

• Original Article

Urinary concentration of 3-phenoxybenzoic acid in elementary students in South Korea

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Objectives Pyrethroid pesticides are among the most commonly using insecticides in South Korean households and have been the subject of considerable interest among public health professionals for their potential health effects. The objective of this study is to examine the level of urinary 3-phenoxybenzoic acid (3-PBA) among elementary students in South Korea.

Methods We conducted a cross-sectional study to evaluate pyrethroid pesticide exposure levels by measuring the urinary metabolites of 3-PBA using a gas chromatography-mass spectrometry method in March 2011. Study participants were 70 Asan-area and Incheon-area elementary students.

Results All respondents had values above the detection limit, and the geometric means of 3-PBA in all children were 1.85 µg/L and 1.46 µg/g creatinine. Children with the top 10% urinary levels of 3-PBA were more likely to be girls, under nine years of age, living in a rural area, and living in a residential type apartment.

Conclusions South Korean children have a higher concentration of urinary 3-PBA compared with those of other countries. Further research identifying exposure pathways and intervention efforts to reduce environmental pesticide use are needed in South Korea.

Keywords Children, Environmental exposure, Pesticide, Pyrethroid insecticide, Urinary metabolites

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Introduction

Pyrethroid pesticides have been used extensively worldwide to control insects in agricultural settings, commerce, public health, and individual households [1]. However, they are known to have potential health effects such as developmental neurotoxicity, immune suppression, endocrine disruption, potential carcinogenesis at high exposure levels, and dopaminergic function interference [1-3].

In South Korea (hereafter Korea), pyrethroid pesticides have been reported as among the most frequently used insecticides [4,5]. Children are at a greater risk than adults for non-occupational pesticide exposure, which can occur through numerous exposure pathways [6]. Biological monitoring in urine has advantages because of the ease of sample collection and is an effective

way to identify low-level pyrethroid metabolites. The 3-phenoxybenzoic acid (3-PBA) is a non-specific and most frequently detected urinary metabolite of major pyrethroid pesticides [6,7].

Examining pesticide exposure level among children would provide important information in children's health perspectives. However, no information is currently available about pyrethroid exposure among children in Korea. The objective of this study, therefore, is to examine the level of urinary 3-PBA levels among elementary students in Korea.

Materials and Methods

Study Participants

This study used the samples collected for the pilot study of the

Korean Environmental Health Survey in Children, which has been reported for its study design and method [8]. The participants were 37 boys and 33 girls aged 6 to 12 in elementary school among 351 children and adolescents who agreed to participate in the pilot study from two regions of Incheon (industrialized area) and Asan (rural area). Information about the children's demographic factors (e.g., birth date, sex, and age), dietary habits, current residence and household characteristics, and parents' socioeconomic information (e.g., income, occupation, and education levels) was obtained via questionnaire administered to their parents or guardians. Spot urine samples were collected from children on the morning before the survey between 9:00 and 11:00 am in March of 2011. Informed consent was obtained from both the children and their parents or guardians before enrollment. This study was approved by the institutional review board of Dankook University Hospital.

Urinary Sample Collection and Analysis

Urine samples were prepared and analyzed for 3-PBA using previously reported methods [9]. The samples were collected using 15 mL polystyrene conical tubes (Sarstedt AG & Co., Numbrecht, Germany). An aliquot (5 mL) of each urine sample was mixed with HCl and heated at 90°C for one hour. After heating, the samples were cooled, and n-hexane and 0.1M NaOH were added to the mixture. The resulting solution was extracted and dried in a nitrogen stream. The dried samples were mixed with toluene and N-(tert-butyl-dimethylsilyl)-N-methyl-trifluoroacetamide and heated at 70°C. After cooling the samples on ice, samples were measured by PerkinElmer Clarus 600T GC-MS (PerkinElmer, Waltham, MA, USA). Internal quality control was achieved through analysis of control urines.

Variation coefficient was 1.1% and the limit of detection was 0.014 mg/L under study. External quality control was achieved through participation in tests of the German External Quality Assessment Scheme operated by the University Erlangen-Nuremberg.

