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

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

By

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List of Abbreviations and Acronyms

3,5,6-Trichloropyridinol	TCPy
3-Phenoxybenzoic Acid	3-PBA
Acetylcholinesterase	AChE
Agency for Toxic Substances and Disease Registry	ATSDR
Alanine Aminotransferase	ALT
Alkaline Phosphatase	Alk Phos
American Conference of Governmental Industrial Hygienists	ACGIH
Arithmetic Mean	AM
Aspartate Aminotransferase	AST
ATSDR Minimal Risk Level	MRL
Attention Deficit Hyperactivity Disorder	ADHD
Benchmark Dose	BMD
Biological Exposure Indices	BEI
Biomarker of Exposure	BOE
Blood Urea Nitrogen	BUN
Butylcholinesterase	BuChE
Center for the Health Assessment of Mothers and Children of Salinas	CHAMACOS

Centers for Disease Control and Prevention	CDC
Centimeter	cm
Central nervous System	CNS
Chemical Abstract Service	CAS
Creatinine-Adjusted Arithmetic Mean	CAAM
Creatinine-Adjusted Geometric Mean	CAGM
Creatinine-Adjusted Mean	CAM
Cumulative Risk Assessment	CRA
Cytochrome P-450	CYP
Diethylphosphate	DEP
Diethylthiophosphate	DETP
Environmental Protection Agency	EPA
Federal Environmental Pesticide Control Act	FEPCA
Federal Food, Drug and Cosmetic Act	FFDCA
Federal Insecticide, Fungicide and Rodenticide Act	FIFRA
Food Additives Amendment	FAA
Food and Drug Administration	FDA
Food Quality Protection Act	FQPA
Gamma-Glutamyl Transferase	GGT
Geometric Mean	GM
Gram per Deciliter	g/dL

Gram per Liter	g/L
Integrated Pest Management	IPM
Integrated Risk Information System	IRIS
Intermediate Syndrome	IS
Kilogram	kg
Lactate Dehydrogenase	LDH
Limit of Detection	LOD
Lower Confidence Limit	LCL
Margin of Exposure	MOE
Methyl Parathion	MP
Microgram per Gram	µg/g
Microgram per Liter	µg/L
Microgram per Kilogram	µg/kg
Microgram per Kilogram per Day	µg/kg/day
Milligram per Deciliter	mg/dL
Milligram per Kilogram per Day	mg/kg/day
Milligram per Cubic Meter	mg/m^3
Millimole per Kilogram	mmol/Kg
Millimole per Liter	mmol/L
Minimal Risk Level	MRL

Mobile Examination Center	MEC
National Health and Nutritional Examination Survey	NHANES
National Institute for Occupational Safety and Health	NIOSH
National Research Council	NRC
Neuropathy Target Enzyme	NTE
Not Available	NA
Number	n
Occupational Exposure Limit	OEL
Occupational Safety and Health Administration	OSHA
Odds Ratio	OR
One-Way Analysis of Variance	ANOVA
Organophosphate	OP
Organophosphate-Induced Delayed Neuropathy	OPIDN
Paranitrophenol	PNP
Paraoxonase	PON1
Part per Million	PPM
Peripheral Nervous System	PNS
Permissible Exposure Limit	PEL
Point of Departure	POD
Primary Sampling Unit	PSU
Recommended Exposure Limit	REL

Reference Value	RV_{95}
Sentinel Event Notification System for Occupational Risk	SENSOR
Short-Term Exposure Limit	STEL
Standard Deviation	SD
Statistical Analysis System	SAS
Threshold Limit Value	TLV
Time Weighted Average	TWA
United States	US
Unit Per Liter	U/L
Upper Confidence Limit	UCL

Abstract

Pesticide use in the United States continues to attract negative public attention. In recent years, this attention has focused on the effects that chronic, low-level pesticides may have, especially on children and various sub-populations. Over the past decade, studies have attempted to correlate negative health effects with detections of pesticide biomarkers in biological media. The current research investigates biomarker of exposure levels in a sample of the United States population. Data from the 2001-2002 NHANES dataset (n=11,039) was evaluated. The detection frequency of urinary biomarkers of exposure and the geometric mean from the NHANES pesticide dataset (n=3,152) were determined. Of the 18 specific pesticide biomarkers, three were detected in more than 50% of the 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). These levels fall within the range of other epidemiological and biomonitoring studies investigating background levels of biomarkers in the general population. The association between the detection of a biomarker and variations in mean height and weight of children aged 6-11 was evaluated. No significant results were found when evaluating these differences for 3,5,6-trichloropyridinol exposure. Paranitrophenol

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)]. Heavier children associated with 3-Phenoxybenzoic Acid at age 7: [Detect=28.61 kg and Non-Detect=25.26 kg (p=0.009)]. Clinical chemistry biochemical concentration comparisons were made between individuals that had a detectable level of the biomarker in urine and those that did not. Two biochemicals had a significant difference across all three biomarkers: cholesterol and sodium. The biochemical levels with significant difference between detects and non-detects for the biomarkers were not elevated above clinical reference values. Overall, there is insufficient evidence to suggest a relationship between background pesticide exposures in this sample and negative health effects.

Chapter 1

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. Research conducted around World War II led to the refinement and discovery of new, inexpensive and effective pesticide products [1, 2]. These new discoveries began to replace the inorganic substances (copper acetoarsenite, sodium chlorate, sulfur) that had been used previously [1, 2]. Consumers in the 1950's did not appear to be concerned with any potential hazards these new pesticides may pose, and consumers in the 1960's were more concerned with the impact that pesticide use had on wildlife than the impact that pesticides had on farmers [2, 3]. This emerging concern originated from the publication of Rachel Carson's *Silent Spring* in 1962 which highlighted the potential environmental dangers of pesticides [1, 2, 3]. Public concern now became focused on the possible detrimental effects pesticide use was having on the environment. While the quality of the research and the conclusions reached in *Silent Spring* have been called into question, the work has been credited with the initiation of the modern environmental movement [4, 5].

Public concern over pesticide use was, in part, responsible for the focus on regulating the use and application of pesticides in the United States. The potential

deleterious effects that pesticides may have on the environment, and on members of the population, spawned a new effort to regulate their use [6-12].

Regulatory agencies began to conduct research into potential exposures to pesticides and determine what might be considered safe exposure levels (i.e., no appreciable increased risk of a deleterious effect). While decades of research have established putative mechanisms for deleterious effects associated with acute, high-level exposures, very little evidence exists to support effects from chronic, low-level exposures. Studies in recent years have attempted to characterize the contribution of chronic, low-level pesticide exposures to birth defects, most notably neurological, behavioral and birth outcomes. The research conducted in the current study will utilize urinary biomarker data gathered during a National Health and Nutrition Examination Survey (NHANES) national sampling event to determine if chronic, low-level exposure to pesticides can be associated with an increased risk of adverse health outcomes.

The objectives of the current study are as follows:

- Characterize low-level exposures in a sample of the general population of the United States.
- Determine mean concentrations of biomarkers of exposure levels in the sample and compare those levels to determine if any subgroup is at an increased risk of a toxicological outcome.
- Examine the feasibility of establishing a biomarker of exposure threshold level based on the concentration of urinary biomarkers of exposure.

This study will attempt to test the following **hypotheses**:

1. Biomonitoring data obtained from NHANES indicate the presence of background biomarkers of exposure in individuals from a sample of the general population.
2. Mean concentrations of biomarkers of exposure are homogeneous across the various subgroups of the sample, indicating that no one subgroup is at an increased risk of an adverse health outcome.
3. Urine sample data from NHANES reveal that biomarker of exposure levels in the sample are not correlated with an increased risk of a negative health outcome.

Chapter 2

Literature Review

Pesticides encompass a large group of chemicals that are used for preventing, destroying, repelling or mitigating pests [13]. This category includes, but is not limited to, insecticides, fungicides, herbicides and rodenticides [14]. A variety of products are available for eliminating or mitigating pests in both residential and agricultural settings. For the purposes of this dissertation, the pesticides being evaluated are listed in Table 1.

Table 1: Pesticides Included In This Research.

Parent Compound	Pesticide Type
Chlorpyrifos	Organophosphate Insecticide
Methyl Parathion	Organophosphate Insecticide
Permethrin	Pyrethroid Insecticide

2.1 Pesticide Use

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 [15]. 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 (Figure 1) [15]. These active ingredients are used to create the more than 20,000 EPA registered pesticides currently available in the US [16].

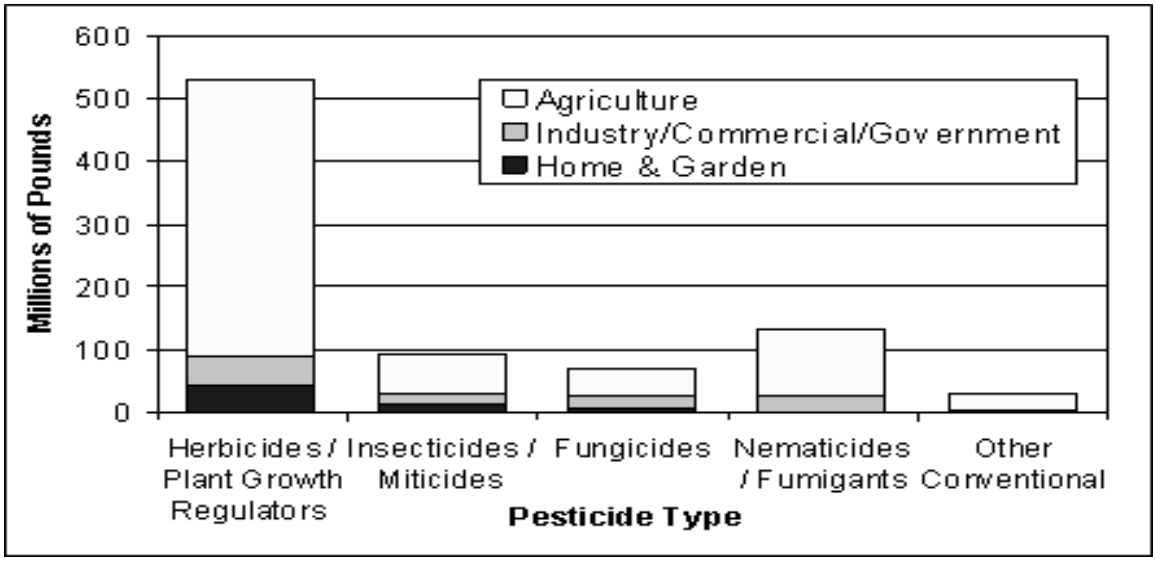

Figure 1: Pesticide Type By Sector. This breakdown from the EPA 2007 market estimate indicates that agricultural applications of herbicides dominate the market. Figure from the EPA [14].

2.2 Regulations

Federal regulations have been established by the federal government in an attempt to make the use and application of pesticide products safe for the end user. The first federal pesticide legislation in the US was the Insecticide Act of 1910 [13]. This Act set standards to protect consumers from misbranded or impure pesticides [17, 18, 19]. The Federal Food, Drug and Cosmetic Act (FFDCA) of 1938 allowed for the establishment of regulatory limits for pesticide residues in food [19]. The Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) of 1947 required all pesticides being sold interstate or to foreign entities to be registered with the US Department of Agriculture [18]. It also required the identification labels on containers to carry information about the manufacturer of the pesticide, the ingredients, directions for use and a protective warning statement [18]. FIFRA was amended in 1959 and 1964 to include additional pesticides. In addition, the 1964 amendment gave authority to the Secretary of Agriculture to refuse

registration of a new pesticide or cancel an existing registration if the pesticide was thought to pose a hazard [17, 18].

The 1954 Miller Amendment to the FFDCA established pesticide residue tolerances and required the seizure of raw agricultural commodities if they contained residues above established tolerance levels [18, 19]. The Delaney Clause, part of the Food Additives Amendment (FAA) of 1958, prohibited the use of a food additive if it was shown to be carcinogenic in humans or laboratory animals [18, 19, 20]. The responsibility of the amended FIFRA was transferred to the EPA at the agency's inception in 1970 [21]. This transfer of power was a paradigm shift from creating safe user conditions in agricultural settings to reducing risk from pesticides in the general population and the environment [22]. FIFRA was amended in 1972 with the passage of the Federal Environmental Pesticide Control Act (FEPCA) [21-23].

The FEPCA required all pesticides to be registered with the EPA and required the pesticide to be classified as general use or restricted use [22]. Under this Act, certification was now necessary for those applying restricted use pesticides and pesticides would now only be registered if they could be demonstrated to not cause unreasonable harm to the environment [22]. In 1996, the FFDCA was amended with the Food Quality Protection Act (FQPA). The FQPA called for the review of all current maximum residue levels of pesticides on food and added the requirement that infants and children must now receive additional protection and focus [24]. The Act also called for an aggregate and cumulative risk assessment for non-occupational exposures to pesticides [24].

2.3 Exposure Assessment

Exposure is the opportunity to contact a substance and absorb a dose [14]. Exposure assessment determines the chemical encountered, the route of exposure, and the magnitude, frequency and duration of the exposure [24]. Assessment methods vary based upon the type of exposure. Occupational exposure evaluations consist of sampling the air surrounding the breathing zone, skin wipe samples or ambient air monitoring to determine an exposure level [25]. In occupational settings, a more sensitive and specific metric for establishing an exposure is to conduct biomonitoring as part of a workplace medical surveillance program [26].

2.4 Biomonitoring

Biomonitoring is the sampling of biological media (blood, urine, tissue, breast milk) for any chemical that may have been absorbed into the body during an exposure [27-32]. History points to the use of biomonitoring for the evaluation of workplace exposures in the 1920's and 1940's with the examination of blood for the presence of lead as well as examining mercury levels in urine [32]. In 1987, the National Research Council (NRC) established three distinct categories for biological markers to be used for the evaluation of an exposure: biomarkers of exposure, biomarkers of effect and biomarkers of susceptibility [28, 31, 32]. Biomarkers of exposure are detectable levels of a parent chemical compound, intermediate biomarker or conjugate in body tissue or biological fluids [29, 32, 33]. Biomarkers of effect are changes in clinical chemistry levels or other biochemical levels that can quantitatively or qualitatively predict an adverse health outcome or an impairment from an exposure [28]. Biomarkers of effect

are used to determine early biological or physiological alterations caused by an exposure to a chemical or substance [32]. Alteration in biochemical enzymes or formation of deoxyribonucleic acid (DNA) adducts due to an exposure would be considered biomarkers of effect. Lastly, biomarkers of susceptibility indicate how an exposure might have an effect based on certain metabolic characteristics of an individual or a population [28, 31].

Biomonitoring provides a measurement of exposure from all routes [30, 32]. Detection of biomarkers in biological media can be influenced by the half-life of a chemical *in vivo*, physical characteristics of the chemical itself and the detection limit of the biomarker in body tissues and fluids by the laboratory conducting the analysis [26, 30, 34]. In order to properly evaluate biomonitoring data, the toxicokinetics of the chemical must be understood [35].

Biomonitoring can be used in the workplace. Occupational biomonitoring is commonly a step in an overall health surveillance program [36]. The program begins with a base-line evaluation that is followed by periodic monitoring to determine if there are any exceedances of established threshold limits [36]. While biomonitoring programs in occupational settings have become more commonplace in recent years, and more accurate due to advances in detection, biomonitoring can also be utilized for establishing exposure in the general population [29].

Population-based studies utilizing biomarker levels in biological samples have attempted to evaluate exposure and to identify any public health trends in order to determine if public health control measures have been effective [32]. Large population based studies evaluating pesticide exposures include: The German Environmental Study (GerES); The Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS); The National Health and Nutrition Examination Survey (NHANES); the Netherlands Generation R study; and the Children's Pesticide Exposure Survey (CPES) of Washington State and Georgia [37-41]. Smaller, localized studies on pesticide exposures have also been conducted.

2.5 Pesticide Exposures

2.5.1 Occupational Exposure

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 [42-44]. It is estimated that approximately 2-2.5 million workers come in contact with pesticides annually as a result of their employment [45, 46]. Dermal contact and inhalation are the primary routes of exposure in occupational settings [47]. 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 [44, 48]. SENSOR data analyzed by Calvert (2008) indicated that, between 1998 and 2005, approximately 3,200 acute occupationally exposed pesticide related illnesses were reported in 10 states [42]. Other identified estimates vary on the

actual number of annual occupational exposures to pesticides. Geer et al. (2004) reported EPA estimates in 1992 that suggested 10,000-20,000 acute occupational excessive exposures occur annually [49]. Occupational excessive exposures may vary depending on a number of factors: The type of pesticide used, the amount used, the route of exposure and the frequency and duration of the exposure to the pesticide [42, 43, 50]. Additional factors when evaluating an occupational exposure to pesticides include: the proper use of personal protective equipment (PPE), proper application of the product, proper training for use, accidental spillage, soiled clothing and the concentration of the pesticide in solution, among others [43, 50, 51].

Occupational exposure threshold limits have been established to protect the health of workers [52]. These limits attempt to protect the worker in exposure situations. Regulatory occupational exposure limits (OELs) are enforceable by law whereas authoritative OELs are suggestions by credible organizations [52]. It is important to note that these regulations are intended to protect worker health. They do not, however, predict whether or not an adverse health outcome will occur if an exposure exceeds the threshold value [14]. Additionally, occupational pesticide exposures are augmented by the EPA Worker Protection Standard, which adds additional margins of safety for employees from occupational exposures to agricultural pesticides [45].

The Occupational Safety and Health Administration (OSHA) publishes regulatory, enforceable Permissible Exposure Limits (PELs) in order to protect worker's health [53]. NIOSH is responsible for the non-regulatory Recommended Exposure

Limits (RELs) [54]. The American Conference of Governmental Industrial Hygienists (ACGIH) publishes both the non-regulatory Threshold Limit Values (TLVs) and Biological Exposure Indices (BEIs), which can be used in conjunction with each other [55]. Not all chemicals have an established BEI. Exposures below the TLV are not expected to result in exceedances of established BEIs. The regulatory and authoritative OELs for the pesticides covered in this research are listed in Tables 2 and 3.

Table 2: Occupational Exposure Limits.

Pesticide	PEL[a] (mg/m^3)[b]		REL[c] (mg/m^3)		TLV[d] (mg/m^3)
	TWA[e]	STEL[f]	TWA	STEL	TWA
Chlorpyrifos	None	None	0.2	0.6 (skin)	0.1
Methyl Parathion	None	None	0.2 (skin)	None	0.02
Permethrin	5.0 (pyrethrum)	None	5.0 (pyrethrum)	None	5.0 (pyrethrum)

[a](PEL) = Permissible Exposure Limit, enforceable by OSHA; [b](mg/m^3) Milligrams per Cubic Meter; [c](REL) = Recommended Exposure Limit, published by NIOSH; [d](TLV) = Threshold Limit Value, published by ACGIH; [e](TWA) = Time Weighted Average, Based on an 8-Hour Work Day, 40 Hours a Week; [f](STEL) = Short-Term Exposure Limit, Based on a maximum 15 Minute Exposure Average. [53-55]

To establish exposure status, occupational exposure assessments can use either direct sampling methods or modeling [52]. As part of a medical surveillance program, biomonitoring can aid in determining an exposure and identifying trends. This includes establishment of a baseline biomarker of exposure (BOE) level and annual or periodic monitoring of biomarkers in conjunction with a physical examination [36].

Table 3: Occupational Biological Monitoring Exposure Limits.

Parent	Biological Exposure Indices (BEI)
Chlorpyrifos	70% AChE[a] of Baseline
Methyl Parathion	70% AChE of Baseline; 0.5 mg/g[b] for Parathion
Permethrin	None

[a](AChE) = Acetylcholinesterase; [b](mg/g) Milligrams per Gram. ACGIH BEI [55].

2.5.2 Residential Exposure

Individuals that are exposed to pesticides outside of an occupational setting are considered general population exposures. Exposures in the general population can be secondary and are generally orders of magnitude lower than occupational exposures [56]. In 2007, domestic use of pesticides accounted for 8 percent of the total conventional pesticides used in the United States [15]. Widespread pesticide use in the United States has equated to ~94,000 reported pesticide exposures in 2008, according to the American Association of Poison Control Centers' National Poison Data System's 26th annual report [57]. This value indicates the number of individuals in the population with suspected pesticide exposures. Public concern has, in part, driven the interest relating to regulation and mitigation of public and environmental contact with these substances [6, 7, 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; and 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 [50, 56, 58-63]. It has

been suggested that ingestion of food products containing pesticides is the primary route of exposure for the general population [64-67].

The FQPA of 1996 called for the safety evaluation of pesticides based on all routes of exposure from non-occupational sources [68, 69]. Additionally, the EPA is required to address the risks pesticides may pose to infants and children. This act also required the re-assessment of pesticide tolerance levels in food products. Based on the FQPA and the National Academy of Science, the EPA established maximum residue levels on food products that vary by commodity [70]. As an additional evaluation of risk, the Agency for Toxic Substances and Disease Registry (ATSDR) has established Minimal Risk Levels (MRL). The MRL (Table 4) is an estimate of the acceptable daily exposure that is likely to be without an increased appreciable, non-cancer risk [71]. The ATSDR MRL was established to evaluate exposures and potential health effects at hazardous waste sites, but can also be used as an evaluation/screening tool for exposures in the general population [71].

Table 4: Oral MRLs from the ATSDR.

Parent	Acute	Intermediate	Chronic
Chlorpyrifos	0.003 mg/kg/day [a]	0.003 mg/kg/day	0.001 mg/kg/day
Methyl Parathion	NA	0.0007 mg/kg/day	0.0003 mg/kg/day
Permethrin	0.3 mg/kg/day	0.2 mg/kg/day	NA

[a](mg/kg/day) Milligrams per Kilogram per Day. These thresholds are based on a milligram per kilogram per day (mg/kg/day) ingestion rate [71]

2.6 Pesticides

As previously noted, pesticides encompass a large group of chemicals aimed at mitigating or destroying pests. Pesticide use has been receding since reaching a plateau in the 1980's, as can be viewed in Figure 2 [13]. This reduction has been, in part, attributed to the manufacture of more efficacious compounds and the establishment of Integrated Pest Management (IPM) systems in developed countries [13]. Mechanisms of toxicity for the pesticides investigated in this study are similar for both pests and mammals [13, 22]. Precautions must be taken to reduce the chance for an overexposure to pesticide products. The two main categories of pesticides that will be covered by this research, organophosphates and pyrethroids, will be detailed in the following sections.

2.6.1 Organophosphate Insecticide

In 2007, organophosphates (OP) accounted for 35% of all insecticides used in the US (33 Million Pounds) [15]. OP were originally synthesized in the 1800's, but their refinement for pest mitigation did not occur until the late 1930's and early 1940's [13, 72]. OP are readily degraded in the environment by way of hydrolysis, photolysis or biodegradation [73, 74]. There is little evidence to suggest that OP bioaccumulate in body tissue or the environment, since metabolism of the parent appears rapid [75]. The research conducted in this study included two organophosphate pesticides: Chlorpyrifos and Methyl Parathion.

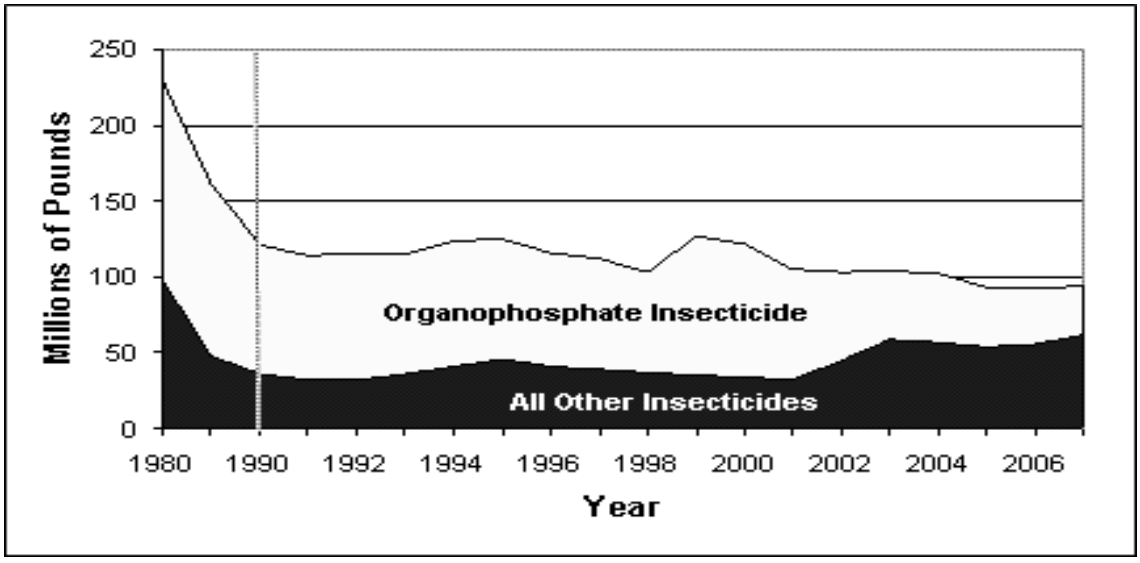

Figure 2: Organophosphate Insecticides Versus Other Insecticides. This EPA graph indicates the application, in pounds, of organophosphates versus all other insecticides since 1980. Figure from the EPA [14]. The dotted line (at year 1990) signifies a change in the scale for time.

2.6.1.1 Organophosphate Health Effects

2.6.1.1.1 Acute

Effects following acute OP exposure are documented and well understood [30]. The toxidrome commonly associated with acute OP overexposure is the inhibition of acetylcholinesterase (AChE) in the Central Nervous System (CNS) and the Peripheral Nervous System (PNS), as AChE is the primary target of OP pesticides [72, 76]. Once a dose of OP has been absorbed, it must undergo biotransformation to produce AChE-inhibitory effects [13]. The majority of the biotransformation takes place in the liver via cytochrome P-450 (CYP) mediated oxidative desulfuration to remove the sulfur that is bound to the phosphorus, replacing it with an oxygen molecule [72]. This forms the reactive oxon form of the parent OP [72]. The oxon analog is responsible for AChE inhibition [77]. Hydrolysis and paroxonase (PON1) activity detoxify the oxon and

reduce it to byproducts [77]. OP can also bypass the desulfuration pathway and can be reduced by CYP-mediated dearylation into excretable metabolites without being transformed into the toxic intermediate [77].

The nervous system is the target for OP AChE inhibition [77]. Acetylcholine is a neurohumoral transmitter that relays nerve impulses across synapses and neuroeffector junctions [73]. Hydrolysis of acetylcholine by AChE at cholinergic nerve terminals terminates the electrical conduction [72, 77]. The inhibition of AChE by the OP oxon results in accumulation of acetylcholine in the CNS and PNS and cholinergic synapses, resulting in the overstimulation of the muscarinic and nicotinic receptors [78]. Overstimulation of these receptors can result in a cholinergic syndrome with symptom presentation based on the receptor type being targeted [76, 79].

Accumulation of acetylcholine in the CNS can result in confusion, hypothermia, tremors, paralysis and coma [76]. PNS muscarinic receptor depression can manifest as bradycardia, hypotension, miosis, gastrointestinal distress and lacrimation [76]. Stimulation of nicotinic PNS receptors present as tachycardia, fasciculation, ataxia, convulsions, and paralysis [76]. Death from acute AChE inhibition is theorized to follow respiratory failure due to bronchoconstriction, increased bronchial secretions, intercostal and diaphragmatic muscle paralysis and inhibition of respiratory centers in the brain stem [13]. While AChE is the most understood cholinesterase when it comes to inhibition, a second type of cholinesterase, butylcholinesterase (also known as plasma pseudocholinesterase; BuChE), also undergoes inhibition, though its function is not well

understood [13]. It is believed that BuChE may be a sensitive biomarker of exposure when acute overexposure is suspected, but inhibition of BuChE alone cannot be considered a biomarker of effect when evaluating exposure to OP [13].

A second manifestation of acute OP excessive exposure is Intermediate Syndrome (IS). This syndrome was first described in 1987 by Sri Lankan physicians observing hospitalizations of attempted suicide patients following ingestion of OP pesticides [13]. Onset of this syndrome is anywhere from 24-96 hours following acute overexposure to OP [81]. The effects observed during this crisis do not appear to originate from AChE inhibition [77]. While the exact disease etiology is unknown, the symptoms are serious and include slowed respiration due to muscle weakness and weak neck muscles, in addition to proximal limb and cranial nerve palsies [13, 77]. IS could lead to death due to paralysis and respiratory failure [13].

A third sequela from excessive op exposure is Organophosphate-Induced Delayed Neuropathy (OPIDN) [13, 14, 82]. Also referred to as a neurotoxicity or a polyneuropathy in the literature, this syndrome manifests itself anywhere from 7-21 days following an acute cholinergic crisis (sources vary on the actual time for the signs to present) [14, 82]. Physiologically, sensory and motor axon degradation occurs in the distal regions of the peripheral nervous system and in the spinal cord [14]. A single exposure to an OPIDN-causing OP can lead to the inhibition of Neuropathy Target Enzyme (NTE) via phosphorylation [83]. Phosphorylation, along with 'aging' of at least 70% of the NTE, is required for the signs of OPIDN to be observed [72]. Aging is a loss

of an alkyl group bound to NTE [80]. Studies have shown that inhibition of NTE is independent of AChE inhibition; however, age of the individual exposed does seem to be a factor on susceptibility, as young animals are at less of a risk to OPIDN following exposure due to the higher percentage of NTE inhibition required for young animals as compared to adult animals (90% versus 70%, respectively) [72]. Symptoms of this toxidrome include paresthesia, sensory loss, muscle weakness in extremities and limb muscles [84]. Not all OP pesticides are capable of causing OPIDN [14]. Only OP that are capable of aging NTE can cause OPIDN [13].

