Journal of Clinical Toxicology
ISSN: 2161-0495

LeBeau et al. ,J Clinic Toxicol 2012, S:5
DOI: 10.4172/2161-0495.S5-003

Research Article Open Access

Evaluation of Urinary Pesticide Biomarkers among a Sample of the Population in the United States

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Abstract

Pesticide use in the United States continues to raise controversy over potential effects on human health. This investigation examined biomarkers of exposure levels in a sample of the United States population from the 2001-2002 NHANES dataset. The detection frequency of urinary biomarkers of exposure and the geometric mean were determined from 3,152 individual samples with stratified analysis for relevant subgroups. The association between the detection of a biomarker of exposure and differences in height and weight of children aged 6-11 was analyzed. Of the 18 specific pesticide biomarkers sampled, three were detected in more than 50% of the population sample: 79% had a detectable level of 3,5,6-trichloropyridinol, a biomarker of chlorpyrifos, with a geometric mean of 2.07 µg/L (C.I: 1.98-2.17); 53% had a detectable level of paranitrophenol, a biomarker of methyl parathion, with a geometric mean of 0.367 µg/L (C.I.: 0.346-0.389); and 77% had a detectable level of 3-phenoxybenzoic acid, a biomarker of permethrin, with a geometric mean of 0.336 µg/L (C.I.: 0.320-0.352). No clear trend emerged when evaluating associations between height and biomarker detection in children, with the absence of significant results for trichloropyridinol; heavier children associated with 3-phenoxybenzoic acid at age 7 [Detect=28.61 kg and Non-Detect=25.26 kg (p=0.009)]; and paranitrophenol being associated with shorter children at age 8 [Non-Detect=134.3 cm and Detect: 130.9 cm (p=0.046)] and taller children at age 11 [Detect=153.7 cm and Non-Detect=149.9 cm (p=0.022)]. A comparative analysis with extant epidemiological and biomonitoring literature is consistent with the findings reported here and suggests that there is insufficient evidence for a relationship between background exposure levels to these common pesticides and measured developmental health effects.

Keywords: Pesticide biomarkers; Organophosphates; Pyrethroids; Pediatric development

Introduction

Public health concerns surrounding the use of pesticides have been a major focus of the United States Environmental Protection Agency (EPA) and the United States Food and Drug Administration (FDA) for decades. On a global scale, approximately $39 billion dollars were spent on pesticides in 2007, with $12.5 billion dollars spent in the Unites States (US) alone [1]. This equates to the use of 5.2 billion pounds of pesticide's active ingredients globally, with 1.1 billion pounds used in the US [1]. These active ingredients are used to create the more than 20,000 EPA registered pesticides currently available in the US [2].

Agricultural workers, exterminators and pesticide manufacturing employees encounter high exposures to pesticides due to their trade and, thus, are at the highest risk for a biologically significant pesticide overexposure in the workplace [3-5]. It is estimated that approximately 2-2.5 million workers come in contact with pesticides annually as a result of their employment [6,7]. The National Institute for Occupational Safety and Health (NIOSH) Sentinel Event Notification System for Occupational Risk (SENSOR) program monitors occupational illnesses and injury in participating states [5,8]. SENSOR data analyzed by Calvert et al. [5] indicated that, between 1998 and 2005, approximately 3,200 acute occupationally exposed pesticide related illnesses were reported in 10 states [3]. Geer et al. [9] reported that the EPA estimates in 1992 suggested that ~10,000-20,000 acute occupational excessive exposures occur annually.

Individuals that are exposed to pesticides outside of an occupational setting are considered general population exposures. Exposures in the general population can be indirect and are generally orders of magnitude lower than occupational exposures [10]. In 2007, domestic use of pesticides accounted for 8 percent of the conventional pesticides used in the United States [1]. Widespread pesticide use in the United States has equated to ~94,000 acute pesticide exposures in 2008, according to the American Association of Poison Control Centers' National Poison Data System's 26th annual report [11].

Residential exposures occur through a variety of sources: handling and application of pesticides in home and garden settings, residues remaining on surfaces in the home following residential application of pesticide products, food and drinking water that contain pesticide residues, aerosol drift, and take-home exposure from either pesticide applications in close proximity to the home or from family members transporting residues home on soiled or contaminated clothing [10,12-18]. It has been suggested that ingestion of food products containing pesticides is the primary route of exposure for the general population [19-22].

While the effects from acute overexposures to organophosphate (OP) pesticides and other pesticides that affect the nervous system (such as pyrethroids) have been well established, there appears to be no consensus on the potential health effects from chronic, low-level exposures [23]. It has been observed that physiological differences in young animals (low detoxification rate by CYP and PON1 enzyme systems) may make them more sensitive to a cholinergic crisis than adult animals [24]. With this increased focus on the young, new

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Received March 27, 2012; **Accepted** April 06, 2012; **Published** April 19, 2012

Citation: LeBeau AL, Johnson GT, McCluskey JD, Harbison RD (2012) Evaluation of Urinary Pesticide Biomarkers among a Sample of the Population in the United States. J Clinic Toxicol S5:003. doi:10.4172/2161-0495.S5-003

concerns arise regarding whether exposures to pesticides could result in teratogenic effects or alter pediatric development. In recent years, this research has focused on the potential effects that chronic, low-level pesticides may have, especially on children and various sub-populations, attempting to correlate adverse health effects with detections of pesticide biomarkers in biological media.

In order to evaluate potential health risk in adult and pediatric populations due to pesticide exposure, it is imperative to characterize the background exposure that is experienced in the population, which can be achieved with national health monitoring data. National health monitoring in the U.S. dates back to the National Health Survey Act of 1956 that gave authorization for the creation of a continuing survey that would give statistical data on the amount, distribution and types of illness and disability in the US [25]. The National Health Examination Survey's of the 1960's (NHES I-III) were multi-year studies that evaluated select chronic diseases and growth and development in a variety of age groups. The impact of nutrition and its relationship to health status was added to the study design in the 1970, thus creating the National Health and Nutrition Examination Survey and resulted in multi-year studies (NHANES I-III and HHANES), with a different focus for each study event. Beginning in 1999, NHANES became a continuous, annual survey event in the US [25]. Sampling for the 2001-2002 NHANES collected data from 11,039 participants aged 6-85+ years within the U.S. and included a subset of urinary pesticide biomarker measurements.

The objective of the current investigation is to use the 2001-2002 NHANES urinary pesticide biomarker measurements to characterize the background exposure burden for common pesticides used within the United States. As well, the association between pediatric growth and the detection of urinary pesticide biomarkers are evaluated to examine potential effects of pesticide exposure on childhood development. A comparative analysis with extant epidemiological literature is presented to interpret the exposure characterization produced in this analysis.

Methods and Materials

Data source

The data evaluated in this research originated from the 2001-2002 NHANES sampling event. Sampling for the 2001-2002 NHANES collected data from 11,039 participants aged 6-85+ years in 30 Primary Sampling Units (PSU) across the US. A PSU is defined as a county or a group of contiguous counties [26]. The PSU can be further divided into blocks or groups of blocks within one of those counties and households on the blocks. The Centers for Disease Control and Prevention (CDC) does not supply specific information on the locations of PSU in order to protect the confidentiality of participants [27]. Demographic information gathered from the examination including participant's age, gender, ethnicity, education level, marital status and household income was assigned to each participant.

Priority non-persistent pesticides and their biomarkers as well as organophosphate pesticide data were supplied in a separate data file. This dataset included information from a subset of 3,152 individuals from the main sampling event and included information for 18 biomarkers. Analysis of spot urine (random) samples to evaluate for the presence of pesticide biomarkers was conducted for a randomly selected sample from the overall NHANES sample population [28]. Detailed laboratory analytical information is available in the NHANES pesticide documentation and NHANES laboratory procedures manual. For each specific biomarker, the dataset indicated whether or not the biomarker was detected and, if detected, the urinary level in micrograms/liter (μg/L). If the biomarker was not detected in the sample, a value of the detection limit (DL) divided by the square root of two (DL/$\sqrt{2}$) was reported [28]. Also included in this data file was the urinary concentration of creatinine in the sampled individual, reported in mg/dL. Information from this subset was matched using a common numerical identifier to the information in the demographic data file.

