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Determination of the pyrethroid insecticide metabolite 3-PBA in plasma and urine samples from farmer and consumer groups in northern Thailand

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Abstract

In this study, the enzyme-linked immunosorbent assays (ELISA) were modified to detect 3-PBA in plasma (including the adducted form) and urine among a large group of consumers and farmers in an agricultural area. The samples were collected on the same day in the morning from 100 consumers (50 females, 50 males) and 100 farmers (50 females, 50 males) in the Fang district, Chiang Mai province, northern Thailand. The ELISA was very sensitive having an IC₅₀ value of 26.7 and 15.3 ng/mL, a limit of quantitation of 5 and 2.5 ng/mL and a limit of detection of 1.08 and 1.94 ng/mL for plasma and urine, respectively. These methods had low (< 5%) intra- and inter-assay coefficients of variation. The extraction technique satisfactorily eliminated the matrix effect from samples before ELISA analysis, yielding good recoveries (85.9–99.4% and 87.3–98.0%, respectively). For the volunteer study, the detection rate for plasma 3-PBA was 24% in consumers and 42% in farmers, but the median and range values were similar (median 5.87 ng/mL, range 5.16–8.44 ng/mL in consumers and 6.27 ng/mL, range 4.29–9.57 ng/mL in farmers). The rate of detection in the urine was similar (76% and 69%, in consumers and in farmers), yet the median concentration was significantly higher in farmers (8.86 µg/g creatinine in consumers vs 16.1 µg/g creatinine in farmers) and the range also much wider in farmers (1.62–80.5 µg/g creatinine in consumers and 0.80–256.2 µg/g creatinine in farmers). There was no correlation between plasma 3-PBA and urinary 3-PBA concentrations in the study presumably because plasma 3-PBA is a measure of cumulative exposures while urinary 3-PBA reflects acute exposures. In addition, metabolism and excretion of pyrethroids varies by individual. Nevertheless, this study demonstrated that these volunteers were exposed to pyrethroids. To our knowledge, this is the first report that compared plasma 3-PBA and urinary 3-PBA in a large

group of volunteers. The ELISA method provided higher sample throughput with lower cost as compared to the instrumental analysis.

Keywords

Pyrethroid insecticide; 3-PBA-adduct; plasma 3-PBA; urinary 3-PBA; farmers; consumers; ELISA

Introduction

The synthetic pyrethroids are derived structurally from the natural pyrethrins that originate from the botanical insecticide pyrethrum, an extract obtained from the flowers of *Chrysanthemum cinerariaefolium*. Synthetic pyrethroids including cypermethrin, deltamethrin and permethrin are registered for use in the United States and many other parts of the world and account for more than \$1.5 billion in pesticide sales.^[1]

Acute toxicity such as headache, dizziness, nausea, irritation of the skin and nose, and paraesthesia can occur when exposed to an overdose of pyrethroids.^[2,3] In the case of chronic toxicity, the pyrethroid fenvalerate may cause lymph node and splenic damage as well as carcinogenesis^[4] and pyrethroids have a suppressive effect on the immune system.^[5] Permethrin has been classified as a potential carcinogen at high concentrations by the U.S. EPA.^[6,7] Pyrethroids may be considered hormone disruptors from studies on their estrogenic potential in human breast carcinoma cells.^[8] Because of the potential toxicity, the U.S. EPA set a Reference Dose (RfD) of 0.25 mg/kg/day for both acute and chronic dietary exposures to permethrin.^[9] The World Health Organization (WHO) has set a limit of 0.3 mg/L (300 ng/mL) as a guideline for permethrin in drinking water when it is applied to water for mosquito control.^[10] The Agency for Toxic Substances and Disease Registry (ATSDR) determined Minimum Risk Levels (MRLs) for oral exposures to technical grade permethrin of 0.3 mg/kg/day for acute oral exposures (up to 14 days) and 0.2 mg/kg/day for intermediate durations (15–364 days).^[11]

After humans are exposed to pyrethroids, the parent compounds measured in plasma have a half life of 2.5–12 hr.^[12,13] The parent compounds are hydrolyzed by an esterase enzyme to 3-phenoxybenzyl alcohol or 3-phenoxybenzaldehyde. These compounds are rapidly converted to 3-phenoxybenzoic acid (3-PBA). The 3-PBA is conjugated to glucuronic acid that renders the xenobiotic more polar and facilitates its excretion in urine. In mammals, pyrethroid esters are mostly eliminated in urine (93%) during the first 24 hour.^[14] Total 3-phenoxybenzoic acid (3-PBA), has been measured in human urine as a biomarker of exposure.^[15,16] Other metabolites such as *cis*- and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid that derive from parent compounds such as cyfluthrin, permethrin, and cypermethrin^[17] are also formed but were not measured in this study.

