Persistent organic pollutants in human plasma from inhabitants of the Arctic

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Abstract

The high levels of persistent organic pollutants (POPs) in the Arctic have caused serious concern regarding the possible health effects chronic exposure might cause. The highest concentrations of POPs in human plasma, in the Arctic and surroundings, have been observed in indigenous populations, who depend on marine mammals as a source of food. High levels of some POPs have also been detected in non-indigenous people from northwest Russia.

This thesis focuses on filling knowledge gaps related to levels of POPs in human plasma in northern areas of Russia, as well as relating them to traditional diets. Both the short-term and long-term effects of diet have been studied. The aim was further to develop cost efficient methodology for the analysis of conventional POPs, as well as for phenolic organohalogens, in small plasma volumes (0.5 ml).

The clean-up of plasma samples was improved when switching from a GPC column and silica fractionating (Paper I) to a single-step separation using florisil columns (Paper II and III). The method was further developed to determine phenolic organohalogens (Paper IV) in plasma volumes as small as 0.8 ml.

High concentrations of β-HCH and *p,p'*-DDT were found in plasma samples of 27 delivering women from Arkhangelsk (northwest Russia) (Paper I). The geometric mean level of β-HCH (3.08 μg/l plasma) was higher than what has been reported in any of the previously studied indigenous Arctic populations. In addition to the high levels, a DDE/DDT ratio as low as 7 was observed. This finding indicates the presence of fresh sources, and the extent of their presence gives reason for concern. The PCBs concentrations were low and comparable to Norwegian data. None of the other pesticide concentrations were elevated. The sources of β-HCH and *p,p'*-DDT have not been identified and efforts should be made to do so.

The PCBs and *p,p'*-DDE contents were low in the plasma of 33 people from Tromsø (northern Norway) (Paper II). Short term changes in plasma levels of PCBs and *p,p'*-DDE were observed in relation to measured intake of the traditional dish "mølje". The dish is rich in marine lipids and POPs. Both the lipid-weight and wet-weight concentrations of *p,p'*-DDE increased significantly ($p<0.01$) four hours after the meal. This was not the case for any of the PCB congeners, despite the fact that some of the

PCB congeners had concentrations comparable to the *p,p'*-DDE. The PCB congeners with minor prevalence in the food, but prominent in the plasma, decreased significantly ($p<0.01$) on a lipid-weight basis after four hours. The observed changes were independent of the amount consumed and body mass index (BMI). Nine of eleven compounds had significantly higher levels in plasma from non-fasting individuals, when compared to samples from fasting individuals. Thus, the use of plasma samples from fasting individuals and the reporting of both wet- and lipid-weight data were advised.

Significant amounts of PCBs and pesticides, including toxaphene, were found in 50 plasma samples from the indigenous population (98 % Chukchi) of the coastal village of Uelen (Chukotka Peninsula, Russia) (Paper III). Compared to other indigenous populations who depend on marine mammals, the β-HCH concentrations were particularly elevated. Through the use of a dietary questionnaire, the consumption of blubber was found to significantly affect the levels of some of the POPs. Below the age of 40, women had significantly less POPs in their plasma than men, whereas above the age of 40 the gender difference was no longer significant ($p>0.05$). There was no significant age dependence for the POPs among the men whereas for the women it was highly significant ($p<0.01$). BMI did not explain any of the variance in the POPs concentrations for both genders. The consumption of store-bought goods in this community was low.

In the individuals from Uelen with the highest PCBs exposure, high plasma levels of OH-PCBs were also present, with 4-OH-CB 107 (median: 1673 pg/g plasma) as the dominating congener (Paper IV). There was, however, great individual variance in congener pattern. The OH-PCBs were significantly correlated to the PCBs, and the sum OH-PCBs constituted 27 – 76 % of the sum PCBs. The median concentration of pentachlorophenol was low (642 pg/g plasma), compared to levels previously reported in native populations.

The following over-all conclusion can be drawn: There is great variation among populations in the composition of POPs in plasma, and not enough information is available to limit selection of a few indicator compounds when assessing exposure in relation to health effects. To overcome this, larger populations need to be screened. This requires even lower cost analytical methods than those developed.

List of papers

The following four papers are included in this thesis and they will be referred to by the use of their roman numbers.

I. **Persistent organic pollutants in plasma of delivering women from Arkhangelsk**. Sandanger, T.M., Odland, J.Ø., Tkachev, A., Burkow, I.C. Sci Total Environ 2003; 306, 171-178.

II. **Change in human plasma levels of persistent organic pollutants after consumption of a traditional Northern Norwegian fish dish - Mølje (cod, cod liver, cod liver oil and hard roe).** Sandanger, T.M., Brustad, M., Lund, E., Burkow, I.C. J Environ Monit 2003; 5, 160-165.

III. **Human plasma levels of POPs and diet among native people from Uelen, Chukotka.** Sandanger, T.M., Brustad, M., Odland, J.Ø., Doudarev, A.A., Miretsky, G.I., Chaschin, V., Burkow, I.C., Lund, E. J Environ Monit, in press.

IV. **New methodology for the combined analysis of neutral and phenolic organohalogens in human plasma.** Sandanger, T.M., Dumas, P., Berger, U., Burkow, I.C. Manuscript.

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Line, I would like to tell you how extremely grateful I am for putting up with a sometimes (often) extremely stressed and quiet husband. It will now improve!

Introduction

Persistent organic pollutants

There are a great variety of compounds or molecules that are defined as persistent organic pollutants (POPs), and they all have the ability to bio-accumulate. Due to their persistence, they endure in the environment for years depending on their molecular structure and composition and the physical and chemical conditions of the environment. The lipophilicity makes these compounds accumulate in the food chain with higher levels occurring in lipid-rich tissues (AMAP, 1998).

Most research and ongoing monitoring work on POPs in the Arctic have mainly focused on industrial compounds like the polychlorinated biphenyls (PCBs), by-products like polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs) and pesticides like the DDT group, chlordanes, hexachlorocyclohexanes (HCH) and the toxaphenes. Toxic metals like mercury, lead and cadmium are also of major concern (AMAP, 1998; Hansen et al., 1996; Jensen et al., 1997). The production, use and sources of exposure for several of these compounds are listed in Table 1.

The PCBs, consisting of 209 congeners, were produced for use in a large number of industrial processes (additives, flame retardants, dielectric fluids etc.). The production of PCBs began in 1929 and the total worldwide production has been estimated to be 1.3 million tons, with over a million kilograms thought to have been released annually to the environment via mobile environmental reserves. The production and use of the PCBs were banned in most western countries from the late 1970s (Breivik et al., 2002; Lang, 1992).

PCDD/Fs enter the environment mainly as by-products of industrial processes and the two compound groups consist of 75 and 135 congeners, respectively. The most significant sources are low-temperature, incomplete incineration of chlorine containing materials such as plastics. Most PCDD/F congeners are extremely hydrophobic and resistant to biodegradation in soils and sediments (AMAP, 1998).

Table 1: Production, uses and sources of exposure to some of the major persistent organic pollutants (POPs) (modified from Odland et al., 2003).

Contaminant	Production and uses	Sources of Arctic exposure
Dibenzo-*p*-dioxins and furanes (PCDD/PCDFs)	By-product from combustion, bleaching and metallurgic industry.	Mainly long-range transport. Some local industrial sources exist. Incineration processes.
Polychlorinated biphenyls (PCBs)	Thermal and electrical insulators, and industrial used oils. Restricted use. Total world production estimated to 1.3 mill. tons.	Mainly long-range transport. Some local sources identified, including wastegrounds, old electrical equipment and military installations.
Hexachlorocyclohexanes (HCHs, α–, β–, γ–) [γ–HCH is referred to as lindane]	Currently produced as an insecticide for fruit, vegetables and forest crops (lindane). Many countries still use large amounts of lindane. Total world production of 0.7 mill. tons of lindane and 10 mill. tons of the technical HCHs. The use of the technical HCHs is banned/restricted in many western countries.	Long-range transport. Some local use as insecticide and for control of head lice and scabies caused by mites.
Hexachlorobenzene (HCB)	Chemical by-product, limited use as fungicide in the 1960s. HCB is banned or restricted in many countries. Global annual emission is 23 tons.	Mainly long-range transport. Minor local sources including industry and leakage from landfills.
DDT group	Pesticide used extensively until 1970. Banned in most western countries, but still in use for the control of malaria spreading mosquitoes. Total global usage of 2.6 mill. tons.	Long-range transport. Minor local use as insecticide.
Chlordanes	Broad-spectrum insecticide used on agricultural crops and for termite control. Banned in many countries since the early 1980s. Total global usage of 80 000 tons.	Long-range transport.
Toxaphenes (CHB)	Used as pesticide and to control ticks and mites in livestock. Banned in most countries since the early 1980s. Total world usage of 1.3 mill. tons.	Mainly long-range transport.

Cyclodienes (chlordanes, heptachlor, aldrin, dieldrin, endrin), dichlorodiphenyltrichloroethane (DDT), hexachlorocyclohexanes (HCHs) and toxaphenes are all examples of pesticides used in large quantities worldwide. Many of these compounds consist of complex mixtures, where the cyclodiene pesticides consists of at least 147 compounds and the toxaphenes consists of several hundred chlorinated bornanes, polychlorinated camphenes, bornenes, and bornadiens. Several of these compounds are found in the Arctic environment and some at high levels (AMAP, 1998).

During the past five years, more polar organochlorine contaminants and metabolites have received increased attention, both due to improved analytical capabilities and their possible biological activity (AMAP, 2003). Both hydroxylated-PCBs (OH-PCBs) and pentachlorophenol (PCP) cause concern. PCBs are biotransformed by the cytochrome P-450 monooxygenases and in most of the known metabolic pathways the formation of the OH-PCBs is the initial step. Even the more persistent PCB 153 is metabolised to a certain extent, both *in vitro* and *in vivo* to form a number of hydroxylated metabolites (Ariyoshi et al., 1992; Schnellmann et al., 1983; Sipes et al., 1982). The OH-PCBs have also been found to be the main metabolite excreted in faeces and/or urine (Guvenius et al., 2002).

PCP is a less persistent pesticide, widely used as fungicide, especially for wood preservation. It is very persistent in water and sediment under aerobic conditions, but degrades rapidly under anaerobic conditions. The worldwide production is estimated to be less than 30000 tons per year (AMAP, 2003). In addition, PCP may be formed by the metabolism of other pesticides (e.g. HCB) (To-Figueras et al., 2000). The levels of PCP have been reported to be higher than OH-PCBs among various populations from the Quebec region, Canada (Sandau et al., 2002).

The brominated flame retardants (e.g., polybrominated diphenyl ethers and tetrabromobisphenol-A (TBBPA)) are a group of compounds for which the environmental levels seem to increase, and huge quantities are produced worldwide (Thomsen et al., 2002). In this thesis, it is only TBBPA that is further discussed in relation to development of the analytical methodology for phenolic organohalogens.

Sources of contamination in the Arctic

POPs are found everywhere in the environment, even in remote areas like the Arctic. In general, present levels of POPs in the Arctic cannot be related to known use and/or release from local sources, and can therefore only be explained by long-range transport from lower latitudes (Burkow and Kallenborn, 2000; Hansen et al., 1996; Jensen et al., 1997). Depending on geographic location, weather condition and the physical-chemical properties of the contaminant, transport to and within the northern regions can be carried out via the atmosphere, water currents, sea-ice drift and Arctic rivers as shown in Figure 1 (AMAP, 1998). For α-HCH, the Arctic Ocean now also is known to act as a source by revolitalisation of previous long-range transported material (AMAP, 1998; Harner et al., 1999), whereas recent findings indicate that β-HCH is still entering the Arctic Ocean in ocean currents passing through the Bering Strait (Li et al., 2002).

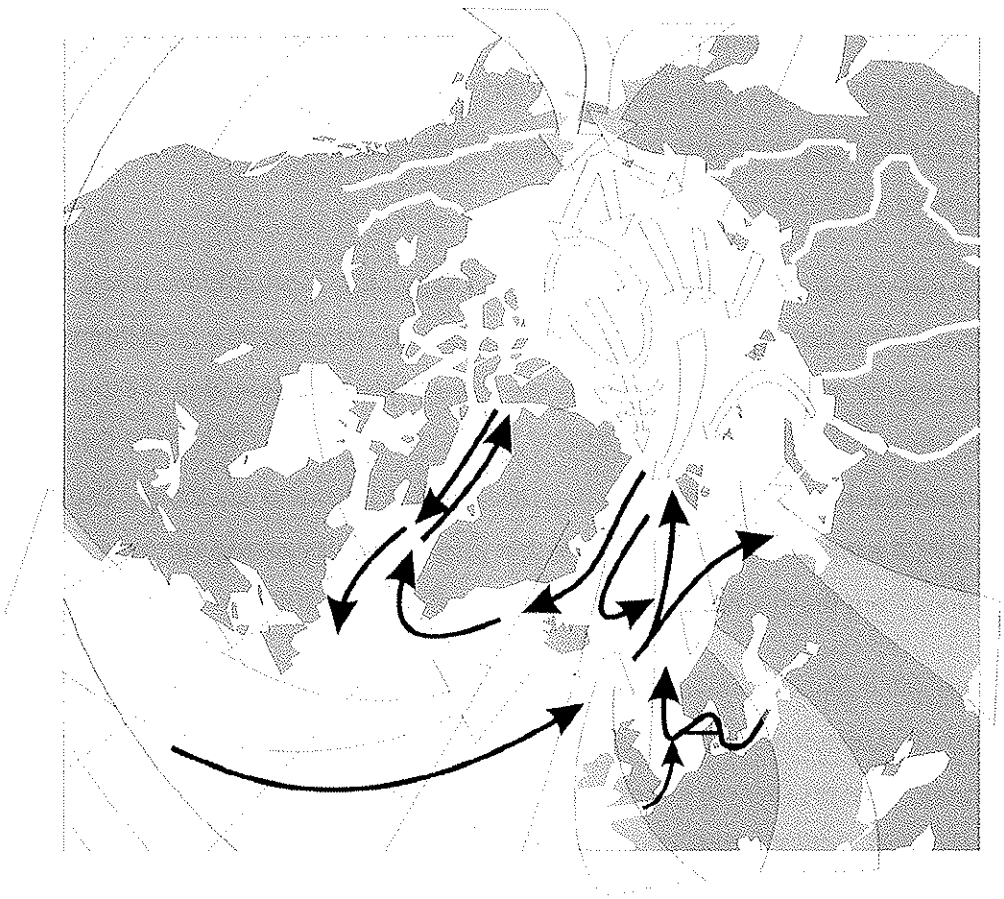


Figure 1: The contaminants are transported to and within the Arctic with air masses, ocean currents and ice drift.

The increasing information on POPs in the Arctic was reviewed in the early 1990s (e.g., Barrie et al., 1992; Muir et al., 1992; Norstrom and Muir, 1994) and later in the Arctic Monitoring and Assessment Reports (AMAP, 1998 and 2003) and by Macdonald et al. (2000). Some of these reviews reported that the levels of POPs were higher in top predators around Svalbard as compared to the Canadian Arctic. Recent data on marine mammals have also indicated that the levels are not as high in northeast Russia (AMAP, 2003). These findings clearly indicate a non-uniform distribution throughout the Arctic.

A number of local sources have also been identified with mineral exploration, coal mining and heavy industry accounting for the highest input. On Svalbard, both Russian and Norwegian communities are local sources of contaminants to the environment. Elevated PCB levels have also been found in sediments of harbours. The levels are high enough compared to the surrounding areas that it can be considered a source for PCBs in the Arctic marine environment (AMAP, 2002).

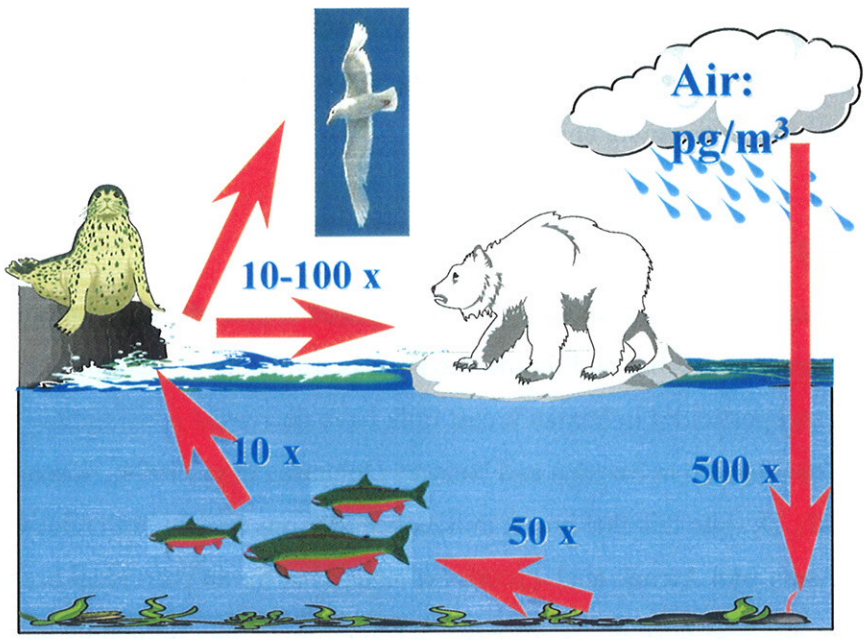


Figure 2: Example of bio-magnification in a food chain. The numbers are rough estimates of the magnification between trophic levels.

Several of the indigenous populations in the Arctic harvest from the local food chain, thereby being exposed to elevated levels of these bio-magnifying and bio-

accumulating compounds. Figure 2 illustrates bio-magnification in a food chain, with rough estimates of magnification factors between trophic levels.

Human exposure

Exposure to organic contaminants begins prior to conception (gametogenesis), continues throughout gestation (organogenesis), the neonatal stage by way of breast-feeding, and subsequently through dietary intake (Feeley and Brouwer, 2000).

Absorption from the diet

The estimates of the absorption of POPs from the diet vary. In one study, an absorption of 80 % for PCB 101 was used in the modelling of lifetime exposure (Alcock et al., 2000). It has also been reported that the absorption is more than 90 % of the amount consumed (Moser and McLachlan, 2001), but there are other papers indicating that food composition influences the rate of absorption and excretion (Morita et al., 1997; Narazaki et al., 1991). In human infants, the dietary absorption of most PCB and dioxin congeners has been found to be more than 95 % (Dahl et al., 1995; McLachlan, 1993). Kreuzer et al. (1997) support the lower absorption rate among adults compared to young children.

Breast-feeding

Through breast-feeding, the human infant can be exposed to considerable amounts of POPs, but the importance it has on lifetime exposure is still being discussed. The levels of PCBs and pesticides in human breast milk have decreased considerably during the last 20-30 years in Sweden and Norway (Johansen et al., 1994; Noren and Meironyte, 2000). Despite this fact breast milk concentrations remain high relative to those in other foods, and Alcock et al. (2000) estimated that for an individual born in 1980, breast-feeding may account for as much as 8 % of total body burden by the age of 25. For an individual born in 1990 the contribution increases to 30 %. Ayotte et al. (1996) estimated breast-feeding to markedly influence the body concentrations of dioxin-like compounds in Inuits mainly during infancy and childhood, but not during adulthood. Kreuzer et al. (1997) on the other hand, claim that the elevated levels in

breast-fed infants decline during a few years, reaching the same concentration as the children not breast fed.

Diet

For humans not occupationally exposed to POPs, food is the most important exposure route and of major concern in some areas of the Arctic (AMAP, 1998). In relation to background exposure, absorption through the skin and through inhalation can be neglected, and thus is discussed no further in this thesis (Alcock et al., 2000).

The diet around the Arctic regions of the world is highly variable, with people in Scandinavia, Iceland and the Faeroe Islands depending on a western diet modified by local influences. Among native people in the Arctic, traditional food is more important, with coastal populations depending heavily on marine mammals. The traditional food is considered very nutritious and the main source of protein, fat, minerals (Fe, Zn, Se, I), vitamin D, and especially of the essential long chain n-3 fatty acids (Kuhnlein et al., 2002; Van Oostdam et al., 1999). For many people these foods, harvested through hunting, are also a source of community spirit, pride, and self-respect. "They can provide health, bodily warmth to withstand the cold climate, and well being in a way that imported food simply cannot" (Van Oostdam et al., 1999). However, the use of traditional diets have declined during the past 50 years, resulting in indigenous people from North America and Greenland getting between 60 and 90 % of their food from the grocery store. This trend is not as clear in Russia, where high prices in combination with low income and strong traditions still make people rely heavily on country foods (AMAP, 2002).

The highest human levels of POPs in the Arctic have been found in populations depending heavily on marine mammals, such as the Inuit of Greenland and Arctic Canada, or among people on the Faeroe Islands consuming pilot whale blubber (Ayotte et al., 1997; Bjerregaard and Hansen, 2000; Deutch and Hansen, 2000; Fangstrom et al., 2002). Assessing the Canadian Arctic diet, the highest levels of POPs were found in the marine mammals, with the Inuit diet being above the tolerable daily intake (TDI) for all compounds reported (Chan, 1998).

The 1998 AMAP Assessment Report (Phase 1) has made it clear that there is a general lack of data on diet and contaminant levels in the Russian Arctic, where high exposure is especially anticipated for indigenous costal populations in northeast Russia who consume marine mammals. Besides the expected high human levels of POPs related to consumption of marine mammals, there have been reports of high concentrations in human plasma of *p,p'*-DDT and β-HCH in the town of Nikel in the Kola Peninsula. These findings have been recognised as indications of current or recent use of these pesticides in that region (AMAP, 1998; Klopov et al., 1998). It is unknown whether this exposure is direct or through consumption of contaminated food.

Plasma levels a good indication of body burden of POPs

The reliability of plasma levels of POPs as a measure of the total body burden is widely discussed. Equilibrium among the organs is expected, and when relating the amounts of POPs to the lipids in each tissue the concentrations are believed to be approximately equal (Alcock et al., 1999; Filser et al., 1997). This was supported by the work of Haddad et al. (2000) who found that, regardless of the identity of the compound and log n-octanol:water partition coefficients, their adipose tissue:blood partition coefficient was equal to the ratio of lipids in adipose tissue and blood. Patterson et al. (1988) found that the ratio of the lipid weight concentration of 2,3,7,8-TCDD in adipose tissue and serum was equal to one. On the other hand, Needham et al. (1990) reported that the lipid-weight levels of different POPs were 1.34 – 2.60 times higher in adipose tissue compared to serum. Further, no correlation for 15 of 17 organochlorines was found between breast adipose tissue and serum, as well as a total absence of the compound residues in serum (Archibeque-Engle et al., 1997).

The importance of fluctuations in plasma levels of POPs following a meal has also been questioned. In one study, the PCB levels in plasma increased more than the lipids after consumption of PCB-contaminated fish (Kuwabara et al., 1979). This was supported by a study on breast cancer where it was found that serum levels were very sensitive to short-term changes in diet, such as fasting, thereby constituting an imperfect surrogate for the levels found in breast adipose tissue (Holford et al., 2000). Phillips et al. (1989) on the other hand claim that when the plasma levels are lipid normalised there is no change four to five hours after consumption of a lipid-rich

meal. Despite this conflicting evidence the major part of the POPs in plasma have been reported to originate from the body's depots, mainly adipose tissue, and that the plasma levels are stabile over a period of 3 months (Gammon et al., 1997; Noren et al., 1999). However, it is not unlikely that the distribution will differ with food intake (Noren et al., 1999). Our current understanding of the transport and fate processes within the body and of the rates/efficiencies of absorption and metabolism are, however, relatively limited (Alcock et al., 1999).

Persistent organic pollutants and human health

"Among the many thousands of man-made bulk-chemicals in use today, only a limited number have been tested or evaluated for their hazard potential. In understanding the possible consequences for human health and the Arctic environment, extensive evaluation is needed. The fact that the consequences often are observed decades after emission to the environment complicates the issue further and should always be kept in mind. Evaluation criteria must include long-range transport ability, persistence, bioaccumulation potential and hazard for human health and the environment" (Odland et al., 2003).

There is no doubt that the persistent organic pollutants discussed in this thesis are toxic compounds, and that they have the potential to affect human health. The question is if the levels of these compounds found in the humans cause adverse effects. The effects are however hard to measure, and if they are observed it is then extremely difficult to state with certainty which chemical or confounding factor is responsible (Van Oostdam et al., 1999). The list of relevant health issues is long and it is beyond the scope of this thesis to include all those suspected and to discuss in depth the suspected contaminants and mechanisms of action.

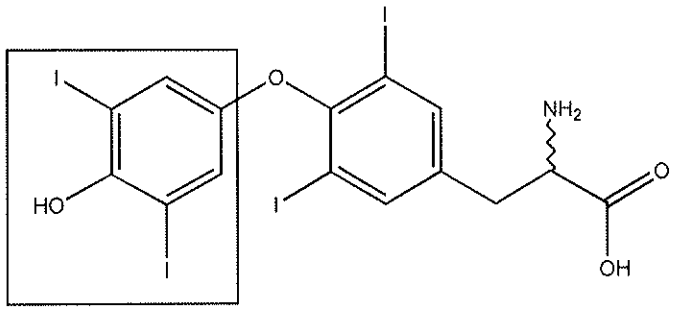
The POPs have, through animal studies and occupational accidents, been shown to have a number of toxic effects. Experimental data from animal studies clearly show that PCBs/PCDD/Fs induce P-450 metabolising enzymes and that the aryl hydrocarbon receptor (AhR) plays a pivotal role in mediating most if not all of the toxic and biochemical effects induced by PCBs/PCDD/Fs (Brouwer et al., 1995 and references therein; Safe, 1994). A wide variety of endocrine systems are affected by the PCBs, including the oestrogen and androgen system, the thyroid hormone system,

retinoid system, corticosteroid system and several other endocrine pathways (Brouwer et al., 1998b; Brouwer et al., 1999; Golden et al., 1998). New information on the mechanisms of the toxic effects of POPs points to disruption of hormone systems. Thyroid hormones have received a particular focus. These hormones control fetal brain development and behaviour, as well as growth, metabolism, and reproduction throughout the life of an animal (AMAP, 2002; Brouwer et al., 1998b). Experimental studies have shown that several PCBs are capable of inducing enzymes in the liver, resulting in increased vitamin K metabolism, which may ultimately lead to deficiency of the vitamin (ten Tusscher et al., 1999). Neurotoxic effects of toxaphene exposure have been reported and the toxaphenes are also believed to represent a potential carcinogenic risk to humans (de Geus et al., 1999). A number of the POPs have been listed as to their carcinogenicity by IARC (Table 2). It is only 2,3,7,8-TCDD that is listed by the IARC as a definite carcinogen.

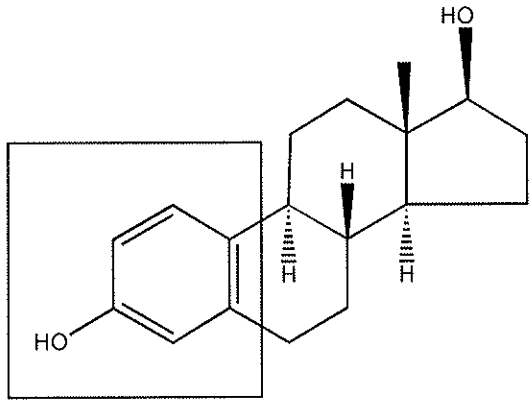
Table 2: POPs listed by the IARC as to their carcinogenicity*.

Group 1: **Carcinogenic to humans**	**Group 2A:** **Probably carcinogenic to humans**	**Group 2B:** **Possibly carcinogenic to humans**	**Group 3:** **Not classifiable as to carcinogenicity to humans**
2,3,7,8-TCDD (Vol. 69; 1997)	Polychlorinated biphenyls (Vol. 18, Suppl. 7; 1987)	Chlordane (Vol. 79; 2001)	Aldrin (Vol. 5, Suppl. 7;1987)
		p,p'-DDT (Vol. 53; 1991)	Dieldrin (Vol. 5, Suppl. 7; 1987)
		Heptachlor (Vol. 79; 2001)	Polychlorinated dibenzo-para-dioxins (other than 2,3,7,8-TCDD) (Vol. 69; 1997)
		Hexachlorobenzene (Vol. 79; 2001)	Polychlorinated dibenzofurans (Vol. 69; 1997)
		Hexachlorocyclohexanes (Vol. 20, Suppl. 7; 1987)	
		Mirex (Vol. 20, Suppl. 7; 1987)	
		Pentachlorophenol (Vol. 53; 1991)	
		Toxaphene (Vol. 79; 2001)	

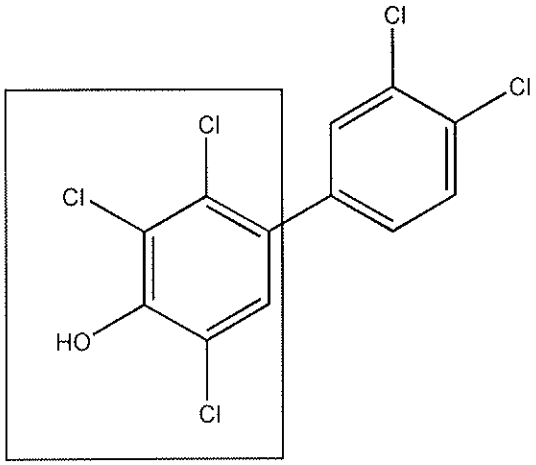
*Volume number and year of the appropriate IARC (International Agency for Research on Cancer) monographs are indicated in parentheses.



Thyroxine (T4)



17β-estradiol (E2)



4-OH-CB107

Figure 3: The structure of thyroxin (T4), 17β-estradiol and 4-OH-CB107, where the aromatic ring with the hydroxyl group is important mechanistically.

The phenolic organohalogens have received increased attention due to the structural similarities to thyroxine (T4) and estrogen (Figure 3), and possible functional mimicry. Phenolic compounds have been found to inhibit the sulfotransferase catalysed E2 sulfation (Kester et al., 2000; Kester et al., 2002). The OH-PCBs have also been shown to be anti-estrogenic (Kramer et al., 1997). With the exception of one compound, the OH-PCBs had the same affinity (Ki = 10-80 nM) for transthyretin as thyroid hormone (thyroxine; T4). Based on these results, OH-PCBs *in vivo* are more

likely to compete for binding to this serum transport protein than binding to the thyroid receptor (Cheek et al., 1999).

The potential of these effects has been demonstrated, but the evidence of their occurrence in relation to background exposure is highly uncertain.

Health effects caused by background levels

Severe health effects have been reported in relation to high accidental exposure to PCBs/PCDD/Fs (Chen et al., 1994; Guo et al., 1999). Examples of effects are abnormalities of liver function, skin (chloracne), and the nervous system (Longnecker et al., 1997). ten Tusscher et al. (1999) found in a Dutch study that current background concentrations of PCBs and dioxins disturb the thyroid hormone metabolism in humans. Longnecker et al. (1997) reported that background exposure to PCBs cause neonatal hypotonia or hyporeflexia. Another study reported higher levels of PCBs, chlordanes and tetrabrominated diphenyl ether in patients with Non-Hodgkins lymphoma (Hardell et al., 2001). The studies reporting significant associations between compounds and different effects are many, but no further descriptions of these are given.

However, several review papers conclude that the results of the various epidemiological studies on the possible effects caused by background exposure are inconclusive.

Swanson et al. (1995) claim that only a few reliable studies exist to suggest that adverse effects in humans have resulted after occupational exposure to PCBs. Further, there was no positive or suggestive evidence from 33 human studies to indicate that environmental exposures other than in the workplace are associated with harmful outcomes.

Adami et al. (2003) concludes that although a modest positive association between organochlorine exposure and breast cancer is difficult to exclude, it is unlikely that exposures to these compounds are important causes of breast cancer.

Kimbrough (1995) claims that the available evidence for cancer and for reproductive effects of PCB exposure is inconclusive. She, however, found that obvious external

clinical signs are observed in the offspring of subhuman primates at dosage levels below those experienced by female capacity workers and members of the general population prior to the control of PCBs.

Independent of the possible effects caused by background exposure, there is no doubt that the developing foetus and breast-fed infant are the age groups at greatest risk in the Arctic (AMAP, 1998; Van Oostdam et al., 1999).

Developing foetus and breast fed infant

Several studies now claim to have observed health effects in children as a result of background exposure. The major developmental endpoints related to *in utero* and lactational exposure to POPs to date in infants are reduced birth weight, altered circulating thyroid hormone levels and altered psychomotor and cognitive function (Brouwer et al., 1995; Feeley and Brouwer, 2000; Golden et al., 1998). A Dutch PCB/dioxin study has illustrated that subtle clinical, endocrine and mental/psychomotor development effects can occur in breast-fed infants (Brouwer et al., 1998a; Patandin et al., 1998). Background exposure might also be associated with a greater susceptibility to infectious diseases (Dewailly et al., 2000; Weisglas-Kuperus et al., 2000).

The most sensitive time period seems to be *in utero* exposure (Brouwer et al., 1995; Hansen, 2000). This is supported by Patandin et al. (1999) who, in a Dutch study, found in that *in utero* exposure, rather than postnatal exposure through breast-feeding, was associated with poorer cognitive functioning in pre-school children.

Feeley and Brouwer (2000), however, describe the mentioned effects as subtle and generally within what can be described as normal population background variation. The only exception they mention is what occurred in two rice-oil poisoning episodes in Asia. Further they claim that little if any clinically adverse health effects have been associated with breast-feeding, stating the observed beneficial effect from breast feeding on neurological measures.

The benefits of breast-feeding appear to outweigh the risks associated with the presence of relatively low levels of PCBs/PCDD/Fs (Brouwer et al., 1995; Hansen, 2000). In a WHO recommendation, it was concluded that the current evidence of

possible health effects does not warrant altering the previous WHO recommendation for promotion/support of breast-feeding (Brouwer et al., 1998a; van den Berg et al., 2000).

Limitations of studies on human health effects

Risk assessments are in general based on evidence collected for individual contaminants at often high concentrations and not for naturally occurring mixtures of contaminants. They are based on extrapolations from single compound animal studies, and when possible combined with data from occupational exposure or accidental poisoning. Very little is known about interactions between different compounds, as additive, synergistic or antagonistic effects might occur (Carpenter et al., 2002). Grandjean et al. (2001) has also reported that limited PCB-related neurotoxicity appears to be affected by correlated methylmercury exposure. The possible effects of nutrients like trace elements and antioxidants present in traditional food must also be considered (Hansen, 2000; Kuhnlein et al., 1995).

The epidemiological studies on effects in human populations and background levels are all based on a limited number of cases or participants. With the low number of participants the statistical power of these studies are limited. The extensive use of a low significance criterion of $p<0.05$ has also been criticised (Sterne and Smith, 2001). One of the main reasons for the low number of participants is the costly analysis of POPs in tissue or plasma. Efforts have been made to reduce costs and one of the possibilities has been the use of possible surrogate indicators of exposure instead of analysing all compounds. One example is PCB congener 153 that in several studies has been reported to be highly correlated to most PCB congeners and in particular the sum PCBs (Furberg et al., 2002; Grimvall et al., 1997). However, because of differential toxicities among the entire class of compounds and the limited information on human health effects, it is recommended to continue to obtain isomer-specific analyses for the PCBs and PCDDs/Fs (Woodruff et al., 1994).

Another important aspect is the sample volume required for valid quantification of the POPs. Most analyses require several millilitres, whereas the amounts available in plasma banks organised by amongst others; the European Prospective Investigation into Cancer and Nutrition (EPIC) and Norwegian Women and Cancer Study

(NOWAC) are kept in special straws and in general between 0.4 and 0.5 ml (Pers. Comm. E. Lund). In relation to EPIC and NOWAC the participants have already submitted dietary and lifestyle information of relevance for possible future studies. There is thus a need to reduce the sample volume, as well as considerable reduction in the time required for the analyses and thus their costs.

Objectives

The principal objective of the present work was to fill knowledge gaps in the research area of human plasma levels of POPs in Arctic regions, and the relation to traditional food consumption. At the same time it was a goal to develop a cost-efficient analytical method allowing small sample volumes.