Data analysis

The frequency of detection was determined for each biomarker in the pesticide dataset. Biomarkers selected for inclusion in this analysis were limited to biomarkers that could be related to a parent compound and those detected in more than 50% of the samples, a criterion commonly used for population biomarker analysis [29]. Three biomarkers met the requirement for inclusion in this research: 3,5,6- trichloropyridinol (TCPy), paranitrophenol (PNP) and 3-phenoxybenzoic acid (3-PBA).

Results for biomarker concentrations were log-transformed to determine the geometric mean. This transformation corrects for the non-normal distribution found in the sample due to the large amount of concentrations below or close to the Limit of Detection (LOD) [30,31]. The overall geometric and arithmetic means for each biomarker were determined and the confidence intervals and quartiles were calculated. Students t-tests were performed to evaluate differences in the geometric means determined for overall biomarker concentrations, the gender subgroups, and height and weight of each of the biomarker detect and non-detect groups for children aged 6-11. Analysis of Variance (ANOVA) was performed for the geometric means determined for the age and ethnicity subgroups for each biomarker; Tukey post-hoc analysis was used to determine which means were significantly different. To account for dilution of urine in the sample, the creatinine-adjusted geometric mean values (in microgram/g creatinine, μg/g) were determined.

Logistic regression was performed to model the relationship between the predictive independent variables (gender, age, and ethnicity) and the dichotomous, dependent outcome (a yes or no detection of the biomarker in the urine sample). The group with the largest amount of individuals was used as the reference group for gender and ethnicity (females and Non-Hispanic Whites). All statistical analysis was performed using SAS (Version 9.2).

Results

Biomarker detection

As indicated in Figure 1, TCPy and 3-PBA were detected in more than 75% of the sample. PNP met inclusion criteria by a small margin (53%). The remaining specific biomarkers were detected in much lower amounts in the sample. The geometric mean was determined for 78.6% of the individuals that had a detectable level of biomarker TCPy in their urine sample (Figure 2). Based on this analysis, the geometric mean for only detectable levels of the biomarker was 1.49 μg/L higher than that of the overall geometric mean.

Stratified analysis

In the TCPy analysis, males had a statistically significant higher geometric mean (GM) when compared to females, but only when using the unadjusted biomarker concentration in urine (μg/L). Adjusting for creatinine negates any significant difference (Table 1). Similar results were observed for concentrations of PNP (Table 2); however in the analysis of 3-PBA (Table 3), creatinine adjusted samples were significantly different by gender while unadjusted samples were not.

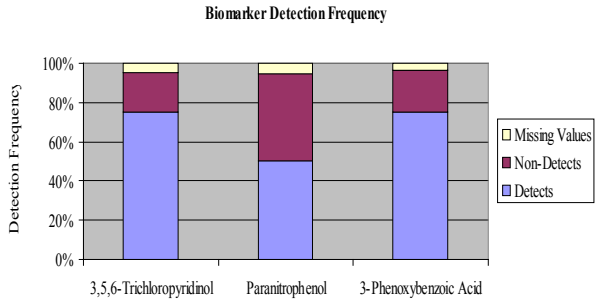


Figure 1: Detection Frequency of Urinary Pesticide Biomarkers.

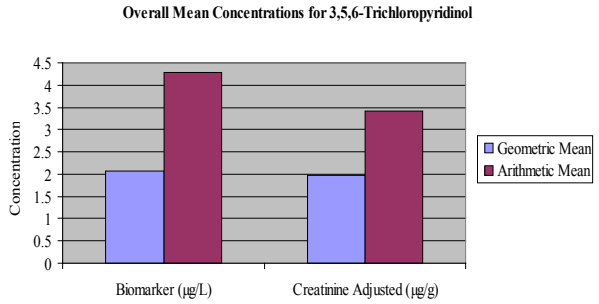


Figure 2: Comparison of Means for 3,5,6- Trichloropyridinol.

When stratified by ethnicity for TCPy analysis, the unadjusted biomarker concentration in urine (µg/L) for non-Hispanic Blacks had a significantly elevated GM as compared with the other ethnic groups. However, when adjusting for creatinine, there were no significant findings (Table 4). Similar results were observed for PNP analysis (Table 5), Mexican Americans, Non-Hispanic Blacks and Non-Hispanic Whites are significantly different compared to each other when using the unadjusted biomarker concentration (µg/L), but no significant difference was observed when adjusting for creatinine. 3-PBA analysis (Table 6) demonstrated that Non-Hispanic blacks have a significantly elevated geometric mean when compared to the other ethnic groups when evaluating the unadjusted urinary concentration of the biomarker (µg/L). When adjusting for creatinine (µg/g), only Mexican American and Non-Hispanic Blacks were significantly different.

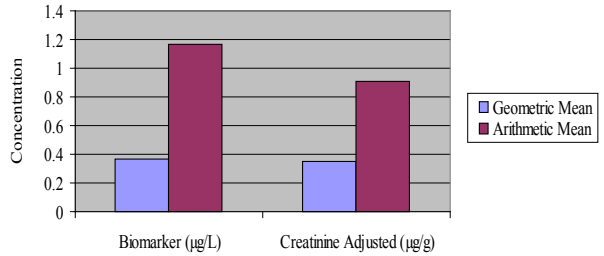
When stratified by age groups (children of 6-11 years of age, adolescents of 12-18 years of age, and adults ≥ 19 years of age), in the TCPy analysis (Table 7) significant differences are present for both the biomarker concentration in urine (µg/L) and when adjusting for creatinine (µg/g). For the unadjusted urinary biomarker concentration, children and adolescents are significantly elevated when compared to adults; however, there is no significant difference between children and adolescents within the same group. All three groups had a significant difference when adjusting for creatinine. In the PNP analysis (Table 8), significant differences exist for both the urinary concentration of the biomarker and when adjusting for creatinine. For the unadjusted biomarker in urine, children and adolescents were significantly different from the adult group, but were not significantly different from each other. Children in the creatinine-adjusted analysis were significantly different from adolescents and adults, but adolescents not significantly different from adults. In the 3-PBA analysis (Table 9), significant differences were present between all three age groups when adjusting for creatinine (µg/g); however there is no significance when evaluating the unadjusted urinary concentration of the biomarker (µg/L).

Evaluation of weight and height

Children of the same year of age where compared by students t-test for the ages of 6-11 for the parameters of weight and height based on the dichotomous outcomes of detectable pesticide biomarker vs non-detectable biomarker. This yielded 36 total comparisons, 6 comparisons of height and 6 comparisons of weight for each of 3 evaluated biomarkers. Of these 36 comparisons, only 3 statistically significant results were produced. A statistically significant result for 3-PBA was found, in that heavier children were associated with 3-PBA at age 7 [Detect=28.61 kg and Non-Detect=25.26 kg (p=0.0009)]. As well, PNP produced two statistically significant results, being associated with shorter children at age 8 [Non-Detect=134.3 cm and Detect: 130.9 cm (p=0.046)] and taller children at age 11 [Detect=153.7 cm and (Non-Detect=149.9 cm p=0.022)]. No significant differences were produced for weight or height related to TCPy detects vs. non-detects.

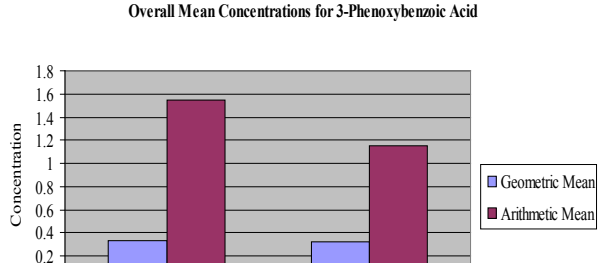
Logistic regression

In TCPy analysis (Figure 5), the overall model fit was significant, with the Likelihood χ^2= 119.4, p <0.0001 and the R^2 Max = 0.0602. The Hosmer and Lemeshow goodness-of-fit test resulted in a χ^2= 12.2, p= 0.144, indicating that the data from the independent variables fit the



µg/l= Micrograms per Liter; µg/g= Micrograms per Gram. Adjusting for creatinine results in means (both geometric and arithmetic) that are lower than if using the concentration of the TCPy biomarker in urine (µg/L) alone. Limit of Detection (LOD) for TCPy in sample was 0.4µg/L

Figure 3: Comparison of Means for Paranitrophenol.



