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REGULAR ARTICLE

Relationship between dietary habits and urinary concentrations of 3-phenoxybonzoic acid in a middle-aged and elderly general population in Japan

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Abstract

Objectives The ingestion of pesticides in the daily diet is assumed to be the main modality of pesticide exposure for most people. A widely used class of pesticides in agricultural or residential settings is pyrethroid. We have examined the relationship between the intake frequency of selected items of vegetables and fruits and urinary metabolites of pyrethroid pesticides in a healthy general population.

Methods A total of 535 residents (184 men and 351 women) who attended a healthcare checkup program conducted in a rural area of Hokkaido, Japan, in August 2005 provided informed consent for their spot urine samples to be used for the determination of 3-phenoxybenzoic acid (3-PBA) levels. They also completed a self-administered questionnaire regarding the intake frequency of 12 food items. The concentrations of creatinine-corrected 3-PBA were predicted by the intake frequency of each item, using analysis-of-covariance models to adjust for age, sex, body mass index, and drinking and smoking status.

Results Both a significant association between the 3-PBA concentration and the frequency of tomato consumption and a significant positive linear trend was found in female subjects. In contrast, no such association was found in the male subjects.

Conclusions The frequency of tomato consumption was confirmed to strongly predict the urinary pyrethroid metabolite levels in the general population—presumably because tomatoes are most often consumed raw and unpeeled (more so than all other vegetables and fruits analyzed in the current study). However, it should be noted that the 3-PBA levels, even among those subjects with the highest consumption of tomatoes, were far below the levels of toxicological significance, although the health consequences from long-term low-level exposure to pyrethroid requires further exploration.

Keywords Analysis of covariance · Food frequency questionnaire · Gas chromatography–mass spectrometry · 3-Phenoxybenzoic acid · Pyrethroid insecticide

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Introduction

Pesticides are widely used in agricultural settings, public health, commercial enterprises and individual households throughout the world [1, 2]. In terms of agricultural applications, any drastic global reduction of pesticide usage is unlikely to occur since this would rapidly result in a serious food crisis due to substantial crop shortages [3]. The amount of pesticides imported into Japan has been declining continually since a peak in 1980 [4].

However, continued dependence on the use of effective pesticides is foreseeable because of the widespread demand to maintain high crop productivity despite the claim of widespread support for more simplified work practices in the face of population ageing in the agricultural industry. The extensive use of pesticides has also been reported in non- agricultural settings for vector control to combat insect-transmitted infectious and parasitic diseases [5, 6] and for pest control in homes, public facilities, restaurants and passenger trains, among others [1, 2]. All of these applications indicate the importance of these compounds in many aspects affecting our daily life [7].

Within this framework of the widespread use of pesticides, there have been multiple reports on the potential toxicological effects of organophosphorus (OP) insecticides on human health, including neurotoxicity [8], immunotoxicity [9] and reproductive toxicity [10, 11]. The toxicity of pyrethroid (PYR) insecticides, which have been increasingly available for consumer use since the banning of OP insecticides [12], has also been the subject of intensive research. For example, permethrin, the most widely used PYR, is suspected of being an endocrine-disrupting chemical and has been classified as a potential carcinogen at high exposure levels [13]. Earlier studies have also suggested that PYR pesticides may have an immunosuppressive effect, causing damage to lymph nodes and the spleen [14]. However, PYRs are known to be much less toxic to humans than other insecticides [15], and their acute toxicity to humans is at least three orders of magnitude lower than that for insects [16] since they are readily cleaved to relatively non-toxic metabolites in mammals [17].

The absorption of pesticides into the human body via indirect routes can occur after oral, inhalative or dermal exposure through air, drinking water, dust and food [18]. Such non-occupational low-level exposure may occur more frequently than that from direct pesticide application in agricultural or occupational settings [7]. Once absorbed into the human body, PYRs are rapidly metabolized by hydrolysis and/or oxidation (phase I metabolism) [19], followed by renal excretion as glucuronide-, glycine-, taurine-, or sulfate-conjugates (phase II metabolism) [20–22]. Some human-volunteer studies have illustrated the rapid metabolism of PYR insecticides [23]; in one study, after inhalative exposure to 160 μg/m^3 of cyfluthrin, 93% of the pesticide was excreted in the first 24 h at peak excretion rates of 0.5–3 h [24].