Specifically:

- Fill knowledge gaps on human plasma concentrations of POPs in northern Russia (Paper I and III).
- Determine the impact of the consumption of traditional foods on the body burden of POPs in a native Russian population (Paper III).
- Study the short-term changes in plasma levels of POPs in relation to high dietary intake of marine lipids and POPs (Paper II).
- Establish a cost-effective analytical method for the determination of both conventional POPs and phenolic compounds in the same small volume of plasma (Paper II and IV).
- Determine hydroxy-PCBs and PCP levels, in a highly exposed subgroup of indigenous people from the Russian eastern Arctic (Paper IV).

Materials and Methods

Details about the study groups and sample collection are thoroughly described in the individual papers. The geographical areas where the different samples were collected are shown in Figure 4. Table 3 summarizes the geographical areas, number of plasma samples collected and analyzed, and the analytes determined in Publications I to IV. In the last three papers, both lipid-weight and wet-weight levels of POPs were reported. In Paper I, with a slightly less sensitive method, the sample amounts available did not allow the determination of lipids as well as POPs.

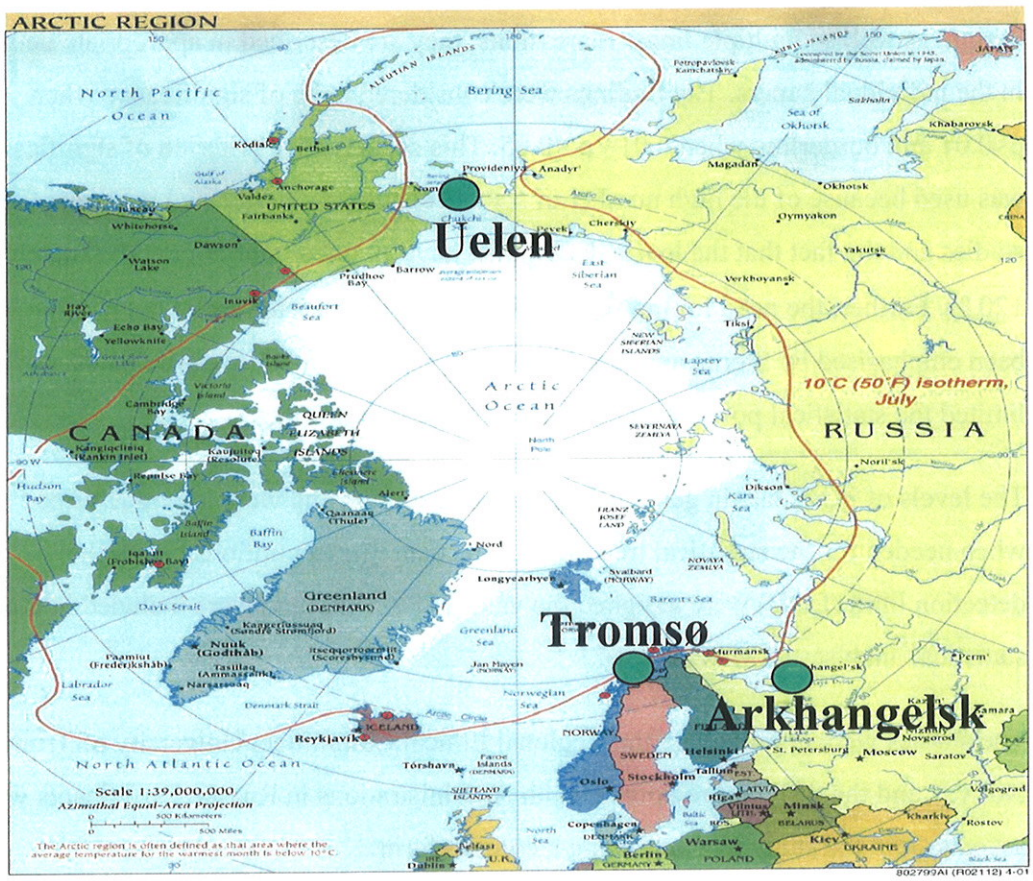


Figure 4: Map of the Arctic and surroundings with the different sampling sites marked.

Table 3: Geographical areas, collection period, subjects and analytes measured.

Place (Period)	Subjects (n), Paper	Analytes
Arkhangelsk (Sept. - Oct. 1996)	Delivering women (27), I	PCBs and pesticides
Tromsø (March 2000)	General population (33 x 4), II	PCBs and *p,p'*-DDE
Uelen (July – Aug. 2001)	General population (50), III + IV	PCBs and pesticides. PCP and OH-PCBs (for 15 highly exposed individuals)

Statistical analyses were carried out employing the SAS software package, version 6.12 (SAS Institute Inc., 1989). The statistical approach was based on univariate analysis, analysis of variance, analysis of variance with a repeated measurement design, as well as multiple linear regressions; they are described in appropriate detail in the individual papers. The findings were considered to be of significance when $p<0.01$ and borderline when $0.01< p <0.05$. This conservative criterion of significance was used because of the high number of statistical analyses performed in some of the studies and the fact that the levels determined have an uncertainty of approximately ±20 %. Further, the need for more powerful studies and stricter use of the p-value has been emphasised by Sterne and Smith (2001). The low number of participants also limited the statistical power of the studies presented in this thesis.

The levels of POPs had in general a skewed distribution and were log transformed when needed for the statistical treatment of the data. Concentrations below the detection limit (LOD) were assigned the values of ½ LOD and were included in the statistical analyses (AMAP, 1998).

The studies were approved by the Regional Ethical Committee, University of Tromsø, Norway and the different regional Health Administrations in Russia. Participants were asked to join the study by completing a consent form.

Main results

Paper I

The analytical method developed to support this study involved gel permeation chromatography and silica gel purification, making inclusion of acid labile compounds possible. Quantification was done using GC-MS instrumentation employing described analyte clean up and separation procedures. The geometric mean plasma concentrations in 27 delivering women from Arkhangelsk, Russia of *p,p'*-DDE, β-HCH and *p,p'*-DDT were 4.52, 3.08 and 0.83 μg/l, respectively. The DDE/DDT ratio was as low as 7. Toxaphene 26 and 50 were the only toxaphenes having levels above the LOD, with geometric mean levels of 0.05 μg/l and 0.06 μg/l, respectively. The concentration of sum PCBs (geometric mean, 1.75 μg/L plasma) were low, with PCB 138/163 as the most abundant congeners (0.42 μg/L plasma). The high β-HCH and *p,p'*-DDT levels as well as low DDE/DDT ratio supported previous indications of the presence of fresh sources of β-HCH and *p,p'*-DDT.

Paper II

In this study, a one step clean-up procedure using florisil columns was developed which replaced the GPC and silica columns employed in Paper I. The plasma levels of *p,p'*-DDE was found to increase significantly four hours after a "mølje" meal was consumed by 33 volunteers, both when expressed as wet-weight (35 % change) and lipid-weight (20 % change). The corresponding changes (0 – 4 h) in wet-weight concentrations of the most prevalent PCB congeners were non-significant. By contrast, PCB congeners with low levels in the food showed a significant drop during the first four hours when lipid-weight adjusted. The observed changes were independent of the amount of cod liver and cod liver oil consumed, age, gender and BMI. Significant differences in concentrations for fasting and non-fasting participants were found for most PCBs and *p,p'*-DDE. The lipid-weight adjusted sum PCBs was significantly lower (16 %) when the participants were fasting.

Paper III

The Chukchi people from Uelen (Chukotka) in eastern Russia consumed extremely high amounts of marine mammals. The combined intake of blubber from walrus, seal and whale was a significant predictor ($p<0.01$) of plasma concentrations of sum PCBs, and borderline ($p<0.05$) for sum CDs and sum DDTs. For males and females combined, the geometric mean concentrations of sum PCBs, *p,p'*-DDE and β-HCH was 1316, 520 and 410 ng/g lipids, respectively. There was a significant gender difference in the plasma levels of POPs for individuals below the age of 40, and among women there was a significant increase with age. PCB 163, which partly co-eluted with PCB 138, was found in high concentrations (40 % of PCB 138). This raises questions regarding the reliability of the previously reported levels of PCB 138 and the use of PCB 138 and 153 to calculate plasma Arochlor 1260 concentrations. Levels of β-HCH were higher than observed for other "high" Arctic native populations who depend on marine mammals.

Paper IV

The method for combined analysis of neutral and phenolic organohalogens developed here was an extension of that employed in Papers II and III. Additional acidification and the use of a more polar solvent (hexane:dichloromethane (3+1)) improved the efficiency of extraction of OH-PCBs and PCP. The use of florisil columns gave complete separation of neutral and hydroxylated compounds and good recovery rates (median; 83 – 116 %). The compounds were methylated using diazomethane, without validating that step in this paper. A single florisil column was used for the final clean up of the methoxylated compounds (median recovery rates; 67 – 90 %). Validating the method using spiked plasma samples gave acceptable recovery rates for all compounds (38 – 75 %), whereas real sample analyses gave good recovery rates for $^{13}C_{12}$-4-OH-CB 187 and $^{13}C_6$-PCP (72 and 64 %, respectively). In the plasma samples from the more highly exposed Chukchi participants (n=15), the median sum OH-PCBs was found to be 5916 pg/g. The major congener was 4-OH-CB107 with a median concentration of 1673 pg/g plasma. The sum OH-PCBs and sum PCBs were significantly correlated ($r=0.7$, $p<0.01$) and the median ratio of sum OH-PCBs to sum PCBs was found to be as high as 0.4. The median PCP concentration was 642 pg/g plasma.

Discussion

Determination of persistent organic pollutants in human plasma

The determination of lipophilic compounds in biota includes several steps in order to obtain reliable data. These steps consist of extraction, lipid removal and determination, clean up, chromatographic separation of different compounds and their quantification. The levels of POPs in human samples are in general low, and complex methods for extraction and clean-up are required to obtain clean samples with low detection limits. For large epidemiological studies the costs of the analyses are still too high and high throughputs at reduced cost is an important objective.

Extraction

The purpose of the extraction step is to extract the compounds of interest quantitatively from the plasma into an organic solvent. Considering the fact that the POPs in general are stored in the lipid fraction of samples, an efficient lipid extraction technique is required. Quantitative lipid extraction has thus been a good estimate of quantitative extraction of the conventional POPs. The phenolic compounds are, however, more polar and a high proportion of these compounds are bound to proteins and especially transport proteins (Letcher et al., 2000). For effective extraction, these proteins must be denatured and the pH must also be low enough to ensure protonation of the phenolic compounds and their transfer to the organic solvent.

As seen in Papers I-III, the use of ethanol, saturated ammonium sulphate and hexane was suitable for the extraction of PCBs and pesticides. But for the extraction of phenolic compounds further acidification was needed, as well as the use of a more polar extractant. Sulphuric acid (9 M) and hexane:dichloromethane (3+1) was used for the third extraction step (Paper IV). Acidification to pH 1-2 was achieved with full protonation of the OH-PCBs, considering that the lowest pK_a values reported for the OH-PCBs is 6.8 (Letcher et al., 2000); the same applies to most protonation sites on proteins.

For phenolic organohalogens, spiking samples with specified amounts of a standard solution was expected to give a poorer estimate of extraction efficiency compared to the neutral non-phenolic compounds, because of the protein binding (Letcher et al., 2000). Because of this protein binding, the amount of phenolic compounds extracted might be highly variable depending on how efficiently the proteins are denatured. The use of ^{14}C-labelled OH-PCBs administered to rats as shown in a study by Hovander et al. (2000), gives a better indication of how efficient the extraction really is. However, metabolism and protein binding might be different in humans and animals (van de Waterbeemd and Gifford, 2003), making the estimate of extraction efficiency in human plasma uncertain. Further work is required for better understanding of protein binding and extractability from human plasma.

Liquid-liquid extraction methods have been criticised because of excessive use of solvent and the possible problems of emulsion and concomitant reduction in extraction efficiency (Thomsen et al., 2001). The use of 12-18 ml of hexane for the extraction of neutral compounds and an additional 6 ml of hexane:dichloromethane (3+1) for the phenolic compounds are however acceptable volumes and do not exceed the volume required for solid phase extraction (SPE) (Thomsen et al., 2001). There were no problems with emulsion formation in all but one sample, for which centrifugation was needed.

As an alternative to liquid-liquid extraction, the SPE methods have given excellent results for the extraction and clean-up of brominated flame retardants including their phenolic derivatives (Thomsen et al., 2001). Especially low detection limits due to reduced blank contamination of the brominated compounds have been reported. These procedures do, however, involve the use of sulphuric acid for lipid removal, which opens up the possibility of selective loss of acid labile compounds.

Clean up and separation of neutral and phenolic organohalogens

Initially, a gel permeation chromatography (GPC) column and silica-column with several fractions was used for the clean up of the plasma samples (Paper I). The use of a GPC column removed the bulk of the lipids in a non-invasive manner allowing acid labile compounds to be quantified. This method was however tedious and needed considerable amounts of solvent (88 ml cyclohexane:ethyl acetate). The single step

clean-up using tandem florisil columns was thus tested, validated and implemented (Paper II). This procedure also allowed the isolation of acid labile compounds, and it was quick and demanded a considerable smaller volume of solvent (11 ml hexane:dichloromethane).

The analysis of large number of samples and an increasing number of compounds are required to obtain comprehensive information on exposure. With the increasing number of compounds to be determined, the probability of interference in the final step of quantification increases. Thus, the different groups of compounds should be split in separate fractions based on their properties. Ideally then, all the lipids should be removed in the same step as the different compound groups of interest are separated.

This was in part accomplished through the use of florisil columns, where the tandem column efficiently removed the lipids from the fraction containing PCBs and pesticides (Papers II-III). The phenolic organohalogens were retained on the first column and could thus be eluted from that column (after discarding the bottom column), thereby affecting separation from the neutral compounds (Paper IV). Efforts were not made to separate the PCBs from the pesticides, considering there were no problems in the quantification step.

The use of the florisil columns also avoided the common approach of using aqueous potassium hydroxide (KOH) solution to separate phenolic and neutral organohalogens (Hovander et al., 2000). The use of florisil columns considerably reduced the time for the analysis of each sample and the reported problems of selective loss of the HCHs with KOH was also avoided (Hovander et al., 2000).

GC-MS analyses

Throughout this work, separation and quantification of the studied compounds were achieved using a gas chromatograph connected to a mass spectrometer (GC-MS) (Papers I-IV). The instrumentation employed and the method of quantification is thoroughly described in the papers. Both positive electron impact ionisation (EI+) and negative chemical ionisation (NCI) have been employed in selected-ion-monitoring (SIM) mode. Problems with poor sensitivity with the NCI source permitted only

detection of tox 26 and tox 50. Thus, we have no information on the amounts of other toxaphene congeners.

Lipid determination

The amount of POPs can either be related to the wet weight of the sample or the amount of lipids in the sample. Normalising the POP concentrations to the amount of lipids is believed to remove the tissue differences caused by the different inherent amounts of lipids (Brouwer et al., 1999). It is thus important to accurately determine the amount of lipids in the plasma sample. This can be done gravimetrically or enzymatically. The disadvantage of the gravimetric determination is the high LOD resulting in high uncertainty or the need of large plasma volumes. The amount determined gravimetrically is strictly the extractable organic material (EOM) with no knowledge of the amount of lipids, lipid composition or the amount of lipoproteins in the extract (Akins et al., 1989).

Enzymatic determination of different lipid classes has become more common, and this procedure employs a summation formula to assess the total amount of lipids (Cheek and Wease, 1969). The preferred formula is: TL = 1.677(TC-FC) + FC +TG+PL (Akins et al., 1989; Phillips et al., 1989). The obvious advantage of this procedure is the fact that the composition of lipids is determined, and it is only lipids that are quantified.

In Papers II, III and IV the lipids were determined using the quoted summation formula or a modification of it (Paper II). In Paper II, it was shown that the EOM determined gravimetrically was 15 % lower than the amount of lipids determined using the summation formula. A comparable difference was reported by Sjodin et al. (2000). For more reliable data and better inter-comparisons, it has been suggested to only use the enzymatic method for determination of lipids (Grimvall et al., 1997). On the basis of our findings in paper II and the problems with the gravimetric determination, we strongly support this recommendation.

Quality assurance

Optimum quality assurance procedures are essential if small differences in health outcomes and exposures are to be detected in epidemiologic studies, especially at the

low POPs levels that may be expected to occur in humans during the next decades (Woodruff et al., 1994). Through the work of AMAP and the monitoring of human levels in the circumpolar regions, the need for an interlaboratory comparison programme became evident (AMAP, 2003). The AMAP Human Health group thus organised a ringtest for the participating laboratories. The Norwegian Institute for Air Research (NILU) and the Centre of Human Toxicology (CTQ) initiated this work, with CTQ being in charge of the continuing programme. We have therefore been participating since the onset of the programme. In Figure 5, our reported levels are compared to the target values for all compounds in Round 3 (2002). Our performance has been excellent through all runs and this is summarized in Table 4. The compounds included in the ringtest were: PCB 28, 118, 138, 153, 170 and 180, and *p,p'*-DDE, *p,p'*-DDT, β-HCH and oxy-chlordane. Mirex was included in one round but then taken out, due to problems in a number of laboratories. The ringtest of today only includes wet-weight data, but efforts should be made to extend it to also include lipid-weight levels. The lipid weight levels are important and quality control is needed for these data as well.

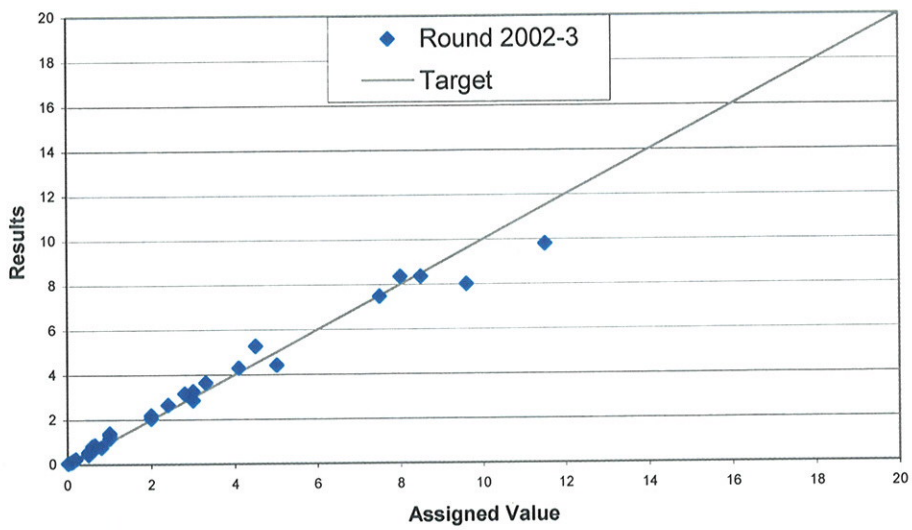


Figure 5: A pseudo Youden plot displaying our performance on Round 3 in 2002. The results for all compounds are indicated as squares, compared to the assigned value indicated by the straight line.

The participation in a ringtest like this is not enough to assure the quality of the data produced. The daily quality controls in the laboratory are important to make sure that performance is continuously good and that possible problems can be detected. Several steps are in place to assure this. Laboratory blanks were analysed with each run, making sure inadvertent contamination is detected. However, standard reference materials with known levels should also be analysed regularly, but that was not done in the present work.

Table 4: Performance (%) in the AMAP human ringtests.

Round	Performance (%)*	Ranking
1- 2001	Performance not calculated	
2- 2001	98.0	4 of 25
1- 2002	85.0	10 of 21
2- 2002	95.0	3 of 23
3- 2002	88.3	7 of 22

*Each result within ± 40 % of the assigned value, gives 1 point. Each result also within ± 20% gives an additional point. Only numerical results are counted. Laboratories must supply data for at least 5 analytes in order to be graded. Scores are expressed as percentages. For example, if a lab reported numerical results for all three samples and all 10 analytes (i.e., a possibility of a total of 60 points) and obtained 48 points, its score would be 80%.

To ensure that compounds are correctly identified, the isotopic ratio method is used. This ratio should not deviate more than 20 %. If that is the case, there is likely some interference and the compound cannot be quantified, and it should not be reported.

The detection limit (LOD) is also important in accurately determining the levels of the different compounds. The LOD indicates the lowest amount that can be reliably reported and by definition provides a confidence limit associated with such values (Woodruff et al., 1994). By applying the International Union of Pure and Applied Chemistry (IUPAC) definition, the LOD is estimated as three times the standard deviation of the blank determinations (Long and Winefordner, 1983). We have, however, chosen to use three times the blank concentration itself as the LOD. Considering the strictness of LOD, and the fact that it may be preferable to retain all determinations, all values below LOD were set to ½ LOD and included in the statistical analyses (Woodruff et al., 1994). The limit of quantification (LOQ) was set to 3 times LOD values.

Despite all efforts to produce reliable data, all reported values have an associated uncertainty. This is evident from the way performance is calculated in the ringtest

(Table 4), where a reported value within ± 20 % of the target (median) gives full score and within ± 40 % gives half the score. As the quantified levels decrease this uncertainty increases. This fact tends to be totally forgotten when differences in concentration are compared among populations and in related health studies.

Evaluation of the total procedure

Initially the aim was to reduce the plasma volume required for the analyses to less than 0.5 ml from about 2 ml, making it possible to analyse samples stored in plasma banks in relation to conducting large epidemiological studies. With the available instruments, the plasma volume could not be reduced too much without losing valuable compound-specific information. However, the use of selected indicator compounds could have enabled further reduction of the plasma volume. PCB 153 has in several studies been shown to correlate extremely well with the other major PCB congeners and sum PCBs (Furberg et al., 2002). The correlations among dioxin like compounds and PCBs has also been found to be fairly good (Gladen et al., 1999; Koopman-Esseboom et al., 1994; Longnecker et al., 2000). Brouwer et al. (1995) claim that when $TEQ_{PCB} >> TEQ_{PCDD/F}$, a single marker PCB analysis gives an accurate prediction of total TEQ concentration in a sample. However, Longnecker et al. (2000) reported that due to different toxic mechanisms for different compounds or compound classes the association might be distorted in studies of health effects associated with background environmental exposure. The proportions may also vary as the source or type of PCB exposure varies across different populations (Longnecker et al., 2003).

The results from Paper I-IV suggest that the use of PCB 153 alone as an indicator compound is not sufficient in estimating total exposure. In Paper I, the levels of *p,p'*-DDT and β-HCH were high, when PCBs were low. The concentration of β-HCH was also high relative to the other compounds in the samples from Uelen (Paper III). The OH-PCBs also seem to be high in the samples from Uelen (Paper IV), and there is little knowledge of the expected ratio to the PCBs. In the samples from Tromsø, only PCBs and *p,p'*-DDE were measured, but they were all at low levels (Paper II). These results clearly indicate differences in mixtures of POPs when comparing populations.

For a quick screening of exposure, it might be possible to use indicator compounds, but they must be from different compound groups to enable detection of a number of exposure scenarios. Based on Papers I-IV, possible candidate compounds are PCB 153, *p,p'*-DDE, *p,p'*-DDT, β-HCH and HCB. These compounds would mostly cover the most prevalent groups of POPs in these areas. However, for studies on human health effects, Longnecker et al. (2000) claim it is not possible to use indicator compounds due to the risk of misclassification.

It must not be forgotten that with a reduction in the sample volume and analysis of a selected number of indicator compounds, compound specific information will be lost. In particular for the brominated flame retardants this would be a problem. The levels of these compounds seem to increase in the environment and at the same time the levels are still low, requiring greater sample volumes (Noren and Meironyte, 2000). This must be considered when compounds and plasma volumes for analysis are selected.

The volumes required were nevertheless reduced to approximately 0.8 ml plasma for the determination OH-PCBs and PCP (Paper IV). This proved to be no problem for highly contaminated samples, but for pesticide analyses at low concentrations, some compound-specific information will be lost with this sample volume and the instrumentation available today.

The effects of a traditional meal on the plasma levels of POPs

It is reasonable to assume that the POPs in blood and adipose tissue are close to being in equilibrium with each other (Moser and McLachlan, 2002). As previously mentioned, there are conflicting conclusions in the literature regarding the effects a meal has on the plasma concentrations of POPs and how much the equilibrium is shifted. Using lipid-weight adjusted levels is believed to prevent differences in estimates after a meal (Phillips et al., 1989). There has, however, been a lack of data corresponding to plasma POPs in relation to meals rich in both lipids and POPs, which is often the case for traditional food.

With the traditional meal "mølje", large amounts of cod liver and fresh cod liver oil were eaten, and with the food considerable amounts of PCBs and *p,p'*-DDE (Paper

II). The plasma levels of *p,p'*-DDE increased significantly four hours after the meal, both on a lipid-weight and wet-weight basis. The lipid-normalised levels of *p,p'*-DDE did, however, fluctuate less than the wet-weight concentrations. In the cod liver oil, the average level of *p,p'*-DDE was 88 ng/g wet-weight, whereas for marine mammals concentrations of *p,p'*-DDE lie in the range of 100-300 ng/g blubber from female ringed seals, and 1000 – 2000 ng/g blubber from beluga whales, with even higher levels in narwhal whales (AMAP, 1998). With the consumption of blubber, a similar or greater increase in plasma concentrations observed for 'mølje' might thus be anticipated. An increase in plasma levels is supported by Juan et al. (2002) who hypothesised that following a potentially PCB-rich meal (e.g. contaminated fish), the PCB concentrations in the blood will be temporarily elevated, but quickly buffered as the lipid-based POPs in blood equilibrate with those in tissue fat.

No increase in plasma levels for PCB 153 and PCB 138/163 was observed four hours after the meal, despite the fact that the levels (lipid-weight) were comparable to the *p,p'*-DDE, both in food and plasma. This indicates that there are compound specific differences in adsorption rate and/or distribution rate, resulting in differences in time to establish new steady states. For the PCB congeners where the lipid-weight levels in the cod liver and cod liver oil were low compared to plasma there was a significant decline in plasma levels (lipid-weight) after four hours. On a wet-weight basis, there was no change. The decrease in lipid-weight adjusted concentrations of congeners could thus only be caused by the increase in amounts of lipids in plasma after four hours. This indicates that the remobilisation of the compounds from the adipose tissue takes longer than the four hours. According to Phillips et al. (1989), the new equilibrium would be established after 4-5 hours, considering the fact that they observed no change in lipid-weight levels. The lipid peak in plasma is expected to occur between four and six hours after the meal (Hansen et al., 1998). In another study, it was found that the concentrations of PCBs and other POPs in various "lipid pools" in the body (e.g., blood lipid, subcutaneous fat, visceral fat, etc.) partitioned with each other fairly rapidly (i.e., over days/weeks) (Juan et al., 2002). Our results indicated that after 12 hours the levels were equilibrated again (Paper II). The exact time for the different compounds to establish equilibrium is not known, but an equilibration time of 7 hours for lipophilic compounds is indicated (Rowland and Tozer, 1995).

It could be argued that the observed changes in Paper II were small and of no practical consequence when human plasma levels are studied and compared. However, some of the changes were of significance and for DDE the changes were considerable. It is nevertheless of uttermost importance to fully understand the processes of absorption and distribution. Several of the populations studied in the Arctic have extreme traditional food dietary patterns and care should therefore be taken to avoid additional errors in estimates, caused by fluctuating lipid concentrations. This is especially so when the data are to be compared to western populations with non-traditional diets. Paper II does show that lipid normalised values also fluctuate following a meal of "mølje".

Based on these findings, we strongly recommend that plasma samples for the analysis of POPs should be obtained from fasting individuals whenever possible. In studies of populations in remote areas where the people are visited at home, this might be difficult to put into practice. Note might then be taken of time passed from the last meal or as in a recent study, the participants can be possibly asked to consume only a light meal before sampling (Hardell et al., 2002). For increased comparability to previous studies, both wet-weight and lipid-weight adjusted values should be reported.

Predictors of POPs in plasma, with a focus on diet

Through the use of a dietary questionnaire, the amount of blubber (seal, whale and walrus) consumed was identified as the only food item that significantly affected the concentrations of POPs in plasma of the participants from Uelen (Paper III). However, it was found only to significantly affect the levels of sum PCBs and did so in a borderline fashion for sum CDs and sum DDTs for males and females combined.

However, the use of dietary questionnaires to estimate the intake of POPs has in several studies been shown to be rather inaccurate (Van Oostdam et al., 1999). There are several reasons for this. Different dietary patterns make it difficult to design a questionnaire that has the right combination of questions for the different populations. People's memory and perception of amounts consumed are subject to inaccuracies. In a study by Deutch and Hansen (2000), the association between reported monthly food frequency and POPs was relatively weak.

The intake of marine mammals was considerable among the participants from Uelen, with little consumption of commercial goods (Paper III). There is reason to believe that this is one of the native populations with the highest intake of marine mammals, considering the fact that the reported intake of commercial goods has been reported to be as high as 60 – 90 % among Inuits of Greenland and northern Canada (AMAP, 2002). Despite the extreme intake of blubber, the plasma concentrations of POPs were not higher than those previously reported in native populations (AMAP, 1998; Ayotte et al., 1997; Deutch and Hansen, 2000; Dewailly et al., 1993). The only exception to this was the level of β-HCH (geometric mean; 410 ng/g lipids); it was higher than in any other native population previously studied. This suggests that the contamination by PCBs and pesticides (except β-HCH) are lower in marine mammals and polar bears from the Chukotka area, compared to the other areas of the circumpolar countries (AMAP, 1998; AMAP, 2002). Environmental assessments indicate that the prevalence of β-HCH seems unusually high in Chukotka/Bering Strait area (AMAP, 2002).

Both age and gender were found to be significant predictors of all the studied compounds. Age was, however, only borderline significant for the sum PCBs and sum tox. For the men, in opposition to the women, there was no significant increase in the plasma concentrations of POPs with age. Several studies have indicated that there is an increase in POP concentrations in plasma with age, particularly in women (Bjerregaard and Hansen, 2000; Grimvall et al., 1997). Ahlborg et al. (1992) state that since the biological half-lives of many chlorinated biphenyls are rather long, the body burden will increase during life, if the dietary intake is constant over time. However, there are several factors that indicate that plasma levels of POPs would not increase continuously with age. One study did show that for a given contamination level in the diet, the net absorption of hexachlorobenzene (HCB), PCBs and PCDD/Fs in human volunteers diminishes as the blood level of the compounds increases (Schlummer et al., 1998). The dietary absorption of POPs appears to some extent be compensated by excretion through the intestinal tract wall into the faeces. Further, a linear relationship between the level of a given compound in blood and in faeces has been observed over a range of blood concentrations covering several orders of magnitude (Moser and McLachlan, 2001). The decline in the amounts of the conventional POPs in the environment has been discussed as a possible explanation for the higher levels

observed in older people (Ahlborg et al., 1995; Grimvall et al., 1997). This is supported by Alcock et al. (2000) who state that the year in which the individual was born, is more important than age for the determination of the shape and magnitude of their exposure profile for a given compound.

Among the women from Uelen, the concentrations of POPs in plasma did increase significantly with age, and the levels of POPs were significantly lower compared to the men. This was also the case after correcting for differences in blubber intake. Breast-feeding has been shown to reduce the mother's stores of POPs considerably (Furberg et al., 2002; Grimvall et al., 1997; Rylander et al., 1997). As much as a 50 % decrease in maternal lipid-weight concentrations has been observed after up to 6 months of breast-feeding (Alcock et al., 2000). Even though the total period of breast-feeding was not a significant factor in the statistical analyses, it was still indicated as the cause of the lower levels and pronounced positive age dependence among the women (Paper III). When breast-feeding ends, the amount of POPs in lactating females are expected to increase again. The total increase is then dependent on the amount consumed and time passed from the last period of breast-feeding. It was not possible to correct for the time passed since the last period of breast-feeding due to lack of information on this, and this might have led to misclassification. The women had been breast-feeding for a median period of 36 months, and the levels of POPs were thus expected to be considerably affected. In the study by Furberg et al. (2002), which showed significant effects of breast-feeding; the women were all of the same age (40-42 years).

BMI and smoking are other factor known to affect the plasma concentrations of POPs (Furberg et al., 2002; Deutch and Hansen, 2000; Long and Winefordner, 1983). BMI was not found to be a significant predictor in the present study, and all the participants but three were smokers. Smoking was therefore not included in the model (Paper III).

Levels of conventional POPs, PCP and OH-PCBs in human plasma

The geometric mean plasma concentrations of the POPs for the three populations studied are listed in Table 5. Since the OH-PCBs were determined only for samples with the highest levels of PCBs, they are not listed, as they would not be representative of the Uelen population (Paper IV).

As observed in Table 5 the concentrations of sum PCBs are composed of different numbers of congeners in the separate studies. This is often the case when different studies are compared and care must be taken if important congeners are missing from the sum values. The lowest numbers of PCB congeners were determined in Tromsø, but in that study only the congeners that had had levels above LOD were selected. Thus, the contribution from the other congeners to the sum value was expected to be small. The additional PCB congeners determined in Uelen compared to Arkhangelsk, all had levels close to or below the LOD values. Thus they did not make much of a difference in the sum value. The participants from Tromsø and Uelen were both men and women, whereas the participants from Arkhangelsk were only women. In addition the age distribution was not the same in the different studies. These factors make the comparisons of levels more uncertain, but it does give an impression of the major differences.

Table 5: Geometric mean concentrations for all the individual POPs determined in the three populations studied

Compound	Non-native delivering women from Arkhhangelsk (n=27) Geometric mean	Non-native population from Tromsø (n=33)* Geometric mean		Native population from Uelen (n=50) Geometric mean	
	µg/l plasma	ng/g lipid	µg/l plasma	ng/g lipid	µg/l plasma
PCB 28				7.3	0.04
PCB 52	0.09			3.6	0.019
PCB 99	0.02	12.1	0.08	85.4	0.44
PCB 101	0.03	6.3	0.04	9.5	0.05
PCB 105	0.08			20.1	0.10
PCB 118	0.29	27.4	0.18	109.6	0.57
PCB 126				0.8	0.004
PCB 128				1.1	0.005
PCB 138/PCB 163	0.42	70.2	0.47	210.5	1.09
PCB 149				1.7	0.01
PCB 153	0.39	100.4	0.67	537.8	2.78
PCB 156	0.05	11.2	0.08	16.4	0.08
PCB 169				0.5	0.002
PCB 170	0.07	39.1	0.26	66.9	0.35
PCB 180	0.13	81.5	0.56	147.2	0.76
PCB 183	0.03	6.8	0.04	15.0	0.08
PCB 187	0.06	21.9	0.15	49.5	0.26
Sum PCBs	**1.75**	**374.6**	**2.50**	**1316.4**	**6.79**
HCB	0.47			167.7	0.86
α-HCH	0.15			3.5	0.02
β-HCH	3.08			409.6	2.10
γ-HCH	0.15			0.8	0.004
Heptachlor	0.10			0.4	0.002
Mirex	0.20			27.0	0.14
Oxy Chlordane	0.18			204.5	1.05
c-Chlordane	0.05				
t-Chlordane				0.6	0.003
c-Nonachlor	0.03			25.1	0.13
t-Nonachlor	0.12			260.6	1.33
Sum CDs	**0.41**			**518.1**	**2.65**
o,p'-DDE				0.9	0.005
p,p'-DDE	4.52	112.2	0.75	520.4	2.69
o,p'-DDT	0.25				
o,p'-DDT/*p,p'*-DDD				2.6	0.01
p,p'-DDT	0.83			33.7	0.17
Sum DDTs	**5.59**			**563.3**	**2.91**
Tox 26	0.05			32.7	0.17
Tox 50	0.06			28.9	0.15
Sum Tox	**0.11**			**62.7**	**0.32**

*The levels reported from the study in Tromsø are from the zero hour samples (non-fasting). Samples from non-fasting individuals were used considering that in the other sampling areas the samples were from non-fasting individuals as well.

