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Levels of chlorinated naphthalenes in mother's milk from first-time mothers in Uppsala, Sweden: results from year 2012 to 2017.

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Sammanfattning
Sedan 1996 har Livsmedelsverket regelbundet samlat in modersmjölk från förstföderskor i Uppsala för analys av persistenta halogenerade organiska miljöföroreningar (POP), i studien POPUP (Persistent Organic Pollutants in Uppsala Primiparas). I följande rapport redovisas halterna av polyklorerade naftalener (PCN) i poolade bröstmjölksprover från 2012 till 2017, ett samlingsprov per år (n=6). Resultaten visar inte på någon statistiskt signifikant trend under tidsperioden. Adderas tidigare analyserad data av polyklorerade naftalener i bröstmjölk från förstföderskor i Uppsala under åren 1997 – 2011 (Haglund, Liljelind et al. 2014) till ovanstående data erhålles en statistiskt signifikant nedgång ($p < 0{,}001$) för total PCN, med en halveringstid på 14 år.

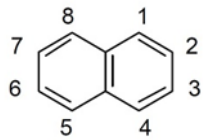
INTRODUCTION

With funding from the Swedish Environmental Protection Agency (EPA), the Swedish National Food Agency (NFA) has made recurrent measurements of persistent halogenated organic pollutants (POP) in mother's milk from primiparous women in Uppsala since 1996. The study is called POPUP (Persistent Organic Pollutants in Uppsala Primiparas), and the aim is to estimate the body burdens of POP among pregnant and nursing women and to estimate temporal trends of the exposure of foetuses and breast-fed infants.

Time trends of chlorinated naphthalenes in mothers milk have previously been reported from mothers in the area of Stockholm during 1972 – 1992 (Lunden and Noren 1998) and Uppsala (POPUP) during 1997 – 2011 (Haglund, Liljelind et al. 2014). The following report presents results of analyses of chlorinated naphthalenes in mother's milk sampled in 2012 to 2017 (according to agreement 2215-15-001).

Polychlorinated naphthalenes

Chlorinated naphthalenes, often called polychlorinated naphthalenes or PCNs, are a group of compounds based on the naphthalene ring system, with one or more hydrogen atoms replaced by chlorine atom(s).They are divided into eight homologue groups, based on the number of chlorine atoms in the molecule, from mono-CNs (one chlorine atom) to octa-CNs (eight chlorine atoms). The generic molecular formula is $C_{10}H_{8-n}Cl_n$, where n = 1 - 8. There are 75 possible PCNs, usually identified using the numbering system shown below:



PCNs are members of the class of chlorinated polycyclic aromatic hydrocarbons. Some of the 75 congeners have been recognised as persistent, bioaccumulative and have toxic properties which characterise these compounds as environmental contaminants. There are also reports showing biological effects in humans and animals (Fernandes, Rose et al. 2017). Due to the

lack of appropriate long-term studies, the toxicological profile of PCNs is experimentally not very well characterized. PCNs are thought to have a mode of action through interacting with the Ah receptor. Therefore, it is expected that exposure to PCNs could result in a pattern of biochemical and toxic responses typical for dioxin-like compounds (WHO 2001).

Historically, PCNs were produced as mixtures of several congeners and the main use was in the electrical industry. They have been used as separators in storage batteries, capacitor impregnates, as binders for electrical grade ceramics and sintered metals and in cable coverings. PCNs have also been used to impregnate wood, paper and textiles, as flame retardants and protection against insects, moulds and fungi. Additionally, they have been used as additives in gear and cutting oils, in lacquers and paints as well as raw material for dyes. Today, PCNs are formed mainly unintentionally during various thermal processes such as non-ferrous metal smelting, iron ore sintering and coking processes (Anonymous 2017). Since 2015 PCNs are listed in annex A in the Stockholm convention and should therefore not be manufactured or used anymore (UNEP 2019).

PCNs can be absorbed via all routes of exposure, are highly lipophilic and with a half-life in the human body of 1.5 to 2.4 years estimated for hexa-CN congeners. Absorption, distribution, metabolism and toxicity are dependent on the isomer. Hexa-CNs are among the most frequently detected congeners in human samples. PCNs have been concluded to be potent foetotoxic and teratogenic agents producing effects similar to those for other toxic dioxin-like compounds and a potential for endocrine disruption is suggested at low exposure concentrations (UNEP 2012).

MATERIALS AND METHODS

Recruitment and sampling

Mothers were randomly recruited among primiparas who were Swedish by birth and delivered at Uppsala University Hospital from 2012 to 2017 (n=180, 30 women per year). The participating mothers sampled milk at home during the third week after delivery (day 14-21 post-partum). Milk was sampled during nursing using a manual mother's milk pump and/or a

passive mother's milk sampler. The women were instructed to sample milk both at the beginning and at the end of the breast-feeding sessions. The goal was to sample 500 mL from each mother during 7 days of sampling. During the sampling week, the milk was stored in the home freezer in acetone-washed bottles. Newly sampled milk was poured on top of the frozen milk. At the end of the sampling week, a midwife visited the mother to collect the bottles. 10 g of breast milk per individual was pooled to an annual pool for each year (Table 1) and 70 ml per pooled sample was sent to Umeå University for PCN analysis.