2.6.1.1.2 Chronic

While the effects from acute overexposures to OP pesticides have been well researched, there appears to be no consensus on the potential health effects from chronic, low-level exposures [72]. Recently, it has been proposed that chronic exposures to pesticides are contributing to neuropsychiatric disorders [85]. However, reviews of the research have found no conclusive evidence that low-level exposures to OP contribute to clinically significant neuropsychological effects or peripheral nerve dysfunction, though research in this area continues [72, 86-88]. Beyond neurological outcomes, public health concern has focused on the effects of pesticide exposures on the young. The FQPA of 1996 called for an evaluation of aggregate pesticide exposures on the general population, with special attention to infants and children [68]. With this increased focus on the young, new concerns arose questioning whether exposures to pesticides could result in teratogenic effects. It has been observed that physiological differences in young animals

(low detoxication rate by CYP and PON1) may make them more sensitive to a cholinergic crisis than adult animals [77].

Epidemiological studies of OP suggest that exposure to the fetus during development may result in biochemical and behavioral abnormalities. These recent studies have begun to evaluate the association of exposures of pregnant mothers to pesticides and negative birth outcomes. Outcomes evaluated in these studies include: decreased head circumference, decreased birth weight, decreased body length, decreased time of gestation, increased incidence of ADHD and memory impairment in children [77].

2.6.1.2 Chlorpyrifos

Chlorpyrifos (*O,O*-diethyl-*O*-(3,5,6-trichloro-2-pyridyl) phosphororthioate; CAS 2921-88-2), a colorless to white solid organophosphate insecticide, has been used for the mitigation of cockroaches, termites, fleas and ticks [73, 77]. It is also used on vegetable, fruit, cotton, tobacco, corn crops and golf course turf to reduce damage from insects [77, 89]. Chlorpyrifos for pesticide applications was first used in 1965 [77, 90]. While chlorpyrifos is still used in the control of pests in agricultural operations, its use in residential settings was phased out in 2001 [77]. The removal from residential settings was based on the findings of the EPA's preliminary human health risk assessment [91]. Current exposures to chlorpyrifos are primarily limited to agricultural settings [77].

2.6.1.2.1 Toxicokinetics

2.6.1.2.1.1 Absorption

Chlorpyrifos is absorbed through ingestion, dermal and inhalation routes [74]. Nolan et al. (1984) reported recovery of urinary biomarkers at 70% following an oral exposure to 0.5 mg/kg of chlorpyrifos and urinary biomarker recovery at 1.3% from a dermal exposure to 5.0 mg/kg from post-dosing samples [92]. A small amount is eliminated in the bile from phase II glutathione conjugations [77]. Timchalk et al. (2002) administered oral doses of 0.5-2.0 mg/kg to human volunteers [93]. Only 20-35% of the oral dose was recovered as 3,5,6-trichloro-2-pyridinol (a primary biomarker detected following exposure to chlorpyrifos; also known as 3,5,6-trichloropyridinol and TCPy) [93]. Chlorpyrifos exposures in the general population are thought to occur via ingestion of residues remaining on food products, though reductions of use in agriculture may reduce exposures from this route [51, 62, 77]. Dermal exposure to chlorpyrifos is not thought to be a major route of exposure in the general population following the restriction of its application, and inhalation exposures from agricultural applications are thought to be low, in relation to the diet [77]. While prior studies have suggested that concentration in ambient air and residues on surfaces may be a potential source of exposure for children, inhalation of ambient air in homes is no longer thought to be a source of exposure following the moratorium of chlorpyrifos use in residential scenarios [73].

2.6.1.2.1.2 Distribution

Animal studies indicate that chlorpyrifos is distributed to all organs of the body following dosing [73]. Bioassay experiments suggest that chlorpyrifos is found in high

concentrations in adipose and fatty tissues [77]. Smith et al. (1967) suggested that the half-life in adipose tissue is 62 hours, while the half-life is reduced to 10-16 hours in the liver, kidney and the heart [94]. Abdel Rhaman et al. (2002) indicated that TCPy was observed in all tissues within 5 minutes following dosing with ^{14}C radiolabeled chlorpyrifos (5mg/kg) [95].

2.6.1.2.1.3 Metabolism

Chlorpyrifos is metabolized to form any of three final biomarkers: 3,5,6-trichloropyridinol, diethylthiophosphate (DETP) or diethylphosphate (DEP) [77]. Chlorpyrifos must be bioactivated/desulfurated and transformed into the chlorpyrifos-oxon analog to inhibit AChE activity in the CNS and PNS [77]. The majority of the transformation occurs in the liver via cytochrome P-450 dependent monooxygenase desulfuration [72]. Detoxication of the oxon takes place through hydrolysis using paroxonase (PON1) as the catalyst to form DEP and TCPy [77]. Chlorpyrifos itself can be directly reduced to DETP and TCPy via cytochrome P-450-mediated dearylation [77]. Animal studies have suggested that TCPy undergoes further metabolism through conjugation with glucuronic acid and sulfate [77].

2.6.1.2.1.4 Elimination

Half-life of elimination in humans has been estimated at 27 hours following an oral or dermal exposure [92]. Timchalk et al. (2007) recovered most of the administered dose in the urine 72 hours after dosing rats with ~50 mg/kg chlorpyrifos [96].

2.6.1.2.2 Mechanism of Toxicity

Chlorpyrifos, by itself, is an inefficient inhibitor of AChE; however, the chlorpyrifos oxon, created by oxidative desulfuration, is an excellent inhibitor of the enzyme [77]. Metabolism of the parent and oxon metabolite is rapid, as can be seen in Figure 3.

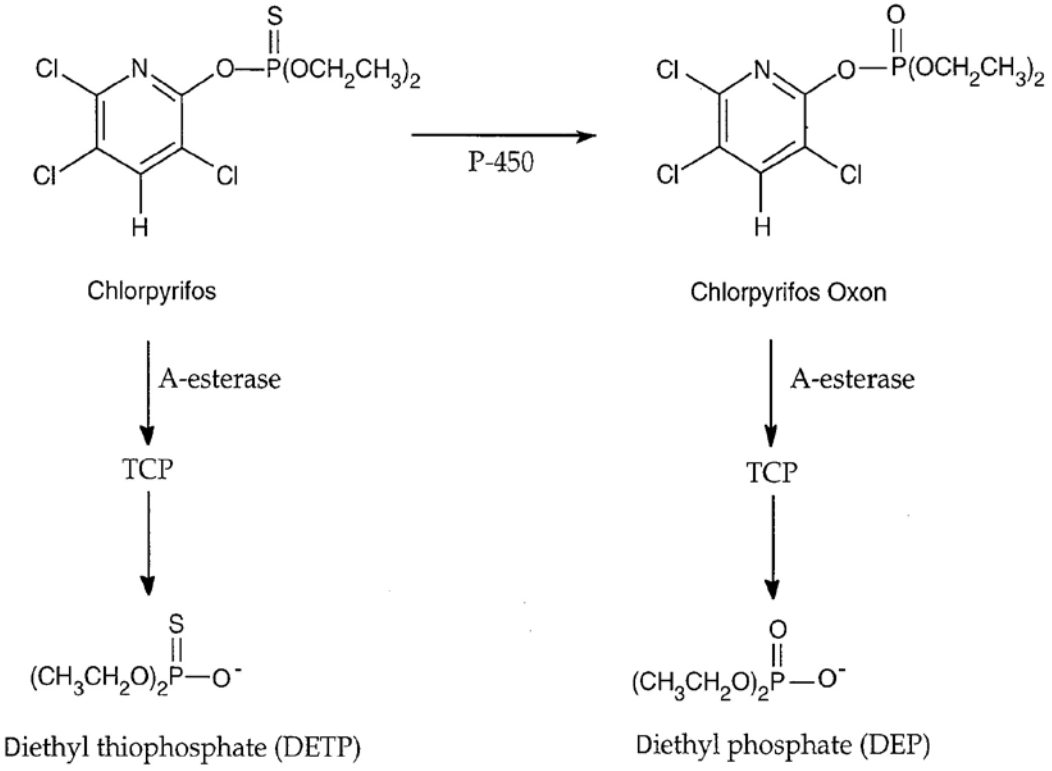

Figure 3: Chlorpyrifos Metabolism. Chlorpyrifos can be either metabolized directly to TCP(y) and DETP or it can undergo further metabolism via Cytochrome P-450 to form chlorpyrifos oxon. The oxon analog is detoxified via Paroxonase 1 to form TCP(y) and DEP. Figure from ATSDR [73]. P-450= Cytochrome P-450; A-esterase= Paroxonase 1; TCP= 3,5,6- trichloropyridinol; DETP= diethylthiophosphate; DEP= diethylphosphate

2.6.1.2.3 Toxicity Assessment

The assessment for chlorpyrifos will be limited to select outcomes. This assessment is focused on the more important public health risks. Studies addressing human data are presented when available.

2.6.1.2.3.1 Body Weight

Male and female rats with whole body exposure to 5,300 mg/m^3 of a solution of Pyrenone Dursban for 4 hour lost a mean 10% body weight, however no weight variations were observed in rats dosed with 2,500 mg/m^3 of Pyrenone Dursban pressurized spray [73]. Corley et al. (1989) dosed Fischer 344 rats with a maximum concentration of 287 µg/m^3 for 6 hours a day, 5 days a week for 13 weeks [97]. No body weight differences were noted for any dosing group [97]. Rodents with acute oral exposure to chlorpyrifos at high doses have recorded decreases in mean body weight [73]. Moser (1995) orally dosed rats with a one-time 100 mg/kg of technical grade chlorpyrifos in corn oil had a 13.3% decrease in body weight 24 hours post dosing [98]. No alterations in body weight have been observed from intermediate exposures in rodents [73].

2.6.1.2.3.2 Neurological

Much of the literature pertaining to neurological effects following exposure to chlorpyrifos centers around the inhibition of cholinesterase activity. An animal study by Breslin et al. (1996) evaluated pregnant Fischer 344 rats orally dosed with 15 mg/kg/day of technical grade Dursban F via gavage on gestation days 6-15 [99]. Erythrocyte AChE

activity was reduced ~80% as compared to controls [99]. An evaluation of OPIDN in hens by Capodicasa et al. (1991) resulted in maximum inhibition of NTE 5-7 days following a one time dosing of 60-150 mg/kg chlorpyrifos [100].

A study by Albers et al. (2004) evaluated the effects of chlorpyrifos on the central nervous system of occupationally exposed individuals [101]. The study evaluated employees at Dow Chemical Company where pesticides were manufactured. Cases and controls were identified from the facility. A baseline examination was conducted as well as a follow-up examination approximately one year later. Average creatinine-adjusted TCPy in the exposed group was 251 (µ)g/L (urine) and 192.2 µg/g (creatinine-adjusted) [101]. The study concluded that chronic, low-level occupational chlorpyrifos exposures were not associated with clinically evident or subclinical peripheral neuropathy when compared to controls [101].

Steenland et al. (2000) conducted a cross-sectional study with 191 termiticide applicators (both current and former) [102]. Recently exposed applicators had a mean urinary concentration of TCPy at 629.5 µg/L [102]. When the termiticide applicators were compared with a control group, few exposure-related effects were significant. Many were homogenous for effects on clinical examination. However, significant differences were found when comparing the performance of the exposed on the length of sway and pegboard test to controls [102].

2.6.1.2.3.3 Developmental Effects

Deacon et al. (1980) evaluated physical abnormalities in a group of pregnant CF-1 mice orally dosed with up to 25 mg/kg/day chlorpyrifos on gestation days 6-15; the study concluded that chlorpyrifos was not teratogenic [103].

Whyatt et al. (2004), along with Perera, 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 [104]. 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 [104]. Plasma TCPy levels were determined and a negative association was found between detection of TCPy and birth weight and length [104]. These data were further analyzed to determine the impact of chlorpyrifos exposure on neurodevelopment. Rauh et al. (2006) determined that "highly exposed" (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) [105]. 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. in 2011 re-assessed these children at age 7 [106]. 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 [106].

Eskenazi et al. (2004) investigated chlorpyrifos exposure and negative birth outcomes in the Center for the Health Assessment of Mothers and Children of Salinas Study (CHAMACOS) [107]. 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) [107].

Berkowitz et al. (2004) investigated the relationship between pesticide exposure and negative birth outcomes in the Children's Environmental cohort study at Mount Sinai Hospital, New York [108]. Races were mixed in this study with approximately 50% of the participants identified as Hispanic [108]. 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 [108].

2.6.1.3 Methyl Parathion

Methyl parathion (*O,O*-dimethyl-*O*-(p-nitrophenyl)-phosphororthioate; CAS 298-00-0), first manufactured in the USA in 1954, is an insecticide used on crops, such as cotton and soybeans [74, 109]. It is a white crystalline solid or brownish liquid containing 80% methyl parathion as the active ingredient [74, 109]. Exposures to the general population are thought to be low [74]. Inhalation exposures are thought to be the route of exposure to individuals living near agricultural application of methyl parathion (MP) [74]. Dermal may be the most relevant exposure route based on the fact that the

EPA has accepted voluntary cancelation of the use of MP on a variety of food products and residential application is prohibited [74, 110]. The EPA determined that MP posed an unacceptable dietary risk to children aged 1-6 [110].

2.6.1.3.1 Toxicokinetics

2.6.1.3.1.1 Absorption

Humans appear to readily absorb MP via ingestion, inhalation and dermal exposures [74]. Oral absorption is limited by first pass metabolism in the small intestine [109]. Human exposures to methyl parathion have shown that AChE inhibition takes place following dermal absorption [74]. The ATSDR indicated that exposure can occur through inhalation, though no human studies identified absorption via inhalation [74].

2.6.1.3.1.2 Distribution

Distribution studies involving human exposures are limited. A human dosing study by Morgan et al. (1977) indicated that ingestion of either 2 mg or 4 mg of MP in food resulted in urinary excretion of biomarkers, with the highest concentrations 4 and 8 hours after ingestion [111]. Garcia-Repetto et al. (1997) reported that MP distributed to various body compartments of the rat following gavage and results indicated that, depending on the tissue, the half-life of elimination is anywhere from 8 to 16 days [112]. Bioassay studies have suggested that MP distributed to the blood, brain, liver and adipose tissue [109].

2.6.1.3.1.3 Metabolism

Like other OP pesticides, MP can be either detoxified or bioactivated after absorption. Detoxication takes place via dearylation by oxidatively cleaving the compound to form paranitrophenol (PNP) and dimethyl thiophosphate [109]. Oxidative desulfuration via Cytochrome P-450 transforms MP to the more toxic analog, Methyl Paraoxon [109]. The oxon is detoxified by paraoxonase (PON1) to form either paranitrophenol or dimethylphosphate and can be further reduced to form conjugates with glucuronic acid and sulfate [74, 109]. Methyl parathion itself is not effective at inhibiting AChE [74]. Studies have indicated that oral absorption of MP limits its toxicity due to first pass metabolism [74].

2.6.1.3.1.4 Elimination

Abu-Qare et al. (2000) used pregnant rats to apply a dermal dose of a radiolabeled MP solution. Urinary recovery of was 91% within 96 hours [113]. Excretion from an oral exposure occurs mainly through urine and appears to be rapid. A human volunteer study by Morgan et al. (1977) suggested that 86% of 4-nitrophenol had been eliminated 8 hours following dosing, with the highest recovery during the first 4 hours [111].

2.6.1.3.2 Mechanism of Toxicity

Like other organophosphate pesticides, MP must be bioactivated to form methyl paraoxon in order to elicit AChE inhibitory effects, as seen in Figure 4 [112].

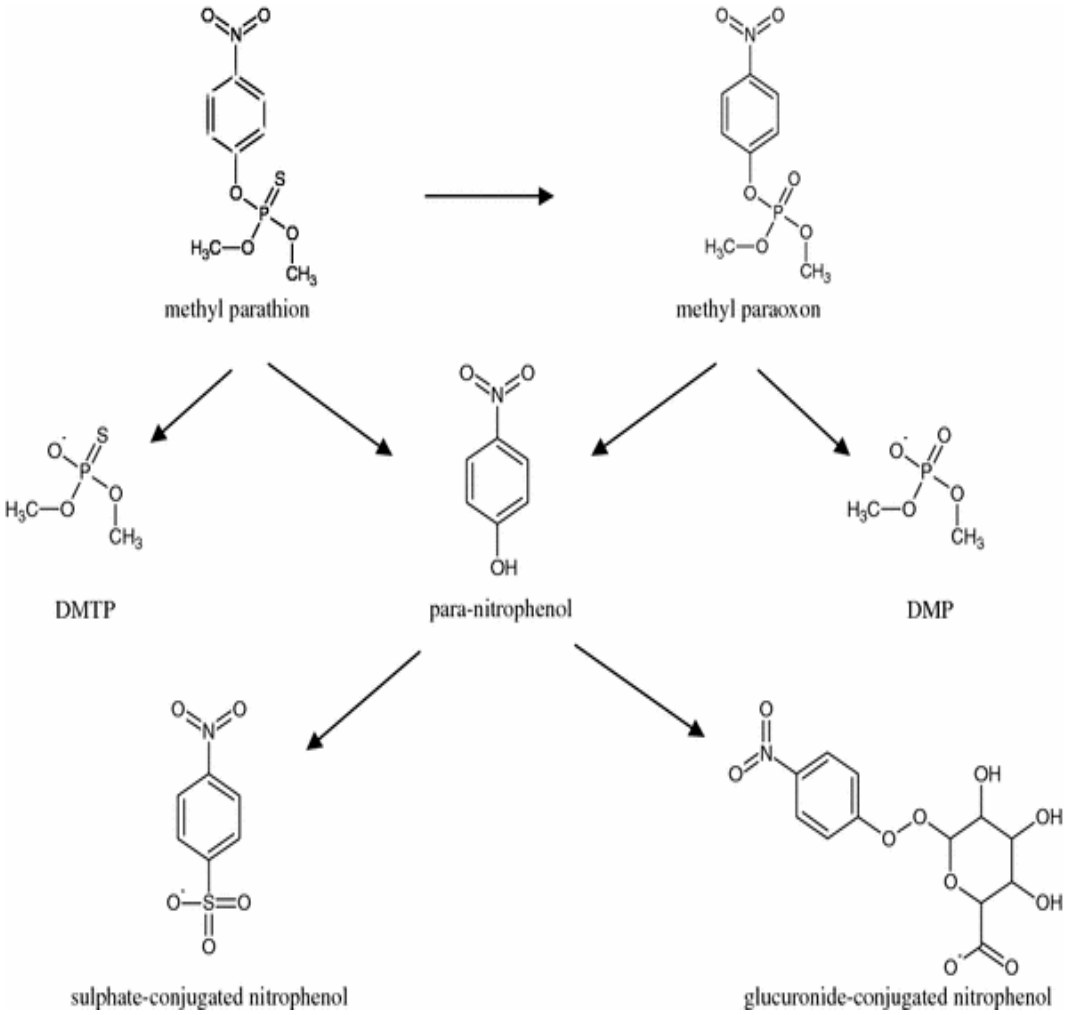

Figure 4: Methyl Parathion Metabolism. Methyl Parathion can be reduced directly to form PNP and DMTP or it can undergo activation via cytochrome P-450 to form methyl paraoxon. The oxon analog is detoxified via PON1 to form PNP and DMP. Figure from Panuwet et al. (2009) [114]. DMTP= dimethyl thiophosphate; DMP= dimethylphosphate. Reprinted with permission.

2.6.1.3.3 Toxicity Assessment

The assessment for methyl parathion will be limited to select outcomes. This assessment focuses on relevant public health outcomes. Studies addressing human data are presented when available.

2.6.1.3.3.1 Body Weight

Studies have evaluated variations in body weight following acute, intermediate and chronic oral exposures. Tian et al. (1997) did not observe any significant body weight variations in mice dosed with 5 mg/kg/day for 15 days [115]. Two of eight dogs dosed with 3.0 mg/kg/day for 13 weeks were reported to be emaciated and dehydrated; these effects were not noted in dogs dosed with concentrations that were orders of magnitude lower [74]. Rats orally dosed with methyl parathion at concentrations of 2.5 mg/kg/day for 2 years showed significant reductions in body weight in comparison to controls [74]. However, this variation was not consistent during the study duration and variations were not observed in animals dosed with concentrations that were orders of magnitude lower [74].

2.6.1.3.3.2 Neurological

The neurotoxic effects of MP center around the inhibition of AChE [112]. Rodnitzky et al. (1978) orally dosed two male volunteers with 2 mg/day of MP for 5 days, and, 8 weeks later, were orally exposed to 4 mg/day of MP for 5 days. No significant reductions in AChE-activity were observed for either dosing [116]. Male rats orally dosed with 5 mg/kg via gavage displayed cholinergic signs seven minutes post dosing, resulting in an approximately 44% reduction in plasma cholinesterase when compared to controls [74]. Crittenden et al. (1998) dosed mice with MP at 1, 3 and 6 mg/kg/day recorded decreased brain AChE activity at various points over the 28-day study in the groups dosed with 3 mg/kg and 6 mg/kg [117].

In the 1990's, MP was illegally applied in residences in at least nine Midwestern and Southern states [118]. 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 (PNP) 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 [118]. 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 [118]. 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 [118].

2.6.1.3.3.3 Developmental Effects

Numerous studies have used rodents to identify the effects of methyl parathion on pregnant dams during gestation. A mouse study by Gupta et al. (1985) identified increased observed visible signs of toxicity in dams and fetuses exposed to 1.5 mg/kg/day of MP [119].

The Eskenazi et al. (2004) study referenced in the chlorpyrifos section (Page 26) also evaluated the association of urinary concentrations of PNP to negative birth

outcomes [107]. When comparing women with detects of the biomarker to those that did not have a detect for PNP, women with levels of PNP above the median (median=0.05µg/L) had children with increased head circumference when compared with women without a detect (β =0.29cm, p=0.06) [108]. Additionally women with PNP levels below the median had children with increased length as compared to women with no detects (β=0.60 cm; p=0.03) [107].

2.6.2 Pyrethroids

Pyrethrum, a natural extract used as an insecticide, is obtained from the *chrysanthemum cinerariaefolium* and *chrysanthemum cineum* flowers [120]. Use of these extracts for insect mitigation can be traced back at least 2,000 years to China and Persia [13, 121]. While effective at controlling insects, degradation of pyrethrum occurs quickly in sunlight and the environment [120]. Pyrethroids are synthetic analogs of pyrethrums [13, 120]. The synthetic pyrethroids are similar in structure to pyrethrum but are more resistant to rapid degradation [120].

Pyrethroids are insecticides used in agricultural settings and household applications for the control of scabies, lice and mosquitoes and, in addition, are among the most frequently used pesticides [13, 122]. Pyrethrum, as a group, contains six active compounds referred to as pyrethrins [123]. In comparison, there are over 1,000 synthetic pyrethroids, though only a few of those are commonly used in the US [120]. Several examples of these compounds can be seen in Table 5. The mechanism of action for pyrethroids is similar for insects and mammals; however, because of a faster metabolism

and lower sensitivity at the target site, mammals are relatively resistant to the actions of pyrethroids [121].

Pyrethroids are classified as either Type I or Type II, depending on their physical properties and toxicological outcomes in rats, though other rodents have also been used to classify the effects of an overexposure [64, 124]. Type I compounds elicit aggressive behavior, increased sensitivity to external stimulation, startle response, fine and whole body tremors as well as prostration in rats [124]. These sequalae are referred to as T-syndrome [124]. Type II pyrethroids are characterized by burrowing, salivation, coarse tremors, choreoatetosis and clonic seizures in rats [124]. In addition, Type II pyrethroids are chemically differentiated from Type I compounds by the presence of a cyano (CN) group on the alpha carbon [124]. Outcomes from exposure to Type II compounds are referred to as CS-syndrome [124]. Pyrethroids contain two chiral carbons and, in some cases, an additional carbon on the alcohol moiety to total three [13, 120]. In addition to existing in cis- and trans- conformations, pyrethroids can result in up to eight different isomers [13, 120]. The conformation of the compound can have an effect on toxicity [13].

Table 5: Partial List of Pyrethrins and Pyrethroids.

Pyrethrins	Type I Pyrethroids	Type II Pyrethroids
Pyrethrin I	Allethrin	Cyfluthrin
Pyrethrin II	Bifenthrin	Cyhalothrin
Cinerin I	Permethrin	Deltamethrin
Cinerin II	Resmethrin	Fenvalerate
Jasmolin I	Tefluthrin	Flumetrhin
Jasmolin II	Tetramethrin	Tralomethrin

Adapted from ATSDR Toxicological Profile [120].

Pyrethrins and pyrethroids are degraded by sunlight and typically do not persist in the environment, though permethrin and cyhalothrin persist longer due to structural characteristics [120]. Environmental half-life ranges from days to a few weeks [120]. The compounds are typically immobile in soil and are biodegradable in the environment, though it has been shown that this insecticide does bioaccumulate in aquatic organisms [120]. Permethrin is the most frequently used pyrethroid in the US and is the pyrethroid that will be focused on in this study [64, 120].

2.6.2.1 Health Effects

2.6.2.1.1 Acute

While their use as an insecticide is effective and potent, they pose little hazard to humans due to their low mammalian toxicity (three orders of magnitude lower than that of insects) [125]. The adverse health effect of pyrethroids depends on their classification, though their primary target is disruption of the voltage-gated sodium channels of the nervous system [121, 24, 126]. Type I pyrethroids function by prolonging the opening of the sodium ion channel to cause repetitive firing of the action potential [13]. Type II compounds also work on the sodium channel; however, instead of rapid fire, the sodium channel remains open which results in depolarization of the channel and does not allow for the generation of an action potential [13].

2.6.2.1.2 Chronic

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 [120]. In 1999, the Cancer Assessment Review Committee (EPA) classified pyrethrins as "*likely to be a human carcinogen by the oral route*" based on a study finding increases in thyroid follicular cell tumors in male rats and increased incidence of hepatocellular adenomas and carcinomas in female rats fed high doses of pyrethroids (1,000 ppm and 3,000 ppm) [120]. However, other animal studies using high dosings have not come to the same conclusions [120]. There has also been an attempt to correlate pyrethroids exposure to reduction in semen and hormone levels in adult men [65, 127].

2.6.2.2 Toxicokinetics

2.6.2.2.1 Absorption

Studies have indicated that pyrethroids are readily absorbed through inhalation exposure and moderately through oral exposure, while less than 2% of the applied dose following dermal exposure is absorbed [120, 128]. Oral absorption takes place in the intestinal tract, though first pass metabolism may underestimate the true absorbed dose [120]. An inhalation study in humans by Leng et al. (1997) indicated that urinary biomarkers were 93% recovered within 24 hours, with peak rates between 30 minutes and 3 hours, indicating rapid metabolism [129]. Human and animal information on dermal absorption is limited [120]. In occupational settings, exposures to pyrethroids are thought to occur from inhalation and dermal exposure, though studies have indicated that the skin is an effective barrier to absorption [120]. General population exposure is suggested to be through the ingestion of agricultural products that have been treated with pyrethroids [120, 125]. It is suggested that general population exposure also originates from

household treatments with pyrethroids [128]. Because of their lower vapor pressure, pyrethroids can form particulates at room temperature and partition into household dusts [128]. Because young children tend to have higher hand-to-mouth activity, this may also be a relevant source of exposure [130].

2.6.2.2.2 Distribution

In humans, once absorbed into the blood stream, pyrethroids are rapidly distributed [128]. Pyrethroids are theorized to distribute to most tissues, especially those high in lipid content [120]. They are also thought to concentrate in the central and peripheral nervous systems [120]. Distribution studies for dermal and inhalation exposures in humans and animals was lacking.

2.6.2.2.3 Metabolism

Many of the studies on the metabolism of pyrethroids are based on mammalian animal models [120]. The parent compound of pyrethroids is the active, toxic form, as metabolism forms byproducts that are thought to have little or no toxicity [13, 120]. Metabolism occurs through two major pathways: hydrolysis of the ester bond and oxidation of the alcohol moiety [13]. One pathway may be the major path of biotransformation, depending on the type of pyrethroids encountered [120]. The biomarker 3-Phenoxybenzoic Acid (3-PBA) will be evaluated in this research. It is a biomarker for 10 out of the 18 common pyrethroids registered in the US [128].

2.6.2.2.4 Elimination

Depending on the specific pyrethroid encountered and the rate of exposure, elimination half-life ranges from 6.4-16.5 hours [128]. Animal studies indicate that Type I and II pyrethroids are eliminated within 4-12 days following oral dosing and the majority is eliminated within 12-48 hours [120].

2.6.2.3 Mechanism of Toxicity

The parent form of the pyrethroid is responsible for interruption in the sodium channel action potential. Metabolism detoxifies the parent pyrethroid, inactivating the Type I and II effects, and forms excretable metabolites, as can be seen in Figure 5.

