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# Nonylphenol and octylphenol in adipose tissue of women in Southern Spain

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ABSTRACT

Alkylphenols (APs) and AP ethoxylates are environmental contaminants with endocrine disrupting activities in wildlife and humans. They have been largely used in industrial, agricultural, and domestic applications. Despite strong concerns about the consequences of human exposure to endocrine disrupters, little information is available on the presence in humans of compounds such as APs. The aim of the present study was to determine 4-nonylphenol (NP) and 4-octylphenol (OP) residues in adipose tissue of non-occupationally exposed women living in Southern Spain. NP was detected in 100% ($n = 20/20$) and OP in 23.5% ($n = 4/20$) of samples, with median levels of 57 and 4.5 ng $g^{-1}$ adipose tissue, respectively. Body mass index emerged as a determinant of exposure since it was associated with NP levels ($p = 0.041$). Adipose tissue NP and OP levels are similar to the few data previously published in other countries. This is the first report on NP and OP levels in a population in Southern Spain. Further research is needed to determine trends in human exposure to these compounds and to investigate their consequences.



## 1. Introduction

Alkylphenol ethoxylates (APEs) have been the most widely used classes of non-ionic surfactants in industrial, agricultural, and domestic applications and are mainly introduced to the environment from wastewaters (Ying et al., 2002). Nonylphenol ethoxylates represent around 80% of APEs, while octylphenol ethoxylates make up most of the remaining 20% (White et al., 1994). In sewage treatment plant (STP) effluents, APEs are degradated to shorter-chain and more resistant alkylphenols (APs), such as 4-nonylphenol (NP) and 4-octylphenol (OP). Many studies have demonstrated the ubiquity of APs in environmental media, especially in the aquatic environment (Ying et al., 2002). Thus, NP and OP have been detected in sediment, STP effluents, surface water, and drinking water in different countries (Ying et al., 2002; Soares et al., 2008). In Spain, studies have mainly focused on overexploited Mediterranean rivers (Sole et al., 2000; Petrovic et al., 2003; Lavado et al., 2004; Cespedes et al., 2005) and on the impact of these compounds on aquatic biota (Petrovic et al., 2002; Carballo et al., 2005).

Humans are largely exposed to APs by the intake of contaminated foods and drinking water. Both NP and OP have been detected in different foods (Guenther et al., 2002; Yang and Ding, 2005; Lu et al., 2007), and it has been suggested that NP and OP present in plastic containers and wrappings may migrate into foods and drinking water (Toyo'oka and Oshige, 2000; Loyo-Rosales et al., 2004). The Danish Environmental Agency proposed 5 μg $kg^{-1}$ body weight as the Tolerable Daily Intake (TDI) of NP (Nielsen et al., 2000) and, although no TDI has been established for OP, a No Observed Adverse Effect Level (NOAEL) of 10 mg $kg^{-1}$ body weight/d was reported by Tyl et al. (1999) based on a two-generation rat study. Other routes of human exposure to these compounds include contact with personal care products and detergents (Talmage, 1994) and the use of spermicides in contraceptives (Brooke et al., 2005). It has been suggested that humans are less exposed to APs than to other ED compounds, such as bisphenol A (BPA) (Calafat et al., 2005, 2008), which have a higher presence in the domestic environment (Wilson et al., 2007).

APs have long been reported to elicit estrogenic activity (Soto et al., 1991). Different *in vitro* (vitellogenin gene expression, E-screen and yeast-screen) and *in vivo* studies (rats and rainbow trout) conducted to assess their effects on endocrine pathways have demonstrated that APs can alter developmental and reproductive functions in animals and humans (reviewed by: Müller et al., 1998a; Bonefeld-Jorgensen et al., 2007). Resulting concerns about their impact on human health led to the prohibition by the European Union of APEs in all detergent applications by 2000 (European Commission-Joint Research Centre, 2002). NP ethoxylates have been replaced by other surfactants, mainly alcohol ethoxylates, in most European countries and in Canada and Japan. However, they are still used as an emulsifier in pesticides and as an antioxidant in plastics (European Commission-Joint Research Centre, 2002).

