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I am submitting herewith a dissertation written by Karyn Ann Casey entitled "Chlorpyrifos in Human Breast Milk?" I have examined the final paper copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor Of Philosophy, with a major in Nursing.

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CHLORPYRIFOS IN HUMAN BREAST MILK?

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Karyn A. Casey
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DEDICATION

This dissertation is dedicated to my husband and love of my life, Dr. Mike Casey (the Real Doctor), the other love in my life, my son, Michael John-Kent Casey, and my family. Thank you for your love, support, guidance and confidence. I am truly blessed to have you all in my life.

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ABSTRACT

The widespread use of pesticides by farmers, pest control operators and even the general public can pose significant risks to children's health. One particular pesticide, chlorpyrifos, was the most widely used pesticide in the United States with total use estimated at approximately 30 million pounds per year. Young children and the developing fetus are far more susceptible to the effects of pesticide exposure as a result of unusual exposure patterns and developmental immaturities. Transplacental transfer and lactational exposure are the pathways exclusive to the developing fetus and infant. Chlorpyrifos exposure is of special concern in this population because of its potential for disruption of normal brain function and cognitive development.

The purpose of this study was to determine the concentration of chlorpyrifos in three different biological specimens: breast milk, plasma, and saliva. The research method employed for this study was a secondary analysis of existing samples collected for the purposes of a prior study. Based on specimen availability, there were a total of 26 subjects each for the lactating group and the non-lactating group. The lactation group had 26 matched breast milk, plasma and saliva samples and the non-lactation group had 26 matched plasma and saliva samples. The biological specimens were tested in the laboratory using an assay specific to chlorpyrifos. The Chlorpyrifos RaPID Assay was developed by Strategic Diagnostics, Inc and had a detection limit of 0.1 ng/ml (ppb) and a linear range of 0.22 to 3.0 ng/ml (ppb).

Overall, for the lactation group, 25/26 samples were positive for chlorpyrifos with milk concentrations significantly higher than plasma or salivary concentrations. 19/26 plasma samples were positive and 10/26 saliva samples were positive for chlorpyrifos. For the non-lactation group, 23/26 plasma samples were positive for chlorpyrifos and 16/26 saliva samples were positive. In addition, the plasma chlorpyrifos concentrations were significantly higher than salivary concentrations in the non-lactation group. Comparing the two groups, salivary chlorpyrifos concentrations were significantly higher in the lactation group.

There is mounting evidence of pesticide contamination in the breast milk of lactating women. This study has contributed to this knowledge by documenting chlorpyrifos concentrations in breast milk, plasma, and saliva of lactating and non-lactating mothers. However, further research needs to be done to determine what these chemicals are doing to our children.

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CHAPTER ONE

INTRODUCTION

The widespread use of pesticides by farmers, pest control operators and even the general public can pose significant risks to human health, especially children. One particular pesticide, chlorpyrifos, is the most widely used pesticide in the United States with total use estimated at approximately 30 million pounds per year (EWG, 1998). Young children and the developing fetus are far more susceptible to the effects of pesticide exposure as a result of unusual exposure patterns and developmental immaturities. Transplacental transfer and lactational exposure are the pathways exclusive to the developing fetus and infant (Byczkowski, Kacew, Miller, Pelkoneu & Saunders, 2001). Because brain growth is very rapid prior to and following birth, chlorpyrifos exposure is of special concern in this population because of its potential for disruption of normal brain growth function and cognitive development. There is mounting concern that repeated low-level exposure to organophosphorus pesticides in utero or postnatally increases the risk of poor performance on neurobehavioral and cognitive examinations during infancy and childhood (Olson, Blank, & Menton, 1998; Weiss & Landrigan, 2000).

Pesticides

Pesticides, which include insecticides, herbicides, and fungicides, are regulated under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). This was the first attempt to address the potential risks to human health posed by pesticide products. The act directs the Environmental Protection Agency (EPA) to

restrict the use of pesticides as necessary to prevent unreasonable adverse effects on people and the environment and prohibits the sale of any pesticide in the US unless it is registered and labeled according to appropriate uses and restrictions. This law specifies that a pesticide may not be marketed until the EPA has registered it. To register a pesticide as an active ingredient, pesticide product, or a new use of a registered pesticide, The EPA requires submission of scientific data on pesticide toxicity and behavior in the environment (this may require data from any combination of more than 100 different tests, depending on the toxicity and degree of exposure).

Toxicity studies are required to assess the potential hazards to humans through varying degrees of exposure (acute, sub-chronic, and chronic) of lab animals to pesticides. Acute toxicity studies provide information on short-term exposures. The first step evaluates oral, dermal, and inhalation toxicities. Sub-chronic (90 day) studies provide information on toxicities that may be a result of repeated exposures to a pesticide over a period up to 30% of the lifetime of a rodent. Chronic (1 to 2 year) studies provide information on toxicities that occur over a large portion of the life span with particular emphasis on carcinogenicity or tumor development. Unfortunately, the EPA does not routinely require neurodevelopmental toxicity testing or functional assessment of the nervous system, even on neurotoxic chemicals (Wallinga, 1998). Nor do they require lifetime testing of those animals exposed in utero or infancy to assess for diseases that might develop later in life. A pesticide is subjected to close scrutiny if the EPA concludes that the chemical poses a significant risk to humans based

on animal studies. However, even if a pesticide is convincingly shown to be teratogenic, the law allows the chemical to be registered if the economic benefits outweigh the risk (Chaffrey, 2000).

Due to the increasing environmental concerns and the vulnerabilities of developing infants and children, Congress specifically requested that the EPA increase the stringency of its requirements for neurotoxic and behavioral testing on prenatal and neonatal animals (Merrill, 2001). Of the 800,000 pesticides on the market, only a few have been evaluated for their cumulative effects on children, and most of the 140 neurotoxic pesticides have not been tested (Anderson, Nielsen, & Grandjean, 2000). The EPA advisory committee has recently identified organophosphate insecticide effects as one of the five areas where children's vulnerabilities have not been adequately assessed by existing EPA regulations (Wigle, 2003).

Because humans are exposed to a variety of chemicals in the environment, US Supreme Court validated the need for a method to determine the inherent health risks associated with chemical exposure. Together with Federal Regulatory Agencies, a framework was developed. This was known as Risk Assessment in the Federal Government: Managing the Process (or simply the Red Book). Risk assessment is defined as the systematic scientific characterization of potential adverse health effects resulting from human exposure to hazardous agents (Hooper, 1989). Because all chemical risk assessment scenarios are not the same, the risk assessment process provides a methodological approach to ensure consistent analysis of different chemicals.

This process involves four steps: hazard identification, dose-response assessment, exposure assessment, and risk characterization or effects assessment (Faustman & Omenn, 2001).

Hazard identification involves the toxicity assessment of chemicals and whether they have the ability to produce adverse health effects in animals or humans. This can involve structure-activity analysis, animal bioassays, and epidemiological approaches. Structure activity relationships (SARS) predict toxic properties from information on molecular structure with the concept that if a particular chemical is known to be a carcinogen then another chemical with the same molecular structure will also be a carcinogen (Gramatica, 2004). Lifetime animal assays were developed as a gold standard to establish such end points as carcinogenicity or teratogenicity. The premise with this test was that it would be extrapolated to humans. The most convincing hazard assessment approach involves the use of epidemiological studies to assess the correlation between exposure and disease.

The second step of the risk assessment process is that of the chemical toxicity or dose-response assessment. This step is important in determining where on the dose-response curve an adverse health effect is expected to occur or. Sometimes it is used to determine the safe exposure level that is sufficiently small enough not to cause any adverse human health effects. The threshold dose is the dose below, which no change in biological response occurs, followed by the no observed adverse effect level (NOAEL) and the lowest observed adverse effect level (LOAEL).

The exposure assessment involves a quantitative estimation of the environmental exposure and the subsequent entry into the human body. This assessment addresses three areas: the exposure (the number of people exposed at specific concentrations for the time period of interest), the resulting dose, and the cause (the contribution of important sources and pathways to exposure or dose) (Sexton et al, 1995). The primary goal is not only to determine the amount of exposure but also to find out how much of a specific agent may be reaching target organs in the body.

Risk characterization is the final step in the risk assessment and is also know as the effects assessment. This step utilizes all of the information obtained in the previous three previous quantitative methods to provide a qualitative conclusion of the likelihood that a particular chemical is able to produce adverse health effects and is therefore, a human health hazard (Sexton et al, 1995). Other questions addressed in risk characterization include: what is the nature and estimated incidence of adverse effects in a given population and how robust is the evidence?

Because there are so many inherent assumptions and extrapolations being made, the risk assessment process can be a source of uncertainty versus actuality. Although some believe that the predominant problem with the risk assessment is over exaggeration or risk estimates, the majority of scientists, toxicologists, and regulatory agencies believe that there are flaws in the process that need to be improved. The extrapolation process is considered a weakness because of the uncertainty associated with the assessment factors.

Extrapolations may be from one species to another or within the same species, from one route of exposure in one species to a different route of exposure in another species, and from continuous exposure conditions in the laboratory to varying human exposures in the environment. Although the debate over the risk assessment is well founded due to the potential inadequacies and uncertainties, the process does provide valuable environmental toxicological information on potential human health hazards.

Insecticides. Insecticides are classified according to three major categories, the organochlorides, the pyrethroids, and the anticholinesterase agents. The differences between the effects of these various chemicals relate primarily to their ability to interfere with the membrane transport of sodium, potassium, calcium, or chloride; ability to inhibit enzyme activities; or the ability to inhibit the breakdown of neurotransmitters at nerve endings (Ecobichon, 2001). The United States government banned several chemicals in the organochloride class because of their high toxicity potential. The year 1962 was known as the "Silent Spring" because of one such chemical. DDT, one of the most powerful pesticides the world has ever known, was developed in 1939 to protect U.S. troops from malaria in the South Pacific Islands. The inventor was awarded the Nobel Prize. It became available for civilian use in 1945. Thirteen years later, a marine biologist with the U.S. Fish and Wildlife Service received a letter from a friend outlining the dangers associated with DDT sprayings. It took four years for Rachel Carson to complete the book entitled "Silent Spring". The book meticulously described the sequential effects associated with DDT sprayings.

Following a single crop application of DDT, it remained toxic in the environment for years even after it was diluted by rainwater. Not only was DDT killing insects but also fish, birds and eventually up the food chain to humans and thereby contaminating the entire world food supply. The book's final chapter illustrates a nameless American town where all life, from fish to birds to apples to human children, had been silenced by the insidious effects of DDT (Carson, 1962). As a result, the EPA banned the use of DDT in 1972. In the late 1980's the EPA also banned other highly toxic organochlorine chemicals like Aldrin, Dieldrin, and Chlordane.

The anticholinesterase insecticides include two chemical classes, the carbamates and the organophosphates. These particular chemicals have become widely used as replacements for the more persistent organochlorides but are receiving more attention because of their acute neurobehavioral effects. Carbamates are primarily used in vector control on lawns and gardens. They are part of a large group of synthetic pesticides that have been developed in the last 40 years.

The organophosphates were first developed for agricultural use for the control of insects. Their acute lethality is due to their ability to inhibit an enzyme that is vital to normal nerve function. Subsequently, organophosphates have been used on humans as well. The Germans used them in WWII as chemical warfare agents (Ecobichon, 1997). Japanese terrorists used them as nerve agents in a Tokyo subway in 1995, and there have been concerns of possible organophosphate use in the first Gulf War.

The organophosphates are esters of phosphoric or phosphorothioic acids with R_1, R_2, and R_3 substituents directly to or through an oxygen to the phosphorus carbon. These substituents can include the alkyl, alkoxy, or aryl groups (Chambers, 1992). Metabolism occurs chiefly by hydrolysis in the liver. Since the breakdown is relatively slow, significant storage in body fat may occur. Some organophosphates are extremely lipid soluble allowing them to be stored in fat with subsequent delayed toxicity due to late release (Ecobichon, 2001).

There are direct and indirect organophosphate inhibitors. Those that are direct (parathion, diazinon, malathion, and chlorpyrifos) require metabolic activation before they can inhibit acetylcholinesterase. This activation is accomplished by the conversion from a thion (P=S) to an oxon (P=O) by the action of the liver microsomes utilizing the cytochrome P450 enzymes (Vale, 1998). Oxidative desulfuration results in the formation of the oxon of the parent compound. Ultimately, both thions and oxons are hydrolyzed at the ester linkage, yielding alkyl phosphates and leaving groups, which are then either excreted or further transformed in the body (Ecobichon, 1997).

Chlorpyrifos. This very common organophosphate gained popularity in 1965 because of the decreased availability of chemicals in the organochlorine class. In the mid 1990's, chlorpyrifos was ranked 12th in frequency of indoor pesticide applications and 5th in frequency of outdoor pesticide applications (Robinson, Pease & Albright, 1994). According to the NHEXAS study by Sexton et al (1995) and the Minnesota Children's Pesticide study by Quackenboss et al (2000), the majority of the U.S. population is exposed to chlorpyrifos. These

epidemiological studies reported measurable concentrations of TCP (3, 5, 6-trichloro-2-pyridinol), the primary urinary metabolite of chlorpyrifos, in 82% of 993 adults and 92% of children. Chlorpyrifos residues were also found in air samples in 83-97% of homes in Florida and in 30-40% of homes in Massachusetts (Whitmore, Immerman, Camann, Bond, Lewis & Schaum, 1994). There are approximately 822 registered products containing chlorpyrifos on the market that are applied in and around homes more than 20 million times a year in the United States (Steenland, Dick, Howell, Chrislip, Hines, & Reed, 2000). Manufacturers of chlorpyrifos earn a reported $350 million a year from chlorpyrifos sales in the United States, not including exports sales to over 88 countries.

