Science of the Total Environment 407 (2009) 4584–4590

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Levels of chlorinated pesticides and polychlorinated biphenyls in Norwegian breast milk (2002–2006), and factors that may predict the level of contamination

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ARTICLE INFO

Article history:
Received 2 December 2008
Received in revised form 6 April 2009
Accepted 21 April 2009
Available online 19 May 2009

Keywords:
Chlorinated pesticides
Polychlorinated biphenyls
Levels
Breast milk
Determining factors
Norway

ABSTRACT

In the present study, polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) were determined in 423 breast milk samples from women living in Norway. Various predictors for the contaminant levels were also investigated. The samples were collected in six counties, representing South, Central and North Norway in 2002–2006. Initial results showed significantly lower levels of OCPs in breast milk from ethnic Norwegians ($N=377$) compared to ethnic non-Norwegians ($N=46$). Median concentrations (range) of PCBs, *p,p′*-DDE, HCB, β-HCH and oxychlordane in breast milk of the Norwegian women, all parities included, were 103 (34–450), 41 (5.4–492), 11 (3.6–24), 4.7 (0.9–37) and 2.8 (0.5–16) ng/g lipid weight, respectively. Results indicated that sum of 18 PCBs, *p,p′*-DDE and β-HCH are good predictors for monitoring of PCB, DDT and HCH levels in Norwegian breast milk. Multivariable linear regression analyses showed that age was strongly associated with increasing OC levels ($P<0.001$), whereas parity was associated with decreasing OC levels ($P<0.001$). Smoking was associated with higher levels of PCBs, *p,p′*-DDE and β-HCH. The models explained from 17 to 35% of the variance. Median levels of OCs in the present Norwegian primparaes seemed to be 29–62% lower than corresponding results found in a Norwegian study from 2000–2002.

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1. Introduction

Persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are substances that because of their high lipohpilicity and persistency can bioaccumulate through the food chain. Humans are exposed to these chemicals mainly through intake of food (Ahlborg et al., 1992; Liem and Theelen, 1997). The human exposure to PCBs is of great concern, due to possible adverse health effects, such as immunotoxicity, reproductive effects, endocrine disruption and carcinogenicity (IPCS–WHO, 2003). Health authorities worldwide are concerned for infants' intake of POPs through nursing and therefore human milk monitoring programs are performed in many countries.

In Norway, breast milk monitoring studies for PCBs and OCPs have been performed periodically since 1970 (Skaare et al., 1988; Clench-Aas et al., 1988; Johansen et al., 1994; Skaare and Polder, 1990). Because of economic and logistic reasons, the numbers of participants in these studies have always been kept at a limited level. Monitoring studies organized by WHO have been based on pooled samples (Becher et al., 2002; Van Leeuwen and Malisch, 2002). In Norway, each WHO pool was based on ten individual breast milk samples, collected in three defined geographic areas (Becher et al., 2002). The results of these studies based on individual and pooled samples showed no significant geographical differences for POPs in breast milk. However, in 2001–2002, significantly higher levels of β-HCH were found in individual primipara breast milk samples from South Norway compared to North Norway (Polder et al., 2008a). In addition, higher maximum levels of *p,p′*-DDE and sum PCBs were found in North Norway compared to South Norway. Regional geographic differences of certain POPs in breast milk have also been observed in other countries (Polder et al., 2003; Kalantzi et al., 2004; Lignell et al., 2006; Mueller et al., 2008). Due to low sample numbers and insufficient personal information it has often been difficult to explain these observed differences (Polder et al., 2008a).

Several factors can affect the level of POPs in the human body, with age, parity, smoking and intake of high contaminated foods as the most important. (Skaare et al., 1988; Skaare and Polder, 1990; Harris et al., 2001; Fängström et al., 2005; Jönsson et al., 2005; Deutch et al., 2007). Settlement in highly industrialised areas is also found to increase levels of PCBs in humans (Koopman-Esseboom et al., 1994). In addition, it seems that more attention should be paid to the ethnic background of participating women. Many countries have become more multicultural during the last decades. It is likely that this will result in a wider variation of POP levels and patterns, because the body burden is a result of historic exposure through breast feeding

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doi:10.1016/j.scitotenv.2009.04.032

combined with dietary intake through life time. Skaare et al. (1988) showed that breast milk of immigrant women from developing countries contained five times higher levels of HCHs and DDTs compared to ethnic Norwegian women. Breast fed children of these immigrant women are therefore suggested to have higher body burdens of POPs compared to children born by Norwegian mothers. A Swedish monitoring study based on a large number of participants showed significantly higher POP levels in breast milk of women that were born in other countries, but had lived in Sweden thereafter (Lignell et al., 2006). Correspondingly, a cohort study, performed by Bradman et al. (2007) reported 1.5–2 times higher levels of OCPs in Californian women born in Mexico, compared to women born in the United States or other countries outside Latin America. Most of the mentioned biological factors are related to each other and it is therefore complicated to predict the influence of a simple factor on the accumulation of POPs in the human body. In larger monitoring studies, more advanced statistical models should therefore be used in order to avoid misinterpretations and misleading conclusions (Tan et al., 2008). In both the Swedish and the Californian studies the results were adjusted for different biological factors (Lignell et al., 2006; Bradman et al., 2007).

The aims of the present study were to describe the recent levels of POPs in breast milk of Norwegian women and secondly to elucidate the influence of age, parity, geography, and living in a rural or urban setting, on levels of POPs in human milk. We utilized a birth cohort, the "Norwegian Human Milk Study" (HUMIS) that was set up for this purpose, and human milk samples from 423 women were selected for analyses of POPs.