Arkhangelsk

High concentrations of β-HCH and *p,p'*-DDT were found in delivering women from Arkhangelsk, with geometric mean values of 3.08 and 0.83 μg/L plasma, respectively (Paper I). The DDE/DDT ratio was calculated to be as low as 7 in these samples. The *p,p'*-DDE concentrations were also high and even higher than in the samples from Uelen, where they consume considerable amounts of marine mammals. The Arkhangelsk data all correspond well with what was reported in human samples from the town of Nikel on the Kola Peninsula of Russia (AMAP, 1998). Further, they suggest the presence of fresh sources of β-HCH and *p,p'*-DDT, but give no indication of their origin. The low PCB levels (sum PCBs, 12 congeners =1.75 μg/L) in the same samples suggest that PCBs and elevated pesticides have different origins. The high plasma levels of β-HCH and *p,p'*-DDT give reason for concern, and further efforts should be made to identify the sources. For 22 of 27 women this was their first delivery, indicating that their levels would not have been greatly affected by previous periods of breast-feeding.

Tromsø

The plasma levels of PCBs and *p,p'*-DDE were low in the participants from Tromsø (Paper II). The geometric mean values of sum PCBs and *p,p'*-DDE were 2.01 μg/L plasma (310 ng/g lipids) and 0.51 μg/L plasma (78.2 ng/g lipids), respectively. The PCB concentrations were comparable to what was found in delivering women from Arkhangelsk, whereas the *p,p'*-DDE levels were considerably lower. By comparison, women who were or had been married to fishermen living on the east Coast of Sweden had geometric mean values of sum PCBs (14 congeners) of 3.01 μg/L plasma (620 ng/g lipids) (Grimvall et al., 1997).

Uelen

The participants from Uelen had rather high plasma levels of PCBs and pesticides. The geometric mean concentrations of sum PCBs (17 congeners) and sum DDTs were 6.79 and 2.91 μg/L plasma, respectively. The geometric mean levels of β-HCH were 2.10 μg/L or 410 ng/g lipids. Compared to other native populations, it is in particularly the amount of β-HCH in these plasma samples that is elevated, and the

levels are not significantly different from levels in the delivering women from Arkhangelsk (3.08 μg/L) (Paper I). The DDE/DDT ratio was 15.4 and gave no indications of fresh sources as compared to the delivering women from Arkhangelsk. High amounts of PCB 163 were found in 20 reanalysed samples, with a median level of 0.31 μg/l plasma as compared to 0.83 μg/l for the PCB 138. The high levels of PCB 163 in human plasma samples have to our knowledge not been reported before. In a comparison of exposures to this congener, care must be taken that comparable separation from the companion congener 138 occurs.

OH-PCBs and PCP were quantified in 15 samples with the higher PCB values (sum PCBs; 2010 – 5614 ng/g lipid-weight) samples from Uelen (Paper IV). Sum OH-PCBs were high (median; 5916 pg/g plasma) and constituted as much as 27 – 76 % of the sum PCBs. The sum values were significantly correlated with a Pearson correlation coefficient of 0.7 ($p<0.01$). 4-OH-CB107 was the dominant congener with a median level of 1673 pg/g plasma. There were a number of unidentified OH-PCBs and some peaks had intensities comparable to the most abundant 4-OH-CB107. PCP concentrations were low with median values of 642 pg/g plasma or 117 ng/g lipid weight. PCP has been reported to be the dominant phenolic compound in Inuits from northern Canada, with a geometric mean of 1670 pg/g plasma (Sandau et al., 2002).

The high levels of the OH-PCBs relative to the PCBs clearly indicate the need for future research on these compounds, especially their behaviour in the human body.

Risk evaluation

"The way we live - our lifestyle- can have a great impact on health. It includes the everyday activities of work and leisure, our relationships to people around us, and our food habits. The different components of a lifestyle are often difficult to separate from one another. Weighing risk against benefits is extremely difficult. It is not possible to precisely quantify the degree of risk when there is insufficient scientific information about health outcomes at given contaminant exposure levels, nor is it possible to place defensible quantitative value on the nutritional, social, and economic benefits of traditional food consumption" (Van Oostdam et al., 1999).

One of the most important benefits of a marine diet is the high content of n-3 fatty acids that can protect against coronary heart disease and mortality (Oomen et al., 2000). The n-3 fatty acids are also important in brain development in the growing fetus and for the proper development of vision. Lower intake of these healthy fatty acids and a higher proportion of other fats may also play a role in increasing the risk for diabetes (Hu et al., 2001; Sirtori and Galli, 2002). In addition, cod liver and cod liver oil are rich in vitamin A, D and E (Brustad et al., 2003; Rimestad et al., 2001). Recent data indicate that the intake of liver from saithe and cod might still be of importance for the level of D-vitamin intake by people from northern Norway (Brustad et al., 2003). In Barrow (Alaska), several people have a D-vitamin status below the recommended range and the same people are consuming the smallest amounts of traditional food (Specker, 1994).

Tolerable weekly intake (TWI) of dioxins and dioxin like PCBs constitutes a threshold value frequently used in the risk assessment of food. TWI indicates the amount of a compound a person can consume every week during life without any damages to health. It is calculated in part from the no-observed-adverse-effect level (NOAEL), and it is given in pg Toxic Equivalents (TE) /kg bodyweight. For the calculations of the TE, the WHO model is being used (van den Berg et al., 1998). The TWI is deduced from animal studies employing conversion factors to humans and uncertainty factors such as taking into account interindividual variations in metabolism. The method of calculating TWIs is described in some detail elsewhere (SCF, 2001); the TWI now is set to 14 pg TE/kg bodyweight.

Based on the TWI of 14 pg/kg bodyweight and levels of PCBs and dioxins in cod liver, the Norwegian Food Control Authorities (SNT) issued a dietary advise in March 2003. All women of fertile age and children were advised to totally stop eating cod liver and commercial products based on cod liver (SNT, 2003). The commercial products did not include the commercial cod liver oil that has been distilled and purified to remove pollutants. The dietary advisory did not mention the positive health effects of the cod liver and what alternatives the people might eat to maintain the same intake of vitamins and n-3 fatty acids. If people follow this advice and shift their diet to include more saturated fats there could be several negative impacts that have not been considered. For the citizens of Norway, several nutritional alternatives are available, but what the people actually replace the cod liver with is not known. "It would seem elementary that any risk assessment should consider the health benefits of a product before regulatory actions are taken. Few risk assessors are ready to do that today, but it should be strongly recommended" (Tuomisto, 2001).

For native people in remote areas like Uelen (Chukotka), where the TWI is exceeded because of the consumption of blubber, a dietary advisory like this is impossible. The people of Uelen have no alternative source of food with similar nutritional benefits, and they do not have the financial resources to buy it from commercial sources. The amounts of POPs in the participants plasmas from Uelen (Paper III) are without doubt elevated compared to the participants from Tromsø (Paper II) or even people from Norwegian coastal populations, where fish is an important part of the diet (Furberg et al., 2002). The amounts of POPs in the blubber are also considerably higher compared to that found in cod liver. Blubber is also one of the main food items in the Chukchi diet (Paper III), whereas the intake of cod liver in Norway is considerably lower. Further, the consumption of "mølje" is seasonal, with its availability lasting approximately 3 months (Paper II).

It is therefore appropriate to ask if actions are necessary to reduce the levels of POPs in the people of Uelen. This is the Arctic dilemma and it is extremely difficult to give an answer to this question. As already indicated, some indigenous populations are beginning to consume an increasing proportion of commercial food. In Greenland and northern Canada, as much as 60 – 90 % of the diet is now believed to consist of commercial products (AMAP, 2002). This shift in diet might reduce exposure to

POPs in several native communities, but it also introduces food with more saturated fats and higher amounts of carbohydrates (AMAP, 2002). This might lead to other health problems, like higher cardiovascular disease and mortality, making it hard to estimate if the overall effect is positive or negative. The cultural aspect, as previously mentioned, is also an important consideration.

The body burdens of POPs in human populations of western Europe seem to be declining. Several studies from western and central Europe clearly indicate that human breast milk concentrations of dioxins follow a downward trend (Alcock and Jones, 1996; Wittsiepe et al., 2000). In breast milk samples from Norwegian women, obtained in 1991, the levels of sum PCBs, HCB and sum DDTs had declined between 65 and 75 % during the last nine years (Johansen et al., 1994). A significant reduction in the levels of POPs in umbilical cord blood of newborns from the Quebec region has also been reported (Dallaire et al., 2002). There is, however, a general lack of time-trend data for native populations in the Arctic and this needs to be rectified.

The dietary advisory in Norway regarding the cod liver seems exaggerated considering the small number of meals people have during the year and the fact that the human levels of POPs are not elevated among people from areas where the cod liver is still consumed (Paper II) (AMAP, 1998). Larsen et al. (2001) have also stated that both daily intake and the total body burden of a pregnant woman achieved over time are important for possible health impairments. In the study by Furberg et al. (2002), no association was found between consumption of the different fish items (mainly lean fish and farmed salmon) and human levels of POPs. The dish "mølje" is also of cultural importance along the coast of northern Norway. The consumption of "mølje" also have apparent health benefits (Brustad et al., 2003). In addition, there still seems to be conflicting evidence regarding to what extent the levels increase with age or if the reported age dependence is partly caused by past exposure (Alcock et al., 1999). The estimates of half-lives in the human body also vary greatly for the compounds in question (Noren and Meironyte, 2000). The reported half-life estimates for 2,3,7,8-TCDD in human varies from 5.5 to 11 years (Van der Molen et al., 1998). The apparent half-life is not absolute, but may vary with dose, body composition, age and sex (van den Berg et al., 2000).

The use of additives in the diet to enhance excretion of POPs has been investigated, and it has been reported that through the use of non-absorbable fat like olestra (six to eight fatty acid chains attached to a sucrose core) the absorption of POPs from the diet is reduced and excretion is increased (Moser and McLachlan, 1999). Rice-fibre bran has also been shown to increase excretion, and there is evidence that the fatty acid composition of typical dietary fats may influence the metabolism of organochlorine compounds (Jandacek and Tso, 2001). However, together with the increased excretion of POPs there are reports of an increase in excretion of the fat soluble vitamins A, D, and E in addition to β-Carotene (Koonsvitsky et al., 1997; Peters et al., 1997). The potential loss of other important nutrients makes the use of olestra risky, even though several of these could be added to the olestra. These consequences need proper validation before efforts are made to implement the use of this and other additives. The suggested study on the effects of high-energy or high-fat diets in combination with substances that interfere with enterohepatic circulation seems highly relevant for several native populations (Jandacek and Tso, 2001).

Conclusions

The main findings of this work are itemized below:

- The levels of β-HCH and *p,p'*-DDT are high in delivering women from Arkhangelsk, Russia, indicating the presence of fresh sources or consumption of contaminated food.

- The consumption of blubber is significantly associated with the levels of POPs among people in Uelen, Chukotka, Russia. The levels of POPs are high and in particular β-HCH is elevated.

- Highly PCB-exposed participants from Uelen also exhibited high concentrations of OH-PCBs, including a number of unidentified OH-PCBs.

- There was no significant age dependence of plasma POPs among the men from Uelen.

- Following a traditional meal rich in lipids and POPs ("mølje"), the plasma PCBs and *p,p'*-DDE changed significantly when considering wet-weight or lipid-weight concentrations. For the most accurate determination of levels in plasma, the use of samples from fasting individuals is advised.

- The findings further support the practice of both wet-weight and lipid-weight data to increase comparability to other studies. The lipids should be determined using enzymatic techniques and a summation formula.

- The analytical method for determination of POPs in plasma has been streamlined considerably. Further, it also allows the quantification of phenolic organohalogens in a small plasma volume (0.8 ml).

- The mixture of POPs in plasma varies between populations, thereby complicating the selection of indicator compounds in large screening strategies.

Future perspectives

Based on the conclusions of this thesis there are several recommendations for future work.

- There is a need for an even simpler analytical method for efficient screening of large number of samples at low cost, allowing larger epidemiological studies with improved statistical power.
- Future risk assessments must consider the positive health effects of traditional food.
- The absorption, distribution, metabolism and excretion of POPs in humans as a function of dose and time is still not well understood and more work is needed.
- OH-PCBs and PCP in plasma must be investigated further to understand their uptake, risk factors and toxicokinetics.
- Further studies must be performed in northwest Russia to identify the sources of *p,p'*-DDT and β-HCH.
- Further efforts must be made to ensure appropriate knowledge transfer back to indigenous populations in question.

Abbreviations

AhR:	Aryl hydrocarbon receptor
AMAP:	Arctic Monitoring and Assessment Programme
ANOVA:	Analyses of variance
CDs:	Chlordanes
CYP:	Cytochrome P450 enzyme
DDT-group:	DDT, DDE and DDD
EI+:	Positive electron impact ionisation
EOM:	Extractable Organic Material
EPIC:	European Prospective Investigation into Cancer and Nutrition
FC:	Free cholesterol
GC-MS:	Gas chromatograph-Mass spectrometer
GPC:	Gel Permeation Chromatography
HCB:	Hexachlorobenzene
HCH:	Hexachlorocyclohexane
IARC:	International Arctic Science Committee
INSPQ:	Institut National de Santé Publique du Québec
KOH:	Potassium hydroxide
LOC:	Level of concern
LOD:	Limit of detection
LOQ:	Limit of quantification
NCI:	Negative chemical ionization
NILU:	Norwegian Institute for Air Research
NOAEL:	No observed adverse effect level
NOWAC:	Norwegian Women and Cancer Study
OCs:	Organochlorines
OH-PCBs:	Hydroxylated polychlorinated biphenyls
PCBs:	Polychrorinated biphenyls
PCDD/F:	Polychlorinated dibenzo-*p*-dioxin and dibenzofuran
PCP:	Pentachlorophenol
PL:	Phospholipids
POP:	Persistent organic pollutant
p,p'-DDE:	2,2'-bis(*p*-chlorophenyl)-1,1-dichloroethylene
p,p'-DDT:	1,1'-bis(*p*-chlorophenyl)-2,2,2-trichloroethane
PTS:	Persistent toxic substances
SIM:	Selected Ion Monitoring
SNT:	Norwegian Food Control Authorities
TC:	Total cholesterol
TCDD:	Tetrachlorodibenzo-*p*-dioxin
TDI:	Tolerable daily intake
TE:	Toxic equivalent
TEF:	Toxic equivalency factors
TEQ:	Dioxin toxic equivalents
TG:	Triacylglycerol
TL:	Total lipids
tox:	Toxaphene
TWI:	Tolerable weekly intake
WHO:	World Health Organization

References

Adami HO, Hunter D, Trichopoulos D. Breast cancer. In: Adami HO, Hunter D, Trichopoulos D, editors. Textbook of Cancer Epidemiology Oxford University Press, Inc., New York, 2003; 301-332.

Ahlborg UG, Hanberg A, Kenne K. Risk assessment of polychlorinated biphenyls (PCBs). Nordic Council of Ministers, Nord. Copenhagen, Denmark, 1992; 26.

Ahlborg UG, Lipworth L, Titusernstoff L, Hsieh CC, Hanberg A, Baron J, Trichopoulos D, Adami HO. Organochlorine compounds in relation to breast-cancer, endometrial cancer, and endometriosis – an assessment of the biological and epidemiological evidence. Crit Rev Toxicol 1995; 25: 463-531.

Akins JR, Waldrep K, Bernert JT, Jr. The estimation of total serum lipids by a completely enzymatic 'summation' method. Clin Chim Acta 1989; 184: 219-226.

Alcock RE, Jones KC. Dioxins in the environment: A review of trend data. Environ Sci Technol 1996; 30: 3133-3143.

Alcock RE, Sweetman AJ, Jones KC. The intake and clearance of PCBs in humans - A generic model of lifetime exposure. Abstr Pap Amer Chem Soc 1999; 217: 26.

Alcock RE, Sweetman AJ, Juan CY, Jones KC. A generic model of human lifetime exposure to persistent organic contaminants: development and application to PCB-101. Environ Pollut 2000; 110: 253-265.

AMAP, 1998. AMAP Assessment Report: Arctic Pollution Issues. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway. xii+859.

AMAP, 2003. AMAP assessment 2002: Human Health in the Arctic. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway, xiv+137.

AMAP, 2002. Arctic Pollution 2002. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway, xii+111.

Archibeque-Engle SL, Tessari JD, Winn DT, Keefe TJ, Nett TM, Zheng TZ. Comparison of organochlorine pesticide and polychlorinated biphenyl residues in human breast adipose tissue and serum. J Toxicol Environ Health 1997; 52: 285-293.

Ariyoshi N, Koga N, Oguri K, Yoshimura H. Metabolism of 2,4,5,2',4',5'-hexachlorobiphenyl with liver - microsomes of phenobarbital - treated dog - the possible formation of PCB 2,3-arene oxide intermediate. Xenobiotica 1992; 22: 1275-1290.

Ayotte P, Carrier G, Dewailly E. Health risk assessment for Inuit newborns exposed to dioxin-like compounds through breast feeding. Chemosphere 1996; 32: 531-542.

Ayotte P, Dewailly E, Ryan JJ, Bruneau S, Lebel G. PCBs and dioxin-like compounds in plasma of adult Inuit living in Nunavik (Arctic Quebec). Chemosphere 1997; 34: 1459-1468.

Barrie LA, Gregor D, Hargrave B, Lake R, Muir D, Shearer R, Tracey B, Bidleman T. Arctic contaminants – sources, occurrence and pathways. Sci Total Environment 1992; 122: 1-74.

Bjerregaard P, Hansen JC. Organochlorines and heavy metals in pregnant women from the Disko Bay area in Greenland. Sci Total Environ 2000; 245: 195-202.

Breivik K, Sweetman A, Pacyna JM, Jones KC. Towards a global historical emission inventory for selected PCB congeners - a mass balance approach 1. Global production and consumption. Sci Total Environ 2002; 290: 181-198.

Brouwer A, Ahlborg UG, van Leeuwen FXR, Feeley MM. Report of the WHO working group on the assessment of health risks for human infants from exposure to PCDDs, PCDFs and PCBs. Chemosphere 1998a; 37: 1627-1643.

Brouwer A, Ahlborg UG, Vandenberg M, Birnbaum LS, Boersma ER, Bosveld B, Denison MS, Gray LE, Hagmar L, Holene E, Huisman M, Jacobson SW, Jacobson JL, Koopmanessenboom C, Koppe JG, Kulig BM, Morse DC, Muckle G, Peterson RE, Sauer PJJ, Seegal RF, Smitsvanprooije AE, Touwen BCL, Weisglaskuperus N, Winneke G. Functional-aspects of developmental toxicity of polyhalogenated aromatic-hydrocarbons in experimental-animals and human infants. Eur J Pharmacol (Environ Toxicol Pharmacol Sect) 1995; 293: 1-40.

Brouwer A, Longnecker MP, Birnbaum LS, Cogliano J, Kostyniak P, Moore J, Schantz S, Winneke G. Characterization of potential endocrine-related health effects at low-dose levels of exposure to PCBs. Environ Health Perspect 1999; 107: 639-649.

Brouwer A, Morse DC, Lans MC, Schuur AG, Murk AJ, Klasson-Wehler E, Bergman A, Visser TJ. Interactions of persistent environmental organohalogens with the thyroid hormone system: Mechanisms and possible consequences for animal and human health. Toxicol Ind Health 1998b; 14: 59-84.

Brustad M, Sandanger T, Wilsgaard T, Aksnes L, Lund E. Change in plasma levels of vitamin D after consumption of cod liver and fresh cod-liver oil as part of the traditional north Norwegian fish dish "Mølje". Int J Circumpolar Health 2003; 62: 40-53.

Burkow IC, Kallenborn R. Sources and transport of persistent pollutants to the Arctic. Toxicol Lett 2000; 112-113: 87-92.

Carpenter DO, Arcaro K, Spink DC. Understanding the human health effects of chemical mixtures. Environ Health Perspect 2002; 110: 25-42.

Chan HM. A database for environmental contaminants in traditional foods in northern and Arctic Canada: development and applications. Food Addit Contam 1998; 15: 127-134.

Cheek AO, Kow K, Chen J, McLachlan JA. Potential mechanisms of thyroid disruption in humans: Interaction of organochlorine compounds with thyroid receptor, transthyretin, and thyroid-binding globulin. Environ Health Perspect 1999; 107: 273-278.

Cheek CS, Wease DF. A summation technic for serum total lipids. Comparison of methods. Clin Chem 1969; 15: 102-107.

Chen YC, Yu ML, Rogan WJ, Gladen BC, Hsu CC. A 6-year follow-up of behavior and activity disorders in the Taiwan Yu- cheng children. Am J Public Health 1994; 84: 415-421.

Dahl P, Lindström G, Wiberg K, Rappe C. Absorption of polychlorinated-biphenyls, dibenzo-*p*-dioxins and dibenzofurans by breast-fed infants. Chemosphere 1995; 30: 2297-2306.

Dallaire F, Dewailly E, Laliberte C, Muckle G, Ayotte P. Temporal trends of Organochlorine concentrations in umbilical cord blood of newborns from the lower north shore of the St. Lawrence River (Quebec, Canada). Environ Health Perspect 2002; 110: 835-838.

de Geus HJ, Besselink H, Brouwer A, Klungsoyr J, McHugh B, Nixon E, Rimkus GG, Wester PG, de Boer J. Environmental occurrence, analysis, and toxicology of toxaphene compounds. Environ Health Perspect 1999; 107: 115-144.

Deutch B, Hansen JC. High human plasma levels of organochlorine compounds in Greenland - Regional differences and lifestyle effects. Dan Med Bull 2000; 47: 132-137.

Dewailly E, Ayotte P, Bruneau S, Gingras S, BellesIsles M, Roy R. Susceptibility to infections and immune status in Inuit infants exposed to organochlorines. Environ Health Perspect 2000; 108: 205-211.

Dewailly E, Ayotte P, Bruneau S, Laliberte C, Muir DCG, Norstrom RJ. Inuit exposure to organochlorines through the aquatic food-chain in Arctic Quebec. Environ Health Perspect 1993; 101: 618-620.

Fangstrom B, Athanasiadou M, Grandjean P, Weihe P, Bergman A. Hydroxylated PCB metabolites and PCBs in serum from pregnant Faroese women. Environ Health Perspect 2002; 110: 895-899.

Feeley M, Brouwer A. Health risks to infants from exposure to PCBs, PCDDs and PCDFs. Food Additives and Contaminants 2000; 17: 325-333.

Filser JG, Baur C, Csanady GA, Kessler W, Kreuzer PE. Toxicokinetic modeling as a tool for risk estimation: 2,3,7,8- tetrachlorodibenzo-*p*-dioxin. Int J Toxicol 1997; 16: 433-448.

Furberg AS, Sandanger T, Thune I, Burkow IC, Lun E. Fish consumption and plasma levels of organochlorines in a female population in Northern Norway. J Environ Monit 2002; 4: 175-181.

Gammon MD, Wolff MS, Neugut AI, Terry MB, Papadopoulos K, Levin B, Wang Q, Santella RM. Temporal variation in chlorinated hydrocarbons in healthy women. Cancer Epidemiol Biomarkers Prev 1997; 6: 327-332.

Gladen BC, Longnecker MP, Schecter AJ. Correlations among polychlorinated biphenyls, dioxins, and furans in humans. Am J Ind Med 1999; 35: 15-20.

Golden RJ, Noller KL, Titus-Ernstoff L, Kaufman RH, Mittendorf R, Stillman R, Reese EA. Environmental endocrine modulators and human health: An assessment of the biological evidence. Crit Rev Toxicol 1998; 28: 109-227.

Grandjean P, Weihe P, Burse VW, Needham LL, Storr-Hansen E, Heinzow B, Debes F, Murata K, Simonsen H, Ellefsen P, Budtz-Jorgensen E, Keiding N, White RF. Neurobehavioral deficits associated with PCB in 7-year-old children prenatally exposed to seafood neurotoxicants. Neurotoxicol and Teratol 2001; 23: 305-317.

Grandjean P, Weihe P, Debes F, Steuerwald U, White R. Neurobehavioral development up to 5.5 years after prenatal exposure to methylmercury and PCB. Epidem 2000; 11: 79.

Grimvall E, Rylander L, Nilsson-Ehle P, Nilsson U, Stromberg U, Hagmar L, Ostman C. Monitoring of polychlorinated biphenyls in human blood plasma: methodological developments and influence of age, lactation, and fish consumption. Arch Environ Contam Toxicol 1997; 32: 329-336.

Guo YL, Yu ML, Hsu CC, Rogan WJ. Chloracne, goiter, arthritis, and anemia after polychlorinated biphenyl poisoning: 14-year follow-Up of the Taiwan Yucheng cohort. Environ Health Perspect 1999; 107: 715-719.

Guvenius DM, Hassanzadeh P, Bergman A, Noren K. Metabolites of polychlorinated biphenyls in human liver and adipose tissue. Environ Toxicol Chem 2002; 21: 2264-2269.

Haddad S, Poulin P, Krishnan K. Relative lipid content as the sole mechanistic determinant of the adipose tissue:blood partition coefficients of highly lipophilic organic chemicals. Chemosphere 2000; 40: 839-843.

Hansen JB, Grimsgaard S, Nilsen H, Nordoy A, Bonaa KH. Effects of highly purified eicosapentaenoic acid and docosahexaenoic acid on fatty acid absorption, incorporation into serum phospholipids and postprandial triglyceridemia. Lipids 1998; 33: 131-138.

Hansen JC. Environmental contaminants and human health in the Arctic. Toxicol Lett 2000; 112: 119-125.

Hansen JR, Hansson R, Norris S. (ed.) The State of the European Arctic Environment. Luxembourg, Office for official publications of the European Communities (EEA Environmental Monograph No 3) 1996.

Hardell L, Eriksson M, Lindström G, Van Bavel B, Linde A, Carlberg M, Liljegren G. Case-control study on concentrations of organohalogen compounds and titers of antibodies to Epstein-Barr virus antigens in the etiology of non-Hodgkin lymphoma. Leukemia & Lymphoma 2001; 42: 619-629.

Hardell L, Lindström G, Van Bavel B. Is DDT exposure during fetal period and breast-feeding associated with neurological impairment? Environ Res 2002; 88: 141-144.

Harner T, Kylin H, Bidleman TF, Strachan WMJ. Removal of alpha- and gamma-hexachlorocyclohexane and enantiomers of alpha-hexachlorocyclohexane in the eastern Arctic Ocean. Environ Sci Technol 1999; 33: 1157-1164.

Holford TR, Zheng TZ, Mayne ST, Zahm SH, Tessari JD, Boyle P. Joint effects of nine polychlorinated biphenyl (PCB) congeners on breast cancer risk. Int J Epidem 2000; 29: 975-982.

Hovander L, Athanasiadou M, Asplund L, Jensen S, Wehler EK. Extraction and cleanup methods for analysis of phenolic and neutral organohalogens in plasma. J Anal Toxicol 2000; 24: 696-703.

Hu FB, van Dam RM, Liu S. Diet and risk of Type II diabetes: the role of types of fat and carbohydrate. Diabetologia 2001; 44: 805-817.

International Agency for Research on Cancer. IARC Monographs on the evaluation of carcinogenic risks to humans. IARC. Lyon, France.

Jandacek RJ, Tso P. Factors affecting the storage and excretion of toxic lipophilic xenobiotics. Lipids 2001; 36: 1289-1305.

Jensen J, Adare K, Shearer R. (ed.) Canadian Arctic contaminants assessment report, Northern contaminants Program. Ottawa, Minister of Indian affairs and Northern Development. 1997; 460.

Johansen HR, Becher G, Polder A, Skaare JU. Congeners-specific determination of Polychlorinated-biphenyls and organochlorine pesticides in human-milk from Norwegian mothers living in Oslo. J Toxicol Environ Health 1994; 42: 157-171.

Juan CY, Thomas GO, Sweetman AJ, Jones KC. An input-output balance study for PCBs in humans. Environ Int 2002; 28: 203-214.

Kester MHA, Bulduk S, Tibboel D, Meinl W, Glatt H, Falany CN, Coughtrie MWH, Bergman A, Safe SH, Kuiper GGJM, Schuur AG, Brouwer A, Visser TJ. Potent inhibition of estrogen sulfotransferase by hydroxylated PCB metabolites: A novel pathway explaining the estrogenic activity of PCBs. Endocrinology 2000; 141: 1897-1900.

Kester MHA, Bulduk S, van Toor H, Tibboel D, Meinl W, Glatt H, Falany CN, Coughtrie MWH, Schuur AG, Brouwer A, Visser TJ. Potent inhibition of estrogen sulfotransferase by hydroxylated

metabolites of polyhalogenated aromatic hydrocarbons reveals alternative mechanism for estrogenic activity of endocrine disrupters. J Clin Endocrinol Metabol 2002; 87: 1142-1150.

Kimbrough RD. Polychlorinated-Biphenyls (PCBs) and human health – an update. Crit Rev Toxicol 1995; 25: 133-163.

Klopov V, Odland JO, Burkow IC. Persistent organic pollutants in maternal blood plasma and breast milk from Russian Arctic populations. Int J Circumpolar Health 1998; 57: 239-248.

Koonsvitsky BP, Berry DA, Jones MB, Lin PYT, Cooper DA, Jones DY, Jackson JE. Olestra affects serum concentrations of alpha-tocopherol and carotenoids but not vitamin D or vitamin K status in free- living subjects. J Nutrition 1997; 127: S1636-S1645.

Koopman-Esseboom C, Huisman M, Weisglaskuperus N, Vanderpaauw CG, Tuinstra LGMT, Boersma ER, Sauer PJJ. PCB and Dioxin levels in plasma and human-milk of 418 Dutch women and their infants – predictive value of PCB congener levels in maternal plasma for fetal and infants exposure to PCBs and dioxins. Chemosphere 1994; 28: 1721-1732.

Kramer VJ, Helferich WG, Bergman A, KlassonWehler E, Giesy JP. Hydroxylated polychlorinated biphenyl metabolites are anti-estrogenic in a stably transfected human breast adenocarcinoma (MCF7) cell line. Toxicol Applied Pharm 1997; 144: 363-376.

Kreuzer PE, Csanady GA, Baur C, Kessler W, Papke O, Greim H, Filser JG. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and congeners in infants. A toxicokinetic model of human lifetime body burden by TCDD with special emphasis on its uptake by nutrition. Arch Toxicol 1997; 71: 383-400.

Kuhnlein HV, Chan HM, Leggee D, Barthet V. Macronutrient, mineral and fatty acid composition of Canadian Arctic traditional food. J Food Comp Anal 2002; 15: 545-566.

Kuhnlein HV, Receveur O, Muir DCG, Chan HM, Soueida R. Arctic and indigenous women consume greater-than acceptable levels of organochlorines. J Nutrition 1995; 125: 2501-2510.

Kuwabara K, Yakushiji T, Watanabe I, Yoshida S, Yoyama K, Kunita N. Increase in human blood PCB levels promptly following ingestion of fish containing PCBs. Bull Environ Contam Toxicol 1979; 21: 273-278.

Lang V. Polychlorinated-biphenyls in the environment. J Chrom 1992; 595: 1-43.

Larsen JC, Grandjean P, Joffe M, van den Akker E, Bro-Rasmussen F, Skakkebaek N, Bontoux L. Exposure levels of endocrine disruptors in Nordic Countries - Discussion. Apmis 2001; 109: S461-S462.

Letcher RJ, Klasson-Wehler E, Bergman A. Methyl sulfone and hydroxylated metabolites of polychlorinated biphenyls. In: Paasivirta J, (ed.) The Handbook of Environmental Chemistry: New Types of Persistent Halogenated Compounds. Springer-Verlag, Berlin, 2000; 315-359.

Li YF, Macdonald RW, Jantunen LMM, Harner T, Bidleman TF, Strachan WMJ. The transport of β-hexachlorocyclohexane to the western Arctic Ocean: a contrast to α-HCH. Sci Total Environ 2002; 291: 229-246.

Long G, Winefordner J. Limit of detection: A closer look at the IUPAC definition. Anal Chem 1983; 55: 712A-724A.

Longnecker MP, Rogan WJ, Lucier G. The human health effects of DDT (dichlorodiphenyl-trichloroethane) and PCBS (polychlorinated biphenyls) and an overview of organochlorines in public health. Ann Rev Publ Health 1997; 18: 211-244.

Longnecker MP, Ryan JJ, Gladen BC, Schecter AJ. Correlations among human plasma levels of dioxin-like compounds and polychlorinated biphenyls (PCBs) and implications for epidemiologic studies. Arch Environ Health 2000; 55: 195-200.

Longnecker MP, Wolff MS, Gladen BC, Brock JW, Grandjean P, Jacobson JL, Korrick SA, Rogan WJ, Weisglas-Kuperus N, Hertz-Picciotto I, Ayotte P, Stewart P, Winneke G, Charles MJ, Jacobson SW, Dewailly E, Boersma ER, Altshul LM, Heinzow B, Pagano JJ, Jensen AA. Comparison of polychlorinated biphenyl levels across studies of human neurodevelopment. Environ Health Perspect 2003; 111: 65-70.

Macdonald RW, Barrie LA, Bidleman TF, Diamond ML, Gregor DJ, Semkin RG, Strachan WMJ, Li YF, Wania F, Alaee M, Alexeeva LB, Backus SM, Bailey R, Bewers JM, Gobeil C, Halsall CJ, Harner T, Hoff JT, Jantunen LMM, Lockhart WL, Mackay D, Muir DCG, Pudykiewicz J, Reimer KJ, Smith JN, Stern GA, Schroeder WH, Wagemann R, Yunker MB. Contaminants in the Canadian Arctic: 5 years of progress in understanding sources, occurrence and pathways. Sci Total Environ 2000; 254: 93-234.

McLachlan MS. Digestive-tract absorption of polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls in a nursing infant. Toxicol Appl Pharmacol 1993; 123: 68-72.

Morita K, Matsueda T, Iida T. [Effect of green tea (matcha) on gastrointestinal tract absorption of polychlorinated biphenyls, polychlorinated dibenzofurans and polychlorinated dibenzo-*p*-dioxins in rats]. Fukuoka Igaku Zasshi 1997; 88: 162-168.

Moser GA, McLachlan MS. A non-absorbable dietary fat substitute enhances elimination of persistent lipophilic contaminants in humans. Chemosphere 1999; 39: 1513-1521.

Moser GA, McLachlan MS. The influence of dietary concentration on the absorption and excretion of persistent lipophilic organic pollutants in the human intestinal tract. Chemosphere 2001; 45: 201-211.

Moser GA, McLachlan MS. Partitioning of polychlorinated biphenyls and hexachlorobenzene into human faeces. Chemosphere 2002; 46: 449-457.

Muir DCG, Wagemann R, Hargrave BT, Thomas DJ, Peakall DB, Norstrom RJ. Arctic marine ecosystem contamination. Sci Total Environ 1992; 122: 75-134.

Narazaki Y, Morita K, Fukamachi K, Tokiwa H, Takahashi K. [Concentration profile of PCBs in the digestive tract of rat fed with cholestyramine and rice bran fiber diet]. Fukuoka Igaku Zasshi 1991; 82: 305-309.

Needham LL, Burse VW, Head SL, Korver MP, McClure PC, Andrews JS, Rowley DL, Sung J, Kahn SE. Adipose-tissue serum partitioning of chlorinated-hydrocarbon pesticides in humans. Chemosphere 1990; 20: 975-980.

Noren K, Meironyte D. Certain organochlorine and organobromine contaminants in Swedish human milk in perspective of past 20-30 years. Chemosphere 2000; 40: 1111-1123.

Noren K, Weistrand C, Karpe F. Distribution of PCB congeners, DDE, hexachlorobenzene, and methylsulfonyl metabolites of PCB and DDE among various fractions of human blood plasma. Arch Environ Contam Toxicol 1999; 37: 408-414.

Norstrom RJ, Muir DCG. Chlorinated-hydrocarbon contaminants in Arctic marine mammals. Sci Total Environ 1994; 154: 107-128.

Odland, JO, Hansen, JC, Burkow, IC. (ed.) The importance of diet on exposure and effects of persistent organic pollutants on human health in the Arctic. Scientific Report. Kjeller, Norway, Norwegian Institute for Air Research, 2003; 1-40

Oomen CM, Feskens EJM, Rasanen L, Fidanza F, Nissinen AM, Menotti A, Kok FJ, Kromhout D. Fish consumption and coronary heart disease mortality in Finland, Italy, and the Netherlands. Am J Epidem 2000; 151: 999-1006.

