		0.26	0.15-0.73	0.17	0.09 (1)	0.01-0.28	0.06
PCB-118	0.5	7.95	3.53-22.03	5.25		8.34	4.04-25.04	6.95	7.30	2.03-16.14	3.43
PCB-114	0.5	0.70	0.17-2.70	0.61		0.64	0.03-2.84	0.80	0.32	0.12-0.78	0.16
PCB-105	0.5	1.41 (1)	0.0004-4.52	1.03		1.55	0.92-4.54	1.21	1.93	0.42-10.88	2.17
PCB-126	0.5	0.03 (8)	0.0003-0.09	0.03		0.03 (4)	0.0003-0.14	0.03	0.04 (2)	0.01-0.08	0.02
PCB 167	0.5	1.18	0.49-2.49	0.64		1.00	0.41-2.40	0.58	1.52	0.39-14.18	2.93
PCB 156	0.5	4.41	1.91-10.43	2.65		3.51	0.82-13.16	3.62	2.56	1.28-5.68	1.07
PCB 157	0.5	0.85	0.31-2.05	0.48		0.85	0.24-3.50	0.96	0.51	0.25-1.26	0.23
PCB 169	0.5	0.06	0.01-0.31	0.08		0.08	0.01-0.04	0.11	0.02 (4)	0.01-0.04	0.01
PCB-170	0.5	13.91	1.35-39.91	10.65		7.48	1.74-19.18	5.23	7.16	2.18-16.76	3.56
PCB-189	0.5	0.47	0.10-1.55	0.35		0.32	0.11-0.64	0.18	0.27	0.08-0.77	0.16
Indicator PCBs											
PCB 28	0.3	1.20	0.04-3.28	0.85		1.58	0.14-9.67	2.89	0.22 (3)	0.001-0.65	0.18
PCB-52	0.3	0.27	0.08-0.57	0.15		0.28	0.02-0.58	0.17	0.15 (2)	0.001-0.49	0.15
PCB-101	0.3	0.36	0.15-1.49	0.30		0.45	0.14-0.65	0.15	0.25	0.03-0.93	0.21
PCB-118	0.3	7.95	3.53-22.03	5.25		8.34	2.43-25.04	6.95	7.27	2.00-16.10	3.43
PCB-153	0.3	37.82	1.37-94.64	27.02		21.85	1.15-84.02	26.92	33.10	13.39-70.62	12.11
PCB-138	0.3	20.81	1.34-56.05	14.07		10.72	1.21-44.54	12.87	15.86	0.01-32.40	7.67
PCB-180	0.3	31.36	5.88-90.70	24.15		14.77	5.23-39.60	10.72	15.57	4.05-39.82	8.56
Classes of PCBs											
Total tri-CBs	0.3	1.31	0.32-3.28	0.77		2.49	0.46-9.67	3.37	0.84	0.02-2.94	0.75
Total tetra-CBs	0.5	6.10	0.09-15.19	4.46		5.66	0.36-19.94	5.65	5.32	1.94-16.12	3.83
Total penta-CBs	0.5	14.89	0.64-28.56	7.36		15.77	0.88-50.52	13.48	13.37	0.49-33.11	8.03
Total hexa-CBs	0.3	83.87	25.42-193.32	52.61		62.82	14.10-207.96	58.54	121.81	22.09-414.38	234.65
Total hepta-CBs	0.3	53.41	11.48-146.91	35.09		25.67	5.86-62.36	17.92	30.74	9.21-119.11	24.20

Table 18 Concentration of PCDDs/F and PCB congeners and classes of these compounds in human milk collected in Piacenza Milan and Giugliano. In parentheses is the number of samples below the limit of detection

7. PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELLING AND HUMAN BIOMONITORING STUDIES

Physiologically based pharmacokinetic (PBPK) models describe the human body as a network of compartments representing organs (e.g., blood, kidney, liver, brain, muscle and skin, placenta etc). They are defined by mathematic functions related to biochemical and physiological processes: absorption, distribution, metabolism and excretion. A PBPK model combines three categories of data: “the system’s data (physiological and anatomical properties); drug-specific data (e.g., absorption or metabolic rates); and the model structure, which refers to the arrangement of tissues and organs included in the model” (Bois et al 2010, Rowland et al 2004). Today PBPK models are commonly used in drugs development or risk assessment to predict the fate of substances in humans and the internal levels in the target tissues, i.e. where the effects of the substance arise. Recently, PBPK models have been introduced to interpret biomonitoring data (e.g., blood or urine concentrations) observed in the general human population. This association results in a powerful tool for the health risk assessment at individual or population level. Over the last decades, a number of researchers have investigated the range of internal doses that occur in humans from environmental exposures (Bois et al 1996, Spear and Bois 1994, Clewell et al 1999, 2000, 2004).

PBPK models can be used to estimate internal doses based on known external exposure (this process is called forward dosimetry), or can be used to estimate the exposure levels in the environment that could give rise to measured biomarker concentrations in a population and this process is called reverse dosimetry. (Clewell et al 1999, Aylward et al 2005, Stern et al 2005). These two approaches are complementary to each other and provide different perspectives on the implications of biomonitoring data from the perspective of human health risk. In this study, both approaches are used: reverse dosimetry to reconstruct the mother dose to PCB-153 and forward dosimetry to predict the tissues concentration in mother and the PCB-153 intake of breastfed children.

8. LIFETIME WHOLE-BODY PBPK MODEL of MOTHER and INFANT

The development of a PBPK model follows a four-step approach: model representation, parameterization, simulation and validation. The PBPK model used in this study was described previously (Beaudouin et al 2010), but was modified to integrate refined information on the chemistry and physiology of breast milk during lactation. Additionally prenatal transfer mother-fetus via placenta was improved thank to application of new placenta composition during fetus development, and new partition coefficient placenta: blood.

8.1 Model presentation

The structure of the lifetime model for mother and infant was developed by Beaudouin et al (2010). The human body is described as a tissues network and consists of 22 compartments representing organs (lungs, adipose tissue, adrenals, bones, brain, breast, pancreas, spleen, stomach, gut, liver, sexual organs, heart, skin, marrow, muscles, thyroid, urinary tract, kidneys). To describe the pregnancy and fetal developments (placenta, amniotic fluid and fetus), 19 compartments were added. All compartments represent a specifi organ or tissue exept the urinary track and sexual organs compartments. The urinary track includes the bladder, the ureters and the urethra. The sexual organs compartments for woman includes ovaries the fallopian tubes and the uterus. The scheme of the model is presented in Figure 24. All organs are assumed to be well mixed and blood-flow limited. Exchanges with the fetus occur via the sexual organs compartment. Absorption of PCB 153 was assumed to be 100%. Elimination occurs via metabolism, urinary and fecal excretion exhalation and milk production.

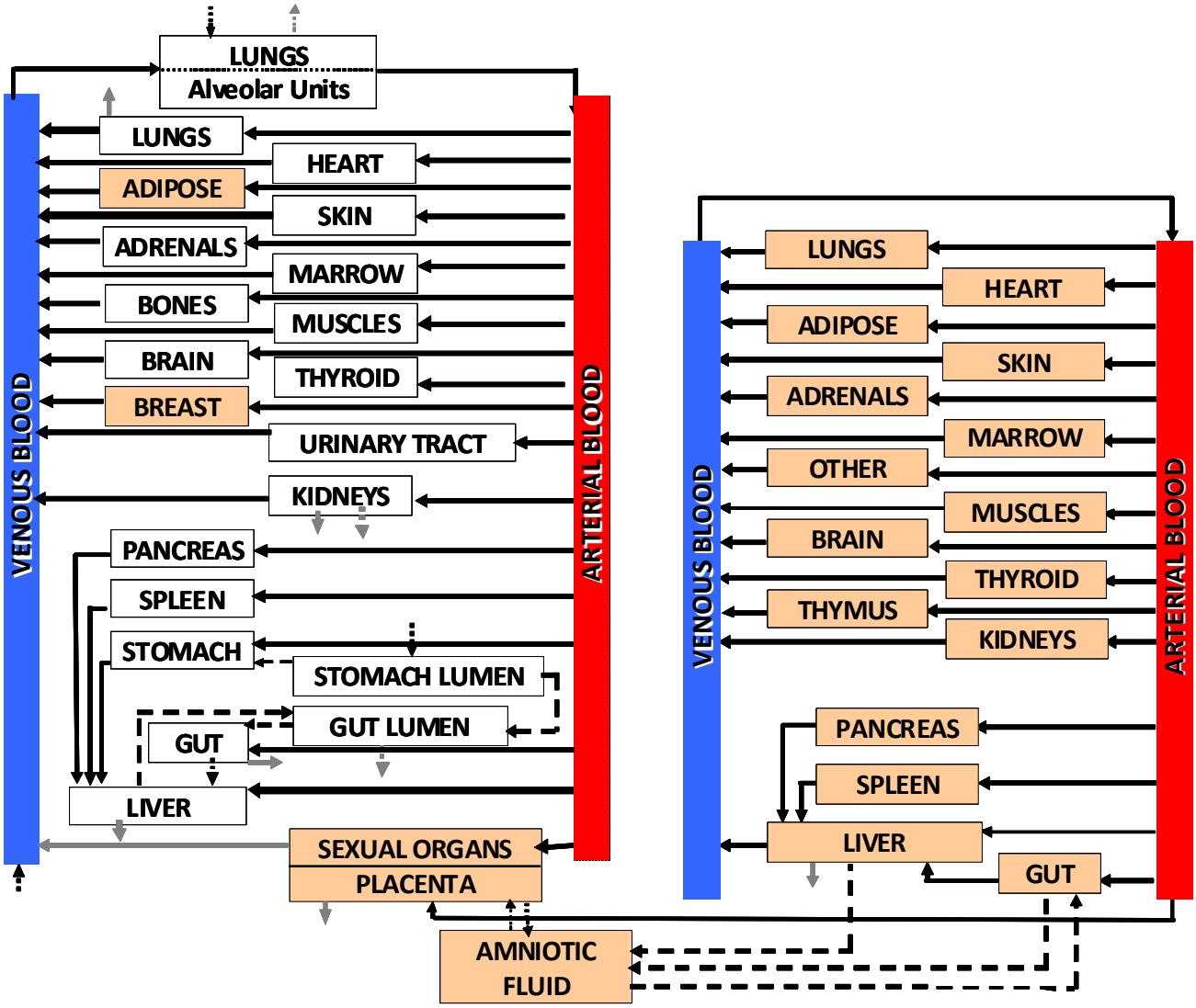


Figure 23 Scheme of PBPK model (Beaudouin et al 2010)

Absorption

PCB153 inputs directly to the liver, and absorption in gut was assumed to be 100%. Inhalation takes place in arterial compartment via lungs, however in case of persistent organic pollutants is the minor exposure pathway.