Pyrethroids form various metabolites in the human body that are excreted in the urine; of these, 3-phenoxybenzoic acid (3-PBA) and *cis*/*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (DCCA) are the most frequently detected, non-specific metabolites of major PYRs, including permethrin and cypermethrin [25]. As these metabolites are known to reliably indicate PYR exposure levels and as urine samples are readily, noninvasively available, the determination of the urinary concentrations of these metabolites has been considered a suitable approach for the biological monitoring of those individuals occupationally exposed to PYR [15, 23]. Assessments of PYR exposure levels by monitoring urinary metabolites in large-sized general populations have occasionally been reported in studies performed outside of Japan [26, 27], whereas no such studies have been conducted in Japan. To date, analytical techniques have become so sophisticated that urinary metabolites can be used as biomarkers to assess the low-level, environmental exposure to PYR that can occur when members of the general population are carrying out their normal daily activities [27]. To evaluate non-occupational PYR exposure levels in the healthy general population, we have recently analyzed the urine of middle-aged and elderly subjects with the aim of determining the levels of 3-PBA, which is the most frequently detected breakdown product (metabolite) of several PYR compounds extensively used in Japan.

Among the general population, the ingestion of pesticide residues in contaminated food has been commonly assumed to be the main pesticide exposure pathway [16, 27]. In a recent longitudinal intervention study conducted in the USA, the substitution of organic food items for conventional diets resulted in a significant reduction of urinary concentrations of PYR metabolites among 23 children aged 3–11 years [13], although the magnitude of that reduction was not as drastic as that observed for urinary OP metabolites [28]. Another study conducted in Germany among 396 children aged 2–11 years demonstrated that urinary PYR metabolite levels were significantly associated with the frequency of the consumption of boiled vegetables [27]. Whereas both studies focused on the children's exposure to PYR pesticides, no analysis for the relationship between each food item and PYR metabolite levels was conducted. In addition, no other earlier studies investigated the relationship between dietary habits and urinary PYR metabolites, particularly among adults in Japan. We have examined the association between urinary 3-PBA concentrations and diets. To this end, we surveyed the diets of healthy subjects of the general Japanese population using a food frequency questionnaire (FFQ) to determine the item-by-item intake frequency of vegetables and fruits.

Materials and methods

Study subjects

A total of 619 residents of a rural area of Hokkaido (the northernmost main island of Japan) attended a healthcare checkup program conducted in August 2005. All participants were asked to fill out a self-administered questionnaire to address such lifestyle characteristics as smoking and drinking status, dietary habits and engagement in agricultural or pest control occupations. Dietary habits were elicited using a food frequency questionnaire of 12 food items: green-leafy vegetables, carrots, squash, tomatoes, cabbage and lettuce, Chinese cabbage, beans, potatoes, edible wild plants, citrus, other fruits and vegetable juice. The intake frequency of each item was categorized into five levels, i.e. one to two times a month (infrequent), one to two times a week, three to four times a week and almost every day. These foods were selected from the 32-item FFQ developed for the Japan Collaborative Cohort Study for Evaluation of Cancer Risk [29]. Subjects were also asked to donate a spot urine specimen to be shipped and stored at −30°C until analysis in our laboratory. The Ethics Committee of the Nagoya University School of Medicine, Nagoya, Japan, approved the study protocol.