The disadvantage to measuring 3-PBA in the urine is that it is considered a biomarker of acute exposure, since the metabolite is so rapidly eliminated. On the other hand, it is well known that protein adducts of xenobiotics measured in plasma are persistent biomarkers

having half lives up to several months.^[18] The compound 3-PBA-glucuronide has also been shown to form protein adducts.^[19] Two mechanisms of adduct formation from glucuronidation of carboxylic acids have been demonstrated. In the transacylation mechanism, acyl glucuronides that are potentially electrophilic can react with nucleophilic residues in proteins. According to the glycation mechanism, an initial internal acyl migration occurs and also reacts with amino groups of the protein, leading to Schiff base adducts or Amadori rearrangement.^[20] The most likely protein in humans for adduction of acyl glucuronides is albumin due to its abundance (60%) in blood.^[21]

Analytical methods for the detection of pyrethroid metabolites such as high-performance liquid chromatography (HPLC) or gas chromatography (GC) with mass spectrometry (MS) are expensive.^[14, 22–24] Enzyme-linked immunosorbent assays (ELISA) have been presented as a cost effective alternative to instrumental methods.^[25] Because of the potential health implications, it is important to monitor populations in order to form strategies to minimize exposure. Thus, we opted to determine both plasma and urinary 3-PBA in populations consisting of consumers and farmers in Thailand utilizing an immunoassay to monitor 3-PBA in urine ^[26] that had been further adapted and validated to measure 3-PBA in plasma following hydrolysis of adducts.^[27] To our knowledge this is the first study to assess 3-PBA protein adducts in humans.

Materials and methods

Materials and instrumentation

Bovine serum albumin (BSA), fetal bovine serum, goat anti-rabbit immunoglobulin G (whole molecule) peroxidase conjugate (GAR-HRP), H₂O₂, Tween 20, dimethyl sulfoxide, 3,3',5,5'-tetramethylbenzidine (TMB), N,N-dimethyl-formamide (DMF), N-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were reagent grade or better from Fisher Scientific (Pittsburgh, PA, USA) or Merck (Darmstadt, Germany). The samples were extracted using Strata Screen-A mixed mode solid phase extraction columns (8B-S019-EAK, Phenomenex, Torrance, CA, USA). The ELISA method was performed on Nunc Maxisorp Immunoplates (96F, 442404, Roskilde, Denmark) and read spectrophotometrically with a microplate reader (Tecan Sunrise, Männedorf, Switzerland). A creatinine kit (Sysmex Corporation, Kobe, Japan) was used to measure the concentration of creatinine in urine according to the manufacturer's instructions.

Study site and population characteristics

The present study was a cross-sectional study designed to assess pyrethroid exposure among consumers and farmers. Farmer volunteers were assumed to be occupationally exposed. The Fang district of Chiang Mai province, one of the most intensive agricultural areas in northern Thailand, is composed of eight sub-districts. The four sub-districts with the most intensive agriculture were selected as study sites. These were Mae Kha, Mae Ngon, Wiang, and Mae Ka sub-districts. These four sub-districts contained 56% (63,879) of the total Fang district population (112,402).^[28] The subjects included 100 consumers (50 females, 50

males) and 100 farmers (50 females, 50 males). Farmers and consumers in these selected sub-districts were sampled using a random number of their household numbers in order to provide the same probability of being selected into the study. Numbers of farmers and consumers participated in the study described by sub-district is shown in Table 1. This study was approved by the Human Experimentation Committee, Research Institute for Health Sciences (RIHES), Chiang Mai University (No. 32/2006).