Agriculturally, chlorpyrifos is used on a variety of food crops such as citrus crops, vegetable crops, and tree fruits. It is also used as a mosquitocide and as an insecticidal ear tag for cattle. Professionally, chlorpyrifos is used in residential and commercial buildings, schools, daycare centers, hotels, restaurants, hospitals, and food manufacturing plants. The majority of urban use is involved in termite control around baseboards. It is also injected into cracks and crevices for roach control and as a broadcast application in the treatment of fleas.

The most common use of this pesticide occurs in the home by the so-called "do-it-yourselfers" as it can be found under the sinks or in the garages of most homes. Chlorpyrifos-containing products are used in the kitchen, bathroom, and other living spaces (Whitmore, Kelly, & Reading, 1992). It is used as a spray to kill roaches or ants, and it is used as a fogger to get rid of fleas. Such products include: Black Flag Roach and Ant Killer, Ortho Home Pest Insect Control and

Lawn Insect Spray, Raid Ant Controller and Home Insect Killer Formula, Sergeant's Fast-Acting Flea and Tick Collar, and Hartz 30 Day Flea and Tick Collar for Dogs. Indoor use by unlicensed or untrained applicators has resulted in excessive human exposure with most of the incidents occurring as a result of misuse (Blondell, Suhre & Smegal, 1999). Among the general population, people who use the insecticide in homes and gardens and people who ingest food exposed to chlorpyrifos are at higher risks of exposure (U.S. Department of Health and Human Services, 1997).

Chlorpyrifos, [O, O-diethyl O- (3, 5, 6-trichloro-2-pyridinyl)-phosphorothionate] is a broad-spectrum organophosphate insecticide. It acts primarily as a contact poison and is available in a variety of formulations (granules, powder, and liquid spray). Chlorpyrifos enters the environment as a result of its widespread use as an insecticide. It is released into the air by volatilization during foliage or soil application or by broadcast application. Chlorpyrifos is considered a moderately volatile chemical meaning that it will exist primarily in the vapor phase but will also partition to available airborne particulate suggesting that it may travel long distances in the air (Racke, 1993).

Chlorpyrifos is considered to be practically insoluble in water, but persistence in water has been measured between 35 and 140 days (McDougall, Wan & Harris, 1994). It has a strong tendency to partition into the soil and sediment and has an affinity for sorption. Once in the soil, chlorpyrifos degrades under ultraviolet light via chemical hydrolysis and by the action of soil microbes. The persistence of chlorpyrifos in soil metabolism half-life ranges from 11 to 180

days; however, much longer soil half-lives of 175 to 1576 days has been reported following termiticide applications (Howard, 1991). Once in the environment, chlorpyrifos can then accumulate into living creatures resulting in bioaccumulation up the food chain. Chlorpyrifos has been found to be extremely toxic to freshwater fish (Srivasta, Tiwari & Srivastava, 1990), birds (Simpson, 1984), cats (Jaggy & Oliver, 1990), and monkeys (Brack & Rothe, 1982).

Exposure Assessment

Chlorpyrifos exposure can occur through several pathways. Oral exposure occurs through the ingestion of residues on food crops, fish, meat, or dairy products. The U.S. Food and Drug Administration (1994) found that chlorpryifos was the fifth most common detected pesticide on food crops. Chlorpyrifos residues have been found in channel catfish following waterborne exposure (Barron, Plakas & Wilga, 1991). Residues have also been found in beef (Ivey, Mann & Oehler, 1972) and pork (Ivey & Palmer, 1979) following treatment for ticks and lice. In all cases fat appeared to have higher chlorpyrifos concentrations than other tissues (Cox, 1995). Residues were also found in dairy cow milk and cream (McKellar, 1976).

Dermal exposure can occur through skin contact with contaminated surfaces. Chlorpyrifos residues were detectable on soft, absorbent surfaces, such as stuffed toys, furniture, and carpet for up to two weeks following a fogging application (Gurnunathan, Freeman, Buckley, Roy, Meyer, Bukowski & Lioy, 1998). Transferable chlorpyrifos residues have been found on children following direct contact with their treated pets (Boone, Tyler & Chambers, 2001).

Inhalation exposure can occur from the aerosols and vapors associated with spraying or fogging techniques. Crack and crevice treatments are applied where the insect is most likely to be found. Chlorpyrifos residues have been found in air samples for up to eight years following termite application (Wright, Leidy & Dupree, 1994). Broadcast applications have found chlorpyrifos residues up to 10 days following treatment (Currie, McDonald & Chung, 1990). Air residues were also collected for up to 30 days following the use of chlorpyrifos pest control strips (Jackson & Lewis, 1981).

Because chlorpyrifos is extremely lipid soluble and therefore poorly eliminated, there is great concern that this chemical can be transferred from the pregnant woman to the developing fetus. In the developing fetus, the exclusive exposure pathway is transplacental transfer from the mother's blood circulation to the placenta. Because the placenta is a lipid membrane, lipid soluble chemicals that are nonionized with low molecular weights rapidly cross the placenta to the fetus. Evidence of anticholinesterase insecticide transfer (eg. organophosphates and carbamates) has been substantiated in the literature by measurements of cholinesterase inhibition in fetal tissues (Gupta & Sastry, 1995; Lassiter, Barone, Moser & Padilla, 1999). Whyatt and Barr (2001) also provided evidence of organophosphate placental transfer in their study of newborn meconium. Chlorpyrifos metabolites were found in 95-100% of meconium samples collected from the diapers of 20 newborns.

Lactational transfer pathway. The lactational transfer pathway is another possible route of infant exposure to chlorpyrifos. Lipophilic chemicals can

accumulate in maternal fat reserves and then be mobilized through lactation and transferred to the nursing infant through the process of depuration (Groer, Humenick & Wilson, 2002). In this process, lipophilic compounds can be released from storage depots or fat deposits and then released back into circulation, allowing them to be taken up into the fat of the mammory gland (Clewell & Gearhart, 2002). There are approximately 50 environmental contaminants that have been detected in human milk (Giroux, Lapointe & Baril, 1992). Since breast tissue has a high fat content, it is possible that chlorpyrifos is transferred to the infant during lactation, thus becoming an excretion pathway for the mother. The transfer of chemicals from maternal blood to breast milk is dependent upon the following factors: the chemical, the mother, the milk, and the infant.

The factors associated with chemical transfer include: 1) lipid solubility, 2) molecular weight, 3) protein binding, and 4) degree of pH ionization (Pons, Rey & Matheson, 1994). Lipid soluble chemicals partition into cell membranes more rapidly than water soluble chemicals in that they rapidly dissolve into the lipid bilayer. This process is known as the lipid (or octanol): water partition coefficient. Unlike plasma, breast milk contains emulsified fat (approximately 3.2 to 3.6%) that can concentrate lipid soluble molecules (Byczkowski et al, 2001).

The mammary epithelium membrane is semipermeable in that it allows chemicals of low molecular weights passage through the membrane. Generally, chemicals that have a molecular weight of 200 or greater have difficulty crossing the lipid membrane but studies have shown that large molecules can cross over

into breast milk (Black, 1996). Larger chemical molecules may be transported biologically active into the alveolar cell by pinocytosis (engulfing of liquids or particles) and then released into the milk by apocrine secretion (Institute of Medicine, 1991).

Chemicals are transported in the bloodstream unbound or partly bound to blood components (plasma or proteins). Only those chemicals that are not protein bound can leave the maternal circulation and diffuse across the cell membrane. By binding to proteins, chemical molecules become trapped and unavailable for lactational transfer. Breast milk contains several proteins; however, none of the major proteins tend to bind chemicals (Clewell and Gearhart, 2002). The lipid soluble chemicals are most often stored in adipose tissues and are in exchange with the circulatory system to reach equilibrium. This results in the toxicant being mobilized into the blood for further distribution and elimination or being redeposited in other adipose tissue.

The degree of ionization is the final factor associated with the chemicals transferability into breast milk. Most chemicals are either weak acids or weak bases and exist in either an ionized or non-ionized form (Harvey & Champe, 1992). Weak bases are nonionized in plasma which increases their lipid solubility and transferability into breast milk. This degree of ionization determines the percentage of un-ionized molecules that can cross the membrane (Wilson, 1981). Distribution of an ionized molecule is determined by its p*Ka* (the pH at which concentrations of non-ionized and ionized forms of the chemical are

equal). For a weak acid, the higher the pH, the lower the ratio of unionized to ionized forms. The opposite is true for a weak base. The lower pH of milk (6.8 compared to plasma at 7.4) allows some weak bases to become ionized preventing them from diffusing back across the membrane (Byczkowski, 2001). The chemical then becomes trapped with the possibility of achieving an even higher concentration in the milk. Therefore those chemicals that are lipid soluble with weak bases generally pass through the membrane easily, concentrate in the milk fat globules that then lead to a high ratio of the chemical in milk as compared to the plasma (Dorman et al, 2001)

Several maternal factors influence the risk of chemical concentration in the breast milk. These factors include the route of exposure, the amount of chemical absorbed into maternal circulation, maternal age and weight, number of births, and timing and duration of lactation (Barrett, 2001). The route of exposure will determine how much of the chemical will be absorbed and distributed to the target sites. Because most toxicants are accumulated over the lifespan, bioaccumulation would be expected to increase with age. Maternal weight or body fat contributes to the concentration of chemicals in the breast milk as persistent lipophilic toxicants are stored in fat deposits. First-born children who were breast-fed would be exposed to a higher concentration of toxicants than subsequent children (Furst, Kruger, Meemke & Groebel, 1989). Since the lipid content in breast milk varies according to feedings, those who nursed for longer periods at a time would be exposed to a higher concentration than those that

nurse for short periods of time (Clewell & Gearhart, 2002). Lactation may be an efficient way to excrete the maternal body burden of such toxicants in that some species can reduce their body burden by as much as 60-80% (Byczkowski, 2001).

Lactational factors include milk composition, milk to plasma ratio, and blood milk barrier. The composition of milk changes according to the gestational age of the baby. Lactogenesis occurs around the time of delivery and is defined as the onset of copious milk secretion (Neville, Morton & Umemura, 2001). During stage 1 alveolar cells are differentiated from secretory cells. In stage 2 (occurs day 2 or 3 to day 8 following birth) endocrine control switches to autocrine (supply-demand) control and milk secretion begins (Neville et al, 2001). The first milk that is secreted is known as colostrum and contains large quantities of protective substances needed for the newborn. It is full of proteins, vitamins, and immunoglobulins. The next milk stage is commonly called transition milk as it occurs days 6-10. During this stage, immunoglobulin and protein content falls while fat and sugar contents rise (IOM, 1991). Fat content during lactogenesis increases from 2.9% in the first week to approximately 4% in mature milk. In addition, the fat content in hindmilk is higher than foremilk, 4-6% and 1-2%, respectively. (Needham & Wang, 2002).

Galactopoiesis (later than 9 days after birth) is referred to as the mature milk stage. Milk volume increases from a mean of 50 ml per day to approximately 500 ml per day. The milk that comes at the beginning of the feeding is known as

the foremilk and has a high level of sugar, protein, vitamins and minerals and a lower level of fat. The hindmilk comes towards the end of the feeding and is much richer in fat. This diurinal variation is more likely due to the varying degrees of breast emptying as opposed to a variation in lipid synthesis (IOM, 1991).

The milk to plasma (M/P) ratio refers to the amount of chemical milk concentration compared to maternal plasma (Ito & Koren, 1994). The amount of chemical concentration in milk depends on its half-life in the plasma and the rate of transfer across the mammary epithelium membrane. Those chemicals with a short plasma half-life may not have time to equilibrate across the epithelium resulting in higher plasma concentrations and lower milk concentrations. The milk to plasma ratio is used to determine the extent of chemical excretion in the milk. Those chemicals with a low M/P ratio are less likely to cause adverse effects in the nursing infant; however, most environmental chemicals have higher ratios which is due to their lipophilicity, poor water solubility and small ionization potential (Berlin & Kacew, 1997).

The infant factors associated with the risk of chemical toxicity include the frequency and volume of feedings, absorption and distribution of the chemical, and clearance of the chemical by the infant. Those infants that breastfeed eight times or more a day are more likely to have a higher chemical concentration than those that breast-feed only once or twice a day. For infants one to six months old, the EPA (1997) suggests using an average breast milk intake of 742 ml/day.

Once inside the body, the chemical is distributed to target organs or storage depots via the bloodstream. Since adipose and breast milk are considered lipid rich matrices, lipophilic chemicals tend to partition among the lipid stores in blood, breast milk, and adipose tissue on a 1:1:1 basis (Clewell & Gearhart, 2002). Chemicals can also be deposited in secretory structures such as tears, saliva, sweat, and milk (Barr, Wang, & Needham, 2005).