Determination of urinary metabolites

We determined urinary concentrations of 3-PBA, which is a non-specific metabolite of major PYR ingredients, including permethrin, cypermethrin, deltamethrin, sumithrin, etofenprox and cyhalothrin. Urine samples were hydrolyzed and derivatized using 1,1,1,3,3,3-hexafluoroisopropanol and *N*, *N*-diisopropylcarbodiimide; the concentration of 3-PBA was then quantified by gas chromatography–mass spectrometry equipped with the electroionization system (Agilent 5975 MDS system; Agilent, Santa Clara CA) using 8 μg/ml of 2-PBA as an internal standard. The analytical method used in this study has been detailed elsewhere [30]. Urine specimens collected and pooled from healthy volunteers were used for quality control. Our method was sensitive enough to detect as little as 0.02 μg/l of urinary 3-PBA; levels below this limit of detection (LOD) were recorded as 0.01 μg/l [31]. Urinary creatinine concentration was determined in accordance with the alkaline picrate method. Informed consent was elicited for participation in our study, and a sufficient volume of urine specimens for metabolite determination were obtained from 535 health-checkup participants (184 men and 351 women).

Statistical analysis

In terms of determining the intake frequency of vegetables and fruits, if the number of subjects in a certain category made up less than 10% of the sex-specific total, that category was integrated with the neighboring one. Applying this approach, we eventually re-categorized the five-level categories of food frequency into either three or four ranked groups for each of the 12 items. Moreover, to yield a cumulative measure of green and yellow vegetables, the category number of each of the green-leafy vegetables, carrots, squash and tomatoes (1 assigned to the lowest category, and 2 through 5 assigned to successively increasing categories) was added to produce a summary value of 5–25, which was itself quartiled for statistical analyses.

Throughout the statistical analysis, 3-PBA concentrations corrected for urinary creatinine concentration (3-PBA_c) were used instead of the uncorrected 3-PBA level. Because of a substantial difference in the creatinine concentration, which was confirmed to exist between the sexes, this correction was likely to affect the results in a sex-specific manner. consequently, subsequent statistical inferences were drawn separately for men and women. To model the association between dietary habits and urinary PYR metabolites, we performed an analysis of covariance (ANCOVA) to estimate the least-squares means of 3-PBA_c concentrations for comparison across the categories of intake frequency for each item of vegetables and fruits. Multivariate adjustments were made for age, sex, body mass index (BMI), drinking status (never, past, current) and smoking status (never, past, current). To test the linear trend for 3-PBA_c concentrations according to the rank of intake frequency, we used a linear contrast on the assumption that the categories were equally spaced from the lowest to highest intake frequency. Since a Kolmogorov–Smirnov test revealed that the distribution of urinary 3-PBA_c concentrations deviated from normality, logarithmically transformed values were used in the statistical analysis. Furthermore, subjects were dichotomized (pesticide sprayers and non-sprayers) according to their occupational pesticide use. All statistical analyses were conducted using the SPSS statistical package for Windows ver. 11.0 J (SPSS, Chicago, IL), and two-sided *P* values of <0.05 were considered to be statistically significant.

Results

Urinary 3-PBA of >LOD was detected in 98.3% of all samples, with a range of 0.03–15.34 μg/g creatinine. The women in our study, who accounted for 65.6% of all the subjects, were significantly younger than the men (Table 1). There were no statistically significant differences in the BMI of either gender. Although the uncorrected 3-PBA concentration in the men tended to be higher than that in the women ($P = 0.11$), correction for urinary creatinine resulted in a significantly higher concentration of urinary metabolites in women than in men. A comparison of 3-PBA_c concentrations between those who

Table 1 Characteristics of the study population

Characteristics	Total study population (n = 535)	Men (n = 184)	Women (n = 351)	P for difference[a]
Age (years)	61.5 ± 0.4[c]	63.6 ± 0.7[c]	60.4 ± 0.5[c]	<0.01
Body mass index (BMI; kg/m^2)	23.7 ± 0.1[c]	23.8 ± 0.2[c]	23.7 ± 0.2[c]	0.66
Urinary creatinine (g/l)	0.74 (1.03)[d]	1.03 (1.05)[d]	0.63 (1.04)[d]	<0.01
Urinary 3-PBA (μg/l)	0.31 (1.05)[d]	0.34 (1.09)[d]	0.29 (1.07)[d]	0.11
Urinary 3-PBA$_c^b$ (μg/g creatinine)	0.41 (1.04)[d]	0.33 (1.07)[d]	0.46 (1.05)[d]	<0.01
Smoking status (frequency, n)				
Never	336	43	293	<0.01
Past	123	92	31	
Current	76	49	27	
Drinking status (frequency, n)				
Never	331	61	270	<0.01
Past	19	12	7	
Current	185	111	74	