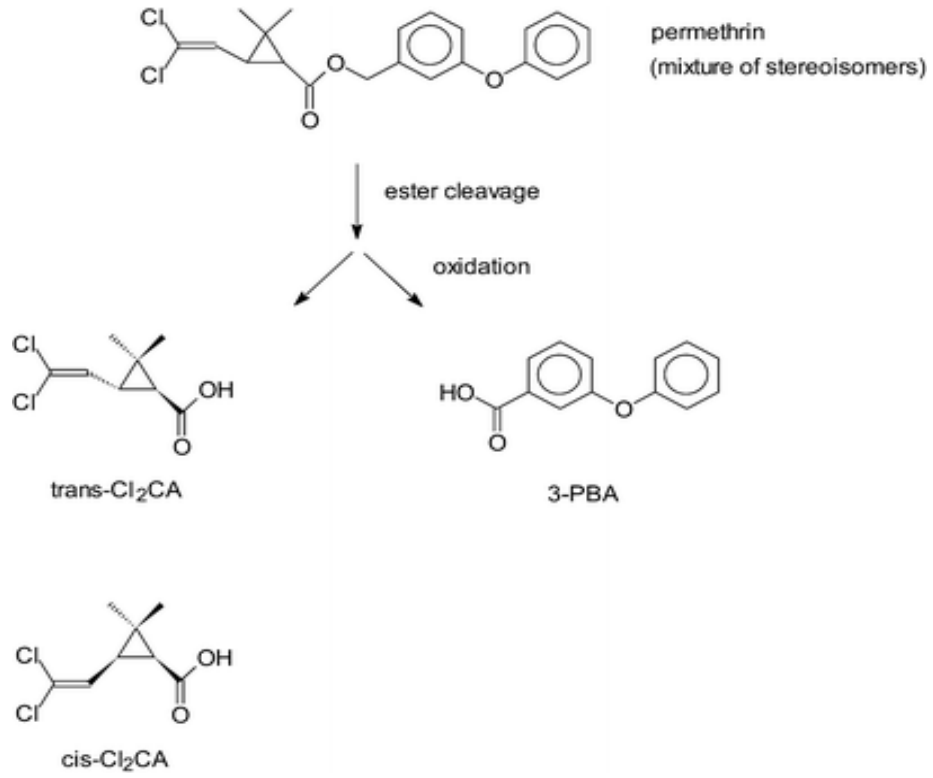

Figure 5: Permethrin Metabolism. Permethrin undergoes detoxication to form 3-Phenoxybenzoic Acid, the metabolite of interest in this research. Figure from Hardt et al. (2003) [131]. Cis/Trans-Cl_2CA= 2,2- Dimethylcyclopropane Carboxlic Acid; 3-PBA= 3-Phenoxybenzoic Acid. Reprinted with permission.

2.6.3.3 Toxicity Assessment

Because there are a variety of synthetic mixtures and conformations of pyrethroids available, the information in this section will not be limited to one specific pyrethrin or pyrethroid. This assessment is limited to relevant public health risks.

2.6.3.3.1 Body Weight

Various animal studies evaluated and associated pyrethroid and pyrethrin exposure to reductions in body weight and food consumption. Parker et al. (1984) orally dosed female dogs with fenvalerate at 1,000 ppm in the diet for 6 months; dosed dogs had

significant decreased body weight and food consumption when compared to controls [132].

2.6.3.3.2 Neurological

In occupational exposure scenarios, paresthesia is reported following dermal contact to pyrethroids [121]. Other acute signs include muscular fasciculations, headache and convulsions [120]. Signs of neurotoxicity seem to dependent on whether the exposure was to Type I or Type II pyrethroids. Neurological signs in laboratory animals from an exposure include aggressive behavior, increased sensitivity to external stimulation, startle response, tremors and prostration for Type I compounds and burrowing, salivation, coarse tremors, choreoatetosis and clonic seizures for Type II compounds [124]. Oral dosing with permethrin in rats resulted in a significant decrease in motor activity at 200 mg/kg [133]. Hypersensitivity to sound has been reported in other bioassay experiments [120].

2.6.3.3.3 Developmental Effects

Pregnant rats exposed to a maximum of 600 mg/kg/day of total pyrethrins (extract) on gestation days 6-15 did not result in developmental toxicity, though maternal toxicity was observed in rats dosed with 75 mg/kg/day [134]. The type of pyrethroids may have an effect on the toxicity outcomes following oral exposure. Pregnant dams dosed with a maximum of 8 mg/kg/day of cypermethrin on gestation days 6-15 did not have any significant differences between control animals [135].

Berkowitz et al. (2004) evaluated the association of phenoxybenzoic acid in urine and decreases in birth weight, length and head circumference in the Mount Sinai study (referenced on page 26). No significant associations were found between the exposure and birth outcomes [108].

Chapter 3

Methods

3.1 Data Source

The data evaluated in this research originated from the 2001-2002 NHANES sampling event. National health monitoring 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 [136]. The National Health Examination Survey's of the 1960's (NHES I-III) were multi-year studies that evaluated either select chronic diseases, or growth and development, in a variety of age groups [136]. 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 [136]. Beginning in 1999, NHANES became a continuous, annual survey event in the US [136].

Sampling for the 2001-2002 NHANES collected data from 11,039 participants aged 6-85+ in 30 Primary Sampling Units (PSU) across the US. A PSU is defined as a county or a group of contiguous counties [137]. The PSU can be further divided into blocks or groups of blocks within one of those counties and households on the blocks. The PSUs were visited over a 12-month period [137]. Examinations, interviews and

specimen collections took place at Mobile Examination Centers (MEC) set-up in the PSU for easy data collection [137]. 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 [138]. Demographic information gathered from the examination was entered into a database file by NHANES researchers. The information found in this file included participant's age, gender, ethnicity, education level, marital status and household income. This information was imported into a Microsoft Access (2003) database for matching to other data files from the NHANES database.

Priority non-persistent pesticides and their biomarkers as well as organophosphate pesticide data were supplied in a separate data file within the 2001-2002 NHANES Laboratory subsection of the NHANES website. 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 [139].

After collection of the samples at each MEC, specimens were shipped to the Division of Environmental Health Laboratory Sciences for analysis using the appropriate shipping and storage methods [139]. The urinary levels of the biomarkers were measured using one of two mass spectrometric methods [139]. 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/√2) was reported [139]. 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. This allows all samples collected from one individual to be matched across multiple, individual data files.

3.2 Data Analysis

The pesticide subfile was imported into SAS (Version 9.2) to undergo basic statistical analysis. The frequency of detection was determined for each biomarker in the pesticide dataset. Biomarkers selected for inclusion in this research were those that were detected in more than 50% of the samples. This criterion has been used in other population biomarker analysis [140]. Three biomarkers met the requirement for inclusion in this research: 3,5,6-trichloropyridinol, paranitrophenol and 3-phenoxybenzoic acid. The analysis was limited to biomarkers that could be related to a parent compound. The non-specific biomarkers for OP compounds were not analyzed in this study.

After using Access to match the pesticide biomarker concentrations to demographic information (age, ethnicity, and gender) from NHANES using a common identifier, data was stratified into individual Access queries for each biomarker. The queries also contained information on each child's and adolescent's height (in

centimeters, cm) and weight (in kilograms, kg) that were also matched in the Access database using the NHANES common identifier. Biomarkers of clinical chemistry (biochemical concentrations) were related to individuals included in the pesticide data file. From the Access database, individual files were created in Microsoft Excel (2003) for import into SAS for analysis. The creation of Excel files also allowed for the data to be examined. There were numerous instances where values were missing across all of the NHANES databases used in this research. The number of missing individual values is observable when examining the number of individuals involved in each analysis across the data.

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) [141-142]. The overall geometric and arithmetic means for each biomarker were determined and the confidence intervals and quartiles were calculated. The geometric mean for each participant that had a detectable level of the biomarker in their urine was also calculated. Once the data was imported into SAS, a Students t-test was performed and the geometric mean determined for overall biomarker concentrations and for the gender subgroup. An Analysis of Variance (ANOVA) was performed and the geometric mean was determined for the age and ethnicity subfiles for each biomarker; the 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 and supplied along side the

biomarker concentration (µg/L) for comparison. Additionally, the correlation of biomarker detects was conducted using Systat 13 in an attempt to determine if the exposures were associated with each other across the overall exposure group. Level of significance was reported based on the variance findings from the SAS analysis (either pooled or satterthwaite).

Logistic regression was performed on the data from the pesticide subsample. This regression used multiple independent variables to model the relationship between the predictive independent variables and the dichotomous, dependent outcome (a yes or no detection of the biomarker in the urine sample). Logistic regression predicted the change in the coefficient (β) in relation to a change in the reference group based on the independent variables in the model: gender (dichotomous data), age (continuous data) and ethnicity (categorical data). The group with the largest amount of individuals was used as the reference group for gender and ethnicity. In these regression models, females and Non-Hispanic Whites were the largest groups. Additionally, the model supplied odds ratios to indicate which independent variable had a higher odds of having a detect for the biomarker in the urine sample in relation to the reference group.

A Students t-test was conducted and the arithmetic mean was determined for the height and weight of each of the biomarker detect and non-detect groups for children aged 6-11. This analysis determined if children with a recorded detect had a statistically significant difference in either height or weight when compared to those children that did

not have a recorded exposure to the biomarker. Level of significance was reported based on the variance findings from the SAS analysis (either pooled or satterthwaite).

An additional data file supplied by the NHANES contained biochemical concentrations for participants aged 12-85+. Included in this file were the concentrations of liver and kidney enzymes, as well as electrolyte concentrations, among others. The biochemical concentrations were imported into an Access database. This information was used to create Excel files for overall male and female concentrations and overall participant concentrations. Student's t-tests were conducted on each break-down group and the arithmetic means were determined. These divisions would determine if age or gender differences show variations in the comparison of individuals that had a coded detect for the biomarker versus those with a non-detect. As with the other analyses, the level of significance was reported based on the variance findings from the SAS analysis (either pooled or satterthwaite).

The NHANES demographic database file, as well as the pesticide subsample file, contained values for interview weight, MEC exam weight and a value for masked-variance pseudo-PSU [137]. These values were not incorporated into this data analysis.

Chapter 4

Results

Results are grouped by pesticide and biomarker. Analysis for each biomarker was conducted to determine:

1. The overall and creatinine-adjusted geometric and arithmetic mean for all individuals in the sample.
2. Geometric mean for individuals coded as a detect for the biomarker.
3. Geometric mean comparison for males versus females.
4. Geometric mean comparison between ethnic groups.
5. Geometric mean comparisons for age groupings.
6. Height and weight comparisons for children based on detects versus non-detects.
7. Biochemical arithmetic mean comparisons for males, females and the biomarker overall.
8. Logistic regression comparison for all groups involved in the research.

4.1 Overall Detection Frequency

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.

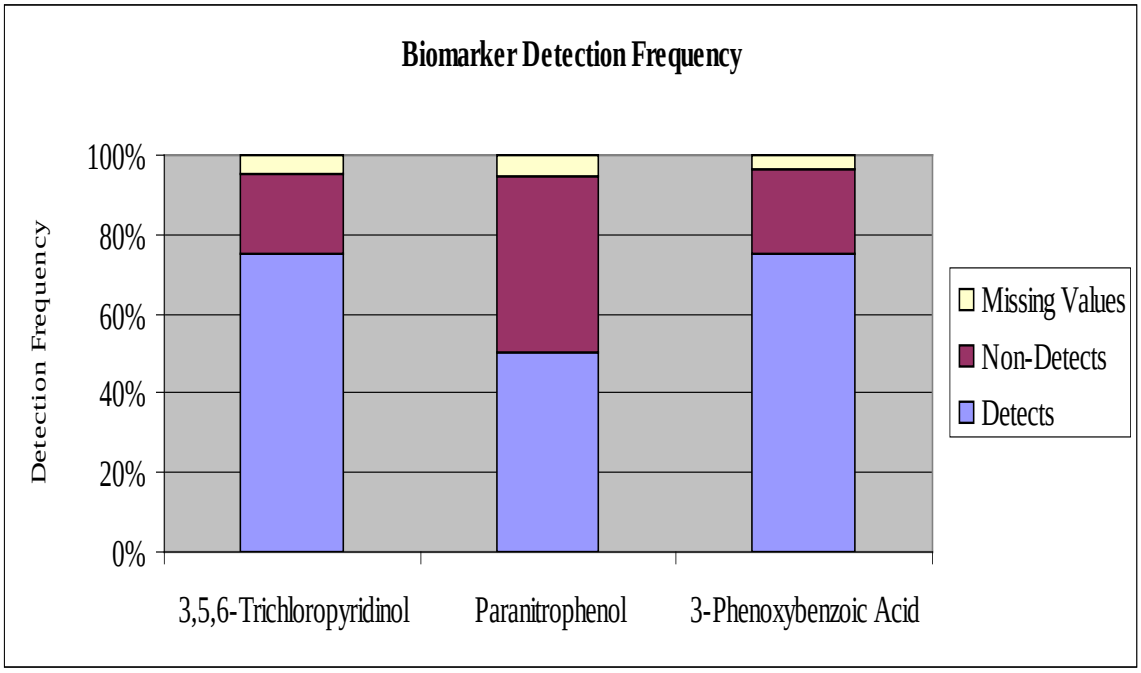

Figure 6: Detection Frequency of Urinary Pesticide Biomarkers. Detection frequencies for specific pesticide biomarkers can be viewed in Table 6.

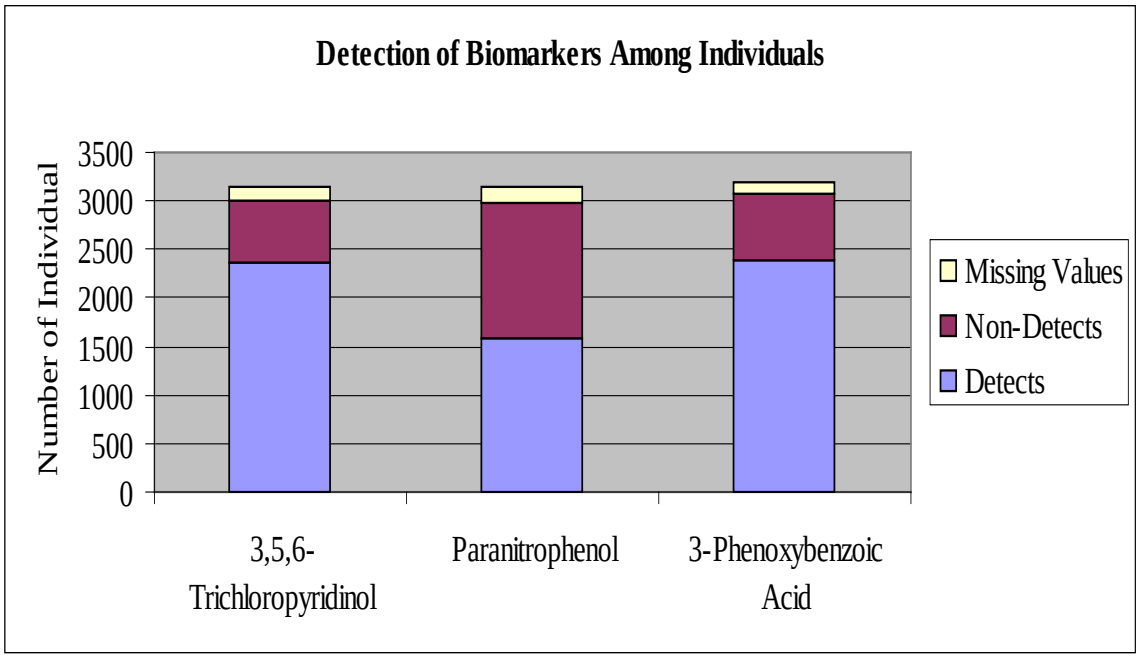

Figure 7: Number of Detections Among Individuals.

Table 6: Detections Among Individuals in the Dataset.

	Detects	Non-Detects	Missing Values	Total Analyzed	Detection Frequency
3,5,6-Trichloropyridinol	2368	643	141	3011	78.6%
Paranitrophenol	1580	1395	177	2975	53.1%
3-Phenoxybenzoic Acid	2395	689	104	3048	77.4%

4.2 Correlation of Exposures

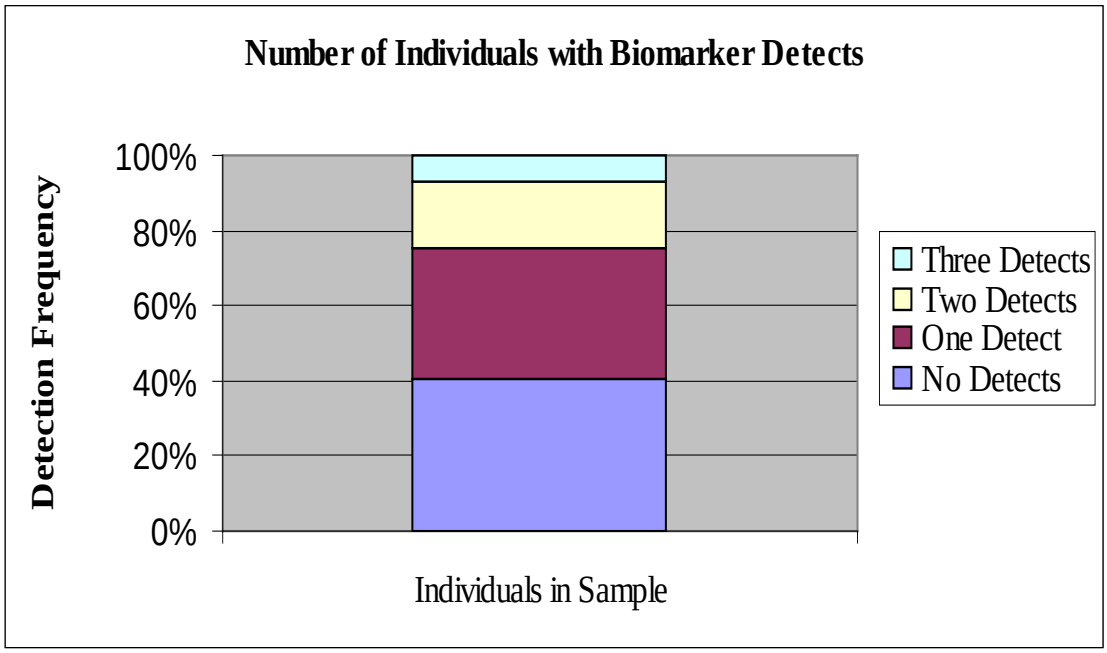

Figure 8: Detection Frequency of Biomarkers Among Individuals.

Table 7: Evaluation of Multiple Exposures Among Individuals in the Sample.

	No Detects	One Detect	Two Detects	Three Detects	Total
Number of Individuals	1205	1030	536	204	2975
Percentage of Individuals	40.50%	34.60%	18%	6.90%	100%

When evaluating individuals with detectable levels of the biomarker in their urine, 536 individuals had recorded detects for two biomarkers, while only 204 individuals were exposed to all three biomarkers.

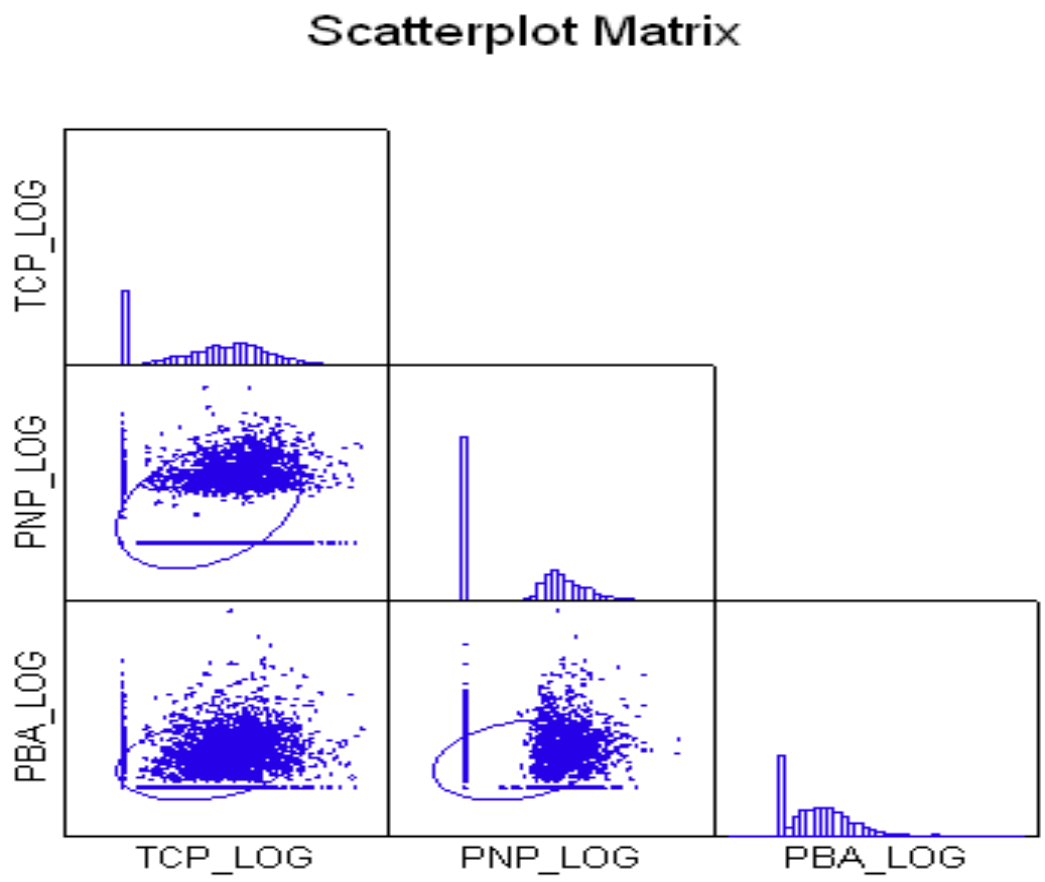

Figure 9: Pearson Correlation of Log-Transformed Biomarker Concentrations in Urine.

Table 8: Pearson Correlation Matrix.

	TCP_LOG	PNP_LOG	PBA_LOG
TCP_LOG	1		
PNP_LOG	r =0.357	1	
PBA_LOG	r= 0.267	r= 0.28	1

All values listed in Table 8 reflect the linear correlation between the biomarkers (r), as seen in Figure 9.

Table 9: Spearman Correlation Matrix.

	TCP_LOG	PNP_LOG	PBA_LOG
TCP_LOG	1		
PNP_LOG	r_s= 0.37	1	
PBA_LOG	r_s= 0.299	r_s= 0.291	1

This correlation examines the nonparametric correlation between the biomarkers (r_s).

Correlation analysis conducted on the log transformed data does not indicate that the levels are highly correlated. Both the Spearman and Pearson correlation was conducted to determine if the results varied. The results appear to be relatively close.

4.3 Organophosphate

4.3.1 Chlorpyrifos Biomarker: 3,5,6-Trichloropyridinol (TCPy)

4.3.1.1 Descriptive Statistics

The information covered in the following figures and tables characterizes the exposures to chlorpyrifos in the overall sample and in various subgroups.

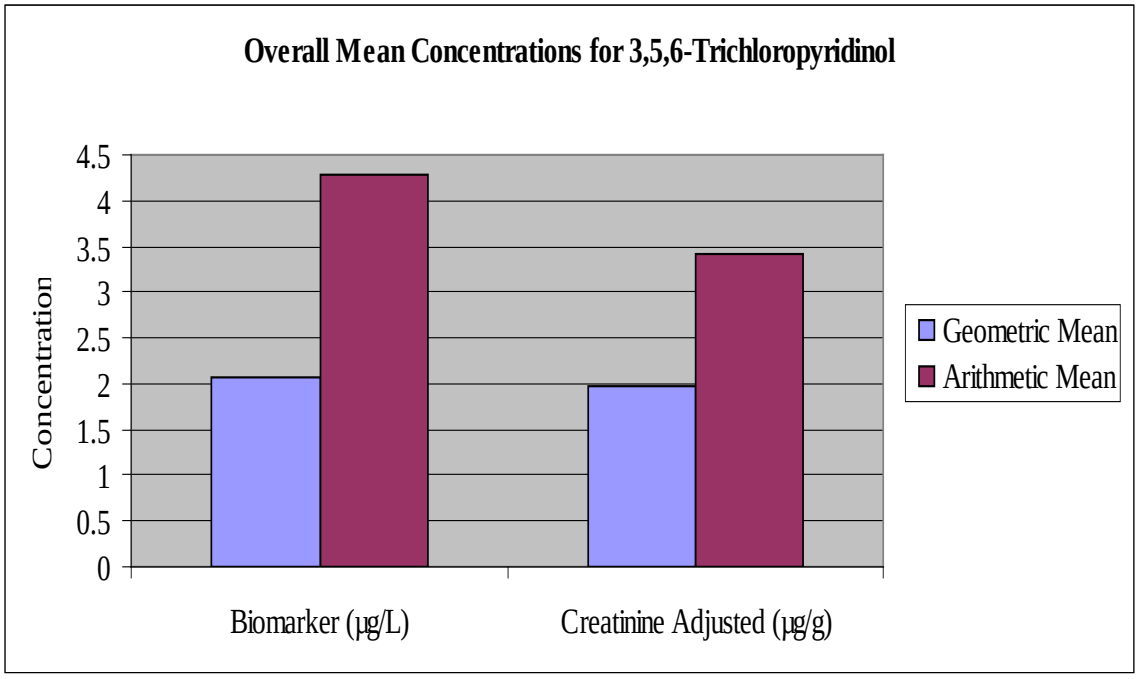

Figure 10: Comparison of Means for 3,5,6-Trichloropyridinol. µg/l= Micrograms per Liter; µg/g= Micrograms per Gram. Adjusting for creatinine results in means (both geometric and arithmetic) that are lower than if using the concentration of the TCPy biomarker in urine (µg/L) alone. Limit of Detection (LOD) for TCPy in sample was 0.4 µg/L.

Table 10: Descriptive Statistics for 3,5,6-Trichloropyridinol.

Biomarker (µg/L)[a]		
	Geometric Mean	Arithmetic Mean
n[c]	3011	3011
Mean	2.07	4.28
LCL[d]	1.98	4.06
UCL[e]	2.17	4.50
SD[f]	3.69	6.06
P25[g]	0.77	0.77
P50[h]	2.61	2.61
P75[i]	5.33	5.33
P90[j]	9.34	9.34
P95[k]	13.87	13.87
Min[l]	0.28	0.28
Max[m]	79.59	79.59

Creatinine Adjusted (µg/g)[b]		
	Geometric Mean	Arithmetic Mean
n	3009	3009
Mean	1.98	3.42
LCL	1.91	3.25
UCL	2.06	3.58
SD	2.98	4.75
P25	1.08	1.08
P50	2.23	2.23
P75	4.08	4.08
P90	6.89	6.89
P95	10.27	10.27
Min	0.0362	0.0362
Max	97.29	97.29

[a](µg/L) = Micrograms per Liter; [b](µg/g) = Micrograms per Gram; [c](n) = Number in Sample; [d](LCL) = Lower Confidence Limit; [e](UCL) = Upper Confidence Limit; [f](SD) = Standard Deviation; [g](P25) = Lower Quartile; [h](P50) = Median; [i](P75) = Upper Quartile; [j](P90) = 90th Percentile; [k](P95) =95th Percentile; [l](Min) = Minimum [m](Max) = Maximum

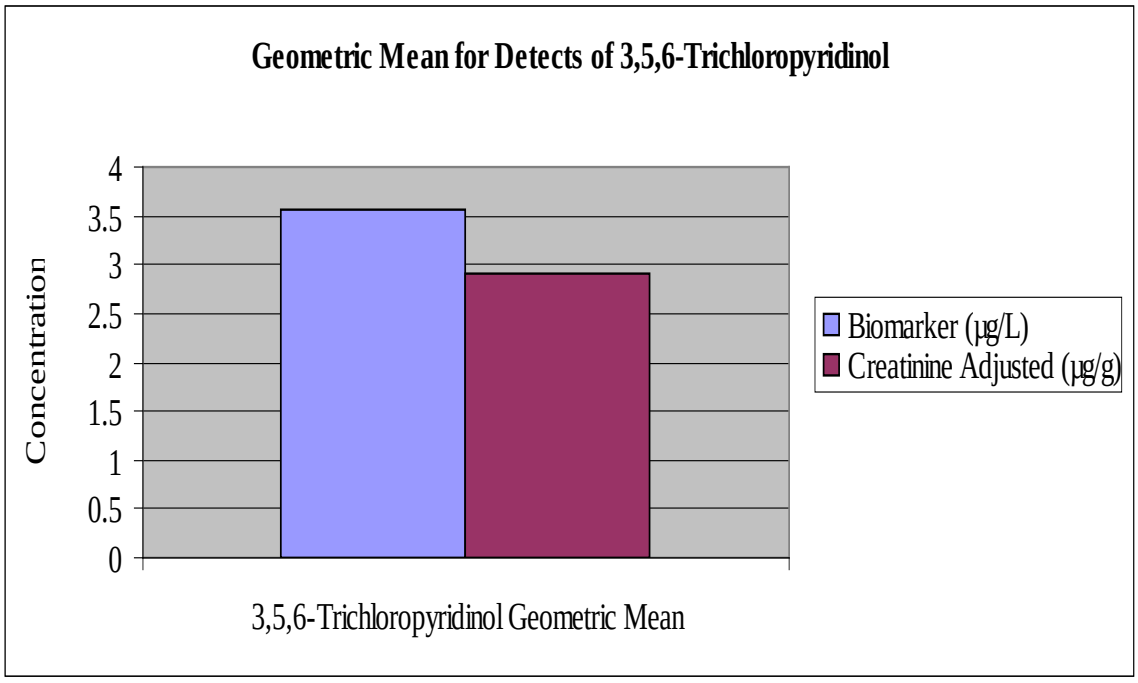

Figure 11: Comparison of Detectable Geometric Mean Concentrations for 3,5,6-Trichloropyridinol. µg/l= Micrograms per Liter; µg/g= Micrograms per Gram.

Table 11: Descriptive Statistics for Detectable Levels of 3,5,6-Trichloropyridinol.

	GM[c]	LCL[d]	UCL[e]	SD[f]	Median	n[g]
Biomarker (µg/L)[a]	3.56	3.44	3.69	2.43	3.68	2368
Creatinine Adjusted (µg/g)[b]	2.91	2.82	3.01	2.23	2.87	2366

[a](µg/L) = Micrograms per Liter; [b](µg/g) = Micrograms per Gram; [c](GM) = Geometric Mean; [d](LCL) = Lower Confidence Limit; [e](UCL) = Upper Confidence Limit; [f](SD) = Standard Deviation; [g](n) = Number in Sample

The geometric mean was determined for 78.6% of the individuals that had a detectable level of biomarker in their urine sample. Based on this analysis, the geometric mean for only detectable levels of the biomarker was 1.49 µg/L higher that the overall geometric mean.