Effects Assessment

Toxicokinetics is the study of how a substance gets into the body and what happens to it once in the body. The processes involved include absorption, distribution, biotransformation, and excretion. Toxicants that are absorbed through the gastrointestinal tract are carried by the blood directly to the liver and are subject to biotransformation or excretion by the liver. Those that are absorbed through the lung or skin enter the bloodstream with blood filtered by the liver and kidneys. Once chlorpyrifos is absorbed into the bloodstream it may be excreted, stored, biotransformed into a different chemical or metabolite, its metabolites may be excreted or stored, and/or it or its metabolites may interact or bind with cellular components (Ecobichon, 2001). If a chemical is biotransformed into hydrophilic metabolites then it becomes detoxified, or less toxic, and is more easily excreted by the kidneys. However, sometimes it can be biotransformed into metabolites that are more toxic than the parent compound, which is known as bioactivation (Ecobichon, 2001).

The liver is the primary biotransforming organ due to its high concentration of enzymes. The primary enzyme system is the cytochrome P450 enzymes, a

microsomal superfamily of isoenzymes that transfer electrons and thereby catalyze the oxidation of many chemicals (Wexler, 1998). Phase 1 reactions involve the formation of a new or modified functional group or a cleavage utilizing the following transformation reactions: oxidation, reduction, hydrolysis, and/or acetylation (Vale, 1998). Because most of the phase 1 chemicals do not possess enough hydrophilicity to be eliminated from the body, they must now go through a second phase. In phase 2 the new modified chemical is conjugated (joined together) with another substance to decrease its ability to cross cell membranes and increase its polarity, water solubility and thus excretion from the body (Vale, 1998).

Chlorpyrifos utilizes the cytochrome P450 monooxygenases to bioactivate the parent compound to the oxon or phosphate metabolites. Through oxidation, the parent compound can be oxidized to form the oxon derivative, remain unchanged, or form des-ethyl chlorpyrifos (Wessels, 2003). Each of these can inhibit cholinesterase to form trichloropyridonol (TCP) as a by-product, which is then usually conjugated and excreted (Wessels, 2003). However, in order for this insecticide to biotransform into a potent anticholinesterase inhibitor, it must convert from a thion (P=S) to an oxon (P=O) (Vale, 1998). If the phase 2 process gets over saturated with too much of the chemical, the pathway is unable to sufficiently transform the chemical and the level of the parent toxin builds or enters different pathway that can result in the production of a more toxic metabolite (Figure 1).

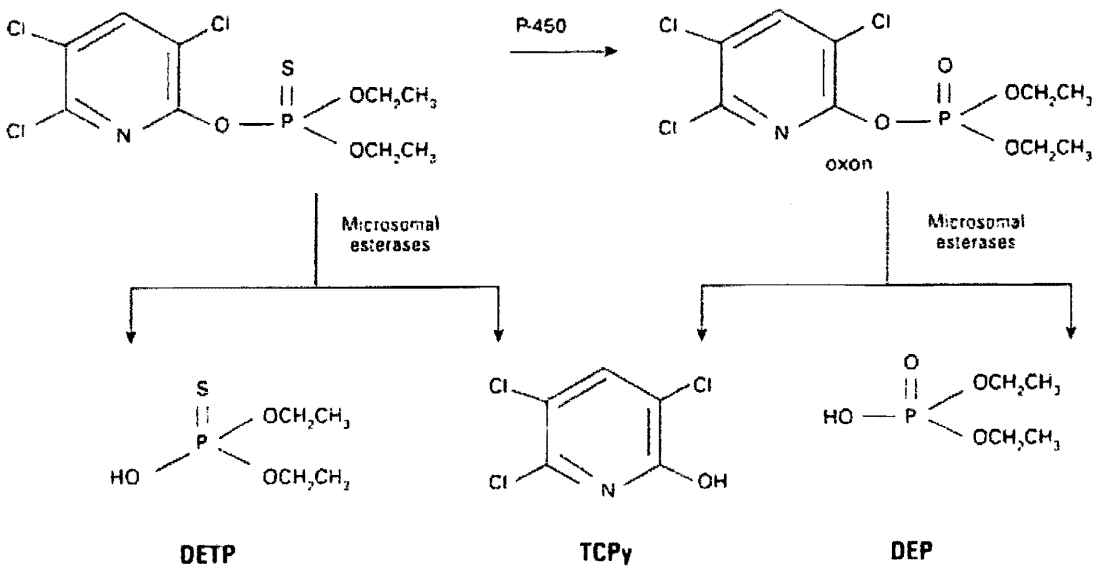


Figure 1. The metabolic activation from chlorpyrifos to chlorpyrifos oxon

Chlorpyrifos is a neurotoxic agent in that it causes damage to the cells of the central nervous system and the peripheral nervous system. The toxic action of chlorpyrifos is acetylcholinesterase inhibition (Joesten, Netterville & wood, 1993). This chemical inhibits the enzyme acetylcholinesterase in the nerve endings, resulting in the accumulation of the neurotransmitter, acetylcholine. If cholinesterase activity is inhibited, acetylcholine does not break down in the synaptic cleft leading to an excessive transmission of nerve impulses at the neuromuscular junctions, both centrally and peripherally (Joesten, 1993). In the central nervous system, excessive accumulation can cause sensory and behavioral disturbances, incoordination, depressed motor function, and respiratory depression (Ecobichon, 2001). Peripherally, overaccumulation can

result in bronchospasm, dyspnea, bradycardia, muscle fasciculations, motor weakness, and paralysis (Ecobichon, 2001).

In animal studies, chlorpyrifos inhibition of acetylcholinesterase activity has been measured at doses that are much smaller than the LD_{50} (dose producing 50% lethality). The inhibition of acetylcholinesterase has been measured two weeks, one month, and six weeks after exposure (Cox, 1994). According to Cox (1994), "researchers believe that this is because chlorpyrifos is lipophilic (attracted to the non water soluble, fatty parts of body tissues) meaning that chlorpyrifos is stored but then released and transformed to chlorpyrifos oxon so that effects (measured in behavioral changes) occur over a long interval" (p.3).

Infants and children have unique characteristics that make them more vulnerable to the toxic effects of pesticides. First, they grow and develop at a rapid rate and any chemical exposure could result in permanent or irreversible dysfunction (Bearer, 1995). Second, the immaturity of their metabolic pathways leaves them unable to detoxify such chemicals (Eskenazi, Bradman & Castorina, 1999). Finally, chemical exposures early in life can result in lifelong diseases like cancer. Of particular concern is the belief that exposure to neurotoxic pesticides like chlorpyrifos can lead to chronic neurological diseases such as dementia or Parkinson's disease (National Research Council, 1992). The underlying molecular and cellular processes are vulnerable to disruption by toxicants and can cause irreversible adverse effects on body structure and function (National

Resource Council, 1993). The scientific community has taken a keen interest in the adverse effects of developmental exposures and outcomes.

Developmental toxicity refers to adverse effects to the developing embryo, fetus, or neonate. The manifestations of developmental toxicity vary according to the timing of the chemical exposure and the underlying processes that are occurring at that time. Those manifestations include: death of the developing organism (embryolethality), malformation (teratogenicity), growth retardation (embryotoxicity) and/or functional deficits (Schmidt & Johnson, 1997).

Functional deficits are of great concern because of the sensitivity of the developing brain and central nervous system. Chemicals reach this system by the blood circulation. Because the central nervous system is so well perfused, lipid soluble chemicals enter the brain and exert their toxicities rapidly. Exposure to toxins can result in a magnitude of effects from mental retardation to significant learning disabilities. Behavioral, mental, and motor deficits are among the possible adverse outcomes (Rogers & Kavlock, 2001).

Because chlorpyrifos is a neurotoxic chemical, the anatomical development of the fetus and infant is extremely vulnerable to its harmful effects. The developing nervous system begins at three weeks of gestation and is not complete until approximately six months after birth (Eliot, 2000). With different regions of the brain developing at different times, there are vulnerable periods throughout neurogenesis. For example, neural tube closure occurs during early gestation, diencephalon and cerebellum growth peak at birth and 2 years of age,

synapse formation continues until age five, and myelination continues through adolescence (Eliot, 2000).

Equally as important, the blood-brain barrier is not fully developed until six months of age and even then is only partially protective to toxins (Rodier, 1995). Prenatal chlorpyrifos exposure in rodents has been shown to inhibit DNA synthesis and reduce brain anticholinesterase activity, resulting in significant motor and learning deficits (Whitney, Seidler & Slotkin, 1995). Other studies have shown that low dose exposures to chlorpyrifos during pregnancy or immediately after birth can adversely affect the central nervous system, resulting in behavioral changes and/or learning problems later in life (Chanda, Harp, Liu & Pope, 1995; Hunter, Lassiter & Padilla, 1999; Muto, Lobelle, Bidanset & Wurpel, 1992; Timchalk, Nolan, Mendrala, Dittenber, Brzak & Mattsson, 2002).

Statement of the Problem

Infants and children differ profoundly from adults and are extremely vulnerable to environmental health hazards such as chlorpyrifos and other pesticides. Disastrous health effects can include mental retardation, behavioral changes, developmental delays and cognitive deficits. Unfortunately, very little is known about the acute or chronic effects of chemicals, such as pesticides used in the environment. As a result, the safety and well being of infants and children may be compromised. This situation is further complicated by the multiplicity of environmental contaminants, various exposure pathways and underlying disease processes. Due to major knowledge gaps and a lack of consensus on causal relationships, little is being done to study adverse health effects in humans, not to

mention the ethical constraints associated with the use of human subjects. The result is an uneducated public and an exacerbation of chemical exposures. Pregnant women and nursing mothers can expose their babies without even knowing it. Although breast-feeding is the recommended method of nutrition, there is limited information on the types of pesticides that can be absorbed and excreted into the breast milk. Collecting and analyzing breast milk during the nursing stage of the infant will help in quantifying chlorpyrifos exposure.

In June 2000, the EPA announced the decision to restrict the sale of chlorpyrifos because of the potential danger to children. As part of the phase out agreement, retailers were to cease the sale of chlorpyrifos-containing products after May of 2002. Unfortunately, they were free to put these products on sale to get them off their shelves. Most importantly, retailers were not required to recall the product or warn consumers of the potential dangers associated with product use. In addition, farmers can obtain this product for use on certain food crops (apples, grapes, and tomatoes) and professional pest control operators are able to use chlorpyrifos containing products for severe termite infestations and as a mosquitocide around homes and on golf courses.

Purpose of the Study

The purpose of the study was to determine the concentration of chlorpyrifos in three different biological specimens: breast milk, plasma, and saliva.

Theoretical Framework

Environmental health hazards, such as poor air quality, lead exposure, and pesticide exposure, can affect the quantity and quality of life. Potential outcomes include cancer, neurotoxic effects, and functional limitations. To understand the complex relationship between health effects and environmental hazards, a theoretical approach is needed to guide nurse researchers toward the goal of a health-promoting environment. An integrative model of environmental health research has been developed by Dixon and Dixon (2002) to provide researchers with a framework for environmental health research. The model contains four domains of knowledge: physiological domain, vulnerability domain, epistemological domain, and health protection domain. Each domain has a unique interdisciplinary focus, yet all the domains are interrelated so that a change in one domain can initiate a change in another.

The physiological domain is considered the starting point of the model. This domain is concerned with the physiological processes and associated human exposure effects from chemical agents in the environment. This domain asks the question "what is the problem?" and it utilizes toxicological and epidemiological methods of inquiry. The domain includes four elements: agents, exposure, incorporation, and health effects. Anything capable of causing disease is defined as an *agent*. Chemicals such as pesticides fall into this category. *Exposure* involves contact with an agent meaning that without an exposure there cannot be adverse effects. Pesticide exposures can occur through oral ingestion, dermal absorption, and inhalation. In the environment, there can be multiple

exposures to multiple agents however; most researchers focus on one chemical and one type of exposure at a time. *Incorporation* refers to the accumulation of the agent into human body compartments. Researchers use biomarkers to measure accumulation and subsequent biochemical changes in the body. *Health effects* are the primary area of interest in the field of environmental health. Heavily researched areas include asbestos exposure and lung disease, lead exposure and encephalopathy, and ozone exposure and lung cancer effects.

The vulnerability domain is concerned with the individual and community characteristics that lead to variation in environmental health risks. This domain asks the question "who is affected?" Individual characteristics that can affect risk include health or disease state, gender, and development. Those persons with preexisting disease states are particularly vulnerable since any type of chemical exposure can exacerbate their current disease state, especially those with chronic respiratory or cardiopulmonary conditions. Women may be especially vulnerable due to pregnancy and reproductive physiology. Infants and children are extremely vulnerable due to their immature organ development.

The epistemological domain is concerned with personal thought and social knowledge as it relates to effects of the environment on health. This domain asks the question "how does everyone know about this?" Personal thought is associated with cognition and begins with an awareness that a potential environmental health risk exists. Some have a lack of knowledge and are unaware of potential risks while others have an alternate attitude in that

such an event could never happen to them. This domain utilizes qualitative (ethnographies) and quantitative (surveys) as methods of inquiry.