3-PBA, 3-Phenoxybenzoic acid; 3-PBA$_c$, 3-phenoxybenzoic acid corrected for creatinine concentration (μg/g creatinine)

[a] Comparison between men and women by t test for continuous variables and chi-square test for categorical variables

[b] Corrected for creatinine concentration

[c] Values indicated as mean ± SEM

[d] Values indicated as geometric mean (geometric SEM)

reported being engaged in agricultural and/or pest control work and those who did not produced no statistical significance (Table 2).

Table 3 presents the observed relationship between food intake frequency and urinary 3-PBA$_c$ concentration by food items. In men, none of the 12 food items showed a significant association or linear relationship between those two variables. This was also true for women—with the exception of tomatoes, the consumption of which was demonstrated to be a strong predictor of their urinary metabolite levels. A trend analysis also revealed a statistically significant increase in 3-PBA$_c$ in proportion to the frequency of tomato consumption among women. However, the quartiled summary measure for the combined consumption of green and yellow vegetables, including tomatoes, was not associated with 3-PBA$_c$ in either men or women (data not shown).

Discussion

We have determined urinary 3-PBA level, which has often been used as a biological marker reflecting the individual's level of exposure to PYR pesticides [32]. Of particular interest in this study was the relationship between 3-PBA concentrations and the intake frequency of vegetables and fruits in the general population. It should be noted that the 3-PBA$_c$ concentrations of women are subject to overestimation because urinary creatinine levels depend on the volume of muscle mass; the statistically significant difference in 3-PBA$_c$ concentrations versus a lack of such significant difference in uncorrected 3-PBA concentrations between the sexes may be largely attributable to this bias, leading us to conclude that the statistical analyses of data based on spot urine samples should be conducted separately for men and women.

Table 2 Multivariate-adjusted mean of urinary 3-PBA$_c$ concentrations among those engaged in agricultural and/or pest control work and other work

	Agricultural and/or pest control work	Other work	P for difference[a]
Men, n	0.43 (1.19), 41	0.36 (1.13), 143	0.28
Women, n	0.51 (1.24), 46	0.46 (1.16), 305	0.59

Values indicated as geometric mean (geometric SEM)

[a] Analysis of covariance adjusted for age, BMI, smoking status and drinking status

The urinary 3-PBA levels among Japanese pest control operators (PCOs) have been previously reported by our colleagues [33], who found that the geometric means of 3-PBA$_c$ concentrations in their study population of PCOs, who had been occupationally exposed to PYRs, were 12.2 μg/g creatinine in the summer and 3.9 μg/g creatinine in the winter. These levels clearly indicate that such workers were exposed to higher doses of PYRs than the individuals of our study population. Contrary to our expectations, however, engagement in agricultural and/or pest control occupations was not significantly associated

Table 3 Multivariate-adjusted mean of urinary 3-PBA$_c$ concentrations according to intake frequency of vegetables and fruits