Patandin S, Koopman-Esseboom C, De Ridder MAJ, Weisglas-Kuperus N, Sauer PJJ. Effects of environmental exposure to polychlorinated biphenyls and dioxins on birth size and growth in Dutch children. Ped Res 1998; 44: 538-545.

Patandin S, Lanting CI, Mulder PGH, Boersma ER, Sauer PJJ, Weisglas-Kuperus N. Effects of environmental exposure to polychlorinated biphenyls and dioxins on cognitive abilities in Dutch children at 42 months of age. J Ped 1999; 134: 33-41.

Patterson DG, Needham LL, Pirkle JL, Roberts DW, Bagby J, Garrett WA, Andrews JS, Falk H, Bernert JT, Sampson EJ, Houk VN. Correlation between serum and adipose-tissue levels of 2,3,7,8-tetrachlorodibenzo-para-dioxin in 50 persons from Missouri. Arch Environ Contam Toxicol 1988; 17: 139-143.

Peters JC, Lawson KD, Middleton SJ, Triebwasser KC. Assessment of the nutritional effects of olestra, a nonabsorbed fat replacement: Summary. J Nutrition 1997; 127: S1719-S1728.

Phillips DL, Pirkle JL, Burse VW, Bernert JT, Henderson LO, Needham LL. Chlorinated-hydrocarbon levels in human-serum – effects of fasting and feeding. Arch Environ Contam Toxicol 1989; 18: 495-500.

Rimestad AH, Borgejordet Å, Vesterhus KN, Sygnestveit K, Løken EB, Trygg K, Pollestad ML, Lund-Larsen K, Omholt-Jensen G, Nordbotten A. Den store matvaretabellen. Gyldendal, Oslo, 2001; 1-156.

Rowland M, Tozer TN. Clinical pharmakokinetics concepts and applications. Lippincott Williams & Wilkins, Philadelphia, 1995; 144.

Rylander L, Dyremark E, Stromberg U, Ostman C, Hagmar L. The impact of age, lactation and dietary habits on PCB in plasma in Swedish women. Sci Total Environ 1997; 207: 55-61.

Safe SH. Polychlorinated-biphenyls (PCBs) – Environmental-impact, biochemical and toxic responses, and implications for risk assessment. Crit Rev Toxicol 1994; 24: 87-149.

Sandau CD, Ayotte P, Dewailly E, Duffe J, Norstrom RJ. Analysis of hydroxylated metabolites of PCBs (OH-PCBs) and other chlorinated phenolic compounds in whole blood from Canadian Inuit. Environ Health Perspect 2000; 108: 611-616.

Sandau CD, Ayotte P, Dewailly E, Duffe J, Norstrom RJ. Pentachlorophenol and hydroxylated polychlorinated biphenyl metabolites in umbilical cord plasma of neonates from coastal populations in Quebec. Environ Health Perspect 2002; 110: 411-417.

SAS Institute Inc. SAS/STAT User's guide, version 6. SAS Institute Inc., Cary, NC, 1989.

SCF. Opinion of the scientific committee on food on the risk assessment of dioxins and dioxin-like PCBs in Food. Scientific Comittee on Food. Brussel, Belgium, European Commission, Health & Consumer Protection Directorate-general, 30.05.2001, 1-29.

Schlummer M, Moser GA, McLachlan MS. Digestive tract absorption of PCDD/Fs, PCBs, and HCB in humans: Mass balances and mechanistic considerations. Toxicol Appl Pharmacol 1998; 152: 128-137.

Schnellmann RG, Putnam CW, Sipes IG. Metabolism of 2,2',3,3',6,6'-hexachlorobiphenyl and 2,2',4,4',5,5'-hexachlorobiphenyl by human hepatic microsomes. Biochem Pharmacol 1983; 32: 3233-3239.

Sipes IG, Slocumb ML, Perry DF, Carter DE. 2,4,5,2',4',5'-hexachlorpbiphenyl – distribution, metabolism, and excretion in the dog and the monkey. Toxicol Appl Pharmacol 1982; 65: 264-272.

Sirtori CR, Galli C. N-3 fatty acids and diabetes. Biomedicine & Pharmacotherapy 2002; 56: 397-406.

Sjodin A, Hagmar L, Klasson-Wehler E, Bjork J, Bergman A. Influence of the consumption of fatty Baltic Sea fish on plasma levels of halogenated environmental contaminants in Latvian and Swedish men. Environ Health Perspect 2000; 108: 1035-1041.

SNT, 2003. http://www.snt.no/nytt/ferskvare/notis.html/784.html

Specker BL. Do North American women need supplemental vitamin D during pregnancy or lactation? Am J Clin Nutr 1994; 59: 484-490.

Sterne JAC, Smith GD. Sifting the evidence - what's wrong with significance tests? B Med J 2001; 322: 226-231.

Swanson GM, Ratcliffe HE, Fischer LJ. Human exposure to Polychlorinated-biphenyls (PCBs) – a critical-assessment of the evidence for adverse health-effects. Reg Toxicol Pharm 1995; 21: 136-150.

ten Tusscher GW, Ilsen A, Koppe JG. Background exposure to dioxins and polychlorinated biphenyls in Europe and the resulting health effects: a review. Prenatal and Neonatal Medicine 1999; 4: 261-270.

Thomsen C, Janak K, Lundanes E, Becher G. Determination of phenolic flame-retardants in human plasma using solid-phase extraction and gas chromatography-electron-capture mass spectrometry. J Chrom B 2001; 750: 1-11.

Thomsen C, Lundanes E, Becher G. Brominated flame retardants in archived serum samples from Norway: A study on temporal trends and the role of age. Environ Sci Technol 2002; 36: 1414-1418.

To-Figueras J, Barrot C, Sala M, Otero R, Silva M, Ozalla MD, Herrero C, Corbella J, Grimalt J, Sunyer J. Excretion of hexachlorobenzene and metabolites in feces in a highly exposed human population. Environ Health Perspect 2000; 108: 595-598.

Tuomisto J. When will we get a holistic risk assessment of food? Eur J Lip Sci Technol 2001; 103: 643.

van de Waterbeemd H, Gifford E. ADMET in silico modelling: Towards prediction paradise? Nat Rev Drug Discovery 2003; 2: 192-204.

van den Berg M, Birnbaum L, Bosveld ATC, Brunstrom B, Cook P, Feeley M, Giesy JP, Hanberg A, Hasegawa R, Kennedy SW, Kubiak T, Larsen JC, van Leeuwen FXR, Liem AKD, Nolt C, Petrson RE, Poellinger L, Safe S, Schrenk D, Tillitt D, Tysklind M, Younes M, Waern F, Zacharewski T. Toxic

equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ Health Perspect 1998; 106: 775-792.

van den Berg M, van Birgelen A, Birnbaum L, Brouwer B, Carrier G, Dragan Y, Farland W, Feeley M, Furst P, Galli CL, Greig J, Hayashi Y, Kogevinas M, Kurokawa Y, Larsen JC, Liem AKD, Matsumura F, Mocarelli P, Moore MR, Newhook RC, Peterson RE, Poellinger L, Portier C, Rogan WJ, Schrenk D, Sweeney MH, Tohyama C, Tuomisto J, Waters J, Zeilmaker M. Consultation on assessment of the health risk of dioxins; re-evaluation of the tolerable daily intake (TDI): Executive summary. Food Addit Contam 2000; 17: 223-240.

van der Molen GW, Kooijman SALM, Michalek JE, Slob W. The estimation of elimination rates of persistent compounds: A re-analysis of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin levels in Vietnam veterans. Chemosphere 1998; 37: 1833-1844.

Van Oostdam J, Gilman A, Dewailly E, Usher P, Wheatley B, Kuhnlein H, Neve S, Walker J, Tracy B, Feeley M, Jerome V, Kwavnick B. Human health implications of environmental contaminants in Arctic Canada: a review. Sci Total Environ 1999; 230: 1-82.

Weisglas-Kuperus N, Patandin S, Berbers GAM, Sas TCJ, Mulder PGH, Sauer PJJ, Hooijkaas H. Immunologic effects of background exposure to polychlorinated biphenyls and dioxins in Dutch preschool children. Environ Health Perspect 2000; 108: 1203-1207.

Wittsiepe J, Schrey P, Ewers U, Wilhelm M, Selenka F. Decrease of PCDD/F levels in human blood - trend analysis for the German population, 1991-1996. Environ Res 2000; 83: 46-53.

Woodruff T, Wolff MS, Davis DL, Hayward D. Organochlorine exposure estimation in the study of cancer etiology. Environ Res 1994; 65: 132-144.

PAPER I

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Persistent organic pollutants in plasma of delivering women from Arkhangelsk

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Abstract

The high levels of persistent organic pollutants have caused concern about human health, especially the health of the foetus and newborn child. This has especially been the case for Greenlandic and Canadian Inuits, where elevated levels of PCB and *p,p'*-DDE have been reported. In recent studies from arctic Russia the levels of β-HCH and the DDT-group have been reported to be high, whereas the levels of PCB are low. However, the information from Northern Russia is, so far, incomplete. In this study, 27 delivering women from the city of Arkhangelsk, Russia, participated. They completed a questionnaire before delivery and plasma samples were collected after delivery. The analytical method developed to support this study involved gel permeation chromatography and silica gel purification, in addition to a traditional GC-MS method, and thus include acid labile compounds. The arithmetic mean levels of *p,p'*-DDE, β-HCH and *p,p'*-DDT were 5.42, 3.59 and 1.17 μg/l, respectively. Toxaphene 26 and 50 were the only toxaphenes above the limit of detection, with arithmetic mean levels of 0.05 and 0.09 μg/l, respectively. Among the PCB congeners, PCB 138/163 was the most abundant with an arithmetic mean of 0.53 μg/l. The elevated levels of β-HCH and *p,p'*-DDT as well as a low DDE/DDT ratio is a strong indication of fresh and maybe local sources in this area.

Keywords: Persistent organic pollutants; POP's; Plasma; Delivering women; β-HCH; DDE/DDT ratio; Russia; Fresh sources

1. Introduction

The Arctic has some of the cleanest environments in the world. Despite this fact high levels of persistent organic pollutants (POPs) have been found in environmental samples from this region. The high levels found in the Arctic are in general a result of long range transportation of contamination from higher contaminated areas (Burkow and Kallenborn, 2000), with few local sources present.

Due to their persistence the POPs bioaccumulate, resulting in transfer to humans harvesting from the top of the food chain. Use of local and indigenous food is a common characteristic of people from Arctic communities, and a wide range

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of animals and plants remain important dietary and cultural resources (AMAP, 1998). Of the local food items, animals from the marine food chains have been found to be the most important sources of exposure to POPs (Hansen, 2000). Therefore, it is generally believed that people depending on local food are more exposed to POPs. This diet is, however, considered very nutritious and important for the health and well being of the people.

In Greenland and northern Canada, where marine mammals are an important part of the diet, the levels of polychlorinated biphenyls (PCB) and the metabolite dichlorodiphenyletylene (DDE) have been found to be high (AMAP, 1998; Van Oostdam et al., 1999; Deutch and Hansen, 2000; Bjerregaard and Hansen, 2000). In men from Scoresbysund in Greenland the average level of sum DDT was found to be 11.1 μg/l plasma and sum PCB was 41 μg/l plasma (Deutch and Hansen, 2000). In Scandinavia, where people depend little on this kind of locally harvested food, the levels are low. In Norwegian women the level of sum PCB was measured to be 4.0 μg/l plasma while sum DDT was 0.9 μg/l plasma (AMAP, 1998). In samples from Northern Russia the levels of *p,p'*-DDT and β-HCH have been found to be high, while the levels of PCB are comparable to Scandinavian samples. In women from Nikel the level of β-HCH was found to be 1.9 μg/l plasma (AMAP, 1998). Based on this it has been indicated that local fresh sources of selected organochlorine pesticides must be present in Russia (Klopov et al., 1998; AMAP, 1998).

The high levels found in human samples from some areas in the Arctic have caused concern about human health effects (Hansen, 2000). However, assessing the human health impacts of exposure to POPs is a very difficult task. Human populations are always exposed to mixtures of POPs in the ecosystem, never to single compounds. A number of the risk assessment tests done in animal studies, are based on single compounds and the applicability to humans may often be questioned. Even though several of the compounds have shown to be carcinogenic in animal studies, their potential as human carcinogens remains unclear (Hansen, 1998). Toxic effects of these compounds are of major public concern, especially for the foetus and breast-fed infant (Bjerregaard and Hansen, 2000; Hansen, 1998, 2000).

Early development of the foetus is the period of greatest susceptibility to the effects of toxic chemicals (Hansen, 1998). Effects such as low birth weight and small head circumference, adverse neurobehaviour, and impaired immune response have been associated with high PCB concentrations (Rogan et al., 1987; Rogan and Gladen, 1990; Gladen and Rogan, 1991). For a number of POPs the concern also extends to male and female fertility (Hansen, 1998). Elevated levels of DDT and DDE found in humans have also shown to reduce the length of lactation (Gladen and Rogan, 1995, 1996).

The mean birth weight and newborn child's body mass index (BMIC) have been found to be statistically significant lower in Russian neonates, compared to Norwegian newborn babies (Odland et al., 1999a,b). The factors determining the birth weight are complex and many, but POPs cannot be excluded as a contributor. However, the information on both POP exposure and possible health effects is still scarce. One objective of this study is to improve our knowledge of POP exposure and body burden in arctic Russia.

2. Materials and methods

Personal contact with the colleagues at the city hospital delivery department was established, and all procedures and protocols were provided in English and Russian language. Information based on questionnaire, medical records, and blood was collected from 27 consecutive patients coming to the obstetric department for delivery. The enrolment and sampling were performed in the period September–October 1996. The women were asked to join the study by completing a written consent form. Enrolment, completion of the informed consent form and the questionnaire implementation were done before the delivery process started in order to minimise stress. Blood samples were obtained 2–5 days after the delivery process was finished, at the normal morning procedure between 08.00 and 09.00 h. Specimens of antecubital vein blood from the mother were taken in EDTA tubes with standard vacutainer equipment. The blood

was centrifuged and immediately stored at −20 °C. The blood samples were transported frozen to Norway and kept frozen until analysis.

All reference materials used for quantification were purchased as single standards from Promochem (Wesel, Germany). All solvents used were of Suprasolv quality and Purchased from Merck (Darmstadt, Germany), except ethanol (absolute alcohol from Arcus, Norway) and water (deionised). The plasma samples were extracted using a liquid–liquid extraction with ethanol, *n*-hexane and deionised water saturated with ammonium sulphate. This is a slight modification of the method developed by Centre de Toxicologie du Quebec, Canada (Romieu et al., 2000). Internal standards were added to 4 ml of plasma, 4 ml of ethanol and 4 ml of deionised water saturated with ammonium sulphate. This solution was extracted three times with 12 ml of *n*-hexane in a small glass-tube. The organic fraction was collected and evaporated to 0.5 ml using isooctane as a keeper. Evaporation was done on a Zymark Turbovap 500 Closed Cell Concentrator (Hopkinton, USA). After extraction, approximately 90% of the lipids were removed using gel permeation chromatography (GPC) column. This GPC system consisted of a glass column (105 cm, 1.0 cm i.d.) from LATEK (Eppelheim, Germany) packed with 35 g Biobeads S-X3 from Biorad, (Hercules, USA). Cyclohexane/ethyl acetate (1:1) was used as eluent with a flow of 0.8 ml/min. The fraction for further analyses was collected between 60 and 110 min.

The remaining lipids were removed using small silica columns with internal diameter of 1.0 cm (Gatermann et al., 1999). They were prepared by packing 2 g of activated (120 °C for 12 h) silica (average particle size of 40 μm, particle size distribution; 20–60 μm and an average pore diameter of 60 Å) in the columns with a glass frit fitted at top and bottom. The columns were conditioned with 10 ml of *n*-hexane just before the sample was added to the column. The following solvent combination was used as eluent and collected for further analyses: 10 ml *n*-hexane, 10 ml *n*-hexane/dichloromethane (9:1), 10 ml *n*-hexane/dichloromethane (4:6), 10 ml dichloromethane, 10 ml dichloromethane/ethyl acetate (1:1). After evaporation to 0.5 ml the volume was further reduced to 100 μl using a gentle flow of nitrogen.

Two microlitres of the sample was injected into a Fisons 8060 Mega gas chromatograph (Milan, Italy), using an on-column injector and an AS800 autosampler from CE instruments (Milan, Italy). All analyses were done using a 30 m DB-5 MS column (0.25 mm i.d. and 0.25 μm film thickness) from J&W Scientific (CA, USA) connected to a deactivated guard column (2.5 m and 0.53 mm i.d.). The GC was connected to a low resolution MD 800 mass spectrometer from Fisons (Milan, Italy). The compounds were quantified using internal standards added to the extraction tubes. The internal standards used were C-13 marked PCB 77, 101, 118, 144 and 178. Octachloronaphtalene was added to the extracts before quantification to calculate recovery rates for the internal standards.

The quantification was done using both negative chemical ionisation (NCI) and positive electron impact ionisation (EI+), both in selected ion monitoring (SIM) mode. PCBs and the DDT group were calculated using EI+, while the remaining pesticides were quantified using NCI. Methane was used as reagent. Helium (99.995%) was used as carrier gas with a flow of 1 ml/min (constant flow control). The following temperature program was used: 60 °C (2 min), then 15 °C/min to 180 °C (0 min) and 5 °C/min to 280 °C (5 min). With the EI mode, the transfer line temperature was held at 220 °C and the quadropole at 200 °C, using NCI the temperatures were 200°C and 180 °C, respectively.

The different compounds were identified based on their SIM masses and retention times. Peaks with differences in isotopic ratio greater than 20% compared to the quantification standard were rejected and not quantified. For every 10 samples a blank was analysed for lab contamination.

Limit of detection (LOD) was calculated using three times the area of noise or if peaks were found in blanks, three times the area of the blank. The limit of quantification was set to three times LOD.

Statistical tests included *t*-test (small samples, equal standard deviations), confidence intervals using Student's *t*-distribution, significance testing and linear regression analyses.

Table 1
Levels (μg/l) of polychlorinated biphenyls (PCBs) in human plasma samples from Arkhangelsk

Compound	Arithmetic mean (μg/l)	Geometric mean (μg/l)	Standard deviation (μg/l)	Range (μg/l)
PCB 52	0.11	0.09	0.05	0.04–0.20
PCB 99	0.02	0.02	0.01	0.00–0.05
PCB 101	0.05	0.03	0.03	0.01–0.11
PCB 105	0.09	0.08	0.06	0.03–0.28
PCB 118	0.34	0.29	0.21	0.10–1.03
PCB 138/163	0.53	0.42	0.40	0.12–1.51
PCB 153	0.49	0.39	0.38	0.11–1.74
PCB 156	0.05	0.05	0.03	0.03–0.13
PCB 170	0.10	0.07	0.09	0.04–0.37
PCB 180	0.17	0.13	0.18	0.04–1.00
PCB 183	0.04	0.03	0.03	0.03–0.15
PCB 187	0.08	0.06	0.08	0.02–0.39

3. Results

The mean age of the delivering women was 23.8 years (range 17–38). Twenty-two of the women delivered their first child (range 1–5). Only one of the mothers smoked during pregnancy (<10 cigarettes per day). The mean birth weight was 3303 g (range 2170–4400). No congenital malformations were observed.

Levels of polychlorinated biphenyls (PCB), dichlorodiphenyltrichloroethane (DDT) and metabolites, toxaphenes (Tox) and other pesticides found in blood plasma are presented in Tables 1 and 2. The samples were analysed for a total of 18 PCBs, 11 toxaphenes and 16 other pesticides. Several compounds were not detected above their detection limit (LOD) and therefore not included in Tables 1 and 2. All analysed compounds are listed in Table 3 with their LOD. For statistical purposes, all levels below LOD are set as 0.5×LOD. Recovery rates were calculated for the C-13 marked PCB congeners 101, 118, 141 and 178, all of them being 59% for the entire analytical method.

The method developed for analysing plasma samples did not involve destruction of lipids with acids or bases. Although time consuming, the GPC column efficiently removes 90% of the lipids from the POPs and the recovery rate is above 80% for all compounds for this step. Acid labile compounds can therefore be easily included in the method, allowing all compounds to be included in the same

Table 2
Levels of pesticides (μg/l) in human plasma samples from Arkhangelsk

Compound	Arithmetic mean (μg/l)	Geometric Mean (μg/l)	Standard deviation (μg/l)	Range (μg/l)
o,p-DDT*	0.30	0.25	0.20	0.08–0.89
p,p'-DDT*	1.16	0.83	1.19	0.28–5.07
p,p'-DDE*	5.42	4.52	3.36	1.76–13.11
cis-Chlordane	0.07	0.05	0.06	0.02–0.33
trans-Nonachlor	0.16	0.12	0.13	0.03–0.66
cis-Nonachlor	0.04	0.03	0.05	0.01–0.23
oxy-Chlordane	0.20	0.18	0.12	0.15–0.67
Heptachlor	0.13	0.10	0.17	0.09–0.95
Mirex	0.22	0.20	0.11	0.14–0.51
γ-HCH*	0.16	0.15	0.07	0.08–0.37
β-HCH*	3.59	3.08	2.36	1.27–11.59
α-HCH*	0.13	0.09	0.13	0.03–0.52
Hexachlorobenzene	0.57	0.47	0.38	0.18–1.40
Toxaphene 26	0.05	0.05	0.02	0.04–0.10
Toxaphene 50	0.09	0.06	0.13	0.02–0.70

* *p,p'*-DDT [1,1'-bis(*p*-chlorophenyl)-2,2,2-trichloroethane], *o,p'*-DDT [1-(*o*-chlorophenyl)-1'-(*p*-chlorophenyl)-2,2,2-trichloroethane], *p,p'*-DDE [2,2'-bis(*p*-chlorophenyl)-1,1-dichloroethylene], *o,p'*-DDE [2-(*o*-chlorophenyl)-2'-(*p*-chlorophenyl)-1,1-dichloroethylene], HCH (hexachlorocyclohexane).

Table 3
All compounds analysed with their detection limits (LOD) calculated from three times the area found in blind samples

Compound	LOD (μg/l)	Compound	LOD (μg/l)	Compound	LOD (μg/l)
PCB 52	0.06	*o,p*-DDT	0.19	Tox 26	0.05
PCB 99	0.01	*p,p'*-DDT	0.25	Tox 32	0.09
PCB101	0.03	*p,p'*-DDE	0.17	Tox 38	0.15
PCB105	0.04	*o,p*-DDE	0.04	Tox 40	0.75
PCB118	0.05	*cis*-Chlordane	0.04	Tox 42a,b	0.10
PCB126	0.04	*trans*-Chlordane	0.02	Tox 44	0.15
PCB128	0.06	Heptachlorepoxide	0.30	Tox 50	0.08
PCB138/163	0.06	*trans*-Nonachlor	0.05	Tox 51	0.18
PCB149	0.06	*cis*-Nonachlor	0.03	Tox 58	0.09
PCB153	0.05	*oxy*-Chlordane	0.35	Tox 62	0.15
PCB156	0.05	Heptachlor	0.15	Tox 69	0.11
PCB169	0.04	Mirex	0.25		
PCB170	0.08	γ-HCH	0.22		
PCB180	0.03	β-HCH	0.71		
PCB183	0.06	α-HCH	0.08		
PCB187	0.04	Hexachlorobenzene	0.08		
PCB194	0.05				

fraction throughout the extraction and clean-up procedure.

Among the PCB congeners (Table 1) the PCB 138/163 was the most abundant one with an arithmetic mean of 0.53 μg/l (range 0.19–1.51). As for the pesticides in Table 2 the level of β-hexachlorocyclohexane (β-HCH) was high with an arithmetic mean of 3.59 μg/l (range1.27–11.59). The arithmetic mean of *p,p'*-DDE (2,2'-bis(*p*-chlorophenyl)-1,1-dichloroethylene) and *p,p'*-DDT (1,1'-bis(*p*-chlorophenyl)-2,2,2-trichlorethane) was 5.42 μg/l (range 1.76–13.11) and 1.17 μg/l (range 0.28–5.07), respectively. As for the other pesticides the levels are low with hexachlorobenzene (HCB) slightly above the others, with an arithmetic mean of 0.57 μg/l (range 0.18–1.40). The toxaphenes were barely detectable with only Tox 26 and Tox 50 above LOD. The arithmetic mean levels of these were 0.05 μg/l (range 0.04–0.10) and 0.09 μg/l (range 0.02–0.70), respectively. Due to contamination of 10 samples with *o,p'*-DDT and *p,p'*-DDT the level of these compounds are only calculated for the remaining 17 samples. Based on, amongst other factors, a high percentage of *o,p'*-DDT in the contaminated samples and the presence of the same contamination profile in some other samples, the source of contamination was identified in the laboratory and removed.

4. Discussion

The level of PCB was found to be low in the samples from Arkhangelsk. For sum PCBs the geometric mean was 1.75 μg/l. This level is comparable to what is previously found in Russian, Norwegian and Swedish samples from the Arctic (AMAP, 1998; Klopov et al., 1998; Polder et al., 1998; Sjodin et al., 2000). For comparison of data with previous studies, see Table 4. According to AMAP (AMAP, 2000), the arctic region of Russia is not supposed to have a high number of PCB containing equipment or hardly any PCB in PCB containing wastes, resulting in few local sources. There are minor differences in the number of PCB congeners included in the sum PCB in this study compared to other studies. However, the congeners that are different do not contribute significantly to the total, which should make the sum-values comparable.

The PCB pattern reveals that the PCB 138/163 is more abundant than the PCB 153 in the samples from Arkhangelsk. As previously found in samples from Nikel (AMAP, 1998), PCB 118 contributes

Table 4
PCB, DDT and β-HCH levels found in this study compared to levels found in previous studies

Country; region	Sum PCB[e] (μg/l) (G)[e]	Sum DDT[d] (μg/l)		DDE/ DDT Ratio	β-HCH (μg/l)	
		(A)[f]	(G)		(A)	(G)
Russia; Arkhangelsk	1.75	5.8	4.8	4.7	3.6	3.1
Russia; Nikel[a]	1.70	3.9	3.4	8.2	1.9	1.7
Canada[a]	1.64	1.9	1.4	16.8	0.1	0.1
Greenland; Disko Bay[b]	5.50		3.8	27.1[a]		0.2
Sweden[a]	2.23	1.1	0.9	35.0	0.1	0.1
Norway[a]	1.36	0.9	0.7	26.0	0.1	0.1
Iceland[a]	1.79	1.1	0.9	28.6	0.3	0.3

[a] Data from AMAP (1998).
[b] Data from Deutch and Hansen (2000).
[c] Sum PCB: sum of PCB 28, 52, 99, 101, 105, 118, 128, 138/163, 153, 156, 170, 180, 183 and 187. (PCB 28 not included in the data from Arkhangelsk.)
[d] Sum DDT: sum of *p,p'*-DDT and *p,p'*-DDE.
[e] Geometric mean.
[f] Arithmetic mean.

more to the sum than in Scandinavian samples. This is not the case for PCB 99, which was found in low levels in the samples from Arkhangelsk. The level of PCB 52 was also relatively higher which is in accordance with results from Monchegorsk (Polder et al., 1998), where the lower chlorinated PCBs contributed relatively more to the sum PCB. The greater contribution of the lower chlorinated PCBs in Arkhangelsk, might be explained by the different composition of the Russian commercial mixtures (AMAP, 2000). The levels of PCB found in this study are low and possible health effects might not be expected.

p,p'-DDT was detected in all the samples with an average level of 1.2 μg/l. The level of DDT is comparable to what was found in human samples from Veracruz, Mexico where they have up to recently been applying DDT (Waliszewski et al., 2000a). In the Veracruz study *p,p'*-DDT was detected in only 41% of the mothers analysed with an average level of 1.8 μg/l plasma. *p,p*-DDE was detected in 98% of the samples in high levels (14.5 μg/l plasma) (Waliszewski et al., 1999).

The average sum DDT (*p,p'*-DDT+*p,p'*-DDE) in the samples from Arkhangelsk was 5.81 μg/l with a 95% confidence interval of 3.8–7.9 (geomean; 4.8). This level is comparable to the highest levels reported in the AMAP report (AMAP, 1998), where the Inuits from Greenland were reported to have arithmetic mean levels of 5.2 μg/l (sum DDT). Levels as high as 11.1 μg/l (sum DDT) in some areas of Greenland have later been reported by Deutch and Hansen (2000). Even though the two sums are similar, the samples from Arkhangelsk have a higher percentage of DDT. In the women from Arkhangelsk the DDE/DDT ratio was found to be 4.7 (95% CI 3.7–5.6) compared to 27.1 in the samples from Greenland (AMAP, 1998). The ratio of 4.7 is significantly (*t*-test and $P<0.05$) lower than 8.2 found in human samples from Nikel in 1995 (AMAP, 1998). This might indicate more recent sources in Arkhangelsk compared to Nikel or a different application of DDT. In the samples from Veracruz the DDE/DDT ratio was approximately 12 (Waliszewski et al., 1999).

The concentration of *p,p'*-DDE, the metabolite of *p,p'*-DDT, is expected to be higher than the DDT because of the longer half life. The half life of both *o,p'*-DDT and *p,p'*-DDT has been reported to be approximately 7 years in soil (Woodruff et al., 1994). These estimates of half-lives vary depending on medium (abiotic or biotic).

In the samples from Arkhengelsk the *o,p'*-DDT congener contributes to the total body burden of sum DDT, lifting the average sum from 5.8 μg/l to 6.1 μg/l plasma. The *o,p'*-DDT was detected

above LOD in 83% of the samples. The *o,p'*-DDE was detected in 40% of the samples, but only just below LOD, and therefore not quantified. Considering the presence of the *o,p*-congeners and the fact that the estrogenic and uterotropic activities of DDT and DDE are most pronounced for the *o,p'*-isomers (Bulger and Kupfer, 1983), these isomers must also be included and elaborated further in future studies.

The mean level of β-HCH (3.6 μg/l plasma, 95% confidence interval of 2.7–4.5) was significantly higher than what is previously reported from Nikel (1.9 μg/l plasma) ($P<0.05$) (AMAP, 1998). The levels of the other pesticides were generally low in the samples from Arkhangelsk. The low toxaphene amount is interesting in relation to the high levels of this pesticide found in seal samples from the Barents Sea indicating their presence in arctic Russia (Wolkers et al., 2000).

p,p'-DDE and PCB 138/163 were significantly positively correlated ($r^2=0.422$, $P<0.001$). Sum DDT and β-HCH were also positively correlated ($r^2=0.265$, $P<0.05$). *p,p'*-DDT and β-HCH were also correlated but with no statistical significance. The weak correlation seen for *p,p'*-DDT and β-HCH makes it hard to conclude whether these compounds come from the same source or not.

No correlation between any of the compounds and parity was found, but cannot be excluded as the spread in this data set was low (first delivery for 22 of 27 women).

The high levels of DDT and HCH found in these samples support the assumptions that there is a continuous use of DDT and β-HCH in Russia. The exposure can either be direct from local use of these compounds or it can be from contaminated food from other contaminated regions of Russia. Whether the exposure is a continuos exposure to high levels or several single exposures to even higher levels have implications for the toxicology of the compound. Tests on rats have shown that appropriately timed toxicant exposure can initiate a cascade of changes that can alter the reproductive outcome, whereas a continuos exposure to same compound do not alter the outcome (Stoker et al., 2001).

There are several aspects to take into account when the possible health effects of the high levels of β-HCH and DDT-group found in the samples from Arkhangelsk are to be considered. β-HCH, *p,p'*-DDT and *p,p'*-DDE concentrations in blood of babies have been found to lie within the same range as in the mothers, pointing out a free passage through the placenta barrier (Klopov et al., 1998; Waliszewski et al., 2000a,b). DeKoning and Karmaus (2000) claim that when assessed on a lipid basis, placental transfer of PCBs seems to be underestimated as a source of foetal exposure. The size of newborns has also been found to be negatively and statistically significant associated with organochlorine exposure (Dewailly et al., 1992). Gladen et al. (2000) claim that prenatal exposures to high background level may affect body size at puberty. For the health of the newborn these findings are concerning and the need for further details on sources of exposure in Russia and possible health effects of the levels found are urgently needed. Correlation to pregnancy outcome factors was not possible due to the small number of samples in our study. It is noteworthy that this was the first delivery for 22 of the delivering women, probably due to the low birth rate in Russia in the period after the 'perestroijka' (Odland, 2000).

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References

AMAP. AMAP assessment report: Arctic pollution issues. Arctic monitoring and assessment programme. Oslo, Norway: AMAP, 1998. (xii+859 pp).

AMAP. State Commitee of the Russian Federation for environmental protection, and Center for International Projects (CIP). PCB in the Russian Federation: inventory and proposals for priority remedial actions. Moscow, 3. 2000. (27 pp).

Bjerregaard P, Hansen JC. Organochlorines and heavy metals in pregnant women from the Disko Bay area in Greenland. Sci Total Environ 2000;245:195–202.

Bulger WH, Kupfer D. Estrogenic action of DDT analogs. Am J Ind Med 1983;4:163–173.

Burkow IC, Kallenborn R. Sources and transport of persistent pollutants to the Arctic. Toxicol Lett 2000;112–113:87–92.

DeKoning EP, Karmaus W. PCB exposure in utero and via breast milk. A review. J Expo Anal Environ Epidemiol 2000;10:285–293.

Deutch B, Hansen JC. High human plasma levels of organochlorine compounds in Greenland. Regional differences and lifestyle effects. Dan Med Bull 2000;47:132–137.

Dewailly E, Bruneau S, Laliberté C, Bélanger D, Gingras S, Ayotte P, Nantel A. Weight, size, head circumference and TSH of Inuit newborn prenatally exposed to high levels of organochlorines. Organohalogen Compd 1992;10:257–259.

Gatermann R, Hellou J, Huhnerfuss H, Rimkus G, Zitko V. Polycyclic and nitro musks in the environment: a comparison between Canadian and European aquatic biota. Chemosphere 1999;38:3431–3441.

Gladen BC, Ragan NB, Rogan WJ. Pubertal growth and development and prenatal and lactational exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene. J Pediatr 2000;136:490–496.

Gladen BC, Rogan WJ. Effects of perinatal polychlorinated biphenyls and dichlorodiphenyl dichloroethene on later development. J Pediatr 1991;119:58–63.

Gladen BC, Rogan WJ. DDE and shortened duration of lactation in a northern Mexican town. Am J Public Health 1995;85:504–508.

Gladen BC, Rogan WJ. DDE and insufficient breast milk—response. Am J Public Health 1996;86:887–888.

Hansen JC. AMAP human Health Group. The human health programme under AMAP. Arctic Monitoring and Assessment Program. Int J Circumpolar Health 1998;57:280–291.

Hansen JC. Environmental contaminants and human health in the Arctic. Toxicol Lett 2000;112–113:119–125.

Klopov V, Odland JO, Burkow IC. Persistent organic pollutants in maternal blood plasma and breast milk from Russian arctic populations. Int J Circumpolar Health 1998;57:239–248.

Odland JO, Nieboer E, Romanova N, Thomassen Y, Brox J, Lund E. Self-reported ethnic status of delivering women, newborn body mass index, blood or urine concentrations of toxic metals, and essential elements in sera of Norwegian and Russian Arctic populations. Int J Circumpolar Health 1999a;58:4–13.

Odland JO, Nieboer E, Romanova N, Thomassen Y, Lund E. Blood lead and cadmium and birth weight among sub-arctic and arctic populations of Norway and Russia. Acta Obstet Gynecol Scand 1999b;78:852–860.

Odland JO. Doctoral Thesis: environmental and occupational exposure, life-style factors and pregnancy outcome in arctic and sub-arctic populations of Norway and Russia. ISM skriftserie nr. 50. Institute of Community Medicine, University of Tromsø, 2000. (xii+59 pp).

Polder A, Becher G, Savinova TN, Skaare JU. Dioxins, PCBs and some chlorinated pesticides in human milk from the Kola Peninsula, Russia. Chemosphere 1998;37:1795–1806.

Rogan WJ, Gladen BC. Perinatal exposure to polychlorinated-biphenyls (PCBs) and child development at 18 and 24 months. Ped Res 1990;27:A97.

