

## Prenatal exposure to nonylphenol-an environmental hormone

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### Introduction

Environmental hormones have raised public concern recently; some of them act by binding to the estrogen receptor and regulating the activity of estrogen responsive genes.<sup>1</sup> Nonylphenol (NP), an important representative of the alkylphenols, was reported to have estrogenicity.<sup>2,3,4,5</sup> Alkylphenols are used as antioxidants and in the form of their ethoxylates as non-ionic surfactants used as detergents, emulsifiers, wetting agents and dispersing agents in agriculture and industrial applications.<sup>6</sup> Lu Y. Y. demonstrated that NP, 4-tert-octylphenols (OP) and 2,4-di-tert-butylphenols (BP) are ubiquitous in daily foodstuffs.<sup>7</sup> The author's previous study indicated the significant levels of nonylphenols in both plasma and urine from textile and housekeeping workers.<sup>8</sup> Ding W. H. and his colleagues showed the higher NP ethoxylate residues in Taiwanese rivers and sediments than in other countries owing to the deficient municipal wastewater treatment in Taiwan.<sup>9,10,11,12</sup> They also found 0.2 to 21% levels of NPEOs in 41% of 90 household detergents.<sup>13</sup> Accordingly, Taiwanese are expected to significant exposure of nonylphenols. The exposure routes are diverse. Exposure via contaminated foods and drinking water, but also via dermal absorption or inhalation could occur.<sup>14,15,16</sup> The question arises whether the nonylphenols that are circulating in the expectant mother's body will pass through the placenta and elicit possible estrogenic effects on developing fetuses. The placenta is an effective barrier against fetal exposure to proteins or xenobiotics that might cause harm. It may also protect the developing embryo against hormones circulating in the maternal blood that might adversely affect its development. Therefore, the placenta barrier is impervious to most sex hormones, including estrogen. However, there are studies that show the presence of 2,3,7,8-TCDD, PCB's, Bisphenol A, nonylphenol, octylphenol, and phthalates in cord blood.<sup>17,18,19</sup> This means that a proportion of chemicals in maternal body may be passed to her child. This study compares human umbilical cord blood samples collected from two regions with different alkylphenol exposure level. Comparisons between maternal and cord blood are also made among some of the samples. The purposes of this study are to determine the prenatal exposure levels for Taiwanese and to explore the protection of the placental barrier against nonylphenol exposure. To our knowledge, this is the first study on fetal exposure by comparison of nonylphenol levels in maternal and cord blood samples.

### Materials and Methods

The Ethics Committee of the Veteran General Hospital, Taipei (VGH) approved the study of pollution in human umbilical cord blood. Before delivery, the expectant mother gave their written informed consent. One hundred and seventy-four human umbilical cord blood samples were examined. Among the specimens, one hundred and twenty-four were from a teaching hospital in central Taiwan and the other fifty from the VGH in metropolitan Taipei (north Taiwan). Forty-two expectant mothers in central Taiwan also donated their blood. The cord blood was collected in a 10mL glass EDTA Vacutainer upon delivery at hospital and mother's blood was obtained by venous puncture before delivery. All samples were immediately chill transported to the laboratory. Plasma was fractioned and kept frozen until analysis. Plasma samples were then homogenized by using a sonicator, followed by enzymatic deconjugation<sup>20</sup> 1.000g of blood was brought to pH 5.5 with acetic acid and mixed with 1mL of 1M ammonium acetate solution and 125µL β-glucuronidase/arylsulfatase. The mixture was incubated for 15 h at 37°C in a shaker bath and then was acidified to pH 3. Following deconjugation<sup>20</sup>, samples were cleaned up with Varian PH solid-phase extraction cartridges. The SPE cartridge was first preconditioned with 20mL of methanol followed by 3mL of pure water (adjusted to pH 3.0 using 1.0M HCl). After sample application, the cartridge was washed with 5mL of pure water, and the analytes were eluted with 3mL of methanols.<sup>8</sup> The analytes were determined by using a reversed-phase HPLC-fluorescence detection. The analytical conditions and validity of the method were described in details by Chen M. L., Lee W.P., Chung H.Y., Guo B.R. and Mao I. F..<sup>8</sup>

### Results and Discussion

The alkylphenols that were detected in the cord blood samples were nonylphenols and 2,4-di-tert-butylphenol. Only

nonylphenol measurements are reported here. Nonylphenol were present in 86% of the samples collected in north Taiwan much lower percentages, 33.1%, were positively detected for samples collected in central Taiwan. The concentrations of nonylphenol ranged from n.d. (not detectable, below 0.91ng/g) to 210.5ng/g. Significantly higher levels were found in north Taiwan samples (48.1±42.8ng/g; ranges: n.d. to 182.3ng/g) compared to those in central Taiwan samples (21.5±45.9ng/g; ranges: n.d. to 210.5ng/g). Previous studies by the authors have found a consistently higher level of nonylphenol in plasma samples in north Taiwan workers than those in south Taiwan workers.<sup>8</sup> An obviously high pollution level is concluded in metropolitan Taiwan. For the forty-two pairs of maternal venous plasma and umbilical-cord plasma, nonylphenol were detectable in 52.4% of both maternal and cord plasma samples. Mean concentrations of nonylphenol were one time higher in maternal venous plasma(34.1±58.0ng/g; ranges: n.d. to 267.9ng/g) than those in cord plasma (15.8±25.0ng/g; ranges:n.d. to 99.6ng/g), however, the difference was not significant in statistics (p=0.06). This study demonstrates the placenta barrier is not impervious to nonylphenol. The prenatal exposure to nonylphenol and a relatively high exposure level for newborn baby in metropolitan area are concluded. It is deemed necessary to take measures to reduce the pollution levels of nonylphenol in the environment.

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