Food item	Sex, *n*	Intake frequency[a]					*P* for difference[b]	*P* for trend[c]
		Infrequently, *n*	1-2/month, *n*	1-2/week, *n*	3-4/week, *n*	Almost every day, *n*		
Green-leafy vegetables	Men, 184	0.42 (1.27), 19		0.34 (1.15), 89	0.42 (1.19), 48	0.38 (1.23), 28	0.65	0.92
	Women, 347	0.48 (1.23), 43		0.45 (1.17), 133	0.46 (1.19), 104	0.51 (1.21), 67	0.85	0.76
Carrot	Men, 184	0.48 (1.20), 36		0.35 (1.15), 89	0.37 (1.17), 59		0.24	0.21
	Women, 347	0.43 (1.23), 45		0.46 (1.17), 149	0.47 (1.19), 105	0.47 (1.22), 48	0.97	0.65
Squash	Men, 184	0.40 (1.24), 26	0.41 (1.15), 73	0.32 (1.17), 62	0.39 (1.27), 23		0.58	0.71
	Women, 344	0.43 (1.18),183		0.53 (1.19), 115	0.41 (1.23), 46		0.19	0.74
Tomatoes	Men, 183	0.43 (1.22), 29		0.37 (1.16), 73	0.41 (1.18), 51	0.28 (1.23), 30	0.33	0.15
	Women, 347	0.34 (1.17), 136			0.53 (1.18), 122	0.58 (1.18), 89	< 0.01	0.01
Cabbage and lettuce	Men, 183	0.45 (1.25), 21		0.32 (1.16), 77	0.41 (1.17), 60	0.40 (1.25), 25	0.33	0.92
	Women, 344	0.45 (1.17), 127			0.51 (1.18), 152	0.50 (1.20), 65	0.63	0.54
Chinese cabbage	Men, 183	0.38 (1.17), 61		0.34 (1.16), 85	0.45 (1.20), 37		0.36	0.38
	Women, 344	0.46 (1.17), 142		0.48 (1.17), 149	0.44 (1.23), 53		0.86	0.77
Beans	Men, 182	0.41 (1.22), 34		0.37 (1.17), 70	0.38 (1.19), 46	0.38 (1.22), 32	0.98	0.86
	Women, 348	0.39 (1.20), 69		0.48 (1.18), 107	0.55 (1.21), 74	0.47 (1.18), 98	0.26	0.16
Potatoes	Men, 184	0.40 (1.21), 30		0.37 (1.16), 89	0.37 (1.19), 45	0.38 (1.27), 20	0.98	0.88
	Women, 345	0.42 (1.21), 49		0.47 (1.17), 155	0.46 (1.18), 141		0.80	0.62
Edible wild plants	Men, 182	0.31 (1.19), 45	0.41 (1.16), 81		0.39 (1.17), 56		0.32	0.26
	Women, 348	0.42 (1.18), 106	0.48 (1.17), 178		0.49 (1.20), 64		0.45	0.31
Citrus	Men, 184	0.45 (1.24), 25	0.33 (1.17), 64	0.39 (1.16), 74	0.37 (1.26), 21		0.59	0.64
	Women, 348	0.45 (1.17), 133		0.43 (1.18), 135	0.57 (1.20), 80		0.14	0.11
Other fruits	Men, 179	0.44 (1.19), 45		0.41 (1.17), 70	0.31 (1.19), 41	0.37 (1.26), 23	0.41	0.33
	Women, 342	0.40 (1.21), 57		0.44 (1.20), 92	0.51 (1.18), 102	0.53 (1.20), 91	0.37	0.09
Vegetable juice	Men , 184	0.35 (1.15), 75	0.37 (1.19), 53	0.48 (1.22), 30	0.38 (1.25), 26		0.50	0.47
	Women, 345	0.45 (1.17), 155	0.44 (1.20), 78	0.53 (1.22), 55	0.52 (1.22), 57		0.62	0.23

Values indicated as geometric mean (geometric SEM)

[a] Re-categorized so as to have the number of subjects falling into every category make up >10% of sex-specific total

[b] Analysis of covariance adjusted for age, BMI, smoking habit and drinking habit