Table 1. Composition of the pooled serum samples used for analyses of PCNs.

Sampling year	No of pools	N in each pool	Age range (yrs)
2012	1	30	20-38
2013	1	30	22-39
2014	1	30	20-38
2015	1	30	21-37
2016	1	30	24-36
2017	1	30	21-34

Chemical analysis

The method used for the determination of the concentrations of PCNs at the Trace Analysis Facility, Umeå University, included sample extraction, clean-up and analysis using gas chromatography (GC 6890N, Agilent Tech., Santa Clara, CA, USA) coupled to high resolution mass spectrometry (Waters Autospec Ultima NT, Milford, MA, USA). The 70 ml samples were poured over to separatory funnels, where isotopically labelled ^{13}C-PCNs, as well as pure ethanol, saturated with sodium oxalate, was added prior to extraction. Liquid-liquid extraction was performed adding a 7/10 mix of diethyl ether and n-hexane, followed by shaking. The organic solvent phase was collected, and the extraction was repeated three times. After concentration, the clean-up was performed first using a multi-layer silica column, including layers with acid and base impregnated as well as neutral silica. Following elution with n-hexane, and concentration, a carbon column was used to further purify the samples. The resulting toluene fraction was concentrated, and a recovery standard was added to finalise the sample treatment. The GC separation was made on a DB-5ms column (Agilent Tech., Santa

Clara, CA, USA). MS analysis was performed using electron impact ionisation and monitoring of selected molecular ions, highly specific for PCNs. The quantification was performed according to the isotope dilution principle, where the ratio of known amounts of naturally occurring and labelled compounds were compared to the samples' ditto. The procedure was adopted from the standard document EN 1948-3:2006. The overall method is analogous to the one used for the determination of the structurally related polychlorinated dioxins and dibenzofurans (PCDD/Fs), for which it has been well validated. The measurement uncertainty was estimated to be 30%.

Statistical analysis

Temporal trends were investigated for the study period (2012 - 2017) as well as with a merged data set for the years 1997 – 2011 (Haglund, Liljelind et al. 2014) and 2012 - 2017. In order to reflect the reported levels of PCNs for 2012 – 2017, the total PCN level for the years 1997 – 2011 was recalculated using the reported values for tri-, tetra, penta- and hexa-PCNs. Linear regression (MINITAB 15® Statistical Software for Windows) were used to analyse associations between concentrations of total PCNs in mother's milk and sampling year.

RESULT AND DISCUSSION

The results of the test series have been reported for chlorinated naphthalenes with three to eight chlorine atoms (Table 2). The sum for TriCN is however not complete and the mono- and dichloride CNs could not be determined accurately due to a contamination of the internal standard. The hepta- and octachlorinated CNs were under the quantification limit (Table 2).

Table 2. Levels of different chlorination grade and total PCN, expressed as pg/g fat, in pooled samples of mothers milk (1 pool/year) from primiparas in the Uppsala region, Sweden.

PCN	Year					
	2012	**2013**	**2014**	**2015**	**2016**	**2017**
TriCN	68	65	41	55	39	35
TetraCN	212	203	109	272	181	165
PentaCN	172	46	122	127	152	116
HexaCN	66	77	61	46	57	60
HeptaCN [a]	<29	<34	<28	<35	<38	<33
OctaCN [a]	<10	<11	<8	<9	<8	<9
Total PCN[b]	518	491	332	501	429	375

[a] Under the quantification limit.
[b] Mono-, di-, hepta- and octaCNs not included.

Time trends of PCNs in mothers milk have previously been reported from mothers in the area of Stockholm during 1972 – 1992 (Lunden and Noren 1998) and Uppsala during 1997 – 2011 (Haglund, Liljelind et al. 2014). The levels of total PCNs in Stockholm were reported at about 3000 pg/g fat in 1972, decreasing to about 500 pg/g fat in 1992 (Lunden and Noren 1998). In Uppsala (POPUP) the reported levels of total PCNs were about 850 pg/g fat and was more or less stable over the time period 1997 to 2011 (Haglund, Liljelind et al. 2014).

When fitting the data for year 2012 – 2017 to total PCNs and to a linear equation (Figure 1), the time trend was not statistically significant (β = -0.046, p = 0.3). However, when adding the previously reported data from year 1997 – 2011 (Haglund, Liljelind et al. 2014) with PCNs analysed in mothers milk from primiparas from the Uppsala region (Figure 2), there is a statistically significant trend (β = -0.051, p = <0.001, 5 % decrease per year), with a half life of total PCN of 14 years.