Data Analysis

Deviation from the normality of the data distribution was examined using a Kolmogorov–Smirnov test. Because of the asymmetric distribution of 3-PBA, we presented the geometric mean to describe the results. The significance of the differences among individual characteristics for 3-PBA levels was estimated using non-parametric tests, i.e., the Mann-Whitney U-test and Kruskal-Wallis test. To identify the characteristics of highly exposed children, the children with the top 10% 3-PBA concentration were also identified. A 0.05 level of probability was used as the criterion of significance (two-tailed). All data analyses were performed with SPSS version 20.0 (IBM Co., Armonk, NY, USA).

Results

The geometric mean of urinary 3-PBA concentration was 1.85 µg/L (Figure 1A), and the geometric mean of creatinine-adjusted 3-PBA concentration was 1.46 µg/g creatinine (Figure 1B). The concentrations of 3-PBA were detected in all participants and were right-skewed for both male and female.

There was no significant difference in 3-PBA level with demographic factors, although rural children showed significantly higher mean levels of 3-PBA concentration before creatinine correction (Table 1). Children with the top 10% urinary levels

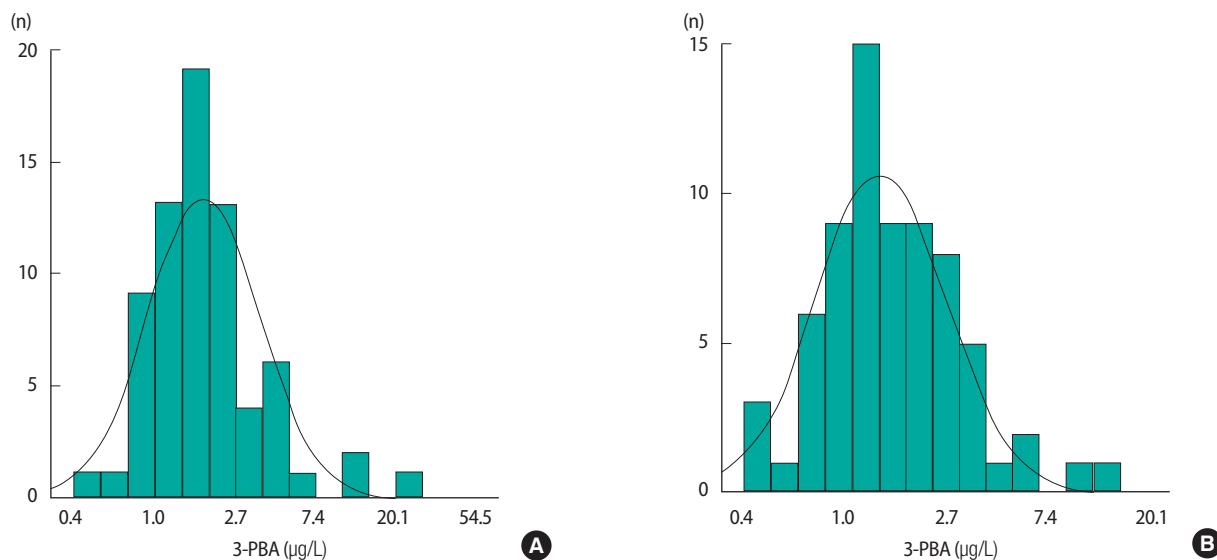


Figure 1. (A) Uncorrected geometric mean of 3-phenoxybenzoic acid (3-PBA) level (µg/L), (B) creatinine-corrected geometric mean of 3-PBA level (µg/g Cr).

Table 1. Geometric mean of urinary 3-PBA levels and sociodemographic characteristics in South Korean children