Sample collection and preparation

A consent form was introduced and signed by individual participants prior to collection of personal data, blood, and urine samples. Blood and urine samples were taken in the morning of the same day. Approximately, 10 mL of venous blood samples in vacutainer heparinized tubes and 50 mL of midstream morning void urine samples in 250 mL polypropylene bottles were collected and placed in the ice chest. The samples were then transported to Toxicology Laboratory, Research Institute for Health Sciences, Chiang Mai University (about 150 km away). On the next day, blood samples were centrifuged at 2500 rpm for 15 min at 4°C to collect plasma. Plasma and urine samples were aliquoted into 1 mL and 10 mL, respectively, and then frozen (-20°C) until analysis. On the day of analysis, samples were thawed at room temperature, gently mixed to homogeneity and then sampled for analysis. The analyses of all samples were finished within 1 year.

The plasma 3-PBA [27] and urinary 3-PBA analyses [15,29] were modified from previous studies. To prepare plasma samples, briefly, 100 µL sodium hydroxide (6 N) was added to the plasma (0.5 mL) and placed in a heating block at 100°C for 1 hr to hydrolyze the 3-PBA adduct. After cooling, 1 mL of 0.2 M sodium acetate buffer, pH 4.5 was added to adjust the pH to around 12. For further cleanup, liquid-liquid extraction (LLE) was employed by adding 2 mL of ethyl acetate and the sample shaken vigorously for 10 min. The ethyl acetate was discarded. The ethyl acetate removes organic contaminants while the 3-PBA remains in the aqueous phase. To the remaining aqueous phase was added 120 µL of hydrochloric acid (6 N) to adjust the pH to around 3. This was extracted twice with 2 mL of ethyl acetate. The organic phases were evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved in 200 µL of methanol. Two milliliters of 0.2 M sodium acetate buffer was added to adjust the pH to around 5 and mixed thoroughly. The sample was then ready for solid phase extraction (SPE). The SPE cartridge used was the Strata Screen-A (55 µm, 70 Å), 100 mg/mL containing a mixed mode silica-based sorbent consisting of mixed-mode SPE hydrophobic (C8) and ionic (anion exchange) phases. The SPE method was described previously.[15]

For measurement of 3-PBA in urine, we used a common acid hydrolysis method to release any 3-PBA that may have been in the conjugated form such as glucuronides, sulfates or amino acids. A 0.5 mL aliquot of urine was added into a glass vial. The urine sample was treated with 100 µL of 6 N hydrochloric acid and heated at 100°C for 1 hr. The hydrolyzed urine was neutralized by adding 1 mL of 0.2 M sodium acetate buffer (pH 4.5) followed by 100 µL of sodium hydroxide (6 N) and mixing thoroughly to adjust the pH to around 4.5. To reduce the matrix effect of the hydrolyzed urine, the same SPE method as for the plasma 3-PBA was used.

Sample analysis

The specific antibody and hapten for the target analyte (3-PBA) and the preparation of coating antigen, the buffers and the procedure for the indirect competitive ELISA was previously described.^[29] Briefly, 96-well plates were coated overnight at 4°C with 100 µL of coating antigen, 3-PBA-BSA, (0.5 µg/mL). The following day, plates were blocked for 30 min with 200 µL 0.5% BSA in PBS (phosphate buffered saline) at room temperature. After 30 min the blocking solution was removed. Then 50 µL of extracted samples (plasma or urine) and 50 µL of antiserum (1:7000) were added to each well and mixed for 1 hr at room temperature. The plates were washed five times. The goat anti-rabbit IgG-horseradish peroxidase conjugate diluted 1/10000 (100 µL) was added for 1 hr. Following a wash, the TMB substrate (100 µL) was added for 15 min then the reaction stopped and absorbance measured at 450 nm. The concentrations of unknown samples were calculated from the calibration curve using a 4-parameter fit equation and expressed as 3-PBA equivalents. Urinary 3-PBA concentrations were normalized to creatinine levels to correct for dilution of urine and is often used to normalize when spot samples are taken rather than 24 hr samples.^[30] Although plasma 3-PBA may be referred to as the 3-PBA-adduct, the concentrations reported here are a measure of the total of bound and free 3-PBA in the plasma.

