The association of nonylphenol exposure with development of secondary sex characteristics

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Abstract

This study examined the urinary NP concentration of adolescent students and explored its association with the development of secondary sex characteristics. Seven hundred and eighty-two students from grades 5 to 8 were recruited. Pre-school urine samples were collected from every student. The development of secondary sex characteristics was reported through a self-administered questionnaire. Urine samples were analyzed with HPLC coupled with fluorescence detection. Demographic data showed the students aged 12.3±1.1 years old; the averaged stature and body weight were 151.7±9.8 cm and 44.9±11.7Kg. The onset of secondary sex characteristics for boys ranged from the lowest in nocturnal emission (18.9%) to the highest in facial acne (50.0%). For school girls, the onset ranged from the lowest in fat tissue increase (28.8%) to the highest in breast development (86.5%). The averaged age of menarche was 11.5 years old. The urinary NP concentrations ranged from N.D. (below 1.60μg/L urine) to 178.3μg/g Cr. Logistic regression showed that the likelihood of development of secondary sex characteristics is as a function of demographic characteristics, including age, stature and body weight, rather than internal NP levels. The effect of NP exposure on the development of secondary sex characteristics was not concluded in this study.

Keywords: urinary nonylphenol, environmental hormone, secondary sex characteristics

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.[1] Nonylphenol (NP), an important representative of the alkylphenols, was reported to have estrogenicity.[2,3,4,5] These environmental estrogenic chemicals may cause precocious sexual development;[6] recently these chemicals have been hypothesized to account for the growing frequency of infertility and related disorders of the male reproductive system in humans.[7] NP is used as antioxidants and in the form of their ethoxylates (NPEOs) as non-ionic surfactants used as detergents, emulsifiers, wetting agents and dispersing agents in agriculture and industrial applications. Lu Y. Y. demonstrated that NP, 4-tert-octylphenols (OP) and 2,4-di-tert butylphenols (BP) are ubiquitous in daily foodstuffs.[8] The authors previous study indicated the significant levels of NP in both occupationally exposed workers and non-occupationally exposed individuals.[9] 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.[10,11,12,13] They also found NPEOs were detected in 41% of 90 household detergents at concentrations from 0.2 to 21%.[14] 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.[15,16,17] The Japanese Environmental Agency listed nonylphenol as a suspected endocrine disruptor and initiated risk assessments in 1998.[18] Several measures have been taken to reduce risk exposure in other countries. For example, the use of NPEOs has been banned or restricted in many European countries because of growing concern about the toxicity of NP in aquatic organisms.[19] In spite of the ubiquity of NP in the environment, currently no restriction of NPEOs has been adopted by the Taiwan government. This study examined the urinary NP concentration of adolescent students and explored its association with the development of secondary sex characteristics.

Materials and Methods

The Ethics Committee of National Yang-Ming University approved the study. A stratified random sampling strategy was conducted to determine four schools (including 2 schools for respective primary and junior high schools) by geographic region (North, Central, South and East Taiwan). One class of respective grades 5 and 6 of

the sampled primary school as well as grades 1 and 2 of the sampled junior high school were randomly selected. All students in the selected class were invited for study. Pre-school urine samples were collected. An extensive questionnaire was designed to collect data on each subject. Besides general statistical data like sex, age, stature, body weight and eating habits were asked for in the questionnaire; the development of secondary sex characteristics was also reported. Urine samples were immediately chill transported to the laboratory and kept frozen until analysis. Samples were then homogenized by using a sonicator, followed by enzymatic deconjugation.[20] 10.0mL of urine 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℃ in a shaker bath and then was acidified to pH 3. Following deconjugation, 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. The analyte was determined by using a reversed-phase HPLC fluorescence detection. The analytical conditions and validity of the method were described in details by Chen et al..[9]