Characteristics	All children			Children with top 10% of 3-PBA			
	n (%)	µg/L ^a (GSD)	µg/g Cr ^b (GSD)	n (%)	µg/L (GSD)	n (%)	µg/g Cr (GSD)
All	70 (100.0)	1.85 (2.01)	1.46 (1.93)	7 (10.0)	8.26 (2.00)	7 (10.0)	5.53 (7.70)
Sex							
Boys	37 (52.9)	1.78 (1.73)	1.40 (1.75)	2 (28.6)	5.39 (1.16)	4 (57.1)	3.87 (1.26)*
Girls	33 (47.1)	1.93 (2.33)	1.53 (2.13)	5 (71.4)	9.80 (2.15)	3 (42.9)	8.90 (1.52)
Age (yr)							
6-7	20 (28.6)	1.58 (1.81)	1.46 (1.73)	2 (28.6)	5.39 (1.16)	3 (42.9)	3.75 (1.31)
8-9	27 (38.6)	2.19 (2.37)	1.68 (2.28)	3 (42.9)	16.56 (1.46)	4 (57.1)	7.39 (1.66)
10-11	23 (32.9)	1.73 (1.72)	1.25 (1.63)	2 (28.6)	4.46 (1.02)	-	-
Area							
Urban	33 (47.1)	1.45 (1.55)	1.33 (1.59)	-	-	1 (14.3)	4.23
Rural	37 (52.9)	2.30 (2.26)**	1.59 (2.20)	7 (100.0)	8.26 (2.00)	6 (85.7)	5.78 (1.76)
Parental education level (yr)							
≤12	25 (35.7)	1.77 (1.91)	1.41 (1.75)	3 (42.9)	6.96 (1.74)	2 (28.6)	4.31 (1.48)
≥13	45 (64.3)	1.89 (2.08)	1.49 (2.03)	4 (57.1)	9.38 (2.32)	5 (71.4)	6.10 (1.79)
Mother's occupation							
Non-manual worker	37 (52.9)	2.02 (2.12)	1.61 (2.00)	3 (42.9)	11.72 (2.31)	5 (71.4)	5.51 (1.70)
Manual worker	3 (4.3)	1.92 (1.07)	1.18 (1.30)	-	-	-	-
Unemployed	28 (40.0)	1.70 (1.95)	1.36 (1.91)	4 (57.1)	6.36 (1.70)	2 (28.6)	5.57 (2.13)
Household income per month (x10 ⁴ KRW)							
<200	5 (7.1)	2.03 (2.14)	1.78 (1.94)	1 (14.3)	5.97	1 (14.3)	3.26
200-400	31 (44.3)	1.64 (1.89)	1.33 (1.73)	2 (28.6)	7.63 (2.10)	3 (42.9)	4.98 (1.16)
400-600	21 (30.0)	2.08 (2.06)	1.52 (2.10)	2 (28.6)	10.63 (3.47)	1 (14.3)	13
>600	13 (18.6)	1.96 (2.26)	1.58 (2.18)	2 (28.6)	8.17 (2.08)	2 (28.6)	5.49 (2.18)
Residential types							
Detached house	11 (15.7)	2.04 (1.43)	1.53 (1.77)	-	-	1 (14.3)	5.11
Apartment house	59 (84.3)	1.81 (2.11)	1.45 (1.96)	7 (100.0)	8.26 (2.00)	6 (85.7)	5.60 (1.79)
Length of residence (yr)							
≤1	17 (24.3)	1.56 (1.68)	1.41 (1.72)	1 (14.3)	4.41	1 (14.3)	4.23
2-4	37 (52.9)	2.06 (2.32)	1.43 (2.13)	6 (85.7)	9.17 (2.01)	5 (71.4)	5.92 (1.88)
≥5	16 (22.9)	1.73 (1.57)	1.60 (1.71)	-	-	1 (14.3)	5.11
Passive smoking							
Yes	21 (35.0)	2.23 (1.88)	1.70 (1.85)	2 (28.6)	8.17 (2.08)	3 (42.9)	5.36 (1.74)
No	39 (65.0)	1.74 (2.20)	1.43 (2.06)	5 (71.4)	8.30 (2.15)	4 (57.1)	5.65 (1.82)

3-PBA, 3-phenoxybenzoic acid; Cr, creatinine; GSD, geometric standard deviation; KRW, Korean won.

^aVolume-based concentrations (µg/L).

^bCreatinine-adjusted concentrations (µg/g Cr).

* $p < 0.05$, ** $p < 0.01$.

of 3-PBA were more likely to be girls, under nine years of age, living in a rural area, and living in a residential type apartment; however, only gender difference in 3-PBA level was statistically significant.