Previous work from this laboratory described suitable conditions for the ELISA analysis of plasma 3-PBA.^[27] An IC₅₀ value of 26.7 ng/mL and reproducibility (%CV < 15%) was obtained. The IC₅₀ value is expressed as the sensitivity of the immunoassay and approximates the concentration of analyte giving 50% inhibition. The recovery of spiked free 3-PBA in plasma was 85.9–99.4%. More than 80% 3-PBA was recovered from the adducted form (3-PBA-BSA) when subjected to hydrolysis. The limit of quantitation (LOQ) was 5 ng/mL, 30- to 40-fold more sensitive than a previous study (150–200 ng/mL) that used HPLC analysis.

Quality assurance and control

For laboratory quality assurance and control, precision measurements were conducted prior to the analysis of real samples. Plasma and urine controls (10 tubes) were analyzed for day-to-day variation of samples to obtain an optimum condition variance (OCV) range (mean ± 2SD). Each plasma analysis batch consisted of 15 samples, 1 sample blank and 2 controls using pooled plasma that were placed in the first and last tubes. For urine, 25 samples were analyzed in each batch with 3 pooled urine controls placed in the first, middle and last tubes. The controls in each batch were analyzed for intra-batch variation of samples to obtain within-day CV (%). Inter-batch variation of samples was analyzed from controls in all batches analyzed (n = 14 and 9, respectively) to obtain between-day CV (%).

Statistical analysis

Data from laboratory results were analyzed by SPSS Statistical Software Package (SPSS, Thailand) version 17 as follows; (1) the concentrations of 3-PBA were tested for normal distribution (parametric or nonparametric), (2) % detection, median and range of 3-PBA concentrations were computed, (3) the plasma 3-PBA and urinary 3-PBA concentrations were compared between consumers and farmers using the paired sample t-test, (4) the

correlation between plasma 3-PBA and urinary 3-PBA concentrations was analyzed using the Spearman test.

Results and discussion

Optimization of the ELISA method

The optimal conditions for the ELISA analysis of plasma 3-PBA was previously developed in this laboratory and described above.^[27] The assay for 3-PBA in urine was also previously reported. The IC₅₀ value in that study was reported at 1.65 ± 0.7 ng/mL.^[29] In our hands, the optimized assay using antiserum 294 (diluted 1:7000, final dilution in well) and a coating antigen concentration of 0.5 µg/mL gave an IC₅₀ value of 15.3 ± 0.77 ng/mL (Fig. 1). Although the IC₅₀ was different, it was highly reproducible, so no further optimization was done. It is not unusual for there to be systematic differences in IC₅₀ between laboratories. Minor differences between operations, preparation of calibrators, or reagent degradation are among some of the causes of such variations.^[31]

Most immunoassays exhibit some cross reactivity to structurally related compounds. The assay used here does not cross react with a variety of parent pyrethroids (permethrin, cypermethrin, esfenvalerate, deltamethrin and cyfluthrin) that contain the phenoxybenzyl moiety. However, 4-fluoro-3-phenoxybenzoic acid, a metabolite of cyfluthrin does cross react at 72%^[29] while 4-hydroxy-3-phenoxybenzoic acid and 3-phenoxybenzaldehyde cross react at 103 and 75%, respectively. Using the same methods as reported here, urine samples collected from forestry workers were analyzed by both LC/MS/MS and immunoassay for 3-PBA. The methods correlated well with slope, intercept and r² of 1.14, 0.24 and 0.964, respectively.^[15] Thus, these other cross-reacting metabolites likely did not greatly impact the determination of urinary 3-PBA for that study population and were not considered significant in the study reported here.

Sample preparation

The 3-PBA immunoassay has been assessed for matrix effects from plasma. Recovery of a laboratory-generated 3-PBA-protein adduct (the 3-PBA was adducted to bovine serum albumin) spiked into fetal bovine serum were greater than 80%. In addition, we found that an additional LLE cleanup was necessary before SPE extraction to eliminate the matrix effect of plasma samples. This resulted in high recovery values (85.9–99.4%) with a limit of quantitation (LOQ) at 5 ng/mL.^[27] In this study, for urinary 3-PBA, SPE extraction was necessary to determine 3-PBA metabolites. The SPE cleanup technique gave high recovery values (87.3–98.0%) with limit of quantitation at 2.5 ng/mL (Table 2), comparable to values obtained earlier.^[15]