Results and discussion

The subjects consisted of 407 primary school and 375 junior high school students. Table 1 shows the physical development of the students. The averaged age was 12.3 years old. The stature ranged from 120 cm to 175 cm; the body weight ranged from 20.7 kg to 119.0 kg. The onset of development of secondary sex characteristics for boys were 50.0% in acne, 34.7% in voice box (Adam's apple) growth, 39.6% in body hair, 36.3% in facial hair, 32.4% in widen shoulder, 18.9% in nocturnal emission and 40.4% in voice change (Table 2). The onset of development of secondary sex characteristics for girls were 66.4% in acne, 86.5% in breast development, 61.2% in body hair, 28.8% in fat tissue increase, 29.3% in pelvis widen, and 55.6% in menarche (Table 3). NP was detected in 30.8% of the primary school students and 29.2% of the junior high school students. The concentrations of NP ranged from n.d. (not detectable, below 1.6μg/L urine) to 178.3μg/g Cr. Geometric mean concentrations of NP were 1.27μg/g Cr for the students (Table 4). Logistic regression showed that the likelihood of development of secondary sex characteristics was as a function of demographic characteristics, including age, stature and body weight, rather than internal NP levels. The effect of NP exposure on the development of secondary sex characteristics was not concluded in this study.

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Table 1. The physical development of students.

		Grade					P value
		5	6	7	8	Total	
Age	Sample No.	199	208	203	172	782	0.000
	Mean±SD	11.1±0.6	11.6±0.6	13.1±0.6	13.6±0.5	12.3±1.1	
	Min.	10.0	10.0	11.2	12.3	10.0	
	Max.	12.6	13.2	14.8	14.5	14.8	
Stature (cm)	Sample No.	192	198	194	171	755	0.000
	Mean±SD	144.4±8.1	147.8±8.2	156.6±7.7	158.9±7.0	151.7±9.8	
	Min.	120.0	127.0	130.0	137.0	120.0	
	Max.	168.0	167.0	173.0	175.0	175.0	
Body Weight (kg)	Sample No.	188	199	188	171	746	0.000
	Mean±SD	38.4±9.4	42.2±10.7	49.1±10.1	50.5±12.2	44.9±11.7	
	Min.	21.0	23.0	30.0	31.0	20.7	
	Max.	84.0	74.0	80.0	119.0	119.0	

Table 2. The development of secondary sex characteristics for adolescent boys.

(%)

	Grade					P value
	5	6	7	8	Total	
Acne	27.5	24.8	72.2	87.2	50.0	0.000
Voice box growth	9.8	20.2	51.5	66.7	34.7	0.000
Body hair	6.9	5.5	77.3	83.3	39.6	0.000
Facial hair	23.5	23.9	47.4	56.4	36.3	0.000
Widen shoulder	27.5	21.1	39.2	46.2	32.4	0.001
Nocturnal emission	12.7	4.6	34.0	28.2	18.9	0.000
Voice change	16.7	22.9	60.8	70.5	40.4	0.000

Table 3. The development of secondary sex characteristics for adolescent girls.

(%)

	Grade					P value
	5	6	7	8	total	
Acne	49.0	58.6	70.1	88.4	66.4	0.000
Breast development	74.5	85.9	90.7	94.7	86.5	0.000
Body hair	25.5	50.5	78.5	89.5	61.2	0.000
Fat tissue increase	20.4	23.2	36.4	34.7	28.8	0.023
Widen pelvis	17.3	19.2	41.1	38.9	29.3	0.000
Menstruation	21.4	38.4	77.6	84.2	55.6	0.000
Age of menarche						
N	21	36	70	68	195	0.000
Mean ± SD	10.9±0.5	11.1±0.9	11.7±0.9	11.8±0.9	11.5±0.9	
Min.	9.9	9.3	9.1	9.0	9.0	
Max.	11.7	13.1	13.9	13.7	13.9	

Table 4. Concentrations of nonylphenol in adolescent students by grade.

(unit: μg/g Cr)

Grade	Min.	Max.	GM	Detection rate (%)	P value
5	nd	178.25	1.63	31.30	0.010
6	nd	59.53	1.34	28.57	
7	nd	16.52	1.14	32.67	
8	nd	57.00	0.97	26.35	
Total	nd	178.25	1.27	29.81	