Discussion

We found that our children have a higher concentration of urinary 3-PBA compared with those of other countries, and the urinary 3-PBA level was not significantly associated with sociodemographic factors. This study provides some descriptive data for the level of urinary metabolites among children and there is a clear need to facilitate a comprehensive evaluation of non-occupational pesticide exposure in Korea.

Comparing the 3-PBA levels found in other countries, our

finding was higher than that in general populations of the US [10,11], Germany [12], China [13], Thailand [14], and Poland [15] but similar to that in children with farm worker family households in California (1.97 µg/g Cr) (Table 2) [16]. The finding from the Korea National Environmental Health Survey in adult (1.84 µg/g Cr) [17] was also higher than in the general populations from other countries such as the US [10], Japan [18], and Poland [19].

The high level of 3-PBA in this study may be related to frequent indoor pesticide use in Korea. Although there is no official report for using pesticide use at home, the majority of domestic insecticides on the market are known to be pyrethroid products, including permethrin and cypermethrin [5]. These pyrethroid insecticide are used at home, in apartment complex landscape gardening, and in parks and public spaces where peo-

Table 2. Summary of previous studies for geometric mean of 3-phenoxybenzoic acid urinary levels in children

Country	Author (published year)	Survey year	Children's age (yr)	No. of children	Urine collection	µg/L ^a	µg/g Cr ^b
United States	Barr et al. (2010) ^c	1999-2000	6-11	483	Spot urine (one convenience)	0.42	0.45
		2001-2002	6-11	580	Spot urine (one convenience)	0.33	0.42
	Morgan et al. (2007)	2001	2-5	127	Spot urine (48 hr)	0.39	0.50
	Trunnelle et al. (2014)	2009	2-8	103	Spot urine (24 hr)	1.11	1.97
Germany	Becker et al. (2009) ^d	2001-2002	2-17	363	Spot urine (morning)	0.31	0.24
China	Ding et al. (2012)	2010-2011	0-14	180	Spot urine (one convenience)	-	0.39
Thailand	Panuwet et al. (2009)	2007	12-13	207	Spot urine (morning)	0.20	0.23
Poland	Wielgomas et al. (2013)	2012	<18	184	Spot urine (morning)	0.29	0.25

^aVolume-based concentrations (µg/L).

^bCreatinine-adjusted concentrations (µg/g Cr).

^cFrom National Health and Nutritional Examination Survey.

^dFrom German Environmental Survey.

ple spend time and play; these environmental exposures may contribute to increases in the level of the 3-PBA found in children in this study. In addition, as commonly used insecticides are installed in narrowly confined spaces such as living room floors and kitchens, children can consume insecticidal ingredients through hand-to-mouth activity [20]. However, because the concentration difference of 3-PBA can occur due to usage patterns of the pyrethroid pesticide, exposure pathways, and other pyrethroid metabolites [7,12], further studies are needed to identify the detailed reason for the high level of 3-PBA and to reduce the pesticide exposure in homes in Korea.

We found no statistically significant differences of 3-PBA concentration with demographics. The important limitation of this cross-sectional study may hinder investigation of the differences in urinary 3-PBA levels among different demographics. Previously, some surveys showed concentration difference depending on season [21,22], suggesting a possibility that pyrethroid metabolite levels in urine vary depending on the exposure or collection time. Thus, studies for characterization of this possible variability by repeated measurements according to various time and season are needed. Children's eating habits or other environmental factors as potential exposures may also influence the results [6]. Dietary intake of vegetables or fruits was also reported to be related to urinary levels of 3-PBA [23,24]. However, the higher proportions of rural children among the top 10% of 3-PBA and the significant increased level in rural children over urban suggest that living in a rural area may be related with high exposure to pyrethroid insecticide.

Despite of these limitations, the high level of 3-PBA concentration among children in this study emphasizes the importance of environmental pesticide exposure in Korea. Further research should examine pesticide exposures and urine collection times as well as other potential factors that may relate with 3-PBA level. It is also necessary to apply intervention efforts to reduce pesticide exposure at home in Korea.

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Conflict of Interest

The authors have no conflicts of interest with material presented in this paper.

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