4.3.1.2 Comparative Statistics

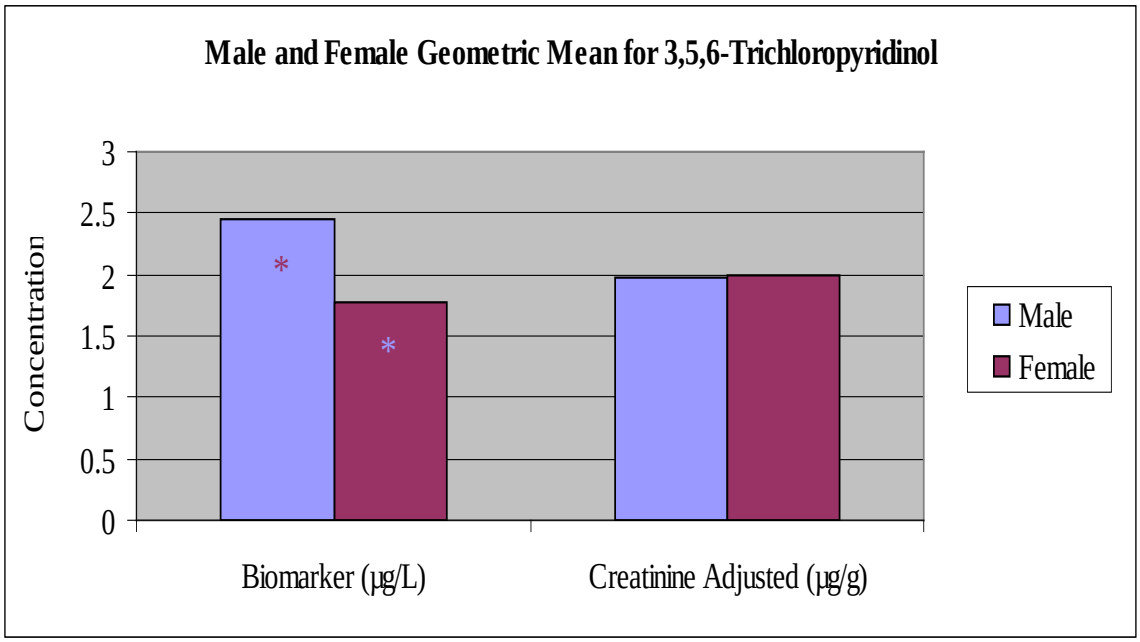

Figure 12: Comparison of Geometric Mean Values of Males Versus Females for 3,5,6-Trichloropyridinol. µg/l= Micrograms per Liter; µg/g= Micrograms per Gram. Color-coded "*" indicates males are significantly different from females.

Table 12: 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	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)[i]							
	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

Males have 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.

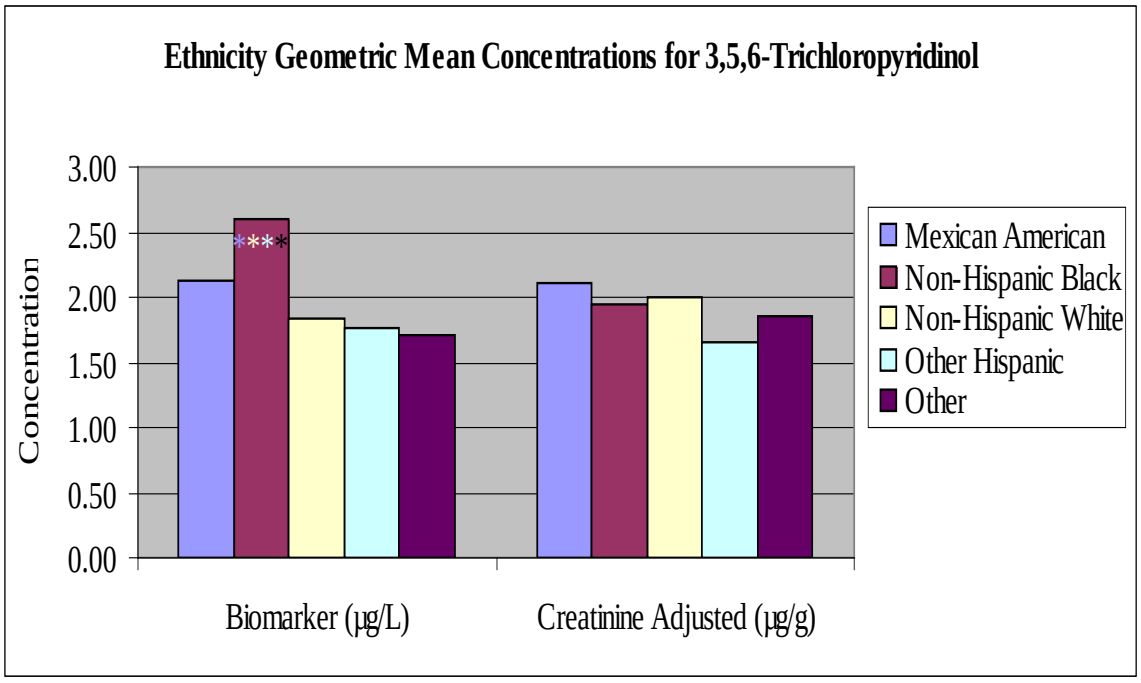

Figure 13: Comparison of Geometric Mean Values of Ethnic Groups for 3,5,6-Trichloropyridinol. µg/l= Micrograms per Liter; µg/g= Micrograms per Gram. Color-coded "****" indicates this groups is significantly different from all other groups.

Table 13: 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]	P[h]
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.

The unadjusted biomarker concentration in urine (µg/L) for non-Hispanic Blacks had a significantly elevated GM as compared with the other ethic groups. However, when adjusting for creatinine, there were no significant findings.

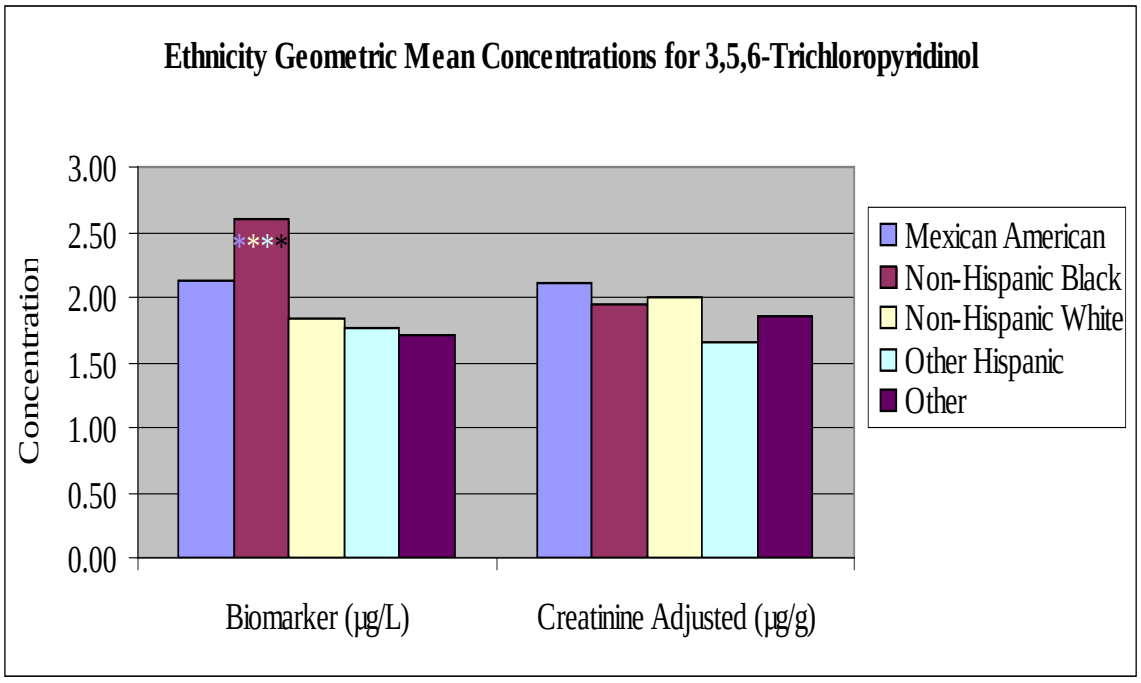

Figure 14: Comparison of Geometric Mean Values of Age Groups for 3,5,6-Trichloropyridinol. µg/l= Micrograms per Liter; µg/g= Micrograms per Gram. Color-coded "****" indicates which groups are significantly different from each other.

Table 14: 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]	P[h]
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.

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.

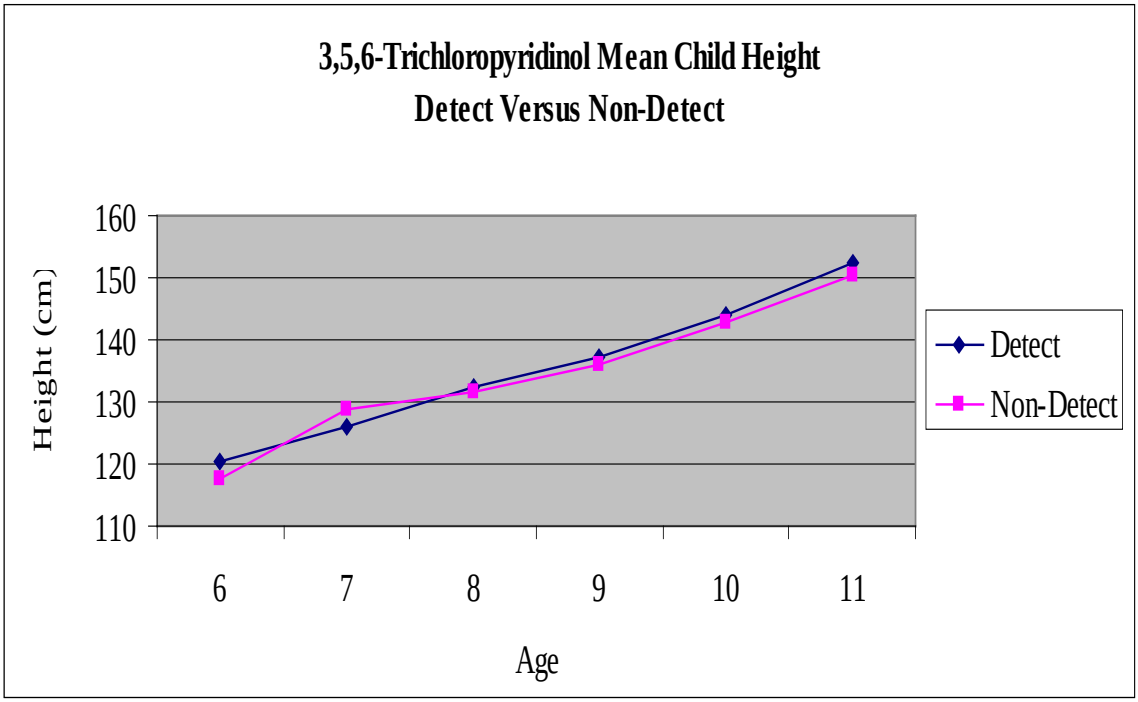

Figure 15: Graph of Arithmetic Mean Height for Detects Versus Non-Detects for 3,5,6-Trichloropyridinol, Ages 6-11. cm= Centimeter.

Table 15: Student's t-test Comparing Detects Versus Non-Detects for Arithmetic Mean of Height for 3,5,6-Trichloropyridinol.

Age	Detect[a]	Mean (cm[b])	LCL[c]	UCL[d]	SD[e]	n[f]	p[g]
6	Yes	120.4	119	121.7	5.90	74	0.096
	No	117.5	114.6	120.4	5.05	14	
7	Yes	125.9	124.7	127.1	5.54	86	0.165
	No	128.8	124.7	132.8	7.90	17	
8	Yes	132.5	130.7	134.3	8.02	77	0.682
	No	131.4	126.9	135.9	6.30	10	
9	Yes	137.2	135.7	138.7	7.45	98	0.492
	No	135.8	132.7	138.9	5.28	14	
10	Yes	144	141.9	146	8.24	67	0.685
	No	142.9	138.8	147	6.04	11	
11	Yes	152.6	150.9	154.3	8.14	88	0.360
	No	150.6	146.6	154.5	7.14	15	

[a](Detect) = Detectable Level of Biomarker in the Urine Sample; [b](cm) = Centimeters; [c](LCL) = Lower Confidence Limit; [d](UCL) = Upper Confidence Limit; [e](SD) = Standard Deviation; [f](n) = Number in Sample; [g](p) = Level of Significance at p= 0.05 Level

There are no significant differences at the $p < 0.05$ level between detect and non-detect urinary concentrations of 3,5,6-trichloropyridinol when evaluating variations in children's height.

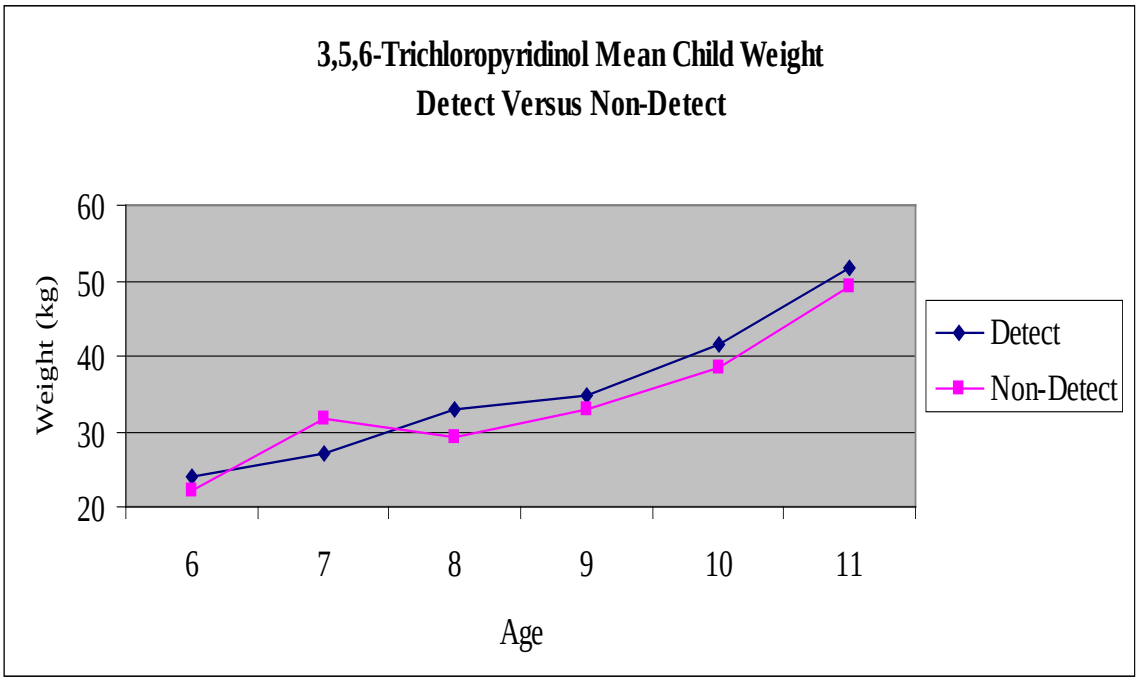

Figure 16: Graph of Arithmetic Mean Weight for Detects Versus Non-Detects for 3,5,6-Trichloropyridinol, Ages 6-11. Kg= Kilograms

Table 16: Student's t-test Comparing Detects Versus Non-Detects for Arithmetic Mean of Weight for 3,5,6-Trichloropyridinol.

Age	Detect[a]	Mean (Kg[b])	LCL[c]	UCL[d]	SD[e]	n[f]	p[g]
6	Yes	23.86	22.91	24.81	4.09	74	0.201
	No	22.29	19.59	24.98	4.66	14	
7	Yes	27.07	25.91	28.23	5.37	85	0.080
	No	31.56	26.58	36.55	9.69	17	
8	Yes	33.07	30.69	35.45	10.34	75	0.258
	No	29.23	24.37	34.09	6.79	10	
9	Yes	34.83	32.78	36.87	10.10	96	0.532
	No	33.06	28.50	37.61	7.89	14	
10	Yes	41.49	38.22	44.77	13.42	67	0.453
	No	38.34	32.74	43.93	8.32	11	
11	Yes	51.65	47.52	55.77	19.11	85	0.640
	No	49.20	40.58	57.82	15.56	15	

[a](Detect) = Detectable Level of Biomarker in the Urine Sample; [b](Kg) = Kilograms; [c](LCL) = Lower Confidence Limit; [d](UCL) = Upper Confidence Limit; [e](SD) = Standard Deviation; [f](n) = Number in Sample; [g](p) = Level of Significance at p= 0.05 Level

There are no significant differences at the $p < 0.05$ level between detect and non-detect urinary concentrations of 3,5,6-trichloropyridinol when evaluating variations in children's weight.

Table 17: 3,5,6-Trichloropyridinol Female Biochemical t-test Comparisons.

Biochemical	Detect[a]	Mean	LCL[b]	UCL[c]	SD[d]	n[e]	Min[f]	Max[g]	p[h]
Alanine Transaminase (U/L)[i]	Yes	18.76	18.15	19.38	9.29	874	5	102	0.177
	No	19.58	18.52	20.64	9.90	340	5	102	
Albumin (g/dL)[j]	Yes	4.17	4.14	4.19	0.34	874	2.6	5	0.661
	No	4.16	4.12	4.20	0.36	340	2.6	5	
Alkaline Phosphatase (U/L)	**Yes**	**83.76**	**80.85**	**86.67**	**43.90**	**874**	**30**	**424**	**0.002**
	No	**76.11**	**72.35**	**79.87**	**35.25**	**340**	**24**	**269**	
Aspartate Transaminase (U/L)	Yes	21.56	21.07	22.04	7.34	874	9	114	0.296
	No	22.05	21.26	22.83	7.35	340	7	84	
Bicarbonate (mmol/L)[k]	**Yes**	**22.76**	**22.61**	**22.90**	**2.22**	**874**	**16**	**29**	**0.001**
	No	**23.24**	**23.01**	**23.47**	**2.18**	**340**	**18**	**29**	
Bilirubin (mg/dL)[l]	Yes	0.66	0.64	0.67	0.22	874	0.2	2.1	0.876
	No	0.66	0.64	0.68	0.20	340	0.2	1.6	
Blood Urea Nitrogen (mg/dL)	Yes	11.99	11.58	12.41	6.23	874	2	122	0.912
	No	11.95	11.37	12.53	5.45	340	3	53	
Total Calcium (mg/dL)	Yes	9.43	9.40	9.45	0.39	874	8.1	11	0.095
	No	9.47	9.43	9.51	0.39	340	8.3	10.7	
Chloride (mmol/L)	**Yes**	**103.20**	**103.00**	**103.40**	**2.61**	**874**	**89**	**111**	**0.034**
	No	**102.80**	**102.50**	**103.10**	**2.87**	**339**	**89**	**113**	
Cholesterol (mg/dL)	**Yes**	**186.90**	**184.10**	**189.70**	**42.13**	**874**	**91**	**460**	**<.0001**
	No	**202.50**	**197.50**	**207.60**	**47.30**	**340**	**90**	**476**	
Creatinine (mg/dL)	**Yes**	**0.72**	**0.71**	**0.74**	**0.22**	**874**	**0.3**	**4.4**	**0.019**
	No	**0.76**	**0.73**	**0.78**	**0.23**	**340**	**0.3**	**3.1**	
γ Glutamyl Transferase (U/L)	Yes	19.54	18.28	20.80	18.96	874	4	266	0.087
	No	22.27	19.41	25.14	26.88	340	6	369	
Globulin (mg/dL)	Yes	3.18	3.15	3.21	0.40	874	2.3	5.4	0.062
	No	3.13	3.09	3.17	0.39	340	2.2	4.6	
Glucose (mg/dL)	Yes	89.21	87.47	90.95	26.25	874	34	453	0.171
	No	92.16	88.30	96.03	36.20	340	30	521	
Iron (mg/dL)	Yes	82.01	79.56	84.47	37.03	874	7	240	0.638
	No	83.14	79.11	87.16	37.75	340	12	268	
Lactate Dehydrogenase (U/L)	Yes	130.80	129.00	132.70	28.33	874	58	470	0.745
	No	130.20	127.20	133.30	28.62	339	51	329	
Osmolality (mmol/Kg)[m]	Yes	275.50	275.10	275.90	5.53	874	248	310	0.989
	No	275.50	274.90	276.10	5.55	340	252	294	
Phosphorus (mg/dL)	**Yes**	**4.00**	**3.96**	**4.04**	**0.58**	**874**	**2.3**	**6.1**	**0.019**
	No	**3.92**	**3.87**	**3.98**	**0.53**	**340**	**2.3**	**5.8**	
Potassium (mmol/L)	Yes	3.98	3.96	4.00	0.33	874	2.6	6	0.179
	No	4.01	3.97	4.05	0.36	340	2.9	5.3	
Total Protein (g/dL)	Yes	7.35	7.31	7.38	0.48	874	5.5	9.5	0.068
	No	7.29	7.24	7.34	0.50	340	5.9	9.1	
Sodium (mmol/L)	Yes	138.30	138.20	138.50	2.51	874	124	147	0.551
	No	138.30	138.00	138.50	2.53	340	125	145	
Triglycerides (mg/dL)	**Yes**	**116.00**	**110.80**	**121.30**	**78.40**	**874**	**23**	**742**	**0.013**
	No	**131.60**	**120.60**	**142.70**	**103.70**	**340**	**28**	**1077**	
Uric Acid (mg/dL)	**Yes**	**4.50**	**4.42**	**4.58**	**1.19**	**874**	**0.4**	**10.9**	**0.018**
	No	**4.70**	**4.55**	**4.85**	**1.39**	**340**	**2.4**	**13.9**	

[a](Detect) = Detectable Level of Biomarker in the Urine Sample; [b](LCL) = Lower Confidence Limit; [c](UCL) = Upper Confidence Limit; [d](SD) = Standard Deviation; [e](n) = Number in Sample; [f](Min) = Minimum; [g](Max) = Maximum; [h](p) = Level of Significance at p= 0.05 Level (highlighted in **Bold**); [i](U/L) = Units per Liter; [j](g/dL) = Grams per Deciliter; [k](mmol/L) = Millimole per Liter; [l](mg/dL) = Milligram per Deciliter; [m](mmol/Kg) = Millimole per Kilogram

Table 18: 3,5,6-Trichloropyridinol Male Biochemical t-test Comparisons.

Biochemical	Detect[a]	Mean	LCL[b]	UCL[c]	SD[d]	n[e]	Min[f]	Max[g]	p[h]
Alanine Transaminase (U/L)[i]	Yes	26.67	25.42	27.93	19.08	892	7	243	0.861
	No	26.46	24.42	28.50	13.93	181	9	109	
Albumin (g/dL)[j]	Yes	4.39	4.37	4.41	0.31	892	2.1	5.3	0.750
	No	4.38	4.34	4.42	0.29	181	3.2	5.3	
Alkaline Phosphatase (U/L)	Yes	104.70	99.81	109.50	73.70	892	19	589	0.112
	No	94.94	83.22	106.70	79.95	181	37	617	
Aspartate Transaminase (U/L)	Yes	26.41	25.57	27.24	12.68	892	9	187	0.426
	No	27.23	25.34	29.12	12.89	181	13	118	
Bicarbonate (mmol/L)[k]	Yes	23.97	23.83	24.11	2.14	892	15	29	0.084
	No	24.27	23.97	24.58	2.08	181	18	30	
Bilirubin (mg/dL)[l]	Yes	0.82	0.80	0.84	0.32	892	0.3	3.5	0.349
	No	0.80	0.76	0.84	0.27	181	0.3	2.4	
Blood Urea Nitrogen (mg/dL)	Yes	14.16	13.81	14.50	5.26	892	3	48	0.204
	No	13.61	12.80	14.42	5.52	181	4	42	
Total Calcium (mg/dL)	Yes	9.55	9.53	9.58	0.40	892	7.2	11.3	0.664
	No	9.54	9.48	9.60	0.40	181	7.6	10.6	
Chloride (mmol/L)	Yes	102.50	102.30	102.70	2.57	892	83	112	0.235
	No	102.20	101.80	102.60	2.69	181	93	108	
Cholesterol (mg/dL)	Yes	184.90	182.10	187.80	43.86	892	71	566	0.308
	No	188.60	182.30	194.90	42.80	181	86	407	
Creatinine (mg/dL)	Yes	0.96	0.95	0.98	0.26	892	0.4	4.4	0.303
	No	0.99	0.95	1.02	0.25	181	0.4	2.6	
γ Glutamyl Transferase (U/L)	Yes	30.12	28.04	32.19	31.59	892	6	482	0.387
	No	32.39	27.24	37.54	35.10	181	6	394	
Globulin (mg/dL)	Yes	3.08	3.05	3.11	0.43	891	1.6	5.6	0.377
	No	3.04	2.97	3.11	0.48	181	2	5.9	
Glucose (mg/dL)	Yes	95.24	93.19	97.29	31.18	892	58	707	0.669
	No	96.33	91.75	100.90	31.25	181	69	382	
Iron (mg/dL)	Yes	96.50	93.94	99.05	38.84	892	12	333	0.298
	No	99.81	93.96	105.70	39.89	181	18	223	
Lactate Dehydrogenase (U/L)	**Yes**	**138.50**	**136.30**	**140.80**	**34.58**	**892**	**52**	**399**	**0.037**
	No	**133.70**	**129.80**	**137.60**	**26.88**	**181**	**72**	**247**	
Osmolality (mmol/Kg)[m]	Yes	278.00	277.60	278.30	5.30	892	218	299	0.061
	No	277.20	276.40	277.90	5.40	181	261	298	
Phosphorus (mg/dL)	Yes	3.92	3.88	3.97	0.71	892	2.4	6.6	0.332
	No	3.87	3.77	3.96	0.64	181	2.4	6.6	
Potassium (mmol/L)	Yes	4.12	4.10	4.14	0.35	892	3.1	5.5	0.523
	No	4.14	4.09	4.19	0.34	181	3.2	5.4	
Total Protein (g/dL)	Yes	7.47	7.44	7.50	0.46	891	5.3	9.4	0.269
	No	7.42	7.35	7.50	0.49	181	6.2	9.8	
Sodium (mmol/L)	Yes	139.10	138.90	139.20	2.58	892	108	147	0.077
	No	138.70	138.40	139.00	2.34	181	131	144	
Triglycerides (mg/dL)	Yes	145.00	132.30	157.70	193.60	892	21	3854	0.617
	No	137.20	110.10	164.20	184.50	181	28	2337	
Uric Acid (mg/dL)	Yes	6.00	5.91	6.09	1.32	892	1.5	13.4	0.905
	No	5.99	5.79	6.19	1.37	181	2.6	11	

[a](Detect) = Detectable Level of Biomarker in the Urine Sample; [b](LCL) = Lower Confidence Limit; [c](UCL) = Upper Confidence Limit; [d](SD) = Standard Deviation; [e](n) = Number in Sample; [f](Min) = Minimum; [g](Max) = Maximum; [h](p) = Level of Significance at p= 0.05 Level (highlighted in **Bold**); [i](U/L) = Units per Liter; [j](g/dL) = Grams per Deciliter; [k](mmol/L) = Millimole per Liter; [l](mg/dL) = Milligram per Deciliter; [m](mmol/Kg) = Millimole per Kilogram

Table 19: 3,5,6-Trichloropyridinol Overall Biochemical t-test Comparisons.