The limit of blank, limit of detection and limit of quantitation as defined by Armbruster and Pry^[32] are shown in Table 3. The urine spiked with levels lower than the limit of quantitation were not detected (ND, Table 2) as expected. Analysis of 3-PBA spiked into fetal bovine serum at 4 ng/mL gave values nearly identical to 5 ng/mL.^[27] This higher recovery in serum may be due to a matrix effect hence the LOQ was set to 5 ng/mL. Samples with concentrations lower than 5 and 2.5 ng/mL, in serum and urine respectively,

were not quantitated. The methods reported here are selective and sensitive enough to assess samples from both acute and chronic exposures to pyrethroids. For example the RfD for pyrethroids is 0.25 mg/kg/day.^[9] Assuming an average person weighs 70 kg, has a blood volume of 5 L, and consumes, in a single day, 0.25 mg of pyrethroid and all was absorbed and circulating in the blood, then the expected concentration in the blood would be about 3.5 µg/mL, well above the LOQs reported here.

Quality assurance and control

For quality control, pooled samples of plasma or urine were used. Two or three replicates of the pooled samples were run with each batch of samples. The coefficient of variation (% CV) was less than 5% for both plasma and urine. Moreover, all control concentrations were within the OCV range (mean ± 2SD; 5.79–6.83 and 14.8–17.8 ng/mL, respectively) as shown in Table 4.

Exposure study

From the questionnaire, data showed that the volunteers in this study used pesticides in the home and in agriculture practices. Of the total study population (consumers and farmers; n = 200), 56% used pesticides indoors. The concentration of plasma 3-PBA in the volunteers who used indoor pesticides (n = 106) was significantly higher ($P < 0.005$) than who did not use them (n = 94). Twenty-five percent of the farmers (n = 100) applied pesticides agriculturally. The farmers (n = 100) used a variety of pesticide types: 83% insecticides including synthetic pyrethroids, 75% herbicides, 72% chemical fertilizers, 56% organic fertilizers and 41% plant growth substances.

The % detection of plasma 3-PBA was less than 50% overall while the detection of urinary 3-PBA was greater than 50% for both groups (Table 5). Although the rates of detection in plasma or urine were similar between the groups, the range of urinary concentrations was generally higher for the farmer group and the median concentrations were significantly different a $P < 0.01$ likely reflecting recent acute exposures in the farmer group. Longer-term exposure seems more similar between the groups since the median and range of plasma 3-PBA concentration was similar between groups.

In Table 6, the % detection of plasma 3-PBA and urinary 3-PBA for consumer and farmer groups are broken out by concentration ranges. Plasma concentrations for all subjects were less than 10 ng/mL although the rate of detection was nearly double in farmers compared to consumers in the 5.1–10 ng/mL range. Similarly more farmers than consumers had urinary concentrations of 3-PBA greater than 10 µg/g creatinine and the upper range of concentrations were much higher for farmers. Additionally, the concentrations of urinary 3-PBA in farmers in Chiang Mai at selected percentile values in the present study were higher than found by previous researchers (Fig. 2).^[33,34] This may be due to different agricultural practices.

Thus, the farmer group appears to have greater acute exposure most likely due to their agricultural activities.^[33] Consumers that lived in communities where crops were produced also likely had more potential for exposure to insecticides.^[24] However, there are other

pathways and routes by which both farmers and consumers may be exposed to these insecticides. Consumption of pesticide-contaminated vegetables and fruits was thought to be the major route by which volunteers were exposed to pesticides. The other routes were dermal contact and the respiratory route from insecticide drift in the air from vector control programs [35] and from usage in the household for controlling insects.

Interestingly, there was no correlation between plasma 3-PBA and urinary 3-PBA concentrations in the study population. However, the farmer population likely had more recent acute exposures that would lead to transient high urinary 3-PBA concentrations. Also, metabolism and urinary elimination of pyrethroids varies by individual.^[36] Further studies are necessary to determine the precise reasons for the difference.

