Environmental Pollution 207 (2015) 145–151

Contents lists available at ScienceDirect

Environmental Pollution

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

# Prenatal nonylphenol exposure, oxidative and nitrative stress, and birth outcomes: A cohort study in Taiwan

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ARTICLE INFO

*Article history:*
Received 5 June 2015
Received in revised form
22 August 2015
Accepted 23 August 2015
Available online xxx

*Keywords:*
Nonylphenol
Oxidative and nitrative DNA damage
Lipid peroxidation
Birth outcomes
Gestational age

ABSTRACT

Data concerning the effects of prenatal exposures to nonylphenol (NP) and oxidative stress on neonatal birth outcomes from human studies are limited. A total of 146 pregnant women were studied (1) to investigate the association between prenatal NP exposure and maternal oxidative/nitrative stress biomarkers of DNA damage (8-hydroxy-2'-deoxyguanosine (8-OHdG), 8-nitroguanine (8-$NO_2$Gua)) and lipid peroxidation (8-iso-prostaglandin $F_{2\alpha}$ (8-iso$PF_{2\alpha}$), 4-hydroxy-2-nonenal-mercapturic acid (HNE-MA)) and (2) to explore the associations among oxidative stress biomarkers, NP exposure, and neonatal birth outcomes, including gestational age, birth weight, length, Ponderal index, and head and chest circumferences. NP significantly increased the 8-OHdG and 8-$NO_2$Gua levels. All infants born to mothers with urinary 8-OHdG levels above the median exhibited a significantly shorter gestational duration ($B_{adjusted} = -4.72$ days; 95% CI: −8.08 to −1.36 days). No clear association was found between NP levels and birth outcomes. Prenatal 8-OHdG levels might be a novel biomarker for monitoring fetal health related to NP exposure.



## 1. Introduction

In recent years, the maternal transfer of environmental pollutants to neonates has raised much concern. Xenoestrogen exposure may have serious consequences, especially for pregnant women, who are particularly vulnerable, and for their fetuses. Nonylphenol (NP), a degradation product of nonylphenol polyethoxylates (NPEOs), is an intermediate chemical that is widely used for the production of surfactants, detergents, emulsifiers, pesticides, lubricants and oil additives that are used in daily life (Ying et al., 2002). Humans may be exposed to NP not only in drinking water but also via other pathways, including the ingestion of contaminated foods, inhalation of air, and dermal absorption (Ahel et al., 1993; Clark et al., 1992). High NP levels were found in raw and treated water in Taiwan (Chen et al., 2013). The average daily intake of NP in Taiwan (28.04 μg/day) was higher than that in Germany or New Zealand (Guenther et al., 2002; Lu et al., 2007; Thomson et al., 2003). We also reported that the daily intake of NP by three month-old babies via breast milk (8.6 μg/kg bw/day) exceeded the tolerable daily intake of 5 μg/kg bw/day, indicating a potential risk for infants in northern Taiwan (Huang et al., 2014). Our previous study showed that NP was positively detected in cord plasma at significantly higher levels in samples from northern Taiwan (metropolitan Taipei) than in samples from central Taiwan (Chen et al., 2008). Relatively high NP exposure has been identified in metropolitan Taipei. Based on Müller's pharmacokinetic study, the half-life of NP in blood is 2–3 h, and the bioactivity of NP is 20% after oral or intravenous ingestion (Muller et al., 1998). Despite the rapid metabolism of NP, significant NP levels can be detected in samples from mothers or infants, suggesting that pregnant women or infants are repetitively and persistently exposed to NP.

The structure of NP is similar to that of estrogen, and NP has

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http://dx.doi.org/10.1016/j.envpol.2015.08.044
0269-7491/

been shown to bind to estrogen receptors in human estrogen-sensitive MCF7 breast tumor cells and to exhibit weak estrogenic properties (Soto et al., 1991). NP exhibits reproductive toxicity (De Jager et al., 1999; Fan et al., 2001), in addition to developmental, immune and thyroid, and nervous system effects in animals (California EPA 2009). In rodents, gestational NP exposure may influence the body weight and some reproductive organ weight, possibly even inducing neurotoxic and reproductive toxic effects in offspring (Jie et al., 2010, 2013; Kimura et al., 2006); however, very few studies have explored the effects of NP in humans. Other authors have shown that NP can cross the placenta (Balakrishnan et al., 2011; Huang et al., 2014), and maternal NP levels during the second trimester are associated with small for gestational age (SGA), decreased body length at birth and low neonatal weight (Chang et al., 2013; Tsai et al., 2013); however, Tang et al. (2013) found no such associations (Tang et al., 2013). Thus, more evidence is needed.