The health protection domain focuses on those actions taken to reduce an environmental health risk. This domain asks the question "what do people do about it?" The elements associated with this domain are: concerns and a sense of threat about risk and potential health effects, efficacy and confidence in the ability to eliminate the potential risk or environmental health hazard either through avoidance or acting as a change agent to reduce the hazard.

This model (figure 2) proposes a circular pattern in that each domain impacts the others. Due to the enormity of environmental concerns, Dixon and Dixon suggest concentrating on one or two domains for a research study. Therefore, this study focused primarily on the physiological and the vulnerability domains. With respect to the physiological domain, chlorpyrifos was the chemical agent of choice and maternal breast milk, plasma, and saliva were the specimens used for the exposure pathway. In the vulnerability domain, lactating and non-lactating women were the targeted sample population.

Toxicokinetics is the study of how a chemical is absorbed into the body and what happens following distribution of the chemical to other body organs. This phase involves four processes: absorption (chemical enters the body), distribution (chemical moves from the site of entry to other areas of the body), biotransformation (the body transforms the chemical into metabolites), and elimination (chemical or its metabolites leave the body).

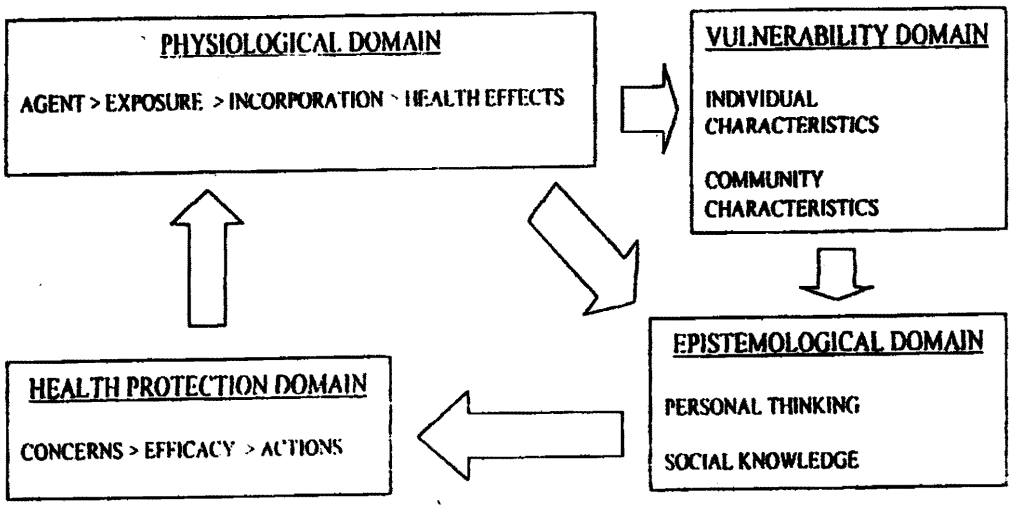


Figure 2. Conceptual model by Dixon and Dixon

Toxicology concepts were incorporated into the study for better understanding of the dynamics concerning the use of pesticides in the environment. Toxicokinetics is the study of how a chemical is absorbed into the body and what happens following distribution of the chemical to other body organs. This phase involves four processes: absorption (chemical enters the body), distribution (chemical moves from the site of entry to other areas of the body), biotransformation (the body transforms the chemical into metabolites), and elimination (chemical or its metabolites leave the body).

Absorption varies greatly with different chemicals and with different exposure pathways. The primary routes of exposure are the gastrointestinal (GI)

tract (exposure to residues on food products or contaminants in drinking water), the lungs (inhalation exposure), and the skin (dermal absorption). Once a chemical is in the bloodstream it may be excreted, biotransformed into metabolites, which then may be excreted or moved into fat stores, or the chemical or its metabolites may interact or bind with cellular components in various organs. For a chemical to move within the body, it must pass across cell membranes. Cell membranes consist of a phospholipid bilayer so that each molecule consists of a phosphate head (hydrophilic) and a lipid tail (lipophilic). Chemicals that are lipophilic, non-polar, and of low molecular weight are readily absorbed through the cell membranes of the GI tract, lungs, and skin. Lipophilic chemicals are hard for the body to eliminate and can accumulate to hazardous levels. Most lipophilic chemicals can be transformed into hydrophilic metabolites that can be excreted by the kidneys. When biotransformation results in metabolites of lower toxicity, the process is known as detoxification; however, when the metabolites are more toxic than the parent compound, the process is known as bioactivation. Once reaching the target site, the toxicodynamic phase occurs. This leads to a molecular biochemical response, which produces a physiological response leading ultimately to the adverse effect in the target organ.

Based on the above information and an extensive literature review, the model below was developed to simplistically illustrate the various potential pathways of chlorpyrifos exposure to the mother and the fetus or infant. In this model there are two pathways of concern. Both pathways begin with maternal

chlorpyrifos exposure, which is then absorbed and distributed to various parts of the body. In the transplacental pathway, chlorpyrifos is metabolized by the liver and then distributed to the placenta via the uterine artery. In the breast milk pathway, chlorpyrifos is absorbed and then distributed to fat stores which are later mobilized for milk production in the duct glands of the breast and excreted through the breast milk. In both pathways chlorpyrifos is delivered to infant blood circulation and then distributed to the immature brain, possibly resulting in acute or chronic neurobehavioral effects. The following framework was used to guide this study (figure 3).

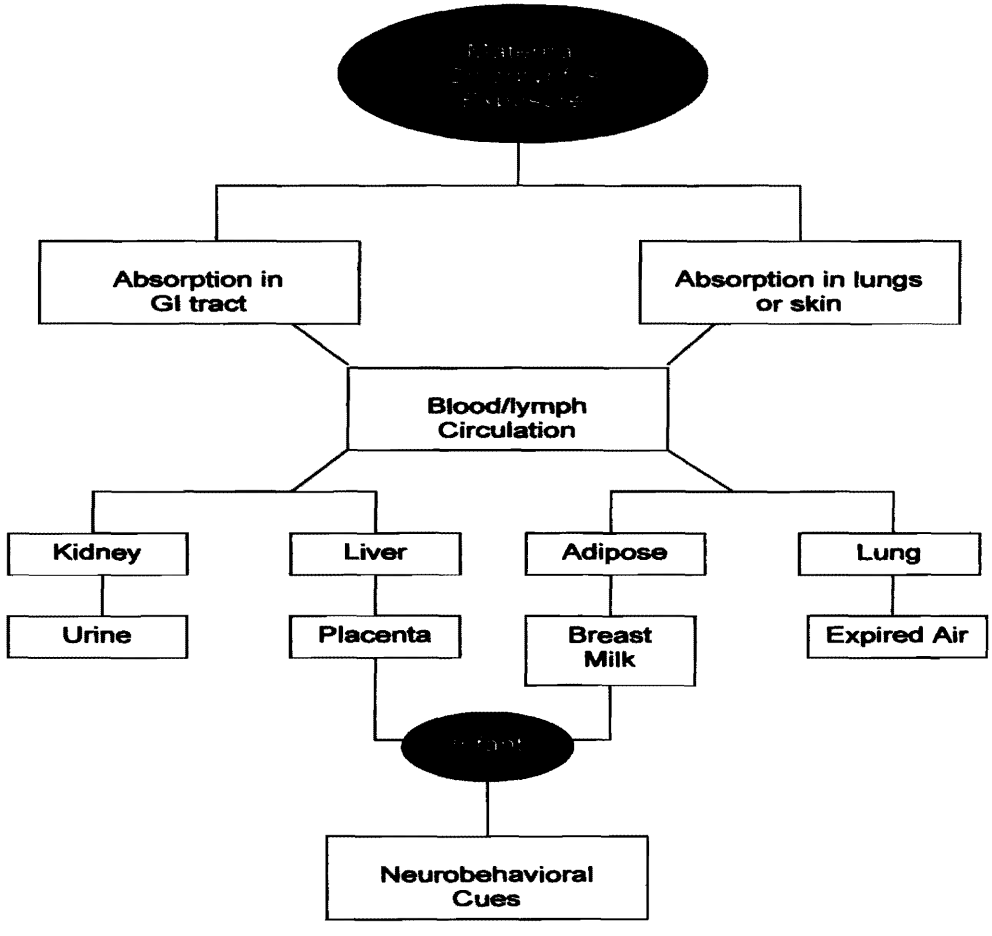


Figure 3. Conceptual model by Casey

Delimitations

Because the study employed a form of research known as secondary analysis, this particular study was confined to the biological specimens that were collected for the previous research project.

Limitations

1. The majority of the participants were Caucasian. Thus the findings are not generalizable to all lactating and non-lactating women.
2. The specimens were collected over a two year time frame and then used for different types of analysis, therefore, subsequent laboratory storage technique might have impacted the integrity of the specimens.
3. Due to the associated costs of using gas chromatography, a chlorpyrifos assay that was designed for water samples was used for the study.
4. Because women are exposed over the lifespan to many organophosphorus compounds that have similar chemical structures, the assay may not be able to differentiate between chlorpryifos and other related compounds.
5. The chlorpyrifos assay is a screening test only for which sampling error may significantly affect testing reliability.

6. Chlorpyrifos measurement is recorded in parts per billion, so it is possible that the amounts in the three biological specimens were below the level of detection.

Significance

This study attempted to determine whether the pesticide, chlorpyrifos can be transferred into human milk and subsequently nursing infants. The enormity of such a find is insurmountable in that nothing like this has been done to date. There is clear evidence that these substances can accumulate and remain in the body fat for prolonged periods of time; therefore, it is imperative that steps be taken to prevent such exposures from occurring. The onus is on the healthcare community to educate the public regarding the risks and potential dangers of pesticide use.

CHAPTER TWO

LITERATURE REVIEW

The purpose of this study was to determine the range of concentrations of chlorpyrifos in human breast milk, plasma, and saliva. This was a concern because of the potential of this chemical to interfere with infant development. Few studies have assessed pesticide exposure in infants and children, and no studies have examined potential adverse effects as a result of chlorpyrifos exposure. Therefore, a broad approach to the literature was taken to establish an empirical linkage between these concepts. The literature reviewed for this research concentrates on two distinct areas. The first section consists of literature that addresses the exposure pathways, specifically for the organophosphate chlorpyrifos. The second section will address the potential health effects associated with such an exposure. A summary of the literature review will conclude this chapter.

Exposure Assessment

Chlorpyrifos exposure can occur through multiple routes. Exposure to such a chemical is due to residues in food and water (oral exposure), skin contact with contaminated articles (dermal exposure), and dispersal of aerosols and vapors in the atmosphere (inhalation exposure). Other routes of exposure include the transfer of chemicals from a mother's placenta (transplacental exposure) and through the consumption of breast milk (lactational exposure).

Oral exposure. Oral exposure occurs by children eating foods contaminated by chlorpyrifos residues. A major breakthrough in the analysis of

pesticide dietary exposure came from a report entitled "Pesticides in the diets of infants and children". This report was published in 1993 by the National Research Council. According to the report, not only were children being exposed to high levels of pesticide residues but they also had proportionately greater dietary exposures than adults. From this report, Congress enacted the passage of the Food Quality Protection Act in which pesticide residue levels in foods must be found to be safe for infants and children. This act also required the government to apply a ten-fold margin of safety to food tolerances for the pesticide. The organophosphate class of insecticides was one of the first groups of chemicals to be scrutinized under this new law. However, an analysis published in 1998 by the Environmental Working Group revealed that out of the 80,000 samples of food inspected, 13 organophosphate insecticide residues were found. Chlorpyrifos was one of the thirteen.

The Environmental Working Group study utilized surveillance data collected by the FDA and USDA on pesticide residues from the years 1992 through 1996. The FDA has two separate surveillance programs, the FDA Pesticide Surveillance and Monitoring Program (PSMP) and the FDA Total Diet Study (TDS). In the PSMP program, approximately 12,000 to 16,000 food samples are tested from all regions of the country. Unfortunately, none of the food samples were obtained from crop growers known to have previous violations. The second program (TDS) tests 234 different foods quarterly from four grocery stores in one of four regions in the country. Unfortunately, the sample size is so small that it does not appear to be representative of pesticide

contaminants. Interestingly enough, out of 16 samples of wheat bread, all of them were positive for chlorpyrifos, malathion (another organophosphate), or both (EWG, 1998). The USDA Pesticide Data Program (PDP) monitors pesticide levels in fresh fruits and vegetables. Approximately 400-700 samples are taken from 12-14 crops per year. Unfortunately, all of the samples were washed and peeled prior to testing which may not accurately reflect actual consumer behavior prior to consumption. The samples with the most toxic levels of organophosphates were apples, peaches, grapes and pears (EWG, 1998).

Chlorpyrifos has also been found as an illegal residue on food products for which its use has been prohibited. In 1990, the FDA found detectable levels of chlorpyrifos on fresh lettuce, a crop for which it was not registered for use. There were nine other pesticide residues found in domestically produced lettuce samples but the most frightening find was the chemical DDE, a byproduct from the breakdown of DDT. Even though the U.S. banned DDT in 1972, its persistence in the environment may account for its presence on certain food crops (Vandeman, Shank, Chandran, & Vasavada, 1992).

