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Nonylphenol and octylphenol in human breast milk

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ABSTRACT

Human milk is the most important form of nourishment for newborn children. Its consumption is strongly recommended by health authorities also for other important advantages. Unfortunately, in the last three decades a great number of investigations have shown the occurrence of several environmental contaminants in human milk, especially those with lipophilic properties.

This study investigates the presence of nonylphenol, octylphenol (OP), nonylphenol monoethoxylate (NP1EO) and two octylphenol ethoxylates (OPEOs) (namely OP1EO and OP2EO), in human breast milk of Italian women. NP was the contaminant found at the highest levels with mean concentrations of 32 ng/mL, about two orders of magnitude higher than OP (0.08 ng/mL), OP1EO (0.07 ng/mL) and OP2EO (0.16 ng/mL). In the group of study a positive correlation among fish consumption and levels of NP in the milk was observed, in accordance with the evidence that seafood represents one of the most important sources of exposure to this group of contaminants in Italy.

On the basis of the concentrations found in the breast milk samples, a maximum NP daily intake of 3.94 μg/kg/day can be calculated, which is close to the Tolerable Daily Intake (TDI) of 5 μg/kg body weight (bw) proposed by the Danish Institute of Safety and Toxicology.

In the cases of OP no TDI is available, but its intake is at least six orders of magnitude lower than the NOAEL of 10 mg/kg/day derived from a two generation study on rats.

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1. Introduction

Human milk is the most important form of nourishment for newborn children. Its consumption is strongly recommended by health authorities also for other important relevant advantages (WHO and FAO, 2007). Indeed, breastfeeding has been associated with immunological benefits conferred to newborn children who experience fewer respiratory and ear infections, reduced child mortality and other health advantages which extend into adulthood (WHO and FAO, 2007).

On the other hand in the last three decades a great number of investigations has shown the occurrence of several persistent organic pollutants (POPs) in human milk from different parts of the world (Fabietti et al., 2004; Kunisue et al., 2006; Lederman, 1996; Paumgartten et al., 2000; Zanieri et al., 2007). Due to their physico-chemical characteristics, POPs biomagnify along the food chain (Wolkers et al., 2006, 2004) and reach the highest concentrations in tissues with high fat contents. During the lactation period, maternal body fat is mobilized together with residues of contaminants and incorporated into breast milk (Nickerson, 2006). It is well known that lactation is an important route for contaminants' excretion (Jonker et al., 2005; Rogan and Gladen, 1985; Vrecl et al., 2005). Infants' blood levels of polychlorinated dibenzo-dioxins (PCDDs) and furans (PCDFs) 1.5–3.6 times higher than those found in maternal blood at the end of the first lactation year, have been reported (Abraham et al., 1998). This means that these contaminants have been accumulated as a consequence of milk ingestion. Indeed in infants during the first 3–6 months, milk is the only source of nourishment, furthermore, with respect to adults, they endow some physiological characteristics of neonates and infants which can enhance the absorption of lipophilic contaminants (de Zwart et al., 2004).

The exposure to POPs occurring in human milk has been associated with effects on the hypothalamic-pituitary-thyroid regulatory system (Koppe et al., 1991; Pluim et al., 1994). In particular PCBs and some PCDDs induce alteration of the thyroid hormone status (Huisman et al., 1995).

Slightly elevated concentrations of endocrine disrupters in milk of mothers with a seafood-rich diet have been associated with adverse effects on neurological development, foetal and postnatal growth, and memory functions on breastfed infants (Jacobs et al., 1996; Lunden and Noren, 1998). Most of these contaminants may interfere with the endocrine system.

A group of priority environmental contaminants belonging to endocrine disrupting chemicals is that of alkylphenols (APEs). They are used as non-ionic surfactants in a number of industrial, household application and personal care products; nonylphenol (NP) has even been used as the active substance in spermicides in contraceptive

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applications (Brooke et al., 2005). NP and octylphenol (OP) bind to estrogen receptors and can block or alter endogenous estrogen functions in various reproductive and developmental stages (Hong et al., 2004; Korach et al., 1991). In a study on rats 4-tert-octylphenol and 4-nonylphenol administered by gavage or subcutaneous injection exhibited similar estrogenic potency, higher than that of bisphenol A, a well known endocrine disrupter (Laws et al., 2000).