µg/l= Micrograms per Liter; µg/g= Micrograms per Gram. Adjusting for creatinine results in means (both geometric and arithmetic) that are lower than if using the concentration of the PNP biomarker in urine (µg/L) alone. Limit of Detection (LOD) for PNP in sample was 0.1µg/L.

Figure 4: Comparison of Means for 3-Phenoxybenzoic Acid.

Biomarker (µg/L)[a]							
	GM[b]	LCL[c]	UCL[d]	SD[e]	Median	n[f] (D/ND)[g]	P[h]
Male	2.45	2.29	2.61	3.56	3.05	1416 (1180/236)	**<0.0001**
Female	1.78	1.67	1.90	3.75	2.27	1595 (1188/407)	
Total						3011	
Creatinine Adjusted (µg/g)[j]							
	GM	LCL	UCL	SD	Median	n	P
Male	1.98	1.87	2.10	2.97	2.20	1416	0.9423
Female	1.99	1.88	2.10	2.99	2.27	1593	
Total						3009	

[a](µg/L) = Micrograms per Liter; [b](GM) = Geometric Mean; [c](LCL) = Lower Confidence Limit; [d](UCL) = Upper Confidence Limit; [e](SD) = Standard Deviation; [f](n) = Number in Sample; [g](D/ND) = Number of Detects/Number of Non-Detects; [h](p) = Level of Significance at p= 0.05 Level (Highlighted in **Bold**); [i](µg/g) = Micrograms per Gram

Table 1: Student's t-test Comparing Geometric Means of Males Versus Females for 3,5,6- Trichloropyridinol.

Biomarker (µg/L)[a]							
	GM[b]	LCL[c]	UCL[d]	SD[e]	Median	n[f] (D/ND)[g]	P[h]
Male	0.447	0.410	0.487	5.14	0.830	1395 (823/572)	**<0.0001**
Female	0.308	0.284	0.333	4.99	< LOD[i]	1580 (757/823)	
Total						2975	
Creatinine Adjusted (µg/g)[j]							
	GM	LCL	UCL	SD	Median	n	P
Male	0.363	0.336	0.392	4.27	0.519	1395	0.276
Female	0.343	0.320	0.368	4.35	0.333	1578	
Total						2973	

[a](µg/L) = Micrograms per Liter; [b](GM) = Geometric Mean; [c](LCL) = Lower Confidence Limit; [d](UCL) = Upper Confidence Limit; [e](SD) = Standard Deviation; [f](n) = Number in Sample; [g](D/ND) = Number of Detects/Number of Non-Detects; [h](p) Level of Significance at p= 0.05 Level (Highlighted in **Bold**); [i](LOD) = Limit of Detection, at 0.1 µg/L; [j](µg/g) = Micrograms per Gram

Table 2: Student's t-test Comparing Geometric Means of Males Versus Females for Paranitrophenol.

Biomarker (µg/L)[a]							
	GM[b]	LCL[c]	UCL[d]	SD[e]	Median	n[f] (D/ND)[g]	pH
Male	0.341	0.319	0.365	3.58	0.320	1429 (1131/298)	0.514
Female	0.331	0.310	0.353	3.81	0.290	1619 (1228/391)	
Total						3048	
Creatinine Adjusted (µg/g)[j]							
	GM	LCL	UCL	SD	Median	n	P
Male	0.278	0.261	0.296	3.38	0.250	1429	**<0.0001**
Female	0.370	0.349	0.392	3.31	0.325	1617	
Total						3046	

[a](µg/L) = Micrograms per Liter; [b](GM) = Geometric Mean; [c](LCL) = Lower Confidence Limit; [d](UCL) = Upper Confidence Limit; [e](SD) = Standard Deviation; [f](n) = Number in Sample; [g](D/ND) = Number of Detects/Number of Non-Detects; [h](p) = Level of Significance at p= 0.05 Level (Highlighted in **Bold**); [i](µg/g) = Micrograms per Gram

Table 3: Student's t-test Comparing Geometric Means of Males Versus Females for 3-Phenoxybenzoic Acid.

model moderately well. When stratifying for individual groups, age does not seem to have an effect on the detection of a biomarker (β=-0.0145 and OR=0.986, p< 0.0001). Mexican Americans (β=0.221 and OR=1.31, p=0.0275) and Other Hispanics (β=0.272 and OR= 1.38, p=0.007) had a slightly higher change in the regression coefficient and higher odds of having a detectable level of the biomarker than Non-Hispanic Whites (reference group). Males had a higher change in the regression coefficient and higher odds of having a detectable level of biomarker than females (β=0.28 and OR= 1.75, p< 0.0001).

For PNP analysis (Figure 6), the overall model fit was also significant, with Likelihood χ^2= 71.3, p <0.0001 and the R^2 Max =0.0316. The Hosmer and Lemeshow goodness-of-fit test resulted in a χ^2= 16.01, p= 0.0414, indicating that the data from the independent variables did not fit the model well. When stratifying for individual groups, age does not seem to have an effect on the detection of a biomarker (β=-0.005 and OR=0.995, p=0.003). Other Hispanics (β=0.183 and OR= 1.45, p= 0.026) had a slightly higher change in the regression coefficient and a higher odds of having a detectable level of biomarker than Non-Hispanic Whites (reference group). Males had a higher change in the regression coefficient and a higher odds of having a detectable level of biomarker than females (β=0.227 and OR= 1.57, p< 0.0001).

Similar to previously evaluated biomarkers, in the 3-PBA analysis the overall model fit was significant, with Likelihood χ^2= 84.8, p <0.0001 and the R^2 Max =0.0418. The Hosmer and Lemeshow goodness-of-fit test resulted in a χ^2= 7.59, p= 0.475, indicating that the data from the independent variables fit the model well. When stratifying for individual groups, age does not seem to have an effect on the detection of a biomarker (β=-0.006 and OR=0.994, p= 0.001). Other Hispanics (β=0.630 and OR= 2.43, p< 0.0001) had a higher change in the regression coefficient and a higher odds of having a detectable level of

Biomarker (µg/L)[a]						
	GM[b]	LCL[c]	UCL[d]	SD[e]	n[f] (D/ND)[g]	pH
Mexican American	2.12	1.88	2.37	3.36	744 (611/133)	
Non-Hispanic Black *[all]	2.60	2.35	2.85	3.52	762 (635/127)	
Non-Hispanic White	1.84	1.62	2.05	3.88	1255 (936/319)	**<0.0001**
Other Hispanic	1.77	1.09	2.45	3.96	129 (94/35)	
Other	1.70	0.99	2.41	3.97	121 (92/29)	
Total					3011	
Creatinine Adjusted (µg/g)[i]						
	GM	LCL	UCL	SD	n	P
Mexican American	2.10	1.91	2.30	2.74	744	
Non-Hispanic Black	1.94	1.73	2.15	2.94	761	
Non-Hispanic White	1.99	1.82	2.16	3.10	1254	0.1847
Other Hispanic	1.66	1.09	2.23	3.30	129	
Other	1.86	1.32	2.41	3.06	121	
Total					3009	

[a](µg/L) = Micrograms per Liter; [b](GM) = Geometric Mean; [c](LCL) = Lower Confidence Limit; [d](UCL) = Upper Confidence Limit; [e](SD) = Standard Deviation; [f](n) = Number in Sample; [g](D/ND) = Number of Detects/Number of Non-Detects; [h](p) = Level of Significance at p= 0.05 Level (Highlighted in **Bold**); [i](µg/g) = Micrograms per Gram. *[all] indicates that this group is significantly different than all other ethnic groups.

Table 4: One-Way ANOVA and Tukey Analysis Comparing Geometric Means of Ethnic Groups for 3,5,6- Trichloropyridinol.