Chlorpyrifos residues were also found in Cheerios. In 1994, the FDA discovered the residues on a sample from General Mills cereal. Further investigation revealed that a commercial pesticide applicator had illegally sprayed 21 million bushels of raw oats with a chlorpyrifos formulation that had not been registered for use. The fumigator pocketed $85,000 from the deal by using chlorpyrifos since it was less expensive. General Mills sold 110 million

boxes of the contaminated cereal before the rest was destroyed (Natural Resources Defense Council, 1994).

In 2000, the EPA announced restrictions on the use of chlorpyrifos for certain food crops in the U. S.; however, many other countries have no such ban. From 1998 to 2003, chlorpyrifos residues were found on apples, oranges, grapes, pears, and grapefruits imported from the United Kingdom. Residues were found on 16% of supermarket apples and 33% of citrus fruit tested (Pesticides Residues Committee, 2004). Tomatoes imported from Mexico also had detectable chlorpyrifos residues in over 20% of samples tested (Benbrook, 2000).

Finally, chlorpyrifos residues were detected in 26% of freshwater fish, which included trout and catfish. The EPA Office of Water published a report citing the study in which fish were collected from 388 sites. The majority of the sites were near agricultural areas, paper mills and industrial plants (1992).

Children may also be exposed to chlorpyrifos residues while exploring their environment by putting fingers, toys, and other objects into their mouths. In a study by Gurunathan et al (1998), two apartments were treated with a broadcast treatment of chlorpyrifos by a licensed pesticide applicator. Plastic and plush toys were placed on the floor one hour following the application. Residual analyses were performed at 8, 24, 72, 168, and 336 hours. According to the results, chlorpyrifos functions initially as an aerosolized particle, which is then deposited onto various surfaces. In the next phase, it releases from the surfaces as a vapor and is then absorbed onto the toys and furniture. The levels on the

plastic toys peaked immediately while the plush toys sustained increasing levels over a two-week period. The ventilation methods employed for this study were thought to coincide with those of a homeowner following label directions. The windows were kept closed immediately following application plus two hours. They were then opened for four hours and then remained closed for the duration of the study. Unfortunately, the researchers did not utilize different ventilation schemes to determine the various chlorpyrifos concentrations if a homeowner did not follow the label directions. Regardless, chlorpyrifos remained on absorbent surfaces for up to two weeks following application.

Dermal exposure. Dermal exposure occurs through skin contact with contaminated surfaces, such as carpets, house dust, and lawns or gardens. Lu and Fenske (1999) conducted a study in which chlorpyrifos residues were compared from carpet and furniture surfaces following broadcast and fogger applications. Two identical dormitory rooms were treated with chlorpyrifos, one with a broadcast application by a licensed pesticide operator and the other with an aerosol fogger. The ventilation techniques employed were according to labeled instructions. The twelve subjects recruited for the study were asked to press their hands into the treated carpet for one sample and then drag their hands on the treated carpet for another sample. Both samples were obtained three and a half hours following applications. These samples were then compared to wiped samples and cloth roller samples used on furniture in the rooms. According to the results, the transfer of chlorpyrifos from hand press and hand drag techniques were comparable between the two applications. The

residues from the carpet were substantially higher for the broadcast application, but the residues found in the fogged room were substantially higher on the furniture. Unfortunately, this study did not attempt to determine the amount of chlorpyrifos absorbed into the body from dermal transfer.

Another potential route of exposure that can occur is through pets treated with chlorpyrifos containing products. In a landmark study by Boone, Tyler and Chambers (2001), two protocols were employed to determine the amount of potential transferable residues following chlorpyrifos flea and tick dips. The first group of twelve dogs was dipped according to the manufacturer's instructions for four consecutive treatments at three week intervals. The other group of twelve were dipped as above but were shampooed immediately after dipping. Chlorpyrifos samples were obtained by rubbing the dogs with cotton gloves. Both groups had fur samples collected prior to dipping, 4 hours after, and at 1, 7, 14, and 21 days post treatment. According to the results, for group one, the dogs averaged 971, 157, 70, and 26 µg of chlorpyrifos compared to the shampooed dogs that averaged 459, 49, 15, and 10 µg, respectively. Most of the residues dissipated quickly after the first dipping (73-87%); however, the researchers concluded that if a 55-pound child pets the dog for just 5 minutes within 24 hours of the dipping, the child could be exposed to a potential dosage of 0.027-0.057 mg of chlorpyrifos or 0.0011-0.0023 mg/kg absorbed residues. The EPA has a safety limit of 0.003 mg/kg/day for all routes of chlorpyrifos exposure (EPA, 1988). The above predictions were calculated according to a geometric mean established in another study so these results reflect potential

estimates at best. This estimate does not take into account other variables such as surface area, the amount of clothing the child has on, or hand to mouth behaviors of the child; not to mention the potential for inhalation vapors associated with pet playing. Of course, most parents would not let their child play with the family pet within 24 hours of getting dipped?!

Inhalation exposure. Inhalation exposure can occur in homes, day care centers, schools, playgrounds, and lawns treated with chlorpyrifos. Foggers, bombs, and aerosols appear to be the most readily inhaled due to their smaller particle size (National Research Council, 1993). Fenske, Black, Elkner, Lee, Methner, and Soto (1990) measured chlorpyrifos residues following a household broadcast application for fleas. In this study, a three-bedroom apartment was treated with a hand held broadcast nozzle by a licensed pest control operator. Opening windows and doors ventilated two of the three rooms. Air samples were obtained pre-application and then at five and 30 minutes, one, one and a half, three, five, seven, and twenty-four hours post application. Chlorpyrifos vapor concentrations (measured at 100cm above the carpet) were significantly higher at five hours (>60μg/m^3) to 24 (>40μg/m^3) hours post application in the non-ventilated room and highest at seven (>10μg/m^3) hours post application in the ventilated rooms. At 25cm above the carpet (considered the infant zone), the concentrations were significant for all three rooms at five (>60μg/m^3 ventilated and >90μg/m^3 non-ventilated) to seven hours (>50μg/m^3 ventilated and >90μg/m^3 non-ventilated) post application. Of most concern is that vapor concentrations appear to peak at five to seven hours whether the rooms are

ventilated or not, suggesting that infant/child exposure is most critical from five to greater than 24 hours following treatment.

In 1998, Byrne, Shurdut, and Saunders were employed by DowAgrosciences to conduct a study evaluating chlorpyrifos exposure following crack and crevice treatment. In this particular type of application, an injector tip was used to streamline the insecticide directly to baseboards and cracks. For the study, the researchers used three different homes of comparable sizes and styles. The same licensed applicator treated all three rooms. Chlorpyrifos was applied to cracks between the baseboards and walls, under sinks, and behind large appliances. The windows remained closed during the application. Chlorpyrifos vapor samples were collected from the kitchen (treated) and family room (untreated) every day for up to ten days post application. At each location, the samples were collected at 40cm (child crawling zone) and 130 cm (standing adult) above the floor. In house #1, levels peaked between zero and two hours (0.76µg/m^3) at 130 cm above the floor in the kitchen. For house # 2, the peak concentration occurred at 60-72 hours (2.3 µg/m^3) and house # 3 at six to eight hours (1.7 µg/m^3) post application. Both peak concentrations occurred at the 40 cm sampling height. In two of the three houses, the air concentrations declined eight hours after the application; however, in the third house, the levels peaked on day three and day six. The researchers are suggesting that this was due to the fluctuation of internal temperatures; however, according to the methods section, the air conditioning and heating remained off in an effort to maintain constant temperatures in all three homes. The results are difficult to interpret, but

the researchers conclude that the daily 24 hour time-weighted average for the air concentrations at the 40 cm level were less than 1.6 $\mu g/m^3$ which is at least five times lower than the National Academy of Sciences guideline of 10 $\mu g/m^3$. Because the highest concentration lasted for only 12 hours and the air measurements decline over a seven-day period coupled with the fact that any actual inhaled dose would be rapidly metabolized and excreted, the researchers concluded that it is unlikely that there would be any cumulative effects for children following a crack and crevice treatment. Remember, the actual ceiling dose for children is 3 µg/kg/day from all sources!

Take-home exposures. Take-home exposures are a route of exposure that occurs from chlorpyrifos residues that are brought home on parent's work clothes or via farm proximity. Lu, Fenske, Simcox, and Kalman (2000) analyzed residential contamination of organophosphates in the homes of agricultural workers. Families were assigned to one of two groups. The first group consisted of families with at least one member employed as a pesticide applicator (#49) or as a farm worker (#13). The reference families comprised the second group and their eligibility criteria stipulated that no one could work or live closer than one-quarter mile to a pesticide-treated orchard (14). All samples were obtained following the May- July spray season for tree fruit production. To analyze the take-home exposure pathway, wipe samples were obtained from the workers boots and the steering wheel. Hand wipe samples were obtained from the children with ages ranging from nine months to six years. According to the results, 38 agricultural families had detectable diethyl organophosphate residues

on one or more wipe samples. The majority of residues were found on the work boots. Ten out of 61 agricultural children had measurable levels of the same residues. None of the reference families had detectable residues on any of the samples collected. Unfortunately, there did not appear to be any determination of time between sample collection and pesticide application. Nor was there an effort to determine if working boots had been washed prior to the wipe sample.

A similar study was conducted by Quandt (2004) in which 41 farm families were analyzed for pesticide residues. All of the families were Spanish and all had at least one child under the age of seven. Wipe samples were obtained from uncarpeted flooring, toys, and children's hands. The data collection occurred between June and December of 2001. According to the results, 44% of the floor samples, 29% of the toy samples and 22% of the hand samples had detectable levels of pesticide residues. The data collection used for this study was limited to peak spraying times.

Transplactental exposure. Transplacental exposure is a new concern in infant/child exposures in that the potential transfer of chemicals from the pregnant mother to the fetus occurs in utero. In a landmark study by Whyatt and Barr (2000), meconium samples were collected from the diapers of 20 newborns. All of the samples were analyzed for six organophosphate metabolites: DEP (diethylphosphate), DETP (diethylthiophosphate), DEDTP (diethyldithiophosphate), DMP (dimethylphosphate), DMTP(dimethylthiophosphate) and DMDTP (dimethyldithiophosphate). According to the results, DEP and DETP metabolites were detected in 95% and

100% respectively in the meconium samples. Both of these are metabolites of chlorpyrifos and diazinon. Virtually none of the other metabolites were detectable. Interestingly, the researchers had no prior knowledge of prenatal pesticide use as the samples were collected randomly over a three-week period. This study suggests that exposures were most likely from the second trimester through delivery since meconium begins to collect around 16 weeks of gestation.

Bradman (2003) wanted to determine early fetal exposures at approximately 15-20 weeks gestation by the use of amniotic fluid obtained during amniocentesis procedures. Amniotic fluid production begins immediately following embryo implantation and increases in volume throughout the pregnancy (Hytten & Chamberlain, 1991). Amniotic fluid samples were obtained from 100 women who were referred for an amniocentesis in an effort to address potential genetic disorders. Twenty samples were used for method validation, and the remaining samples were divided into four sets of 20. The samples were then analyzed for synthetic pyrethroids, organophosphates, and other pesticides such as carbamates, herbicides, and chlorinated phenol metabolites. Twenty-five percent of the organophosphate group had detectable levels of DEP, DMP, or DMTP. Ninety percent of the samples had detectable levels of at least one chlorinated pesticide. There were not any detectable levels of any of the synthetic pyrethroids or herbicides in the samples. This study suggests that fetal exposures can occur as early as 15-18 weeks gestation which correlates with central nervous system development (Selevan, Kimmel, & Mendola, 2000). Again, the researcher had no prior knowledge of prenatal pesticide use.

Finally, in the Children's Environmental Cohort study (2004), pregnant women were recruited from a prenatal clinic and two private clinics. Only primiparas with singleton births were selected for the study, which totaled 404 participants. The researchers were interested in assessing the relationship between prenatal pesticide exposure, paraoxonase (enzyme) activity, and infant growth and neurodevelopment. Paraoxonase (PONI) is an enzyme that is important in the hydrolysis and deactivation of organophosphates (Costa, Cole & Furlong, 2003). A questionnaire was administered during the third trimester to ascertain potential pesticide exposures. Maternal blood samples and urine were also obtained during the third trimester, and cord blood samples were obtained at birth. The blood samples were used to analyze paraoxonase activity in the mother and infant. The urine samples were used to assess for the chlorpyrifos metabolite 3,5,6-trichloro-2-pyridinol (TCP). During hydrolysis, chlorpyrifos is detoxified and excreted in the urine as TCP. The infant birth outcomes used for the study included weight, length, head circumference and gestational age. Almost half of the participants (46.2%) reported indoor pesticide use during the pregnancy. The only significant finding was the correlation between maternal paraoxonase activity, detectable levels of TCP in maternal urine and small infant head circumference. It was then hypothesized that since small head circumference has been associated with cognitive ability, chlorpyrifos could have a detrimental effect on infant neurodevelopment.