[c] Test for trend using linear contrast on the assumption of equal spacing from lowest to highest category

with high urinary 3-PBA$_c$ concentrations in our subjects. Although the reason for this lack of difference remains unclear, we speculate that such factors as pesticide application methods and/or work practices specific to our study area were involved. For example, most of the pesticide sprayers among our subjects were engaged in dairy farming, which calls for less frequent handling and spraying in the summer. Alternatively, our study areas may not be as plagued by destructive insects as other areas of Japan, thereby requiring less frequent spraying of insecticides. Moreover, unlike the questionnaire designed to assess pesticide exposure in terms of frequency and length of operation of selected pesticides [33], the self-administered questionnaire used in our healthcare checkup program was not designed to specifically reveal detailed information on the practices of pesticide use. This may have caused a nondifferential misclassification in terms of occupational PYR exposure, thus possibly leading to diminished statistical power to detect true differences. Nonetheless, we assume that the effect of such a misclassification would be quite limited since overall 3-PBA$_c$ concentrations in our subjects were distributed over a much lower range than those reported in previous PCO data [33], indicating that high-dose PYR exposures were rare among our subjects. For these reasons, we aggregated the data of sprayers and non-sprayers in our statistical analyses.

We also assumed that the ingestion of food contaminated by pesticide residue is a major route of background environmental exposure to PYRs among the general population [27]. Our results demonstrate a significant association between the frequency of tomato consumption and the urinary 3-PBA$_c$ concentrations in women—but not in men—and no other items of vegetables or fruits under consideration were found to be associated with urinary 3-PBA$_c$ concentrations irrespective of gender. A previous model experiment indicated that the most effective means for eliminating agrochemical food residues is peeling, followed by deep frying, sautéing and boiling [34]. In terms of PYR applications, the use of cypermethrin has been officially recommended over other approaches as a means to effectively control *Liriomyza sativae*, a verminous insect to which cultivated tomatoes are vulnerable. Because tomatoes are customarily eaten raw and unpeeled, and are less likely than other vegetables to undergo these cooking processes, we suggest that the significant association between tomato consumption and 3-PBA$_c$ concentration results largely from this specific way tomatoes are consumed. Another means of removing agrochemicals is by rinsing, but the rates of fenitrothion and cypermethrin elimination were as low as 14.8 and 25.3%, respectively, when tomatoes were hand-rinsed in running water for about 5 s [34]. Moreover, although such tomato products as puree or ketchup are so thoroughly processed that they seem to contain little trace of agrochemicals, such products were seldom likely to be counted as tomato consumption by subjects in our self-administered questionnaires and thus unlikely to have affected the results of the statistical analyses.

We speculate that one possible reason for the lack of a significant relationship between the frequency of tomato consumption and urinary 3-PBA$_c$ concentrations in men was a recall-bias misclassification due to an important difference in dietary behaviors between the sexes; i.e. men were more likely to wrongly classify their intake frequency of food consumption because, as is commonly recognized, they generally do not purchase food themselves or fix meals as routinely as most women do.

To deal with the reliability issue concerning the self-administered FFQ, we measured the test–retest reliability with kappa (κ) statistics to assess the agreement of responses to the questions on the 12 food items among 295 subjects (103 men and 192 women) who participated in the healthcare checkup program in both 2005 and 2006. We found the κ values to be 0.40–0.75 for carrots, citrus and vegetable juice and <0.40 for the remaining nine items. Although the estimated levels of agreement did not meet the criterion of being excellent [35], the obtained κ values were all significantly different from null in this reliability test.

In extrapolating our results, interpretations should be made with caution. Although a relationship of statistical significance between the frequency of tomato consumption and urinary 3-PBA$_c$ concentrations was confirmed in women, the estimated mean of 0.58 μg/g creatinine measured in the highest frequency tomato consumers was far below that found in PCOs in the earlier study [33]—and even among those highly exposed PCOs, toxicological signs or symptoms were barely detectable. Any causal relationship between long-term, low-level PYR exposure and any consequences of clinical significance have yet to be explored.