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Although much is known about the estrogenic effects of APs and their occurrence and fate in the environment, there have been few reports on their concentrations in human tissues (Ademollo et al., 2008; Calafat et al., 2005, 2008; Chen et al., 2005, 2008; Mao et al., 2004; Müller et al., 1998b; Smeds and Saukko, 2003; Tan and Mohd, 2003). The present study is part of an extensive characterization of the exposure of women in Southern Spain to environmental chemicals. Data have already been published on the presence in the same population of organochlorine (OC) pesticides (Cerrillo et al., 2006), polybrominated diphenyl ethers (PBDE), polybrominated biphenyls (PBB) (Fernandez et al., 2007a), BPA and chlorinated derivatives ($Cl_x$BPA) (Fernandez et al., 2007b), polychlorinated biphenyls (PCBs) (Fernandez et al., 2008), and polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) (Lopez-Espinosa et al., 2008). In order to expand these reference data for biomonitoring planning, the present study was designed to investigate the concentration of NP and OP residues in the adipose tissue of these women and to compare levels with other populations.

## 2. Materials and methods

### 2.1. Subjects

NP and OP were determined in 20 human adipose tissue samples collected from women undergoing surgery for malignant and benign diseases at the San Cecilio University Hospital of Granada (Spain) during 2003. All of these women lived in Granada. After signing informed consent, the women were interviewed by a trained interviewer using a structured questionnaire on their socio-demographic characteristics, reproductive history, and life-style factors. All women reported a mixed diet including meat and fish and no occupational exposure to AP compounds. The study was approved by the Ethics Committee of the San Cecilio University Hospital.

### 2.2. Sample collection and storage

Adipose tissues were placed into a glass vial on ice, coded, and frozen to −86 °C, always within 30 min of being excised, and samples were stored at the same temperature at the Laboratory of Medical Investigations in the San Cecilio University Hospital of Granada (Spain) until their dispatch for analysis to the Department of Analytical Chemistry in the University of Granada, where NP and OP concentrations were determined.

### 2.3. Reagents and standards

All reagents were of analytical grade unless otherwise specified. Water (18.2 MΩ $cm^{-1}$) was purified with a Milli-Q plus system (Millipore, Bedford, USA). Methanol, hexane, ethanol, ethyl acetate, and diethyl ether were supplied by Panreac (Barcelona, Spain). 4-Nonylphenol (NP) and 4-octylphenol (OP) were supplied by Sigma–Aldrich (Madrid, Spain). Stock standard solutions (100 mg $L^{-1}$) of each chemical compound were prepared in *n*-hexane and stored in dark glass bottles at 4 °C until use, remaining stable for at least three months. These solutions were used to spike the adipose tissue samples. SPE cartridges $C_{18}$ AccuBONDII ODS-$C_{18}$ were supplied by Agilent Technologies, Waldbron, Germany). A mixture of *N,O*-bis(trimethylsilyl) triflouroacetamide (BSTFA) and trimethylchloro silane (TMCS) (99:1, v/v), supplied by Supelco (Bellefonte, PA, USA), was used as silylation reagent.

### 2.4. Sample preparation, extraction, and derivatization

Two hundred milligrams of adipose tissue were homogenized with 6 mL of *n*-hexane. Then, 2 mL of acetonitrile were added to the *n*-hexane solution. After shaking for 3 min, the phase containing the acetonitrile was separated and dried under a gentle stream of nitrogen. Prior to the extraction, adipose tissue samples were spiked with bisphenol F (BPF) as internal standard. SPE cartridges were conditioned with 3 mL of diethylether, 3 mL of methanol, and 3 mL of deionized water on an SPE manifold at a rate of 1–2 mL $min^{-1}$.

Sample extracts were resuspended using 15 mL of deionized water and passed through SPE cartridges at a flow rate of 1–2 mL $min^{-1}$. SPE was carried out in a Supelco 12-port vacuum manifold connected to a Supelco vacuum tank and a vacuum pump. Then, cartridges were dried under vacuum for 20 min. APs were eluted from sorbents with 3 mL of a mixture of diethyl ether/methanol (9:1 v/v) at a flow rate of 1 mL $min^{-1}$. Finally, eluents were evaporated to dryness under a stream of nitrogen, and 120 μL of ethyl acetate and 30 μL of BSTFA/TMCS (1:1, v/v) were added to the reaction vial in order to resuspend the residue and carry out the derivatization. Next, vials were closed and heated at 60 °C for 30 min. Once the derivatization process was completed, 2 μL of the reaction mixture was injected into the gas chromatography–mass spectrometry (GC–MS) system.