Other studies have been done to determine pesticide exposures in pregnant women. In 2002, Whyatt et al. conducted a study to determine pesticide

use in pregnant women. Three hundred and fourteen African-American women were recruited for the study of which 72 consented to personal air monitoring. A pesticide questionnaire was administered to each woman in her home by the same researcher. Information was obtained on demographics, smoking and alcohol use and residential pesticide use. The 72 women that agreed to air sampling during the third trimester was asked to wear an ambient air monitor during the daytime hours for two consecutive days. From the questionnaire, 85% reported use of a pesticide during pregnancy. Most household uses were by sticky traps and gels; however, 35% reported the use of exterminators in the home and half of the homes were sprayed more than once a month. Alarmingly, 9% reported using alternative illegal street pesticides, such as "tempo" (a pyrethroid) and "tres pasitos" (a carbamate). As for the personal ambient air monitoring, 100% of the women monitored had detectable levels of three pesticides: chlorpyrifos (range 0.7-193 ng/m^3), diazinon (another organophosphate ranging from 2.0-6,010 ng/m^3) and propoxur (a carbamate ranging from 3.8-1,380 ng/m^3). Unfortunately, there was no prior determination of when the pesticide was used in the home prior to the personal air monitoring. Regardless, this is a significant finding for pregnant women!

A similar study by Berkowitz et al (2003) investigated pesticide use by 386 pregnant women. The questionnaire was also administered to the women during the third trimester to assess the use of pesticides by a household member, exterminator, fumigator, and/or pesticide applicator. Urine samples were also obtained and assessed for organophosphate metabolites. According to the

results, almost half (46%) stated that they or another household member had applied pesticides in the home during the pregnancy. The urinary metabolite TCP was (average 11.3 µg/g creatinine) higher than previously reported in other studies (2.2 µg/g in the NHANES III survey by Hill et al and 4.6 µg/g in the NHEXAS-MD study by Macintosh et al).

Lactational exposure. Lactational exposure is a significant route of potential exposure in that infants can be exposed through contaminated breast milk. Lipophilic chemicals partition into maternal adipose tissue, and are then stored there for many years. Due to the rapid weight loss following birth, these chemicals can then be released back into circulation and taken up into the fat of the mammary gland (Clewell & Gearhart, 2002). Due to decades of toxic chemicals in the environment, contamination of breast milk is widespread throughout the country. These chemicals are stored in the fat component of the breast milk through the process of depuration. In this process, the fat comes from maternal fat stores and diet.

In a landmark study by Brilliant et al. (1978), polybrominated biphenyls (PBBs) were measured in the breast milk of nursing mothers. PBBs and PBDEs (polybrominated diphenyl ethers) belong to a family of brominated flame-retardants that are used in diverse products including electrical appliances and computers as well as in carpets and furniture upholstery. They most frequently enter the environment directly from broken capacitors. For instance, following the result of an industrial accident, several thousand Michigan dairy farms were contaminated with PBBs. These chemicals then entered the food chain through

meat and dairy products over a two-year period. The researchers conducted a random sample survey from 95 nursing mothers from the Upper and Lower Michigan peninsula. According to the findings, 43% of the women from the Upper Peninsula and 96% from the Lower Peninsula had detectable levels of PBBs in their breast milk. The authors estimate that approximately eight million of Michigan's nine million residents had detectable body burdens of PBBs.

In 2003, Schecter, Pavuk, Papke, Ryan, Birnbaum and Rosen analyzed 47 breast milk samples from a milk bank and community health clinic in Texas. The milk samples were from nursing mothers between the ages of 20 and 41 years. The samples were analyzed for PBDE concentrations. According to the findings, all of the milk samples contained various levels of PBDE concentrations (6.2 to 419 ng/g) with a mean of 73.9 ng/g (parts per billion). The PBDE levels did not appear to vary according to maternal age, number of previous births, or length of lactation.

Most recently (2004), the Environmental Working Group (EWG) conducted a survey to evaluate flame-retardants in breast milk. In their study, 20 healthy primiparous women were recruited to participate. The women were asked to collect a milk sample within several months following the birth of their first child and complete an exposure assessment questionnaire intended to obtain information concerning their lifestyle and home and work environments. The demographic information was as follows: 1) the participants were from 14 different states in the US, 2) 18 were Caucasian, one Vietnamese, and one of mixed races, 3) ages ranged from 29 to 40, 4) 19 were college graduates, and 5)

five were former smokers but none smoked during pregnancy. According to the results, every breast milk sample tested was contaminated with varying levels of PBDEs (9.5 to 1078 ng/g). In addition, the researchers reported finding two of the highest levels of PBDEs ever reported in human beings in the world (one worked in computer related research and the other was a registered nurse). One had recently replaced furniture cushions and the other had reupholstered dining room chairs. None of the participants reported unusual PBDE exposure so the variability in the PBDE levels was not easily explained by their diet, occupation, age, body mass or amount of time they breast-fed their infants. Interestingly, 17 of the 20 participants had reported eating fish at least once per week, and 13 had reported eating fish in the week prior to collecting the sample.

Cousins to the polybrominated biphenyl family are the polychlorinated biphenyls (PCBs) and the polychlorinated dioxins (PCDDs) and furans (PCDFs). PCBs were previously used mainly as heat transfer fluids in transformers while PCDDs and PCDFs were formed primarily as byproducts following incineration and metal smelting and refining. Wickizer, Brilliant, Copeland and Tilden (1981) analyzed PCB levels in 1,057 nursing mothers. The samples were obtained from 68 of 83 Michigan counties of which all the samples contained PCB residues ranging from trace amounts to 5,100 ppm (parts per million). The mean PCB level was 1,496 ppm.

In a four-year study of PCB contaminants, Rogan and Gladen (1985) collected breast milk samples from 856 mothers of which 43% were primiparous. The breast milk contained almost universal contamination of PCBs and DDE.

They also reported a decline in the PCB levels by approximately 20% after six months with an average time for breast-feeding of 29 weeks. Unfortunately, they did not describe the data collection or data analysis in the paper.

Additionally, in a study by Hong, Xiao, Casey, Bush, Fitzgerald and Hwang (1994), 50 breast milk samples were analyzed for PCBs from 20 women from the Mohawk reservation, which is in close proximity to an industrial facility, and 30 controls who resided elsewhere. The authors reported an association between breast milk PCB concentrations and age, parity, duration of lactation, and cigarette smoking. Those that were older, smoked and breast-fed their first child had higher levels of PCBs in the breast milk.

Schecter, Ryan and Papke (1998) also reported finding a decrease in PCB concentration over the lactation period. In this study, the researchers collected 13 breast milk samples from a mother who was nursing twins and analyzed them for PCBs, PCDDs and PCDFs. In their findings, the PCB concentration decreased from 285 to 63 ng/g over two and a half years whereas the PCDD and PCDF concentrations decreased from 309 to 173 ng/kg (75%) and 21 to 9 ng/kg (83%) respectively. The authors concluded that based on mathematical calculations, approximately two-thirds of the PCDD concentration was transferred to the twins. The study is obviously limited by sample size. Other persistent organochlorine chemicals have also been found in human breast milk such as dichlorodiphenytrichloroethane (DDT) and its metabolite (DDE), dieldrin, heptachlor, and hexachlorobenzene (HCB). In the New York State Angler Cohort study (1996), researchers studied breast-feeding women who consumed fish

from Lake Ontario. The women were either licensed anglers or spouses of male licensed anglers, and their milk samples were analyzed for PCBs, DDE, and HCB. Nine out of 77 PCB congeners and DDE were found in all of the 100 breast milk samples with those that consumed fish from Lake Ontario having a significantly higher level of PCB residues than the non-fish eaters (Vena, Buck, Kostyniak, Mendola, Fitzgerald, Sever, Freudenheim, Greizerstein, Zielezny, McReynolds, & Olson, 1996).

Schecter, Ryan and Papke (1998) not only reported PCBs, PCDDs and PCDF concentrations in their milk samples of the mother nursing twins but also DDE and HCB. The DDE concentration (246 to 46 ng/g) declined by 81% over the 30-month lactation period. The HCB concentrations (10.7 to 1.8 ng/g) decreased 92% over the same time period.

The only reported chlorpyrifos study was performed on maternal rats. This report (1998) was an analysis of twelve developmental neurotoxicity studies evaluated by the Office of Prevention, Pesticides, and Toxic Substances (OPPTS). The results of the studies were summarized and compared to related studies to evaluate prenatal development, reproduction, and neurotoxicity in adult rats. In one of the studies, pregnant rats were administered chlorpyrifos from the time of implantation until day ten of the lactation period. Blood and milk samples were collected on gestation day 20 and lactation days one, five, and eleven and then analyzed for chlorpyrifos and the two metabolites (chlorpyrifos oxon and TCP). According to the findings, chlorpyrifos milk concentrations on lactation

days one and five were at least ten-fold higher than maternal blood concentrations (Makris, Raffaele, Sette, & Seed, 1998).

A recent study by Sanghi, Pillai, Jayalekshmi, and Nair (2003) of human breast milk samples found several organophosphates including chlorpyrifos. In this Indian study, breast milk samples were collected from 12 mothers aged 19 to 45 years. Among the mothers, three were primigravidae, and the rest had two to five children. The breast milk samples were collected by manual expression once the infant had nursed. Unfortunately, the authors did not provide any time line as to when the samples were collected. The samples were then frozen at –20°C. Data analysis was performed with the use of gas chromatography to determine t the presence of three organochlorines and three organophosphates. Among the various pesticide residues present, chlorpyrifos was the second highest concentration found in the breast milk samples. In addition, all 12 samples contained varying residue levels of chlorpyrifos.

Biomarkers of Exposure

Because fetuses, infants and children have so many potential exposure routes, biomarkers have now become the benchmark for determining such an exposure. Biomarkers for determining organophosphate exposure typically include cholinesterase inhibition (De Peyster et al, 1993; Lessenger & Reese, 1999; Vandekar, 1980) in blood and metabolites in urine (Adgate et al, 2001; Castorina et al, 2003; Fenske, 2002; Loewenherz et al, 1997; Lu et al, 2001; Mills, 2001). As noted earlier, organophosphates undergo chemical changes in the human body. The first reaction for chlorpryifos occurs in the liver where the

double bond of the central phosphorus atom transforms from sulfur to oxygen. It is now a considered an "oxon" and is a much more potent inhibitor of cholinesterase enzymes. This process can be measured in red blood cells by determining the reduced cholinesterase activity or AChE in the blood. Unfortunately, AChE levels can vary between individuals and can fluctuate within individuals as a result of health conditions, such as pregnancy, diseases, medications, and illegal drugs (Lessenger & Reese, 1999). In addition, large doses of chemicals are required for significant inhibition to occur resulting in an insensitivity to low exposure levels (He, 1999). Chlorpyrifos can be measured directly in the blood by way of plasma or serum; however, because chlorpyrifos metabolizes in 24-48 hours, it is unlikely that sample collection would coincide with the exposure (WHO, 1996).

The second chlorpyrifos reaction that occurs is hydrolysis which yields a diakylphosphate and a leaving group. This metabolic reaction is known as detoxification as it is easily excreted in urine as the metabolite TCP. The difficulty in measuring this metabolite and other organophosphate metabolites is related to the concentration of the urine. Most laboratories will normalize the concentration by using creatinine clearance or specific gravity; however, in children, this can be quite variable (O'Rourke et al., 2000).

Newer methods such as that of testing meconium (Whyatt & Barr, 2002), amniotic fluid (Bradman, 2003), saliva (Timchalk et al, 2004) and breast milk are serving as biomarkers of the future. "The use of meconium shows promise and further research could firmly establish it as a biomarker for organophosphate

exposure" (Wessels, 2003). Breast milk is an appropriate biomarker for exposure because chemicals (especially those that are extremely lipophilic) can accumulate in the fatty tissues for a number of years.

Effects Assessment

Due to the vulnerability of fetal, infant and child development, the brain and the nervous system are especially sensitive to the effects of neurotoxic chemicals, such as the organophosphate chlorpyrifos. Other manifestations of toxicity includes fertility problems, spontaneous abortion, fetal death, birth defects and delayed growth. Most of this evidence comes from animal studies that examined developmental neurotoxicity. The precise nature of these effects depends on the amount of the dose received, the toxicological properties of the chemical and the timing of the exposure with the critical windows of fetal, infant and child development.

Animal studies are done by exposing various animals to a chemical and then evaluating the effects of such an exposure. In a landmark study by Muto, Lobelle, Bidanset and Wurpel (1992), pregnant rats were given chlorpyrifos on days 0 to 7 or days 7 to 21. For both groups, the rats were randomly assigned to receive 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg or saline. Following delivery, the rat pups were evaluated for viability, birth weight, and general physical characteristics. They were then re-evaluated on day 16 for behavioral abnormalities and neurotoxicity. In another study, rat pups were either given 0.1mg/kg or 0.3 mg/kg on postnatal days 3, 10, or 12 for group one and daily on

days 6-10 for the second group. Both groups were then observed on day 16 for behavioral abnormalities and neurotoxicity.