Distribution

Distribution is managed by blood flow and tissue:blood partition coefficients. Several mass balance equations were established to describe the quantity of chemical in each compartment which based on general dependence (Eq. 1):

$$\frac{dQ(t)}{dt} = F_i \times \left(C_{art}(t) - \frac{C_i(t)}{PC_i} \right) \quad \text{(Eq. 1)}$$

where: Q is the quantity of chemical in each compartment, F_i the blood flow entering compartment *i*, C_{art} – the concentration in arterial blood, C_i *the* concentrations in the tissue *i*, PC_i – the tissue *i*: blood partition coefficient

Metabolism and Excretion

PCBs are poorly metabolized and elimination of unchanged chemicals was assumed. The elimination in liver via biliary excretion is considered as the main excretion route and the urinary excretion, in case of PCBs, can be skipped. Metabolism and biliary excretion are parameterized from half-life values (for PCB-153: 27.5 years). These processes were assumed to depend on age but not on gender. A first order model was defined. The intrinsic clearance value per kilogram of liver (CL_{intC}) is given by:

$$CL_{\text{int}_C} = \left(\frac{Eh_{age} \times Ql_{age}}{1 - Eh_{age}} \right) / Vl_{age} \quad \text{(Eq. 2)} \qquad \text{Where: } Eh_{age} = CL_{age} / Ql_{age} \quad \text{(Eq2.1)}$$

$$Vd_{age} = \left(\sum P_{age} \times Vt_{age} \right) + Vb_{age} \quad \text{(Eq2.2)}$$

$$CL_{age} = \left(\ln 2 / HL(h) \right) \times Vd_{age} \quad \text{(Eq2.3)}$$

And:
Ql - the blood flow to the liver,
Vl - volume of the liver,
CL - clearance for the studied compound,
Vd - the volume of distribution,
P - tissue:blood partition coefficient,
Vt - the volume of tissues
Vb - the volume of blood.

Additionally excretion through the breast milk was managed by milk flow out of the mammary tissue compartment and milk:blood partition coefficient.

$$\frac{dQ_{milk}}{dt} = F_{milk} \times \frac{C_{breast}(t)}{PC_{breast}} \times PC_{milk} \quad \text{(Eq.4)}$$

8.2 Parameterization

Quantitative relationships between the tissues volumes, BW and other parameter with age were described previously (Beaudouin et al 2010), and included effects of aging and athropy on muscles or adipose tissues.

Partition coefficients tissue:blood

Tissues:blood partition coefficients represent an important set of input parameters for PBPK models. They describe the concentration of the substance in a target tissue to its concentration in blood under equilibrium conditions. For PCB-153, partition coefficients tissue: blood were estimated using Parham's approach (Parham et al 1997). This Quantitative Structure-Activity Relationship (QSAR) has been developed only for PCBs and relies on the structural information of the molecules (See Eq.5-8). Several structural parameters such as UNS - an indicator of whether the congener has a ring on which adjacent *meta* and *para* carbons are not chlorine- substituted or NPL - a variable describing the nonplanarity of the PCB, were selected as predictors of the affinity of PCBs to tissues (see Table 20 for the set of the structural parameters). The first step was to estimate, from the structural descriptors, the fat:plasma partition coefficient (PC_{fp}), and than to convert it to fat:blood partition coefficient (PC_{fb}).The fat:plasma partition coefficient is obtained from:

$$\text{Log}(PC_{fp}) = 1.9988 - 0.5004 \times \text{UNS} + 0.1793 \times \text{NPL} + 0.05931 \times \text{DIFF}^2 \qquad \text{(Eq. 5)}$$

The ratio plasma:blood (r_{pb}) is used to convert the fat:plasma partition coefficient into the fat:blood partition coefficient:

$$PC_{fb} = PC_{fp} \times r_{pb} \qquad \text{(Eq. 6)}$$

$$r_{pb} = \frac{1.65 - 0.31 \times NUNMP}{1.24 + 0.07 \times NUNMP} \qquad \text{(Eq. 7)}$$

$$PC_{fb} = 10^{\left(1.9988 - 0.5004 \times \text{UNS} + 0.1793 \times \text{NPL} + 0.05931 \times \text{DIFF}^2\right)} \times \frac{1.65 - 0.31 \times NUNMP}{1.24 + 0.07 \times NUNMP} \qquad \text{(Eq. 8)}$$

Parameters	Descritption	Values	Parameters	Descritption	Values
NOC	Number of *ortho* (2, 6, 2*, or 6*) chlorines	2	R1	is 1 if there is a ring with 0 or 1 chlorines, 0 otherwise	0
OC	is 1 if NOC >0, 0 otherwise	1	NUNMP	Number of adjacent non-chlorine-substituted *meta–para* carbon pairs	0
NPL	is NOC if NOC <2,is 2 otherwise	2	UNS	is 1 if NUNMP >0, 0 otherwise	0
NMC	Number of *meta* (3, 5, 3*, or 5*) chlorines	2	UNMP	is 1 if NUNMP>1, 0 otherwise	0
NPC	Number of *para* (4 or 4*) chlorines	2	ACARB	Largest number of adjacent non-chlorine-substituted carbons	4
NCL	Total number of chlorines	6	AC	Largest number of adjacent chlorines	4
R MAX	Number of chlorines on most-substituted	3	ACM	is AC-1 if ACM>0, 0 otherwise	3
R MIN	Number of chlorines on least-substituted	3	NUNMP	Number of adjacent non-chlorine-substituted *ortho–meta* carbon pairs	0
DIFF	RMAX-RMIN	0			

Table 20. Structural parameters for Parham's approach (Parham et al 1997)

The partition coefficients for tissues other than fat were determined by multiplying PC_{fb} with an adjustment factor depending on the lipid content of the tissue. The adjustment factor was proposed by Poulin and Krishnan (1995). They considered that the lipophilicity – hydrophilicity behaviour of phospholipids might be approximated by the fractional additivity of neutral lipids 30% and water 70% solubility characteristics (Poulin Krishnan 1995, Williams and Tung 1979). The quantity of phospholipids can then be transformed into neutral lipids equivalent and water equivalents according to equation below:

$$L_{\text{tot tissue}} = F_{NL} + 30\%\ F_{PL} \qquad \text{(Eq. 9a)}$$

$$W_{\text{tot tissue}} = F_{W} + 70\%\ F_{PL} \qquad \text{(Eq. 9b)}$$

$$AF_{\text{adj.tissue}} = L_{\text{tot tissue}}/L_{\text{tot adipose}} \qquad \text{(Eq. 10)}$$

Where:

$AF_{\text{adj.tissue}}$	Adjustment Factor for tissue
$L_{\text{tot tissue}}$	Total lipid content of tissue
$L_{\text{tot adipose}}$	Total lipid content of adipose tissue
F_{NL}	Fraction of neutral lipids
F_{W}	Fraction of water
F_{PL}	Fraction of phospholipids

Since the composition of tissues changes from childhood to adulthood, the values of the partition coefficients tissue:blood may be modified. A literature overview was performed in order to extract the most accurate lipids concentration with the special attention putted on

young infant tissues. The innovation in this study is the application the Parham approach to the tissues in early age after birth.

Figure 24 presents the lipid content of several tissues and corresponding partition coefficients.

