Introduction

Background level studies of persistent organic pollutants (POPs) such as polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) in human are of concern since they represent potential health risks. Among the available matrices for human, adipose tissues and blood are the most analyzed since they are quite easily—especially for blood—available. Considering that infants are proportionally more exposed to these toxicants than adults, it is of prime interest to monitor their exposure and body burden. Knowing that the principal source of dioxin exposure for these infants is constituted by the mother breast milk and that blood and adipose tissues sampling in babies is not as easy than for adults, studies of breast milk levels are generally conducted to estimate the body burden of breast-feed infants.

From last 20 years studies, it has been shown that lactation could be an important process for the elimination of POPs from the mother organism which can concentrate significant levels of dioxins in its breast milk lipids [1]. These lipids constituting the primary source of infant’s adipose tissues. It’s then important to monitor the levels in breast milk among the population to estimate the evolution of the exposure for the infants. This study presents some results obtained from samples issued of volunteer mothers living in industrial area (Wallonia, Belgium).

Materials and methods

Samples

Breast milk samples (N=20) issued from primi and multiparae women between the ages of 26 and 38. They were collected between August 2000 and April 2001 in Belgium (most of them in the city of Liege, Wallonia) at different times of lactation. Volunteers were asked to fill out a personal questionnaire including parameters of interest. Decontaminated collection vials and breast-pump were used by volunteers for sampling and samples were stored less than one day at 4°C before freezing at -20°C until analysis. Portions between 15 and 50 ml were used for this study.

Extraction and Clean-up

Automated extraction and clean-up were performed on the new Power-Prep™ system generation (Fluid Management Systems, Waltham, MA, USA) using disposable columns (octadecyl bonded (C₁₈), multi-layer silica and PX-21 carbon) after sample pre-treatment [2]. Since the solid phase
extraction technique does not allow the isolation of the fat, lipid content determinations were carried out on the side of the analysis using small volume (10 ml) of milk.

Analysis
Analysis were performed (isotopic dilution method) using a validated method routinely used for dioxin monitoring in foodstuffs. An Autospec Ultima high-resolution mass spectrometer (Micromass, Manchester, UK) operating at a resolution of 10,000 in the selected ion monitoring mode (SIM) was used. Gas chromatography was carried out on an Agilent (Palo Alto, CA, USA) 6890 Series gas chromatograph equipped with a RTX-5 (30m x 0.25mm x 0.25μm) capillary column (Restek, Interscience, Louvain-la-neuve, Belgium). Samples were analyzed for 7 PCDDs, 10 PCDFs and 3 cPCBs using WHO TEFs [3].

Results and Discussion
The representative mean TEQ value for all samples was 30.2 ±11.5 pg TEQ/g of fat. The relative contribution of PCDDs was 15.5 ±6.1 pg TEQ/g of fat (51%) and 14.8 ±5.6 pg TEQ/g of fat (49%) for PCDFs. If cPCBs are included in the TEQ, the mean value is 40.9 ±14.9 pg TEQ/g of fat, the relative contributions are represented in Fig. 1. This TEQ profile is similar to the profile observed in an other study reporting cPCBs values [4]. These values actually include both primiparae and multiparae mothers. If only primiparae are considered (mothers between 20 and 33 years old, mean age 28.2 ±3.6, samples collected between week 1 and week 22 after delivery, lipid content range of 0.5%-5.9%), the mean TEQ value for PCDD/Fs is 28.6 ±9.4 pg TEQ/g of fat, which does not make a significant difference with the previous value. This slightly lower value for primiparae mothers is probably due to the combination of such parameters as donor's age and duration of breast-feeding. The mean value obtained in this study is higher than the one obtained in a recent study carried out in a border country where the mean value was 19.6 pg TEQ/g of fat [5]. Even if this was obtained for a rather rural area and if different analytical methods have been used, the present results show that levels in Belgium tend to be higher than in neighbouring countries as previously reported [6,7].

The congeners pattern for PCDD/Fs and cPCBs is illustrated in the Fig.2. This congener distribution is typical for breast milk issued from industrialized countries [8,9]. The most prominent PCDDs congeners being the more chlorinated such as 1,2,3,6,7,8-HxCDD and OCDD on a concentration basis. For the PCDFs, a decrease in concentration is observed coming from the less chlorinated congeners such as 2,3,4,7,8-PeCDF to 1,2,3,4,7,8,9-HpCDF and OCDF which were even no present in samples or in concentration lower to the LOQ. For cPCBs, PCB-126 is more abundant than PCB-77 and PCB-169. Variations inside the cPCBs distribution is however
dependent of the lactation period and can significantly vary from a sample to an other, accounting for the fairly elevated standard deviation observed in this study [10].

Over the analyzed samples, two multiparae mothers of 37 (6th baby) and 39 (4th baby) years old breast-feeding their babies (both of them having twin) for respectively 624 and 897 days were not included in the mean calculations. These two particular cases were however of interest since they constituted exceptional cases of breast-feeding mothers. These samples actually illustrate previously reported results concerning the evolution of POPs levels following consecutive lactations and breast-feeding duration after delivery [11,12,13]. Both mothers, which were still producing milk containing fairly high amount of fat (4.4% and 3.2%, respectively), presented very low levels (11.8 and 2.0 pg TEQ/g of fat, respectively). One can also note that the congener distribution of these samples is the same than the mean one.

Finally, one sample (excluded from the mean calculations) was completely out of the range (74.5 pg TEQ/g of fat) without any significant differences in the donor's parameters. The questionnaire completed by this volunteer did not indicate any particular or occupational exposure to PCDD/Fs and the pattern was the same but all the congeners were present in higher concentration.

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Conclusions
These results seem to indicate relatively higher background levels for the studied population. However, it's clear that data obtained for breast milk are quite difficult to compare due to the number of parameters which have an effect on the PCDD/Fs content. Further studies using similar analytical tools and procedures are needed to increase the number of results and to allow easier inter-comparisons between laboratories in order to permit continuous monitoring of dioxins levels in breast milk [14,15]. Since levels measured in nonoccupationally exposed women are still relatively high and that they can not only be attributed to dietary habits, continuous efforts have to be done to reduce emission from industry in order to reduce general population body burden and preserve this first choice quality food for newborn infants.

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