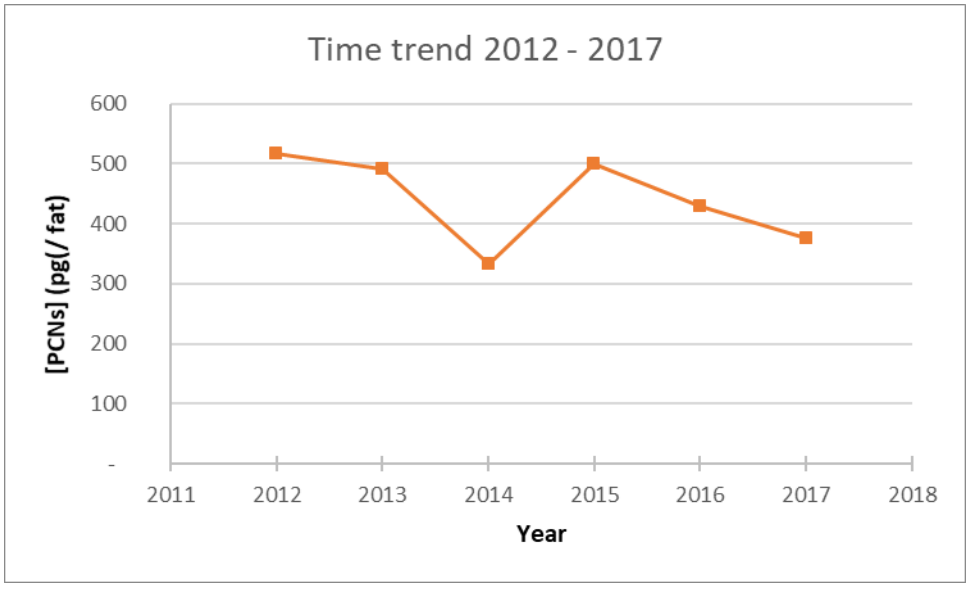


Figure 1. Time trends of the sum of the detected PCNs during 2012-2017.

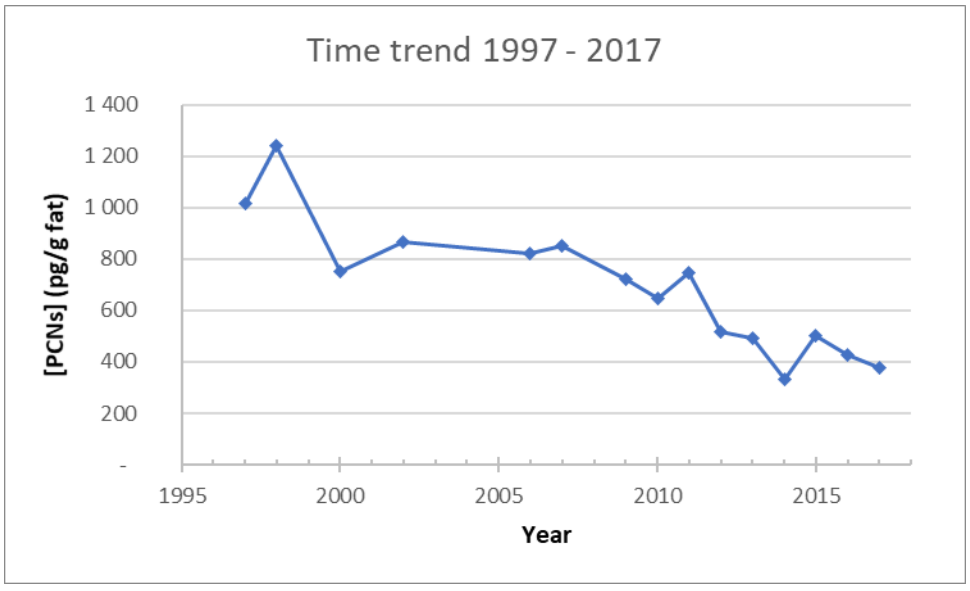


Figure 2. Time trends of the sum of the detected PCNs during 1997-2017.

However, care should be taken when interpreting different data sets from different studies, as it is not always clear if all possible congeners were analysed for in all samples. As stated in a

report from WHO, data have in some cases been summarized into classes of PCNs. This might reflect the volume of data if all individual congeners were to be reported and also the differences in resolution between congeners in specific methodologies analysed at different times or in different laboratories (WHO 2001).

Nevertheless, the time trend for PCNs in mother's milk from primiparas in Uppsala seems to decrease during the time period from 1997 to 2017. The rate at which the levels of PCNs drops by seems to start to level out when compared with the levels reported from 1972 to 1992 in a population from the Stockholm area (Lunden and Noren 1998).

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