Biomarker (µg/L)[a]						
	GM[b]	LCL[c]	UCL[d]	SD[e]	n[f] (D/ND)[g]	pH
Mexican American*	0.353	0.012	0.695	4.75	744 (402/342)	
Non-Hispanic Black*	0.486	0.095	0.877	5.42	738 (439/299)	
Non-Hispanic White*	0.319	0.034	0.603	5.12	1247 (605/642)	**<0.0001**
Other Hispanic	0.306	-0.537	1.15	4.83	126 (62/64)	
Other	0.424	-0.418	1.27	4.71	120 (72/48)	
Total					2975	
Creatinine Adjusted (µg/g)[i]						
	GM	LCL	UCL	SD	n	p
Mexican American	0.350	0.066	0.634	3.95	744	
Non-Hispanic Black	0.364	0.036	0.692	4.54	737	
Non-Hispanic White	0.344	0.113	0.576	4.16	1246	0.131
Other Hispanic	0.292	-0.443	1.03	4.21	126	
Other	0.461	-0.161	1.08	3.48	120	
Total					2973	

[a](µg/L) = Micrograms per Liter; [b](GM) = Geometric Mean; [c](LCL) = Lower Confidence Limit; [d](UCL) = Upper Confidence Limit; [e](SD) = Standard Deviation; [f](n) = Number in Sample; [g](D/ND) = Number of Detects/Number of Non-Detects; [h](p) = Level of Significance at p= 0.05 Level (Highlighted in **Bold**); [i](µg/g) = Micrograms per Gram.
*Indicates groups that are significantly different from each other

Table 5: One-Way ANOVA and Tukey Analysis Comparing Geometric Means of Ethnic Groups for Paranitrophenol.

biomarker than Non-Hispanic Whites (reference group). Males had a change in the regression coefficient and a higher odds of having a detectable level of biomarker than females (β=0.0927 and OR= 1.2, p= 0.036).

Comparative analysis

A PubMED/Medline literature search was conducted to aggregate extant literature evaluating the presence of the three salient pesticide biomarkers evaluated in this investigation. Results of the literature search are summarized in Table 10. The unadjusted and, when available, adjusted creatinine values for measured urinary concentrations of TCPy, PNP, and 3-PBA were aggregated and compared to the biomarkers measured in the current study. While the frequency of detection of other investigations was typically higher in other published findings, the nominal background levels were consistent with means reported here. In some cases, where populations with suspected exposures were sampled, measures of central tendency were higher than those observed in the current study.

Discussion

As demonstrated by the analysis of the NHANES 2001-2002 dataset containing urinary pesticide biomarkers, there is evidence of a nominal background pesticide exposure the sample of the U.S. general population. However, significant differences among subgroups varied when examining the biomarker concentrations in urine versus correcting for dilution with creatinine. Metabolism rates and intake of water can vary among individuals [32,33]. Dilution of urine may have an effect on the concentration of the biomarker. Creatinine adjustment has been used to normalize analyte concentrations due to the relatively constant excretion rate of creatinine, reporting the result as a weight of analyte per gram of creatinine. Barr et al. [34] suggests that there may be urine dilution variability between groups based on gender, ethnicity and age, and suggests establishing and using reference ranges for creatinine concentrations for the individual being investigated, as those values may be a more appropriate comparison. Analysis of both urinary biomarker level and creatinine-adjusted levels should be

Biomarker (µg/L)[a]						
	GM[b]	LCL[c]	UCL[d]	SD[e]	n[f] (D/ND)[g]	pH
Mexican American	0.284	0.047	0.520	3.34	767 (580/187)	
Non-Hispanic Black*[all]	0.489	0.251	0.727	3.35	762 (667/95)	
Non-Hispanic White	0.298	0.083	0.513	3.91	1269 (920/349)	**<0.0001**
Other Hispanic	0.320	-0.339	0.980	3.82	129 (103/26)	
Other	0.335	-0.434	1.10	4.32	121 (89/32)	
Total					3048	
Creatinine Adjusted (µg/g)[i]						
	GM	LCL	UCL	SD	n	p
Mexican American*	0.283	0.068	0.497	3.03	767	
Non-Hispanic Black*	0.367	0.148	0.585	3.08	761	
Non-Hispanic White	0.323	0.123	0.524	3.64	1268	**0.0007**
Other Hispanic	0.301	-0.339	0.942	3.71	129	
Other	0.367	-0.341	1.08	3.98	121	
Total					3046	

[a](µg/L) = Micrograms per Liter; [b](GM) = Geometric Mean; [c](LCL) = Lower Confidence Limit; [d](UCL) = Upper Confidence Limit; [e](SD) = Standard Deviation; [f](n) = Number in Sample; [g](D/ND) = Number of Detects/Number of Non-Detects; [h](p) = Level of Significance at p= 0.05 Level (Highlighted in **Bold**); [i](µg/g) = Micrograms per Gram. *[all] Indicates that group is significantly different than all other groups.
*Indicates that groups are significantly different from each other

Table 6: One-Way ANOVA and Tukey Analysis Comparing Geometric Means of Ethnic Groups for 3-Phenoxybenzoic Acid.

Biomarker (µg/L)[a]						
	GM[b]	LCL[c]	UCL[d]	SD[e]	n[f] (D/ND)[g]	pH
Children*	2.72	2.44	2.99	3.39	573 (491/82)	
Adolescent*	2.84	2.59	3.08	3.38	741 (643/98)	**<0.0001**
Adult**	1.65	1.47	1.83	3.77	1697 (1234/463)	
Total					3011	
Creatinine Adjusted (µg/g)[i]						
	GM	LCL	UCL	SD	n	p
Children***	3.26	3.03	3.49	2.78	573	
Adolescent***	2.09	1.90	2.28	2.66	740	**<0.0001**
Adult***	1.76	1.61	1.90	3.02	1696	
Total					3009	

[a](µg/L) = Micrograms per Liter; [b](GM) = Geometric Mean; [c](LCL) = Lower Confidence Limit; [d](UCL) = Upper Confidence Limit; [e](SD) = Standard Deviation; [f](n) = Number in Sample; [g](D/ND) = Number of Detects/Number of Non-Detects; [h](p) = Level of Significance at p= 0.05 Level (Highlighted in **Bold**); [i](µg/g) = Micrograms per Gram. * Indicates group is significantly different from Adults.
** Indicates that group is significantly different from Adolescents and Children.
***Indicates that all groups are significantly different from each other

Table 7: One-Way ANOVA and Tukey Analysis Comparing Geometric Means of Age Groups for 3,5,6- Trichloropyridinol.

Biomarker (µg/L)[a]						
	GM[b]	LCL[c]	UCL[d]	SD[e]	n[f] (D/ND)[g]	pH
Children*	0.455	0.038	0.873	5.06	565 (338/227)	
Adolescent*	0.399	0.043	0.755	4.91	732 (414/318)	**<0.0001**
Adult**	0.329	0.081	0.576	5.18	1678 (828/850)	
Total					2975	
Creatinine Adjusted (µg/g)[i]						
	GM	LCL	UCL	SD	n	p
Children***	0.549	0.210	0.887	4.10	565	
Adolescent	0.295	0.004	0.586	4.02	731	**<0.0001**
Adult	0.328	0.129	0.527	4.16	1677	
Total					2973	

[a](µg/L) = Micrograms per Liter; [b](GM) = Geometric Mean; [c](LCL) = Lower Confidence Limit; [d](UCL) = Upper Confidence Limit; [e](SD) = Standard Deviation; [f](n) = Number in Sample; [g](D/ND) = Number of Detects/Number of Non-Detects; [h](p) = Level of Significance at p= 0.05 Level (Highlighted in **Bold**); [i](µg/g) = Micrograms per Gram. * Indicates group is significantly different from Adults.
**Indicates that group is significantly different from Adolescents and Children.
***Indicates that Children were significantly different from Adolescents and Adults

Table 8: One-Way ANOVA and Tukey Analysis Comparing Geometric Means of Age Groups for Paranitrophenol.

Biomarker (µg/L)[a]						
	GM[b]	LCL[c]	UCL[d]	SD[e]	n[f] (D/ND)[g]	pH
Children	0.349	0.047	0.650	3.71	580 (453/127)	
Adolescent	0.363	0.169	0.556	3.40	749 (613/136)	0.0742
Adult	0.321	0.140	0.501	3.17	1719 (1293/426)	
Total					3048	
Creatinine Adjusted (µg/g)[i]						
	GM	LCL	UCL	SD	n	p
Children***	0.418	0.139	0.697	3.43	580	
Adolescent***	0.269	0.049	0.490	3.08	748	**<0.0001**
Adult***	0.321	0.159	0.483	3.43	1718	
Total					3046	

[a](µg/L) = Micrograms per Liter; [b](GM) = Geometric Mean; [c](LCL) = Lower Confidence Limit; [d](UCL) = Upper Confidence Limit; [e](SD) = Standard Deviation; [f](n) = Number in Sample; [g](D/ND) = Number of Detects/Number of Non-Detects; [h](p) = Level of Significance at p= 0.05 Level (Highlighted in **Bold**); [i](µg/g) = Micrograms per Gram. *** Indicates that all three groups are significantly different from each other.