Biochemical	Detect[a]	Mean	LCL[b]	UCL[c]	SD[d]	n[e]	Min[f]	Max[g]	p[h]
Alanine Transaminase (U/L)[i]	Yes	22.76	22.03	23.48	15.56	1766	5	243	0.218
	No	21.97	20.94	22.99	11.91	521	5	109	
Albumin (g/dL)[j]	**Yes**	**4.28**	**4.26**	**4.29**	**0.34**	**1766**	**2.1**	**5.3**	**0.011**
	No	**4.23**	**4.20**	**4.26**	**0.36**	**521**	**2.6**	**5.3**	
Alkaline Phosphatase (U/L)	**Yes**	**94.31**	**91.43**	**97.19**	**61.68**	**1766**	**19**	**589**	**<.0001**
	No	**82.65**	**77.86**	**87.45**	**55.71**	**521**	**24**	**617**	
Aspartate Transaminase (U/L)	Yes	24.01	23.51	24.50	10.66	1766	9	187	0.755
	No	23.85	22.99	24.70	9.94	521	7	118	
Bicarbonate (mmol/L)[k]	**Yes**	**23.37**	**23.26**	**23.47**	**2.26**	**1766**	**15**	**29**	**0.041**
	No	**23.60**	**23.41**	**23.79**	**2.20**	**521**	**18**	**30**	
Bilirubin (mg/dL)[l]	**Yes**	**0.74**	**0.73**	**0.76**	**0.28**	**1766**	**0.2**	**3.5**	**0.010**
	No	**0.71**	**0.69**	**0.73**	**0.24**	**521**	**0.2**	**2.4**	
Blood Urea Nitrogen (mg/dL)	Yes	13.09	12.81	13.36	5.86	1766	2	122	0.053
	No	12.53	12.05	13.00	5.53	521	3	53	
Total Calcium (mg/dL)	Yes	9.49	9.47	9.51	0.40	1766	7.2	11.3	0.910
	No	9.49	9.46	9.53	0.39	521	7.6	10.7	
Chloride (mmol/L)	Yes	102.80	102.70	103.00	2.61	1766	83	112	0.103
	No	102.60	102.40	102.80	2.82	520	89	113	
Cholesterol (mg/dL)	**Yes**	**185.90**	**183.90**	**187.90**	**43.01**	**1766**	**71**	**566**	**<.0001**
	No	**197.70**	**193.70**	**201.70**	**46.22**	**521**	**86**	**476**	
Creatinine (mg/dL)	Yes	0.84	0.83	0.86	0.27	1766	0.3	4.4	0.529
	No	0.84	0.81	0.86	0.26	521	0.3	3.1	
γ Glutamyl Transferase (U/L)	Yes	24.88	23.64	26.13	26.64	1766	4	482	0.539
	No	25.79	23.18	28.40	30.34	521	6	394	
Globulin (mg/dL)	Yes	3.13	3.11	3.15	0.42	1765	1.6	5.6	0.203
	No	3.10	3.07	3.14	0.42	521	2	5.9	
Glucose (mg/dL)	Yes	92.26	90.90	93.61	29.00	1766	34	707	0.416
	No	93.61	90.64	96.59	34.59	521	30	521	
Iron (mg/dL)	Yes	89.33	87.53	91.13	38.63	1766	7	333	0.835
	No	88.93	85.55	92.31	39.28	521	12	268	
Lactate Dehydrogenase (U/L)	**Yes**	**134.70**	**133.20**	**136.20**	**31.86**	**1766**	**52**	**470**	**0.024**
	No	**131.40**	**129.00**	**133.90**	**28.05**	**520**	**51**	**329**	
Osmolality (mmol/Kg)[m]	**Yes**	**276.70**	**276.50**	**277.00**	**5.56**	**1766**	**218**	**310**	**0.015**
	No	**276.10**	**275.60**	**276.50**	**5.55**	**521**	**252**	**298**	
Phosphorus (mg/dL)	**Yes**	**3.96**	**3.93**	**3.99**	**0.65**	**1766**	**2.3**	**6.6**	**0.042**
	No	**3.90**	**3.85**	**3.95**	**0.57**	**521**	**2.3**	**6.6**	
Potassium (mmol/L)	Yes	4.05	4.03	4.07	0.35	1766	2.6	6	0.826
	No	4.05	4.02	4.09	0.36	521	2.9	5.4	
Total Protein (g/dL)	**Yes**	**7.41**	**7.38**	**7.43**	**0.47**	**1765**	**5.3**	**9.5**	**0.003**
	No	**7.34**	**7.29**	**7.38**	**0.50**	**521**	**5.9**	**9.8**	
Sodium (mmol/L)	**Yes**	**138.70**	**138.60**	**138.80**	**2.57**	**1766**	**108**	**147**	**0.017**
	No	**138.40**	**138.20**	**138.60**	**2.47**	**521**	**125**	**145**	
Triglycerides (mg/dL)	Yes	130.70	123.70	137.60	148.90	1766	21	3854	0.680
	No	133.50	121.70	145.30	137.10	521	28	2337	
Uric Acid (mg/dL)	Yes	5.26	5.19	5.32	1.47	1766	0.4	13.4	0.137
	No	5.15	5.02	5.28	1.51	521	2.4	13.9	

[a](Detect) = Detectable Level of Biomarker in the Urine Sample; [b](LCL) = Lower Confidence Limit; [c](UCL) = Upper Confidence Limit; [d](SD) = Standard Deviation; [e](n) = Number in Sample; [f](Min) = Minimum; [g](Max) = Maximum; [h](p) = Level of Significance at p= 0.05 Level (highlighted in **Bold**); [i](U/L) = Units per Liter; [j](g/dL) = Grams per Deciliter; [k](mmol/L) = Millimole per Liter; [l](mg/dL) = Milligram per Deciliter; [m](mmol/Kg) = Millimole per Kilogram

4.3.1.3 Logistic Regression

Table 20: Logistic Regression for 3,5,6-Trichloropyridinol.

	Detect[a] (DF[b]=1)	Detect, Non-Hispanic White (DF=1)	Detect, Female (DF=1)
Age	**β=-0.0145 Wald χ^2=53.74 p<0.0001 OR=0.986**		
Mexican American		**β=0.221 Wald χ2=4.86 p=0.028 OR=1.31**	
Non-Hispanic Black		β=-0.317 Wald χ2=3.50 p=0.062 OR=0.765	
Other Hispanic		**β=0.272 Wald χ2=7.20 p=0.007 OR=1.38**	
Other		β=-0.128 Wald χ2=0.500 p=0.479 OR=0.924	
Male			**β=0.28 Wald χ2=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.**

The overall model fit was significant, with the Likelihood χ2= 119.4, p <0.0001 and the R^2_{Max} = 0.0602. The Hosmer and Lemeshow goodness-of-fit test resulted in a χ2= 12.2, p= 0.144, indicating that the data from the independent variables fit the model moderately well. The listed detects at the row headers are the reference groups for each sub-category. 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=0.028) 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).

4.3.2 Methyl Parathion Biomarker: Paranitrophenol (PNP)

4.3.2.1 Descriptive Statistics

The information covered in the following figures and tables characterizes the exposures to methyl parathion in the overall sample and in various subgroups.

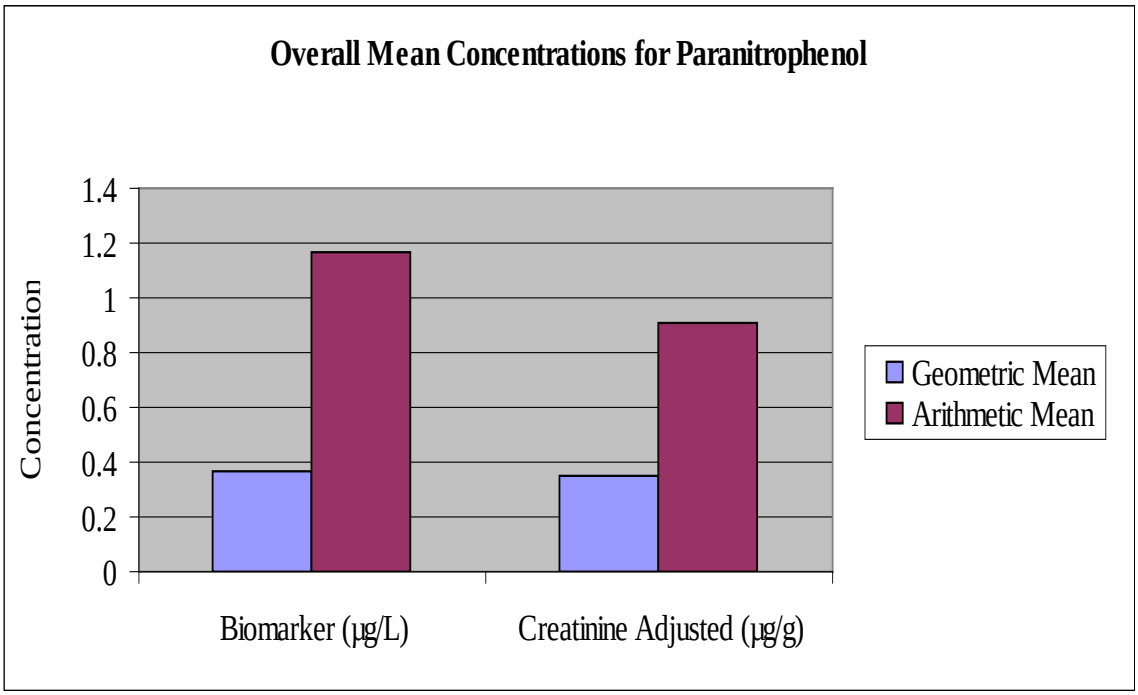

Figure 17: Comparison of Means for Paranitrophenol**.** µg/l= Micrograms per Liter; µg/g= Micrograms per Gram. Adjusting for creatinine results in means (both geometric and arithmetic) that are lower than if using the concentration of the PNP biomarker in urine (µg/L) alone. Limit of Detection (LOD) for PNP in sample was 0.1 µg/L.

Table 21: Descriptive Statistics for Paranitrophenol.

Biomarker (µg/L)[a]		
	Geometric Mean	Arithmetic Mean
n[c]	2975	2975
Mean	0.367	1.16
LCL[d]	0.346	1.08
UCL[e]	0.389	1.25
SD[f]	5.11	2.35
P25[g]	< LOD[h]	< LOD
P50[i]	0.7	0.7
P75[j]	1.46	1.46
P90[k]	2.82	2.82
P95[l]	3.92	3.92
Min[m]	0.07	0.07
Max[n]	59.89	59.89

Creatinine Adjusted (µg/g)[b]		
	Geometric Mean	Arithmetic Mean
N	2973	2973
Mean	0.352	0.905
LCL	0.335	0.839
UCL	0.371	0.970
SD	4.18	1.83
P25	0.096	0.096
P50	0.427	0.427
P75	1.08	1.08
P90	2.10	2.10
P95	3.20	3.20
Min	0.0124	0.0124
Max	51.26	51.26

[a](µg/L) = Micrograms per Liter; [b](µg/g) = Micrograms per Gram; [c](n) = Number in Sample; [d](LCL) = Lower Confidence Limit; [e](UCL) = Upper Confidence Limit; [f](SD) = Standard Deviation; [g](P25) = Lower Quartile; [h](LOD) = Limit of Detection, at 0.1 µg/L.; [i](P50) = Median; [j](P75) = Upper Quartile; [k](P90) = 90th Percentile; [l](P95) =95th Percentile; [m](Min) = Minimum [n](Max) = Maximum

Adjusting for creatinine results in means (both geometric and arithmetic) that are lower than if using the concentration of the biomarker in urine (µg/L) alone, but not by a large margin.

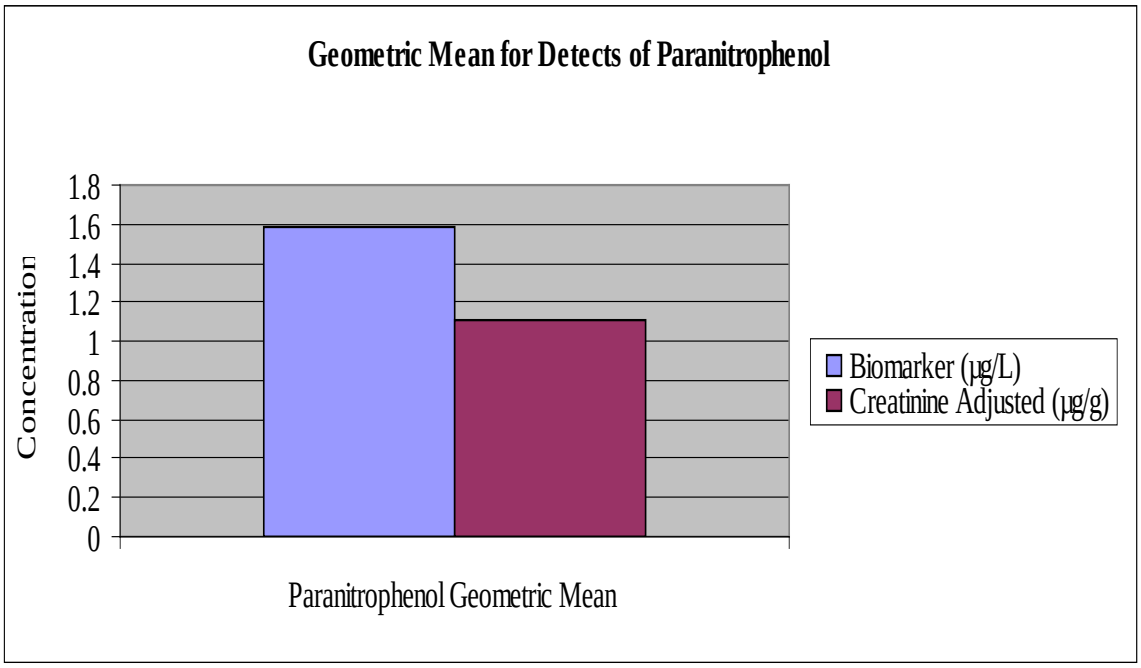

Figure 18: Comparison of Detectable Geometric Mean Concentrations for Paranitrophenol. µg/l= Micrograms per Liter; µg/g= Micrograms per Gram.

Table 22: Descriptive Statistics for Detectable Levels of Paranitrophenol.

	GM[c]	LCL[d]	UCL[e]	SD[f]	Median	n[g]
Biomarker (µg/L)[a]	1.58	1.53	1.64	1.97	1.39	1580
Creatinine Adjusted (µg/g)[b]	1.11	1.06	1.15	2.16	1.02	1578

[a](µg/L) = Micrograms per Liter; [b](µg/g) = Micrograms per Gram; [c](GM) = Geometric Mean; [d](LCL) = Lower Confidence Limit; [e](UCL) = Upper Confidence Limit; [f](SD) = Standard Deviation; [g](n) = Number in Sample

The geometric mean was determined for the 53.1% of the individuals that had a detectable level of biomarker in their urine sample. Based on this analysis, the geometric mean for only detectable levels of the biomarker was 1.2 µg/L higher that the overall geometric mean.

4.3.2.2 Comparative Statistics

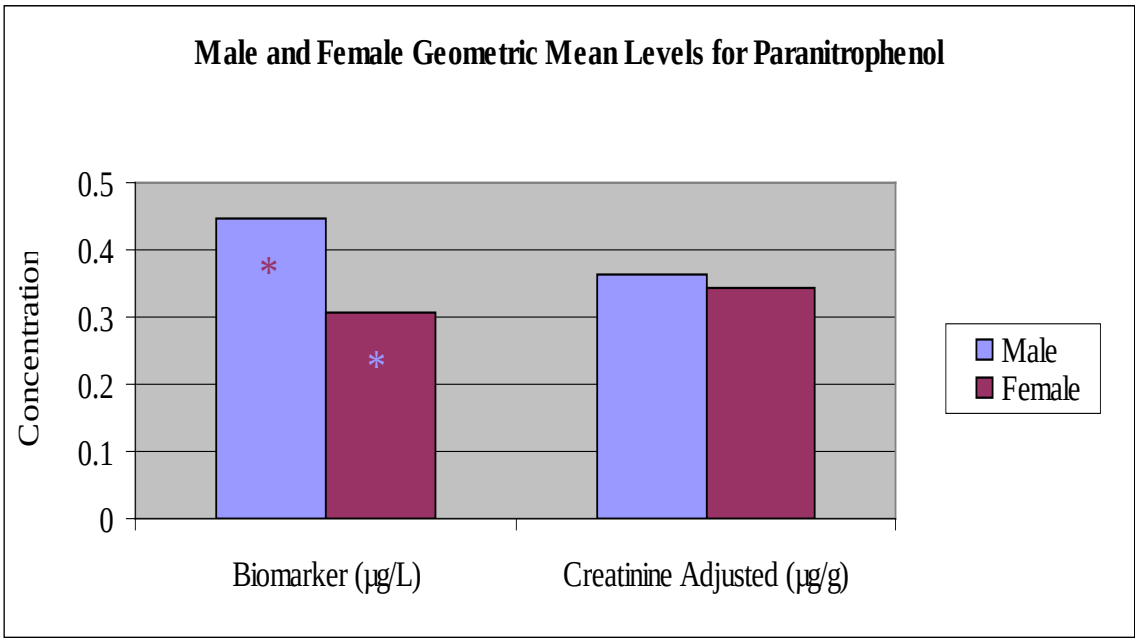

Figure 19: Comparison of Geometric Mean Values of Males versus Females for Paranitrophenol. µg/l= Micrograms per Liter; µg/g= Micrograms per Gram. Color-coded "*" indicates males are significantly different from females.

Table 23: 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]	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

Males have a statistically significant higher GM when compared to females, but only when using the unadjusted biomarker concentration in urine (µg/L). Adjusting for dilution negates any significant difference.

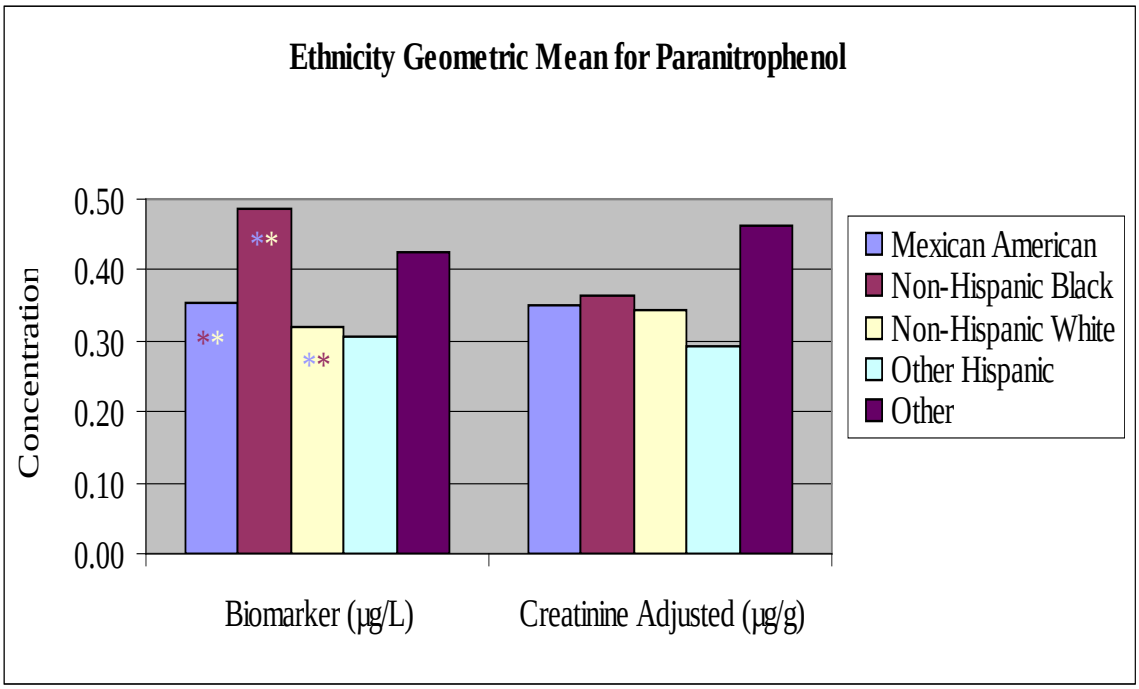

Figure 20: Comparison of Geometric Mean Values of Ethnic Groups for Paranitrophenol. µg/l= Micrograms per Liter; µg/g= Micrograms per Gram. Color-coded "**" indicates which groups are significantly different from each other.

Table 24: One-Way ANOVA and Tukey Analysis Comparing Geometric Means of Ethnic Groups for Paranitrophenol.

Biomarker (µg/L)[a]						
	GM[b]	LCL[c]	UCL[d]	SD[e]	n[f] (D/ND)[g]	P[h]
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.

Mexican Americans, Non-Hispanic Blacks and Non-Hispanic Whites are significantly different compared to each other when using the unadjusted biomarker concentration (µg/L). No difference was observed when adjusting for creatinine.

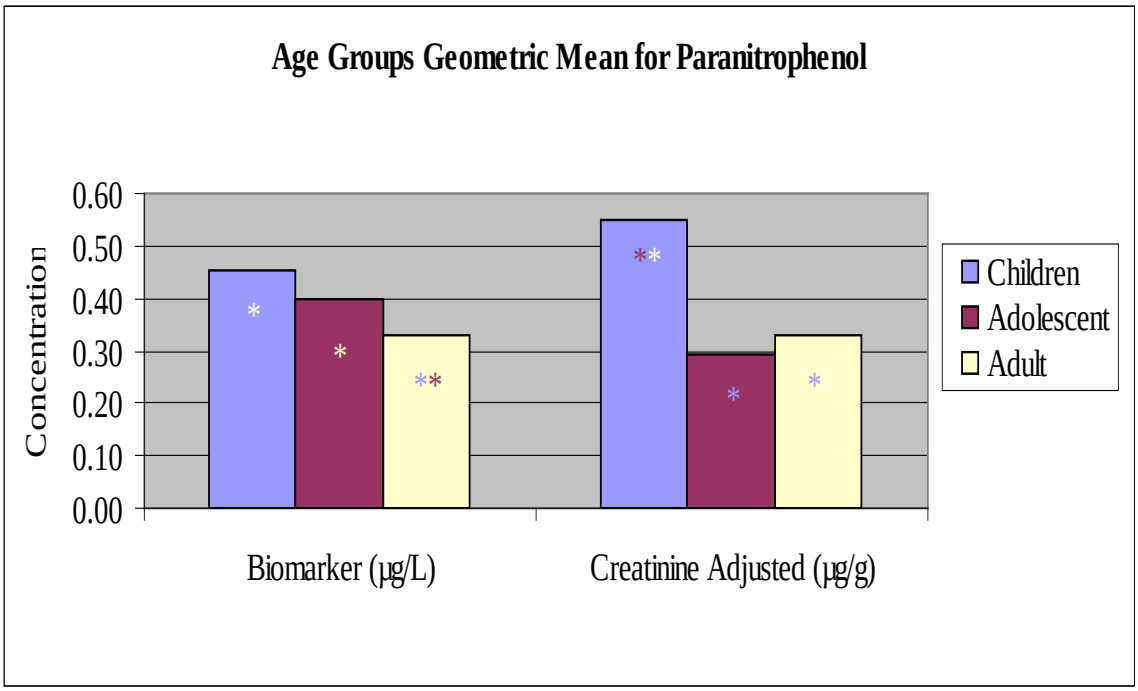

Figure 21: Comparison of Geometric Mean Values of Age Groups for Paranitrophenol. µg/l= Micrograms per Liter; µg/g= Micrograms per Gram. Color-coded "*" indicates which groups are significantly different from each other.

Table 25: 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]	P[h]
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.

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.

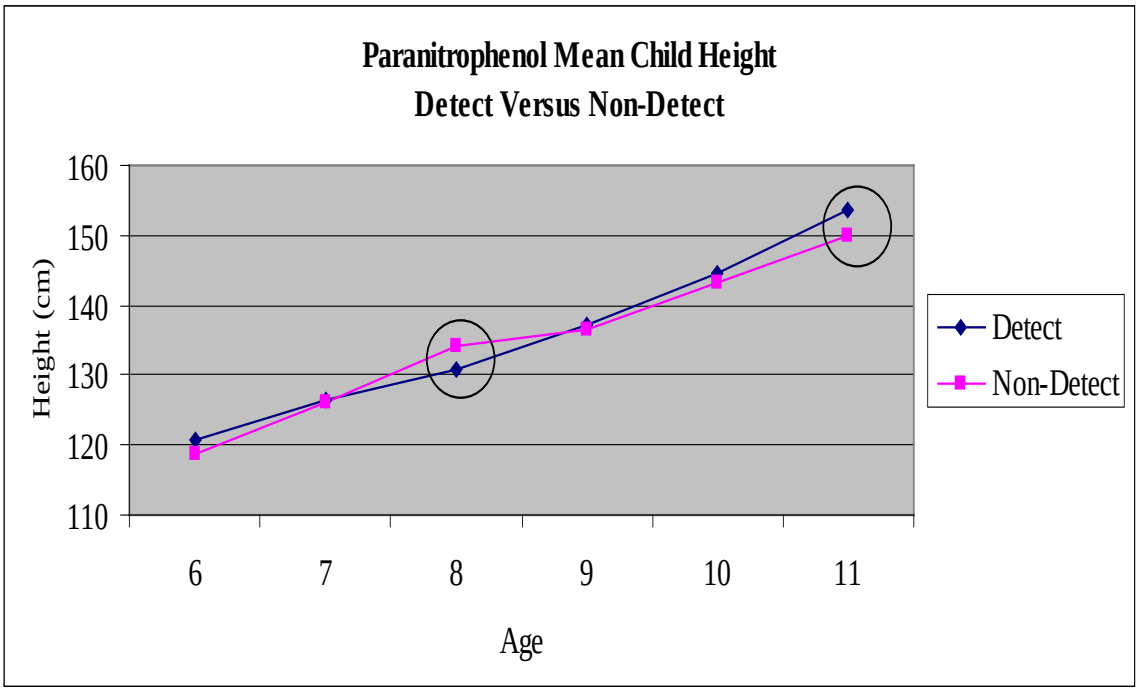

Figure 22: Graph of Arithmetic Mean Height for Detects Versus Non-Detects for Paranitrophenol, Ages 6-11. cm= Centimeter. ⬭ = Significantly different means.

Table 26: Student's t-test Comparing Detects Versus Non-Detects for Arithmetic Mean of Height for Paranitrophenol.

Age	Detect[a]	Mean (cm[b])	LCL[c]	UCL[d]	SD[e]	n[f]	p[g]
6	Yes	120.7	118.9	122.4	6.43	54	0.100
	No	118.7	117.1	120.3	4.60	34	
7	Yes	126.5	125	128	5.90	62	0.759
	No	126.1	124	128.1	6.37	40	
8	**Yes**	**130.9**	**128.7**	**133.2**	**7.83**	**49**	**0.046**
	No	**134.3**	**131.8**	**136.7**	**7.48**	**38**	
9	Yes	137.3	135.4	139.3	7.63	62	0.619
	No	136.6	134.7	138.6	6.67	48	
10	Yes	144.4	141.9	146.9	8.56	47	0.512
	No	143.2	140.5	145.8	7.04	29	
11	**Yes**	**153.7**	**151.6**	**155.8**	**8.34**	**63**	**0.022**
	No	**149.9**	**147.6**	**152.3**	**7.04**	**37**	

[a](Detect) = Detectable Level of Biomarker in the Urine Sample; [b](cm) = Centimeters; [c](LCL) = Lower Confidence Limit; [d](UCL) = Upper Confidence Limit; [e](SD) = Standard Deviation; [f](n) = Number in Sample; [g](p) = Level of Significance at p= 0.05 Level (highlighted in **Bold**)

There is a significantly higher mean height for age 8 non-detects as compared to those with a detect for the biomarker. Children with a recorded detect age 11 had a significantly higher mean height than those children in the same age group that did not have a detect.

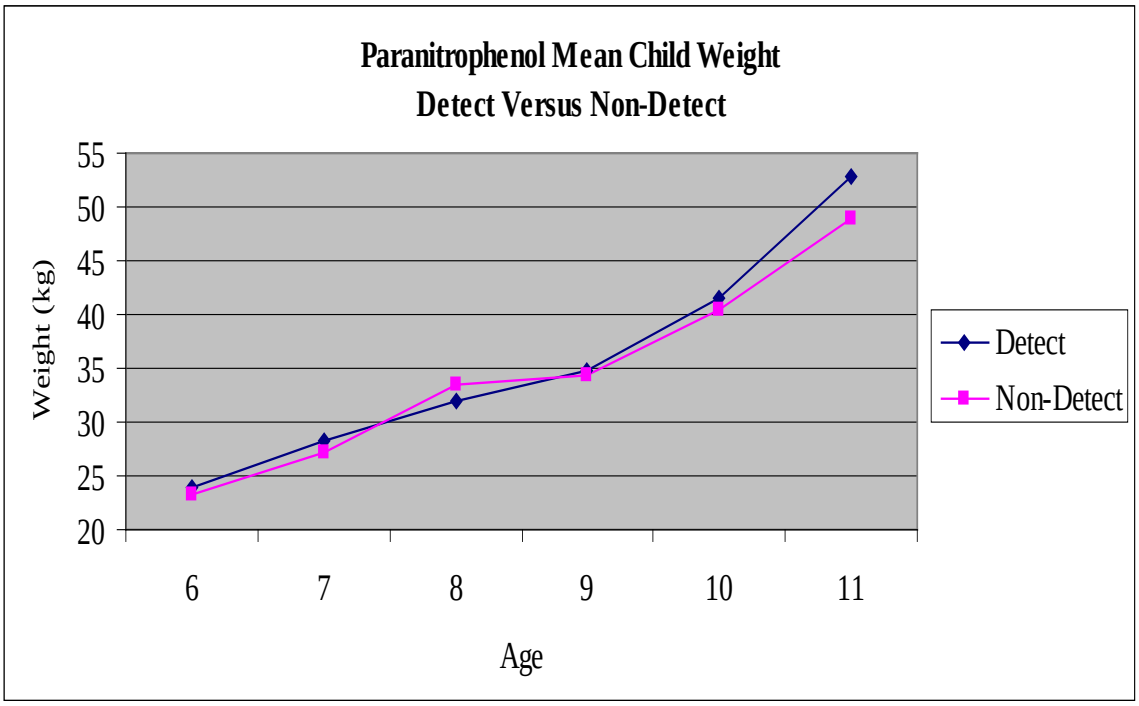

Figure 23: Graph of Arithmetic Mean Weight for Detects Versus Non-Detects for Paranitrophenol, Ages 6-11. Kg= Kilograms

Table 27: Student's t-test Comparing Detects Versus Non-Detects for Arithmetic Mean of Weight for Paranitrophenol.

Age	Detect[a]	Mean (Kg[b])	LCL[c]	UCL[d]	SD[e]	n[f]	p[g]
6	Yes	23.86	22.62	25.11	4.56	54	0.476
	No	23.20	21.95	24.45	3.58	34	
7	Yes	28.19	26.64	29.75	6.11	62	0.470
	No	27.23	24.93	29.53	7.09	39	
8	Yes	31.96	28.92	34.99	10.46	48	0.491
	No	33.48	30.30	36.66	9.54	37	
9	Yes	34.70	31.94	37.45	10.68	60	0.864
	No	34.37	31.79	36.94	8.86	48	
10	Yes	41.56	37.73	45.38	13.03	47	0.711
	No	40.41	35.41	45.41	13.14	29	
11	Yes	52.89	47.76	58.02	20.02	61	0.319
	No	48.93	43.31	54.54	16.60	36	

[a](Detect) = Detectable Level of Biomarker in the Urine Sample; [b](Kg) = Kilograms; [c](LCL) = Lower Confidence Limit; [d](UCL) = Upper Confidence Limit; [e](SD) = Standard Deviation; [f](n) = Number in Sample; [g](p) = Level of Significance at p= 0.05 Level

There are no significant differences at the $p < 0.05$ level between detect and non-detect urinary concentrations of paranitrophenol when evaluating variations in children's weight.