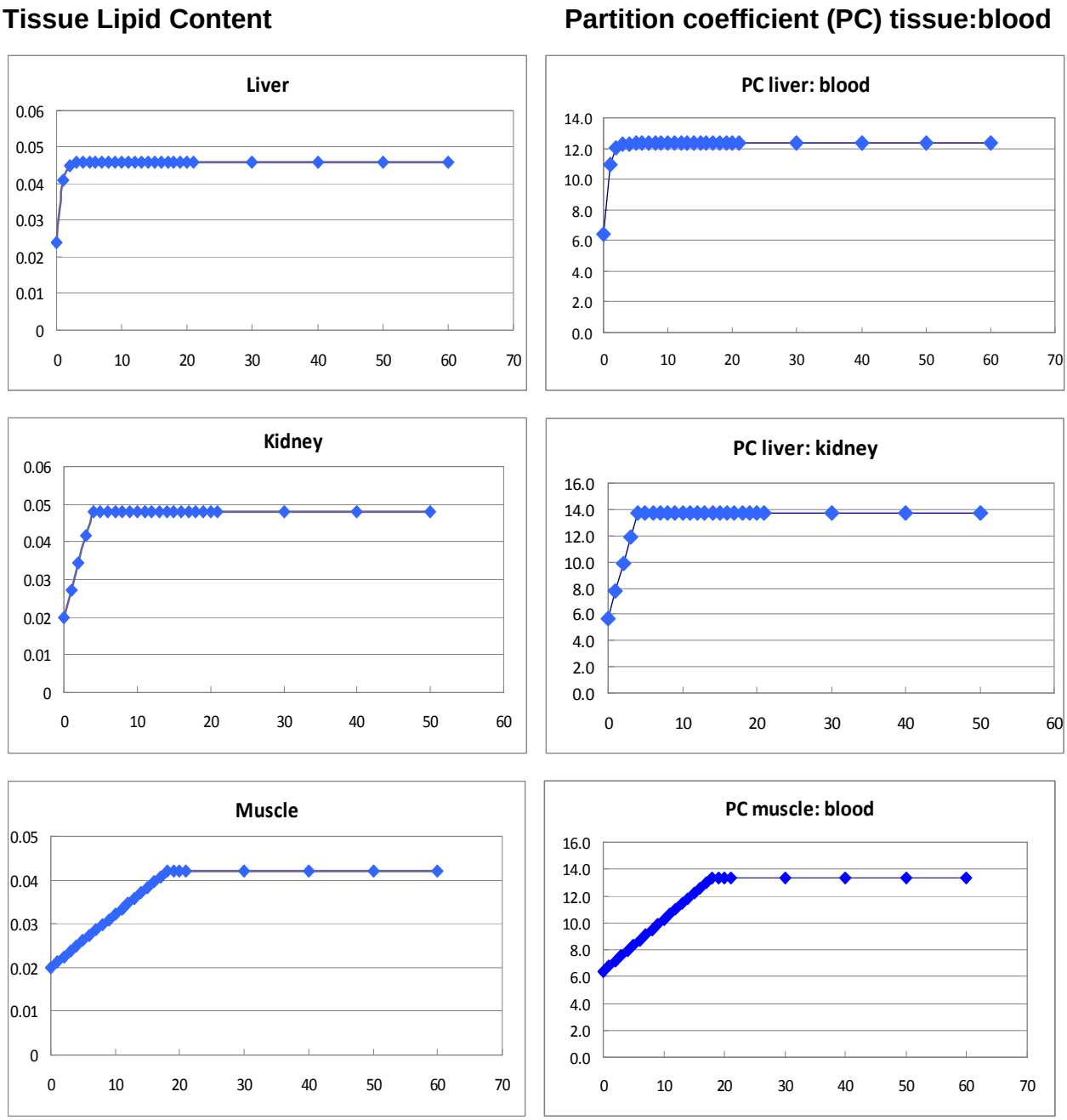


Figure 24. Fat content and partition coefficient for kidney, muscle and liver tissues

Maternal parameter and model inputs for nursing variables

Maternal parameters were taken from the previously published model (Beaudouin et al 2010) with important modification of secretion of milk during lactation. Lactogenesis in a limited way appears already during pregnancy. Secretion before delivery is called stage I, and copious secretion after delivery - stage II. Initiation of the secretion does not depend on suckling by the infant, but its rate declines when milk subtracted is not practiced (Wooldridge et al 1985). During stage I, approx. 30 mL of colostrum per day can be secreted but as this secretion cannot be removed by suckling, the components are reabsorbed into the mother's blood stream. Stage I of lactogenesis was not then modelled. During stage II, the composition of milk and the volume of milk excreted per day evolve. Thus several parameter were modified form our previous model (Beaudouin et al 2010). Milk excretion from the mother and intake by infant can be expressed as a function of infant's age (Neville et al 1988, Arcus-Arth et al 2005 and Verner et al 2009). Figure 25 presents the milk yield and infant milk intake during 400 days after delivery (Neville et al 1988)). We considered a 6 months period of exclusively breastfeeding and partial weaning for another 6 months. The evolution of the milk production is in agreement with a decrease in milk volume when the infant is weaning or supplementary food is included in his diet.

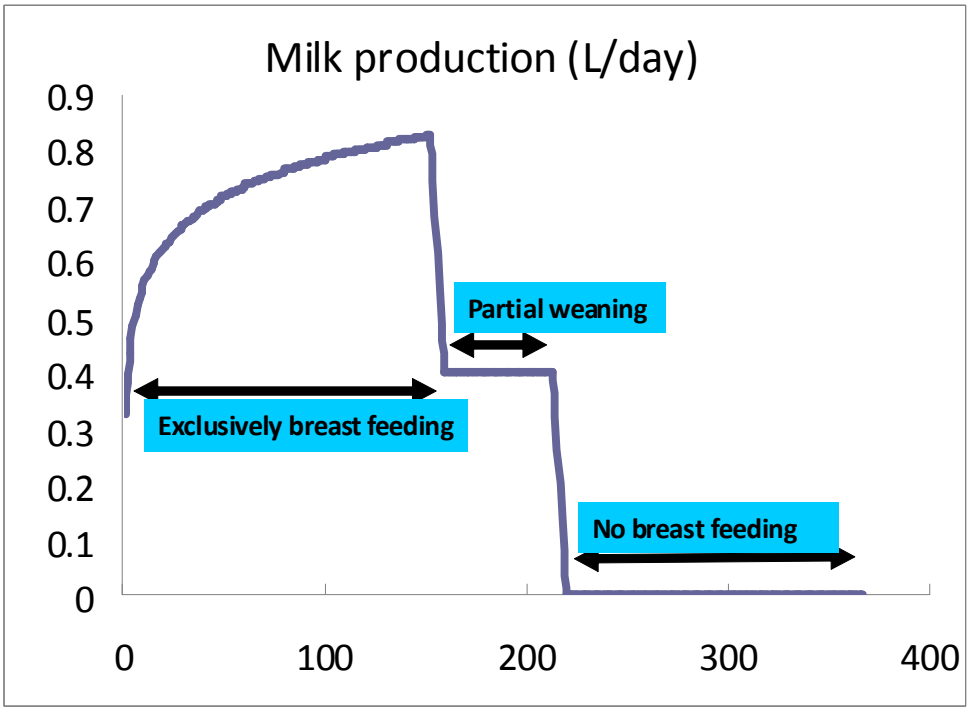


Figure 25. Correlation between infants age (in age) and milk intake during lactation

Similar modifications were applied to describe the milk composition during lactogenensis (Paul et al 1997, Michaelsen et al 1990,1994, Guerrini et al 1981, Dewey et al 1984, Mitoulas et al 2002, Bitman et al 1984, 1986, 1989, Kent et al 1999, 2006 , Underwood et al 1970, Crawford et al 1976, Larnkjaer et al 2006, Mandel et al 2005, Woodward et al 1989). The lipids and phospholipids concentrations in milk change during lactogenensis, and then may impact the affinity of PCB-153 to milk (partition coefficient milk:blood). Information on the milk composition was integrated in our model to describe these changes. Figure 26 presents the lipid content during lactation as a function of time (days), and the impact on the partition coefficient.

Tissue Lipid Content **Partition coefficient (PC) tissue:blood**

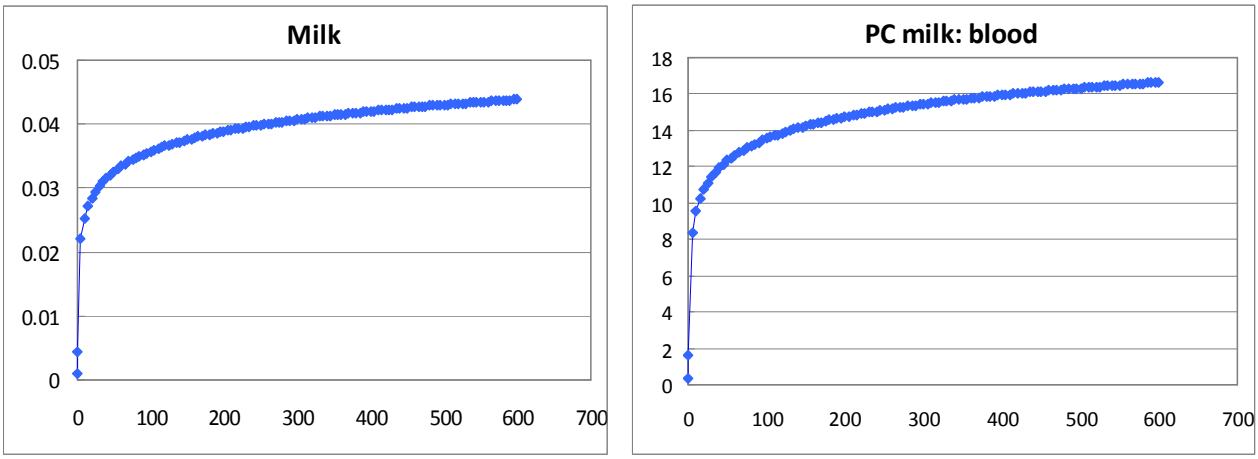


Figure 26 Milk fat content and partition coefficient milk: blood in function of time in days

8.3 Bayesian analysis to estimate the exposure and some key parameters

8.3.1 Estimation of key parameters from an independent dataset

Before using the PBPK model to reconstruct the exposure of the Italian women, we compared the model's predictions to an independent dataset obtained from literature: German survey on couples: mother child from 1996 (Abraham et al 1996). This study was selected because it contains a complete description of the infants' exposure and the sampling of the different tissues (venous and cord blood, milk, and placenta). This study contained also wide range of tissues measurements performed for individuals (couple mother child), which is crucial to verify the behavior of the PBPK model.