Rogan WJ, Gladen BC, McKinney JD, Carreras N, Hardy P, Thullen J, Tingelstad J, Tully M. Polychlorinated biphenyls (PCBs) and dichlorodiphenyl dichloroethene (DDE) in human milk: effects on growth, morbidity, and duration of lactation. Am J Public Health 1987;77:1294–1297.

Romieu I, HernandezAvila M, LazcanoPonce E, Weber JP, Dewailly E. Breast cancer, lactation history, and serum organochlorines. Am J Epidemiol 2000;152:363–370.

Sjodin A, Hagmar L, KlassonWehler E, Bjork J, Bergman A. Influence of the consumption of fatty Baltic Sea fish on plasma levels of halogenated environmental contaminants in Latvian and Swedish men. Env Health Persp 2000;108:1035–1041.

Stoker TE, Goldman JM, Cooper RL. Delayed ovulation and pregnancy outcome: effect of environmental toxicants on the neuroendocrine control of the ovary. Environ Toxicol Pharmacol 2001;9:117–129.

Van Oostdam J, Gilman A, Dewailly E, Usher P, Wheatley B, Kuhnlein H, Neve S, Walker J, Tracy B, Feeley M, Jerome V, Kwavnick B. Human health implications of environmental contaminants in Arctic Canada: a review. Sci Total Environ 1999;230:1–82.

Waliszewski SM, Aguirre AA, Benitez A, Infanzon RM, Infanzon R, Rivera J. Organochlorine pesticide residues in human blood serum of inhabitants of Veracruz, Mexico. Bull Environ Contam Toxicol 1999;62:397–402.

Waliszewski SM, Aguirre AA, Infanzon RM, Siliceo J. Carryover of persistent organochlorine pesticides through placenta to fetus. Salud Publ Mex 2000b;42:384–390.

Waliszewski SM, Aguirre AA, Infanzon RM, Siliceo J. Partitioning coefficients of organochlorine pesticides between mother blood serum and umbilical blood serum. Bull Environ Contam Toxicol 2000a;65:293–299.

Wolkers H, Burkow IC, Lydersen C, Witkamp RF. Chlorinated pesticide concentrations, with an emphasis on polychlorinated camphenes (toxaphenes), in relation to cytochrome P450 enzyme activities in harp seals (*Phoca groenlandica*) from the Barents Sea. Environ Toxicol Chem 2000;19:1632–1637.

Woodruff T, Wolff MS, Davis DL, Hayward D. Organochlorine exposure estimation in the study of cancer etiology. Environ Res 1994;65:132–144.

PAPER II

Change in levels of persistent organic pollutants in human plasma after consumption of a traditional northern Norwegian fish dish—Mølje (cod, cod liver, cod liver oil and hard roe)

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The traditional northern Norwegian fish dish "mølje", consisting of boiled cod, cod liver, cod liver oil and hard roe, is still consumed frequently during the winter months January to March. The liver of the cod is rich in lipids and the levels of persistent organic pollutants (POPs) are relatively high. To better understand the short-term consequences of this traditional meal on the plasma levels of PCBs and *p,p'*-DDE, individual intake of liver and cod liver oil during one meal was measured. Blood samples were collected from 33 participants before the meal, and then 4 h, 12 h and 5 days after it. Lipid-weight and wet-weight levels of 10 PCB congeners and *p,p'*-DDE were determined in the plasma samples and the food. The plasma levels of *p,p'*-DDE was found to increase significantly from 0 to 4 h, both when expressed as wet-weight (35% change) and lipid-weight (20% change). The corresponding changes (0–4 h) in wet-weight levels of the most prevalent PCB congeners were non significant. By contrast, PCB congeners with low levels in the food showed a significant drop in lipid-weight levels during the first 4 h. The observed changes were independent of amount consumed. Significant differences in fasting and non-fasting samples were found for most PCBs and *p,p'*-DDE. For the lipid weight levels of sum PCBs there was a significant decrease of 16% from non-fasting to fasting samples. To obtain reliable data on human levels of POPs it is, on the basis of these findings, recommended that blood samples should be collected from fasting individuals and both wet-weight and lipid-weight levels should be reported.

Introduction

The Arctic has some of the cleanest environments in the world. Despite this fact high levels of persistent organic pollutants (POPs) have been found in environmental samples from this region. Long-range transportation of contamination from more contaminated areas is believed to be responsible, considering the few local sources.[1] Due to their persistence, the POPs bioaccumulate, permitting transfer to humans harvesting from the top of the food chain. Use of local and indigenous food is a common characteristic of people in Arctic communities, and a wide range of animals and plants are consumed.[2] Of these local food items, animals from the marine food chains have been found to be the most important sources of exposure to POPs.[3] A considerable part of the traditional diet is rich in marine lipids and also POPs.

"Mølje" consisting of boiled cod, cod liver, hard roe, and fresh cod liver oil from the boiling of the cod liver, was traditionally a considerable part of the diet during the winter season among people living in the coastal areas of northern Norway. Cod liver and cod liver oil are rich in vitamin D and a dietary survey from northern Norway in 1931 did show that cod liver and fresh cod liver oil was the most important vitamin D and fat source.[4] Although the cod is a lean fish, the liver is lipid rich containing up to 60% in raw liver.[5] Today, the contribution of "mølje" in the daily northern Norwegian diet is less pronounced, but recent data from the nationwide Norwegian Women and Cancer Study (NOWAC) have shown that there are still places in northern Norway where it is frequently consumed (Lund, E. personal communication).

Considering the high lipid content of this meal, a short-term increase in plasma lipids following the meal is expected. This increase is further expected to affect the equilibrium of POPs between adipose tissue and plasma.[6] This is supported by Haddad *et al.*,[7] who have shown that the adipose tissue:blood-partitioning coefficient is equal to the ratio of lipids in adipose tissue and blood. The levels of POPs found in plasma are considered to reflect the body burden,[8] but there have been discussions whether to use lipid-weight data or wet-weight data.[6,7,9,10] The levels of POPs have thus been reported in different ways in different studies and some samples have been from fasting and some from non-fasting individuals, making comparison between studies difficult. Different methods for determining lipids in plasma complicate the issue more. Lipids might be quantified by gravimetric determination of the extractable organic material (EOM) or by a summation of the different enzymatically determined lipid classes.[6] These methods appear to give different results and several studies are recommending the enzymatic summation method.[6,11,12]

Due to the high lipid content as well as "high" levels of POPs in cod liver, the intake of a meal of "mølje" is a good opportunity to study the short-term consequences on the equilibrium of POPs in plasma, both lipid-weight and wet-weight.

The aim of this study was to determine how much an intake of large amounts of marine lipids by consuming "mølje", affects the levels of POPs in plasma and if they are influenced differently depending on their relative amounts in food.

Materials and methods

Study group

The majority (60.6%) of the 33 people participating were women; the average age was 42 (range: 28–65) years with an average body mass index (BMI) of 24.2 (range: 19.5–28.5). One participant (of Eastern European origin) was excluded from

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the statistical analyses of the data, since the person had *p,p'*-DDE levels four times higher than the average values (significant outlier). The participants were all working and living in Tromsø.

The meal and the sampling

The participants were served the traditional hot northern Norwegian fish dish consisting of cod with liver, hard roe, fresh cod liver oil, and potatoes. The meal was prepared by boiling the cod and the hard roe separately in water. Fresh cod liver oil was obtained by boiling liver (in small pieces) in small amounts of water. Participants could eat as much as they wanted. The amount of liver and cod liver oil consumed by each participant was weighed and recorded separately.

The study was carried out in the beginning of April 2000. The meal was served between 6 and 7 pm on Day 1. Blood samples were collected just before the meal (0 h), after 4 h, 12 h, and 5 days. The 12 h and 5 d blood specimens were taken in the morning before breakfast thus designated as fasting specimens compared to the 0 and 4 h as non-fasting. Participants were asked to maintain their ordinary diet during the study period, except not to have any meals between lunch and the cod dish served on Day 1. Body weight was measured on Day 5.

Analytical procedures

Blood samples were drawn from a cubital vein into 7 ml Vacutainer with ethylenediaminetetracetic acid (EDTA) as anticoagulant (Hemoguard, Becton Dickinson, Sweden). Plasma was separated by centrifugation at 2000 rev min^{-1} for 10 min (Kubota 2010 centrifuge, Kubota Corporation, Tokyo, Japan), and transferred to pre-cleaned vials, which were coded and kept frozen (−20 °C) until analysis.

p,p'-DDE and 10 PCBs were included in this study. Because of the expected abundance below the detection limits, no other compounds like *p,p'*-DDT with metabolites, other pesticides and toxaphenes were included in the study. Inclusion of these components would have reduced the statistical power (data not shown) or our ability to detect changes.

The method employed for extraction and clean up was a slight modification of that developed at Centre de Toxicologie du Quebec, Canada.[13] Plasma samples were extracted using liquid–liquid extraction with internal standards added before the first extraction. Specifically, 3 ml of plasma, 3 ml of ethanol and 3 ml of deionised water saturated with ammonium sulfate were extracted twice with 10 ml of n-hexane in a small glass tube. POPs were separated from the lipids using a tandem florisil column manually packed with 1.5 g of 0.5% deactivated florisil with 2 g of granulated sodium sulfate on top. Hexane:dichloromethane (3:1) was used as eluent. The columns were pre-washed using 10 ml of eluent before the sample was applied to the column, and the POPs were eluted using 11 ml of eluent.[13] The collected fraction was evaporated to 0.5 ml using a Zymark Turbovap 500 Closed Cell Concentrator (Hopkinton, USA), followed by a gentle flow of nitrogen for reduction to 100 µl. Gas chromatography (GC) was performed using a Fisons 8060 Mega gas chromatograph (Milan, Italy). A 30 m DB-5 MS column (0.25 mm id and 0.25 µm film thickness; J&W Scientific, CA, USA) and a deactivated guard column (0.53 mm id, 2.5 m, J&W Scientific, CA, USA) were used for all analyses. The GC was further connected to a low-resolution Fisons MD 800 mass spectrometer (Milan, Italy). The internal standards used for quantification were C-13 labelled *p,p'*-DDE, PCB 101, 118, 141 and 178. Octachloronaphthalene (OCN) was added to calculate the recovery. The quantification was done using both negative chemical ionization (NCI) and positive electron-impact ionization (EI+) sources in the same MS. In both cases selected ion monitoring (SIM) mode was used. The different compounds were identified from their SIM masses and retention times. Peaks with differences in isotopic ratio greater than 20% compared with the quantification standard were rejected and not quantified. For every 10 samples, a blank was analysed to assess laboratory-derived (*i.e.*, inadvertent) sample contamination. The limits of detection (LOD) were calculated using three times the area of the noise in 8 s (estimated peak width) at the given retention time, or if peaks were found in the blanks, three times the area of the blank. To account for the additional uncertainty with matrix effects, the limits of quantification were set to 3 times the LOD values.

Our laboratory participates in the AMAP's Human Health Ring test for human plasma samples. We have been participating in this programme from its outset. We have performed well throughout the participation performing a score of 98, 85 and 95 of a 100 in the last three inter-laboratory comparisons. We are still participating in this program.

The cod liver and cod liver oil was analysed according to a method published by Herzke *et al.*[14] For the plasma samples, the amount of lipids were determined using the following summation formula: TL = 2.27 × TC + TG + 62.6 (mg dl^{-1}).[15] Here TL is total lipids, TC is total cholesterol, and TG is triacylglycerols. The levels of lipids were determined gravimetrically for 22 samples making comparison of the two methods possible.

Statistical analyses

Statistical analyses were carried out employing the SAS software package, version 6.12 (SAS Institute, 1996).[16] One-way ANOVA was used when trying to determine the predictors of change in plasma levels of the different compounds. Change in plasma PCB and DDE levels by time was assessed by ANOVA with repeated measurement design, as well as paired sample *t*-test for comparison of two means for different sampling times.

The data was not log transformed since most of the statistics were based on differences that did show a normal distribution.

The criteria of significance has conservatively been set to 99%, due to multiple comparisons and the high number of components, as well as the uncertainties in these types of analyses.

Results

Analytical aspects

For the analyses of the plasma samples the recovery rates were good for all internal standards with a range of 65–95% (results not shown). The calculated LOD and LOQ values are shown in Table 1. Some values were below the LOQ but they were included in the statistical analyses as they were, considering the strict definition of LOQ and the few levels in this range. The few values below LOD were set to half the LOD value before included. As for the PCB congeners the PCB 138 is not fully

Table 1 Limit of detection (LOD) and limit of quantfication (LOQ) for the different compounds

Component	LOD/µg l^{-1} plasma	LOQ/µg l^{-1} plasma
p,p'-DDE	0.121	0.363
PCB 99	0.021	0.063
PCB 101	0.010	0.030
PCB 118	0.040	0.120
PCB 138/163	0.053	0.159
PCB 153	0.063	0.189
PCB 156	0.015	0.045
PCB 170	0.018	0.054
PCB 180	0.030	0.090
PCB 183	0.016	0.048
PCB 187	0.018	0.054

Table 2 Measured intake of cod liver, cod liver oil, *p,p'*-DDE and of sum PCBs

	Boiled cod liver/g	Freshly boiled cod liver oil/g	*p,p'*-DDE/μg	Sum PCBs[a]/μg
Arithmetic mean	116	22	6.3	23.9
Median	107	5.5	5.2	20.8
Range	43–281	0–152	1.6–24	6.3–89

[a]Sum PCBs = PCB (99 + 101 + 118 + 138/163* + 149 + 153 + 156 + 170 + 180 + 183 + 187) *PCB 138 and PCB 163 are co-eluting.

Table 3 Lipid levels in plasma and average differences with corresponding *p*-values

	0 h/mg dl^{-1}	4 h/mg dl^{-1}	12 h/mg dl^{-1}	5 d/mg dl^{-1}	0–4 h % difference (*p*-value)	4–12 h % difference (*p*-value)	12 h–5 d % difference (*p*-value)	0–12 h % difference (*p*-value)
Arithmetic mean	680.8	753.2	611.4	653.1	10.9 ($p < 0.01$)	−18.3 ($p < 0.01$)	7.2 ($p = 084$)	−9.8 ($p < 0.01$)
Geometric mean	669.2	739.9	602.1	644.3				
Range	464.4–954.5	460.9–1040.2	434.7–820.1	474.0–854.1	−5.6–35.6	−33.5– −3.2	−3.6–27.2	−27.5–7.4
Standard deviation	128.8	143.1	108.5	108.7	8.9	7.5	7.3	6.3

resolved from PCB 163, and thus reported as PCB 138/163. PCB 163 however accounts for less than 20% of the total peak area (results not shown).

Consumption

The amount of cod liver and cod liver oil eaten by the participants is shown in Table 2, together with the calculated amounts of PCBs and *p,p'*-DDE consumed. The fresh fish liver oil contained 98% lipids, whereas the pieces of cooked liver itself contained 65% lipids (results not shown).

Lipids in plasma

The average amount of lipids in the plasma samples and the differences in levels are listed in Table 3. From 0 to 4 h, the amount of lipids increased significantly by an average of 11% and from 4 to 12 h the amount of lipids dropped significantly by 18%. From 12 h to 5 d there was no significant change in the levels. The difference of 10% observed between 0 and 12 h was also significant. The changes observed in the amount of total lipids are mainly caused by the changes in levels of triacylglycerols. All PCBs and *p,p'*-DDE did however show a better correlation with the total lipids than the triacylglycerols (data not shown).

POPs in the meal and plasma

In the fish liver and liver oil, *p,p'*-DDE was the most abundant compound with wet-weight levels of 37.5 ng g^{-1} and 88.0 ng g^{-1} respectively (Table 4). Of the PCBs, PCB 138/163 and PCB 153 dominated, followed by 118 > 101 > 99. In human plasma, *p,p'*-DDE and PCB 153 were the most abundant compounds followed by PCB 180 > 138/163 > 170 > 118, 187. Wet-weight data are given in Table 5 and lipid-weight data in Table 6.

Table 4 Fresh-weight levels of PCBs and *p,p'*-DDE in cod liver and cod liver oil (after boiling)

	Boiled cod liver/ng g^{-1}	Fresh cod liver oil/ng g^{-1}
p,p'-DDE	37.5	88.0
PCB 99	11.9	28.2
PCB 101	14.7	35.2
PCB 118	27.0	54.7
PCB 138/163	35.2	71.6
PCB 149	6.6	14.2
PCB 153	33.1	70.7
PCB 156	2.0	4.4
PCB 170	3.4	7.3
PCB 180	7.1	15.4
PCB 183	1.8	3.5
PCB 187	4.4	9.5
Sum PCB	147	315

The most pronounced difference in congener pattern between human plasma and fish liver and oil samples was observed for PCB 180. It was the second most abundant PCB congener in human plasma, constituting 23% of the sum PCB value. In liver and fish liver oil, PCB 180 was the 6th most abundant congener constituting only 5% of the sum value. The

Table 5 Wet-weight levels of PCBs and *p,p'*-DDE in plasma

	0 h/μg l^{-1} plasma		4 h/μg l^{-1} plasma		12 h/μg l^{-1} plasma		5 d/μg l^{-1} plasma	
	AM[a] (SD[b])	GM[c]	AM[a] (SD[b])	GM[c]	AM[a] (SD[b])	GM[c]	AM[a] (SD[b])	GM[c]
p,p'-DDE	0.87 (0.47)	0.75	1.17 (0.71)	0.99	0.67 (0.44)	0.56	0.59 (0.31)	0.51
PCB 99	0.09 (0.04)	0.08	0.09 (0.04)	0.08	0.07 (0.04)	0.06	0.07 (0.03)	0.06
PCB 101	0.05 (0.02)	0.04	0.03 (0.02)	0.03	0.02 (0.01)	0.02	0.01 (0.00)	0.01
PCB 118	0.20 (0.08)	0.18	0.18 (0.08)	0.16	0.15 (0.07)	0.13	0.14 (0.07)	0.13
PCB 138/163	0.54 (0.27)	0.47	0.58 (0.29)	0.51	0.49 (0.28)	0.42	0.45 (0.23)	0.39
PCB 153	0.78 (0.42)	0.67	0.82 (0.44)	0.72	0.68 (0.39)	0.58	0.65 (0.33)	0.57
PCB 156	0.09 (0.05)	0.08	0.08 (0.04)	0.07	0.07 (0.04)	0.05	0.07 (0.04)	0.06
PCB 170	0.30 (0.15)	0.26	0.27 (0.14)	0.24	0.24 (0.15)	0.20	0.25 (0.14)	0.21
PCB 180	0.65 (0.36)	0.56	0.58 (0.31)	0.50	0.52 (0.32)	0.41	0.49 (0.27)	0.42
PCB 183	0.05 (0.03)	0.04	0.05 (0.02)	0.04	0.04 (0.03)	0.04	0.04 (0.02)	0.04
PCB 187	0.17 (0.09)	0.15	0.17 (0.09)	0.15	0.14 (0.11)	0.12	0.13 (0.07)	0.12
Sum PCB	2.86 (1.44)	2.50	2.84 (1.40)	2.52	2.44 (1.38)	2.14	2.30 (1.14)	2.01

[a]AM = arithmetic mean. [b]SD = standard deviation. [c]GM = geometric mean.

Table 6 Lipid normalised levels of PCBs and *p,p'*-DDE in plasma

	0 h/μg kg^{-1} lipids		4 h/μg kg^{-1} lipids		H/μg kg^{-1} lipids		5 d/μg kg^{-1} lipids	
	AM[a] (SD[b])	GM[c]	AM[a] (SD[b])	GM[c]	AM[a] (SD[b])	GM[c]	AM[a] (SD[b])	GM[c]
p,p'-DDE	124.38 (54.59)	112.17	149.49 (71.95)	132.32	103.54 (51.43)	90.61	87.72 (40.90)	78.22
PCB 99	12.89 (4.49)	12.09	11.08 (4.37)	10.25	10.27 (4.46)	9.06	9.97 (4.01)	9.20
PCB 101	7.31 (3.42)	6.34	4.41 (2.46)	3.81	3.09 (1.24)	2.79	1.81 (0.56)	1.73
PCB 118	29.01 (10.35)	27.38	23.52 (9.94)	21.14	23.32 (9.12)	21.51	21.71 (9.96)	19.65
PCB 138/163	77.77 (35.57)	70.20	73.89 (30.60)	67.91	74.82 (32.05)	68.87	67.13 (31.64)	60.08
PCB 153	112.43 (54.12)	100.38	105.10 (46.48)	95.47	103.93 (45.73)	94.83	98.17 (46.44)	87.49
PCB 156	12.50 (6.12)	11.24	10.64 (4.84)	9.54	10.44 (5.71)	8.70	9.88 (4.88)	8.73
PCB 170	43.34 (20.26)	39.08	35.05 (16.07)	31.65	36.70 (17.62)	27.87	36.83 (19.89)	32.11
PCB 180	91.59 (47.10)	81.46	74.97 (35.80)	67.21	79.46 (39.16)	67.30	74.04 (38.48)	65.04
PCB 183	7.46 (3.34)	6.79	6.40 (2.64)	5.87	6.67 (2.96)	5.83	6.34 (2.90)	5.66
PCB 187	24.05 (21.85)	21.85	21.63 (9.57)	19.71	21.82 (10.42)	19.44	20.17 (9.38)	18.05
sum PCB	412.10 (185.05)	374.63	366.17 (150.55)	336.77	375.81 (151.94)	347.15	345.20 (159.65)	310.13

[a]AM = arithmetic mean. [b]SD = standard deviation. [c]GM = geometric mean.

Table 7 Average % change in levels of the different compounds between the different sampling hours

	0–4 h % change		4–12 h % change		12 h–5 d % change		0–12 h % change		0 h–5 d % change	
	Wet weight	Lipid weight	Wet weight	Lipid weight	Wet weight	Lipid weight	Wet weight	Lipid weight	Wet weight	Lipid weight
p,p'-DDE	35[a]	20[a]	−40[a]	−27[a]	−12	−16[a]	−20[a]	−13[a]	−32[a]	−30[a]
PCB 99	−2	−13[a]	−21[a]	−4	2	−3	−25[a]	−18[a]	−25[a]	−22[a]
PCB 101	−20[a]	−28[a]	−27[a]	−10[a]	−32[a]	−36[a]	−46[a]	−41[a]	−68[a]	−67[a]
PCB 118	−8	−18[a]	−18[a]	1	0	−5	−28[a]	−22[a]	−29[a]	−26[a]
PCB 138/163	10	−2	−17[a]	1	−4	−10[a]	−10	−2	−16[a]	−13[a]
PCB 153	8	−4	−18[a]	0	0	−6	−12[a]	−5	−15[a]	−12[a]
PCB 156	−1	−11	−16[a]	2	12	5	−16[a]	−9	−17[a]	−14
PCB 170	−3	−13[a]	−10	9	5	−2	−13[a]	−6	−13	−10
PCB 180	−6	−16[a]	−10	9	−3	−9	−15[a]	−9[a]	−21[a]	−18[a]
PCB 183	2	−12[a]	−10	9	1	−5	−7	−5	−12	−12
PCB 187	1	−8[a]	−11	6	4	−3	−10	−3	−17[a]	−14[a]
Sum PCB	2	−9[a]	−16[a]	2	−1	−7	−15[a]	−8[a]	−18[a]	−16[a]

[a]$p \leqslant 0.01$.

food contained a higher proportion of lower chlorinated PCBs compared to the plasma samples.

Comparing the lipid-weight levels of the major compounds in plasma with the levels in liver and oil indicates that they are slightly lower in the food. For PCB 138/163 the levels were comparable.

Observed changes in plasma levels of *p,p'*-DDE and the PCBs

Using paired sample *t*-tests, several of the changes in the levels of PCB and *p,p'*-DDE were found to be significant. The average differences together with *p*-values are listed in Table 7. The *p,p'*-DDE showed the greatest fluctuations in levels (Fig 1). On a wet-weight basis all changes observed were significant except from 12 h to 5 d. From 0 to 4 h there was an increase of 35% followed by a decrease of 40% from 4 h to 12 h. Comparing the 0 h samples to the 12 h and 5 d samples showed a drop of 20 and 32% respectively. On a lipid-weight basis the changes were less pronounced even though they were all of significance.

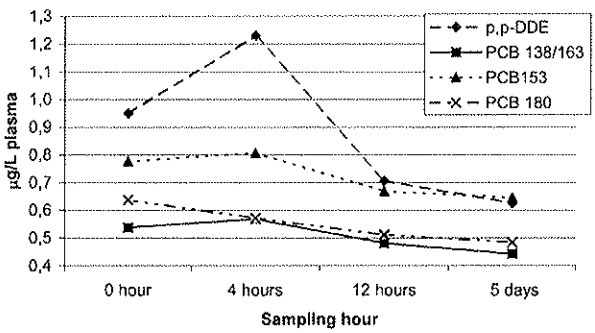

Fig. 1 Wet-weight levels of the major compounds in plasma at the different sampling hours.

The PCB congeners appear to behave differently and the fluctuations are smaller than for the *p,p'*-DDE (Table 7, Fig. 1). Looking at the wet-weight levels first, we found no significant changes for any of the major congeners or the PCBs sum from 0 to 4 h. From 4 to 12 h all concentrations drop between 10 and 27%, with the higher chlorinated congeners showing non-significant changes. Differences observed between 12 h and 5 d are in general non significant. From 0 h to 12 h and 5 d most congeners drop significantly. On a lipid-weight basis most congeners and sum PCBs drop significantly from 0 to 4 h with the PCB 138/163 and 153 as the only exception. No significant differences were observed for any of the major congeners from 4 to 12 h or 0 to 12 h. The only exception was for PCB 180 and sum PCBs from 0 to 12 h.

No significant predictors of the changes in plasma levels of POPs and lipids were found using ANOVA with a repeated measurement design. Individual intake, age, gender and BMI were included in the statistical model. As expected, the correlations between lipids in plasma and levels of POPs were significant (results not shown).

Discussion

Changes in plasma levels of PCBs and *p,p'*-DDE

Significant changes in levels of most of the studied compounds were found in connection to a meal of "mølje" even when the

plasma levels were lipid adjusted. The study further found differences in levels of most compounds from fasting and non-fasting individuals. As for the set-up of the study the 0 h plasma sample should ideally have been fasting. This was however not possible when attempts were made to serve this in a traditional way and a blood sample 12 hours after the meal was required for D-vitamin analyses. It would also be hard to find volunteers to eat this heavy meal for breakfast. Further the full summation formula for the lipid determination in plasma should have been used, but due to missing free cholesterol values this was not possible (discussed further below).

Based on ANOVA with a repeated measurement design significant ($p < 0.01$) changes with time were found for all compounds studied in this paper. There was however no relationship between individual consumption and concentration changes. Inclusion of BMI, age, and gender in the statistical model did not strengthen it. BMI, age and gender are factors known to affect the levels of POPs.[17,18]

The fact that changes in levels of either POPs or lipids were not at all related to individual intake, is surprising. There might be several reasons for this. Small sample size giving low statistical power might be one. There also seem to be great individual variation in rate of uptake. Another factor is the time before the second sample was taken. After a meal, the maximum amount of lipids in plasma is normally reached between five and six hours. Based only on one plasma sample 4 h after the meal it is impossible to conclude whether this was the peak concentration of the studied compounds. Further it is not possible to conclude if the peak concentration of POPs occurs at the same time as the lipid peak or if the concentration curves were different.

Previous studies have shown that absorption from the diet is >90% for persistent lipophilic compounds. For coefficients exceeding $\log K_{ow} = 7.5$, absorption diminishes with increasing hydrophobicity.[19] It has also been found that high intake of lipids and POPs increases uptake from the gastrointestinal tract.[19,20] The relative uptake also seems to depend on the type of lipids consumed.[21] In the present study the average amounts of *p,p'*-DDE and sum PCBs consumed was 6 μg and 23.8 μg respectively. In comparison the average daily intake of sum PCBs in Scandinavia is estimated to be 15–20 μg.[22] Further, the concentration of *p,p'*-DDE in plasma was approximately 1 μg l^{-1} and there is about 3 l of plasma in an average body.[23] This should give a total amount of approximately 3 μg of *p,p'*-DDE in the plasma before consumption of the meal (6 μg *p,p'*-DDE). This intake is thus expected to significantly alter the adipose tissue–plasma equilibrium. The fact that the level of *p,p'*-DDE actually increased with 35% on a wet-weight basis confirms this assumption. The level even increased more than the lipid levels, confirmed by the 20% lipid-weight increase. There was no significant increase for any of the PCB congeners with similar levels in plasma and food. For the higher chlorinated PCBs there was even a significant decrease on a lipid weight basis. These compounds also had lower concentrations in the food. No significant changes (0–4 h) on wet weight basis were observed. Explanations for these differences in concentration curves might be different uptake, distribution and different excretion rates (into bile) for the PCBs and *p,p'*-DDE. Several factors have been found to affect uptake, adsorption and distribution.[21] Our findings are further supported by Schlummer *et al.*[20] who found that absorption cannot be explained only on the basis of the gradient between the lipid-based food and blood concentrations. Kuwabara *et al.*[24] found that digestion of PCB contaminated fish gave a significant increase after 5 h in the levels of PCBs that was not related to a similar increase in the amount of lipids.

For the PCB congeners the relative amounts in the food was of importance to the observed changes. From 0 to 4 h the levels of the three major PCB congeners in plasma, PCB 153, 180 and 138 developed differently. The lipid concentrations of PCB 153 and 138 were comparable in plasma, cod liver and cod liver oil, but no significant change in plasma levels was found. The lipid level of PCB 180 in the liver and oil was only 17% of the level in the plasma and a significant drop (−16%) from 0 to 4 h was observed. The other higher chlorinated PCBs with low levels in the food also exhibit significant decreases in plasma levels. Further evidence that the congener pattern in the food is reflected in the plasma, is given by a Finnish study where it was found that the PCDD/F pattern in plasma was dependent on the patterns in the fish consumed.[25]

The percent differences observed for the PCB congeners are small. The immediate changes caused by diet should, however, be considered when trends in human levels are monitored making sure that plasma samples are collected in the same manner under standardised dietary conditions.

Independent of the lipid rich meal, the 0 h and the 5 d samples were considered as non-fasting and fasting samples respectively. From Table 7 it is evident that the concentrations are different (in both units) in the two sample sets. These findings are in contrast to the findings by Phillips *et al.*[6] and Longnecker *et al.*[9] who found no significant differences in lipid-weight levels of POPs, between fasting and non-fasting samples. The study by Phillips *et al.*[6] did however not take into consideration the levels of POPs in the meal itself and the effect that has on the plasma levels. In that study the meal was not expected to have "high" levels of POPs. Our results (0 h–4 h) clearly indicate that there will be fluctuations in levels of POPs depending on the food, the amount of POPs in the food and the physicochemical properties of the compounds consumed. The use of lipid-weight concentrations does however reduce the fluctuations for *p,p'*-DDE. Bergman *et al.*[10] on the other hand claim that the lipid content in human plasma varies depending on the diet and how soon after the meal the blood sample is taken, and it is therefore more correct to express the concentration on a fresh-weight basis.

POPs in plasma in relation to other studies

Comparing the plasma levels in this study with levels in other studies yielded different results, depending on whether the samples were from the fasting or non-fasting individuals. In a study on 47 women in Vestvågøy, a small fishing village in northern Norway, the average levels of sum PCBs (7 congeners) and *p,p'*-DDE was 2.34 and 1.20 μg l^{-1} plasma respectively.[18] The samples from the Vestvågøy study were from non-fasting individuals. The 0 h samples from the present study had sum PCB and *p,p'*-DDE levels of 2.88 and 0.87 μg l^{-1} respectively. In the 4 h samples, the levels were 2.86 and 1.17 μg l^{-1}. As we see the concentrations in the two studies with non-fasting samples are comparable. The average wet-weight levels in the 5 d samples (fasting) of sum PCBs and *p,p'*-DDE were 2.31 μg $l^{-1}$and 0.59 μg l^{-1} respectively. For *p,p'*-DDE it appears that the level is now only half of that in the Vestvågøy study.[18]

The average concentrations of *p,p'*-DDE, PCB 153 and PCB 138/163 found in human plasma (5 d) were 88, 98 and 67 μg kg^{-1} lipid-weight respectively. These levels are comparable to Swedish and Norwegian data in the AMAP report 1998,[2] but lower than what was found in Swedish men (*p,p'*-DDE, PCB 153, PCB 138; 290, 220, 160 μg kg^{-1} lipid-weight respectively) who reported no or low intake of fish from the Baltic Sea.[26] By contrast for Swedish men reporting high intake of fish from the Baltic Sea, the average levels of DDE and PCB 153 were 4500 and 1000 μg kg^{-1} lipid.[27]

The levels of POPs found in this study are low and a meal of "mølje" gives a significant increase (after 4 h) only in the levels of *p,p'*-DDE. No association between fish consumption and levels of POPs was found in the northern-Norwegian fishing village Vestvågøy.[18]Based on this study, the findings of Furberg *et al.*[18] and the discussion of possible health effects

in the AMAP report[2] there is no reason to believe that the levels found in these people and the intake of "mølje" can cause negative health effects related to POPs.

Lipids in plasma

Plasma lipids determined gravimetrically were 15% lower than the levels assessed using the summation formula. This is comparable to the 20% difference reported by Sjödin *et al.*[26] They used the summation formula that included phospholipids and free cholesterol. One of the main advantages of using the summation method is that a defined total lipid value of known composition is obtained, rather than a simple weight of substances extracted by organic solvents.[11] The correct working definition of what is determined gravimetrically refers to extractable organic material (EOM) rather than lipids. The gravimetric determination also requires greater sample volumes for accurate determinations. Thus, several studies are recommending the enzymatic summation method,[11,28] with the following formula being the preferred one; TL = 1.677(TC − FC) + FC + TG + PL.[6,11,12,28] Here FC is free cholesterol.

The differences observed in lipid normalised levels of PCBs and *p,p'*-DDE between fasting and non-fasting samples, might have been reduced if the full summation formula had been used.[6] For the free cholesterol Akins *et al.*[11] have shown that this fraction ranged from 21.1% to 30.1% in their study, showing the importance of including it in the summation formula. Nevertheless, we suspect that the use of the complete formula would not have removed the statistically significant differences observed. The reason why the short formula for calculating lipids was being adopted was that data on the free cholesterol was not available for any of the samples.

POPs in cod liver

The sum PCBs levels found in the boiled cod liver (147 ng g^{-1} wet-weight) and cod liver oil (315 ng g^{-1} wet-weight) are comparable to levels previously reported in raw cod liver (240 ng g^{-1} wet-weight) from northern Atlantic cod by Kallenborn *et al.*[29] but slightly lower than 660 ng g^{-1} wet-weight in liver from northern Atlantic cod, by SNT.[5]

Conclusions

The concentrations of PCBs and *p,p'*-DDE in plasma change significantly after a meal rich in marine lipids and relatively high levels of these compounds. This was in particular the case for *p,p'*-DDE.

As observed for the PCB congeners, this study indicates that the relative congener pattern of POPs in the food is reflected in the plasma sample, when this sample is taken 4 h after the meal.

Consequently, it seems essential to collect plasma specimens under fasting conditions and to continue to endorse the practice of reporting both unadjusted and lipid adjusted concentrations. One can then test when examining associations which units explains most of the variability.

A common approach for determining lipids in plasma should be employed to facilitate better comparison of data. The enzymatic summation method is here recommended.

Further studies are needed for better understanding of the kinetics of POPs during digestion and remobilisation from adipose tissue.