2. Materials and methods

2.1. Study population

We used data from the "Human milk study" (HUMIS), which is an ongoing multi-centre birth-cohort of mothers who have recently given birth in Norway. The research protocol for this study was approved by the Norwegian Data Inspectorate and the Regional Ethics Committee for Medical Research. All women signed an approved consent before they were enrolled in the study.

The sampling locations are shown in Fig. 1. Participants in Telemark, Rogaland, Oppland, Troms and Finnmark County were recruited at a home visit approximately within 2 weeks after birth by health visitors. This visit is part of routine follow up of all mothers in Norway. Participants in the county of Østfold ($N = 50$) were recruited in a slightly different manner. In this county, recruitment was done by a paediatrician at the maternity wards among mothers who had given birth to term babies. Overall 36% of the invited women declined to participate in the study. The design of the study has been described in details elsewhere (Eggesbo et al., in press).

The results in this paper are based on the combined data from 3 subgroups of the HUMIS population that had their milk analyzed for POPs: 1) 350 mothers that were randomly selected from the HUMIS cohort, stratified on maternal county of residence 2) 27 samples were selected randomly among women with a high intake of traditional foods. 3) 46 samples were analyzed as part of the Fourth WHO-Coordinated Survey of Human Milk for Persistent Organic Pollutants in Cooperation with UNEP (www.who.int/entity/foodsafety/chem/POPprotocol.pdf) and therefore selected at random from primiparous mothers born in Norway and who had lived in Norway for the last 5 years. The study sample thus consisted of in total 423 subjects.

The mothers received containers for breast milk and a questionnaire as soon as they had given their consent. The guidelines and questionnaire for collecting and storing of the breast milk provided by WHO were used, but modified and further developed for the HUMIS study (Eggesbø et al., in press). We asked the mothers to save a 25 ml milk sample on the morning of each of eight days, in specially cleaned containers, before the child was two months of age, although milk sampled otherwise was also accepted. The milk samples were kept frozen during collection, but were returned by regular mail service. After reception, the samples were kept at −20 °C until analyses.

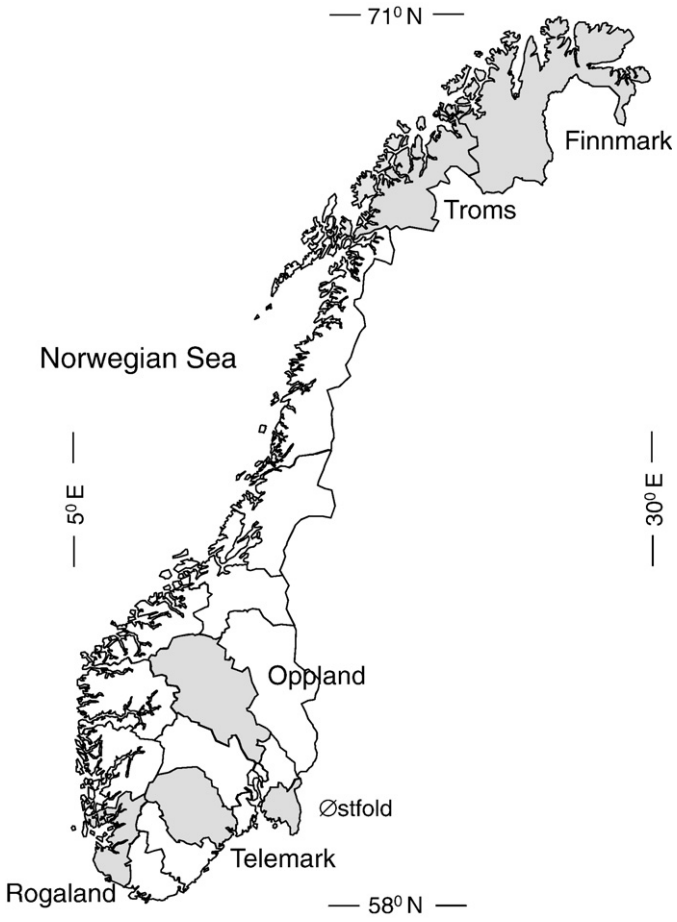

Fig. 1. Map of Norway, showing the different counties in which the breast milk was sampled.

In the present study, information on maternal age, pre-pregnancy BMI, parity, smoking habits and living conditions were included, in addition to sex of the infants.

2.2. Extraction, separation and detection

The analyses of organochlorines (OCs) were performed at the Norwegian School of Veterinary Science. Concentrations of 10 indicator polychlorinated biphenyls (PCBs) IUPAC numbers, PCBs 28, 52, 74, 99, 101, 138, 153, 170, 180, 194, 8 mono-*ortho* PCBs 105, 114, 118, 123, 156, 157, 167, 189, 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene (*p,p′*-DDE), hexachlorobenzene (HCB), beta-hexachlorocyclohexane (β-HCH) and oxychlordane were analysed in 346 samples. In 77 of the samples, PCBs 31, 47, 66, 56, 87, 136, 110, 151, 149, 141, 137, 187, 183, 128, 199, 196, 206 and 209, 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane (*p,p′*-DDD), 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (*p,p′*-DDT), α -HCH, γ -HCH, *cis*-chlordane, *trans*-nonachlor and mirex were analysed in addition to the above mentioned OCs.

The method of extraction was a modification of a method described by Brevik (1978). The samples (~10 g) were spiked with the internal standards PCBs 29, 112 and 207. Lipid extraction was done twice using cyclohexane and acetone (3:2) and an ultrasonic homogenizer (4710 Series, Cole Parmer Instruments Co., Chigaco, IL, USA). Concentrated H_2SO_4 (purity 96%) (Scanpure, Chemscan AS,

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