Table 28: Paranitrophenol Female Biochemical t-test Comparisons.

Biochemical	Detect[a]	Mean	LCL[b]	UCL[c]	SD[d]	n[e]	Min[f]	Max[g]	p[h]
Alanine Transaminase (U/L)[i]	Yes	18.70	17.87	19.53	9.78	538	7	102	0.375
	No	19.18	18.51	19.86	8.87	666	5	83	
Albumin (g/dL)[j]	Yes	4.15	4.12	4.18	0.33	538	2.6	5	0.196
	No	4.18	4.15	4.20	0.36	666	2.6	5	
Alkaline Phosphatase (U/L)	Yes	83.90	80.06	87.75	45.43	538	30	424	0.084
	No	79.63	76.69	82.57	38.64	666	24	341	
Aspartate Transaminase (U/L)	Yes	21.48	20.83	22.13	7.70	538	9	114	0.399
	No	21.84	21.31	22.37	6.93	666	7	84	
Bicarbonate (mmol/L)[k]	Yes	23.01	22.83	23.19	2.16	538	16	29	0.083
	No	22.79	22.61	22.96	2.27	666	16	29	
Bilirubin (mg/dL)[l]	Yes	0.65	0.63	0.66	0.20	538	0.2	2.1	0.083
	No	0.67	0.65	0.68	0.22	666	0.2	1.9	
Blood Urea Nitrogen (mg/dL)	Yes	11.99	11.57	12.41	4.96	538	2	45	0.973
	No	11.98	11.46	12.49	6.79	666	2	122	
Total Calcium (mg/dL)	Yes	9.43	9.40	9.46	0.38	538	8.1	10.7	0.516
	No	9.45	9.42	9.48	0.40	666	8.3	11	
Chloride (mmol/L)	Yes	103.20	102.90	103.40	2.60	538	93	111	0.375
	No	103.00	102.80	103.20	2.76	665	89	113	
Cholesterol (mg/dL)	**Yes**	**187.10**	**183.60**	**190.50**	**41.28**	**538**	**91**	**334**	**0.003**
	No	**194.60**	**191.10**	**198.10**	**46.08**	**666**	**90**	**476**	
Creatinine (mg/dL)	Yes	0.73	0.72	0.75	0.19	538	0.3	2.3	0.820
	No	0.73	0.71	0.75	0.25	666	0.3	4.4	
γ Glutamyl Transferase (U/L)	Yes	20.59	18.82	22.36	20.94	538	5	266	0.705
	No	20.12	18.44	21.80	22.08	666	4	369	
Globulin (mg/dL)	Yes	3.17	3.14	3.21	0.40	538	2.2	5.4	0.478
	No	3.16	3.13	3.19	0.39	666	2.2	4.8	
Glucose (mg/dL)	**Yes**	**92.03**	**89.15**	**94.91**	**33.99**	**538**	**50**	**521**	**0.044**
	No	**88.48**	**86.56**	**90.40**	**25.23**	**666**	**30**	**453**	
Iron (mg/dL)	Yes	81.59	78.38	84.81	37.99	538	8	249	0.493
	No	83.07	80.29	85.85	36.54	666	7	268	
Lactate Dehydrogenase (U/L)	Yes	129.40	127.10	131.70	26.96	538	51	277	0.230
	No	131.40	129.10	133.60	29.49	665	58	470	
Osmolality (mmol/Kg)[m]	Yes	275.80	275.30	276.20	5.37	538	250	297	0.100
	No	275.30	274.80	275.70	5.67	666	248	310	
Phosphorus (mg/dL)	Yes	4.02	3.97	4.07	0.59	538	2.3	5.7	0.053
	No	3.95	3.91	3.99	0.55	666	2.3	6.1	
Potassium (mmol/L)	Yes	3.99	3.96	4.02	0.33	538	3.1	5.5	0.924
	No	3.99	3.96	4.01	0.35	666	2.6	6	
Total Protein (g/dL)	Yes	7.32	7.28	7.37	0.49	538	5.5	9.5	0.737
	No	7.33	7.30	7.37	0.48	666	5.9	9.1	
Sodium (mmol/L)	Yes	138.40	138.20	138.60	2.42	538	125	145	0.229
	No	138.20	138.00	138.40	2.60	666	124	147	
Triglycerides (mg/dL)	Yes	121.90	114.20	129.50	90.47	538	23	742	0.644
	No	119.50	113.20	125.90	83.24	666	28	1077	
Uric Acid (mg/dL)	Yes	4.57	4.46	4.67	1.26	538	0.4	10.9	0.826
	No	4.55	4.46	4.64	1.25	666	1.8	13.9	

[a](Detect) = Detectable Level of Biomarker in the Urine Sample; [b](LCL) = Lower Confidence Limit; [c](UCL) = Upper Confidence Limit; [d](SD) = Standard Deviation; [e](n) = Number in Sample; [f](Min) = Minimum; [g](Max) = Maximum; [h](p) = Level of Significance at p= 0.05 Level (highlighted in **Bold**); [i](U/L) = Units per Liter; [j](g/dL) = Grams per Deciliter; [k](mmol/L) = Millimole per Liter; [l](mg/dL) = Milligram per Deciliter; [m](mmol/Kg) = Millimole per Kilogram

Table 29: Paranitrophenol Male Biochemical t-test Comparisons.

Biochemical	Detect[a]	Mean	LCL[b]	UCL[c]	SD[d]	n[e]	Min[f]	Max[g]	p[h]
Alanine Transaminase (U/L)[i]	Yes	26.54	25.04	28.04	18.97	620	8	243	0.954
	No	26.61	24.97	28.24	17.40	436	7	165	
Albumin (g/dL)[j]	Yes	4.38	4.35	4.40	0.30	620	2.1	5.3	0.200
	No	4.40	4.37	4.43	0.31	436	2.6	5.3	
Alkaline Phosphatase (U/L)	Yes	102.10	96.44	107.70	71.20	620	19	617	0.872
	No	102.80	95.69	109.90	75.50	436	33	515	
Aspartate Transaminase (U/L)	Yes	26.55	25.50	27.59	13.28	620	9	187	0.950
	No	26.50	25.36	27.63	12.05	436	12	118	
Bicarbonate (mmol/L)[k]	Yes	24.00	23.82	24.17	2.16	620	15	29	0.903
	No	24.01	23.81	24.21	2.10	436	17	30	
Bilirubin (mg/dL)[l]	Yes	0.82	0.79	0.84	0.30	620	0.3	2.9	0.518
	No	0.83	0.80	0.86	0.32	436	0.3	3.5	
Blood Urea Nitrogen (mg/dL)	Yes	14.25	13.84	14.67	5.28	620	3	48	0.103
	No	13.72	13.24	14.20	5.10	436	3	41	
Total Calcium (mg/dL)	**Yes**	**9.53**	**9.50**	**9.56**	**0.38**	**620**	**7.2**	**10.6**	**0.022**
	No	**9.59**	**9.55**	**9.63**	**0.42**	**436**	**7.6**	**11.3**	
Chloride (mmol/L)	**Yes**	**102.60**	**102.40**	**102.80**	**2.63**	**620**	**83**	**111**	**0.003**
	No	**102.20**	**101.90**	**102.40**	**2.55**	**436**	**93**	**112**	
Cholesterol (mg/dL)	Yes	183.60	180.00	187.20	45.40	620	71	566	0.078
	No	188.40	184.50	192.40	41.69	436	86	402	
Creatinine (mg/dL)	Yes	0.97	0.95	0.99	0.26	620	0.4	4.4	0.871
	No	0.97	0.94	0.99	0.24	436	0.4	2.5	
γ Glutamyl Transferase (U/L)	Yes	30.34	27.74	32.93	32.89	620	6	482	0.788
	No	30.88	27.90	33.87	31.73	436	6	394	
Globulin (mg/dL)	Yes	3.08	3.04	3.11	0.47	620	1.6	5.9	0.765
	No	3.07	3.03	3.10	0.39	435	2	4.4	
Glucose (mg/dL)	Yes	94.51	92.73	96.29	22.60	620	65	295	0.312
	No	96.65	92.89	100.40	39.97	436	68	707	
Iron (mg/dL)	Yes	95.80	92.76	98.84	38.58	620	12	276	0.180
	No	99.07	95.34	102.80	39.60	436	18	333	
Lactate Dehydrogenase (U/L)	Yes	136.30	133.80	138.80	31.70	620	52	355	0.205
	No	139.00	135.60	142.40	35.74	436	75	399	
Osmolality (mmol/Kg)[m]	Yes	278.00	277.60	278.40	5.51	620	218	299	0.126
	No	277.50	277.00	278.00	4.99	436	261	298	
Phosphorus (mg/dL)	Yes	3.93	3.88	3.99	0.69	620	2.5	6.6	0.234
	No	3.88	3.81	3.95	0.70	436	2.4	6.6	
Potassium (mmol/L)	Yes	4.12	4.09	4.15	0.36	620	3.1	5.5	0.937
	No	4.12	4.09	4.15	0.34	436	3.2	5.3	
Total Protein (g/dL)	Yes	7.45	7.42	7.49	0.48	620	5.3	9.8	0.569
	No	7.47	7.43	7.51	0.44	435	5.9	8.7	
Sodium (mmol/L)	Yes	139.10	138.90	139.30	2.72	620	108	147	0.146
	No	138.90	138.60	139.10	2.28	436	131	146	
Triglycerides (mg/dL)	Yes	140.90	127.00	154.70	175.90	620	21	2677	0.550
	No	148.30	128.00	168.60	215.60	436	27	3854	
Uric Acid (mg/dL)	Yes	5.98	5.88	6.08	1.26	620	1.5	13.4	0.643
	No	6.02	5.89	6.15	1.40	436	2.6	11	

[a](Detect) = Detectable Level of Biomarker in the Urine Sample; [b](LCL) = Lower Confidence Limit; [c](UCL) = Upper Confidence Limit; [d](SD) = Standard Deviation; [e](n) = Number in Sample; [f](Min) = Minimum; [g](Max) = Maximum; [h](p) = Level of Significance at p= 0.05 Level (highlighted in **Bold**); [i](U/L) = Units per Liter; [j](g/dL) = Grams per Deciliter; [k](mmol/L) = Millimole per Liter; [l](mg/dL) = Milligram per Deciliter; [m](mmol/Kg) = Millimole per Kilogram

Table 30: Paranitrophenol Overall Biochemical t-test Comparisons.

Biochemical	Detect[a]	Mean	LCL[b]	UCL[c]	SD[d]	n[e]	Min[f]	Max[g]	p[h]
Alanine Transaminase (U/L)[i]	Yes	22.90	21.98	23.81	15.88	1158	7	243	0.208
	No	22.12	21.33	22.91	13.43	1102	5	165	
Albumin (g/dL)[j]	Yes	4.27	4.25	4.29	0.33	1158	2.1	5.3	0.646
	No	4.27	4.24	4.29	0.36	1102	2.6	5.3	
Alkaline Phosphatase (U/L)	Yes	93.62	90.09	97.16	61.26	1158	19	617	0.053
	No	88.80	85.41	92.18	57.29	1102	24	515	
Aspartate Transaminase (U/L)	Yes	24.19	23.54	24.84	11.32	1158	9	187	0.247
	No	23.68	23.12	24.25	9.57	1102	7	118	
Bicarbonate (mmol/L)[k]	**Yes**	**23.54**	**23.41**	**23.66**	**2.22**	**1158**	**15**	**29**	**0.005**
	No	**23.27**	**23.14**	**23.41**	**2.29**	**1102**	**16**	**30**	
Bilirubin (mg/dL)[l]	Yes	0.74	0.72	0.75	0.27	1158	0.2	2.9	0.612
	No	0.73	0.72	0.75	0.28	1102	0.2	3.5	
Blood Urea Nitrogen (mg/dL)	**Yes**	**13.20**	**12.90**	**13.50**	**5.25**	**1158**	**2**	**48**	**0.028**
	No	**12.67**	**12.30**	**13.04**	**6.23**	**1102**	**2**	**122**	
Total Calcium (mg/dL)	Yes	9.48	9.46	9.51	0.38	1158	7.2	10.7	0.287
	No	9.50	9.48	9.53	0.41	1102	7.6	11.3	
Chloride (mmol/L)	Yes	102.90	102.70	103.00	2.63	1158	83	111	0.073
	No	102.70	102.50	102.80	2.71	1101	89	113	
Cholesterol (mg/dL)	**Yes**	**185.20**	**182.70**	**187.70**	**43.55**	**1158**	**71**	**566**	**0.0002**
	No	**192.20**	**189.50**	**194.80**	**44.48**	**1102**	**86**	**476**	
Creatinine (mg/dL)	**Yes**	**0.86**	**0.84**	**0.87**	**0.26**	**1158**	**0.3**	**4.4**	**0.002**
	No	**0.82**	**0.81**	**0.84**	**0.27**	**1102**	**0.3**	**4.4**	
γ Glutamyl Transferase (U/L)	Yes	25.81	24.17	27.45	28.39	1158	5	482	0.218
	No	24.38	22.79	25.96	26.83	1102	4	394	
Globulin (mg/dL)	Yes	3.12	3.10	3.15	0.44	1158	1.6	5.9	0.967
	No	3.12	3.10	3.14	0.39	1101	2	4.8	
Glucose (mg/dL)	Yes	93.36	91.72	95.00	28.48	1158	50	521	0.198
	No	91.71	89.81	93.61	32.12	1102	30	707	
Iron (mg/dL)	Yes	89.20	86.95	91.44	38.94	1158	8	276	0.902
	No	89.40	87.12	91.68	38.57	1102	7	333	
Lactate Dehydrogenase (U/L)	Yes	133.10	131.40	134.80	29.78	1158	51	355	0.323
	No	134.40	132.50	136.30	32.31	1101	58	470	
Osmolality (mmol/Kg)[m]	**Yes**	**277.00**	**276.60**	**277.30**	**5.56**	**1158**	**218**	**299**	**0.0004**
	No	**276.10**	**275.80**	**276.50**	**5.52**	**1102**	**248**	**310**	
Phosphorus (mg/dL)	Yes	3.97	3.93	4.01	0.64	1158	2.3	6.6	0.075
	No	3.92	3.89	3.96	0.62	1102	2.3	6.6	
Potassium (mmol/L)	Yes	4.06	4.04	4.08	0.36	1158	3.1	5.5	0.170
	No	4.04	4.02	4.06	0.35	1102	2.6	6	
Total Protein (g/dL)	Yes	7.39	7.36	7.42	0.49	1158	5.3	9.8	0.768
	No	7.39	7.36	7.42	0.47	1101	5.9	9.1	
Sodium (mmol/L)	**Yes**	**138.80**	**138.60**	**138.90**	**2.60**	**1158**	**108**	**147**	**0.008**
	No	**138.50**	**138.30**	**138.60**	**2.49**	**1102**	**124**	**147**	
Triglycerides (mg/dL)	Yes	132.00	123.80	140.30	143.00	1158	21	2677	0.858
	No	130.90	122.00	139.80	150.80	1102	27	3854	
Uric Acid (mg/dL)	**Yes**	**5.32**	**5.24**	**5.41**	**1.45**	**1158**	**0.4**	**13.4**	**0.002**
	No	**5.13**	**5.04**	**5.22**	**1.49**	**1102**	**1.8**	**13.9**	

[a](Detect) = Detectable Level of Biomarker in the Urine Sample; [b](LCL) = Lower Confidence Limit; [c](UCL) = Upper Confidence Limit; [d](SD) = Standard Deviation; [e](n) = Number in Sample; [f](Min) = Minimum; [g](Max) = Maximum; [h](p) = Level of Significance at p= 0.05 Level (highlighted in **Bold**); [i](U/L) = Units per Liter; [j](g/dL) = Grams per Deciliter; [k](mmol/L) = Millimole per Liter; [l](mg/dL) = Milligram per Deciliter; [m](mmol/Kg) = Millimole per Kilogram

4.3.2.3 Logistic Regression

Table 31: Logistic Regression for Paranitrophenol.

	Detect[a] (DF[b]=1)	Detect, Non-Hispanic White (DF=1)	Detect, Female (DF=1)
Age	**β=-0.005 Wald χ2=8.78 p=0.003 OR=0.995**		
Mexican American		β=-0.0239 Wald χ2=0.0863 p=0.77 OR=1.18	
Non-Hispanic Black		β=-0.213 Wald χ2=2.00 p=0.158 OR=0.976	
Other Hispanic		**β=0.183 Wald χ2=4.96 p=0.026 OR=1.45**	
Other		β=0.242 Wald χ2=2.39 p=0.122 OR-1.54	
Male			**β=0.227 Wald χ2=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.**

The overall model fit was significant, with Likelihood $\chi 2 = 71.3$, $p < 0.0001$ and the $R^2_{Max} = 0.0316$. The Hosmer and Lemeshow goodness-of-fit test resulted in a $\chi 2 = 16.01$, $p = 0.0414$, indicating that the data from the independent variables did not fit the model well. The listed detects at the row headers are the reference groups for each sub-category. 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).

4.4 Pyrethroid

4.4.1 Pyrethroid Biomarker: 3-Phenoxybenzoic Acid (3-PBA)

4.4.1.1 Descriptive Statistics

The information covered in the following figures and tables characterizes the exposures to pyrethroids in the overall sample and in various subgroups.

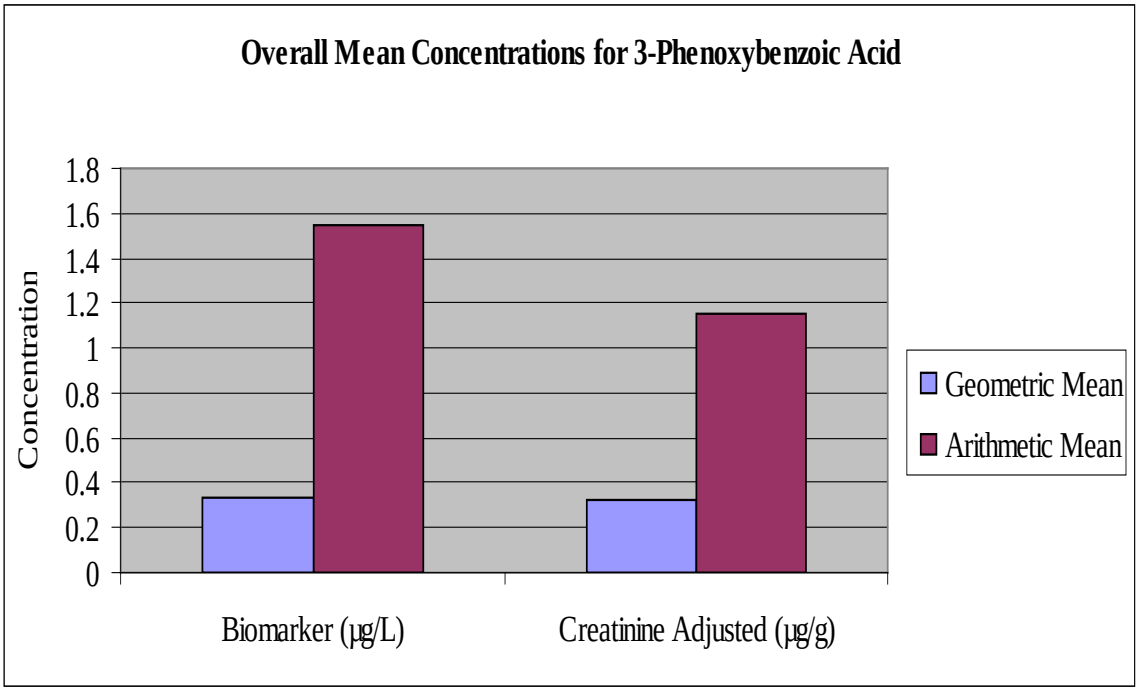

Figure24: Comparison of Means for 3-Phenoxybenzoic Acid. µg/l= Micrograms per Liter; µg/g= Micrograms per Gram. Adjusting for creatinine results in means (both geometric and arithmetic) that are lower than if using the concentration of the 3-PBA biomarker in urine (µg/L) alone. Limit of Detection (LOD) for 3-PBA in sample was 0.1 µg/L.

Table 32: Descriptive Statistics for 3-Phenoxybenzoic Acid.

Biomarker (µg/L)[a]				Creatinine Adjusted (µg/g)[b]		
	Geometric Mean	Arithmetic Mean			Geometric Mean	Arithmetic Mean
n[c]	3048	3048		N	3046	3046
Mean	0.336	1.55		Mean	0.323	1.15
LCL[d]	0.320	0.864		LCL	0.310	0.819
UCL[e]	0.352	2.24		UCL	0.338	1.48
SD[f]	3.70	19.31		SD	3.37	9.27
P25[g]	0.110	0.110		P25	0.143	0.143
P50[h]	0.300	0.300		P50	0.292	0.292
P75[i]	0.740	0.740		P75	0.612	0.612
P90[j]	1.73	1.73		P90	1.46	1.46
P95[k]	3.38	3.38		P95	2.82	2.82
Min[l]	0.07	0.07		Min	0.0201	0.0201
Max[m]	999.6	999.6		Max	421.8	421.8

[a](µg/L) = Micrograms per Liter; [b](µg/g) = Micrograms per Gram; [c](n) = Number in Sample; [d](LCL) = Lower Confidence Limit; [e](UCL) = Upper Confidence Limit; [f](SD) = Standard Deviation; [g](P25) = Lower Quartile; [h](P50) = Median; [i](P75) = Upper Quartile; [j](P90) = 90th Percentile; [k](P95) =95th Percentile; [l](Min) = Minimum [m](Max) = Maximum

Like the organophosphates, adjusting for creatinine results in means (both geometric and arithmetic) that are lower than if using the concentration of the biomarker in urine (µg/L). While the maximum urinary biomarker concentration was 999.6 µg/L, the next two highest values in the dataset were 253.8 and 160 µg/L.

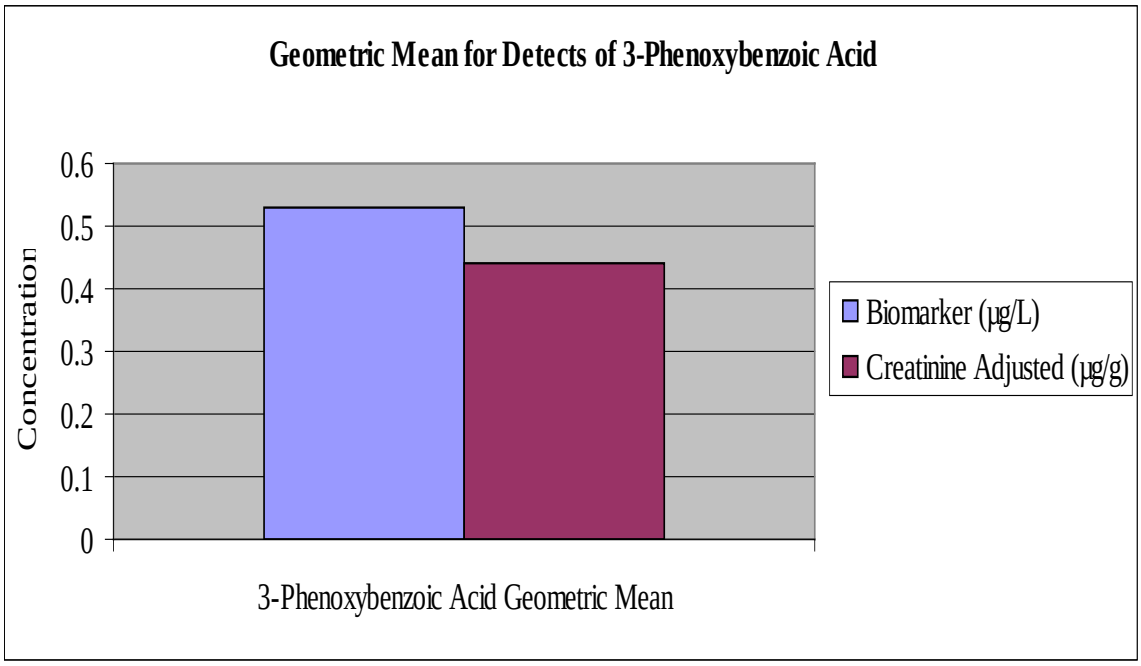

Figure 25: Comparison of Detectable Geometric Mean Concentrations for 3-Phenoxybenzoic Acid. µg/l= Micrograms per Liter; µg/g= Micrograms per Gram.

Table 33: Descriptive Statistics for Detectable Levels of 3-Phenoxybenzoic Acid.

	GM[c]	LCL[d]	UCL[e]	SD[f]	Median	n[g]
Biomarker (µg/L)[a]	0.531	0.507	0.556	3.10	0.450	2359
Creatinine Adjusted (µg/g)[b]	0.442	0.422	0.463	3.12	0.375	2359

[a](µg/L) = Micrograms per Liter; [b](µg/g) = Micrograms per Gram; [c](GM) = Geometric Mean; [d](LCL) = Lower Confidence Limit; [e](UCL) = Upper Confidence Limit; [f](SD) = Standard Deviation; [g](n) = Number in Sample

The geometric mean was determined for 77.4% of the individuals that had a detectable level of biomarker in their urine sample. Based on this analysis, the geometric mean for only detectable levels of the biomarker was ~0.2 µg/L higher that the overall geometric mean.

4.4.1.2 Comparative Statistics

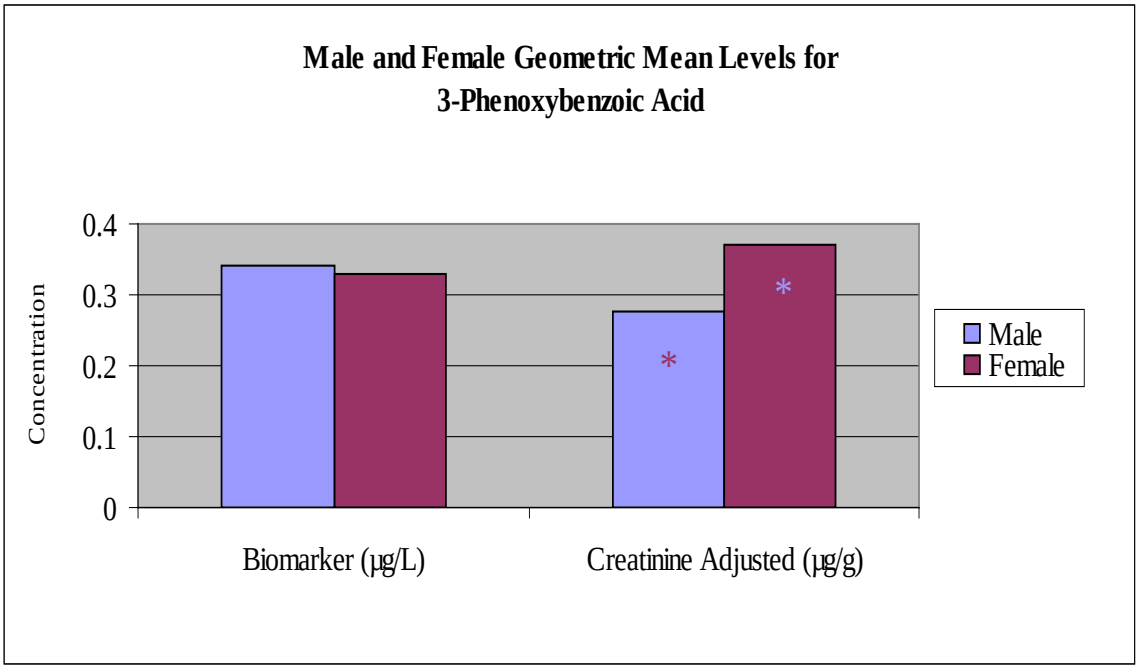

Figure 26: Comparison of Geometric Mean Values of Males Versus Females for 3-Phenoxybenzoic Acid. µg/l= Micrograms per Liter; µg/g= Micrograms per Gram. Color-coded "*" indicates females are significantly different from males.

Table 34: Student's t-test Comparing Geometric Means of Males Versus Females 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	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)[i]							
	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

Females had a significantly higher geometric mean when compared to males when adjusting for creatinine (µg/g). However, the significance is not present when using the unadjusted urinary concentration of the biomarker.