First we parameterized the PBPK model and the exposure model as defined above and run the models for the German women. However the predictions were far from the concentrations observed in those women (results not shown). We then selected few parameters to estimate, for which a high uncertainty affects the estimates from previous knowledge (*e.g.,* literature). Four parameters were selected: the diffusion rate between the mother's uterus and the placenta, scaling factors for blood:milk and blood:placenta partition coefficients and a scaling factor for the daily intake of PCB-153. We chose to estimate scaling factors and not the parameters themselves to keep the time evolution of these parameters during the pregnancy, lactation or lifetime. The individual concentration of PCB-153 in venous and cord blood, milk, placenta were used to calibrate the model

8.3.2 Reverse dosimetry for the Italian women

The reverse dosimetry for the Italian women was performed with the PBPK model parameterized with the estimated values of the diffusion rate between the mother's uterus and the placenta, and the scaling factors for blood: milk and blood: placenta partition coefficients obtained from the analysis of the Abraham's dataset (Abraham et al 1996). For each Italian woman, we estimate the scaling factor for the daily intake of PCB-153 to keep its time evolution during the last century.

8.3.3 Bayesian analysis

The analysis of the German and Italian datasets was performed in a Bayesian framework. Non-informative prior distributions for each parameter were defined and are described in Table 21. Markov Chain Monte Carlo (MCMC) methods were used to numerically obtain a sample of parameter values from their joint posterior distribution. The MCSim software was used to perform the analyses. For each dataset, two independent MCMC chains were run until convergence was reached

Parameters	Prior distribution
diffusion rate between uterus and placenta	Uniform (0, 0.1)
scaling factor for blood: milk partition coefficient	Uniform (0.001, 10)
scaling factors for blood: placenta partition coefficient	Uniform (0.001, 10)
scaling factor for the daily intake	Uniform (0, 100)

Table 21. Prior distributions for the PBPK model parameters to estimate

8.4 Results and discussion

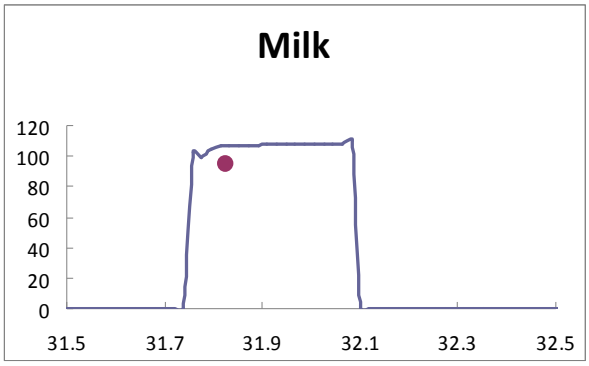
8.4.1 Model verification

The PBPK model was fitted to the Abraham data. The parameters estimates are given in Table 22. Our results suggest that the partition coefficients for milk and placenta predicted with the Parham's approach are over-estimated (38% for milk and 54% for placenta). The estimates are quite precise, since the coefficient of variation is quite low. We also observed that the intake for the German population derived from studies on food contamination and consumption was too high. Our results conclude that only 5% to 8% was necessary to obtain the internal levels observed in these women

Parameters	**Mean**	**CV (%)**	**Confidence interval 95%**
Diffusion rate between uterus and placenta	0.00027	34	0.000151 – 0.000375
Scaling factor for milk:blood partition coefficient	0.62	15	0.52 – 0.78
Scaling factor for placenta:blood partition coefficient	0.46	6	0.40 – 0.51
Scaling factor for the daily intake			
Mother I	0.0516	6	0.0467 - 0.058
Mother III	0.0794	7	0.0703 - 0.0919
Mother IV	0.073	5	0.0656 - 0.0786

Table 22 Parameters' estimates from Abraham data

The fits of the model are in good agreement with the experimental data (see Figure 27A-D for tissue dosimetry correlation for one woman). The decrease in the placenta and cord blood concentrations and the increase in milk correspond to the delivery. The decrease in milk corresponds to the end of the lactation period. The model was not able to reproduce a high decrease in the concentration in the venous blood of the woman. However, considering all the results, the verification step allows to consider our PBPK model to reconstruct the exposure of Italian women with a single sample in breast milk.



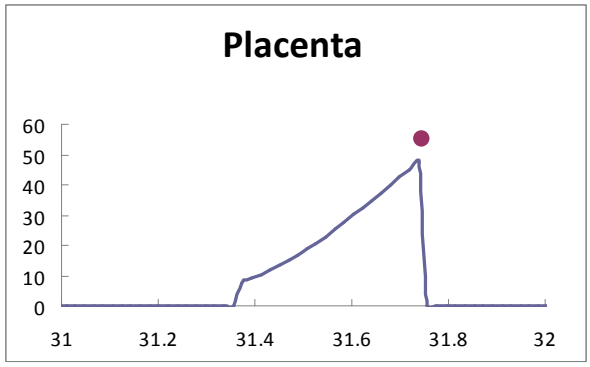
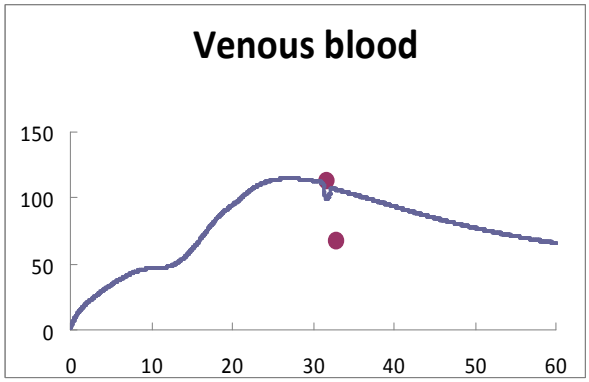


Figure 27A milk PCB-153 concentration

Figure 27 B placenta PCB-153 concentration



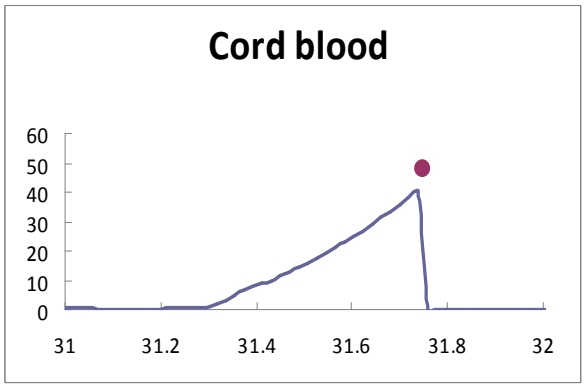


Figure 27C venous blood PCB-153 concentration

Figure 27 D cord blood PCB-153 concentration

Good close agreement was obtained also for reverse dosimetry, here the special attention was putted on the character of the curve representing the history of dietary intake during the post ban period. Our model was able to predict the decreasing tendency over the lifetime of the German woman (1959-1993). The verification step gave the confidence to our model.

8.4.2 Reverse dosimetry

Using our PBPK model, reverse dosimetry simulation was carried out to estimate the possible exposure scenarios leading to breast milk contamination with PCB-153 in Italian women in 2009**.** As mentioned before reverse dosimetry was performed with the PBPK model parameterized the estimated values of the diffusion rate between the mother's uterus and the placenta, and the scaling factors for milk: blood and placenta: blood partition coefficients. For each Italian woman, the scaling factor for the daily intake of PCB-153 was estimated to keep its time evolution during the last century (Figure 29B).

The estimated values of the scaling factors vary among the women from 0.04 to 40. This high variability only reproduces the variability observed in the milk concentrations. However three women have PCB-153 levels in the milk that significantly differ from the other women. Two of these women are from Piacenza and exhibit very high levels of PCB in milk (26.21 and 17.42ng/g fat in milk for women born in 1987), and one form Milano exhibit very low levels in milk (1.37 ng/g fat in milk for a woman born in 1972). If these three women are excluded, the scaling factors are estimated between 0.1 and 11 (thus reducing the variability by a factor 10). Table 23 presents the average scaling factor according to the age of the women. We clearly observed that, the predicted intake for women born in the 70's is close to the initial intake predicted from food studies (See chapter 4.2) (the minimum intake). However for women born in the 80's, the initial intake is too low since the scaling factors are around 7. It means that a higher exposure to PCB-153 is needed to reach the levels in milk.

Year of birth	Average scaling factor	Year of birth	Average scaling factor
1971	1.6	1982	7.3
1972	1.3	1983	6
1973	1	1984	8.9
1974	1.7	1985	8.2
1975	1.1	1986	5.7
1976	1.5	1987	8.1
1977	2.2		

Table 23 Average scaling factor according to the age of the women.