### 2.5. Apparatus: gas chromatographic–mass spectrometric analysis

GC–MS analysis was performed using a 6890 Agilent (Agilent Technologies, Wilmington, USA) gas chromatograph with a 7683 series injector and a quadruple mass filter 5976 network mass selective detector (MSD) following a previously published method (Ballesteros et al., 2006). A ZB-5 MS Zebron capillary column (30 m × 0.25 mm i.d.; 0.25 μm film thickness) from Phenomenex (Torrance, CA, USA) was used for qualitative determinations, applying the selected ion-monitoring (SIM) mode for quantitative determinations. The injector port of the GC was set at 280 °C. Samples were automatically injected using the splitless-injection mode. The transfer line of the GC to the MS was set at 270 °C, the electron impact (EI) ion source of the MS at 250 °C, and the electron impact (EI) ion source of the MS at 250 °C. The ionization energy was 70 eV. The GC oven temperature program was: initial temperature at 120 °C for 2 min and then increased to 230 °C at 30 °C $min^{-1}$ and maintained at 230 °C for 2 min, then increased to 270 °C at 40 °C $min^{-1}$ and maintained at 270 °C for 6 min. The carrier gas was high-purity helium (99.999%) with a constant flow of 1 mL $min^{-1}$. A solvent delay time of 4 min was used to protect the ion multiplier of the MS instrument from saturation. Fig. 1 shows a chromatogram of a standard mixture of NP, OP, and BPF in a spiked adipose tissue sample.

In SIM mode, the qualifier ions for NP and OP were 179 *m/z* and 207 *m/z*, respectively, corresponding to loss of OTMS group. The base peaks for NP and OP were 292 and 278 *m/z*, respectively, corresponding to molecular ion. Fragments 263 (OP) and 277 (NP) *m/z*

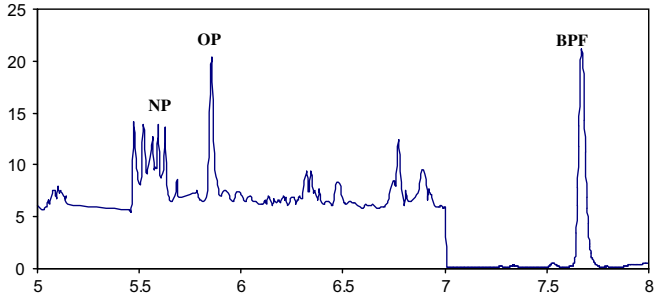


**Fig. 1.** Chromatogram of a standard mixture of 4-nonylphenol (NP), 4-octylphenol (OP), and bisphenol F (BPF) in a spiked adipose tissue sample.

were also used, corresponding to loss of methyl group. The ions used for the internal standard, BPF, corresponded to *m/z* 344 (molecular ion), 329 (loss of methyl group) and 179 (loss of OTMS groups). Fig. 1 shows a chromatogram of a standard mixture of NP, OP, and BPF in a spiked adipose tissue sample.

### 2.6. Analytical performance

Six concentrations were prepared for the calibration, applying the above extraction procedure (duplicate preparations and triplicate analyses of the central calibration sample). Calibration curves were constructed plotting the analyte/internal standard peak area ratio against the concentration of analyte. Linearity of the calibration graphs was tested using the lack-of-fit test, according to the Analytical Methods Committee (1994). Recoveries of tested compounds were 95–105% in all cases. Limits of detection (LOD) were 10.5 and 2.8 ng $g^{-1}$ for NP and OP, respectively. Recoveries for internal standards were 70%.