Conclusion

A sensitive and specific ELISA for plasma and urinary 3-PBA had been developed. The modified methods were successfully applied to the detection of 3-PBA, a urinary metabolite of some pyrethroid insecticides among a rather large population in an agricultural area. These assays could analyze 20 and 37 samples per day for plasma and urine samples, respectively. The ELISA generated data rapidly (within 3 hr) and was suitable for routine analysis. We estimate that each sample cost about \$12.00 to analyze, compared to the relatively high cost (\$50–150 per sample) of instrumental analysis such as a liquid chromatography-tandem mass spectrometry (LC/MS/MS). To our knowledge, this is the first report that compares plasma 3-PBA, including the adducted form and urinary 3-PBA in a large group of volunteers. Although there was no correlation between plasma 3-PBA and urinary 3-PBA concentrations in the study population, it may be due to differences in metabolism and urinary elimination of pyrethroids among individual as well as the higher likelihood those farmers have recent acute exposures. Further studies will be needed to validate the 3-PBA in plasma as a biomarker of cumulative exposures to pyrethroids. However, the applications of the methods for plasma and urinary 3-PBA analyses in this study showed confirmation that these volunteers are exposed.

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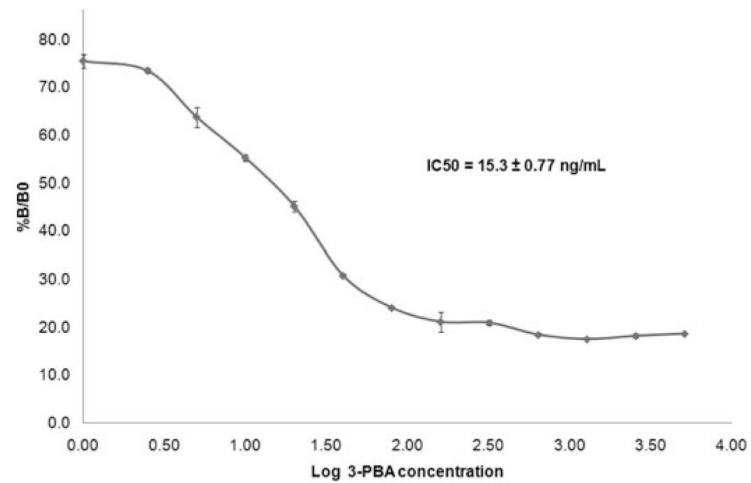


Fig. 1. ELISA inhibition curve for urinary 3-PBA using antiserum 294 (diluted 1:7,000, final dilution in well), coating antigen 3-PBA-BSA (0.5 $\mu\text{g}/\text{mL}$), and GAR-HRP (1:10,000). IC_{50} ($15.3 \pm 0.77 \text{ ng}/\text{mL}$) approximates the concentration of analyte giving 50% inhibition. The X axis is the log of the 3-PBA concentration and the Y axis is %B/B0 calculated from (absorbance of sample/absorbance of blank) \times 100. The standard deviation was calculated in duplicate ($n = 2$) at each point.

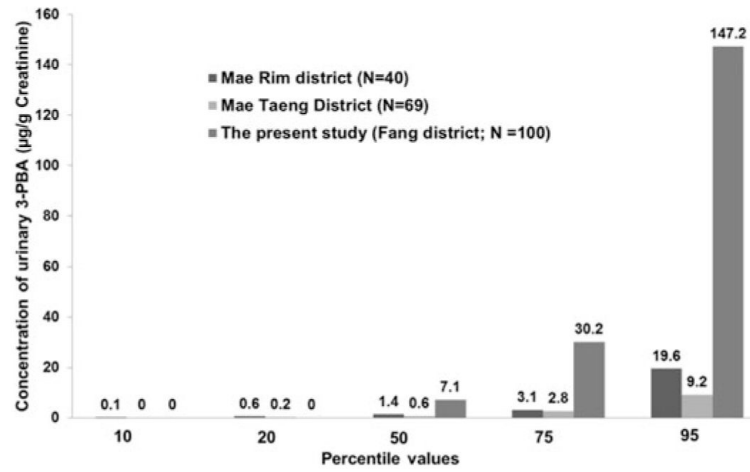


Fig. 2.

The concentrations of urinary 3-PBA in farmers in Chiang Mai province at Mae Rim district (N = 40 ^[34]) and Mae Taeng District (N = 69 ^[33]) at selected percentile values compared to the present study (Fang district).

Table 1

Participant distribution through 4 sub-districts in the Fang district of Chiang Mai, Thailand.

Subdistricts	Consumer (n)	Farmer (n)
Mae Kha	37	20
Mae Ngon	19	15
Wiang	26	36
Mae Ka	18	29
Total	100	100

Consumer and farmer groups consisted of 50 males and 50 females.