Results of the dose reconstruction are presented in the Figures 29 A-B. Model was able to reflect the decreasing tendency of daily intake, what can be noticed in the reconstruction of the dose for mothers born in 1971-74 (See Figure 29A). The characteristic shape of curve representing intake is a results of the changing food contamination during mother's life and changing dietary habits. Strong decrease around 1980-1982 reflects the ban of PCBs production. This profile can be still noticed in intake of woman born in 1976-1978, however disappears in group of women born in 1982-1987. Intake predicted for the youngest woman slopes gently and reflects diminished contamination of the food. It is important to notice that during this period several EU directive were set in order to reduce the introduction of POPs (i.e. PCBs) to environment (European Directives on the reduction of the emission limits of PCDDs/Fs and PCBs from

waste incineration set by EU Directive 2000/76/EC, or Commission Regulations such as (EC) No 466/2001 and 1881/2006 setting maximum levels for certain contaminants in foodstuffs) and effectively diminished human exposure to contaminants. For each Italian woman, was estimated also the scaling factor for the daily intake of PCB-153 to keep its time evolution during the last century. Scaling factors represent the variability of the population.

Predicted intake can be compared with the assessment studies prepared for Italian population and focused on the exposure to non-dioxin-like PCBs. (Baldassarri et al 1995, Zuccato et al 1999, Fattore et al 2008). Assessment study conducted in the late eighties and early nineties (Badassarri et al 1995) indicated the daily intake ca 2000 ng/day based on food measurements. Similar study in the middle of nineties noted strong decrease and estimated the daily intake 370±300 ng/day (Zuccato et al 1999). Model prediction of the intake for this period is in a good agreement with study of Zuccato, however lower of one order of magnitude compared with study of Baldassari. In the most recent study of Fattore et al (2008) authors estimated the daily intake of total NDL-PCBs 11.5 ng/day/kg b.w for population aged 0.5-95 years for the beginning of 2000. The body weight used to calculate this value is not known. The average intake of one PCB-153 predicted by model for these years was 0.6 ng/day /kg b.w (for adult woman of 60 kg after delivery). In this comparison, it seems that PCB-153 account for ca 5% of total NDL-PCB intake, this result seems to be slightly underestimated.

To explain these underestimations several reasons may be suggested: first that the real dietary intake of PCBs in Italian population in the late eighties and early nineties was lower and the individual effective dose of PCBs was far from estimates calculated for average population in study of Baldassarri (1995). The fact is that studies from 1995 did not provide any range, neither standard deviation for intake calculation. Second possibility might be that the estimation of Fattore is burdened with some uncertainty due to the methodology utilised in the study (i.e. body weight), the third explanation might be that PBPK model underestimated the population intake. However there is a fundamental difference between methodology used in reproduced investigations and methodology used in this work: PBPK model bases on physiology and anatomy of human body to calculate the dose which gives a target effect in the tissue, while the calculation in mentioned studies based on the

combination of consumption rate and food contamination. Therefore in order to obtain the individual intake, a PBPK model needs to be used.

Beside this, reverse dosimetry results were confirmed by prediction of PCB milk contamination. Very close agreement with measurement data was obtained. Figure 29 below presents the predicted and measured concentration of PCB-153 in breast milk. A mean ratio measurement / prediction was 1.06±0.17 (median 1.04, range 0.80-1.84). This clearly shows accuracy and precision of the PBPK model predictions, and gives confidence in the PBPK model used in this study.

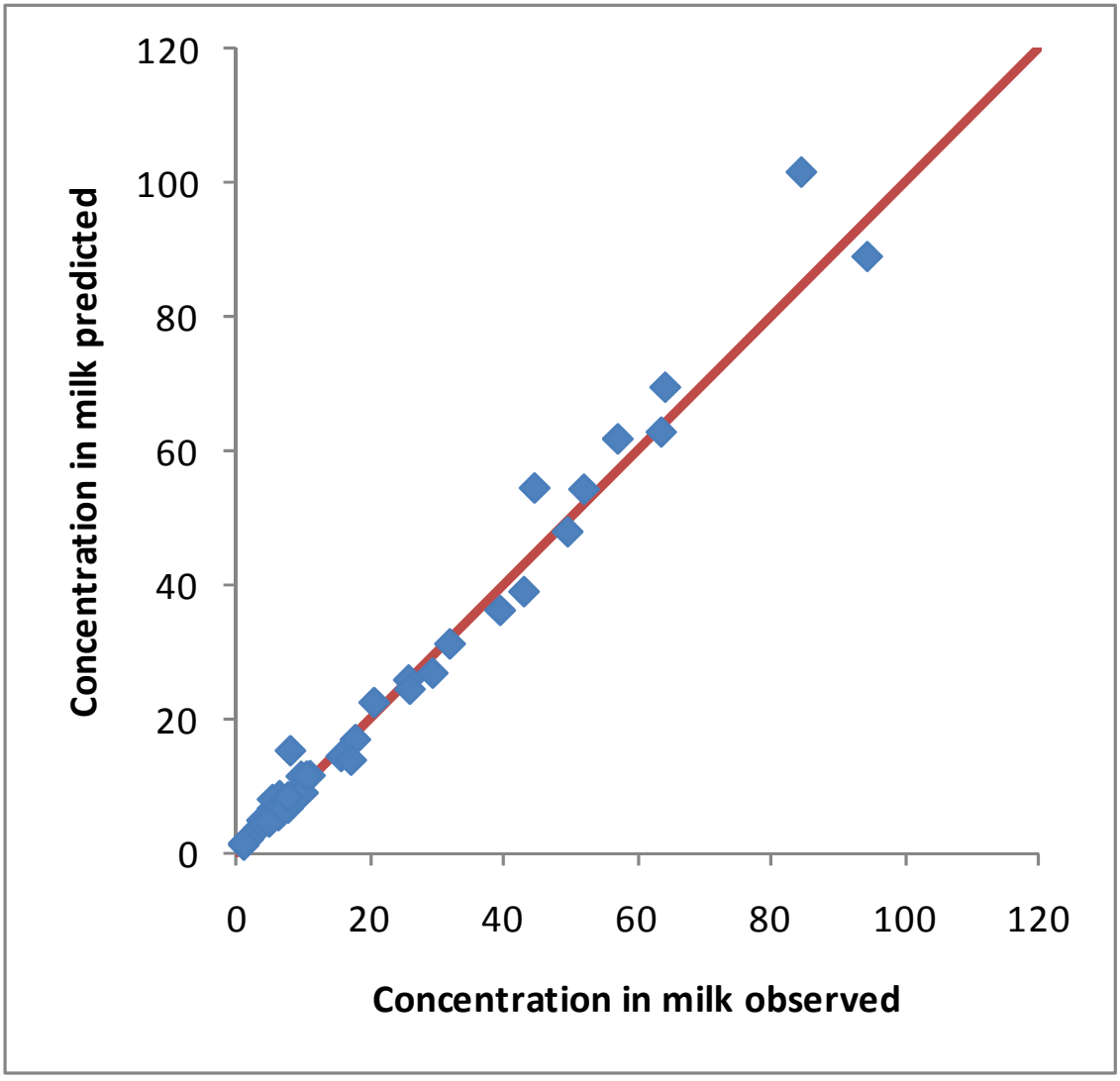
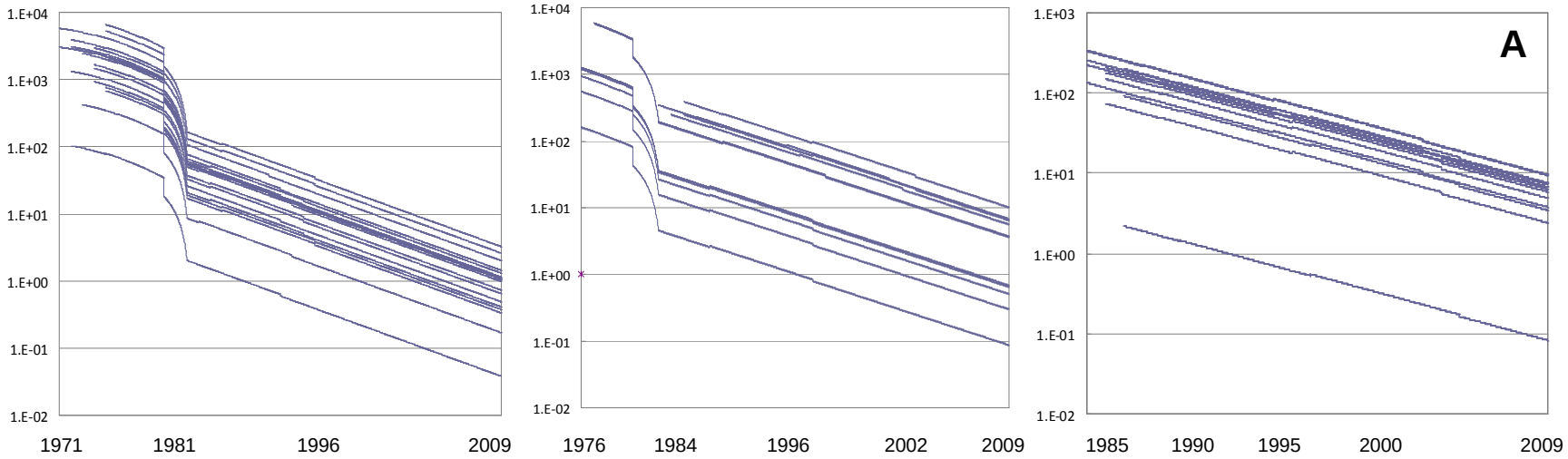