Table 9: One-Way ANOVA and Tukey Analysis Comparing Geometric Means of Age Groups for 3-Phenoxybenzoic Acid.

Author	n[a]	Sample Source	Metabolite	DF[b]	Central Tendency
Adgate (2001) [16]	102	Minnesota Child	TCPy	93%	GM[c]=6.4 µg/L[d]; AM[e]= 9.2 µg/L
Aprea (1999) [55]	42	General Population Italy	TCPy	88%	CAM[f]=3.5 µg/g[g]
Arcury (2007) [56]	60	Latino Age 1-6	TCPy	83.3%	GM=1.92µg/L; CAGM[h]=2.38 µg/g
			PNP	90%	GM=1.0 µg/L; CAGM= 1.25 µg/g
			3-PBA	40%	NA[i]
Barr (2005) [53]	1994	All	TCPy	91% (weighted)	GM=1.77µg/L; CAGM=1.58 µg/g
Barr (2010) [19]	3048 (3046 for CA)	NHANES 1999-2002	3-PBA	75.4% (weighted)	GM=0.318µg/L; CAGM=0.324 µg/g
Berger-Preiss (2002) [57]	145	Adults and Children	3-PBA	28%	Mean=0.25 µg/L
Berkowitz (2004) [37]	404	Pregnant Females	TCPy	NA	Median=7.6µg/L; CA Median= 11.5 µg/g
Eskenazi (2004) [45]	488	Pregnant Females	TCPy	76.3%	Median=3.3 µg/L
		in Agricultural Community	PNP	54.4%	Median= 0.5 µg/L
Hill (1995) [55]	993	USA NHANES III	TCPy	82%	Mean=4.5 µg/L; CAM=3.1 µg/g
	980		PNP	41%	Mean=1.6 µg/L, CAM=1.2 µg/g
Macintosh (2001) [44]	80	NHEXAS- Maryland	TCPy	96%	GM=5.1 µg/L; CAGM= 4.5 µg/g
Morgan 2005 [58]	128	Children	TCP	NA	GM=5.2 ng/ml; Mean=7.3 ng/ml
	110 (Creatinine)				CAGM 8 ng/mg; CAM= 10.5 ng/mg
Naeher (2010) [59]	203	Children Age 4-6	3-PBA	99.5%	Mean=5.0 µg/L
Olsson (2003) [60]	140	NA	TCPy	56%	GM= 9.7 µg/L
			PNP	99%	GM= 2.1 µg/L
Panuwet (2008) [61]	136	Thailand General Population	PNP	99.3%	GM= 2.8 µg/L; CAGM=2.1 µg/g
	104		TCPy	76.5%	GM=1.7 µg/L; CAGM=1.3 µg/g
	118		3-PBA	86.8%	GM=1.1 µg/L; CAGM=0.86 µg/g
Panuwet (2009) [62]	207	Thailand Age 12-13	PNP	98%	GM=2.68 ng/ML; CAGM=3.07 µg/g
					AM=4.07 ng/ml; CAAM[j]=3.81 µg/g
			TCPy	92%	GM=2.35 ng/ml; CAGM=2.7 mg/g
					AM=4.02 ng/ml; CAAM=3.74 mg/g
			3-PBA	47%	GM=0.2 ng/ml; CAGM=0.23 µg/g
					AM=1.0 ng/ml; CAAM=0.95 µg/g
Steenland (2000) [63]	65	Termiticide Applicator (Recent App)	TCPy	NA	Mean=629.5 µg/L; CAM= 331 µg/g
	40	Termiticide Applicator	TCPy	NA	Mean= 119.0 µg/L; CAM= 55 µg/g
	52	Non-Exposed Control	TCPy		Mean=6.2 µg/L; CAM=3µg/g
Ueyama (2009) [64]	448	Japanese General Population	3-PBA		GM=0.29 µg/L; CAGM=0.4 µg/g
					AM=0.63 µg/L; CAAM= 0.73 µg/g
	87	Japanese Farmers			GM=0.38 µg/L; CAGM=0.45 µg/g
					AM=0.76 µg/L; CAAM=0.81 µg/g
Ye (2008) [65]	9778	Mothers	TCPy	100%	GM=1.2 µg/L; CAGM=1.9 µg/g

[a](n) = Number in Sample; [b](DF) = Detection Frequency; [c](GM) = Geometric Mean; [d](µg/L) = Micrograms per Liter; [e](AM) = Arithmetic Mean; [f](CAM) = Creatinine-Adjusted Mean; [g](µg/g) = Micrograms per Gram; [h](CAGM) = Creatinine-Adjusted GM; [i](NA) = Not Available; [j](CAAM) = Creatinine-Adjusted AM

Table 10: Summary of Results from Other Biomarker Studies.

conducted to determine if significance is eliminated or elucidated due to the correction with creatinine, as observed with some groups in this study.

For instance, variations in the mean concentrations for gender depended on whether or not the biomarker concentration in urine (µg/L) was used or whether it was corrected for dilution with creatinine (µg/g). Mean concentrations of biomarkers among ethnicity groups were consistently significantly varied. Non-Hispanic Blacks, followed by Mexican Americans, appeared to have significantly higher means than the other members of the group. Children and, in some cases, adolescents had significantly higher mean values as compared to adults. These variations could be due to the biological differences between children and adults, as children may metabolize xenobiotics at a slower rate than adults [35,36]. As well, increased instances of hand-to-mouth contact and pica in children may also result in an increase in exposure and explain the findings in this research [37,38,22].

Some of the statistically significant outcomes reported in this analysis could also be due to the oversampling of minorities by NHANES as certain subgroups that were the target of a specific health interest were oversampled to ensure their inclusion into the dataset [39]. This oversampling of certain minority or at-risk groups can overestimate the true nominal background exposure that exists in the general population.

Based on the analysis in this study, there were no consistent results to suggest that exposed individuals had an increased risk of an adverse health outcome. When comparing the weight and height of study participants ages 6-11 among those with a recorded detect versus non-detect of urinary biomarkers, most of the comparisons were not statistically significant, indicating that there was no appreciable difference between the exposed versus non-exposed. Even among results that yielded statistically significant differences, no clear trend emerged with heavier children associated with 3-phenoxybenzoic acid detection at age 7 (Figure 4) [Detect=28.61 kg and Non-Detect=25.26 kg (p=0.0009)]; and paranitrophenol being associated with shorter children at age 8 (Figure 3) [Non-Detect=134.3 cm and Detect: 130.9 cm (p=0.046)] and taller children at age 11 [Detect=153.7 cm and (Non-Detect=149.9 cm p=0.022)]. Out of the 36 height and weight comparisons made between detectable levels of biomarker and non-detectable levels, 33 did not have significant findings at the $p \leq 0.05$ level. It appears that exposure in this group of children does not have an overall association with childhood growth development. It should be noted that other indicators for developmental outcomes not present in the NHANES data may provide additional insight into the broader characterization of background levels of pesticide exposure and developmental effects. However, the pre-adolescent measures of height and weight provided in the current study provide important indicator data to assess any potential relationship that might have existed between background level pesticide exposure and prominent childhood development outcomes.

The logistic regression performed in this study allowed for modeling to determine how the independent variables (age, ethnicity and gender) had an effect on the detection of the biomarkers in the sample. This was then used to determine, based on the detection, which groups had a higher odds of having a detectable biomarker when compared to a reference group. All models were significant; however, only 3,5,6- trichloropyridinol and 3-phenoxybenzoic acid (Figure 7) demonstrated acceptable model fit. The independent variables for ethnicity and gender in the 3,5,6- trichloropyridinol model confirmed the significant differences observed when comparing the geometric means of biomarker concentrations. Gender was the only significant predictor of 3-phenoxybenzoic acid.