Several studies have reported that NP induces oxidative/nitrative stress *in vitro* and *in vivo* by generating reactive oxygen and nitrogen species (ROS/RNS) (Gong and Han, 2006; Korkmaz et al., 2011; Okai et al., 2004; Zhang et al., 2008). Free radical-mediated oxidative/nitrative products of DNA and lipids are thought to be the key biomarkers of oxidative/nitrative stress, including products of oxidative and nitrative DNA damage (8-hydroxy-2'-deoxyguanosine (8-OHdG) and 8-nitroguanine (8-$NO_2$Gua)) and lipid peroxidation (8-iso-prostaglandin $F_{2\alpha}$ (8-iso$PF_{2\alpha}$) and 4-hydroxy-2-nonenal-mercapturic acid (HNE-MA)) (Chen et al., 2015). However, little information is available on oxidative/nitrative stress biomarkers that are induced by NP exposure in humans. Monitoring oxidative stress in pregnant women is important for improving our understanding of the connection between oxidative stress and adverse effects in pregnant women and neonates. Increases in oxidative stress in pregnant women, as evidenced by 8-OHdG and 8-iso$PF_{2\alpha}$ levels, have been associated with preeclampsia, preterm birth, low birth weight, birth weight reduction, SGA, and fetal growth restriction (FGR) (Chen et al., 2012; Kim et al., 2005; Longini et al., 2005; Negi et al., 2014; Peter Stein et al., 2008; Potdar et al., 2009); however, the mechanisms involved remain unclear.

For these reasons, we investigated whether prenatal NP exposure is associated with maternal oxidative/nitrative stress biomarkers of DNA damage and lipid peroxidation (including 8-OHdG, 8-$NO_2$Gua, 8-iso$PF_{2\alpha}$, and HNE-MA). Additionally, we explored the associations among multiple oxidative/nitrative stress biomarkers, prenatal NP exposure, and neonatal birth outcomes, including gestational age, birth weight, birth length, Ponderal index, and head and chest circumferences.

## 2. Material and methods

### 2.1. Ethics statement

The Ethics Committee of Taipei City Hospital, Taipei (TCH) approved this study. Before participating in the study, all expectant mothers provided written informed consent for themselves and their babies.

### 2.2. Study populations and data collection

A cohort of pregnant women was studied at an obstetrics clinic in northern Taiwan. The women were followed throughout the duration of the third trimester of pregnancy; they provided urine samples, completed structured questionnaires and also provided birth outcome data upon delivery. The questionnaire was employed to collect data on socio-demographic characteristics (age, weight, height, and education level), lifestyle (smoking status, alcohol and coffee consumption, exercise habits, frequency of detergent and plastic product use, consumption of healthy foods and vitamins (such as vitamins C and E)), and medical conditions. In total, 150 mother–infant pairs from the TCH in metropolitan Taipei in northern Taiwan agreed to participate in this study, and 146 mother–infant pairs completed the follow-up until delivery from March to August 2014.

### 2.3. Birth outcomes

All of the neonatal anthropometric parameters were measured by the obstetrician at time of birth. These parameters included birth weight (g), birth length (cm), head circumference (cm), and chest circumference (cm). The Ponderal index (PI) [birth weight (g)/birth height ($cm^3$)] was also calculated. Gestational age was based on the date of the last menstrual period and corrected using fetal crown-rump length, which was obtained from the early ultrasound scan. Apgar scores were calculated at 1 min and 5 min after delivery.

### 2.4. Urinary NP analysis

Urine samples were collected during the 3rd trimester of pregnancy and stored at −20 °C until analysis. 4-n-NP was analyzed using high-performance liquid chromatography (HPLC) coupled with fluorescence detection, as previously described (Chen et al., 2008, 2005). Briefly, 10 mL of urine sample was adjusted to pH 5.5 using 1 M acetic acid and then mixed with 1 mL of 1 M ammonium acetate solution. Next, urine samples were deconjugated using β-glucuronidase/arylsulfatase (Sigma–Aldrich, USA) and incubated at 37 °C for 15 h and then acidified to pH 3 using 1.0 M hydrochloric acid. Following enzymatic deconjugation, the samples were cleaned up using solid phase extraction. Finally, analytes were eluted in 3 mL methanol. The analytical method employed in this study exhibited good accuracy and precision. No plastic products were used during sample pretreatment. The average recovery was 77–105% for NP levels of 6–235 ng/mL. The limit of detection (LOD) of NP in urine was 0.20 ng/mL, and the regression coefficient ($R^2$) for the standard curve was greater than 0.995. The urinary NP level was adjusted with creatinine and is expressed as μg/g creatinine.

### 2.5. Simultaneous analysis of multiple biomarkers for oxidative/nitrative stress and lipid peroxidation

Urinary 8-OHdG, 8-$NO_2$Gua, 8-iso$PF_{2\alpha}$, and HNE-MA were simultaneously analyzed using our newly established LC-electrospray ionization (ESI)-MS/MS method (Chen et al., 2015). The LODs for 8-OHdG, 8-$NO_2$Gua, 8-iso$PF_{2\alpha}$, and HNE-MA in urine were 0.02 ng/mL, 0.03 ng/mL, 0.008 ng/mL, and 0.01 ng/mL, respectively. Excellent linearity over the concentration range of 0.1–50 ng/mL was observed, with $R^2 > 0.9982$. To validate the method performance, four mixtures of the standards were spiked in the urine at three levels (0.5, 5, and 25 ng/mL) and then analyzed. Mean accuracy, defined as the percentage ratio of the calculated level of the four standards to the expected spiked concentration, ranged from 97.8 to 102.2%. Intra- and inter-day variations, expressed as relative standard deviations (RSDs), were 3.0–8.1% and 3.1–9.3%, respectively. The urinary 8-OHdG, 8-$NO_2$Gua, 8-iso$PF_{2\alpha}$, and HNE-MA levels were adjusted with creatinine and are expressed as μg/g creatinine.

### 2.6. Creatinine measurement

Urinary creatinine concentrations were measured based on the Hinegard and Tiderstrom modification of the Jaffe reaction (Jaffe,