For the neurotoxicity evaluation, a rotating rod on which pups had to balance was used to determine the number of falls, with comparison between the two groups of chlorpyrifos versus saline. The incline test was used to determine motor activity, and general motor behavior was assessed using open field observation. The pregnant females that received the chlorpyrifos injections did not exhibit any overt signs of toxicity; however, all three chlorpyrifos doses caused a greater number of abnormalities than those pups that received saline. For the 0 to 7 day group, chlorpyrifos caused 77%, 57% and 68% lethality and 15%, 66% and 55% physical abnormalities. For the 7 to 21 day group, chlorpyrifos caused 17%, 22% and 38% lethality with only the latter dose causing any physical abnormalities. None of the pups from the saline infused groups had any lethalities or abnormal physical characteristics. Rat pups given 0.3 mg chlorpryifos /kg prenatally demonstrated significant neurotoxicity on postnatal day 16 as well as those pups that received chlorpryifos on postnatal days 3, 10, or 12.

Chanda, Harp, Liu and Pope (1995) also demonstrated neurobehavioral effects in rat pups following chlorpyrifos injections. In their study, pups were place in a supine position and then measured to determine their ability to turn over (known as the righting reflex). Chlorpyrifos exposure greatly altered this reflex on postnatal day one. In a different study, Chanda and Pope (1996) found that repeated chlorpyrifos exposures in rats during pregnancy resulted in

inhibited levels of AChE. Whitney, Seidler and Slotkin (1995) demonstrated that a single dose of chlorpyrifos given to one-day-old rats could cause significant inhibition of DNA synthesis in all brain regions within four hours of treatment.

Human studies are done to assess the potential effects of chemicals after the exposure has occurred. Steenland et al. (2000) conducted neurologic tests on pest control operators who had worked as termiticide applicators from 1987-1997 in 12 counties of North Carolina. Those that had worked for a year or more were recruited for the study until 200 participants were selected (exposed group). Half of these subjects were asked to bring a friend to serve in the nonexposed group. Another 100 state employees were recruited as well for a total of 200 in the nonexposed group. All participants were given the Neurobehavioral Evaluation, which consisted of a vibrotactile test, arm/hand tremor test, postural sway test, manual dexterity test, eye-hand coordination test, vision test, olfaction test, nerve conduction velocity, and neurological exam. In addition, the participants were given a questionnaire to assess neurologic symptoms. Urine and buccal swabs were taken to measure TCP and paraoxanase, respectively.

In the exposed group, 191 termiticide applicators who had an average of 2.4 years applying chlorpyrifos were compared to 189 non-exposed participants. The two groups did not differ significantly in the neurological exam; however, the exposed group did not function as well as the non-exposed group on a few of the neurobehavioral tests (postural sway test and the pegboard turning test). In addition, the exposed group reported significant differences on the questionnaire complaining more memory problems, emotional mood states, fatigue, and loss of

muscle strength. Those applicators that had more recent chlorpyrifos exposures had higher levels of TCP (629.5 ug/ L) in their urine than the other applicators (4.5 ug/L).

Kaplan, Kessler, Rosenberg, Pack and Schaumburg (1993) conducted a retrospective analysis of eight people exposed to chlorpyrifos following commercial application by professional exterminators. All eight report peripheral neuropathy while five of the eight experienced memory loss and cognitive slowing. The authors concluded that environmental contact with chlorypyrifos could cause sensory neuropathy and central nervous system dysfunction. This study is extremely limited by the small sample size.

Zweiner and Ginsburg (1988) reviewed the medical records of 37 children that had been diagnosed with organophosphate toxicity. The ages of the patients ranged from one month to 11 years. A little more than half of the children were boys, and 62% of the poisonings occurred from April through September. All of the poisonings occurred at home and six cases involved chlorpyrifos. Ingestion of stored liquid was involved in three-fourths of the cases, and playing on the carpet or floor following application was involved in 14% of the cases. The most common signs and symptoms included miosis (73%), excessive salivation (70%), muscle weakness (68%), respiratory depression (59%), lethargy (54%), and tachycardia (49%). Three of the six chlorpyrifos cases were life threatening due to coma or respiratory arrest (Blondell, 1999). There were no deaths, and everyone recovered promptly following therapy.

Sherman (1996) reported four cases of birth defects associated with chlorpyrifos poisoning. The obstetrical history was reviewed in all four cases for incidence of infections, alcohol and/or illicit drug use, medications and smoking. Other evaluations included chromosomal analyses, family pedigrees and records of treating physicians. The only common element found in all four cases involved chlorpyrifos exposure during the pregnancy. Three of the mothers were exposed at home, and the fourth was exposed at work. Child #1 and child #3 are siblings and were both exposed to chlorpyrifos after the family used a flea fogger with child #1 and used a commercial operator who applied chlorpyrifos in the home with child #3. The mother of child #2 was exposed late in the first trimester after treating the home with chlorpyrifos, and the mother of child #4 was exposed at work early in her first trimester. According to the author, all four children had ventricular, eye, and palate defects as well as growth retardation. Three of the four children had hydrocephaly, microcephaly, mental retardation, blindness, hypotonia and other limb defects. Brain defects were remarkable for structural defects of the corpus callosum in two children, an enlarged chloroid plexus in one child and absence of the septum pellucidum in the fourth child. The author suggests that the pattern of defects in all four children is conclusive enough to warrant further investigation of chlorpyrifos as a common cause of teratogenic effects.

Summary

There is ample evidence in the literature that chlorpyrifos poses a significant threat to fetuses, infants and children. The various pathways of exposure are well documented as well as the potential for developmental neurotoxicity in animals and humans. However, what is lacking is the documentation of chlorpyrifos as a vehicle for transport from the lactating mother to the nursing infant and the subsequent effects, if any, in the infant or child. Even though the EPA has banned chlorpyrifos for home use, it is still being used agriculturally and by professional pest control operators as they see fit. Until more is known about the developmental effects of these chemicals, continued research is imperative. Although most experts agree that breast -feeding is the preferred nutrition for nursing infants, studies are needed to evaluate pesticide exposures through breast milk in order to protect the infant from adverse effects. With this type of information, breast milk monitoring programs could be put in place to educate those populations at risk for chlorpyrifos exposure and potential toxicity.

CHAPTER THREE

METHOD

The purpose of this study was to determine the concentration range of chlorpyrifos in human breast milk, plasma, and saliva. The research method employed for this study was an analysis of existing samples collected for the purposes of a prior study. The study design, sample, setting, protection of human subjects, instruments used, data collection, and data analysis will be described below.

Study Design

Evidence suggests that women are being exposed to environmental chemicals such as chlorpyrifos; therefore a quantitative methodology was to guide this study. With this approach, biological samples can be collected (empirical evidence) to obtain numeric information about chlorpyrifos concentrations that can then be analyzed by statistical procedures. Due to the ethical issues associated with testing humans for chemical effects, non-experimental studies are the most common research methods for collecting data of this nature. The most logical design for this type of study would be that of a longitudinal nature. Once the basic question of exposure has been answered, then effects of chemicals on factors such as neurobehavioral indicators could be measured over a specified unit of time. Theorizing that chlorpyrifos exposures will have developmental effects on fetal organs, such as the brain, there is a possibility that negative cognitive and behavioral indicators might not present themselves for months or even years to come. Although this researcher would

favor this type of research design, due to time constraints associated with a dissertation proposal, such a study was not feasible. Taking into consideration the element of time, a more feasible option was that of the cross-sectional design. This type of design is used when there is a desire to obtain information in an area about which little previous research has been done. According to Polit and Hungler (2002), "cross-sectional data can most appropriately be used to infer temporal sequencing under two circumstances 1) when there is evidence or logical reasoning indicating that one variable preceded the other, and 2) when there is a strong theoretical framework guiding the analysis" (p. 162). This particular type of method fit this research design well in that logical reasoning implies that a mother must first have been exposed to chlorpyrifos so that the concentration can then be measured in the breast milk.

However, for chemical exposure studies, the route, timing, and extent of the exposure are often difficult to determine (Clewell & Gearhart, 2002). Virtually all exposure studies collect historical information regarding pesticide use, which are usually answered through questionnaires or interviews. Fenske, Bradman, Whyatt, Wollfe, and Barr (2005) found that subjects were best able to provide general information regarding the use of products, as in controlling weeds or insects, but not necessarily able to provide detailed information on specific chemicals. According to these researchers,

> In preliminary analyses of questionnaires administered by the Columbia center, women provided a pesticide product name for only 39% of the pest control methods reported to be used in the home during pregnancy

and, in particular, were very rarely able to identify the pesticide products used by an exterminator. Further, pesticide products can have the same brand name but contain different active ingredients, further complicating use of questionnaire data in pesticide exposure assessments. In addition, the Mount Sinai center collected urine samples in the 3rd trimester of pregnancy, and found that approximately 70% of the women in the cohort had been exposed to pesticides, but no associations were found between the biological levels and pesticide questionnaire data.

The researchers concluded that it is highly unlikely that questionnaire data alone can prove adequate for identifying pesticide exposure of women during pregnancy (2005).

According to the National Children's Study (2005), pesticide exposures to the general population may be very difficult to assess because humans are generally exposed to low levels of environmental chemicals all the time, the exposures may occur without prior knowledge, and they may occur through a multitude of routes or pathways. Therefore, when assessing pesticide exposures, there is no single method that will capture all the needed exposure information all the time (Needham et al, 2005).

Another concern is related to the subject matter of this particular study, in that the determination of chlorpyrifos residues present in maternal breast milk may be of extreme concern to nursing mothers. Mothers will want to know not only if the chemical is present but also the potential ramifications associated with such a find. In addition, the potential horror those mothers may experience when

learning that they may have inadvertently poisoned their babies could be in and of itself a potential crisis. Due to the above concerns, a less obtrusive research method known as secondary analysis was employed for this study.

Secondary analysis involves the use of existing samples, collected for the purposes of a prior study in order to ask a new research question that is distinct from that of the original work (Hinds, Vogel, & Clarke-Steffen, 1997). This type of analysis can be used to generate new knowledge or new hypotheses. The advantages to using secondary analysis are that it reduces the burden of subject recruitment and data collection and it allows wider use of data from potentially inaccessible respondents. The study participants were told that the purpose of the original study was to learn more about stress and health during the early postpartum period. The benefits of the original study were to gain more knowledge about stress, health and immune function in lactating versus non-lactating postpartum mothers.

Sample Design

The original research study explored the influence of lactation on stress and immunity in postpartum mothers. This was a non-experimental, cross sectional study funded by the National Institutes of Health. The specific aims of the study were: 1) to analyze perceived stress, stressful life events, mood states, levels of stress-associated hormones, and relationships among these variables in lactating compared to non-lactating women; 2) to analyze immune variables and occurrence and severity of symptoms of common infections in lactating compared to non-lactating women; 3) to analyze relationships between stress

and mood variables, immune variables, and incidences and severity of symptoms of common infections in lactating and non-lactating women. The researchers measured levels of stress hormones (oxytocin, prolactin, cortisol), type 1 and 2 cytokines, enumeration of selected lymphocyte subsets, proliferative responses of lymphocytes, levels of stress hormones, neopterin and immunoglobulin A concentrations in saliva.

Sample

A power analysis was conducted to determine the appropriate sample size needed to increase the likelihood of demonstrating significant results. For a power of 0.80, a significance level of 0.05 and a medium size effect (r=. 3), a sample size of 80 subjects for each group would be sufficient. Therefore, the researchers recruited 100 lactators and 100 non-lactators for the study.

Subjects were recruited from the postpartum unit of a local hospital, through the health department, and participating OB/GYN offices at the time of their first postpartum visit. The subjects were healthy mothers between the ages of 18 and 45 years old who were four to six weeks postpartum after delivery of a full term infant by an uncomplicated vaginal or cesarean delivery. Exclusion criteria included the following: under 18 or over 45 years of age, chronic mental or physical illnesses, medications that could influence immune function, serious complications during pregnancy, labor, or delivery. The subjects were visited either in their homes or at their health care providers office between four to six weeks postpartum. At the visit, the investigators collected the study questionnaires, breast milk (from lactating group), saliva, and blood samples.

The milk, saliva, and serum samples were placed on ice and transported to the lab within two hours of attainment. The serum was spun, aliquoted, and frozen at –20°C. The milk was spun at 6000 rpm, aliquoted and also frozen at –20°C.

For the purposes of this dissertation, this researcher chose 52 subjects from the original study for use in a secondary analysis. The subjects were selected based on the availability of biological specimens. In the lactating group there were 26 each of matched breast milk, plasma and saliva samples and in the non-lactating group there were 26 matched plasma and saliva samples. A two group design was chosen to compare differences in plasma and saliva chlorpyrifos concentrations. Based on the literature, these two concentrations should be higher in the lactation group.

Setting

As mentioned previously, this researcher chose 26 lactating subjects and 26 non-lactating subjects for secondary analysis of breast milk samples, serum samples and saliva samples. The analysis was conducted at the Walker Research Laboratory in the College of Nursing, University of Tennessee, Knoxville, under the direct supervision of Dr. Maureen Groer. This researcher performed all the analysis of the biological specimens.