When compared to extant epidemiological literature using similar markers in biological media, the mean biomarker levels from this study fall within the ranges of other investigations. Whyatt et al. [40], used data obtained from the Columbia Center for Children's Environmental Health in New York to determine the association of exposure of African American or Dominican pregnant women to pesticides. Women in this study participated in biomonitoring at the time of birth. Blood samples were collected from the umbilical cord at birth and from the mother within two days after giving birth [40]. Plasma chlorpyrifos levels were determined and a negative association was found between detection of TCPy and birth weight and length [40]. These data were further analyzed to determine the impact of chlorpyrifos exposure on neurodevelopment. Rauh et al. [41] determined that "highly exposed"

	Detect[a] (DF[b]=1)	Detect, Non-Hispanic White (DF=1)	Detect, Female (DF=1)
Age	**β=-0.0145 Wald χ^2=53.74 p<0.0001 OR=0.986**		
Mexican American		**β=0.221 Wald χ^2=4.86 p=0.0275 OR=1.31**	
Non-Hispanic Black		β=-0.317 Wald χ^2=3.50 p=0.062 OR=0.765	
Other Hispanic		**β=0.272 Wald χ^2=7.20 p=0.007 OR=1.38**	
Other		β=-0.128 Wald χ^2=0.500 p=0.479 OR=0.924	
Male			**β=0.28 Wald χ^2=36.28 p<0.0001 OR=1.75**

[a](Detect) = Detectable Level of Biomarker in the Urine Sample, used as a reference category in the Model; [b](DF)= Degree of Freedom. Significant differences are highlight in **Bold.**

Figure 5: Logistic Regression for 3,5,6- Trichloropyridinol**.**

	Detect[a] (DF[b]=1)	Detect, Non-Hispanic White (DF=1)	Detect, Female (DF=1)
Age	**β=-0.005 Wald χ^2=8.78 p=0.003 OR=0.995**		
Mexican American		β=-0.0239 Wald χ^2=0.0863 p=0.77 OR=1.18	
Non-Hispanic Black		β=-0.213 Wald χ^2=2.00 p=0.158 OR=0.976	
Other Hispanic		**β=0.183 Wald χ^2=4.96 p=0.026 OR=1.45**	
Other		β=0.242 Wald χ^2=2.39 p=0.122 OR-1.54	
Male			**β=0.227 Wald χ^2=36.82 p<0.0001 OR=1.57**

[a](Detect) = Detectable Level of Biomarker in the Urine Sample, used as a reference category in the Model; [b](DF)= Degree of Freedom. Significant differences are highlight in **Bold.**

Figure 6: Logistic Regression for Paranitrophenol.

	Detect[a] (DF[b]=1)	Detect, Non-Hispanic White (DF=1)	Detect, Female (DF=1)
Age	**β=-0.006 Wald χ^2=10.2 p=0.001 OR=0.994**		
Mexican American		β=-0.175 Wald χ^2=3.44 p=0.064 OR=1.09	
Non-Hispanic Black		β=0.076 Wald χ^2=0.171 p=0.679 OR=1.4	
Other Hispanic		**β=0.630 Wald χ^2=33.5 p<0.0001 OR=2.43**	
Other		β=-0.273 Wald χ^2=2.46 p=0.117 OR=0.986	
Male			**β=0.0927 Wald χ^2=4.40 p=0.036 OR=1.2**

[a](Detect) = Detectable Level of Biomarker in the Urine Sample, used as a reference category in the Model; [b](DF)= Degree of Freedom. Significant differences are highlight in **Bold.**

Figure 7: Logistic Regression for 3-Phenoxybenzoic Acid.

(chlorpyrifos >6.17 pg/g plasma) children 3 years of age scored lower on the Bayley Psychomotor Developmental Index and the Bayley Mental Development Index when compared to those exposed to a lower level (<6.17 pg/g). These levels are based on the chlorpyrifos detected in the umbilical cord plasma collected at the time of birth. A follow-up study by Rauh et al. [42] re-assessed these children at age 7. This study, using the original biomarker level at birth, reported that children exposed to chlorpyrifos in utero show deficits in the working memory index and the full scale IQ test [42].

Eaton et al. [24], in a review of these studies, observed that other environmental factors, including tobacco and alcohol consumption, have been associated with negative birth outcomes in other studies. These exposures could have resulted in the negative outcomes discovered in the Columbia study. While the cotinine levels of participants in the study were evaluated, the short half-life of the biomarker and the time of sample collection (after admission to the hospital) could have resulted in cotinine levels that underestimated actual exposure [24]. Additionally, alcohol consumption was self reported at the time of the interview and, while they were used as a covariate in the analysis in some part, underreporting of alcohol use could introduce bias into the evaluations. Another limitation of these studies is the use of TCPy as the specific biomarker for chlorpyrifos. It has been determined that chlorpyrifos-methyl is also a parent of this biomarker [24]. Degradation of the parent compound can also lead to environmental TCPy exposure [43]. Because of the multiple sources of TCPy, the exposure to AChE inhibiting chlorpyrifos may be overestimated in certain instances. With regard to adverse health effects from prenatal exposure, Eaton points out that scientific evidence does not suggest adverse neurodevelopment effects in infants from in utero dietary exposures to chlorpyrifos, if the neurodevelopment effects are from inhibition of AChE [24]. The article does point out that the results from studies finding associations cannot be ignored and further epidemiological investigation is warranted to fully elucidate the associations.

The studies evaluating developmental effects from prenatal pesticide exposure attempted to associate low-level exposures with the risk of a negative health outcome. The results are determined by a cross-sectional examination with simultaneous evaluation of both exposure and outcome. While this method may produce associations, these associations cannot be viewed as causal. No studies were identified that attempted to characterize exposure over a period of time (none more than a few days or multiple sampling events over a period of time) with negative health effects. A longitudinal prospective study may allow urinary biomarker concentrations to be better characterized. However, because other chemicals are capable of causing teratogenic and neurotoxic outcomes, controlling for the many covariates that are involved in everyday life may be difficult.

In recent evaluations of exposures to pesticides, an increased amount of consideration has been placed on the small number of human studies that found associations between prenatal pesticide exposures and negative birth outcomes, including those studies associating chlorpyrifos exposure and reductions in birth outcomes and cognitive abilities later in life. While chlorpyrifos is still applied on a limited number of agricultural commodities, no recent studies were identified that attempted to re-evaluate current chlorpyrifos exposures and negative health effects. Recently published articles appear to be based on original, decade-old cross-sectional data and use various analytical techniques and different covariates to determine if any associations can be found. Because chlorpyrifos is no longer registered for residential application, the current primary exposure route of exposure is oral. Previous studies included evaluation of cumulative exposures. If inhalation is no longer a viable exposure route for residential application (except for certain populations residing in close proximity to applications in agricultural scenarios), it would be expected that the current exposure is lower than those previously documented.

Eskenazi et al. [44] investigated chlorpyrifos exposure and negative birth outcomes in the Center for the Health Assessment of Mothers and Children of Salinas Study (CHAMACOS). After sampling and analyzing maternal urine for chlorpyrifos biomarkers, no significant association was found between TCPy detection in urine and negative birth outcomes (birth weight, length, head circumference and length of gestation) [44]. Berkowitz et al. [37] investigated the relationship between pesticide exposure and negative birth outcomes in the Children's Environmental Cohort Study at Mount Sinai Hospital, New York. Races were mixed in this study with approximately 50% of the participants identified as Hispanic [37]. Evaluation of urinary TCPy concentration was not found to have a significant association with reductions in birth weight and length; however, this study did find a slight decrease in head circumference when TCPy and PON1 activity were considered jointly [37].

In the 1990's, MP was illegally applied in residences in at least nine Midwestern and Southern states [45]. The target population of one of the studies investigating MP exposure was children who were 6 years of age or younger at the time of MP application. Exposure was established based on either wipe samples from the home (in Ohio and Mississippi) or the detection of paranitrophenol in a urine sample (Ohio only). The study evaluated whether exposed children had an adverse neurological outcome. Based on the results of tests used to evaluate neurobehavior and general intelligence, the exposures were not associated with negative outcomes on most neurobehavioral tests [45]. The findings do suggest that those children exposed may have alterations in short term memory, but the authors note that the findings are not conclusive because the effects are not consistent across both study sites [45]. The delay in time between the application of MP and neurobehavioral testing (2.5 years in Mississippi and 4.5 years in Ohio) and the age of the children at the time of the neurobehavioral assessment may have affected the findings [45].