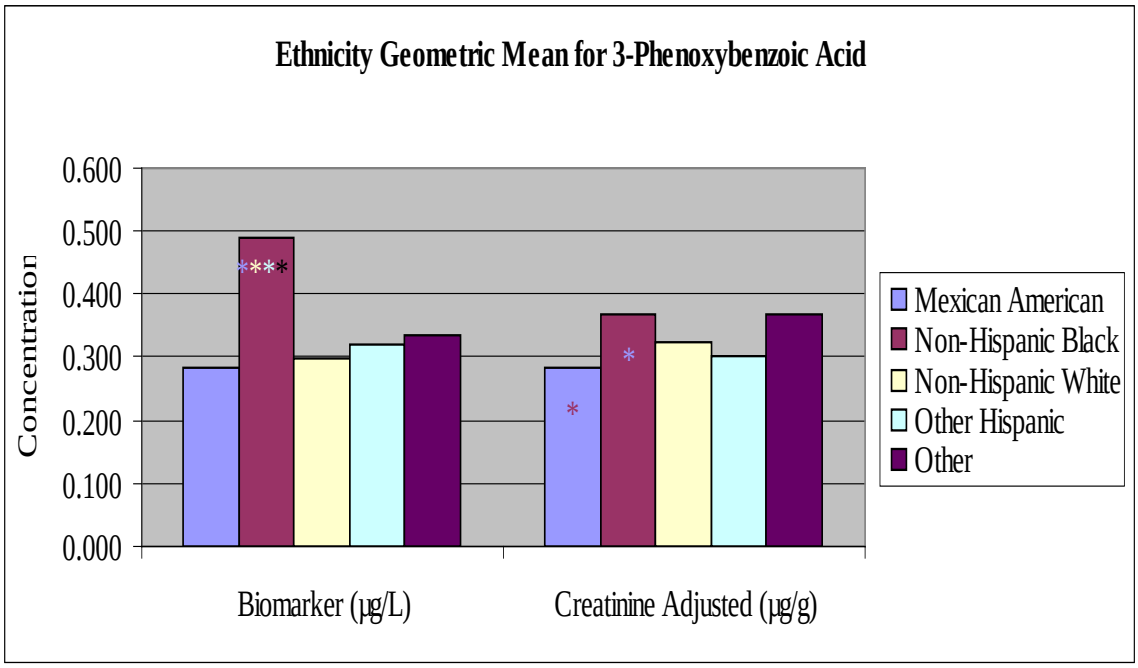

Figure 27: Comparison of Geometric Mean Values of Ethnic Groups for 3-Phenoxybenzoic Acid. µg/l= Micrograms per Liter; µg/g= Micrograms per Gram. Color-coded "****" indicates this groups is significantly different from all other groups. Color-coded "*" indicates these groups are significantly different from each other.

Table 35: 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]	P[h]
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.

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.

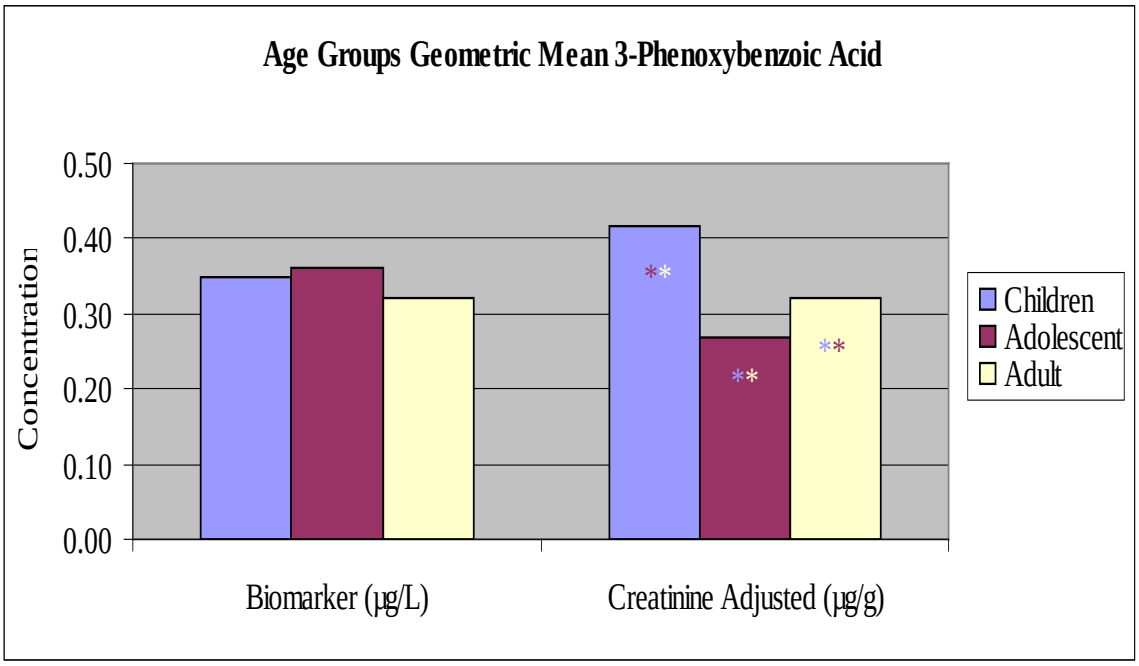

Figure 28: Comparison of Geometric Mean Values of Age Groups for 3-Phenoxybenzoic Acid. µg/l= Micrograms per Liter; µg/g= Micrograms per Gram. Color-coded "*" indicates which groups are significantly different from each other.

Table 36: One-Way ANOVA and Tukey Analysis Comparing Geometric Means of Age Groups for 3-Phenoxybenzoic Acid.

Biomarker (µg/L)[a]						
	GM[b]	LCL[c]	UCL[d]	SD[e]	n[f] (D/ND)[g]	P[h]
Children	0.349	0.047	0.650	3.71	580 (453/127)	0.0742
Adolescent	0.363	0.169	0.556	3.40	749 (613/136)	
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	**<0.0001**
Adolescent***	0.269	0.049	0.490	3.08	748	
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.

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).

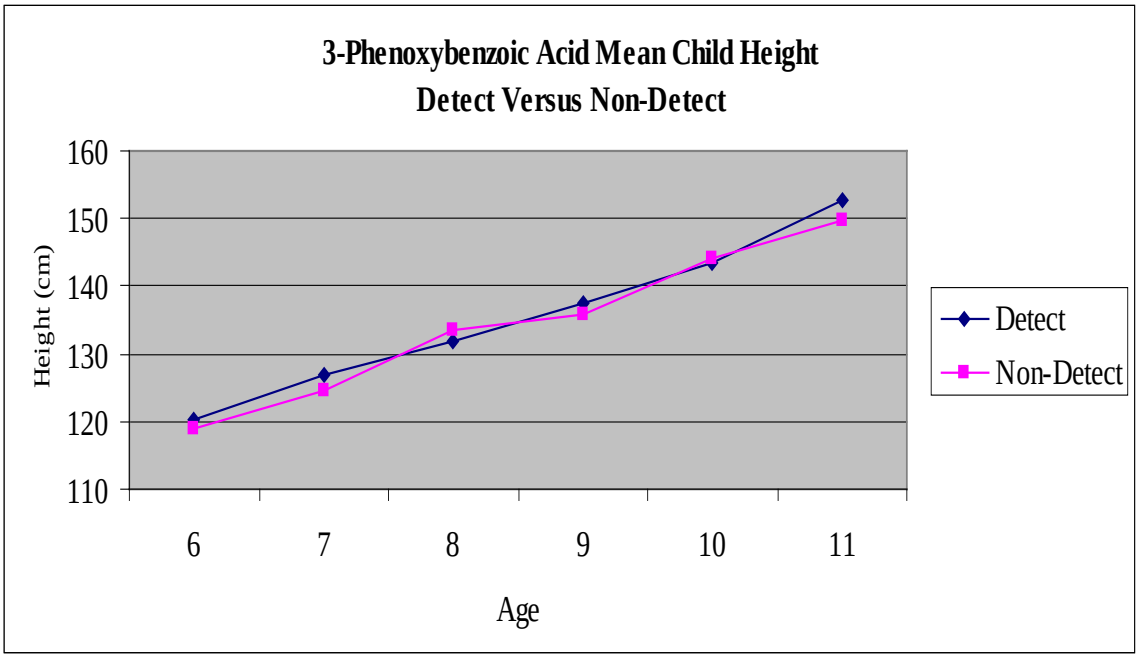

Figure 29: Graph of Arithmetic Mean Height for Detects Versus Non-Detects for 3-Phenoxybenzoic Acid, Ages 6-11. cm= Centimeter.

Table 37: Student's t-test Comparing Detects Versus Non-Detects for Arithmetic Mean of Height for 3-Phenoxybenzoic Acid.

Age	Detect[a]	Mean (cm[b])	LCL[c]	UCL[d]	SD[e]	n[f]	p[g]
6	Yes	120.2	118.7	121.7	6.14	66	0.415
	No	119	116.9	121.2	4.84	22	
7	Yes	127	125.5	128.4	6.36	77	0.081
	No	124.6	122.8	126.5	4.60	27	
8	Yes	132	130.2	133.9	7.85	69	0.441
	No	133.6	129.8	137.5	7.79	18	
9	Yes	137.4	135.9	138.9	7.30	91	0.346
	No	135.8	133	138.7	6.59	23	
10	Yes	143.6	141.8	145.5	7.19	60	0.809
	No	144.2	139.3	149	10.04	19	
11	Yes	152.7	151	154.4	8.11	89	0.154
	No	149.7	146.1	153.3	6.91	17	

[a](Detect) = Detectable Level of Biomarker in the Urine Sample; [b](cm) = Centimeters; [c](LCL) = Lower Confidence Limit; [d](UCL) = Upper Confidence Limit; [e](SD) = Standard Deviation; [f](n) = Number in Sample; [g](p) = Level of Significance at p= 0.05 Level

There are no significant differences at the $p < 0.05$ level between detect and non-detect urinary concentrations of 3-phenoxybenzoic acid when evaluating variations in children's height.

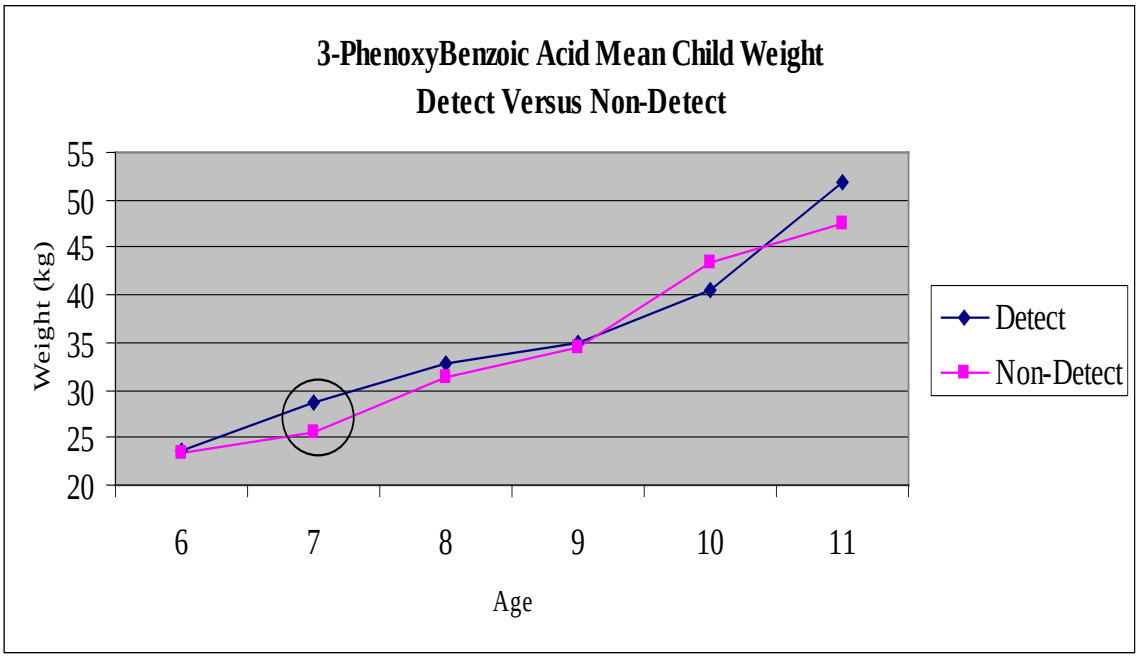

Figure 30: Graph of Arithmetic Mean Height for Detects Versus Non-Detects for 3-Phenoxybenzoic Acid, Ages 6-11. Kg= Kilograms. ⬭ = Significantly different means.

Table 38: Student's t-test Comparing Detects Versus Non-Detects for Arithmetic Mean of Weight for 3-Phenoxybenzoic Acid.

Age	Detect[a]	Mean (Kg[b])	LCL[c]	UCL[d]	SD[e]	n[f]	p[g]
6	Yes	23.72	22.62	24.81	4.45	66	0.672
	No	23.28	21.76	24.80	3.43	22	
7	**Yes**	**28.61**	**27.03**	**30.18**	**6.90**	**76**	**0.009**
	No	**25.56**	**23.89**	**27.22**	**4.22**	**27**	
8	Yes	32.91	30.35	35.46	10.56	68	0.597
	No	31.46	27.47	35.45	7.76	17	
9	Yes	34.88	32.85	36.91	9.64	89	0.867
	No	34.49	29.74	39.25	11.00	23	
10	Yes	40.56	37.32	43.80	12.54	60	0.412
	No	43.35	36.64	50.06	13.92	19	
11	Yes	51.79	47.73	55.84	19.03	87	0.408
	No	47.64	40.26	55.02	13.85	16	

[a](Detect) = Detectable Level of Biomarker in the Urine Sample; [b](Kg) = Kilograms; [c](LCL) = Lower Confidence Limit; [d](UCL) = Upper Confidence Limit; [e](SD) = Standard Deviation; [f](n) = Number in Sample; [g](p) = Level of Significance at p= 0.05 Level (highlighted in **Bold**)

There is a significantly higher arithmetic mean height for the age 7 group that recorded a detect for the biomarker in urine as compared to those with a non-detect for the biomarker.

Table 39: 3-Phenoxybenzoic Acid Female Biochemical t-test Comparisons.

Biochemical	Detect[a]	Mean	LCL[b]	UCL[c]	SD[d]	n[e]	Min[f]	Max[g]	p[h]
Alanine Transaminase (U/L)[i]	Yes	18.95	18.32	19.58	9.69	924	5	102	0.993
	No	18.95	17.98	19.91	8.62	309	5	102	
Albumin (g/dL)[j]	Yes	4.16	4.14	4.19	0.34	924	2.6	5	0.671
	No	4.17	4.13	4.21	0.36	309	2.6	5	
Alkaline Phosphatase (U/L)	**Yes**	**82.87**	**80.07**	**85.66**	**43.33**	**924**	**30**	**424**	**0.036**
	No	**77.54**	**73.40**	**81.67**	**36.92**	**309**	**24**	**330**	
Aspartate Transaminase (U/L)	Yes	21.62	21.12	22.12	7.80	924	7	114	0.442
	No	21.94	21.30	22.57	5.64	309	9	57	
Bicarbonate (mmol/L)[k]	Yes	22.84	22.70	22.97	2.14	924	16	29	0.104
	No	23.08	22.82	23.35	2.38	309	17	29	
Bilirubin (mg/dL)[l]	Yes	0.65	0.64	0.67	0.21	924	0.2	1.9	0.119
	No	0.68	0.65	0.70	0.22	309	0.2	2.1	
Blood Urea Nitrogen (mg/dL)	**Yes**	**11.76**	**11.39**	**12.14**	**5.82**	**924**	**2**	**122**	**0.015**
	No	**12.78**	**12.05**	**13.50**	**6.50**	**309**	**2**	**53**	
Total Calcium (mg/dL)	Yes	9.44	9.41	9.46	0.39	924	8.3	11	0.897
	No	9.44	9.40	9.48	0.39	309	8.1	10.8	
Chloride (mmol/L)	Yes	103.10	102.90	103.30	2.63	924	90	111	0.065
	No	102.80	102.40	103.10	2.95	308	89	113	
Cholesterol (mg/dL)	**Yes**	**188.60**	**185.80**	**191.40**	**42.99**	**924**	**90**	**476**	**<.0001**
	No	**200.20**	**195.00**	**205.40**	**46.65**	**309**	**91**	**348**	
Creatinine (mg/dL)	**Yes**	**0.72**	**0.71**	**0.73**	**0.18**	**924**	**0.3**	**2.8**	**0.008**
	No	**0.77**	**0.74**	**0.81**	**0.32**	**309**	**0.4**	**4.4**	
γ Glutamyl Transferase (U/L)	Yes	20.19	18.76	21.61	22.11	924	4	369	0.752
	No	20.60	18.45	22.76	19.26	309	5	190	
Globulin (mg/dL)	**Yes**	**3.18**	**3.16**	**3.21**	**0.40**	**924**	**2.3**	**5.4**	**0.006**
	No	**3.11**	**3.07**	**3.15**	**0.37**	**309**	**2.2**	**4.4**	
Glucose (mg/dL)	Yes	90.27	88.25	92.29	31.29	924	34	521	0.761
	No	89.75	87.07	92.42	23.91	309	30	284	
Iron (mg/dL)	Yes	81.67	79.28	84.06	37.04	924	8	268	0.135
	No	85.35	81.05	89.64	38.37	309	7	249	
Lactate Dehydrogenase (U/L)	**Yes**	**129.60**	**127.90**	**131.30**	**26.73**	**923**	**51**	**329**	**0.035**
	No	**134.00**	**130.30**	**137.60**	**32.68**	**309**	**58**	**470**	
Osmolality (mmol/Kg)[m]	Yes	275.40	275.10	275.80	5.26	924	248	310	0.881
	No	275.50	274.80	276.20	6.23	309	252	305	
Phosphorus (mg/dL)	Yes	3.99	3.95	4.02	0.58	924	2.3	6.1	0.401
	No	3.95	3.90	4.01	0.53	309	2.3	5.6	
Potassium (mmol/L)	Yes	3.99	3.97	4.01	0.33	924	2.6	6	0.733
	No	3.98	3.94	4.02	0.36	309	2.9	5.3	
Total Protein (g/dL)	Yes	7.35	7.32	7.38	0.49	924	5.9	9.5	0.056
	No	7.29	7.23	7.34	0.48	309	5.5	9.1	
Sodium (mmol/L)	Yes	138.30	138.20	138.50	2.44	924	124	145	0.329
	No	138.20	137.90	138.50	2.74	309	125	147	
Triglycerides (mg/dL)	**Yes**	**116.10**	**110.50**	**121.70**	**86.44**	**924**	**23**	**1077**	**0.001**
	No	**134.30**	**124.50**	**144.10**	**87.41**	**309**	**34**	**628**	
Uric Acid (mg/dL)	**Yes**	**4.50**	**4.42**	**4.58**	**1.19**	**924**	**1.8**	**10.8**	**0.027**
	No	**4.70**	**4.54**	**4.86**	**1.40**	**309**	**0.4**	**13.9**	

[a](Detect) = Detectable Level of Biomarker in the Urine Sample; [b](LCL) = Lower Confidence Limit; [c](UCL) = Upper Confidence Limit; [d](SD) = Standard Deviation; [e](n) = Number in Sample; [f](Min) = Minimum; [g](Max) = Maximum; [h](p) = Level of Significance at p= 0.05 Level (highlighted in **Bold**); [i](U/L) = Units per Liter; [j](g/dL) = Grams per Deciliter; [k](mmol/L) = Millimole per Liter; [l](mg/dL) = Milligram per Deciliter; [m](mmol/Kg) = Millimole per Kilogram

Table 40: 3-Phenoxybenzoic Acid Male Biochemical t-test Comparisons.

Biochemical	Detect[a]	Mean	LCL[b]	UCL[c]	SD[d]	n[e]	Min[f]	Max[g]	p[h]
Alanine Transaminase (U/L)[i]	**Yes**	**27.44**	**26.12**	**28.75**	**19.56**	**857**	**7**	**243**	**0.0004**
	No	**23.78**	**22.23**	**25.32**	**11.82**	**227**	**8**	**102**	
Albumin (g/dL)[j]	Yes	4.38	4.36	4.40	0.30	857	2.1	5.3	0.772
	No	4.39	4.35	4.43	0.33	227	3	5.1	
Alkaline Phosphatase (U/L)	Yes	102.10	97.32	106.90	71.59	857	19	589	0.675
	No	104.70	93.58	115.80	85.04	227	37	617	
Aspartate Transaminase (U/L)	**Yes**	**27.07**	**26.14**	**27.99**	**13.80**	**857**	**9**	**187**	**0.0003**
	No	**24.71**	**23.83**	**25.59**	**6.71**	**227**	**12**	**61**	
Bicarbonate (mmol/L)[k]	Yes	23.98	23.84	24.12	2.06	857	15	29	0.255
	No	24.17	23.87	24.48	2.33	227	17	30	
Bilirubin (mg/dL)[l]	Yes	0.82	0.80	0.84	0.32	857	0.3	3.5	0.436
	No	0.81	0.77	0.84	0.26	227	0.3	2	
Blood Urea Nitrogen (mg/dL)	Yes	14.02	13.67	14.37	5.24	857	3	48	0.407
	No	14.35	13.62	15.07	5.54	227	3	40	
Total Calcium (mg/dL)	Yes	9.55	9.53	9.58	0.39	857	7.2	11.3	0.658
	No	9.54	9.49	9.60	0.42	227	7.8	10.8	
Chloride (mmol/L)	**Yes**	**102.50**	**102.40**	**102.70**	**2.43**	**857**	**92**	**112**	**0.020**
	No	**102.00**	**101.60**	**102.40**	**3.13**	**227**	**83**	**109**	
Cholesterol (mg/dL)	Yes	185.90	182.90	188.90	44.47	857	71	566	0.931
	No	186.20	180.80	191.70	41.72	227	84	407	
Creatinine (mg/dL)	Yes	0.96	0.94	0.98	0.26	857	0.4	4.4	0.317
	No	0.98	0.95	1.01	0.26	227	0.5	2.5	
γ Glutamyl Transferase (U/L)	Yes	31.21	28.93	33.49	34.03	857	6	482	0.076
	No	27.79	24.77	30.81	23.11	227	6	183	
Globulin (mg/dL)	**Yes**	**3.09**	**3.06**	**3.12**	**0.43**	**856**	**2**	**5.9**	**0.009**
	No	**3.01**	**2.95**	**3.07**	**0.46**	**227**	**1.6**	**4.5**	
Glucose (mg/dL)	Yes	95.09	93.53	96.65	23.30	857	58	317	0.641
	No	96.70	90.09	103.30	50.55	227	67	707	
Iron (mg/dL)	Yes	97.36	94.71	100.00	39.50	857	12	333	0.767
	No	96.49	91.54	101.40	37.86	227	18	223	
Lactate Dehydrogenase (U/L)	**Yes**	**138.90**	**136.60**	**141.30**	**34.90**	**857**	**52**	**399**	**0.004**
	No	**132.90**	**129.40**	**136.30**	**26.43**	**227**	**72**	**220**	
Osmolality (mmol/Kg)[m]	Yes	278.00	277.70	278.30	4.67	857	264	299	0.105
	No	277.20	276.20	278.10	7.20	227	218	298	
Phosphorus (mg/dL)	Yes	3.91	3.86	3.96	0.70	857	2.4	6.6	0.946
	No	3.91	3.82	4.00	0.68	227	2.4	6.6	
Potassium (mmol/L)	**Yes**	**4.14**	**4.12**	**4.16**	**0.35**	**857**	**3.1**	**5.5**	**0.003**
	No	**4.06**	**4.02**	**4.11**	**0.36**	**227**	**3.1**	**5.3**	
Total Protein (g/dL)	**Yes**	**7.47**	**7.44**	**7.50**	**0.45**	**856**	**5.9**	**9.8**	**0.025**
	No	**7.40**	**7.33**	**7.46**	**0.50**	**227**	**5.3**	**9**	
Sodium (mmol/L)	**Yes**	**139.10**	**139.00**	**139.30**	**2.22**	**857**	**131**	**147**	**0.022**
	No	**138.60**	**138.10**	**139.00**	**3.44**	**227**	**108**	**144**	
Triglycerides (mg/dL)	Yes	141.30	128.40	154.30	192.70	857	21	3854	0.372
	No	154.10	129.70	178.50	186.40	227	28	2337	
Uric Acid (mg/dL)	Yes	6.00	5.92	6.09	1.28	857	1.5	10.8	0.880
	No	5.99	5.79	6.18	1.50	227	2.6	13.4	

[a](Detect) = Detectable Level of Biomarker in the Urine Sample; [b](LCL) = Lower Confidence Limit; [c](UCL) = Upper Confidence Limit; [d](SD) = Standard Deviation; [e](n) = Number in Sample; [f](Min) = Minimum; [g](Max) = Maximum; [h](p) = Level of Significance at p= 0.05 Level (highlighted in **Bold**); [i](U/L) = Units per Liter; [j](g/dL) = Grams per Deciliter; [k](mmol/L) = Millimole per Liter; [l](mg/dL) = Milligram per Deciliter; [m](mmol/Kg) = Millimole per Kilogram

Table 41: 3-Phenoxybenzoic Acid Overall Biochemical t-test Comparisons.

Biochemical	Detect[a]	Mean	LCL[b]	UCL[c]	SD[d]	n[e]	Min[f]	Max[g]	p[h]
Alanine Transaminase (U/L)[i]	**Yes**	**23.03**	**22.30**	**23.77**	**15.83**	**1781**	**5**	**243**	**0.001**
	No	**20.99**	**20.11**	**21.87**	**10.37**	**536**	**5**	**102**	
Albumin (g/dL)[j]	Yes	4.27	4.25	4.29	0.34	1781	2.1	5.3	0.803
	No	4.27	4.23	4.30	0.36	536	2.6	5.1	
Alkaline Phosphatase (U/L)	Yes	92.13	89.37	94.89	59.42	1781	19	589	0.299
	No	89.04	83.66	94.42	63.40	536	24	617	
Aspartate Transaminase (U/L)	**Yes**	**24.24**	**23.71**	**24.77**	**11.42**	**1781**	**7**	**187**	**0.003**
	No	**23.11**	**22.58**	**23.64**	**6.26**	**536**	**9**	**61**	
Bicarbonate (mmol/L)[k]	Yes	23.39	23.28	23.49	2.18	1781	15	29	0.171
	No	23.54	23.34	23.75	2.42	536	17	30	
Bilirubin (mg/dL)[l]	Yes	0.74	0.72	0.75	0.28	1781	0.2	3.5	0.755
	No	0.73	0.71	0.75	0.25	536	0.2	2.1	
Blood Urea Nitrogen (mg/dL)	**Yes**	**12.85**	**12.59**	**13.11**	**5.66**	**1781**	**2**	**122**	**0.047**
	No	**13.44**	**12.92**	**13.96**	**6.16**	**536**	**2**	**53**	
Total Calcium (mg/dL)	Yes	9.49	9.48	9.51	0.39	1781	7.2	11.3	0.596
	No	9.48	9.45	9.52	0.41	536	7.8	10.8	
Chloride (mmol/L)	**Yes**	**102.80**	**102.70**	**102.90**	**2.55**	**1781**	**90**	**112**	**0.007**
	No	**102.40**	**102.20**	**102.70**	**3.05**	**535**	**83**	**113**	
Cholesterol (mg/dL)	**Yes**	**187.30**	**185.30**	**189.30**	**43.72**	**1781**	**71**	**566**	**0.001**
	No	**194.30**	**190.40**	**198.10**	**45.12**	**536**	**84**	**407**	
Creatinine (mg/dL)	Yes	0.84	0.82	0.85	0.25	1781	0.3	4.4	0.112
	No	0.86	0.83	0.89	0.31	536	0.4	4.4	
γ Glutamyl Transferase (U/L)	Yes	25.49	24.14	26.84	29.00	1781	4	482	0.108
	No	23.65	21.84	25.45	21.26	536	5	190	
Globulin (mg/dL)	**Yes**	**3.14**	**3.12**	**3.16**	**0.42**	**1780**	**2**	**5.9**	**0.001**
	No	**3.07**	**3.03**	**3.10**	**0.41**	**536**	**1.6**	**4.5**	
Glucose (mg/dL)	Yes	92.59	91.30	93.88	27.83	1781	34	521	0.953
	No	92.69	89.49	95.89	37.69	536	30	707	
Iron (mg/dL)	Yes	89.22	87.40	91.03	39.03	1781	8	333	0.658
	No	90.07	86.80	93.34	38.52	536	7	249	
Lactate Dehydrogenase (U/L)	Yes	134.10	132.70	135.60	31.28	1780	51	399	0.692
	No	133.50	130.90	136.10	30.17	536	58	470	
Osmolality (mmol/Kg)[m]	Yes	3.95	3.92	3.98	0.64	1781	2.3	6.6	0.613
	No	3.93	3.88	3.99	0.60	536	2.3	6.6	
Phosphorus (mg/dL)	**Yes**	**4.06**	**4.05**	**4.08**	**0.35**	**1781**	**2.6**	**6**	**0.008**
	No	**4.02**	**3.99**	**4.05**	**0.36**	**536**	**2.9**	**5.3**	
Potassium (mmol/L)	**Yes**	**7.41**	**7.39**	**7.43**	**0.47**	**1780**	**5.9**	**9.8**	**0.001**
	No	**7.33**	**7.29**	**7.37**	**0.49**	**536**	**5.3**	**9.1**	
Total Protein (g/dL)	Yes	276.70	276.40	276.90	5.14	1781	248	310	0.144
	No	276.20	275.60	276.80	6.70	536	218	305	
Sodium (mmol/L)	**Yes**	**138.70**	**138.60**	**138.80**	**2.37**	**1781**	**124**	**147**	**0.009**
	No	**138.30**	**138.10**	**138.60**	**3.06**	**536**	**108**	**147**	
Triglycerides (mg/dL)	**Yes**	**128.20**	**121.40**	**135.10**	**148.00**	**1781**	**21**	**3854**	**0.045**
	No	**142.70**	**130.90**	**154.40**	**138.50**	**536**	**28**	**2337**	
Uric Acid (mg/dL)	Yes	5.22	5.16	5.29	1.44	1781	1.5	10.8	0.792
	No	5.24	5.11	5.38	1.58	536	0.4	13.9	

[a](Detect) = Detectable Level of Biomarker in the Urine Sample; [b](LCL) = Lower Confidence Limit; [c](UCL) = Upper Confidence Limit; [d](SD) = Standard Deviation; [e](n) = Number in Sample; [f](Min) = Minimum; [g](Max) = Maximum; [h](p) = Level of Significance at p= 0.05 Level (highlighted in **Bold**); [i](U/L) = Units per Liter; [j](g/dL) = Grams per Deciliter; [k](mmol/L) = Millimole per Liter; [l](mg/dL) = Milligram per Deciliter; [m](mmol/Kg) = Millimole per Kilogram

4.4.1.3 Logistic Regression

Table 42: Logistic Regression for 3-Phenoxybenzoic Acid.