Figure 28. Predicted and measured concentration of PCB-153 in breast milk



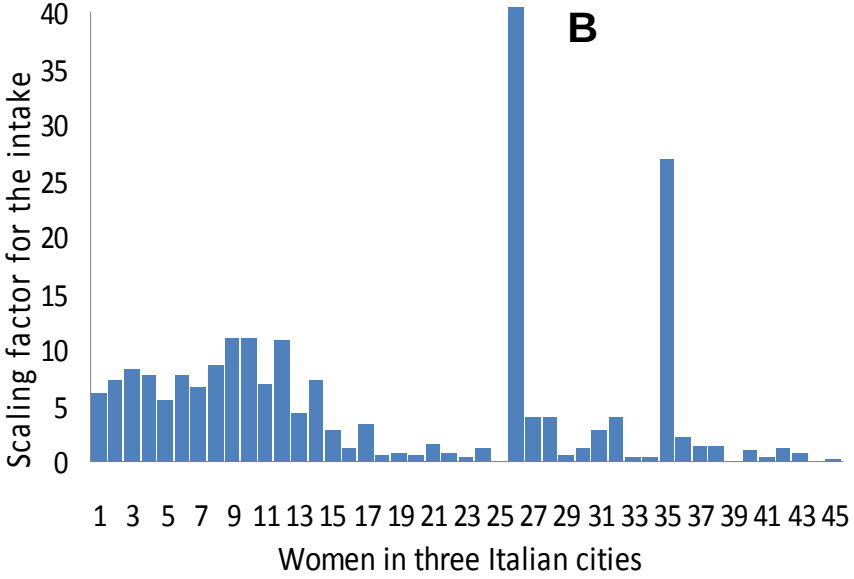


Figure 29
Results of reverse dosimetry simulation.

A- The reconstruction of daily intake for Italian mothers

B- Scaling factor for the intake adjustment

8.4.3 Forward dosimetry

PBPK model simulation of PCB-153 content in blood

In order to test the sensitivity and accuracy of model prediction in mean of forward dosimetry the mother blood was chosen. As mentioned in the beginning PCBs are recognized as endocrine disruptors which may alter hormonal balance, therefore the knowledge of their levels in blood is an important issue. Several studies showed the correlation of endometriosis with higher exposure of PCB (Koninckx et al 1994, De Filip et al 2004, Van Larebeke et al 2001, Gerhard and Runnebaum, 1992; Heilier et al., 2004; Buck Louis et al., 2005, Pauwels et al 2001). An association between PCBs 138, 153 and 180 and endometriosis had been observed in a study carried out in German women (Gerhard and Runnebaum, 1992); similar results were obtained by Heilier et al 2004.

Blood concentrations for woman born between 1971 and 1986 generated by model were within the literature ranges. As presented in the Figure 30, there are notable differences in blood levels between older and younger mothers over their lifetime. The model predicted the blood concentration in mothers born in 70ies higher than in mothers born in mid 80ies, what reflects different environmental exposure. Predicted results are in good agreement with literature. For example Todaka et al 2010 found PCB-153 at level of 26.82±15.4 (range 9.25-120) in 30 years old mother (sampling in 2002), prediction of PBPK model for woman born in 1971-1974 was in range 28-60 ng/g of lipid blood. Another example of accuracy of model prediction is a good agreement with recent Italian study of Porpora et al (2006), where in blood of young Italian woman with and without endometriosis was examinated for concentration of PCBs Indicators. In blood of 29 year healthy nulliparous PCB-153 was found at concentration 95 ng/g lipid blood (SD=55).

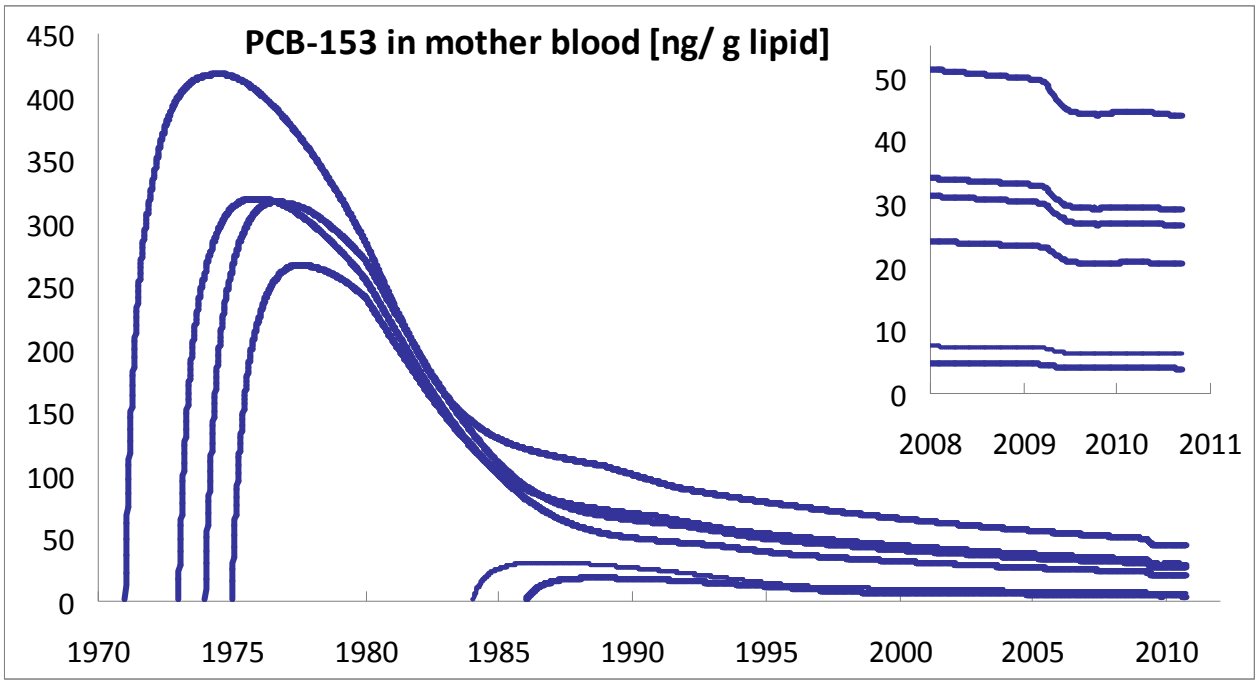


Figure 30. Prediction of mother blood concentration from birth until delivery

In fragmentary enlargement (upper zoom in Figure 31) can be easily noticed the characteristic decrease in blood concentration (around 2009) in all profiles predicted by PBPK model. It is known that delivery and breastfeeding reduce PCB levels in mother. Decrease in PCB blood concentration appears during pregnancy and lactation due to the presence of a foetus and production of milk, what rapidly changes the body burden.

In order to investigate the influence of pregnancy and lactation on kinetic of PCB-153 in blood additional simulations were performed.

Figure 31 below presents results of simulation for three different scenarios. First scenario was performed for woman without pregnancy and without lactation, in order to see the basic level of blood contamination (red line). The second scenario assumed the pregnancy at 23 years followed by 6 months breast feeding (blue line). The third scenario assumed the pregnancy alone (green line).

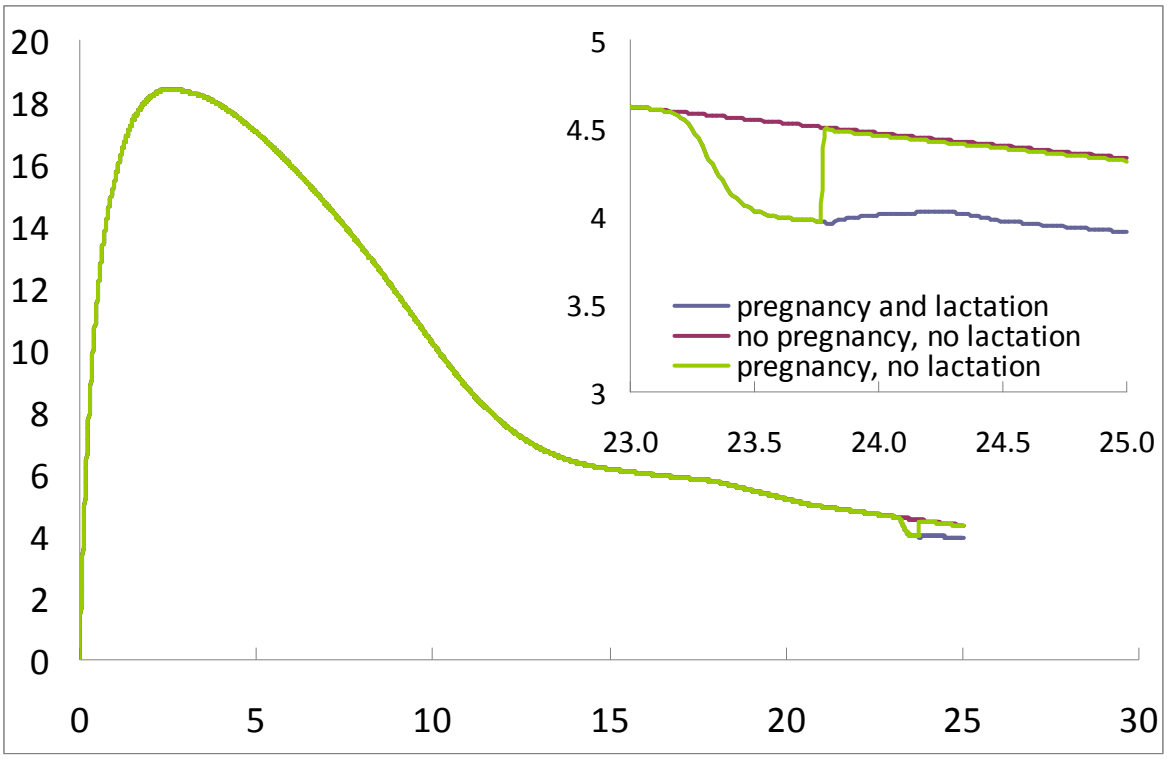


Figure 31. Kinetic profiles for PCB-153 in blood concentration

Simulations show that the pregnancy alone induces a drop in the blood concentration that rapidly returned to pre-pregnancy levels, suggesting that the observed decrease is due to the modification of the physiology of the woman (increased volume of fat, blood and breasts) rather than a significant transfer to the foetus. Simulation for pregnancy followed by breastfeeding showed that lactation has a greater impact on the blood concentration. In fact the excretion of milk is an important route of POPs elimination and exerts major change in kinetic profile.