The reported effects of chronic, low-level exposure to pyrethroids, if any, are limited in the literature. However, because of the rapid metabolism of the compounds, pyrethroids are not believed to result in neurological signs from chronic, low-level exposures [46]. There has also been an attempt to correlate pyrethroids exposure to reduction in semen and hormone levels in adult men [20,47].

Biological Exposure Indices published by the ACGIH established thresholds for biomarkers in occupational settings [48]. However, there are no regulatory health-related thresholds for pesticide biomarkers of exposure in the general population. An attempt has been made by the German Human Biomonitoring Commission to establish a reference value (RV95) from biomonitoring data [49]. Heudorf [50] reports on the use of biomonitoring data from a sample of the German population for non-specific OP biomarkers and biomarkers for pyrethroids. A value was established for 3-PBA and was set at 2 μg/L (for children age 3-14). The RV95 were statistically derived from the 95% percentile within the 95% CI, are not based on toxicological data and are not related to risk assessment [50]. Because these levels are statistically derived, they should not be used to evaluate adverse health effects from biomonitoring data [49,50]. The RV95 is suggested to be used to determine if any one group or population is exposed to a higher degree than another population and to highlight populations for further evaluations where the biomarker levels are elevated. The mean values for children age 6-11 in this study fall below this value.

While various biomonitoring studies have evaluated the presence of biomarkers in biological media, most are unable to assess risk and suggest that the results serve as a reference range and can be used to evaluate trends in public health [51-53]. However, previously mentioned studies have used biomonitoring data to form associations between low-level pesticides and adverse birth outcomes. Statistically significant associations were observed in the reviewed epidemiological studies as well as in the current research. While associations may warrant further investigation, there are no known exposures in extant epidemiological biomonitoring studies that demonstrate adverse health effects to exposures related to the range of biomarkers described in this study. This research, like other cross-sectional research studies, evaluated the exposure and the outcome simultaneously. Without knowing all of the covariates involved in the exposure, it remains difficult to relate the biomarker concentration to a risk factor. As well, there is always some potential for discordance when extrapolating biomonitoring measurements to exposure. For instance Morgan et al. [54] 2011 found that there are potentially other intake sources that can affect TCPy levels in children, and Sudakin 2006 [55] indicated similar issues of specificity regarding 3-PBO for pyrethroid biomonitoring. However, in the absence of a known confounding factor that differentially modulates biomonitoring outcomes between developmental outcome groups, these issues of specificity would not be expected to alter the conclusions reached in the current research [54,55].

Conclusion

This study used urinary biomarker of exposure levels from the 2001-2002 National Health and Nutrition Examination Survey (NHANES) to characterize the nominal background exposure to three common pesticides and determine if chronic, low-level exposure to pesticides can be associated with an appreciable increase in risk of adverse developmental growth in children. The results demonstrated that there were detectable levels of pesticide biomarkers in the urine of individuals that participated in the study, and, depending on the dilution of the analyte concentration in urine, certain subgroups had significantly higher means than others. Analysis of phenotypic variations in children in the study revealed significant differences, but the differences were not consistent across the biomarkers or age groups. Out of the 36 height and weight comparisons made between detectable levels of biomarker and non-detectable levels, 33 did not have significant findings, and two of the associations indicated that detection of a biomarker in urine was positively associated with the height and weight of children. Mean overall biomarker levels were consistent with other studies evaluating background levels of pesticides and the mean levels were lower than those in research that associated adverse health outcomes from exposure.

References

1. http://www.epa.gov/pesticides/pestsales/07pestsales/market_estimates2007.pdf
2. http://ppis.ceris.purdue.edu/
3. Curwin BD, Hein MJ, Sanderson WT, Barr DB, Heederik D, et al. (2005) Urinary and hand wipe pesticide levels among farmers and nonfarmers in Iowa. J Expo Anal Environ Epidemiol 15: 500-508.
4. Jaga K, Dharmani C (2003) Sources of exposure to and public health implications of organophosphate pesticides. Rev Panam Salud Publica 14: 171-185.
5. Calvert GM, Karnik J, Mehler L, Beckman J, Morrissey B, et al. (2008) Acute pesticide poisoning among agricultural workers in the United States. Am J Ind Med 51: 883-898.
6. http://www.epa.gov/pesticides/health/worker.htm
7. http://www.cdc.gov/niosh/topics/pesticides/
8. http://www.cdc.gov/niosh/topics/pesticides/overview.html
9. Geer LA, Cardello N, Dellarco MJ, Leighton TJ, Zendzian RP, et al. (2004) Comparative analysis of passive dosimetry and biomonitoring for assessing chlorpyrifos exposure in pesticide workers. Ann Occup Hyg 48: 683-695.
10. Bouvier G, Seta N, Vigouroux-Villard A, Blanchard O, Momas I (2005) Insecticide urinary metabolites in nonoccupationally exposed populations. J Toxicol Environ Health B Crit Rev 8: 485-512.
11. Bronstein AC, Spyker DA, Cantilena LR Jr, Green JL, Rumack BH, et al.(2009) 2008 Annual Report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 26th Annual Report. Clin Toxicol (Phila) 47: 911-1084.
12. Damalas CA, Eleftherohorinos IG (2011) Pesticide exposure, safety issues, and risk assessment indicators. Int J Environ Res Public Health 8: 1402-1419.
13. Beamer PI, Canales RA, Bradman A, Leckie JO (2009) Farmworker children's residential non-dietary exposure estimates from micro-level activity time series. Environ Int 35: 1202-1209.
14. Doherty JD (2006) Screening pesticides for neuropathogenicity. J Biomed Biotechnol 2006: 70414.
15. Needham LL (2005) Assessing exposure to organophosphorus pesticides by biomonitoring in epidemiologic studies of birth outcomes. Environ Health Perspect 113: 494-498.
16. Adgate JL, Barr DB, Clayton CA, Eberly LE, Freeman NC, et al. (2001) Measurement of children's exposure to pesticides: analysis of urinary metabolite levels in a probability-based sample. Environ Health Perspect 109: 583-590.
17. Lu C, Toepel K, Irish R, Fenske RA, Barr DB, et al. (2006) Organic diets significantly lower children's dietary exposure to organophosphorus pesticides. Environ Health Perspect 114: 260-263.
18. Kimata A, Kondo T, Ueyama J, Yamamoto K, Yoshitake J, et al. (2009) Comparison of urinary concentrations of 3-phenoxybenzoic acid among general residents in rural and suburban areas and employees of pest control firms. Int Arch Occup Environ Health 82: 1173-1178.
19. Barr DB, Olsson AO, Wong LY, Udunka S, Baker SE, et al. (2010) Urinary concentrations of metabolites of pyrethroid insecticides in the general U.S. population: National Health and Nutrition Examination Survey 1999-2002. Environ Health Perspect 118: 742-748.
20. Meeker JD, Barr DB, Hauser R (2009) Pyrethroid insecticide metabolites are

associated with serum hormone levels in adult men. Reprod Toxicol 27: 155-160.

21. Marrs TC, Ballantyne B (2004) Pesticides: An Overview of Fundamentals. John Wiley & Sons, Ltd, UK.

22. Morgan MK, Sheldon LS, Croghan CW, Jones PA, Chuang JC, et al. (2007) An observational study of 127 preschool children at their homes and daycare centers in Ohio: environmental pathways to cis- and trans-permethrin exposure. Environ Res 104: 266-274.

23. Costa LG (2006) Current issues in organophosphate toxicology. Clin Chim Acta 366: 1-13.

24. Eaton DL, Daroff RB, Autrup H, Bridges J, Buffler P, et al. (2008) Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. Crit Rev Toxicol 38: 1-125.

25. http://www.cdc.gov/nchs/nhanes/history.htm

26. National Center for Health Statistics, Centers for Disease Control and Prevention (2005) Analytic and Reporting Guidelines: The National Health and Nutrition Examination Survey.

27. http://www.cdc.gov/nchs/nhanes/nhanes2001-2002/nhanes01_02faqs.htm

28. National Health and Nutrition Examination Survey (2005) 2001-2002 Documentation, Codebook and Frequencies, Urinary Priority pesticides.