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References

1 I. C. Burkow and R. Kallenborn, *Toxicol. Lett.*, 2000, **112–113**, 87–92.
2 AMAP Assessment Report: Arctic Pollution Issues, Oslo: Arctic Monitoring and Assessment Programme (AMAP), 1998.
3 J. C. Hansen, *Toxicol. Lett.*, 2000, **112–113**, 119–125.
4 J. Kloster, *Acta Paediatr.*, 1931, **12**, 1–82.
5 Norwegian Food Control Authority (SNT), Assessment of Environmental Contaminants in Fish and Shellfish in Northern Norway, SNT-rapport 4, Oslo, 1997.
6 D. L. Phillips, J. L. Pirkle, V. W. Burse, J. T. Bernert, L. O. Henderson and L. L. Needham, *Arch. Environ. Contam. Toxicol.*, 1989, **18**, 495–500.
7 S. Haddad, P. Poulin and K. Krishnan, *Chemosphere*, 2000, **40**, 839–843.
8 D. G. Patterson, L. L. Needham, J. L. Pirkle, D. W. Roberts, J. Bagby, W. A. Garrett, J. S. Andrews, H. Falk, J. T. Bernert, E. J. Sampson and V. N. Houk, *Arch. Environ. Contam. Toxicol.*, 1988, **17**, 139–143.
9 M. P. Longnecker, L. Bernstein, C. L. Bird, A. K. Yancey and J. C. Peterson, *Cancer Epidemiol. Biomarkers Prev.*, 1996, **5**, 753–755.
10 A. Bergman, E. Klasson-Wehler and H. Kuroki, *Environ. Health Perspect.*, 1994, **102**, 464–469.
11 J. R. Akins, K. Waldrep and J. T. Bernert, Jr, *Clin. Chim. Acta*, 1989, **184**, 219–226.
12 C. S. Cheek and D. F. Wease, *Clin. Chem.*, 1969, **15**, 102–107.
13 I. Romieu, M. Hernandez-Avila, E. Lazcano-Ponce, J. P. Weber and E. Dewailly, *Am. J. Epidemiol.*, 2000, **152**, 363–370.
14 D. Herzke, G. W. Gabrielsen, A. Evenset and I. C. Burkow, *Environ. Pollut.*, 2002, **121**, 293–300.
15 L. L. Needham, V. W. Burse, S. L. Head, M. P. Korver, P. C. McClure, J. S. Andrews, D. L. Rowley, J. Sung and S. E. Kahn, *Chemosphere*, 1990, **20**, 975–980.
16 SAS Institute Inc., SAS/STAT User's Guide, Version 6, Fourth Edition, Cary, NC: SAS Institute Inc., 1989.
17 B. Deutch and J. C. Hansen, *Int. J. Circumpolar Health*, 1999, **58**, 214–219.
18 S. Furberg, T. Sandanger, I. Thune, I. C. Burkow and E. Lun, *J. Environ. Monit.*, 2002, **4**, 175–181.
19 G. A. Moser and M. S. McLachlan, *Chemosphere*, 2001, **45**, 201–211.
20 M. Schlummer, G. A. Moser and M. S. McLachlan, *Toxicol. Appl. Pharmacol.*, 1998, **152**, 128–137.
21 R. J. Jandacek and P. Tso, *Lipids*, 2001, **36**(12), 1289–1305.
22 P. Weihe, P. Grandjean, F. Debes and R. White, *Sci. Total Environ.*, 1996, **186**, 141–148.
23 M. Rowland and T. M. Tozer, *Clinical Pharmacokinetics Concepts and Applications*, ed. D. Balado, Lippincott, Williams and Wilkins, Philadelphia, 1995, p. 149.
24 K. Kuwabara, T. Yakushiji, I. Watanabe, S. Yoshida, K. Yoyama and N. Kunita, *Bull. Environ. Contam. Toxicol.*, 1979, **21**, 273–278.
25 H. Kiviranta, T. Vartiainen and J. Tuomisto, *Environ. Health Perspect.*, 2002, **110**, 355–361.
26 A. Sjödin, L. Hagmar, E. Klasson-Wehler, J. Bjork and A. Bergman, *Environ. Health Perspect.*, 2000, **108**, 1035–1041.
27 L. Asplund, B. G. Svensson, A. Nilsson, U. Eriksson, B. Jansson, S. Jensen, U. Wideqvist and S. Skerfving, *Arch. Environ. Health*, 1994, **49**, 477–486.
28 E. Grimvall, L. Rylander, P. Nilsson-Ehle, U. Nilsson, U. Stromberg, L. Hagmar and C. Ostman, *Arch. Environ. Contam. Toxicol.*, 1997, **32**, 329–336.
29 R. Kallenborn, I. C. Burkow, M. Schlabach and E. H. Jørgensen, *Organohalogen Compd.*, 1997, **32**, 252–256.

PAPER III

Human plasma levels of POPs, and diet among native people from Uelen, Chukotka

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Abstract

Some of the people living in the Chukotka Peninsula of Russia depend heavily on marine mammals, but little is known of the exact dietary patterns and plasma levels of POPs among these populations. In this study, POPs levels in plasma from 50 participants from the isolated community of Uelen (Bering Strait) were determined and related to dietary information obtained through a food frequency questionnaire. The intake of marine mammals was high and the combined intake of blubber from walrus, seal and whale was a significant predictor ($p<0.01$) of plasma concentrations of sum PCBs and borderline for sum CDs ($p=0.02$) and sum DDTs ($p=0.04$). There was a significant gender difference in the levels of POPs, and among women there was a significant increase with age. Extensive breastfeeding and lower blubber intake among women could be possible explanations for this gender difference. Despite the high intake of blubber the plasma levels of PCBs and DDTs were lower than some of those reported for the East Coast of Greenland. The geometric mean values for sum PCBs (17 congeners) and sum DDTs were 1316 ng/g lipids and 563 ng/g lipids, respectively. PCB 163, which partly co-eluted with PCB 138, was found in high concentrations (40 % of PCB 138). This raises questions regarding the validity of using PCB 138 and PCB 153 to calculate the level of Arochlor 1260. The geometric mean of sum CDs was 518 ng/g lipids. Concentrations of β-HCH (geometric mean; 410 ng/g lipids) were higher than observed for other native populations depending on marine mammals. Transportation of β-HCH by ocean currents through the Bering Strait into the Arctic Ocean or regional point sources might explain these elevated levels.

Introduction

Polychlorinated biphenyls (PCBs) and other halogenated aromatic hydrocarbons are ubiquitous environmental contaminants that accumulate in lipid-rich body tissues [1]. The lipophilicity and resistance to biodegradation are responsible for the bioaccumulation in the food web, and in particular the aquatic food web [2]. In humans, biological half-lives of several years or longer have been reported for many organochlorines [3,4].

Present levels of persistent organic pollutants (POPs) in the Arctic cannot generally be related to known use and/or release from local sources, and can therefore only be explained by long-range transport from lower latitudes [5-7]. The long-range transport of POPs to the Arctic is well-documented [8]. However, for α-HCH the Arctic Ocean appears now also to act as a source by revolatilization of long-range transported material previously deposited [9]. Several review papers and reports have also indicated a non-uniform distribution of POPs in the Arctic [10,2,5,11,12]

High intake of blubber from marine mammals, by humans Greenland, northern-Canada and the Faeroe Island have been found to lead to elevated blood levels of POPs in humans, especially PCBs and the metabolite *p,p'*-dichlorodiphenylethylene (*p,p'*-DDE) [5,13-15]. In men from Scoresbysund in Greenland, the average amount of sum PCBs and sum DDTs in plasma was found to be 41 μg/L and 11.1 μg/L, respectively [13]. In Scandinavia and most areas in northwest Russia, where people eat small amounts of marine mammals, the concentrations of PCBs are low. In Norwegian women, the mean sum PCBs concentration in plasma has been reported to be 4.0 μg/L, while sum DDT was 0.9 μg/L [5]. However, high levels of *p,p'*-dichlorodiphenyltrichloroethane (*p,p'*-DDT) and *β*-HCH have been found in human plasma from residents of the town of Nikel and city of Arkhangelsk indicating the presence of *de nova* local sources at these sites in western Russia [5,16]; their exact origin have thus far not been identified.

The information on plasma levels of POPs and in diet in northeast Russia is very limited in comparison to other Arctic countries. This is also highlighted in the 1998 AMAP report and Chukotka Peninsula is now one of the key areas in the future work of AMAP [5,17]. Several of the communities in the Chukotka Peninsula depend heavily on marine mammals, although the concentrations of POPs in the seals from that

region have been reported to be lower than in other arctic areas such as Spitsbergen, Eastern Greenland and northwest Russia [5]. The levels of β-HCH have, on the other hand, been reported to be high in polar bears from the Chukotka Peninsula [17]. Further, there is no information on the possibility of local point sources in this remote region as compared to northwest Russia.

The data presented here clarify these various conflicting observations. We report plasma concentrations of POPs for 50 participants from Uelen in the Chukotka Peninsula, and examine the link to dietary intake of marine mammals and other traditional foods.

Methods

The joint RAIPON/AMAP/GEF[a] study of pregnant women and their neonates was initiated in 2001. Four regions are involved in the project, the Kola Peninsula, Pechora Basin, Taimyr Peninsula and Chukotka Peninsula. During the fieldwork, additional participants from the general population were interviewed and blood samples were collected from them. In Uelen, situated by the Bering Strait and Chukotka Sea, the intake of marine mammals was known to be considerable. A total of 250 adults were interviewed and who donated blood. The 250 participants were all the people available, from an adult population of 374, at the time of the field work. Of these 250 samples, 50 were selected at random (corresponding to every 5th donor) and analysed to get a first indication of the human POP content among the general population of Uelen. The samples were collected in July and August of 2001 and the weight and the height of the participants were measured at the time of the interview. Specimens of antecubital vein blood were taken in EDTA tubes with standard vacutainer equipment. The blood was centrifuged and plasma was immediately stored at –20^0 C and kept frozen until analyses.

Of the 50 participants 92 % considered themselves Chukchi, whereas the remaining were Eskimos or Evenk. There were 26 women and 24 men with an average age of 37.3 years (range, 20 – 70 years). Of the 50 study subjects, 36 (73.5 %) may be

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[a] RAIPON: Russian Association of Indigenous Peoples of the North
AMAP: Arctic Monitoring and Assessment Programme (Arctic Council/AEPS group)
GEF: Global Environment Facility

considered to have normal weight (body mass index (BMI)= 18.5 – 24.9 kgm^{-2}) and the remaining 14 were overweight (BMI>25 kg m^{-2}), based on WHO criteria. The average height was 161.9 cm (range; 132 to 180 cm). Local health professionals solicited information on breastfeeding, 6 months after the sample collection. Unfortunately, no information on time elapsed from the last period of breastfeeding was obtained.

Dietary information

The food frequency questionnaire was designed to include most food items consumed locally, registering the frequency, the amount eaten and the season for consumption. For the estimate of the frequency the following options were given to the participants: 'daily', '1-3 times per week', '2-3 times per month' or 'once per month or less'. Six trained health personnel carried out the interviews. Use was not made of models describing portion size due to very different dietary habits. For example, the hunters ate much more during the hunting season (June – Sept.). The reported intake per meal was thus estimated to the nearest 50 g. From the reported frequency of intake and portion sizes, the average consumption per day was calculated. This intake in g per day was used in the statistical models.

To facilitate the consideration of intakes of the 11 most common food articles, they were normalised by calculating the corresponding energy intake using the Norwegian Food Composition Table [18]. The energy intake values were compared to energy requirements for high physical activity calculated from 'Energy and protein requirements' according to WHO [19]. These calculations enable comparison of energy intake to other studies independent of food articles.

Despite the energy calculations, no attempts were made to validate the food questionnaire and the self estimated intakes completely. The self reported intake was used only to separate high and low consumers on a relative basis, remembering that the questionnaire has not been validated .

Analytical procedures

The plasma samples were extracted and purified according to a slightly modified method developed at the Centre of Human Toxicology (CTQ), National Institute of Public Health, Quebec [20], and the compounds were quantified according to a method

published previously [21]. In short, the plasma samples were extracted using liquid–liquid extraction with ethanol, deionised water saturated with ammonium sulphate and hexane. The POPs were separated from the lipids using a tandem florisil column manually packed with 1.5 g of 0.5 % deactivated florisil with 2 g of granulated sodium sulphate on top in each of the columns. The POPs were eluted using 11 ml hexane: dichloromethane (3+1). The collected fraction was evaporated to 0.5 ml using a Zymark Turbovap 500 Closed Cell Concentrator (Hopkinton, USA), followed by a gentle flow of nitrogen for reduction to 100 µl. Gas chromatography (GC) was performed using a Fisons 8060 Mega Gas Chromatograph (Milan, Italy). A 30-m DB-5 MS column (0.25 mm i.d. and 0.25 µm film thickness; J&W Scientific, CA, USA) and a deactivated guard column (0.53 mm i.d., 2.5 m, J&W Scientific, CA, USA) were used for all analyses. The GC was further connected to a low-resolution Fisons MD 800 Mass Spectrometer (Milan, Italy).

C-13 labelled PCB 101, 118, 141 and 178, *p,p'*-DDE and *p,p'*-DDT were used as internal standards. Octachloronaphthalene was added as a recovery standard and the recovery rates for the internal standards all were in the range of 65 to 97 % (results not shown). The quantification was achieved using both positive electron-impact ionisation (EI+) and negative chemical ionisation (NCI); both were employed in the selected ion-monitoring (SIM) mode in the same instrument. The different compounds were identified from their SIM masses and retention times. Peaks with differences in isotopic ratio greater than 20% compared to the quantification standard were rejected and not quantified. For every 10 samples, a blank was analysed to assess laboratory-derived (i.e. inadvertent) sample contamination. The limit of detection (LOD) was calculated using three times the area of the noise or, if peaks were found in the blanks, three times the area of the blank. All levels below the LOD were set to half LOD and included as such in the statistical analyses.

PCB 138 was only partially resolved from PCB 163 and integrated as one peak, and thus was reported as PCB 138/163. Twenty samples were, however, reanalysed to determine the presence of additional PCB congeners and possible co-elutions. In these it was possible to quantify these two congeners separately due to better separation caused by minor differences in column performance. The lipid content of the plasma samples was determined enzymatically [22].

In terms of quality control, our laboratory participates three times per year in the AMAP Human Health Ringtest for plasma samples. The ringtest includes 6 PCB congeners, *p,p'*-DDE, *p,p'*-DDT, β-HCH and oxy chlordane, and three plasma samples each round. We have been participating from the outset of this programme and performed well throughout. During 2002 our performance was 85, 95 and 89 % on the three runs. (Each result within ± 40 % of the assigned value, gives one point. Each result also within ± 20 % gives an additional point. Only numerical results are counted. Laboratories must also supply results for at least 5 analytes to be graded. Scores are expressed as a percentage.)

Statistical analyses

Analyses of Variance (ANOVA), t-tests and linear regression models were used when assessing the predictors of POP concentrations in plasma. Shapiro-Wilks test criteria were first used to decide whether the levels of the different compounds were normally distributed. The levels of all compounds had to be log transformed to obtain a normal distribution (results not shown). All statistics was performed on lipid weight data. Pearson correlation coefficients (r) were calculated for linear relationships. Both genders were included in the ANOVA. To better explore the gender differences and the effects of breastfeeding; POPs and lipid levels were compared in men and women below and above the age of 40 years. This was done using an independent sample t-test and ANOVA adjusting for blubber intake. Different combinations of blubber from seal, whale and walrus including their sums were used in the statistical models to find the best predictor of POPs intake.

Because of the uncertainty in data on blubber intake, it was not treated as a continuous variable. Participants were instead divided into two equal sized groups on the basis of blubber consumption: low intake and high intake. They were first divided into tertiles (results not shown), but the moderate and high consumer had very similar levels and it was thus decided to divide them into two groups. Further, they were divided into two age groups, 0-40 years and 41 years and above, and two BMI groups, 0 - 24.99 kg/m^2 and 25 kg/m^2 and above. The women were divided into two equal-sized breastfeeding groups, 0 – 36 months and 37 months and above, because categorical grouping (breastfed or not) was not possible as only one had not been breastfeeding her child. Smoking, also found to affect the levels of POPs [23], was not included in the analyses

because 95 % of the participants were smokers and the remaining 5 % were previous smokers.

All statistical analyses were done using the SAS software package, version 6.12 (SAS Institute, 1996) [24]. The border for significance was set to 99 % instead of more commonly used 95 % due to the high number of variables and uncertainties involved in these types of analyses. Significance between 95 and 99 % was considered as borderline.

Results

Breast feeding

Women reported breastfeeding for a median total of 36 months (range: 0 – 270), and every child was breast-fed a median time of 18 months.

Diet

It must be noted that the food questionnaire has not been validated and the self reported intake is presented here just to give an impression of the relative reported intake values. The most common food items are listed in Table 1 with the median and average amounts consumed. All participants reported eating local food with marine mammals being the most important besides bread. Walrus and seal meat, as well as walrus blubber were most frequently consumed with a frequency of 8 meals per month for all three items. The median intakes were reported to be 105, 79 and 26 g per day, respectively. The intake of blubber and marine meat was highly correlated (r-coefficient of 0.81 and $p<0.001$). The most common fish, saithe was consumed 2.7 times per month corresponding to 26 g per day.

Bread was consumed daily at an intake rate of 200 g per day. Considerable amounts of cereal and macaroni were also consumed with intakes of 100 and 86 g wet weight, respectively. Besides those items, little vegetables and other commercial goods were eaten.

The median energy intake for the 11 most common food items was found for men to be 20360 kJ/day (18 – 30 years) and 16357 kJ/day (31-60 years). For women 18 to 30 years and 30 to 60 years, it was found to be 9096 and 18024 kJ/day, respectively.

Persistent organic pollutants (POPs)

The limits of detection (LOD) for all compounds are shown in Table 2. Wet-weight and lipid-weight plasma concentrations of POPs are shown in Table 3 and Table 4, respectively. In Table 3 the percentage of individuals with values below the LOD is indicated. *cis*- Chlordane was also quantified, but due to isotopic interference in the chromatograms it was not reported. The compounds with the highest concentrations were PCB 153, *p,p'*-DDE, β-HCH, *trans*-nonachlor and oxy-chlordane with geometric means of 538, 520, 410, 261 and 205 ng/g lipids respectively (Table 4). The geometric mean of β-HCH was 521 ng/g lipids for men and 331 ng/g lipids for women (results not shown). Toxaphene 26 (tox 26) and 50 (tox 50) were detected in 82 % and 86 % of the samples, respectively. Geometric mean level of tox 26 + tox 50 (sum tox) was 62.7 ng/g lipids. The DDE/DDT ratio was found to be 15.4. Both mirex and α-HCH were detected in all samples with geometric means of 27 ng/g lipids and 3.5 ng/g lipids.

The concentration of PCB 153 was more than twice as high as that of PCB 138/163, followed in sequence by PCB 180 > 118 > 99 > 170 > 187; PCB 187 levels were considerably higher than the remaining congeners. Reanalyses of 20 samples showed no co-elution for PCB 153, but high amounts of PCB 163 were found to co-elute partly with PCB 138. PCB 163 had a geometric mean of 0.22 μg/L plasma. It must be noted that the separation of PCB 138 and 163 was only partial, adding uncertainty to their relative amounts. PCB 146 was also identified in all 20 plasma samples with a geometric mean of 0.15 μg/L (Table 5). The PCB congeners were all highly inter-correlated. These correlations were all significant, except for some of the lower chlorinated congeners like PCB 28, 52 and 101. The correlation between PCB 170 and 180 had an r-coefficient of 0.994, while that of PCB 138/163 and PCB 153 was 0.984.

Diet, lipids and POPs

No food item except blubber was found to significantly affect the plasma concentrations of POPs in the statistical models. Of the different combinations of blubber intake, the sum of all three types of blubber was the best predictor of intake of POPs. Attempts were also made to use the frequency of intake instead of intake in g/day in the statistical models, and it was found not to be a good predictor of intake of

POPs. The diet is highly seasonal and the whole year needs to be considered for the complete input. The results from the ANOVA are shown in Table 6 where the concentrations of POPs in the low and high blubber intake groups are listed. Here the levels of POPs in both intake groups are adjusted for the other significant predictors, age and gender. Age and gender were significant factors for all the studied compounds and their respective p-values are listed. The intake of blubber was a significant predictor of sum PCBs ($p=0.01$) and borderline for sum CDs ($p=0.02$) and sum DDTs ($p=0.04$). It was not a significant factor for sum tox and β-HCH, even though there seems to be an increase in levels with consumption. For the sum tox and β-HCH there was a significant interaction between age and gender. The interaction factor was however not included in the model because of the low number of participants. BMI and the self-reported domestic use of pesticides were not significant predictor variables for any of the compounds.

Clearly, the amount of lipids in plasma was found to increase significantly with age independent of gender (Table 6). The same significant increase with age was observed for free cholesterol, total cholesterol and phospholipds. The amount of triglycerides did not increase with age nor blubber intake (results not shown). There was a slight increase in the amounts of lipids with the consumption of blubber, but it was not of significance (Table 6).

For men, none of the plasma concentrations of POPs increased significantly with age, not even when age was treated as a continuous variable. The intake of blubber was borderline significant ($p=0.04$) for the plasma sum CDs, otherwise not. BMI was only borderline significant for the sum tox and sum CDs (results not shown).

For the women, the level of POPs increased significantly with age. The levels of POPs did however not increase consistently but seemed to increase slowly at low age with a greater increase at higher age. Below the age of 40 there was no significant correlation between age and the sum PCBs and sum DDTs (lipid weight). Above the age of 40 this association was borderline significant ($p=0.04$) for both compound groups. Intake of blubber was only significant for the concentration of sum PCBs. Duration of breastfeeding was not a significant factor in predicting plasma levels of POPs, but the time spent breastfeeding was highly correlated to age.

In Table 7, the results from an independent sample t-test of the gender differences in two age groups are shown. Below the age of 40, the levels of POPs are significantly ($p<0.01$) lower among the women. The only exception was for β-HCH where the difference was borderline significant ($p=0.03$). Above 40 years of age, there is no longer a significant gender difference ($p>0.05$) in the levels except for the chlordanes ($p<0.01$). As for the lipids, there was no gender difference in any age group. The same results were obtained using ANOVA adjusting for the blubber intake.

Discussion

Methodological issues

Through the food frequency questionnaire we were able to identify the intake of blubber as a significant contributor to increased levels of PCBs and borderline for sum DDTs and sum chlordanes. The dietary issue is, however, extremely complex with great seasonal variations in both intake and physical activity, especially for the hunters. Comparing calculated energy intake (median) to expected energy needs for high physical activity, it seems that the self-estimated food intake was high for some of the participants. Especially among women aged from 30 to 60 years, the estimated intake was 18024 kJ/day compared to a calculated need of 10228 kJ/day. Among men from the age of 18 to 30 years, the estimated energy intake was 20360 kJ/day compared to a calculated need of 14825 kJ/day[19]. It must however be noted that most hunters were in this age group and they eat considerable amounts when hunting, indicating that this discrepancy might be expected. In addition the people live under extreme conditions with high energy needs. The reported energy intake in Greenland in the year 1926, was also reported to be 1.5 times their expected needs [17]. Among men (30 – 60) and women (18-30), the energy intake was comparable to their calculated energy need. These calculations constitute a crude comparison to assess that the self-estimated intake could be used as an approximate measure of intake of the different food items. It should be emphasised that the calculation of energy intake was based only on 11 food items (approx 95 % of the energy intake) and not total food intake. It must also be noted that the numbers in each group was low, adding additional uncertainty to the values.

Levels of POPs

In a comparison of the concentrations of POPs in this study with previous studies, we discuss the different compound groups separately. The geometric mean level of the sum PCBs (17 congeners) found in men and women were 2027 ng/g lipids and 884 ng/g lipids respectively. This is considerable lower than what was found in men from Scoresbysund (6750 ng/g lipids) on the Eastern Coast of Greenland, but in the same range as from the other settlements of that region. As for the women, the levels were in the same range as found in the Disko Bay area of Greenland [13]. The mean sum PCB level of 1755 ng/g lipids found in this study also appears to be lower than what was found in Nunavik (northern Quebec), where the mean sum PCBs (20 congeners) was 4080 ng/g lipids [25].

Of the PCB congeners the PCB 153 is by far the most dominant congener with levels twice that of PCB 138/163. This has also been observed in other populations depending heavily on marine mammals [25]. The reanalyses of 20 samples revealed that PCB 163 constituted about 40 % of the PCB 138 content (Table 5) in all the reanalysed samples, thus increasing the difference in ratio of 153 to 138 further. The high levels of PCB 163 have to our knowledge not been reported in human samples before. The partial separation of PCB 138 and PCB 163, as well as the high levels of PCB 163, questions the conversion of PCB 138 and PCB 153 levels to Arochlor 1260 by using the following summation formula; Arochlor 1260 = 5.2(153 + 138). This formula was deducted from the congener composition in Arochlor 1260 [26] and employed, among other places, in the AMAP report 1998 [5]. Our data suggest that it should perhaps be 5.2 (138 + 153 + 163), if the PCB 138 and PCB 163 are separated. This has, however, not been validated further. The few reports of PCB 163 in the environment might be the consequence of its poor separation from PCB 138 in most previous studies.

It was further found that PCB 146, 157, 163 and 167 constituted about 7 % of the sum PCBs (Table 5). This adds up to a considerable part of the total burden and needs to be considered when analysing individual congeners, especially since PCB 146 and 163 were present in all the analysed samples. As observed in previous studies, most PCB congeners were highly correlated [27-29]. On a lipid weight basis the sum PCBs and PCB 153 had a Pearson correlation coefficient of 0.99 ($p<0.001$).

The β-HCH level in women (1.7μg/L plasma) was lower, but not significantly (t-test; $p>0.05$), than what has been found in delivering women from Arkhangelsk (3.1 μg/L) [30]. On the other hand, it was higher than in any of the native populations from Greenland, Canada and the Faeroe Islands both for men and women [5,13,25,31]. The β-HCH levels in men (331 ng/g lipids) was 4 times higher than the highest levels reported previously (93 ng/g lipids) from Eastern Greenland [13]. Elevated β-HCH levels compared to the PCBs have also been observed in Polar Bears from the Chukotka Peninsula [17]. These findings are also consistent with oceanic transport data, which indicates that β-HCH enters the Arctic Ocean through the Bering Strait [32].

As for the DDTs, chlordanes and toxaphenes, the observed concentrations are comparable to what has been found in other native populations [5,13]. Mirex was also present in 96 % of the samples when analysing Inuit breast milk samples [31]. The ratio of DDE to DDT was found to be 15.4 (range; 5 – 45). This value seems lower than what has been reported in Greenland, Sweden, Norway and Iceland (26.0 –35.0), but comparable to Inuit women from Canada (16.8). The observed ratio is also higher than what has been found in Nikel and Arkhangelsk (8 and 7, respectively) [17]; it is thus not low enough to conclude that fresh local sources are present. It might, however, indicate a difference in metabolite pattern in the diet, or a combination of exposure from marine mammals and another source of DDT.

As noted the amount of lipids in plasma increased significantly with age. That is an important consideration when using wet weight levels in plasma.

POPs and diet

First of all, we emphasise the fact that the traditional diet is rich in essential nutrients and of great cultural importance for the native people [5]. However, as found elsewhere, the consumption of marine mammals had a significant effect on the levels of some POPs [5,31,33]. Despite the uncertainties in estimated intake, there is no doubt that the blubber intake is also higher than what has been published from other populations. The intake of traditional food has declined in most other parts of the Arctic with 60 – 90 % of the diet in northern Canada and Greenland now being from a commercial source [17]. In Ittoqqortoormiit and Tassilaq (Greenland), the median blubber intake was estimated to be 6 g/d in both places (pers. Comm. B. Deutch), compared to a median intake of 49 g/d in Uelen.

In the Faeroe Islands, the estimated daily intake of whale blubber between 1974 and 1980 was estimated to be 11.8 g [34]. By comparison the people from Uelen reported a median daily consumption of 5 g whale blubber, in addition to 26 g of walrus blubber and 18 g of seal blubber. The total daily intake of blubber and marine mammal meat adds up to 253 g per day. This is comparable to a reported daily intake of 300 g of ringed seal, bearded seal, beluga skin and walrus among the Canadian Inuits [31]. Despite the high intake of blubber, the PCB and DDE concentrations in plasma are lower than found in some communities on the East Coast of Greenland by Deutch, B. and Hansen, JC.[13], and slightly lower than among Inuits in northern Quebec [25]. This might indicate that the levels of these compounds are lower in the marine mammals from the Russian eastern Arctic. Lower levels of PCBs and DDTs in marine mammals fron this are of the Arctic has been reported in Arctic Pollution, 2002 [17].

Even though the plasma levels of POPs seem to increase with intake of blubber, it was only for the sum PCBs ($p=0.01$) that blubber was a significant predictor. For sum CDs and for sum DDTs the intake of blubber was of borderline significace ($p=0.02$ and 0.04, respectively). These findings suggest that even though there are apparent uncertainties associated with the questionnaire used, we were still able to predict the levels of some POPs using the dietary information.

As found in other studies, age and gender are significant predicting factors for all POPs in plasma; [23,35] but for sum tox and β-HCH there was also a significant interaction (results not shown). The likely reason for the interaction was that there was no significant age increase in POP plasma levels among men as opposed to the women. Even though this interaction was not significant for the other compounds it adds uncertainty to the statistical analyses of men and women together.

Even after adjusting for differences in blubber intake, which was higher among the young men compared to the young women, the concentrations of POPs were still significantly lower among premenopausal women. The observed difference can possibly be explained by extensive breast-feeding keeping the plasma levels low in that period. Breastfeeding has been found to reduce the levels of POPs in women considerably [35-37]. In the present study, however, breastfeeding was not found to significantly reduce the levels of POPs when it was included in the ANOVA. The reason for this we believe is the fact that age and period of breastfeeding was highly correlated, implying that duration of breastfeeding was also correlated to the time

elapsed since the last period of lactation. Thus the broad age distribution likely masked the effects of lactation thereby reducing the importance of this factor in the model. We had no information on time elapsed since the last period of breast-feeding making it impossible to adjust for this. This is the reason for the age division by considering that women below 40 are normally fertile and closer to the last lactation period. The two age groups could thus be considered as recent breast feeders and old breast feeders, explaining the difference in levels of POPs.

Above the age of 40, the plasma concentrations of POPs among women had increased considerably, and the gender difference was no longer significant. The reason for the lack of significance might be the small numbers in each group and the broad concentration range. The increase in plasma concentrations with age among women could likely be explained by accumulation with time since the last period of breastfeeding (Table 7). The older women also reported eating more blubber than the younger women (comparable to the amount older men consumed (results not shown)). It was only for the chlordanes that the levels were still significantly ($p<0.01$) different in the above-40 group.

The reason for the non-significant age increase among men is not clear, but might indicate that the intake rate is not much above elimination rate. It seems like they are approaching a steady state for these compounds but there appears not to be enough statistical power to discern this in the present study. It has also been shown that for a given contamination level in the diet, the net absorption of hexachlorobenzene (HCB), PCBs and PCDD/Fs in human volunteers diminishes as the blood level of the compounds increases [38]. The amount of lipids also increased significantly with age and the lipid weight data were employed in the analyses. Another explanation for the slow increase in levels might be the observation that men's consumption of wildlife may be inversely correlated with age (Pers. Comm. E. Nieboer). The interviewers also had that impression of the people in Uelen, but the energy intake was only slightly higher among the younger men, and not of significance.

High β-HCH levels might be of special health concern considering the fact that this compound is found in higher levels in the liver compared to adipose tissue [39]. Further the most crucial time window of sensitivity for adverse effects by PCBs appears to be the prenatal and the early postnatal period [40]. As a Dutch PCB/dioxin study has illustrated subtle clinical, endocrine and mental/psychomotor development effects can

occur in breastfed infants [41,42]. It has also been reported that nursing babies absorb more than 90 % of these compounds when present in breast milk [43,44]. In a study by Ayotte and colleagues of dioxin-like compounds, it was found that breastfeeding strongly influenced body burden during childhood, but not after the age of 20 [45]. The women in this study breastfed their children for a long time period. It is reasonable to assume that the first children breastfeeding likely will receive the highest amounts of POPs from their mothers. Nevertheless, the health benefits of breastfeeding do appear to outweigh the negative aspects from POP exposure; only extreme levels should result in advice against breastfeeding [5,46].

Conclusions

Self-reported intake of the sum of seal, walrus and whale blubber was found to be a significant predictor of the plasma concentrations of sum PCBs and borderline for sum CDs and sum DDTs, for males and females combined.

The increase in levels of POPs with age is not significant among the men in this study, whereas age is a highly significant factor for women.

Below the age of 40, the levels of POPs are significantly lower among women compared to men. Above the age of 40 the levels are still different but no longer at significance, except for the chlordanes.

High amounts of PCB 163 were found partly to co-elute with PCB 138

The levels of β-HCH were elevated compared to the PCBs confirming previous findings that ocean currents through the Bering Strait are the main transporters of β-HCH to the Arctic Ocean or indicating the presence of fresh sources.

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Reference List

1 F. Laden, L. M. Neas, D. Spiegelman, S. E. Hankinson, W. C. Willett, K. Ireland, M. S. Wolff, and D. J. Hunter, *Environ. Health Perspect.*, 1999, **107,** 75-81.

2 D. C. G. Muir, R. Wagemann, B. T. Hargrave, D. J. Thomas, D. B. Peakall, and R. J. Norstrom, *Sci. Total Environ.*, 1992, **122,** 75-134.

3 J. F. Brown, R. W. Lawton, M. R. Ross, J. Feingold, R. E. Wagner, and S. B. Hamilton, *Chemosphere*, 1989, **19,** 829-834.

4 D. P. Morgan and C. C. Roan, *Arch. Environ. Health,* 1971, **22,** 301-&.

5 AMAP. AMAP Assessment Report: Arctic Pollution Issues. Oslo: Arctic Monitoring and Assessment Programme (AMAP), 1998. 1998.

6 J. Jensen, K. Adare, and R. Shearer. Canadian Arctic Contaminants Assessment report. 460. 1997. Ottawa, Indian and Northern Affairs Canada.

7 Hansen J.R., R. Hansson, and S. Norris. The State of the European Arctic Environment. 136. 1996. Oslo. EEA Environmental Monograph 3.

8 I. C. Burkow and R. Kallenborn, *Toxicol. Lett,* 2000, **112-113,** 87-92.

9 T. Harner, H. Kylin, T. F. Bidleman, and W. M. J. Strachan, *Environ. Sci. & Technol.*, 1999, **33,** 1157-1164.

10 L. A. Barrie, D. Gregor, B. Hargrave, R. Lake, D. Muir, R. Shearer, B. Tracey, and T. Bidleman, *Sci. Total Environ*, 1992, **122,** 1-74.

11 R. J. Norstrom and D. C. G. Muir, *Sci. Total Environ*, 1994, **154,** 107-128.

12 R. W. Macdonald, L. A. Barrie, T. F. Bidleman, M. L. Diamond, D. J. Gregor, R. G. Semkin, W. M. J. Strachan, Y. F. Li, F. Wania, M. Alaee, L. B. Alexeeva, S. M. Backus, R. Bailey, J. M. Bewers, C. Gobeil, C. J. Halsall, T. Harner, J. T. Hoff, L. M. M. Jantunen, W. L. Lockhart, D. Mackay, D. C. G. Muir, J. Pudykiewicz, K. J. Reimer, J. N. Smith, G. A. Stern, W. H. Schroeder, R. Wagemann, and M. B. Yunker, *Sci. Total Environ*, 2000, **254,** 93-234.

13 B. Deutch and J. C. Hansen, *Dan. Med. Bull*, 2000, **47,** 132-137.

14 P. Bjerregaard and J. C. Hansen, *Sci. Total Environ.*, 2000, **245,** 195-202.

15 J. Van Oostdam, A. Gilman, E. Dewailly, P. Usher, B. Wheatley, H. Kuhnlein, S. Neve, J. Walker, B. Tracy, M. Feeley, V. Jerome, and B. Kwavnick, *Sci. Total Environ.*, 1999, **230,** 1-82.

16 V. Klopov, J. O. Odland, and I. C. Burkow, *Int. J. Circumpolar. Health* , 1998, **57,** 239-248.

17 Arctic Pollution 2002. Oslo: Arctic Monitoring and Assessment Programme (AMAP), 2002. Oslo, Norway, AMAP.

18 A. H. Rimestad, Å. Borgejordet, K. N. Vesterhus, K. Sygnestveit, E. B. Løken, K. Trygg, M. L. Pollestad, K. Lund-Larsen, G. Omholt-Jensen, and A. Nordbotten, *Den store matvaretabellen,* Gyldendal, Oslo (2001).