Some weaknesses of this study should be addressed. No evaluations of PYR dose levels from dietary or environmental exposure were implemented in this study, since such an approach is hugely challenging given that individual exposure patterns during normal daily activities are thought to be extremely diversified. For this reason, accurate evaluations of personal exposure levels have historically relied on the development of monitoring tools for assaying urinary metabolites. As such, exposures from food ingestion as opposed to those from other pathways are usually indistinguishable. Schettgen et al. argued that the oral uptake of the daily diet was the main route of exposure to PYRs based on their findings that the amounts of both dermal uptake and inhalatory absorption of PYRs were relatively small [16]. In contrast, Lu et al. concluded that residential pesticide use represented a highly influential factor in children's exposure to PYR insecticides, while diet also remained a significant predictor of PYR exposure [13]. Since no similar research has been conducted among adult general populations, we have recently initiated a new epidemiological study aimed at capturing the impact of pesticide use in residential settings on the variations in urinary metabolite levels and their possible health consequences.

References

1. Heudorf U, Angerer J. Metabolites of pyrethroid insecticides in urine specimens: current exposure in an urban population in Germany. Environ Health Perspect. 2001;109:213–7.
2. Heudorf U, Angerer J, Drexler H. Current internal exposure to pesticides in children and adolescents in Germany: urinary levels of metabolites of pyrethroid and organophosphorus insecticides. Int Arch Occup Environ Health. 2004;77:67–72.
3. Gunma Prefectural Food Safety Information Center. A booklet on agrichemicals for consumers (in Japanese). 2nd edn. Gunma Prefecture: Japan; 2006.
4. Ito T, Nakamura Y. Deaths from pesticide poisoning in Japan, 1968–2005: data from vital statistics. J Rural Med. 2008;3:5–9.
5. Carr WC Jr, Iyer P, Gammon DW. A dietary risk assessment of the pyrethroid insecticide resmethrin associated with its use for West Nile Virus mosquito vector control in California. Sci World J. 2006;6:279–90.
6. Kulkarni MA, Malima R, Mosha FW, Msangi S, Mrema E, Kabula B, et al. Efficacy of pyrethroid-treated nets against malaria vectors and nuisance-biting mosquitoes in Tanzania in areas with long-term insecticide-treated net use. Trop Med Int Health. 2007;12:1061–73.
7. Alavanja MC, Hoppin JA, Kamel F. Health effects of chronic pesticide exposure: cancer and neurotoxicity. Annu Rev Public Health. 2004;25:155–97.
8. Jamal GA, Hansen S, Julu PO. Low level exposures to organophosphorus esters may cause neurotoxicity. Toxicology. 2002; 181–182:23–33.
9. Galloway T, Handy R. Immunotoxicity of organophosphorus pesticides. Ecotoxicology. 2003;12:345–63.
10. Kamijima M, Hibi H, Gotoh M, Taki K, Saito I, Wang H, et al. A survey of semen indices in insecticide sprayers. J Occup Health. 2004;46:109–18.
11. Okamura A, Kamijima M, Shibata E, Ohtani K, Takagi K, Ueyama J, et al. A comprehensive evaluation of the testicular toxicity of dichlorvos in Wistar rats. Toxicology. 2005;213:129–37.
12. Crow JA, Borazjani A, Potter PM, Ross MK. Hydrolysis of pyrethroids by human and rat tissues: examination of intestinal, liver and serum carboxylesterases. Toxicol Appl Pharmacol. 2007;221:1–12.