### 2.7. Data analysis

NP concentrations were transformed to a natural logarithm scale to improve the normal distribution of concentrations. Linear regression analyses for NP (dependent variable) were performed to examine its association with age (yrs) and body mass index (BMI) (kg $m^{-2}$). Age and BMI were treated as continuous variables in the models. OP concentrations were not evaluated in the regression analysis because of the low frequency of their detection (>LOD = 23.5%). Statistical analysis was performed using SPSS Version 15.0 statistical software (SPSS Inc., Chicago, IL).

## 3. Results and discussion

### 3.1. Characteristics of study population

The mean age (standard deviation [SD]) of this female population was 59.7 yrs (14.1), ranging from 24 to 81 yrs. Mean BMI (SD) was 31.8 kg $m^{-2}$ (11.5), ranging from 19.1 to 65.0 kg $m^{-2}$. This elevated mean BMI is consistent with previous reports (Fernandez et al., 2007a; Sotillo et al., 2007) of a higher BMI in women from Southern Spain than in women from other Spanish regions and central European countries. Thus, Sotillo et al. (2007) reported a mean BMI of 27.6 for 40–49-yr-old and 30.2 kg $m^{-2}$ for 50–60-yr-old in women from the same region, similar to the mean of 32.4 kg $m^{-2}$ for the over-40-yr-old in the present study. All women were Caucasians and none were occupationally exposed to APs.

### 3.2. Levels of NP and OP in adipose tissue samples and comparison with other studies

Similar to previous reports, the frequency of detection in adipose tissue samples of NP (100%) was higher than that of OP (23.5%) (Table 1) (Inoue et al., 2000; Tan and Mohd, 2003; Chen et al., 2005; Ademollo et al., 2008). NP was also present at higher concentrations (median = 57 ng $g^{-1}$ adipose tissue) vs. OP, reaching 567 ng $g^{-1}$ adipose tissue in one sample (Fig. 2), which may be explained by the more frequent use of NP (European Commission-Joint Research Centre, 2002) and by a higher affinity of NP for lipid fractions (Ohta et al., 2002; Ademollo et al., 2008). Müller et al. (1998b) reported that NP is rapidly distributed into the lipid phase of the human body within 2 h of its oral administration. This lipophilicity may contribute to the bioaccumulation of NP observed in algae, fish, and aquatic birds living in or near a contaminated river (Ahel et al., 1993), which can also be attributed to the saturation of detoxification pathways from excessive exposure (Certa et al., 1996).

Although concentrations of NP and OP in different types of human sample have been reported in a few countries, their presence in adipose tissue is poorly documented (reviewed in Table 2). Moreover, comparisons are hampered by differences in study design (e.g., number of samples), country of origin, biological matrix, and the AP isomer studied. Median NP concentrations found in women from Southern Spain were similar to those reported by Müller et al. (1998b) in human adipose tissue from cadavers ($n = 25$) in Switzerland (37 vs. 57 ng $g^{-1}$ adipose tissue in present study). In a Finnish study (Smeds and Saukko, 2003), OP was only detected in two adipose tissue samples ($n = 2/13$: 1.6 and 4.6 ng $g^{-1}$ adipose tissue, respectively) at similar concentrations to those reported by Müller et al. (1998b) (range, 0.58–4.1 ng $g^{-1}$ adipose tissue), and lower than OP concentrations determined in our study (range, 4.2–8.6 ng $g^{-1}$ adipose tissue in 4/20 samples).

There is more information on AP concentrations in human blood, breast milk, and urine than on concentrations in adipose tissue (Table 2). Tan and Mohd (2003) reported that NP was the most frequent ED (86%) in Malaysian cord blood ($n = 180$), finding 4-*n*-OP and 4-*tert*-OP at lower concentrations vs. NP in 53% and 17% of samples, respectively. In Taiwan, NP and OP were found in all plasma samples ($n = 33$) from a population occupationally exposed to these compounds (Chen et al., 2005), and NP was detected in 26% and 76% of umbilical cords from populations in Central

**Table 1**
Concentrations of 4-nonylphenol and 4-octylphenol (ng $g^{-1}$ adipose tissue) in adipose tissue samples from women living in Southern Spain.

| | *n* (%) | >LOD | | | |
|---|---|---|---|---|---|
| | | Mean (SD) | 25th | Median | 75th |
| 4-Nonylphenol | 20/20 (100) | 82 (127) | 40 | 57 | 69 |
| 4-Octylphenol | 4/20 (23.5) | 5.5 (2.1) | 4.2 | 4.5 | 7.7 |

*n* (%) = Number of subjects (percentage of detection); LOD = limit of detection; SD = standard deviation.