19 FAO, WHO, and UNU. Energy and protein requirements. FAO, WHO, and UNU. 1985. Geneva, World Health Organisation. Technical Report Series 724.

20 I. Romieu, M. HernandezAvila, E. LazcanoPonce, J. P. Weber, and E. Dewailly, *Am. J. Epidemiol.,* 2000, **152,** 363-370.

21 T. M. Sandanger, M. Brustad, E. Lund, and I. C. Burkow, *J. Environ. Monit.*, 2003, **5** , 160-165.

22 S. Moorjani, A. Dupont, F. Labrie, P. J. Lupien, D. Brun, C. Gagne, M. Giguere, and A. Belanger, *Metabolism-Clinical and Experimental,* 1987, **36,** 244-250.

23 B. Deutch and J. C. Hansen, *Int. J. Circumpolar. Health,* 1999, **58,** 214-219.

24 SAS Institute Inc., *SAS/STAT User's guide, version 6,* SAS Institute Inc., Cary, NC (1989).

25 P. Ayotte, E. Dewailly, J. J. Ryan, S. Bruneau, and G. Lebel, *Chemosphere,* 1997, **34,** 1459-1468.

26 D. E. Schulz, G. Petrick, and J. C. Duinker, *Environ. Sci. Technol.,* 1989, **23,** 852-859.

27 D. M. Guvenius, P. Hassanzadeh, A. Bergman, and K. Noren, *Environ. Toxicol. and Chem.,* 2002, **21,** 2264-2269.

28 S. L. ArchibequeEngle, J. D. Tessari, D. T. Winn, T. J. Keefe, T. M. Nett, and T. Z. Zheng, *J. Toxicol. Environ. Health* , 1997, **52,** 285-293.

29 S. S. Atuma, C. E. Linder, O. Andersson, A. Bergh, L. Hansson, and A. WicklundGlynn, *Chemosphere,* 1996, **33,** 1459-1464.

30 T. M. Sandanger, J. O. Odland, A. Tkachev, and I. C. Burkow, *Sci. Total Environ.,* 2003, **306,** 171-178.

31 E. Dewailly, P. Ayotte, S. Bruneau, C. Laliberte, D. C. G. Muir, and R. J. Norstrom, *Environ. Health Perspect,* 1993, **101,** 618-620.

32 Y. F. Li, R. W. Macdonald, L. M. M. Jantunen, T. Harner, T. F. Bidleman, and W. M. J. Strachan, *Sci. Total Environ.,* 2002, **291,** 229-246.

33 E. Dewailly, A. Nantel, S. Bruneau, C. Laliberte, L. Ferron, and S. Gingras, *Chemosphere,* 1992, **25,** 1245-1249.

34 M. P. Simmonds, P. A. Johnston, M. C. French, R. Reeve, and J. D. Hutchinson, *Sci. Total Environ.,* 1994, **149,** 97-111.

35 E. Grimvall, L. Rylander, P. Nilsson-Ehle, U. Nilsson, U. Stromberg, L. Hagmar, and C. Ostman, *Arch. Environ. Contam Toxicol.,* 1997, **32,** 329-336.

36 A. S. Furberg, T. Sandanger, I. Thune, I. C. Burkow, and E. Lun, *J. Environ. Monit.,* 2002, **4,** 175-181.

37 U. G. Ahlborg, L. Lipworth, L. Titusernstoff, C. C. Hsieh, A. Hanberg, J. Baron, D. Trichopoulos, and H. O. Adami, *Crit. Rev. Toxicol,* 1995, **25,** 463-531.

38 M. Schlummer, G. A. Moser, and M. S. McLachlan, *Toxicol. App. Pharmacol.,* 1998, **152,** 128-137.

39 E. Dewailly, G. Mulvad, H. S. Pedersen, P. Ayotte, A. Demers, J. P. Weber, and J. C. Hansen, *Environ. Health Perspect.,* 1999, **107,** 823-828.

40 R. J. Golden, K. L. Noller, L. Titus-Ernstoff, R. H. Kaufman, R. Mittendorf, R. Stillman, and E. A. Reese, *Crit. Rev. Toxicol.,* 1998, **28,** 109-227.

41 A. Brouwer, U. G. Ahlborg, F. X. R. van Leeuwen, and M. M. Feeley, *Chemosphere,* 1998, **37,** 1627-1643.

42 S. Patandin, C. Koopman-Esseboom, M. A. J. De Ridder, N. Weisglas-Kuperus, and P. J. J. Sauer, *Ped. Res.,* 1998, **44,** 538-545.

43 M. S. McLachlan, *Toxicol. App. Pharmacol.,* 1993, **123,** 68-72.

44 P. Dahl, G. Lindstrom, K. Wiberg, and C. Rappe, *Chemosphere,* 1995, **30,** 2297-2306.

45 P. Ayotte, G. Carrier, and E. Dewailly, *Chemosphere,* 1996, **32,** 531-542 .

46 W. J. Rogan, P. J. Blanton, C. J. Portier, and E. Stallard, *Regul. Toxicol. Pharmacol.,* 1991, **13,** 228-240.

Table 1: Self-reported daily intake of the 18 most common food articles in Uelen (n=50).

Food article (g/day)	Mean daily intake	Median daily intake	Range
Seal meat	159	79	7 – 1000
Walrus meat	165	105	12 – 800
Whale meat	32	14	0 – 173
Seal blubber	34	18	0 – 200
Walrus blubber	46	26	0 – 370
Whale blubber	5	12	0 – 132
Wheat bread	251	200	3 – 600
Cereal	108	100	8 – 500
Macaroni	113	66	26 – 500
Sugar	50	40	0 – 400
Saithe	57	26	0 – 345
Arctic char	31	21	0 – 164
Duck	26	19	0 – 132
Hunchback salmon	23	8	0 – 263
Conserved beef	17	3	0 – 200
Chum salmon	15	2	0 – 263
Hare	19	2	0 – 575
Polar bear meat	5	2	0 – 77

Table 2: Limit of detection (LOD) for all compounds studied (µg/L plasma)

Compound	LOD µg/L	Compound	LOD µg/L	Compound	LOD µg/L
PCB 28	0.007	PCB 156	0.004	α-HCH	0.005
PCB 52	0.004	PCB 169	0.005	β-HCH	0.012
PCB 99	0.009	PCB 170	0.005	γ-HCH	0.008
PCB 101	0.009	PCB 180	0.004	Heptachlor	0.004
PCB 105	0.007	PCB 183	0.005	Oxy-Chlordane	0.100
PCB 118	0.007	PCB 187	0.007	Mirex	0.001
PCB 126	0.008	*o,p'*-DDE	0.005	*t*-Chlordane	0.004
PCB 128	0.007	*p,p'*-DDE	0.045	*c*-Chlordane	0.005
PCB 138/163	0.009	*o,p'*-DDT/*p,p'*-DDD	0.007	*c*-Nonachlor	0.003
PCB 149	0.009	*p,p'*-DDT	0.010	*t*-Nonachlor	0.004
PCB 153	0.008	HCB	0.006	Tox 26	0.058
				Tox 50	0.050

Table 3: Wet weight levels of POPs in human plasma from people in Uelen (n=50)

Compound	Average (µg/L plasma)	stdev	Median (µg/L plasma)	Geomean (µg/L plasma)	Range (µg/L plasma)	% below LOD
Lipids	5.3 g/L	1.3	5.1 g/L	5.2 g/L	3.1 - 7.9 g/L	
HCB	1.15	0.80	1.08	0.86	0.08 - 3.42	0
α-HCH	0.02	0.01	0.02	0.02	0.01 - 0.05	0
ß-HCH	2.87	1.98	2.65	2.10	0.216 - 8.18	0
γ-HCH	0.005	0.006	0.004	0.004	0.004 - .048	98
Heptachlor	0.002	0.000	0.002	0.002	0.002 - 0.002	100
Mirex	0.22	0.24	0.18	0.14	0.02 - 1.44	0
Oxy Chlordane	1.83	1.85	1.68	1.05	0.05 - 9.71	4
t-Chlordane	0.004	0.004	0.002	0.003	0.002 - 0.017	69
c-Nonachlor	0.19	0.15	0.165	0.13	0.013 - 0.57	0
t-Nonachlor	2.01	1.63	1.68	1.33	0.11 - 6.97	0
Sum CDs	**4.03**	**3.46**	**3.65**	**2.65**	**0.24 - 17.27**	
o,p'-DDE	0.006	0.005	0.005	0.005	0.002 - 0.025	95
p,p'-DDE	3.30	1.97	2.91	2.69	0.46 - 8.34	0
o,p'-DDT/*p,p'*-DDD	0.03	0.04	0.02	0.01	0.003 - 0.19	34
p,p'-DDT	0.23	0.17	0.19	0.17	0.01 - 0.73	3
Sum DDTs	**3.56**	**2.12**	**3.15**	**2.91**	**0.54 - 9.28**	
PCB 28	0.05	0.03	0.04	0.04	0.003 - 0.12	2
PCB 52	0.026	0.022	0.018	0.019	0.002 - 0.108	19
PCB 99	0.57	0.37	0.54	0.44	0.05 - 1.36	0
PCB 101	0.07	0.10	0.05	0.05	0.004 - 0.57	2
PCB 105	0.13	0.08	0.12	0.10	0.02 -0.29	0
PCB 118	0.76	0.50	0.73	0.57	0.07 - 1.84	0
PCB 126	0.006	0.017	0.004	0.004	0.004 - 0.125	98
PCB 128	0.025	0.11	0.004	0.005	0.004 - 0.73	76
PCB 138/PCB 163	1.51	1.14	1.23	1.09	0.14 - 5.28	0
PCB 149	0.01	0.01	0.01	0.01	0.004 - 0.04	45
PCB 153	4.03	3.30	3.30	2.78	0.31 - 15.75	0
PCB 156	0.13	0.12	0.10	0.08	0.01 - 0.59	0
PCB 169	0.002	0.001	0.002	0.002	0.002 - 0.012	98
PCB 170	0.56	0.56	0.41	0.35	0.03 - 2.42	0
PCB 180	1.20	1.14	0.95	0.76	0.07 - 5.14	0
PCB 183	0.10	0.07	0.09	0.08	0.012 - 0.26	0
PCB 187	0.36	0.27	0.28	0.26	0.034 - 1.24	0
Sum PCBs	**9.54**	**7.39**	**8.00**	**6.79**	**0.853 - 33.90**	
Tox 26	0.26	0.21	0.21	0.17	0.03 - 0.83	18
Tox 50	0.22	0.19	0.17	0.15	0.03 - 0.82	14
Sum tox	**0.48**	**0.39**	**0.39**	**0.32**	**0.05 - 1.58**	

Table 4: Lipid-weight levels of POPs in human plasma from Uelen (n=50)

Compound	Average (ng/g lipids)	stdev	Median (ng/g lipids)	Geomean (ng/g lipids)	Range (ng/g lipids)
HCB	211.4	130.4	204.0	167.7	19.7 - 531.4
α-HCH	4.0	2.1	3.5	3.5	1.0 - 10.6
ß-HCH	524.3	319.5	519.7	409.6	51.1 - 1281.5
γ-HCH	1.0	1.2	0.8	0.8	0.5 - 9.2
Heptachlor	0.4	0.1	0.4	0.4	0.3 - 0.7
Mirex	41.1	41.0	28.6	27.0	3.1 - 228.7
Oxy Chlordane	339.2	327.3	281.2	204.5	7.0 - 1537.9
t-Chlordane	0.7	0.6	0.5	0.6	0.3 - 2.7
c-Nonachlor	34.2	23.9	32.8	25.1	2.6 - 106.1
t-Nonachlor	362.2	264.2	315.8	260.6	25.9 - 1123.0
Sum CDs	**736.4**	**592.1**	**683.0**	**518.1**	**56.2 - 2734.6**
o,p'-DDE	1.1	0.8	1.0	0.9	0.3 - 3.5
p,p'-DDE	608.9	322.9	563.2	520.4	90.7 - 1633.3
o,p'-DDT/*p,p'*-DDD	4.7	5.6	2.9	2.6	0.4 - 24.4
p,p'-DDT	42.7	30.6	36.2	33.7	1.0 - 167.2
Sum DDTs	**657.5**	**344.7**	**607.7**	**563.3**	**106.8 - 1726.0**
PCB 28	8.6	4.4	8.1	7.3	0.4 - 19.7
PCB 52	4.9	4.2	3.5	3.6	0.3 - 24.5
PCB 99	105.3	61.4	100.4	85.4	10.8 - 311.9
PCB 101	14.0	20.4	10.4	9.5	0.8 - 123.3
PCB 105	24.2	13.1	23.8	20.1	3.5 - 52.4
PCB 118	139.9	85.0	142.5	109.6	16.5 - 336.8
PCB 126	1.2	2.8	0.8	0.8	0.5 - 20.3
PCB 128	4.8	19.7	0.8	1.1	0.5 - 122.2
PCB 138/PCB 163	275.0	187.9	253.9	210.5	27.6 - 836.6
PCB 149	2.0	1.2	1.7	1.7	0.5 - 6.1
PCB 153	744.0	579.0	645.1	537.8	65.9 - 2645.3
PCB 156	24.0	21.1	20.1	16.4	1.9 - 93.6
PCB 169	0.5	0.3	0.5	0.5	0.3 - 2.3
PCB 170	103.3	98.3	68.4	66.9	6.6 - 431.2
PCB 180	220.1	196.8	164.0	147.2	16.9 - 813.6
PCB 183	18.4	10.5	18.8	15.0	2.7 - 43.4
PCB 187	64.9	43.4	60.2	49.5	7.5 - 196.4
Sum PCBs	**1755.3**	**1262.7**	**1606.2**	**1316.4**	**175.2 - 5614.1**
Tox 26	45.9	32.7	41.4	32.7	4.1 - 144.2
Tox 50	40.0	29.7	33.3	28.9	4.8 - 134.8
Sum tox	**85.9**	**61.6**	**75.5**	**62.7**	**10.3 - 279.0**

Table 5: Additional PCB congeners and PCB 138 and PCB 163, based on repeat analyses of 20 of the plasma samples (µg/L).

Compound	Average (µg/L)	stdev	Median (µg/L)	Geomean (µg/L)	Range (µg/L)
PCB 138	0.820	0.657	0.825	0.562	0.080 – 2.206
PCB 146	0.217	0.199	0.171	0.137	0.017 – 0.660
PCB 157	0.056	0.055	0.042	0.031	0.002 – 0.170
PCB 163	0.376	0.372	0.308	0.220	0.020 – 1.216
PCB 167	0.034	0.030	0.028	0.022	0.004 – 0.117
Sum additional congeners*	0.683	0.650	0.578	0.414	0.043 – 2.136
% of total sum PCBs	7.2	1.4	7.3	7.1	5.1 - 11.1
% of 163 relative to 138	40.9	13.2	37.9	39.1	24.4 - 76.4

* Sum of PCB 146, 157, 163 and 167

Table 6: Lipid weight levels of POPs in two blubber intake groups*, adjusted for age and gender. The p-values for the significant predictors used in the ANOVA are listed. Logarithmic values were used in the model.

Compound	Low blubber intake (n=25)		High blubber intake (n=25)		p-value		
	GM (ng/g lip)	Range (ng/g lip)	GM (ng/g lip)	Range (ng/g lip)	Age group**	Gender	Blubber intake*
Sum PCBs	1127.4	175.2 – 3378.6	1791.0	363.5 – 5614.1	0.04	<0.001	0.01
Sum DDTs	507.7	106.8 – 1379.0	686.3	223.8 – 1726.0	0.02	<0.001	0.05
Sum CDs	463.7	56.2 – 1595.4	742.9	112.0 – 2734.6	0.01	<0.001	0.02
Sum tox	59.9	10.3 – 175.2	78.5	13.3 – 279.0	0.04	0.002	>0.05
β-HCH	390.2	51.1 – 1281.5	533.0	91.6 – 1160.7	0.01	0.006	>0.05
Lipids*** (g/L)	5.07	3.36 – 7.84	5.52	3.06 – 7.85	<0.001	>0.05	>0.05

* Blubber intake in two equal size groups, low and high intake.

** Two age groups, 0 – 40 years and 41+ years.

*** The level of lipids is only adjusted for age.

GM-Geometric mean

Table 7: The lipid weight levels (geometric mean, GM) of POPs for men and women in the two age groups adjusted for blubber intake and the results from the independent sample t-test for the gender differences in the two age groups.

Compound	40 years and below			Above 40 years		
	Men GM n=15 (ng/g lipids)	Women GM n=15 (ng/g lipids)	t-test (p-value)	Men GM n=9 (ng/g lipids)	Women GM n=11 (ng/g lipids)	t-test (p-value)
Sum PCBs*	2046.8	679.1	<0.001	2272.2	1280.3	>0.05
Sum DDTs*	699.4	357.6	0.002	792.5	651.6	>0.05
Sum CDs*	822.1	245.9	<0.001	1204.2	480.7	0.001
Sumtox*	99.1	30.5	<0.01	88.8	90.4	>0.05
β-HCH*	510.6	241.0	0.03	662.9	505.0	>0.05
Lipids (g/L)	4.69	4.51	>0.05	5.94	6.02	>0.05

* The logarithmic values where used for the t-test.

PAPER IV

New methodology for combined analysis of neutral and phenolic organohalogens in human plasma

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Abstract

A trace analytical method is presented for the combined analyses of neutral and phenolic organohalogens in human plasma samples. The method was based on a method already validated for the neutral organohalogens, specifically the PCBs and organochlorine pesticides. In short it includes liquid-liquid extraction with ethanol, water saturated with ammonium sulphate and hexane. Further acidification and hexane:dichloromethane (3+1) was needed for efficient extraction of hydroxylated PCBs (OH-PCBs) and pentachlorophenol (PCP). Use of florisil columns for the clean up allowed complete separation of the neutral and phenolic organohalogens, with good recovery rates (83 – 116 %) for the phenolic organohalogens. The hydroxylated compounds were methylated using diazomethane in ether. Another florisil column was used for the final clean-up of the methoxylated compounds (median recovery rates; 67 – 90 %). The clean up was also validated for tetrabromobisphenol-A (TBBPA). The derivatised analytes were quantified using GC-MS (ECNI) with methoxylated compounds as external standards.

Validating the method using spiked plasma samples gave acceptable median recovery rates for OH-PCBs and PCP (38 – 75 %), whereas plasma sample analyses gave good recovery rates for ^{13}C-labeled internal standards (64 and 72 %). The limit of detection was in the range of 2-20 pg/g plasma for the OH-PCBs, and 2 pg/g plasma for PCP. No matrix interference was observed in the chromatograms.

In high PCB exposed plasma samples from the native population of Uelen, Russia (n=15), the median ratio of sum OH-PCBs to sum PCBs was as high as 0.4 and the sum values were significantly correlated ($r=0.7$, $p<0.01$). The median sum OH-PCBs (10 congeners) was 5916 pg/g plasma with 4-OH-CB107 as the dominating congener (median: 1673 pg/g plasma). The PCP levels were moderate (median: 642 pg/g plasma). The high OH-PCB concentrations clearly indicate the need for further research on the levels and the behaviour of these compounds in humans.

Introduction

Polychlorinated biphenyls (PCBs) are one of the most intensely studied compound groups since they were discovered as environmental pollutants over 30 years ago (Jensen, 1972). They have been found worldwide in high levels in biological and non-biological samples. After the banning of production and use of PCBs in most countries in the late 1970ies, the levels appear to be declining in the environment (AMAP, 02). The knowledge of PCBs behaviour in the environment is considerable, but the levels and effects of the metabolites of PCBs and other phenolic organohalogens have been little studied.

PCBs are biotransformed by the cytochrome P-450 monooxygenases and in most of the known metabolic pathways the formation of the OH-PCBs is the initial step. Even the more persistent PCB 153 is metabolised both *in vitro* and *in vivo* to form a number of hydroxylated metabolites (Ariyoshi et al., 1992; Schnellmann et al., 1983; Sipes et al., 1982). The OH-PCBs can also be formed by direct hydroxylation of the parent PCBs, and they have been found to be the main metabolites excreted in faeces and/or urine (Guvenius et al., 2002; Letcher et al., 2000).

In human plasma a number of OH-PCBs have been identified and some have been found in relatively high levels (Bergman et al., 1994; Sandau et al., 2000). In a study by Klasson-Wehler et al. (1997) the OH-PCB levels in human plasma were comparable to the levels of the PCBs, and as many as 38 different OH-PCB congeners were reported in another study by Hovander et al. (2002). Guvenius et al. (2002) found higher levels of OH-PCBs in human liver compared to adipose tissue. Plasma is, however, the preferred tissue for specific localisation (Letcher et al., 2000).

In experimental studies with OH-PCBs, both estrogenic and antiestrogenic activities have been demonstrated (Brouwer et al., 1998; Fielden et al., 1997). They have also been shown to be more estrogenic than the PCBs (Andersson et al., 1999). OH-PCBs have further been found to have the same affinity for transthyretin as thyroid hormone (thyroxine; T_4) (Cheek et al., 1999). In another study the OH-PCBs competitively displaced T_4 from transthyretin with differential potency (Lans et al., 1993).

Another phenolic compound that have caused concern is PCP, a fungicide mainly used for wood preservation. It is still produced worldwide, however, it is banned in Canada and Scandinavia, and registered only for restricted use in USA and western Europe (AMAP, 1998). Because of its volatility PCP evaporates or leaches to a large extent from wood structures (AMAP, 2002). PCP is also formed during metabolism of hexachlorobenzene (van Raaij et al., 1991). PCP is, like OH-PCBs, retained in blood and binds to transthyretin (van den Berg et al., 1991), and it has been found in higher levels in human plasma than the OH-PCBs (Hovander et al., 2002; Sandau et al., 2000; Sandau et al., 2002). PCP has been shown to be genotoxic (Tisch et al., 2002), and the possible altered thyroid hormone status in newborns, caused by PCP and OH-PCBs, could lead to neurodevelopmental effects in infants (Sandau et al., 2002).

One of the major commercial brominated flame-retardants is the covalently bound tetrabromobisphenol-A (TBBPA). This compound, in addition to several other brominated flame-retardants, has been detected in human plasma (Thomsen et al., 2002). The levels are orders of magnitude lower than the PCBs and OH-metabolites, but they seem to be increasing in environmental samples, indicating the need for close monitoring (Thomsen et al., 2002).

The need to quantify the phenolic organohalogens in addition to the neutral organohalogens is evident. Thus, the aim of this paper was to develop a method for determination of both neutral and phenolic organohalogens in small plasma volumes. The methodology was based on a method already validated for PCBs and organochlorine pesticides (Romieu et al., 2000; Sandanger et al., 2003). Plasma samples from two populations were analysed for verification of the method, and to obtain the first indications of OH-PCBs and PCP status among high exposed native people from the Russian arctic.

Methods

For the analytical procedure a scheme is shown in Figure 1, where numbers in parentheses indicate individual steps. These numbers are referred to in the text.

Materials and reagents

The following solvents were used: acetone (pesticide grade), *n*-hexane (hex; optima), methanol (MeOH; ACS) and sulphuric acid, all from Fisher (Pittsburgh, USA), dichloromethane (DCM; omnisolv) from EM Science (Gibbstown, NJ, USA) and anhydrous ethanol from Pierce (Rockford, USA). In addition anhydrous sodium sulphate was obtained from Baker (Phillipsburg, USA) and florisil (60-100 mesh) and ammonium sulphate (ACS) were purchased from Fisher (Pittsburgh, USA).

$^{13}C_{12}$-labeled 4-hydroxy-2,2',3,4',5,5',6-heptachlorobiphenyl ($^{13}C_{12}$-4-OH-CB 187, chemical >98 %, isotopic 99 %, 50 mg/L in toluene) was obtained from Wellington Laboratories Inc. (Guelph, Ontario, Canada), pentachlorophenol (PCP, 98 %) from Sigma-Aldrich AS (Oslo, Norway) and $^{13}C_6$-PCP (isotopic 99 %, 103 mg/L in nonane) and $^{13}C_{12}$-PCB 141 (isotopic 99 %, 40 mg/L in nonane) from Cambridge Isotope Laboratories (Andover, MA, USA). Tetrabromobisphenol A (TBBPA, technical) was provided by LGC Promochem AB (Borås, Sweden).

Quantitative solutions of the following methoxylated PCBs (5 μg/mL in isooctane) were obtained from the working group of Prof. Åke Bergman (Department of Environmental Chemistry, Stockholm University, Stockholm, Sweden): 4-MeO-CB 107, 4'-MeO-CB 130, 3'-MeO-CB 138, 4-MeO-CB 146, 3-MeO-CB 153, 4-MeO-CB 162, 4'-MeO-CB 172, 3'-MeO-CB 180, 4-MeO-CB 187 and 4-MeO-CB 193. The methoxylated PCBs were cleaved to their corresponding OH-PCBs according to Press (1979). The step was not quantitative and the OH-standards were used for method development only. The MeO-PCBs and OH-PCBs were numbered according to Letcher et al. (2000).

Diazomethane and diazoethane were prepared in ether with respectively solid precursor N-methyl-N-nitroso-p-toluenesulfonamide and 1-ethyl-3-nitro-1-nitrosoguanidine (both from Sigma Aldrich, Canada) in biphasic mixture of sodium

hydroxide and diethyl ether (McKay et al., 1950). The diazo-solutions were then stored in small volume containers at –80 °C until use.

Plasma samples

The plasma samples used for verification of the method were from two native populations; the Chukchi population of Uelen (n=15), situated on the Chukotka Peninsula by the Bering Strait, Russia and the Cree Indians (n=14) living in northern Quebec, Canada. (The results from the Cree Indians could not be presented in full details in this paper, since they belonged to a different project.)

These populations have different dietary patterns, with the people from Uelen depending heavily on marine mammals and the Cree Indians depending heavily on inland fish. The Cree samples were randomly selected for analysis of phenolic compounds, whereas a subset of high PCB exposed samples were selected from the Chukchi samples. The lipid content in the Uelen samples was determined enzymatically according to a method published by Akins et al., (1989).

Based on the different population characteristics and selection of samples, differences in levels and congener patterns of the phenolic compounds were expected. This allowed a better verification of the method compared to using only one sample set, and thus the Cree samples were included.

Extraction

The method of extraction (step 1 in Figure 1) was based on the liquid-liquid extraction previously presented (Sandanger et al., 2003). Specifically, 2 ml of plasma, 2 ml of ethanol and 2 ml of deionised water saturated with ammonium sulphate was extracted twice with 6 ml of *n*-hexane in a small glass tube. $^{13}C_{12}$-labeled 4-OH-2,2',3,4',5,5',6-heptachlorobiphenyl ($^{13}C_{12}$-4-OH-CB187) and $^{13}C_6$ -labeled pentachlorophenol ($^{13}C_6$ -PCP) were used as internal standards for correction of the recovery of the phenolic compounds. 20 µl of a 250 pg/µl solution was added to each sample before the first extraction. A third extraction was done using hexane:DCM (3+1) after acidifying the water/plasma phase to a pH of 1-2 using 100 µl 9 M H_2SO_4. The organic fractions were treated separately, for some samples, to obtain information on distribution of the different phenolic compounds in the different extracts. Recovery

tests were performed with spiked water, spiked plasma and unspiked plasma samples (only ^{13}C-labelled compounds). The organic fractions were evaporated to 0.5 ml on a Speed Vac ® plus No. SC210 A (Savant Instruments Inc., NY, USA). ^{13}C-marked PCB 141 was used as recovery standard (20 µl of a 100 pg/µl solution) and as volume standard for external quantification.

Clean-up and separation on florisil columns

The PCBs and pesticides were separated from the lipids and the phenolic organohalogens using a tandem florisil column manually packed with 1.5 g of 0.5 % deactivated florisil and 2 g of granulated sodium sulphate on top, in each of the two columns (step 2 in Figure 1). The tandem column system was pre-washed using 10 ml of n-hexane: DCM (3+1). The PCBs and pesticides were eluted through both columns using 11 ml of n-hexane: DCM (3+1). After discarding the bottom column, PCP, OH-PCBs and TBBPA were eluted from the top column using 10 mL of 10 % MeOH in DCM followed by 6 mL of 20 % MeOH in DCM.

For recovery tests on the florisil columns, standards were applied directly on the column and the collected fraction was evaporated, derivatised and injected on the GC.

Derivatisation

Diazomethane in ether (250 µL for 1 h at 20 °C) was used for methylation of the phenolic compounds (step 3 in Figure 1). Excess ether and diazomethane was evaporated using a gentle flow of nitrogen (Hovander et al., 2000). Due to the reported problems of ethylation instead of methylation when using diazomethane in ether (Sandau, 2000), one standard solution was ethylated using diazoethane in ether. Injection of the ethylated standard on the GC-MS enabled the determination and monitoring of the ethylated masses during method development. It must be kept in mind that the OH-PCB solutions were only qualitative since they were obtained from demethylation of the methoxylated compounds, thus no exact percentage of ethylation could be determined.

Final clean-up

After derivatisation of phenolic fraction, further clean-up was needed for the analysis of plasma samples, and a single florisil column identical to the ones used in step 2 was used for this purpose (step 4 in Figure 1). Me-PCP and MeO-PCBs were eluted using 15 mL of hexane:DCM (3+1). Me_2-TBBPA was finally eluted with additional 6 mL of 15 % acetone in hexane.

GC-MS analyses

The MeO-PCBs, Me-PCP and Me_2-TBBPA were analysed on a Hewlett Packard 5890 Series II Plus gas chromatograph (GC) equipped with an HP G1512A automatic injector and a Hewlett Packard 5890B (Engine) mass spectrometer (step 5 in Figure 1). The mass spectrometer was used in electron capture negative ionisation (ECNI) mode with methane (99.97 %) as the reagent gas. It was used in selected ion monitoring (SIM) mode and quantification was performed using the masses listed in Table 1 as target and confirmation ions. Retention times for each compound are also given in Table 1. The vacuum in the source was maintained at 1.8 torr and the source temperature was kept at 150 °C. In scan mode, m/z 65 to 550 were scanned at a rate of 0.8 scan/s.

The GC was fitted with a 60 m DB-5 column (5 % phenyl-methylpolysiloxane), 0.25 mm i.d., 0.25 μm film thickness from J&W Scientific (CA, USA). The carrier gas was helium, and all injections were of 2 μL in splitless mode. The injector and transfer line were kept at 275 °C and 280 °C, respectively. The temperature program was as follows: Initial temperature 100 °C (7 min), 20 °C/min to 200 °C (0 min), 1.5°C/min to 245 °C (5 min) and finally at 10 °C/min to 280 (12 minutes), for a total run time of 57.5 minutes.

The methoxylated compounds were used as external standards, and calibration curves were made for the individual compounds and congeners using solutions of 1, 10 and 100 pg/μL. Response factors were calculated relative to the $^{13}C_{12}$-PCB 141. All levels were finally corrected according to the recovery rates of $^{13}C_{12}$-4-OH-CB 187 (for OH-PCBs) and $^{13}C_6$-PCP (for PCP). TBBPA was not analysed in plasma samples.

Results

Extraction

The first results from the extraction tests using spiked water indicated that ethanol, water saturated with ammonium sulphate and hexane was sufficient for the extraction of the phenolic compounds. However, for plasma samples (with ^{13}C-standards) a significant proportion of the OH-PCBs and PCP was only extracted after further acidification and the use of hexane:DCM (3+1) (Figure 2). In Figure 2, the proportions of phenolic compounds extracted using hexane, is compared to the proportions extracted with hexane:DCM (3+1) after acidification of the samples (n=7). 4'-OH-CB130 and 4-OH-CB193 were not included in the figure because the levels were close to LOD in one of the fractions. The error bars show the maximum and minimum values in each fraction. The percentages were calculated on the basis of the total amount extracted (defined as 100 %).

Clean up and separation of phenolic and neutral compounds using florisil columns

The PCBs and organochlorine pesticides are known to elute with good recovery rates in the first fraction of 11 ml hex/DCM (3+1) through a tandem florisil column (Sandanger et al., 2003). After discarding the bottom column, the top column could be washed using 6 ml of 15 % acetone in hexane without loss of the phenolic compounds.

Using 10 ml of 10 % methanol (MeOH) in DCM and 6 ml of 20 % MeOH in DCM all phenolic compounds were eluted with good recovery rates from the top column (83 % to 116 %; Table 2).

Derivatisation

The degree of methylation was not calculated, but it did seem to be satisfactory, even though a certain percentage of the lower chlorinated products was ethylated (estimated to < 10 %). The degree of ethylation seemed to increase with time the

bottle of diazomethane had been out of the freezer (-80 °C), despite the fact that it was being kept capped and in the fridge.

A small percentage (< 10 %) of TBBPA was only mono-methylated. The degree of mono-methylation seemed to be independent of the freshness of the diazomethane.

Final clean-up

Recovery rates for the methoxylated compounds on the final clean-up column are shown in Table 2. They were good for all studied compounds with median values in the range of 72 to 90 %.

GC-MS analyses

All methoxylated standard compounds were completely separated by the employed GC system. The response for all the analysed methoxylated compounds was linear in the range of 1 to 100 pg/μL ($r > 0.99$, $p < 0.001$). A TIC chromatogram recorded in SIM mode of a plasma sample from Uelen is displayed in Figure 3. Low detection limits (LOD), calculated according to three times the signal to noise ratio, were obtained for plasma samples. For the OH-PCBs they were in the range of 2 – 5 pg/g plasma, except for 4'-OH-CB 130 that had a LOD of 20 pg/g plasma. For PCP the LOD was 2 pg/g plasma, whereas for Me_2-TBBPA the LOD was not determined in plasma samples. Instrumental detection limit was 2 pg injected for Me_2-TBBPA.

Validation of the total method

A scheme of the total procedure, indicating the individual steps, is displayed in Figure 1. However, the recovery rates for the individual compounds were variable using spiked plasma samples, with median values in the range of 38 to 75 % for all compounds (Table 2). The recovery rates of the native compounds and the ^{13}C-labeled compounds were comparable. For the unspiked plasma samples the recovery rates were good for the $^{13}C_6$-PCP and $^{13}C_{12}$-4-OH-CB187, with median values of 64 and 72 %, respectively (Table 3). The chromatograms of plasma samples contained little interference and noise.

Low levels of OH-PCBs and PCP were found in the 14 samples from the Cree Indians. The dominant compound was PCP with a median level of 598 pg/g plasma

(range; 161-1590). The median levels of OH-PCBs were in the range of <LOD to 163 pg/g plasma, with 4-OH-PCB187 as the most dominant congener. The median ratio of sum OH-PCBs to sum PCBs was 0.08. The median recovery rates for $^{13}C_6$-labeled PCP and $^{13}C_{12}$-labeled 4-OH-CB 187 were 61 % (30 – 105 %) and 89 % (52 – 113 %), respectively.

Levels of OH-PCBs and PCP in high contaminated plasma samples from the native Chukchi population

The sum PCBs (17 congeners) in the 15 Uelen samples were in the range of 2010 to 5614 ng/g lipid. The levels of hydroxy PCBs were also high, with a median sum OH-PCB (10 congeners) concentration of 5916 pg/g plasma (1098 ng/g lipids). 4-OH-CB107 was the dominant congener in most samples (Table 3). The median level of 4-OH-CB107 was 1673 pg/g plasma (275 ng/g lipid weight) followed by 3- OH-CB153, 4- OH -CB146, 3'-OH-CB138 and 4-OH-CB187. The median level of PCP was 642 pg/g plasma (117 ng/g lipid weight).

The sum OH-PCBs and the sum PCBs were significantly correlated ($r=0.7$, $p<0.01$) and the median ratio of sum OH-PCBs to sum PCBs was 0.40 (range; 0.27 – 0.74). The median recovery rates for ^{13}C-labeled PCP and 4-OH-CB187 were 64 % (43 - 83 %) and 72 % (41 - 90 %), respectively.