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Table 2

Recoveries after hydrolysis, extraction and ELISA analysis of 3-PBA spiked in urine containing no detectable 3-PBA by ELISA, in triplicate (n = 3) on the same day.

<i>Standard addition(ng/mL)</i>	<i>Mean ± SD (n = 3) of 3-PBA (ng/mL)</i>	<i>% Recovery</i>
1	ND	ND
2.5	2.32 ± 0.20	92.8
5	4.47 ± 0.72	89.5
10	8.73 ± 0.16	87.3
20	19.6 ± 0.34	98.0

ND = Not detected, because the signal was not distinguishable from background.

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Table 3

Limit of blank (LOB), limit of detection (LOD) and limit of quantitation (LOQ) of methods for measurement of plasma 3-PBA and urinary 3-PBA.

<i>Value</i>	<i>Plasma 3-PBA Mean \pm SD, n = 2, (ng/mL)</i>	<i>Urinary 3-PBA Mean \pm SD, n = 2 (μg/g Creatinine)</i>
LOB	0.50 \pm 0.03	0.46 \pm 0.01
LOD	1.08 \pm 0.20	1.94 \pm 0.34
LOQ	5.00 \pm 0.19	2.50 \pm 0.37

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Table 4

Intra- and inter-assay variation of pooled controls for plasma and urine.

Batches	Plasma 3-PBA			Batches	Urinary 3-PBA				
	Concentration (ng/mL)		2 tubes; intra-variation % CV of controls		Concentration (ng/mL)			3 tubes; intra-variation % CV of controls	
	First-tube	Last-tube			First-tube	Mid-tube	Last-tube		
1	5.95	5.95	0.01	1	17.3	16.4	17.2	2.88	
2	6.22	6.49	3.03	2	15.6	15.2	16.2	2.95	
3	6.12	5.82	3.52	3	16.5	16.1	15.8	2.13	
4	6.47	6.47	0.00	4	16.1	15.7	16.8	3.58	
5	6.22	6.27	0.57	5	15.9	15.7	16.9	4.08	
6	6.66	6.27	4.34	6	16.1	16.4	15.7	1.90	
7	6.37	6.37	0.01	7	17.3	16.4	17.2	2.88	
8	6.50	6.47	0.38	8	15.6	15.2	16.2	2.95	
9	6.29	6.48	2.21	9	16.5	16.1	15.8	2.13	
10	6.30	6.24	0.74	% CV of controls (27 tubes; inter-variation) = 4.60					
11	6.15	6.09	0.75						
12	6.22	5.96	3.02						
13	6.34	6.14	2.25						
14	6.10	5.91	2.34						

% CV of controls (28 tubes; inter-variation) = 4.14.

Table 5

Descriptive analytical data for plasma 3-PBA and urinary 3-PBA detected in human plasma between consumers (n = 100) and farmers (n = 100).

Metabolites	% Detection		Median		Range	
	Consumer	Farmer	Consumer	Farmer	Consumer	Farmer
Plasma 3-PBA (ng/mL)	24	42	5.82	6.27	5.16–8.44	4.29–9.57
Urinary 3-PBA ($\mu\text{g/g}$ creatinine)	76	69	8.86	16.1*	1.62–80.5	0.80–256.2

* Significantly different between groups at $P < 0.01$ using a paired T test.

Table 6

The % detection of plasma 3-PBA and urinary 3-PBA in consumer and farmer groups by concentration ranges.

<i>Concentration ranges</i>	<i>% Detection</i>			
	<i>Consumer (n = 100)</i>		<i>Farmer (n = 100)</i>	
	<i>plasma 3-PBA (ng/mL)</i>	<i>urinary 3-PBA (µ g/g creatinine)</i>	<i>plasma 3-PBA (ng/mL)</i>	<i>urinary 3-PBA (µ g/g creatinine)</i>
Less than 2.5	76	27	59	36
2.5–5.0	(Less than 5)	15	(Less than 5)	10
5.1–10.0	24	26	42	11
10.1–20.0	–	10	–	12
20.1–30.0	–	7	–	6
30.1–40.0	–	5	–	6
40.1–50.0	–	3	–	6
More than 50	–	7	–	13