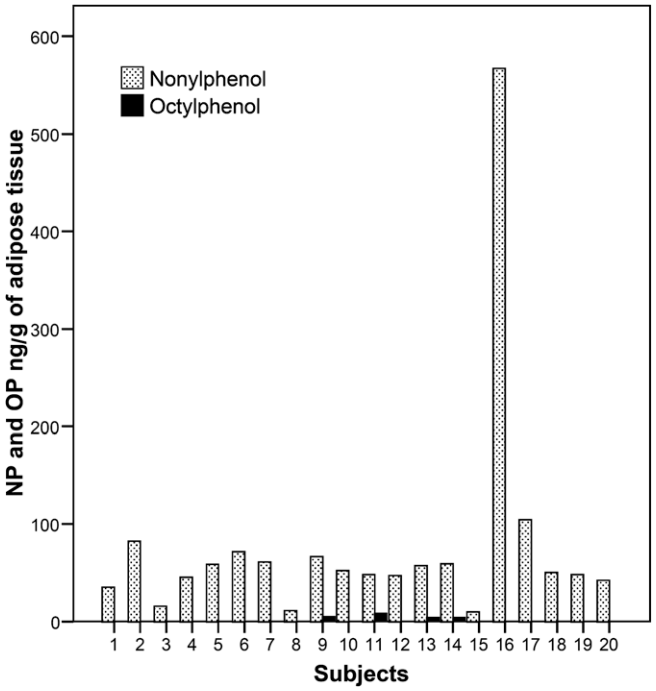


**Fig. 2.** Individual concentrations (ng $g^{-1}$ adipose tissue) of 4-nonylphenol (NP) and 4-octylphenol (OP) in adipose tissue samples.

**Table 2**
Reported concentrations of 4-nonylphenol (NP) and 4-octylphenol (OP) in human samples from different countries.