29. Kieszak SM, Naeher LP, Rubin CS, Needham LL, Backer L, et al. (2002) Investigation of the relation between self-reported food consumption and household chemical exposures with urinary levels of selected nonpersistent pesticides. J Expo Anal Environ Epidemiol 12: 404-408.

30. Whyatt RM, Garfinkel R, Hoepner LA, Andrews H, Holmes D, et al. (2009) A biomarker validation study of prenatal chlorpyrifos exposure within an inner-city cohort during pregnancy. Environ Health Perspect 117: 559-567.

31. Elfman L, Hogstedt C, Engvall K, Lampa E, Lindh CH (2009) Acute health effects on planters of conifer seedlings treated with insecticides. Ann Occup Hyg 53: 383-390.

32. Manno M, Viau C, Cocker J, Colosio C, Lowry L, et al. (2010) Biomonitoring for occupational health risk assessment (BOHRA). Toxicol Lett 192: 3-16.

33. Egeghy PP, Cohen Hubal EA, Tulve NS, Melnyk LJ, Morgan MK, et al. (2011) Review of pesticide urinary biomarker measurements from selected US EPA children's observational exposure studies. Int J Environ Res Public Health 8: 1727-1754.

34. Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, et al. (2005) Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ Health Perspect 113: 192-200.

35. National Research Council (U.S.) Committee on Pesticides in the Diets of Infants and Children (1993) Pesticides in the Diets of Infants and Children. National Academies Press, Washington DC.

36. Wasley A, Lepine LA, Jenkins R, Rubin C (2002) An investigation of unexplained infant deaths in houses contaminated with methyl parathion. Environ Health Perspect 110: 1053-1056.

37. Berkowitz GS, Wetmur JG, Birman-Deych E, Obel J, Lapinski RH, et al. (2004) In utero pesticide exposure, maternal paraoxonase activity, and head circumference. Environ Health Perspect 112: 388-391.

38. Paustenbach DJ (2000) The practice of exposure assessment: a state-of-the-art review. J Toxicol Environ Health B Crit Rev 3: 179-291.

39. http://www.cdc.gov/nchs/tutorials/nhanes/faqs.htmhttp://www.cdc.gov/nchs/tutorials/nhanes/faqs.htm

40. Whyatt RM, Rauh V, Barr DB, Camann DE, Andrews HF, et al. (2004) Prenatal insecticide exposures and birth weight and length among an urban minority cohort. Environ Health Perspect 112: 1125-1132.

41. Rauh VA, Garfinkel R, Perera FP, Andrews HF, Hoepner L, et al. (2006) Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. Pediatrics 118: 1845-1859.

42. Rauh V, Arunajadai S, Horton M, Perera F, Hoepner L, et al. (2011) Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide. Environ Health Perspect 119: 1196-1201.

43. Morgan MK, Sheldon LS, Croghan CW, Jones PA, Robertson GL, et al. (2005) Exposures of preschool children to chlorpyrifos and its degradation product 3,5,6-trichloro-2-pyridinol in their everyday environments. J Expo Anal Environ Epidemiol 15: 297-309.

44. Eskenazi B, Harley K, Bradman A, Weltzien E, Jewell NP, et al. (2004) Association of in utero organophosphate pesticide exposure and fetal growth and length of gestation in an agricultural population. Environ Health Perspect 112: 1116-1124.

45. Ruckart PZ, Kakolewski K, Bove FJ, Kaye WE (2004) Long-term neurobehavioral health effects of methyl parathion exposure in children in Mississippi and Ohio. Environ Health Perspect 112: 46-51.

46. U.S. Department of health and human services (2003) Toxicological Profile for Pyrethrins and Pyrethroids.

47. Xia Y, Han Y, Wu B, Wang S, Gu A, et al. (2008) The relation between urinary metabolite of pyrethroid insecticides and semen quality in humans. Fertil steril 89: 1743-1750.

48. ACGIH (2008) ACGIH 2011 TLVs and BEIs Resources. Cincinnati, Oh.

49. Schulz C, Wilhelm M, Heudorf U, Kolossa-Gehring M (2011) Update of the reference and HBM values derived by the German Human Biomonitoring Commission. Int J Hyg Environ Health 215: 26-35.

50. Heudorf U, Butte W, Schulz C, Angerer J (2006) Reference values for metabolites of pyrethroid and organophosphorous insecticides in urine for human biomonitoring in environmental medicine. Int J Hyg Environ Health 209: 293-299.

51. Paustenbach D, Galbraith D (2006) Biomonitoring and biomarkers: exposure assessment will never be the same. Environ Health Perspect 114: 1143-1149.

52. Barr DB, Allen R, Olsson AO, Bravo R, Caltabiano LM, et al. (2005) Concentrations of selective metabolites of organophosphorus pesticides in the United States population. Environ Res 99: 314-326.

53. Hill RH Jr, Head SL, Baker S, Gregg M, Shealy DB, et al. (1995) Pesticide Residues in urine of adults living in the United States: reference range concentrations. Environ Res 71: 99-108.

54. Morgan MK, Sheldon LS, Jones PA, Croghan CW, Chuang JC, et al. (2011) The reliability of using urinary biomarkers to estimate children's exposures to chlorpyrifos and diazinon. J Expo Sci Environ Epidemol 21: 280-290.

55. Sudakin DL (2006) Pyrethroid insecticides: advances and challenges in biomonitoring. Clin Toxicol (Phila) 44: 31-37.

56. Aprea C, Sciarra G, Sartorelli P, Ceccarelli F, Centi L (1999) Multiroute exposure assessment and excretion of urinary metabolites of fenitrothion during manual operations on treated ornamental plants in greenhouses. Arch Environ Contam Toxicol 36: 490-497.

57. Arcury TA, Grzywacz JG, Barr DB, Tapia J, Chen H, et al. (2007) Pesticide Urinary Metabolite Levels of Children in Eastern North Carolina Farmworker Households. Environ Health Perspect 115: 1254-1260.

58. Berger-Preiss E, Levsen K, Leng G, Idel H, Sugiri D, et al. (2002) Indoor pyrethroid exposure in homes with woollen textile floor coverings. Int J Hyg Environ Health 205: 459-472.

59. Naeher LP, Tulve NS, Egeghy PP, Barr DB, Adetona O, et al. (2010) Organophosphorus and pyrethroid insecticide urinary metabolite concentrations in young children living in a southeastern United States city. Sci Total Environ 408: 1145-1153.

60. Olsson AO, Nguyen JV, Sadowski MA, Barr DB (2003) A liquid chromatography/electrospray ionization tandem mass spectrometry method for quantification of specific organophosphorus pesticide biomarkers in human urine. Anal Bioanal Chem 376: 808-815.

61. Panuwet P, Prapamontol T, Chantara S, Thavornyuthikarn P, Montesano MA, et al. (2008) Concentrations of urinary pesticide metabolites in small-scale farmers in Chiang Mai Province, Thailand. Sci Total Environ 407: 655-668.

62. Panuwet P, Prapamontol T, Chantara S, Thavornyuthikarn P, Bravo R, et al. (2009) Urinary Paranitrophenol, a metabolite of methyl parathion, in Thai farmer and child populations. Arch Environ Contam Toxicol 57: 623-629.

63. Steenland K, Dick RB, Howell RJ. Chrislip DW, Hines CJ, et al. (2000) Neurologic function among termiticide applicators exposed to chlorpyrifos. Environ Health Perspect 108: 293-300.

64. Ueyama J, Kimata A, Kamijima M, Hamajima N, Ito Y, et al. (2009) Urinary excretion of 3-phenoxybenzoic acid in middle-aged and elderly general population of Japan. Environ Res 109: 175-180.

65. Ye X, Pierik FH, Hauser R, Duty S, Angerer J, et al. (2008) Urinary metabolite concentrations of organophosphorous pesticides, bisphenol A, and phthalates among pregnant women in Rotterdam, the Netherlands: the Generation R study. Environ Res 108: 260-267.

This article was originally published in a special issue, **Occupational & Environmental Toxicology** handled by Editor(s). Dr. Masayuki Ohyama, Osaka Prefectural Institute of Public Health, Japan