A SIM chromatogram of one of the Uelen samples is shown in Figure 3 and a full scan chromatogram is shown in Figure 4. As can be seen in Figure 4 many OH-PCB congeners were detected in full scan mode and it is evident that there were several unidentified penta-, hexa-, hepta-, octa- and even a nona- chlorinated OH-PCB in this sample. The mass spectrum of a pentachlorinated OH-PCB eluting just before the $^{13}C_{12}$-PCB 141, is shown in Figure 5. No attempts were made to analyse the TBBPA in the real samples due to low sample volume, 0.8 – 1.8 ml for the Uelen samples, and 2 ml for the Cree samples, and the previously reported low levels in plasma (Thomsen et al., 2002).

Discussion

The analytical procedure was based on a strategy of simultaneously determining PCBs, pesticides and the phenolic organohalogens in low volume (0.8-2 ml) plasma sample.

Extraction

The recovery rates of the extraction step alone were difficult to assess due to the fact that spiked plasma samples demanded clean-up before injection on the GC was possible. Furthermore, spiking experiments might not be a good simulation of the way phenolic compounds are naturally embedded in the matrix. The use of spiked water only, as a test of extraction efficiency was not viable due to the fact that the equilibrium between the organic phase and aqueous phase was shifted towards the organic phase. The high amount of phenolic compounds found in the second, more polar organic extract (Figure 2) clearly indicated that acidification with H_2SO_4 and the use of hex:DCM (3+1) was necessary for improved extraction. The ratio of the compounds in the two organic fractions was consistent for all 7 samples, as shown by the error bars in Figure 2. No explanation was found for the different partitioning of the individual compounds/congeners, and it seemed to be independent of the degree of chlorination. It was not tested if DCM in the solvent or the acid had more influence on the improved extraction. The fact that the ^{13}C-labeled compounds had identical distribution as the corresponding unlabeled compounds, indicated that the more polar solvent was the important factor, and not denaturation of the proteins. However, less polar solvents like hexane and methyl *tert*-butyl ether have been used, with success, for the extraction of phenolic compounds (Hovander et al., 2000).

These results clearly emphasise the importance of the conditions for an efficient extraction of phenolic compounds. Letcher et al. (2000) also described the extraction as a crucial step considering possible protein binding. The use of ethanol in the extraction might also affect the equilibrium between the organic phase and the aqueous phase, and thus the extraction efficiency. However, other working groups used 2-propanol and methanol in the extraction without reporting reduced recovery rates (Bergman et al., 1994; Guvenius et al., 2002; Hovander et al., 2000).

The extraction efficiency can be controlled using ^{14}C-labelled OH-PCBs dosed intravenously to rats as shown in a study by Hovander et al. (2000). The protein binding might, however, be different in humans, making it extremely difficult to assess the extraction step fully for human plasma samples.

Separation of neutral and phenolic organohalogens

The use of florisil columns for the separation of neutral and phenolic compounds was highly efficient. This method also avoided the use of KOH solution for the separation of the compound groups and the possible problems of poor recovery rates for the HCHs (Hovander et al., 2000). The use of KOH solution is also a more tedious procedure than using the florisil columns that actually, in the same step, cleans the fraction containing the PCBs and pesticides from matrix compounds. In addition, the phenolic organohalogens were dissolved in methanol already during elution from the florisil column. The use of methanol has been reported to reduce chances of loss caused by adsorption to glassware (Hovander et al., 2000).

Derivatisation

No attempts were made to improve the method of derivatisation in this paper, and it did seem to be satisfactory for all compounds determined. However, for TBBPA there was a problem of a small percentage being only mono-methylated. Furthermore, the relatively low recovery rates for the spiked plasma sample tests could have been caused by an incomplete methylation. The diazomethane used for these tests was not fresh from the freezer (-80 °C). For the unspiked plasma samples the fresh diazomethane was used and the recovery rates for the ^{13}C-standards were improved compared to the spiking tests. The importance of using freshly distilled diazomethane has previously been reported (Sandau, 2000).

Final clean-up

The final clean up was highly efficient for Me-PCP, and all MeO-PCBs. This was made evident by the good recovery rates (Table 2) and the chromatograms with little interference (Figure 3 and 4). The Me_2-TBBPA did, however, elute later than the other compounds in a different fraction. The advantage of this was that it was

completely separated from the other compounds, but at the same time it was more likely that interferences co-eluted in the TBBPA-fraction. This last step was not validated for TBBPA using plasma samples.

GC-MS analyses

The use of methoxylated compounds as external standards adds an additional uncertainty to the results, caused by the possible variable degree of methylation for the OH-PCBs with different numbers of chlorine atoms. Despite the fact that all levels are corrected for the recovery rates of the internal standard, the recovery rates will be different for individual congeners and in this study only one $^{13}C_{12}$-OH-PCB was used and it was hepta-chlorinated. Efforts should be made to have the underivatised OH-PCBs available as single quantitative standards.

Validation of the total method

Using the method described in this paper clean chromatograms and low detection limits were obtained for OH-PCBs and PCP. Sample extracts injected on the GC with the MS in full scan mode gave chromatograms and mass spectra that made it easy to identify the number of chlorine atoms of different OH-PCBs (Figure 4 and 5). The florisil fractionation was tested over a period of one year with the same results, indicating the robustness of the method. For the neutral compounds the method has been validated earlier (Sandanger et al., 2003).

However, with spiked plasma samples the recovery rates of phenolic compounds were variable, in particular for PCP. The florisil columns were thoroughly validated and not believed to be the cause of the problem. The use of older diazomethane might have reduced the degree of methylation and thus the recovery rates. For real sample analyses, fresh diazomethane (from the freezer) was used, and the recovery rates were improved (Table 2). Poor extraction might also have caused reduced recovery rates, but the fact that the recovery rates improved with fresh diazomethane, with no changes in extraction conditions, indicated that this was not the reason. The extraction step is in any case difficult to validate, and further research is needed.

Levels of OH-PCBs and PCP in high contaminated plasma samples from Chukchi people

The levels of the OH-PCBs identified and quantified were high in the high PCB exposed plasma samples from Chukchi people (Uelen; Table 3). As observed in other studies (Fangstrom et al., 2002; Hovander et al., 2002; Sandau et al., 2000; Sandau et al., 2002) the congener pattern was also here highly variable, with different congeners dominating in different samples. The OH-PCB concentrations were higher than the PCP in contrast to what was previously reported in Inuits from northern Canada (Sandau et al., 2000; Sandau et al., 2002). The concentrations of OH-PCBs in the Chukchi samples were comparable to the plasma levels in pregnant women from the Faeroe Islands, reporting high recent intake of pilot whale blubber (Fangstrom et al., 2002). In the Faroese study the two dominant congener was 4-OH-CB187 and 4-OH-CB146 with a median level of 1.6 and 1.1 ng/g plasma, respectively. The two dominant congeners in the Chukchi samples were 4-OH-CB107 and 3-OH-CB153 with a median level of 1.7 and 1.3 ng/g plasma, respectively. The native population of Uelen also consume considerable amounts of marine mammal blubber.

Even though the levels of the dominant OH-PCBs seemed comparable, the ratio of sum OH-PCBs to sum PCBs were different with a range of 0.27 to 0.76 in the Uelen samples, and a range of 0.05 to 0.2 in the Faroese study (Fangstrom et al., 2002). The sum OH-PCB in the Faroe study did, however, consist of only 5 congeners.

Geometric mean levels of sum OH-PCBs (11 congeners) in whole blood from Canadian Inuits were 1040 and 614 pg/g plasma in males and females, respectively (Sandau et al. 2000). Those levels seemed lower than reported for the high exposed Chukchi samples (median: 5916 pg/g). In both studies the 4-OH-CB107 was the most dominant congener. The individual variation in levels seemed to be less in the Chukchi samples, but again it must be remembered that these samples were chosen because of their high PCB levels. In the Canadian study the ratio of OH-PCBs to PCBs was as low as 0.1 (Sandau et al., 2000). The reason for the differences in the ratio of OH-PCBs to PCBs is not well understood.

It is evident from Figure 4 that there is a high number of OH-PCBs that have not been identified. High numbers of unidentified congeners have also been observed in other studies (Hovander et al., 2002; Klasson-Wehler et al., 1997). Some of the unidentified

congeners were of comparable intensity to the more abundant identified congeners (Figure 4). The pentachlorinated OH-PCB eluting just before the 4-OH-CB107 might be 4'-OH-CB108, reported to co-elute using a DB-5 30 m column (Sandau et al., 2002). Due to lack of standards this was not possible to verify. In addition to penta, hexa, hepta and octa-chlorinated OH-PCBs, one nona chlorinated was also identified in Figure 4. A nona-chlorinated congener has also been reported by Hovander et al. (2002).

The low PCP concentration relative to the OH-PCBs was surprising based on previous studies in northern Canada, where PCP has been the dominant phenolic compound (Sandau et al., 2000; Sandau et al., 2002). Poor extraction and thus an underestimation of the PCP levels might have been one reason for the lower levels in the high exposed Chukchi samples. However, the fact that the amount of PCP was higher than the OH-PCBs in the Cree samples indicated that the extraction was not the reason for the difference. Differences in exposure through the diet might also be a reason for low PCP levels in the Chukchi samples. However, since the samples were not selected randomly from the Chukchi population of Uelen, the pattern might be different among other people from the same population.

Conclusions

On the basis of these findings the following is concluded:

The method developed is suitable for quantifying both neutral and phenolic organohalogens in plasma samples at very low levels.

Florisil columns are efficient in separating PCBs and pesticides from PCP, OH-PCBs and TBBPA.

Future studies must focus on the completeness of the extraction.

Chukchi people from Uelen (Bering Strait) with high PCB levels also had high OH-PCB levels. The median ratio of OH-PCBs to PCBs was 0.4 and a high number of unidentified OH-PCBs were found.

The reason for differences in the ratio of the sum OH-PCBs to sum PCBs among individuals and populations is not well understood and needs to be studied further.

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References

Akins JR, Waldrep K, Bernert JT, Jr. The estimation of total serum lipids by a completely enzymatic 'summation' method. Clin Chim Acta 1989; 184: 219-226.

AMAP, 1998. AMAP Assessment Report: Arctic Pollution Issues. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway. xii+859.

AMAP, 2002. AMAP Human Health Assessment report. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway. (in press)

Andersson PL, Blom A, Johannisson A, Pesonen M, Tysklind M, Berg AH, Olsson PE, Norrgren L. Assessment of PCBs and hydroxylated PCBs as potential xenoestrogens: *In vitro* studies based on MCF-7 cell proliferation and induction of vitellogenin in primary culture of rainbow trout hepatocytes. Arch Environ Contam Toxicol 1999; 37: 145-150.

Ariyoshi N, Koga N, Oguri K, Yoshimura H. Metabolism of 2,4,5,2',4',5'-hexachlorobiphenyl with liver- microsomes of phenobarbital-treated dog - the possible formation of PCB 2,3-arene oxide intermediate. Xenobiotica 1992; 22: 1275-1290.

Bergman A, KlassonWehler E, Kuroki H. Selective retention of hydroxylated PCB metabolites in blood. Environ Health Perspect 1994; 102: 464-469.

Brouwer A, Morse DC, Lans MC, Schuur AG, Murk AJ, Klasson-Wehler E, Bergman A, Visser TJ. Interactions of persistent environmental organohalogens with the thyroid hormone system: Mechanisms and possible consequences for animal and human health. Toxicol Ind Health 1998; 14: 59-84.

Cheek AO, Kow K, Chen J, McLachlan JA. Potential mechanisms of thyroid disruption in humans: Interaction of organochlorine compounds with thyroid receptor, transthyretin, and thyroid-binding globulin. Environ Health Perspect 1999; 107: 273-278.

Fangstrom B, Athanasiadou M, Grandjean P, Weihe P, Bergman A. Hydroxylated PCB metabolites and PCBs in serum from pregnant Faroese women. Environ Health Perspect 2002; 110: 895-899.

Fielden MR, Chen I, Chittim B, Safe SH, Zacharewski TR. Examination of the estrogenicity of 2,4,6,2 ',6 '- pentachlorobiphenyl (PCB 104), its hydroxylated metabolite 2,4,6,2 ',6 '-pentachloro-4-biphenylol (HO-PCB 104), and a further chlorinated derivative, 2,4,6,2 ',4 ',6 '- hexachlorobiphenyl (PCB 155). Environ Health Perspect 1997; 105: 1238-1248.

Guvenius DM, Hassanzadeh P, Bergman A, Noren K. Metabolites of polychlorinated biphenyls in human liver and adipose tissue. Environ Toxicol Chem 2002; 21: 2264-2269.

Hovander L, Athanasiadou M, Asplund L, Jensen S, Wehler EK. Extraction and cleanup methods for analysis of phenolic and neutral organohalogens in plasma. J Anal Toxicol 2000; 24: 696-703.

Hovander L, Malmberg T, Athanasiadou M, Athanassiadis L, Rahm S, Bergman A, Wehler EK. Identification of hydroxylated PCB metabolites and other phenolic halogenated pollutants in human blood plasma. Arc Environ Contam Toxicol 2002; 42: 105-117.

Jensen S. The PCB story. Ambio 1972; 1: 123-131.

Klasson-Wehler E, Hovander L, Bergman A. New organohalogens in human plasma - Identification and quantification. Organohalogen Compd 1997; 33: 420-425.

Lans MC, KlassonWehler E, Willemsen M, Meussen E, Safe S, Brouwer A. Structure-dependent, competitive interaction of hydroxy- polychlorobiphenyls, hydroxy-dibenzo-*p*-dioxins and hydroxy-dibenzofurans with human transthyretin. Chem Biol Interact 1993; 88: 7-21.

Letcher RJ, Klasson-Wehler E, Bergman A. Methyl sulfone and hydroxylated metabolites of polychlorinated biphenyls. In: Paasivirta J, editor. The Handbook of Environmental Chemistry: New Types of Persistent Halogenated Compounds. Springer-Verlag, Berlin, 2000; 315-359.

Mckay AF, Ott WL, Taylor GW, Buchanan MN, Crooker JF. Diazohydrocarbons. Can J Res Sect B-Chem Sci 1950; 28: 683-688.

Press JB. Deethylation of Aryl Ethyl Ethers by Boron Tribromide. Synth Commun 1979; 9: 407-410.

Romieu I, HernandezAvila M, LazcanoPonce E, Weber JP, Dewailly E. Breast cancer, lactation history, and serum organochlorines. Am J Epidemiol 2000; 152: 363-370.

Sandanger TM, Brustad M, Lund E, Burkow IC. Change in levels of persistent organic pollutants in human plasma after consumption of a traditional northern Norwegian fish dish-molje (cod, cod liver, cod liver oil and hard roe). J Environ Monit 2003; 5: 160-165.

Sandau CD, Ayotte P, Dewailly E, Duffe J, Norstrom RJ. Analysis of hydroxylated metabolites of PCBs (OH-PCBs) and other chlorinated phenolic compounds in whole blood from Canadian Inuit. Environ Health Perspect 2000; 108: 611-616.

Sandau CD, Ayotte P, Dewailly E, Duffe J, Norstrom RJ. Pentachlorophenol and hydroxylated polychlorinated biphenyl metabolites in umbilical cord plasma of neonates from coastal populations in Quebec. Environ Health Perspect 2002; 110: 411-417.

Sandau, CD. Analytical chemistry of hydroxylated metabolites of PCBs and other halogenated phenolic compounds in blood and their relationship to thyroid hormone and retinol homeostasis in humans and polar bears. 2000; 1-262. Carleton University, Ottawa, Ontario.

Schnellmann RG, Putnam CW, Sipes IG. Metabolism of 2,2',3,3',6,6'-hexachlorobiphenyl and 2,2',4,4',5,5'-hexachlorobiphenyl by human hepatic microsomes. Biochem Pharmacol 1983; 32: 3233-3239.

Sipes IG, Slocumb ML, Perry DF, Carter DE. 2,4,5,2',4',5'-Hexachlorobiphenyl - distribution, metabolism, and excretion in the dog and the monkey. Toxicol Appl Pharmacol 1982; 65: 264-272.

Thomsen C, Lundanes E, Becher G. Brominated flame retardants in archived serum samples from Norway: A study on temporal trends and the role of age. Environ Sci Technol 2002; 36: 1414-1418.

Tisch M, Bergenthal S, Maier H. Genotoxic effect of PCP and lindane on human epithelial tonsil cells. Hno 2002; 50: 920-927.

van den Berg KJ, van Raaij JA, Bragt PC, Notten WR. Interactions of halogenated industrial chemicals with transthyretin and effects on thyroid hormone levels *in vivo*. Arch Toxicol 1991; 65: 15-19.

van Raaij JA, van den Berg KJ, Notten WR. Hexachlorobenzene and its metabolites pentachlorophenol and tetrachlorohydroquinone: interaction with thyroxine binding sites of rat thyroid hormone carriers *ex vivo* and *in vitro*. Toxicol Lett 1991; 59: 101-107.

Table 1: Masses used for identification and quantification of the MeO-PCBs, Me-PCP and Me_2-TBBPA.

Compound	Retention time (minute)	Target ions (m/z)	Confirmation ions (m/z)
Me-PCP*	12.89	280	282
$^{13}C_6$-Me-PCP	12.89	286	288
4-MeO-CB107	35.21	354	356
3-MeO-CB153	36.35	354	356
4-MeO-CB146	36.59	390	392
3'-MeO-CB138	39.02	354	356
4'-MeO-CB130	39.31	390	392
4-MeO-CB187*	41.19	409	411
$^{13}C_{12}$-4-MeO-CB187	41.19	436	438
4-MeO-CB162	42.99	375	377
3'-MeO-CB180	45.27	424	426
4'-MeO-CB172	45.46	424	426
4-MeO-CB193	46.01	424	426
Me_2-TBBPA	52.25	491	493
$^{13}C_{12}$-PCB 141	30.07	372	374

*For Me-PCP and 4-MeO-CB 187 fragment ions from the ^{13}C-labelled surrogate interfered with the quantification mass. Corrections were performed using the following formulas: area(m/z280) -(0.0127 x area(m/z286)) and (area(m/z409) - (0.015 x area(m/z436)), respectively.

Table 2: Recovery rates for the hydroxylated and the methoxylated compounds on florisil columns, as well as the recovery rates for the whole procedure using spiked plasma samples (n=3).

Compound	Median recovery rate* (%, range)	Median recovery rate** (%, range)	Median recovery rate*** (%, range)
Me-PCP	112 (104-120)	89 (81-97)	50 (41-67)
$^{13}C_6$-Me-PCP	116 (105-119)	90 (82-94)	38 (34-43)
4-MeO-CB107	90 (54-109)	76 (76-84)	47 (37-62)
4'-MeO-CB130	107(51-118)	73 (72-81)	56 (47-72)
3'-MeO-CB138	115 (65-151)	72 (68-79)	75 (62-99)
4-MeO-CB146	96 (56-119)	76 (68-77)	65 (56-81)
3-MeO-CB153	107 (62-130)	77 (76-83)	73 (63-92)
4-MeO-CB162	99 (59-125)	67 (63-77)	46 (37-59)
4'-MeO-CB172	95 (58-125)	81 (79-87)	69 (57-90)
3'-MeO-CB180	101 (60-123)	84 (80-85)	75 (62-97)
4-MeO-CB187	111 (105-130)	82 (75-84)	43 (34-61)
$^{13}C_{12}$-4-MeO-CB187	95 (90-129)	85 (78-92)	54 (48-65)
4-MeO-CB193	83 (77-125)	80 (78-89)	48 (40-64)
Me_2-TBBPA	88 (63-90)	72 (70-81)	

* Recovery rates for the hydroxylated compounds on the first florisil column (step 2 in Figure 1) using 10 ml DCM:MeOH (9+1) and 6 ml DCM:MeOH (8+2).

** Recovery rates for the methylated compounds on a single florisil column as the final clean up step (Step 4 in Figure 1) using 15 ml hexane:DCM (3+1). Me_2-TBBPA was eluted with 6 ml hexane:acetone (85+15), following the first fraction.

***Recovery rates for the hydroxylated compounds through the whole procedure. Plasma samples were spiked with approximately 5 ng of each compound.

Table 3: Wet weight and lipid weight levels of OH-PCBs and PCP in plasma of selected high PCB exposed samples from Chukchi people from Uelen, Chukotka Peninsula (n=15). Recovery rates: $^{13}C_6$PCP = 64% (range 43 – 83 %), $^{13}C_{12}$ 4-OH-CB 187 = 72 % (range 41 – 90 %).

Compound	Median (pg/g wet weight)	Range (pg/g wet weight)	Median (ng/g lipid weight)	Range (ng/g lipid weight)
PCP	642	369-1197	117	51-252
4-OH-CB107	1673	697-3948	275	203-917
4'-OH-CB130	33	10-100	6	1-20
3'-OH-CB138	859	536-1469	174	92-341
4-OH-CB146	841	452-2105	191	87-346
3-OH-CB153	1313	558-2100	194	104-488
4-OH-CB162	85	48-251	18	8-58
4'-OH-CB172	188	90-439	37	17-82
3'-OH-CB180	92	46-227	18	9-53
4-OH-CB187	786	447-1967	136	74-457
4-OH-CB193	71	18-356	15	4-83
Sum OH-PCBs	5916	3304-12215	1098	707-2838

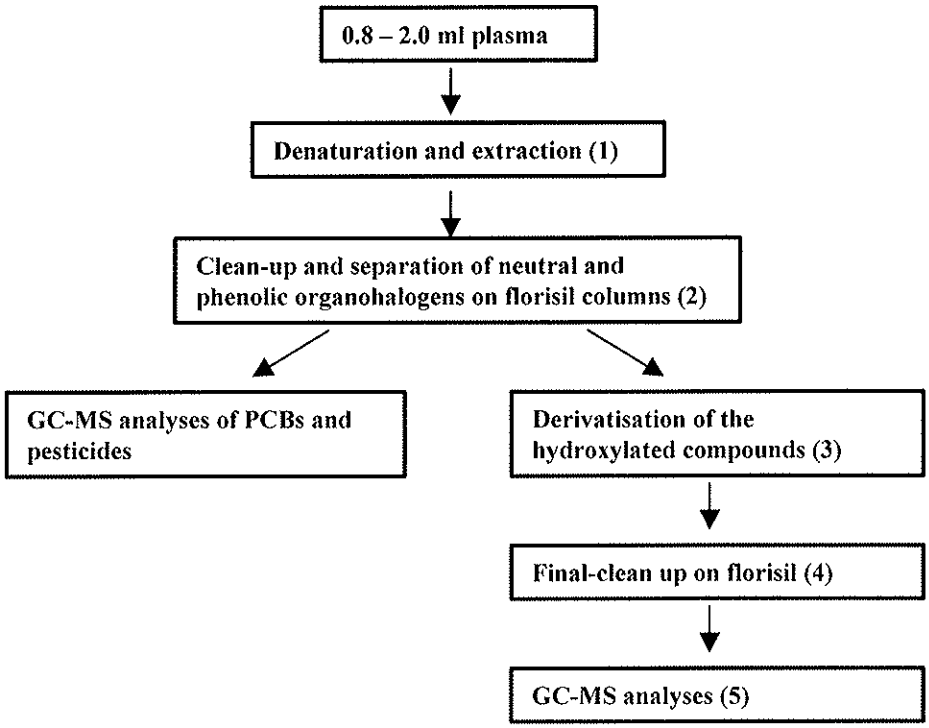

Figure 1: Scheme of the extraction and analyses of neutral and phenolic organohalogens in plasma. The numbers in brackets indicate the different steps referred to in the text.

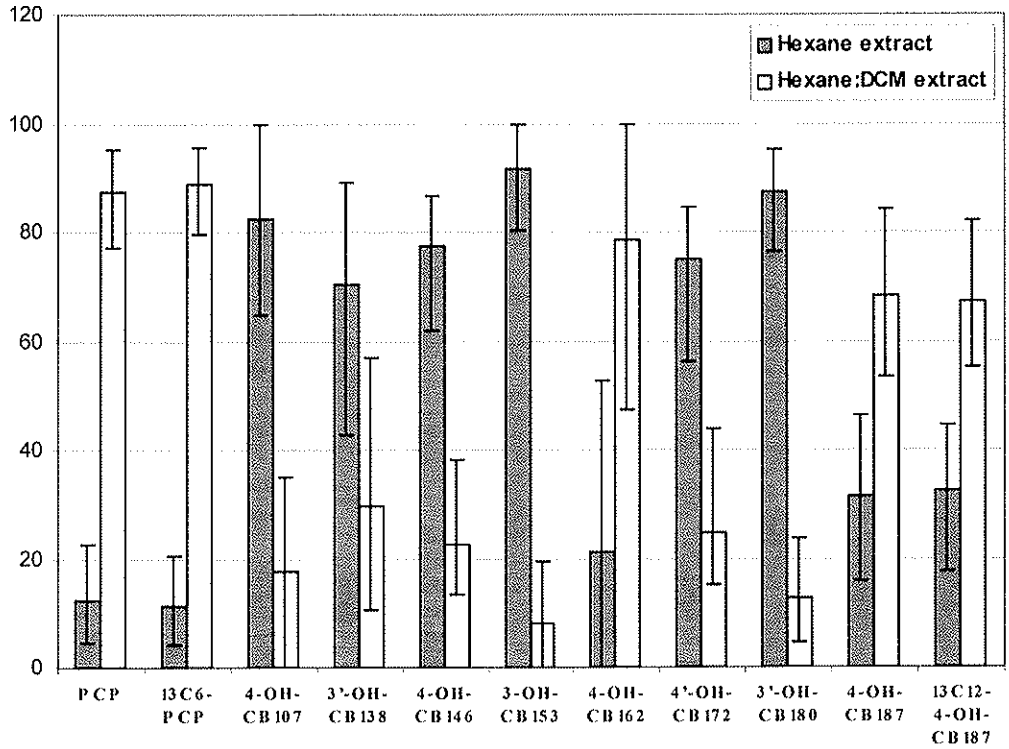

Figure 2: The percentage of each compound in the two organic fractions from the extraction for the same sample; the hexane fraction, and the hexane:DCM fraction from the acidified plasma (n=7). Error bars indicate maximum and minimum values.

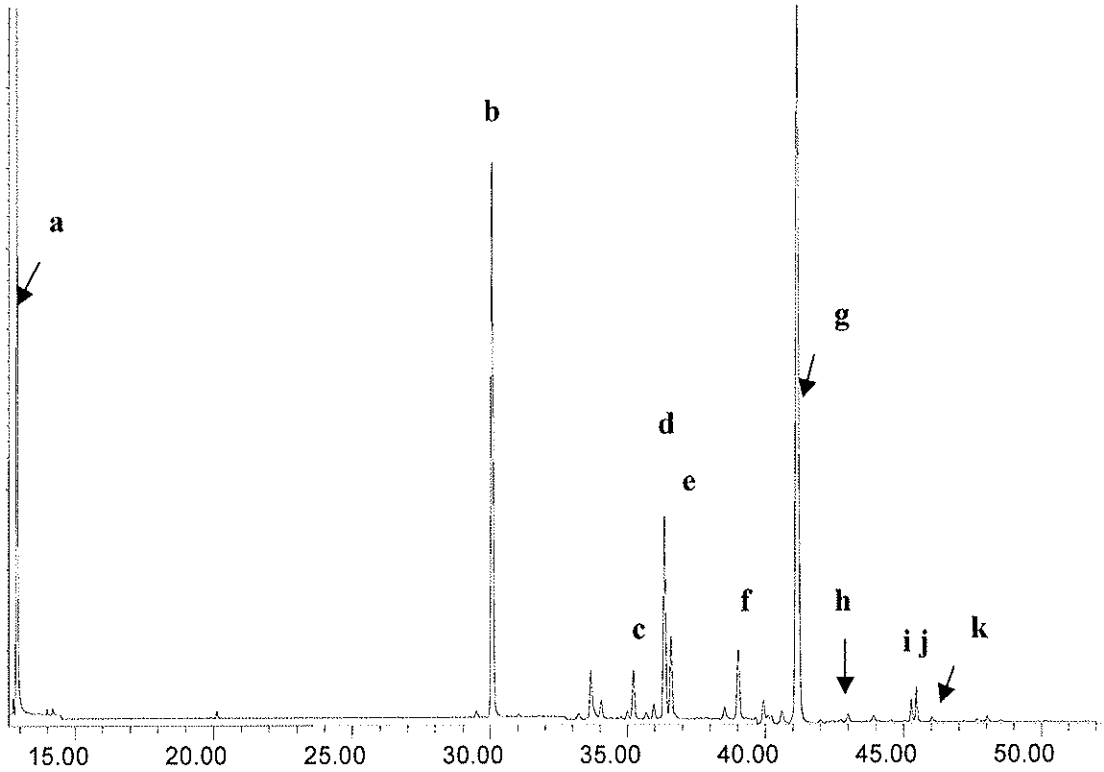

Figure 3: Chromatogram (TIC) of a real sample from the Chukchi population of Uelen analysed in SIM-mode. a, Me-PCP+ $^{13}C_6$-Me-PCP; b, $^{13}C_{12}$-PCB141; c, 4-MeO-CB107; d, 3-MeO-CB153; e, 4-MeO-CB146; f, 3'-MeO-CB138; g, 4-MeO-CB187 +$^{13}C_{12}$-4-MeO-CB187; h, 4-MeO-CB162; i, 3'-MeO-CB180; j, 4'-MeO-CB172; k, 4-MeO-CB193

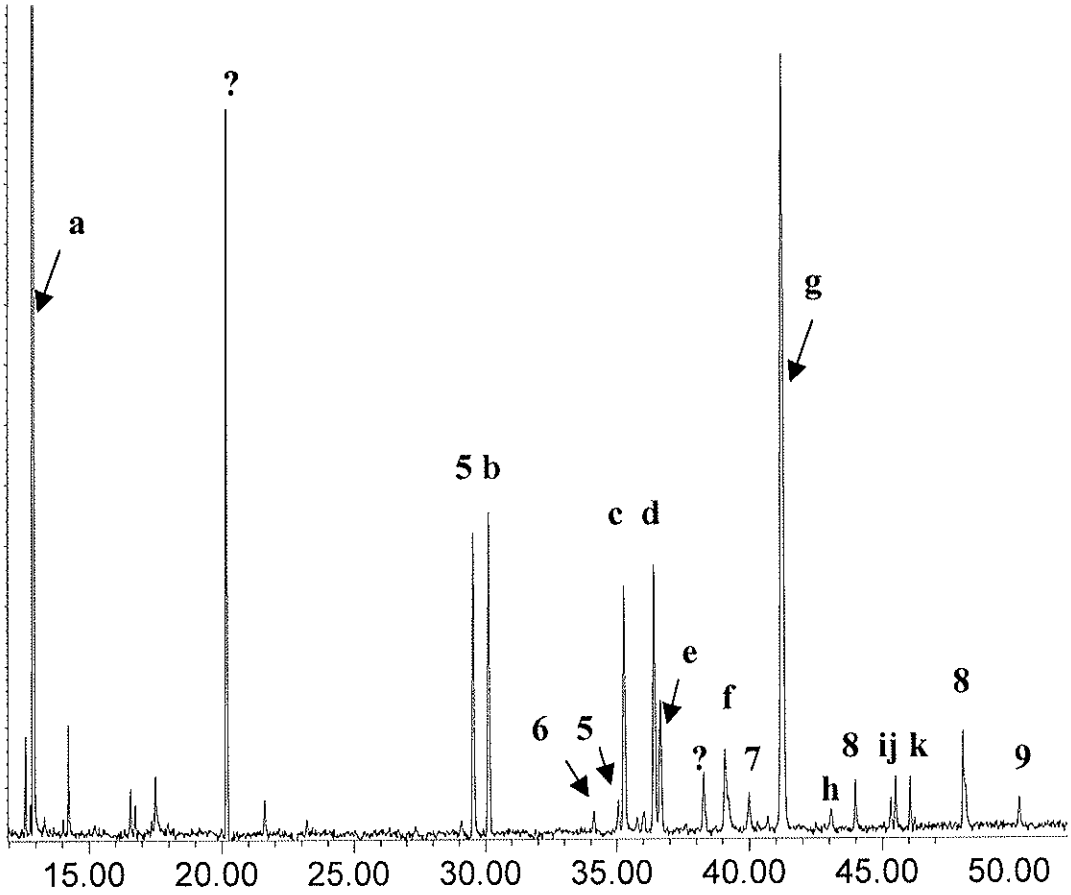

Figure 4: Full-scan chromatogram of one of the samples from the Chukchi population of Uelen. a, Me-PCP+ $^{13}C_6$-Me-PCP; b, $^{13}C_{12}$-PCB 141; c, 4-MeO-CB107; d, 3-MeO-CB 153; e, 4-MeO-CD146; f, 3'-MeO-CB138; g, 4-MeO-CB187 +$^{13}C_{12}$-4-MeO-CB187; h, 4-MeO-CB162; i, 3'-MeO-CB180; j, 4'-MeO-CB172; k, 4-MeO-CB193. The numbers indicate number of chlorine atoms on unidentified OH-PCBs. Unknown compounds that were not identified as OH-PCBs are indicated with a question mark.

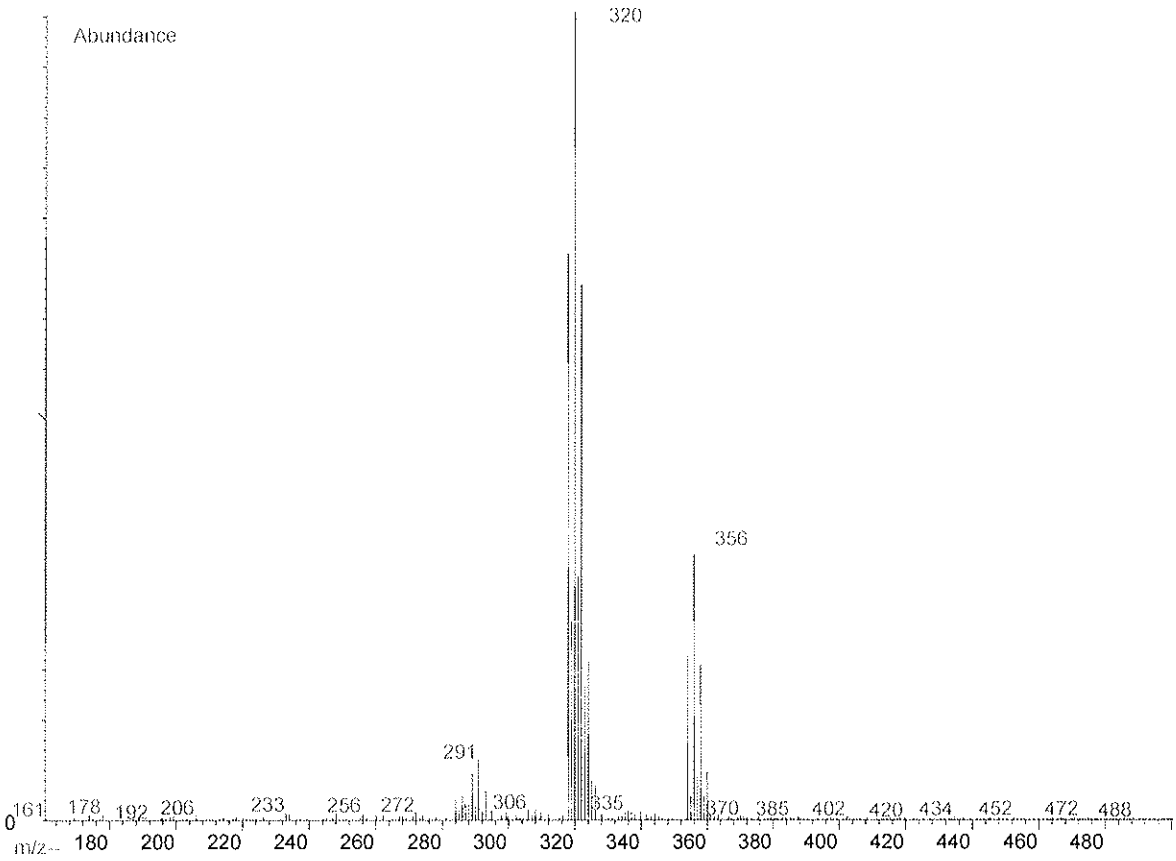

Figure 5: Full-scan mass spectrum of the first peak identified with the number 5 (penta chlorinated OH-PCB; Rt, 29.6 min) in Figure 4.