PBPK model simulation of infant exposure to PCB-153

PBPK model was used to predict the infant exposure to PCB-153. The period breast feeding was set for 9 months, where 6 of full breastfeeding, and 3 of partial feeding. The milk concentrations were set individually for each mother based on measurement from human breast milk monitoring. The volume of milk ingested was modelled with the same equation used for the milk excretion. Model predicted the daily intake for each infant [ng/day/kg b.w.], and results are presented in Figure 32 below.

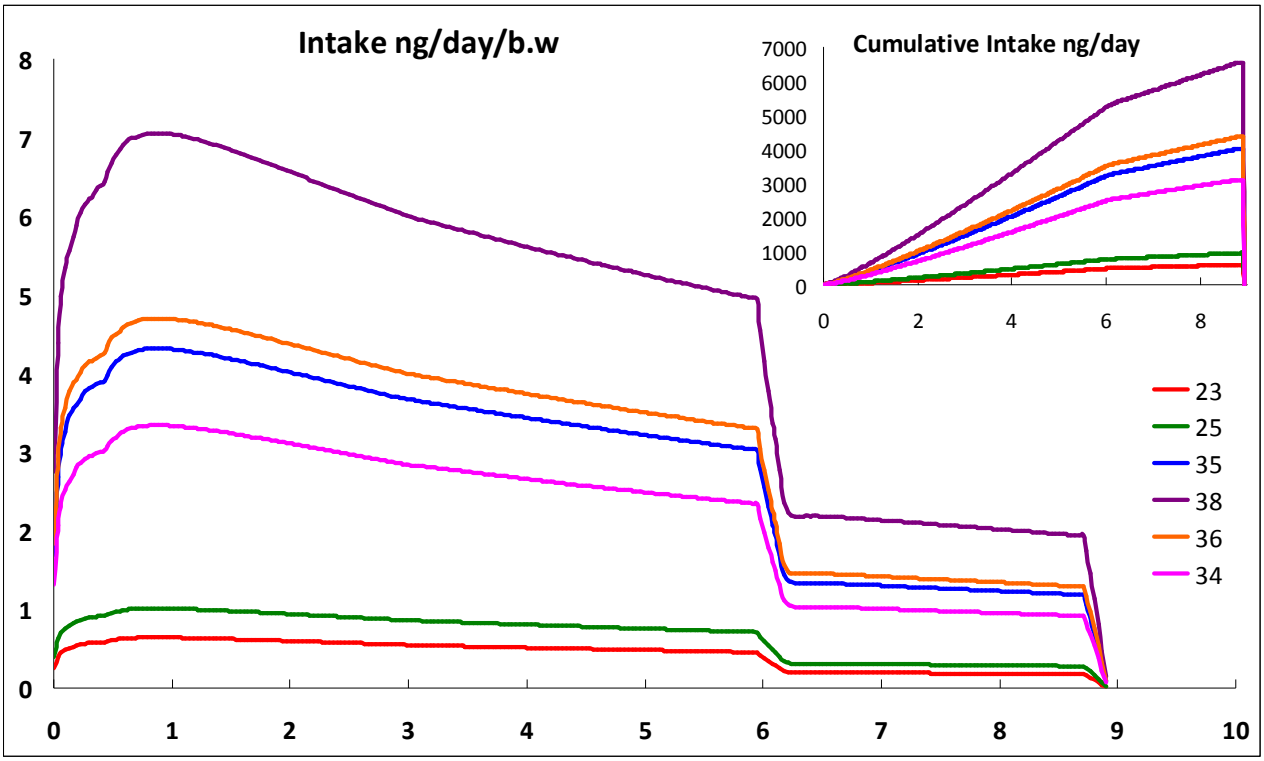


Figure 32. Infant exposure to PCB-153 via breast milk: daily intake and cumulative intake.

The shape of intake curves is determined directly by different breastfeeding periods: 6 months exclusively breast feeding, 3 months of partial weaning. Notable difference can be observed between intake of baby born to younger and older mother, what is a consequence of longer exposition to contaminants. Upper graph shows the cumulative intake after all breastfeeding period.

8.5 Conclusions

This study attempted to interpret the human biomonitoring study in mean of mother and infant exposure characterisation. The model used in this study was a stochastic whole body life-time PBPK model based on detailed description of the human physiology and anatomy (Beaudouin et al 2010) and was examined to predict historical exposure that is associated with specific biomarker in breast milk. Based on measurement data derived from breast milk monitoring conducted recently in Italy, the reverse and forward dosimetry were performed. PBPK modelling was shown to be a relevant method to assess an infant postnatal exposure to PCB-153, to reconstruct the historical exposure of mother during her

life time, and to predict PCB-153 blood concentration from mother's birth until first delivery. This study is the first to propose the reconstruction of life-time intake by PBPK model based on single breast milk measurement.

Reconstruction of dietary intake trend over the mother's life was performed in mean of reverse dosimetry thank to several improvements applied to existing PBPK model. Among the modification important were: new data about the tissues composition and new partition coefficients tissue: blood calculated for young organism (PC placenta: blood, PC milk: blood). Crucial for this study was the parameterisation of several parameters like the diffusion rate between the mother's uterus and the placenta, milk: blood and placenta: blood partition coefficients, which greatly improved a prenatal and postnatal transfer between mother and infant. Moreover the estimation of scaling factor for the daily intake of PCB-153 resulted in accurate reconstruction of intake evolution during the last century and give important information about variability of the population.

It is well known that breast milk is good predictor of an infant exposure, and several studies showed how to use the PBPK model for infant exposure assessment. (Clewell et al 2002, 2008). Only a few articles dealt with mother exposure reconstruction based on breast milk studies. (Lorber and Phillips 2002, Alyward et al 2005), investigations considering PCBs are even less (Redding et al 2008, Verner et al 2009). In all these studies it was assumed that mother exposure is constant. However this assumption is not correct, especially when modelling is performed for POPs such as PCDD/Fs , PCB or DDT. It is well known that occurrence of these compounds is characterized by a decreasing trend. Such a trend appears after use and production of a chemical that has been restricted or banned. This is exactly the case of PCBs, which were being produced intensively in mid 70, and than have been banned. Often such an environmental distribution in time takes the form of bell-curve. Simple decreasing trend is fitted with exponential function (log-linear regression).

An understanding of past exposure is important to place current body burdens in perspective, and also useful in assessing potential future exposure. Very few direct measurements of the main sources of exposure to PCBs (i.e., soil, food) over the past decades are available. Especially the lack of data in food measurement in the past induces a need to develop new methods to reconstruct realistic scenarios of human exposure or human intake. Moreover, very important is to create a scenario that accurately reflects the

situation for specific population, geographic regions or country. Combination of breast milk monitoring with PBPK model allowed to model a dynamic exposure to reflect the evolution of environmental levels of PCBs in Italy.

Simulations included also the prediction of mother blood concentration of PCB-153. PCBs are recognized as endocrine disruptors which may alter hormonal balance. Therefore the knowledge of their levels in blood is an important issue. Several investigations showed the correlations between higher level of PCBs indicators in blood and endometriosis (Buck-Luis et al 2005, Koninckx et al 1994, De Filip et al 2004, Van Larebeke et al 2001, Gerhard and Runnebaum, 1992). Also in this case model estimated realistically the history of mother blood concentration, proving its sensitivity and accuracy.

The modelling outcomes provided an historical perspective on the changing exposure of the Italian population to PCB-153. It was proved also that breast milk is good biomarker of historical exposure and in combination with PBPK modelling can be used to reconstruct the mother dose. So far, this is the first study which reconstructed the dynamic exposure for specific population. Results of this work can dramatically change the way to perform modelling for substances such us POPs, as similar trends to PCBs is observed for PCDD/Fs, DDT, HCB etc. Challenging may be modelling for substances such as PBDE which are characterized by increased tendency, and their amount in environment grows up.