| Study | Location | Study population | *N* | Sample | NP | | | | OP | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | % >LOD | Median | Min | Max | % >LOD | Median | Min | Max |
| Present study[a] | Southern Spain | Women, 24–81 yrs | 20 | Adipose tissue | 100 | 57 | 10 | 567 | 23.5 | 4.5 | nd | 8.6 |
| Müller et al. (1998b)[a] | Switzerland | 15 Males, 10 females, 3–100 yrs | 25 | Adipose tissue (cadavers) | 100 | 37 | 19.8 | 84.4 | – | – | 0.58 | 4.07 |
| Smeds and Saukko (2003)[a] | Finland | 7 Males, 6 females, 43–90 yrs | 13 | Adipose tissue (cadavers) | – | – | – | – | 15.4 | 1.6/4.6 | – | – |
| Inoue et al. (2000)[b] | Japan[c] | Healthy people | 3 | Blood (plasma) | – | – | 0.5 | 1.0 | – | – | nd | <0.25 |
| Tan and Mohd (2003)[b] | Malaysia | Women | 180 | Cord blood (plasma) | 86 | – | nd | 15.2 | 17[g] | – | nd | 1.15 |
| Kawaguchi et al. (2004)[b] | Japan[c] | Healthy people, 24–25 yrs | 3 | Blood (plasma) | 100 | – | 0.2 | 0.3 | 100 | – | 0.1 | 0.2 |
| Chen et al. (2005)[a] | Taiwan | Housekeepers | 33 | Blood (plasma) | 100 | 53 (40)[d] | 12.1 | 285 | 100[g] | 16 (2.8)[d] | 12.68 | 23.9 |
| Liu et al. (2006)[b] | China[c] | Healthy people | 10 | Blood (serum) | – | – | – | – | 20 | 0.2 | 0.2 | 0.2 |
| | | Women, Central Taiwan | 124 | Cord blood (plasma) | 26 | 0.9 | nd | 182 | – | – | – | – |
| | | Women, Northern Taiwan | 50 | Cord blood (plasma) | 76 | 42 | nd | 211 | – | – | – | – |
| Chen et al. (2008)[a] | Taiwan | Expectant women, 18–40 yrs | 42 | Blood (plasma) | 52 | 5.7 | nd | 268 | – | – | – | – |
| | | Women, 18–40 yrs | 42 | Cord blood (plasma) | 52 | 2.9 | nd | 100 | – | – | – | – |
| Otaka et al. (2003)[a] | Japan[c] | Women | 3 | Breast milk | 100 | – | 0.65 | 1.4 | – | – | – | – |
| Ye et al. (2006)[b] | US | Women | 20 | Breast milk | – | – | – | – | 25[g] | 2.7[e] | nd | 7.6 |
| Ademollo et al. (2008)[b] | Italy | Women | 10 | Breast milk | 100 | 34 | 13.4 | 56.3 | 70 | 0.12 | nd | 0.2 |
| Kuklenyik et al. (2003)[b] | US | People painting their homes | 30 | Urine | 0 | nd | – | – | 35[g] | – | 0.4 | 13.9 |
| Kawaguchi et al. (2004)[b] | Japan[c] | Healthy people, 22–24 yrs | 5 | Urine | 0 | nd | – | – | 0 | nd | – | – |
| Mao et al. (2004)[b] | China | Males, 21–29 yrs | 10 | Urine | 50 | 0.38 (0.8)[d] | nd | 2.3 | – | – | – | – |
| | | Females, 21–29 yrs | 10 | Urine | 70 | 0.05 (0.05)[d] | nd | 0.14 | – | – | – | – |
| Calafat et al. (2005)[b] | US | ⩾6 yrs (NHANES) | 371 | Urine | 51 | <0.1 (nd–1.6)[f] | – | – | – | – | – | – |
| | | Housekeepers | 29 | Urine | 97 | 6.2 (4.8)[d] | nd | 36.7 | 83[g] | 6.5 (8.7)[d] | nd | 71.5 |
| Chen et al. (2005)[b] | Taiwan | Textile workers, pre-shift | 40 | Urine | 90 | 21 (18)[d] | nd | 71.9 | 50[g] | 1.4 (1.7)[d] | nd | 7.02 |
| | | Textile workers, post-shift | 40 | Urine | – | 38 (46)[d] | – | – | – | 2.1 (2.5)[d] | – | – |
| Kawaguchi et al. (2005)[b] | Japan[c] | Healthy people | 5 | Urine | 100 | – | 1.04 | 2.0 | 20[g] | – | nd | 0.05 |
| Kawaguchi et al. (2006)[b] | Japan[c] | Healthy people, 22–25 yrs | 6 | Urine | 100[h] | – | <1.1 | 2.1 | – | – | – | – |
| Calafat et al. (2008)[b] | US | ⩾6 yrs (NHANES) | 2,517 | Urine | – | – | – | – | 57[g] | 0.3 (nd–0.4)[f] | – | – |

*N* = sample size; LOD = limit of detection; nd = not detected; NHANES = The National Health and Nutrition Examination Survey.
[a] ng/g.
[b] ng/mL.
[c] Methodological studies.
[d] Mean (standard deviation).
[e] Mean.
[f] Median (95% confidence interval).
[g] tertiary-OP.
[h] NP-glucuronide.

($n$ = 124) and North ($n$ = 50) Taiwan, with higher concentrations in residents of metropolitan areas (Chen et al., 2008). Trace levels of APs in human blood have been detected in various Japanese and Chinese studies (Inoue et al., 2000; Kawaguchi et al., 2004; Liu et al., 2006). Reports on breast milk NP levels have ranged from 0.65–1.4 ng $g^{-1}$ in Japan ($n$ = 3) (Otaka et al., 2003) to 13.4–56.3 ng $mL^{-1}$ in Italy ($n$ = 10) (Ademollo et al., 2008), with OP levels ranging from non-detected (nd) to 0.2 ng $mL^{-1}$ in Italy (Ademollo et al., 2008) and from nd to7.6 ng $mL^{-1}$ for *tertiary*-OP (*tert*-OP) in the USA ($n$ = 20) (Ye et al., 2006).