After treatment with high doses of APEs, the expression of a cytosolic calcium binding protein (calbidin_D_{9k}, CaBP-9k) in rats resulted in a significant increase of this protein in neonatal uterus, suggesting that maternally injected estrogenic compounds may transfer to neonates trough breast milk and affect uterine functions (Hong et al., 2004).

A number of studies have been conducted on the possible association between human health and exposures to APEs, but no clear conclusions have been reached so far from epidemiological ones. The majority of these refer to NP and a few to the other APEs (Nielsen et al., 2000). Among the available toxicological investigations on NP (European Commission—Joint Research Centre et al., 2002), of particular interest is an oral three-generation study in rats, from which the lowest observed adverse effect level (LOAEL) of 15 mg/kg body weight (bw)/day was identified, associated with renal alterations in both sexes (Cunny et al., 1997). A No Observed Adverse Effect Level (NOAEL) of 40 mg/kg bw/day (reduced weight) has been reported from a 90-day feeding study in rats both for nonylphenol 4-ethoxylated (NP4EO) and NP9EO. In a study on dogs, after a technical mixture of NPEOs was orally administered, cardiotoxicity was observed at all the tested doses, even at the lowest of 40 mg/kg/day (Nielsen et al., 2000). In a two generation study on rats, after oral administration of OP, a NOAEL of 10 mg/kg bw/day was reported (Tyl et al., 1999). These studies have been used by the Danish Environmental Agency to derive the Tolerable Daily Intakes (TDI) of 5 and 13 μg/kg body weight for NP and NPEO, respectively (Nielsen et al., 2000).

Diet seems to be the major source of human APEs' intake. In a diet survey in Germany NPs was found in all the investigated food samples with concentrations ranging from 0.1 up to 19.4 μg/kg (Guenther et al., 2002); NP concentrations from 5.8 to 235.8 μg/kg were determined in Taiwan foodstuff samples (Lu et al., 2007). Diet is strongly influenced by its composition, especially with reference to the relative importance of seafood (Ferrara et al., 2005, 2001). Indeed, an NP mean concentration of 354 ng/g in Italian seafood has been reported (Ferrara et al., 2005).

The number of investigations on APEs levels in human milk is very limited (Guenther et al., 2002; Otaka et al., 2003; Ye et al., 2006) and none of them refer to Italian women. The aim of the present study was to define the occurrence of five APEs in milk of Italian women with different diet habits, and to assess the relative risk posed to breastfed infants.

2. Materials and methods

Ten samples of human mature milk, collected during the whole day (24 h) from both breasts, were supplied by children's Hospital "Bambino Gesù" of Rome. The samples were immediately transported to the laboratory inside isothermal bags cooled with dry ice, and there stored at −20 °C until analyses. Each sample was accompanied by a card with the birth date of the mother, information about her job, place of residence (classification of the area, nearness to factories, intensity of road traffic), diet in last 12 month before sampling, and drug use (Table 1).