13. Lu C, Barr DB, Pearson M, Bartell S, Bravo R. A longitudinal approach to assessing urban and suburban children's exposure to pyrethroid pesticides. Environ Health Perspect. 2006;114:1419–23.
14. Repetto R, Baliga SS. Pesticides and immunosuppression: the risks to public health. Health Policy Plan. 1997;12:97–106.
15. Leng G, Kuhn KH, Idel H. Biological monitoring of pyrethroids in blood and pyrethroid metabolites in urine: applications and limitations. Sci Total Environ. 1997;199:173–81.
16. Schettgen T, Heudorf U, Drexler H, Angerer J. Pyrethroid exposure of the general population—is this due to diet? Toxicol Lett. 2002;134:141–5.
17. Dorman DC, Beasley VR. Neurotoxicology of pyrethrin and the pyrethroid insecticides. Vet Hum Toxicol. 1991;33:238–43.
18. Leng G, Gries W. Simultaneous determination of pyrethroid and pyrethrin metabolites in human urine by gas chromatography-high resolution mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2005;814:285–94.
19. Hodgson E. In vitro human phase I metabolism of xenobiotics I: pesticides and related compounds used in agriculture and public health, May 2003. J Biochem Mol Toxicol. 2003;17:201–6.
20. Eadsforth CV, Baldwin MK. Human dose-excretion studies with the pyrethroid insecticide, cypermethrin. Xenobiotica. 1983;13:67–72.
21. Eadsforth CV, Bragt PC, van Sittert NJ. Human dose-excretion studies with pyrethroid insecticides cypermethrin and alphacypermethrin: relevance for biological monitoring. Xenobiotica. 1988;18:603–14.
22. Kim HJ, Ahn KC, Ma SJ, Gee SJ, Hammock BD. Development of sensitive immunoassays for the detection of the glucuronide conjugate of 3-phenoxybenzyl alcohol, a putative human urinary biomarker for pyrethroid exposure. J Agric Food Chem. 2007;55:3750–7.
23. Hardt J, Angerer J. Biological monitoring of workers after the application of insecticidal pyrethroids. Int Arch Occup Environ Health. 2003;76:492–8.
24. Leng G, Leng A, Kuhn KH, Lewalter J, Pauluhn J. Human dose-excretion studies with the pyrethroid insecticide cyfluthrin: urinary metabolite profile following inhalation. Xenobiotica. 1997;27:1273–83.
25. Sudakin DL. Pyrethroid insecticides: advances and challenges in biomonitoring. Clin Toxicol. 2006;44:31–7.
26. Saieva C, Aprea C, Tumino R, Masala G, Salvini S, Frasca G, et al. Twenty-four-hour urinary excretion of ten pesticide metabolites in healthy adults in two different areas of Italy (Florence and Ragusa). Sci Total Environ. 2004;332:71–80.
27. Becker K, Seiwert M, Angerer J, Kolossa-Gehring M, Hoppe HW, Ball M, et al. GerES IV pilot study: assessment of the exposure of German children to organophosphorus and pyrethroid pesticides. Int J Hyg Environ Health. 2006;209:221–33.
28. Lu C, Toepel K, Irish R, Fenske RA, Barr DB, Bravo R. Organic diets significantly lower children's dietary exposure to organophosphorus pesticides. Environ Health Perspect. 2006;114:260–3.
29. Ozasa K, Watanabe Y, Ito Y, Suzuki K, Tamakoshi A, Seki N, et al. Dietary habits and risk of lung cancer death in a large-scale cohort study (JACC Study) in Japan by sex and smoking habit. Jpn J Cancer Res. 2001;92:1259–69.
30. Leng G, Gries W, Selim S. Biomarker of pyrethrum exposure. Toxicol Lett. 2006;162:195–201.
31. Finkelstein MM, Verma DK. Exposure estimation in the presence of nondetectable values: another look. AIHAJ. 2001;62:195–8.
32. Wilkes MF, Woollen BH, Marsh JR, Batten PL, Chester G. Biological monitoring for pesticide exposure—the role of human volunteer studies. Int Arch Occup Environ Health. 1993;65:S189–92.
33. Wang D, Kamijima M, Imai R, Suzuki T, Kameda Y, Asai K, et al. Biological monitoring of pyrethroid exposure of pest control workers in Japan. J Occup Health. 2007;49:509–14.
34. Yoshikawa N, Sugibayashi S, Kataoka J, Kawaguchi Y, Fujimoto Y, Nakanishi H, et al. Rinsing and cooking processes markedly change the content of twelve kinds of agrochemicals, including fenitrothion, added in a model system to green peppers, grapes, or spinach(in Japanese). Jpn J Food Chem. 1996;3:57–63.
35. Zhu S, Toyoshima H, Kondo T, Tamakoshi K, Yatsuya H, Hori Y, et al. Short- and long-term reliability of information on previous illness and family history as compared with that on smoking and drinking habits in questionnaire surveys. J Epidemiol. 2002;12:120–5.