Urinary AP levels were measured in two large NHANES (The National Health and Nutrition Examination Survey) reports, finding NP in 51% and *tert*-OP in 57% of US individuals (Calafat et al., 2005, 2008), with higher concentrations of *tert*-OP (median = 0.3 ng $mL^{-1}$) than of NP. Lower OP concentrations and similar NP levels to US findings were reported in Japanese studies, although the number of samples was very small (Kawaguchi et al., 2004, 2005, 2006). Urinary OP (range = 0.4–14 ng $mL^{-1}$) but not NP was detected in people painting their homes in US (Kuklenyik et al., 2003). A Taiwanese study (Chen et al., 2005) of textile workers exposed to NP and OP found significantly higher urinary AP levels at the end of the shift (mean NP = 38 ng $mL^{-1}$) compared with pre-shift samples (21 ng $mL^{-1}$). Higher urinary NP levels were found in Chinese males ($n$ = 10; mean = 0.38 - ng $mL^{-1}$) than females ($n$ = 10; mean = 0.05 μg $L^{-1}$), although the frequency of detection was higher in the latter (70 vs. 50%) (Mao et al., 2004). Calafat et al. (2008) also found small sex differences in urinary OP levels (median = 0.3 and 0.2 ng $mL^{-1}$ in men and women, respectively).

### 3.3. Associations of NP and OP levels with age and BMI

We found a significant and negative association between NP concentration and BMI ($\beta$ = 0.05; 95%CI = 0.01, 0.09; $p$ = 0.033; $R^2$ = 0.27), which remained significant after controlling for women's age ($\beta$ = 0.05; 95%CI = 0.02, 0.09; $p$ = 0.041; $R^2$ = 0.27). Previous studies of these women showed non-significant associations between body fat content and concentrations of PCBs, PCDD/Fs, PBDE/PBBs, and BPA in adipose tissue (Fernandez et al., 2007a,b, 2008; Lopez-Espinosa et al., 2008). Interestingly, the most obese woman (65 kg $m^{-2}$) in this series had the highest adipose tissue levels of NP as well as of PCBs (Fernandez et al., 2008), PCDD/Fs (Lopez-Espinosa et al., 2008), PBBs (Fernandez et al., 2007a), and $Cl_2$BPA (Fernandez et al., 2008).

NP levels were not associated with age ($\beta$ = −0.01; 95%CI = −0.04, 0.03; $p$ = 0.81), as reported for PBDEs and PBBs (Fernandez et al., 2007a) and in contrast to findings for other persistent and fat soluble compounds (Fernandez et al., 2007b, 2008; Lopez-Espinosa et al., 2008), although the lack of significance may be attributable to the small sample size. Finally, due to the low frequency of detection of OP ($n$ = 4/20), its association with age and BMI was not evaluated.

A major route of exposure to APs may be diet. Ten years ago, Müller et al. (1998a) estimated that the daily oral intake of NP by non-occupationally exposed people was less than 0.16 mg $d^{-1}$. More recently, Guenther et al. (2002), Thomson et al. (2003), and Lu et al. (2007) reported the presence of OP and especially NP in various foods in Germany, New Zealand, and Taiwan and estimated the daily adult intake of NP to be 7.5, 3.3, and 28 μg $d^{-1}$, respectively. In the German diet survey, NP concentrations in food samples ranged from 0.1 to 19.4 μg $kg^{-1}$, regardless of their fat content (Guenther et al., 2002). The above variations in AP intake may be attributable to differences in food habits, although many of the food samples purchased in local supermarkets were internationally marketed products.

## 4. Conclusions

NP was detected in 100% (median = 57 ng $g^{-1}$ adipose tissue, $n$ = 20/20) and OP in 23.5% (median = 4.5 ng $g^{-1}$, $n$ = 4/20) of the present samples at similar levels to the very few reports on their presence in adipose tissue. Although APs have a lower affinity for lipid fractions in comparison to other ED compounds, adipose tissue is a suitable matrix to determine their concentrations. In addition, there is an extensive body of data on the exposure of these women to different groups of persistent and bioaccumulative compounds as measured in adipose tissue, which may better reflect past exposure and may therefore offer a chronological marker of exposure.

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