The following compounds were analysed: nonylphenol (NP), NP monoethoxylate (NP1EO), octylphenol (OP) and two OP ethoxylates (OPEOs) namely OP mono- (OP1EO) and di-ethoxylates (OP2EO). 4-OP, OP1EO, OP1-2E, NP and NP1E, were supplied from ChemService (West Chester, PA, U.S.A.). Dodecylbenzene (DB) was supplied from Fluka (Bucs, SG, Switzerland) and was used as internal standards to perform quantification of the analytes. Their analysis was performed according to the following procedure. Lipids were extracted from human milk according to the official method of the European Community (Folch et al., 1957). Briefly, 10 g of milk were extracted with 25 mL of NH_3 at 25% v/v, 10 mL of ethanol and 25 mL of ethyl ether. After shaking, 25 mL of petroleum ether was added to the sample and the funnel shaken again for 30 min. The upper phase was then recovered in a pre-weighed bowl and the aqueous phase re-extracted twice, with 15 mL ethyl ether and 15 mL of petroleum ether. APE were extracted according to the method of Wahlberg et al. (1990) without the final derivatization. GC-MS analyses were performed on a 6890 plus gas chromatograph coupled with a 5973 network ion trap mass spectrometer (Agilent Instrumentation Inc., Santa Clara, CA, U.S.A.). Analytes were separated in a DB-XLB 30 mt×0.25 mm×0.25 μm column (J&W, Agilent Technologies, Santa Clara, CA, U.S.A.). The column operated at 80 °C (hold 2 min.) to 160 °C at 20 °C/min., then to 240 °C at 8 °C/min., then to 300 °C (hold 10 min.) at 5 °C/min. The splitless injector was set at 280 °C, the transferline at 280 °C and the source was kept at 150 °C. Helium was used as carrier gas (1 mL/min. constant flow) and the electron impact ionization was set at 70 eV. The identification of APEs in the samples was performed with the instrument used in single ion monitoring (SIM). APEs were monitored following the masses (m/z) specific for each compound, and fragment ions were chosen according to the most abundant ions in each oligomer (Table 2). Calibration standard solutions were used to generate response factors in relation to the internal standard. The quantification of APEs was performed using the internal calibration method based on five-point calibration curve. The calibration curves for NP and OP showed high linearity ($R^2 \geq 0.991$). The limits of quantification (LOQ) and the limit of detection (LOD) were calculated adding 10 or 3 times the blank standard deviation (σ) respectively, to the quantitative values of an analytes-free commercial

Table 1
Synthetic view of the questionnaire given to the breastfeeders

Personal information	
	Field of occupation
	Job position
	Drugs/Food supplements
Residence information	
	Area classification
	Proximity of industries
Eating habits	
	Meat oriented
	Fish oriented
	Vegetarian
	Frequency of fish/meat meals
	Most consumed fish species

Table 2
GC/MS fragment ions masses (m/z) of internal standard, octyl-, nonylphenol and their respective ethoxylates

Compound	SIM ion m/z				Detection limit	Quantitation limit	Recoveries %
					ng/mL	ng/mL	Mean±SD
4-OP	107	135			0,019	0,023	82±12
OP1EO	179	250			0,036	0,049	78±19
OP2EO	223	296			0,076	0,112	70±19
$DB_{(internal\ standard)}$	92	246			0,16	0,18	89±6
NP tech	107	121	135	149	9,8	12,1	80±18
NP1EO	165	179	193	264	45,1	86,9	59±15

4-OP: 4-tert-octylphenol; OP1-2E: 4-tert OP mono, di-ethoxylates; NP tech: cluster of peak of 4-nonylphenol isomers; NP1E: clusters of peaks of 4-NP monoethoxylates; DB: dodecylbenzene.

Table 3
Levels of alkylphenols in human breast milk in Italian breast milk samples

	NP ng/ml	OP ng/ml	OP1EO ng/ml	OP2EO ng/ml	Fat content %
LM 1	42.9	nd	0.05	0.17	4.3
LM 2	51.4	0.21	0.10	0.14	5.0
LM 3	15.5	0.10	0.05	0.11	4.6
LM 4	14.7	nd	0.09	0.13	4.0
LM 5	13.4	nd	0.08	0.14	3.5
LM 6	32.8	0.12	0.08	0.12	3.0
LM 7	41.4	0.15	0.07	0.14	2.6
LM 8	35.6	0.02	0.08	0.16	3.2
LM 9	15.9	0.05	0.05	0.19	4.8
LM 10	56.3	0.18	0.09	0.27	3.0
Mean	32.00	0.12	0.07	0.16	3.78
S.D.	16.2	0.07	0.02	0.05	0.85
CV	50.7%	58.1%	27.3%	29.6%	22.4%
Min	13.4	nd	0.05	0.11	2.6
Max	56.3	0.21	0.10	0.27	5.0

nd: < limit of detection (0.01 ng/ml).
CV: coefficient of variation.
note: NP1EO always at levels below the sensitivity method.