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10 ANNEX 1

Questionnaire for human milk donors

"PROGETTO NUOVA VITA"
Studio epidemiologico sui livelli di diossina nel latte materno

REGIONE LOMBARDIA

ISTITUTO "MARIO NEGRI" MILANO
Dipartimento Ambiente e Salute
Dott. Roberto Fanelli

STUDIO SUI LIVELLI DI ACCUMULO DI DIOSSINE NEL LATTE MATERNO QUALI INDICATORI DI INQUINAMENTO AMBIENTALE NELLA CITTA' DI MILANO

Data:

CODICE IDENTIFICAZIONE INDIVIDUALE	CODICE IDENTIFICAZIONE DEL POOL

INFORMAZIONI PERSONALI

Nome: Cognome:

Data e luogo di nascita:

Indirizzo:

Telefono:

e- mail: @

ANAMNESI PATOLOGICA

Patologie diagnosticate in passato:

Patologie in atto:

QUESTIONARIO DI SCREENING

1- Hai programmato di allattare al seno tuo figlio? SI NO

2- E' il tuo primo figlio? SI NO

3- Stai aspettando un solo bambino? (no indica gemelli) SI NO

4- Stai avendo una gravidanza senza problemi di salute? SI NO
Se no, quali:

5- Risiedi nella stessa zona da almeno 10 anni? SI NO
Se no, da quanti anni?

6- Hai meno di 30 anni? SI NO

7- Risiedi nelle vicinanze di aree di discarica, aree industriali, strade ad intenso traffico, zone di incendi di rifiuti all'aria aperta? SI NO
In caso di risposta affermativa precisare le distanze dall'abitazione:
- area di discarica:
- area industriale:
- strada ad intenso traffico:
- zona di incendi di rifiuti all' aria aperta:

8- Qual è la tua attuale condizione lavorativa?

9- Durante lo svolgimento del tuo lavoro sei stata mai esposta ad agenti chimici o fisici? SI NO
Se si, quali?

10- Abitudine al fumo: SI NO
Se si, indicare il numero di sigarette al giorno:

ABITUDINI ALIMENTARI

Come descriveresti le tue abitudini alimentari prima della gravidanza?
Dieta mista Vegetariana ma con latte e uova
Strettamente vegetariana Altro

Quanto spesso hai mangiato i seguenti cibi prima della gravidanza?

DA COMPLETARE A CURA DEL COORDINATORE PER L'INTERVISTA PRE-NATALE

Nome della madre:

Numero di telefono:

Data del parto:

E-mail: @

Indirizzo:

Stato della donatrice in riferimento allo studio:
Selezionata Riserva

Codice d'identificazione individuale:

INFORMAZIONI DA RACCOGLIERSI AL MOMENTO DEL PRELIEVO DEL CAMPIONE

Sei intenzionata a firmare il modello di consenso informato? SI NO
Se si, firma il modello di consenso.
Se no, la madre non è eleggibile per partecipare allo studio.

I neonati devono avere più di tre settimane (21 giorni).
Il coordinatore deve avvisare la madre di ritornare quando il piccolo ha raggiunto le tre settimane per il prelievo del campione di latte.
Il campione deve essere prelevato dalle tre alle otto settimane dal parto.

MODULO PER IL CONSENSO INFORMATO

Certificato di consenso

Sono stata invitata a partecipare al Progetto "Nuova Vita" da….- Studio sui livelli di accumulo nel latte materno di diossine quali indicatori di inquinamento ambientale nella regione Lombardia.
Sono stata informata degli scopi e delle procedure di questo studio, che di seguito si esplicitano in breve:

Obiettivi
Le diossine e le diossine derivate sono un gruppo di sostanze chimiche prodotte dall' uomo che possono essere ritrovate nell' ambiente che ci circonda. Queste sostanze sono abbastanza stabili nel tempo e possono essere ritrovate in cibi contenenti grasso, incluso il latte umano.
Il progetto ha come obiettivo principale la rilevazione dei livelli di accumulo delle diossine e delle diossine correlate nel latte materno delle donne gravide. Tutte le conoscenze scientifiche continuano a evidenziare i vantaggi per la salute dell'allattamento al seno, soprattutto nei primi sei mesi di vita.

Procedure
Vi viene chiesto un campione di 60-100 mL di latte che sarà prelevato sia in maniera naturale che con un tiralatte, sarebbe meglio pero' minimizzare il contatto con la plastica. Il campione può essere prelevato sia in ospedale sia presso la sua abitazione e sarete adeguatamente istruite allo scopo. Lo stesso sarà analizzato per la ricerca delle diossine insieme a quello di altre 50 mamme ed i risultati potranno essere confrontati con quelli di altri studi per una valutazione regionale. **Il campione deve essere sempre conservato in freezer!** Qualora non fosse possibile prelevare 60 ml in una sola volta, si possono effettuare diversi prelievi nello stesso vasetto nell'arco di qualche giorno, avendo cura di conservare sempre il latte in freezer.
Sarà anche distribuito un questionario ad intervista per valutare residenza, condizioni ambientali, abitudini, stato di salute dieta ed eventuali esposizioni a sostanze tossiche o dannose.

Riservatezza
Le informazioni raccolte durante questo progetto di ricerca saranno strettamente riservate. Esse saranno anonime e contrassegnate da un numero cui è associato ogni nome. Il nome associato con il numero assegnato è segreto e tenuto sotto chiave e non può essere rivelato a nessuno. I risultati non includono il nome ma un codice, inoltre solo la media dei risultati di tutti i partecipanti allo studio sarà riportata e non il singolo risultato. Fino a quando il suo campione non sarà unito al pool con gli altri, può in qualsiasi momento rinunciare a partecipare allo studio.
Per domande ed informazioni può contattare le seguenti persone:

Dott.ssa Marynka M. Ulaszewska maria.ulaszewska@unicatt.it 339 381 95 37
Istituto di Ricerche Farmacologiche "Mario Negri" Milano
Dipartimento: Ambiente e Salute
Laboratorio: Tossicologia della Nutrizione

Ho letto, o mi sono state lette, le predette informazioni. Ho avuto l'opportunità di fare domande ed ho avuto risposte che mi hanno soddisfatta. Acconsento volontariamente a partecipare allo studio e sono a conoscenza che ho il diritto a ritirarmi dallo stesso fino al momento in cui il mio campione sarà messo nel pool con gli altri. Se scelgo di ritirarmi dallo studio so che in nessun modo ciò comprometterà le mie cure mediche. Acconsento affinché ogni eccesso di campione di latte materno possa essere utilizzato per futuri studi correlati.

Nome della madre partecipante Data e firma della partecipante

Nome del Sanitario Data e firma del Sanitario

11. ANNEX 2

Questionnaire WHO Fourth WHO-Coordinated Survey of Human Milk for Persistent Organic Pollutants in Cooperation with UNEP

QUESTIONNAIRE FOR POTENTIAL HUMAN MILK DONORS		
Fourth WHO-Coordinated Survey of Human Milk for Persistent Organic Pollutants ***CONFIDENTIAL!***		
Section 1: Personal Information		
Name	Phone number e-mail	Today's Date (dd/mm/yyyy)
Address		

Section for National Coordinator	
Individual Identification Code	Pool Identification Code
Based on established criteria, is the participant eligible? Yes ☐ No ☐	
What is the status of donor in regard to the survey? Selected ☐ Reserve ☐ Not Selected ☐ If this mother has been pre-selected to donate a sample (or is designated as an alternate), the top of Section 4 should be completed and detached from this questionnaire. Section 4 should be sent to the clinic to be completed at the time of sample collection.	

Section 2: Screening Questionnaire	
Name of Interviewer:	Date of interview (dd/mm/yyyy):
Place of interview:	
1. Are you planning to breastfeed your child? Yes ☐ No ☐	
2. Is this your first child? Yes ☐ No ☐	
3. Are you expecting a single child? (not twins) Yes ☐ No ☐	
4. Are you having a normal healthy pregnancy? Yes ☐ No ☐	
5. Have you lived in your current area for 10 years? Yes ☐ No ☐ * If no, actual number of years _____	
6. Are you under 30 years of age? Yes ☐ No ☐ * If no, date of birth _____(dd/mm/yyyy)	
7. Do you live near incinerators, pulp and paper industries, metal industries or where chemicals are produced Yes ☐ No ☐	

***Note that if the answers to questions 5 or 6 was "no", ask what the participant's actual residence time and/or birth date.**
If any answers to questions 1-6 were "no" or if the answer to question 7 was "yes", the participant is not eligible for this survey.

5. Was your mother born in this country? Yes ☐ No ☐
6. Were you breastfed? Yes ☐ No ☐ Do not know ☐ If you know, for how long? _____
7. Were you engaged in work other than housework before pregnancy? Yes ☐ No ☐ If yes, please state the duration and describe type of work :
8. Has the inside of your house been sprayed with DDT in order to prevent mosquitoes? Yes ☐ No ☐ Do not know ☐ If yes, when? _____

Section 3: Health History Questionnaire

Date of Birth (dd/mm/yyyy)	Age
Height (cm)	Weight before pregnancy (kg)

1. What is your expected delivery date (dd/mm/yyyy)?

2. Where have you been residing during last 10 years:

urban (city) ☐ rural (countryside) ☐

3. How would you describe your dietary habits before pregnancy?

Mixed diet ☐ Vegetarian but with milk and eggs ☐

Strictly vegetarian ☐ Other ☐

4. How often, on average, did you eat following foods before pregnancy?

	Fish and fish products (e.g. tuna salad)	Marine mammals (e.g. whales, dolphins)	Seafood other than fish and marine mammals (e.g. shrimps, mussels)	Milk and milk products (e.g. cheese, butter, cream, yogurt)	Meat and poultry and derived products (e.g. sausage)	Eggs
Never						
Less than once a week						
Once a week						
Twice a week						
More than twice a week but not every day						
Every day						

4.1 What types of fish do you consume most often?

Fish from the sea ☐ Freshwater fish ☐ Both ☐

Please state the species if known :