	Detect[a] (DF[b]=1)	Detect, Non-Hispanic White (DF=1)	Detect, Female (DF=1)
Age	**β=-0.006 Wald χ2=10.2 p=0.001 OR=0.994**		
Mexican American		β=-0.175 Wald χ2=3.44 p=0.064 OR=1.09	
Non-Hispanic Black		β=0.076 Wald χ2=0.171 p=0.679 OR=1.4	
Other Hispanic		**β=0.630 Wald χ2=33.5 p<0.0001 OR=2.43**	
Other		β=-0.273 Wald χ2=2.46 p=0.117 OR=0.986	
Male			**β=0.0927 Wald χ2=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.**

The overall model fit was significant, with Likelihood $\chi2= 84.8$, $p <0.0001$ and the $R^2_{Max} =0.0418$. The Hosmer and Lemeshow goodness-of-fit test resulted in a $\chi2= 7.59$, $p= 0.475$, indicating that the data from the independent variables fit the model well. The listed detects at the row headers are the reference groups for each sub-category. 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 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).

4.5 Summary of Mean Biomarker Concentrations

The summary of biomarker concentrations from this research are listed in Table 43.

Table 43: Summary of Means for All Three Biomarkers.

	Biomarker (µg/L)[a]			Creatinine Adjusted (µg/g)[b]		
	Geometric Mean	Arithmetic Mean		Geometric Mean	Arithmetic Mean	DF[c]
TCPy[d]	2.07	4.28		1.98	3.42	78.6%
PNP[e]	0.37	1.16		0.35	0.90	53.1%
3-PBA[f]	0.34	1.55		0.32	1.15	77.4%

[a](µg/L) = Micrograms per Liter; [b](µg/g) = Micrograms per Gram; [c](DF) = Detection Frequency; [d](TCPy) = 3,5,6-Trichloropyridinol; [e](PNP) = Paranitrophenol; [f](3-PBA) = 3-Phenoxybenzoic Acid

The unweighted sample results from this study can be compared to the concentrations determined in other studies. Those studies are listed in Table 44.

Table 44: Summary of Results from Epidemiological Biomarker Studies.

Author	n[a]	Sample Source	Metabolite	DF[b]	Central Tendency
Adgate (2001) [61]	102	Minnesota Child	TCPy	93%	GM[c]=6.4 µg/L[d]; AM[e]= 9.2 µg/L
Aprea (1999) [143]	42	General Population Italy	TCPy	88%	CAM[f]=3.5 µg/g[g]
Arcury (2007) [144]	60	Latino Age 1-6	TCPy	83.3%	GM=1.92 µg/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) [145]	1994	All	TCPy	91% (weighted)	GM=1.77 µg/L; CAGM=1.58 µg/g
Barr (2010) [64]	3048 (3046 for CA)	NHANES 1999-2002	3-PBA	75.4% (weighted)	GM=0.318 µg/L; CAGM=0.324 µg/g
Berger-Preiss (2002) [147]	145	Adults and Children	3-PBA	28%	Mean=0.25 µg/L
Berkowitz (2004) [108]	404	Pregnant Females	TCPy	NA	Median=7.6µg/L; CA Median= 11.5 µg/g
Eskenazi (2004) [107]	488	Pregnant Females in Agricultural Community	TCPy	76.3%	Median=3.3 µg/L
			PNP	54.4%	Median= 0.5 µg/L
Hill (1995) [147]	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 ug/g
Macintosh (2001) [148]	80	NHEXAS-Maryland	TCPy	96%	GM=5.1 µg/L; CAGM= 4.5 ug/g
Morgan 2005 [149]	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) [150]	203	Children Age 4-6	3-PBA	99.5%	Mean=5.0 ug/L
Olsson (2003) [151]	140	NA	TCPy	56%	GM= 9.7 µg/L
			PNP	99%	GM= 2.1 µg/L
Panuwet (2008) [51]	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) [114]	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) [102]	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) [152]	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) [153]	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

4.6 Biochemical Summary

When examining significant alterations in overall biochemical concentrations across all three biomarkers, only two were significant: cholesterol and sodium.

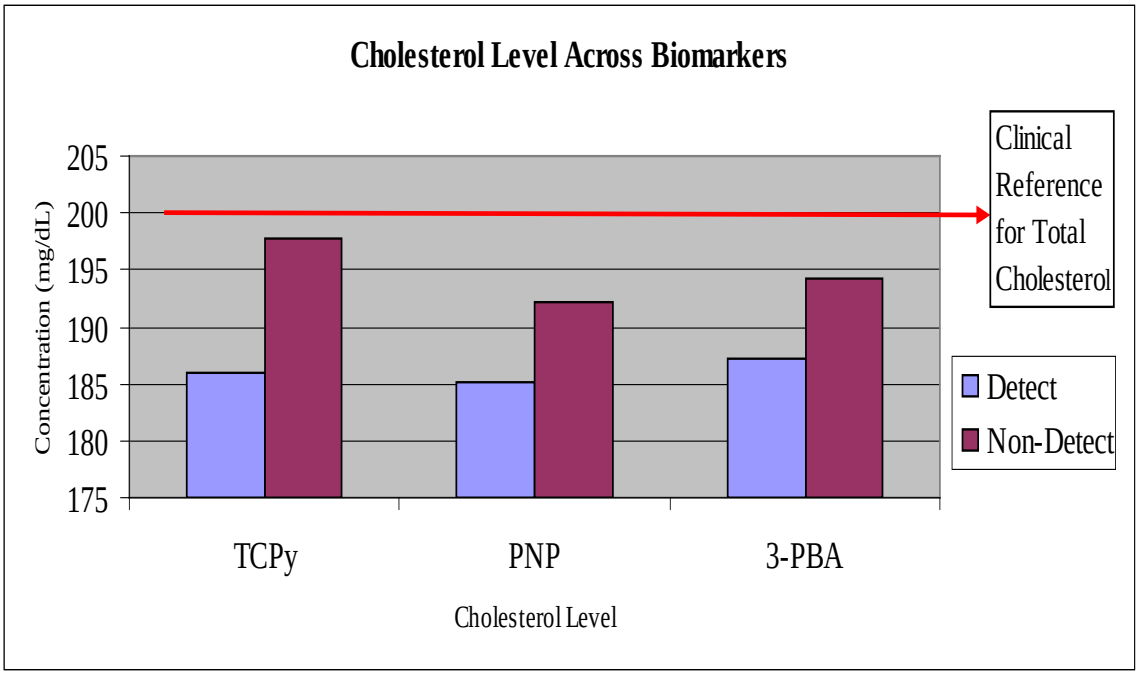

Figure 31: Overall Cholesterol Levels Across All Three Biomarkers. mg/dL= Milligram per Deciliter; TCPy= 3,5,6-Trichloropyridinol; PNP = Paranitrophenol; 3-PBA = 3-Phenoxybenzoic Acid. The clinical reference for serum levels of cholesterol are <200 mg/dL for desirable [154]. Borderline high is defined as 200-239 mg/dL [154].

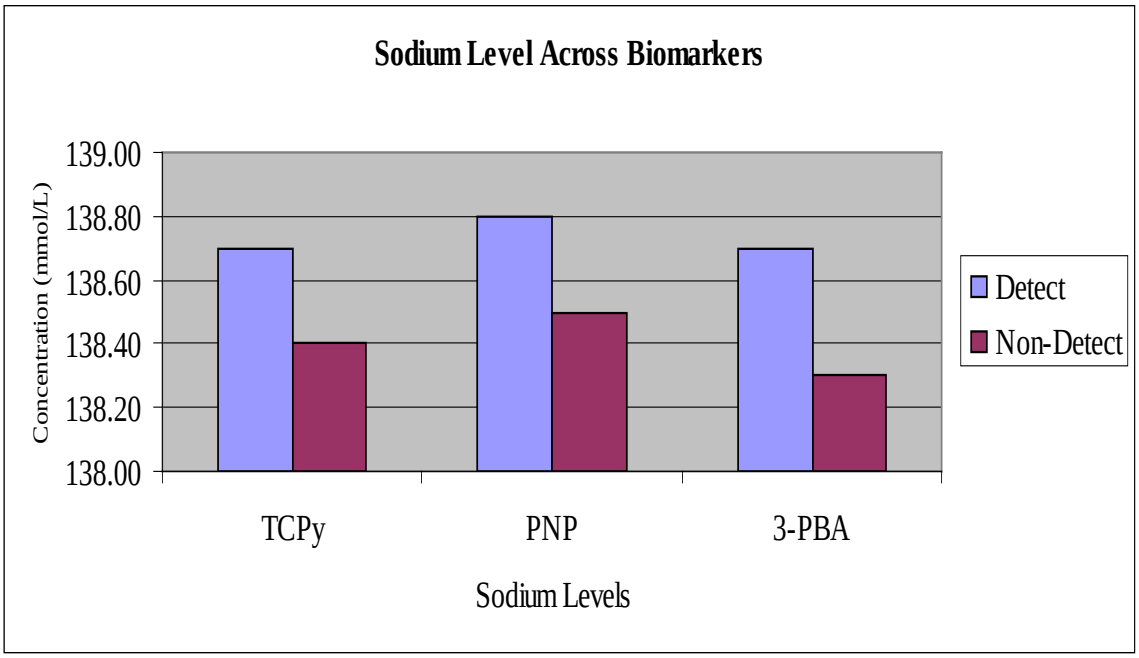

Figure 32: Overall Sodium Levels Across All Three Biomarkers. mmol/L= Millimole per Liter; TCPy= 3,5,6-Trichloropyridinol; PNP = Paranitrophenol; 3-PBA = 3-Phenoxybenzoic Acid. The clinical reference range for serum sodium levels is 135-145 mmol/L [154].

There were more significant findings when comparing the overall biochemical concentrations between the organophosphate pesticides: bicarbonate, cholesterol, osmolality and sodium. None of the significantly different levels were above clinical reference values [154].

Stratification for gender removes most of the consistent significant findings. The only gender specific significantly different biochemical concentrations across all three biomarkers was the cholesterol levels in females.

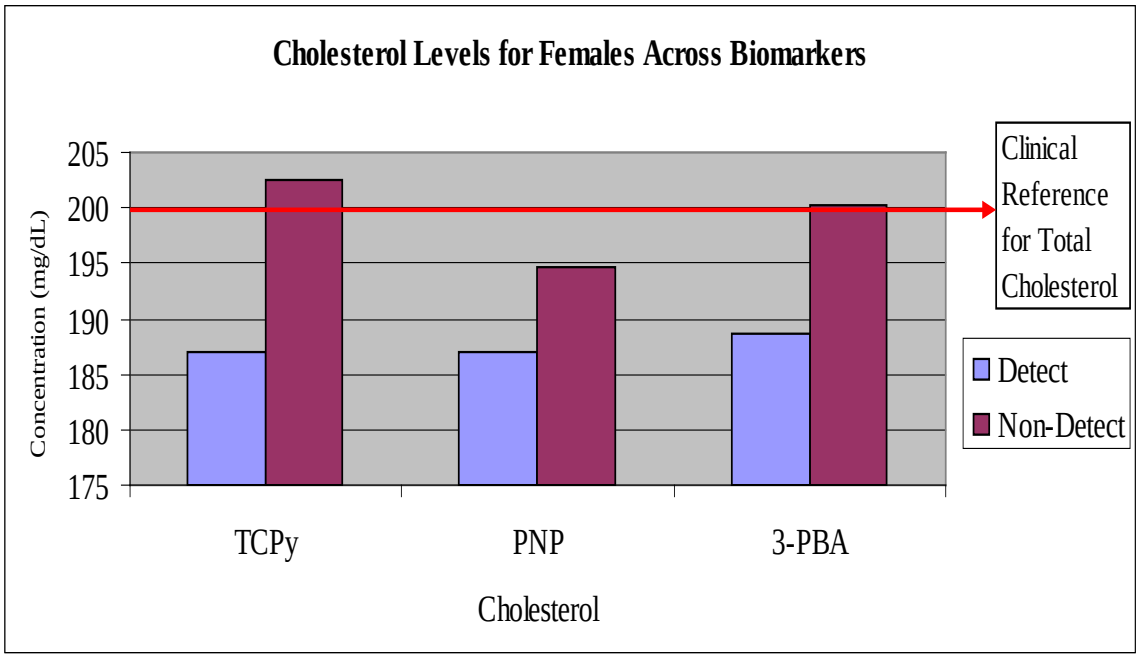

Figure 33: Female Cholesterol Levels Across All Three Biomarkers. mg/dL= Milligram per Deciliter; TCPy= 3,5,6-Trichloropyridinol; PNP = Paranitrophenol; 3-PBA = 3-Phenoxybenzoic Acid. The clinical reference for serum levels of cholesterol are <200 mg/dL for desirable [154]. Borderline high is defined as 200-239 mg/dL [154].

Males did not have a consistent significant alteration for biochemicals across the biomarkers. Differences were limited to a specific biochemical.

Chapter 5

Discussion

5.1 Evaluation of Research Hypotheses

To determine how well the results of this study supported the overall goals of this research, the postulated hypotheses will be examined below:

Hypothesis 1: Biomonitoring data obtained from NHANES indicate the presence of background biomarkers of exposure in individuals from a sample of the general population.

Based on the analysis of the pesticide dataset, there is evidence that pesticide biomarkers are present in a sample of the US general population. However, significant findings varied when examining the biomarker in urine versus correcting for dilution with creatinine. Metabolism rates can vary among individuals [26, 128]. 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 [155]. Barr et al. (2005) suggests that there may be urine dilution variability between groups (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 [155]. Analysis of both urinary biomarker level and creatinine-adjusted levels should be conducted to determine if significance is eliminated or elucidated due to the correction with creatinine, as observed with some groups in this study.

Hypothesis 2: Mean concentrations of biomarkers of exposure are homogeneous across the various subgroups of the sample indicating that no one subgroup is at an increased risk of an adverse health outcome.

Upon examining the mean concentrations of the biomarkers for various subgroups in the study, the mean concentrations are not homogeneous, at least on the surface. Certain groups have a significantly higher mean concentration of the biomarker than other groups. 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 biomarker of exposure concentrations in 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 different rate than adults [156-157]. Different rates of enzyme activity may also affect these results. Increased instances of hand-to-mouth and pica in children may also result in an increase in exposure and explain the findings in this research [108, 158-159].

Significant outcomes discovered in this research could be due to the oversampling of minorities by NHANES researchers in the sample. NHANES oversampled certain subgroups that were the target of a specific health interest [160]. In this study period, NHANES researchers oversampled "*low-income persons, adolescents 12-19 years, persons 60+ years of age, African Americans and Mexican Americans*" [131]. The survey oversampled to increase reliability and precision in the target population [160]. The NHANES documentation does not give a specific reason for not oversampling other subgroups (citing "*cost prohibitive*" or "*operationally not feasible*" as reasons) [160]. This oversampling of certain minority or at-risk groups can overestimate the true exposure. Significant means discovered in this data may be negated if the groups had not been given special focus.

If oversampling had not occurred, and/or if the unused weight supplied by the NHANES documentation for population-based analysis could accurately control for the oversampling, this significance may disappear and the groups may actually be homogenous for mean concentrations of biomarkers. When assessing the population, the results are viewed as non-random and over sampled. As the documentation for the NHANES data suggests, not using appropriate weights may lead to an over-estimate of actual exposure. If this is the case, these data may be viewed as overestimating the actual concentration of the biomarker when comparing to a population. The population levels may actually be lower.

Hypothesis 3: Urine sample data from NHANES reveal that biomarker levels in the sample are not correlated with an increased risk of a negative health outcome.

Based on the overall results of this study, there were no consistent results to suggest that exposed individuals had an increased risk of a negative health outcome. When comparing the weight and height of study participants ages 6-11, those with a recorded biomarker detect versus non-detect, many of the comparisons were not significant, indicating that there was no appreciable difference between the exposed versus non-exposed. No significant results were found when evaluating these differences for 3,5,6-trichloropyridinol exposure. Paranitrophenol 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 ($p=0.022$)]. Heavier children associated with 3-Phenoxybenzoic Acid at age 7: [Detect=28.61 kg and Non-Detect=25.26 kg ($p=0.009$)]. The significant findings are not consistent between all of the exposed 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 at the $p < 0.05$ level. It appears that exposure in this group of children does not have an overall negative association with childhood development. Similar comparisons have been made when evaluating biomarker levels in newborns with negative associations with birth outcomes [104, 107-108].

The biochemical concentrations evaluated in this research can be used to evaluate health status at the time of the sampling. Many of the comparisons were not statistically

significant. For those levels that were significantly different, those groups with a detect for the biomarker were within the normal range. While stratification for gender further elucidated any statistical significance between those with a detectable level of the biomarker versus those without a detectable level, few alterations were consistent across all three biomarkers, or among the biomarkers themselves. The overall biochemical concentrations that had significant findings were cholesterol and sodium. In females, the only consistently significantly different biochemical concentration was cholesterol.

Overall, individuals that recorded a detect for the biomarker had a significantly lower cholesterol level than those without a detect. While establishing a causal relationship is difficult without further investigation, it is possible that those individuals that ate more agricultural commodities had a healthier diet than those that did not have a detect for the biomarker. If a major pathway of exposure to the general population is through the ingestion of residues on fruits and vegetables, then this conclusion may be plausible. However, like the assessment of the biomarkers, the biochemical concentrations are one point in time and do not give a trend to evaluate variations in the levels. Many factors can influence the status of biochemical levels on a physiological level and further research would be necessary to determine if there is a causal relationship [77].

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 correlation/odds of having a detect for a biomarker when related to a reference group. Unlike linear regression, logistic regression does not fully explain the variance of the data. Some groups within each biomarker had a significantly higher correlation and higher odds of having a detect. All models were significant; however, only 3,5,6-trichloropyridinol and 3-phenoxybenzoic acid were the regressions where the independent variables fit the model well. The independent variables did not fit well within the paranitrophenol model. The independent variables for ethnicity and gender in the 3,5,6-trichloropyridinol model reflected the actual odds ratios well. Gender consistency reflected the actual odds ratios for all models.

5.2 Evaluation of Results

Based on the information in Tables 43 and 44, the mean biomarker levels from this study are consistent with measurements of central tendency in other population and sample based research studies. Comparison to other studies can be difficult depending on the statistical test performed or the sample media used, and, as previously noted, creatinine concentrations can vary for a variety of biological reasons. The presentation of the data using multiple forms of descriptive and comparative statistics can make comparison to other studies more plausible.

Data from this NHANES dataset has been partially analyzed previously by Barr et al. in 2010 [64]. The study focused on 3-PBA and used data from both the 1999-2000 and 2001-2001 NHANES data. The study divided participants into only three ethnic groups (Non-Hispanic White, Non-Hispanic Black and Hispanic Americans). The Barr

et al. study used the sample weights found in the NHANES dataset. Weighted geometric mean for the biomarker in urine was determined to be 0.318 µg/L and the creatinine adjusted geometric mean concentration was 0.324 µg/g [64]. Unweighted geometric means from the current research were 0.336 µg/L for the biomarker and 0.323 µg/g when correcting for creatinine. When comparing the data in this research to the Barr et al. study in which the various weights and unequal probabilities of selection as supplied in the NHANES data files were integrated into the analysis, the unadjusted results only overestimate the means by 0.018 µg/L for the biomarker in urine and 0.001µg/g when adjusted for creatinine, suggesting that the results of the unadjusted data reflect the results of the complex study-designed data rather well.

Based on occupational studies, individuals working with pesticides had a chronic exposure and measurable levels of biomarker in their urine. The mean TCPy levels in the Albers et al. (2004) study were ~100 times higher than the mean TCPy concentration found in the current research [101]. The overall lack of significant findings in the Albers et al. study suggest that the individuals in this sample are at less risk of a negative neurological outcome. In addition, the lack of consistency in the studies evaluating exposures and negative birth outcomes gives further support to the final hypothesis of this research. There does not appear to be a consistent correlation between the low-levels observed in this research and an increased risk of an adverse health effect.

5.3 Biomarker of Exposure Research

As mentioned earlier in the literature review, epidemiological studies by Whyatt et al. (Columbia Study), Eskenazi et al. (CHAMACOS) and Berkowitz et al. (Mount Sinai Study) evaluated prenatal exposures to pesticides and negative birth outcomes [104, 107-108]. Results across these studies were not consistent. The Columbia Study indicated an association between chlorpyrifos exposure and reductions in birth weight and length [104]. Further analysis of the Columbia data by Rauh et al. suggested delays in neurodevelopment [105-106]. The studies by Eskenazi et al. and Berkowitz et al. did not find overall significant associations, and in some instances, some associations appeared to be protective [107-108].

Eaton et al., 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 and could have resulted in the negative outcomes discovered in the Columbia study [77]. 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 [77]. 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 [77]. Degradation of the parent compound in the environment

can also lead to TCPy exposure [148]. 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 et al. point 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 [77]. 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 [77].

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 elucidate 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.

Many of the reviewed cross-sectional studies did not evaluate the dose that lead to the detection of the biomarkers in urine. A small sample of studies were identified that attempted to estimate dose based on urinary concentrations of pesticide biomarkers. Fenske et al. (2000) used a deterministic steady state model to estimate doses from urinary concentrations of non-specific OP biomarkers [162]. The spray season creatinine-adjusted dose estimated means were 2.4 to 3.8 µg/kg/day for children with a household relation working in the agricultural field and the single day dose estimated creatinine-adjusted means ranged from 2.5-4.0 µg/kg/day [162]. A second study by Curwin et al. (2007) used a farming community and reference community in Iowa to

estimate a maximum-likelihood geometric mean chlorpyrifos dose of 0.67 µg/kg/day and a maximum dose estimate of 1.96 µg/kg/day [163]. The ATSDR exposure levels from Table 4 for chlorpyrifos put the acute and intermediate threshold dose at 3 µg/kg/day and the chronic dose at 1 µg/kg/day. The average estimate of 0.67 µg/kg/day from the Curwin et al. study is below the chronic threshold dose and the estimated maximum is lower than the acute and intermediate threshold. Because these are estimates, the actual exposed amount may be lower.

Biological Exposure Indices published by the ACGIH established thresholds for biomarkers in occupational settings [55]. 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 (RV_{95}) from biomonitoring data [164]. Heudorf et al. (2006) reports on the use of biomonitoring data from a sample of the German population for non-specific OP biomarkers and biomarkers for pyrethroids [165]. A threshold value was established for 3-PBA and was set at 2µg/L (for children age 3-14). The RV_{95} were statistically derived from the 95% percentile within the 95% CI, are not based on toxicological data and are not related to risk assessment [165]. Because these levels are statistically derived, they should not be used to evaluate adverse health effects from biomonitoring data [164-165]. The RV_{95} 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 value for children age 6-11 in this study fall below this value.

Based on a Pubmed/Medline literature search, it appears research co-authored by Perera at the Columbia Center for Children's Environmental Health has focused on children's prenatal and postnatal exposure to various chemicals and the associated negative developmental outcomes. In addition to pesticides, research at the Columbia Center has associated negative birth outcomes with prenatal exposures to polychlorinated biphenyl ethers (PCBEs) and polycyclic aromatic hydrocarbons (PAHs) [166-167]. The research findings from the Columbia center suggest two possibilities: 1) that the researchers suffer from investigator bias, because they continue to find negative associations between exposure to a variety of chemicals and birth outcomes, or 2) that their research is supporting the fact that there are multiple confounding chemicals that should be considered when investigating negative outcomes and that attributing negative outcomes to pesticides alone is not warranted. It is the unknown exposures that hamper the establishment of causation in biomonitoring exposures scenarios.

5.4 Evaluation of Risk

The FQPA of 1996 required all routes of exposure to be taken into consideration when evaluating risk from a non-occupational exposure to pesticides [68]. This has resulted in the EPA publishing a guidance document on cumulative risk assessment for pesticides. The cumulative risk assessment makes the assumption that the pesticides included in the assessment have a common mechanism of toxicity [168]. Because biomonitoring is an assessment based on all routes of exposure, the cumulative risk assessment is the most appropriate assessment for the current studies researching cumulative exposure data. In 2006, the EPA published an updated cumulative risk

assessment for OP pesticides (an update to the original CRA published in 2002) [169]. This assessment uses a relative potency factor (RPF) to determine joint risk associated with the exposure by using a reference chemical for comparing the toxicity of other OP pesticides [169]. The RPF is the ratio of the toxic potency of an OP to its index chemical (for the EPA CRA, the reference chemical is Methamidophos) [169]. This index pesticide is used to determine toxic potencies and used an exponential dose-response model to determine points of departure (POD) to extrapolate risk from an exposure in human populations [168]. The point of evaluation is the Benchmark Dose (BMD_{10}) in which 10% AChE inhibition takes place in the female rat brain from an oral exposure [169]. This leads to the determination of a Margin of Exposure (MOE). The target MOE is 100 for both one day acute exposure and for a 21 day rolling chronic exposure. The findings from the CRA conclude that:

> "*Taking all of these factors into account, EPA finds that there is a reasonable certainty of no harm to all major, identifiable population subgroups from cumulative exposure to the OPs" [169]*.

A recently published CRA for pyrethroids and pyrethrins finds that there are no cumulative estimated risks of concern [169]. These CRA are based on traditional outcomes from exposure to pesticides. For the pyrethroid CRA, deltametrhin was used as the index chemical [169]. The findings from these CRA are designed to be conservative estimates of risk. This conservative approach adds additional levels of safety into the findings of the assessments.

When reviewing the EPA Integrated Risk Information System (IRIS) database, it was observed that the previous Reference Dose (RfD) of 0.3µg/kg/day for chlorpyrifos had been removed on March 24, 2011 [170]. The RfD remains for both MP and

parathion. It was not clear why the RfD was removed. It may be because the RfD for chlorpyrifos is undergoing re-assessment based on the most recent risk assessment and Interim Registration Eligibility Decision (IRED). The Oral RfD for permethrin remains at 5.0×10^{-2} mg/kg/day [171].

The data obtained from biomonitoring is difficult to relate to risk due to the lack of information on exposure pathway and source of exposure [30]. Information on dose, duration of the exposure, phenotypic and genotypic differences between individuals and the time of the exposure in relation to the biomarker's half-life are important to know when assessing toxicity [29]. However, this information is usually not available for large-scale biomonitoring programs and lack of such information can confound the results. The detection of a biomarker from a biomonitoring event does not mean that the exposed are at an increased risk [29]. There is a dose for a particular chemical at which there will not be an appreciable risk of an adverse health effect.

While various biomonitoring studies have evaluated the presence of biomarkers in biological media, many shy away from assessing risk and suggest that the results serve as a reference range and can be used to evaluate trends in public health [29, 145, 147]. However, previously mentioned studies have used biomonitoring data to form associations between low-level pesticides and negative 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 exposure factors in the current epidemiological biomonitoring studies that may

allow the elucidation of causality. 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.

5.5 Limitations of the Research

It is unknown if the associations found in this research are causal based on the supplied information. A more accurate way to identify health risk would be a longitudinal study [29]. This would assist in establishing a temporal relationship. However, this design also has limitation beyond normal bias. The mechanism for the purported adverse health effects from chronic, low-level exposure, if it exists, is unknown and thus makes evaluating the dose-response relationship difficult.

Statistical comparisons were made in this research between detectable and non-detectable levels of biomarkers of exposure in urine. It is plausible that the individuals with a recorded non-detect were actually exposed, but those exposures were not detected due to the half-life of the biomarker and the laboratory limit of detection. This could have introduced misclassification bias into the research. However, this bias would be expected in other epidemiological studies using the same criteria. The oversampling of certain subgroups may have introduced sampling bias into the analysis as well.

Chapter 6

Conclusion

This research study used urinary biomarker of exposure levels from the 2001-2002 National Health and Nutrition Examination Survey (NHANES) national sampling event to determine if chronic, low-level exposure to pesticides can be associated with an appreciable increase in risk of an adverse health event. The research determined 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 and adolescents and biochemical concentrations across individuals in the study revealed significant differences, but the differences were not consistent across the biomarkers. 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 negative outcomes from exposure. In instances where significant differences between biochemical concentrations where found, the group with detectable levels of a biomarker did not exceed established clinical reference values. In fact, cholesterol levels for overall

biomarker concentration and females were consistently and significantly lower than those with a non-detect for the biomarker.

As previously noted, based on the weight of evidence from the studies investigating low levels of biomarkers of exposure in biological media, there are not enough significant findings to conclude that these exposures would result in adverse health effects or increase the risk of such outcomes. While young individuals may be more sensitive to AChE inhibition from acute exposures, there is little evidence to suggest that the pesticides examined in this research bioaccumulate in any body compartment [75]. Metabolism and elimination of the parent compound and intermediates appears to occur quickly [75, 120].

Biomarker of exposure concentrations from national sampling events can be useful for establishing ranges of background exposures, but only if the data is unbiased. Oversampling of certain subpopulations can skew the results and give an inaccurate baseline for evaluation. Additionally, the physical sampling locations for the national sampling should be made available to determine if one area has an elevated exposure level as compared to another.

Any further studies investigating low-level pesticide exposures and neurodevelopment impairment should seek to further limit investigator bias that skews the research results. Conducting research with a pre-conceived notion that exposures to pesticides, and other chemicals, will be associated with negative outcomes can have an

effect on results and can negatively influence any regulatory decisions based on those results.

Conclusions from this study are:

1. Biomarkers of exposure are present in a sample of the US population and certain subgroups have significantly higher geometric means than others.
2. Due to the cross-sectional nature of the data, there is no clear way of establishing causation based on the associations found.
3. Oversampling of certain at-risk groups may have skewed the findings in the research.
4. Evaluation of health status of the individuals in the sample does not indicate that there is an overall negative health impact associated with exposures.

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