Protection of Human Rights

Subjects were invited to participate in the original study through notices posted at the University of Tennessee Medical Center, the Knox County Health Department, and in participating physician offices. Nurses were available at all locations to describe the purpose of the study and to answer any potential

questions. Once they agreed to participate in the study, a written consent was obtained. The participants were given a packet that included an information sheet about the study, potential risks and benefits, data collection procedures, notice of rights to withdraw from the study at any time, email address and telephone number of the researcher, and a $50 gift certificate. To insure confidentiality, no names were attached to any specimens, questionnaire forms, or instrument packets. All of the completed forms and specimens are kept in the Walker Lab, which is locked at all times.

For purposes of the secondary analysis study, this researcher maintained confidentiality by assigning numbers to the questionnaires and biological specimens. The groups were categorized as lactators and non-lactators. The information from the analysis was kept in a locked cabinet at the researchers home. Any published material will only contain the above information.

Instruments

The demographic tool was the only instrument accessed from the original study for use in this study. This questionnaire included information on age, race, height and weight, yearly income, family size, work status, exercise, presence of any acute or chronic health problems, smoking, medication use. It also included information on labor history, birth history, postpartum complications, and breast-feeding or bottle-feeding status.

Biological testing has become the template for determining human pesticide exposure. Because pesticide exposures have multiple routes, biomarkers tend to integrate all the various pathways. The analysis is done by

estimating the amount that is absorbed into the body and then measuring the pesticide itself, its metabolite, or its reaction products in biological media, such as urine, blood, saliva, meconium, and breast milk (Barr & Needham, 2002). Most persistent pesticides are best measured in such media as a result of their long biological half-lives and ability to sequester into lipid rich matrices. According to Barr and Needham (2002), because of the long half-lives of persistent pesticides, it is usually difficult or impossible to distinguish recent exposures from exposures that occurred decades ago. One possible indicator of a recent exposure may be a level elevated above the range that is normally seen. However, many chemicals and/or their metabolites are present in different concentrations in different types of biological media.

The most common biospecimen for use in analyzing chemical exposures is urine. Such analysis is due to its ease in availability, the volume, and potentially higher concentrations (ug/l or parts per billion) as compared to blood concentrations (ng/l or parts per trillion). Unfortunately, the measurement is most often a metabolite of the parent compound. The other disadvantage to using blood for biosampling relates to the volume variability. Adjustments are required to correct for dilution issues, which often use creatinine concentrations. In the case of chlorpyrifos, there are several metabolites, such as DEP and DEPT, \that are the same metabolites as other organophosphates. The only metabolite that is specific to chlorpyrifos is TCP. The half-lives of chlorpyrifos and its metabolites in urine are 15 to 30 hours (Griffin et at, 1999). In the Minnesota Children's Pesticide Exposure Study (2000), 102 urine samples were collected

from children aged 3 to 13. TCP was present in 93% of the samples. The metabolite TCP has been measured in urine as low as 0.5 ppb with a concentration range of 0.5 to 340 ppb of urine (Barr et al, 2004a; Berkowitz et al, 2004; Chang, Lin, Lo, & Lin, 1996; Eskenazi et al, 2004; Fenske, Lu, Barr, & Needham, 2002; Krieger et al, 2001; Morgan et al, 2004). Unfortunately, urine samples were not obtained from participants in the original study from which samples used in this study were derived.

Measuring pesticides in blood is advantageous in that the parent compound, or its metabolites, can be directly measured in plasma and serum. Furthermore, because blood is a regulated fluid and does not vary markedly with water intake or other factors, no corrections for dilution are necessary (Barr & Needham, 2002). The half-life of chlorpyrifos in blood is 24 hours (Nolan, Rick, Freshour, & Saunders, 1984). Chlorpyrifos concentrations have been detected in maternal and cord blood plasma with a range of <1 to 10 ppt (Whaytt et al, 2003; Whayett et al, 2004). Chlorpyrifos metabolites have also been detected in plasma ranging from 0.1 to 2.8 ppm (Drevenkar et al, 1994).

Because chlorpyrifos is a potent inhibitor of acetylcholinesterase, the inhibition of cholinesterase is believed to be the most sensitive tool for evaluating exposure (Abdollahi, Mood, Akhgari, Jannat, Kebriaeezadeh, & Nikfar, 1996). This effect can be measured in red blood cells (AChE) and plasma (PChE). However, due to fluctuations in acetylcholinesterase levels, a baseline is usually required to determine if suppression has occurred (International Biomonitoring Workshop, 2004). Another disadvantage to AChE monitoring is that high

exposure levels are normally required to indicate acute toxicity and since most exposures are more likely to be intermittent, AChE might not be an appropriate measuring tool for chronic exposures (He, 1999). However, De Peyster, Willis, Molgaard, MacKendrick, and Walker (1993) found a high correlation between self-reported pesticide exposure and plasma cholinesterase activity for 203 pregnant women.

Saliva has recently received attention as a possible matrix for measuring pesticides. Scientists at the Department of Energy's Pacific Northwest National Laboratory found very low concentrations of chlorpyrifos in rat saliva (Kousba, Poet, & Timchalk, 2003). The researchers used a modified assay to assess salivary cholinesterase in male rats. In addition, Denovan, Lu, Hines, & Fenske (2000) reported saliva measurements of the herbicide atrazine in human pesticide applicators. The obvious attraction to this type of analytical measurement is that it is easy, cost-efficient and non-invasive.

Breast milk has been shown to be an excellent source for evaluating pesticide exposures. Many organochlorine compounds have already been documented in human breast milk; however, there is only limited data available on any of the chemicals in the organophosphate class, especially chlorpyrifos. In a study by Mattson, Maurissen, Nolan, and Brzak (2000), chlorpyrifos and chlorpyrifos oxon analyses were conducted on milk from female rats that were exposed to chlorpyrifos via gavage. The samples were taken on lactation days one, five, and eleven. According to the authors, not only were chlorpyrifos milk concentrations up to 200 times those in blood but also nursing pups had a

measurable exposure to chlorpyrifos via the milk. Chlorpyrifos has also been detected in human breast milk according to a study from India. The mean chlorpyrifos concentration in 12 breast milk samples obtained during the first month of lactation from Indian women was 230 ug/L (ppb) with a range of 85 to 355 ug/L (ppb) (Sanghi, Pillai, Jayalekshmi, & Nair, 2003).

Scientists have been using gas chromatography for pesticide analysis because of its ability to detect low levels at extremely low concentrations; however, this particular type of method is expensive and requires skilled analysts for sample preparation. The need for rapid, cost effective monitoring methods to assess human exposures to environmental contaminants such as chlorpyrifos have been brought to the forefront by various research laboratories. Immunochemical methods such as immunoassays provide information regarding the presence and concentration levels of various environmental contaminants. This analysis is more time efficient and cost effective than other laboratory analyses such as gas chromatography.

Immunoassays, used to detect and quantify many various types of molecules, which vary widely in size, chemical properties and biological activity, are sensitive, accurate, and reproducible. Most pesticides are rather small molecules and are typically less than 1,000 atomic mass units. Unless they are bound to an antigen, they will not bind to a specific antibody. Therefore, they must be conjugated to a larger protein molecule before they can be used as antigens. Fortunately, several research laboratories have developed selective enzyme-linked immunosorbent assays (ELISA) for specific insecticides. These

ELISAs are based on selective antibodies combined with enzyme reactions to detect low levels of chemicals and was the method of choice used in this study.

Chlorpyrifos RaPID Assay utilizes the principles of an immunoassay developed for the purposes of chlorpyrifos detection in water, wastewater, cereal grains, bananas, and soil. Even though the method had not been used for biological samples, the ease of the test and the minimal cost of the assay made it extremely attractive. A test kit was purchased from Strategic Diagnostics Inc. and a pilot test was run on five samples each of plasma, saliva and breast milk that were spiked with varying amounts of chlorpyrifos. Based on previous human biological studies of chlorpyrifos, this researcher spiked the samples with 0.1, 0.5, 1.0, and 5.0 nanograms per milliliter (parts per billion) (Drevenkar et al, 1994; Sanghi et al, 2003 & Whyatt et al, 2004). The lowest concentration of chlorpyrifos the assay was able to detect was 0.1 ppb with a linear range was 0.22 to 3.0 ppb. The assay is approximately eight times more sensitive to chlorpyrifos than any other related compound.

The assay applies the principles of an ELISA (enzyme linked immunosorbent assay) by relying on antibodies that specifically detect chlorpyrifos and related compounds. To begin the test, 250 micrograms of standards (0.22, 1.0, and 3.0), control, and samples are placed into labeled test tubes. Chlorpyrifos enzyme conjugate (horseradish peroxidase labeled chlorpyrifos analog of HRP) is added to the test tubes followed by 500 micrograms of paramagnetic particles. The chlorpyrifos antibody (mouse monoclonal anti-chlorpyrifos) is covalently bound to paramagnetic particles,

which are suspended in buffered saline. Both the chlorpyrifos in the sample and the HRP labeled chlorpyrifos compete for the binding sites on the antibody bound to the magnetic particles. Following incubation, a magnetic field is applied to hold the paramagnetic particles in place, which allows the unbound reagents to be decanted. After the decanting, the particles are washed with deionized water. The presence of chlorpyrifos is detected by adding a solution of hydrogen peroxide and chromogen (3,3', 5'5'-tetramethylbenzidine) in an organic base. These react to form a colored product. Following a second incubation, this reaction is stopped and stabilized by the addition of sulfuric acid. In the pilot test, the colorimetry results were evaluated with a photometer, which measures optical density (O. D.). Optical density values were then utilized to estimate chlorpyrifos concentrations using the standard curve O. D.s, with the GraphPad Prism software. All three biological samples responded accordingly in that the concentrations followed an appropriate linear curve based on the amount of chlorpyrifos spiked in the sample. The inter assay coefficients of variation for the breast milk samples were 18.56%. Based on this information, more kits were purchased for analysis of the three biological specimens.

Data Collection

In the original study, once the participants volunteered to participate in the study, a packet of instructions, instruments, and containers were mailed to their home address. Between four and six weeks postpartum the mothers were either visited during the morning in their homes or at their health care providers office by one of the trained investigators. On the morning of the visit, according to the

instructions, mothers were to collect a saliva sample and a milk sample (if breast feeding). For the saliva sample, they were instructed not to eat, smoke or brush their teeth one hour prior to collection. The timed five-minute saliva specimen was collected by drool methods into a container. For the breast milk sample, the mothers were instructed to wash their hands with an antimicrobial soap before collection. They were to collect five milliliters (approximately one teaspoon) of milk, either by hand or by use of a breast pump, after they finished nursing. This sample was to be immediately placed in the refrigerator. Once the trained investigator arrived, ten milliliters of venous blood was obtained by antecubital venipuncture and immediately placed on ice. Once the biological samples and paperwork were obtained, they were transported on ice to the laboratory.

For this study, the assays were run in batches. At the time the assays were run, the chlorpyrifos kit and the specimens were allowed to thaw to room temperature. The standards, control, and specimens were set up in duplicates for all the batches for a total of 26 breast milk samples, 52 plasma samples and 52 saliva samples (26 each for lactating and non-lactating subjects). The Chlorpyrifos RaPID Assay was performed according to the manufacturers instructions. Absorbance was measured using a photometer with a wavelength of 450 nm.

Data Analysis

Descriptive statistics were used to describe the sample characteristics and demographic variables of the sample population. Descriptive statistics were also used to compute the mean, mode and median of the chlorpyrifos concentrations

in all three biological samples. Tests for normality were conducted to determine whether parametric or nonparametric tests were appropriate for statistical analysis. The variables requiring log10 transformation were plasma and saliva chlorpyrifos concentrations for positive skewness.

To examine for relationships between milk, plasma, saliva and the other variables, such as age, weight, height, race, income, work, number of pregnancies, number of live births, marital status, smoking, use of prescription or herbal medications and number of weeks postpartum at the time of data collection, a correlation matrix was performed. The Pearson Product Correlation and the Spearman Rho Correlation were both used according to the level of data measurement. An alpha level of .05 was the criterion used for statistical significance. All data analyses were conducted with the SPSS software program.

To determine if there were any differences between the lactating and non-lactating groups, a t-test between the means was performed using the independent samples t-test. The t-tests were run on plasma and saliva for both groups. An alpha level of .05 was the criterion used for statistical significance.

Summary

Bio monitoring or testing humans for pollutants is performed on urine, meconium, amniotic fluid, blood and saliva. Sometimes those particular samples can be invasive and harder to acquire. Human breast milk is easy to obtain, less intrusive, and can provide much needed information on environmental chemicals deposited and retained in fat reserves. This chapter discussed the methods and

procedures that were used for data collection and analysis of chlorpryifos in breast milk, plasma, and saliva samples.

CHAPTER FOUR

RESULTS

The purpose of this study was to determine the concentration range of chlorpyrifos in human breast milk, plasma, and saliva in lactating mothers as compared with plasma and saliva in non-lactating mothers. In this chapter the findings of the study will be presented in three sections. The first section will describe the sample demographics. The second section will report the chlorpyrifos concentrations in the breast milk, plasma, and saliva samples in the lactating group. The third section will report the chlorpyrifos concentrations in the plasma and saliva samples in the non-lactating group.

Sample Demographics

Based on biospecimen availability, there were a total of 26 subjects each for the lactating and non-lactating groups. For the lactating group there were 26 breast milk samples, 26 plasma samples and 26 saliva samples. The non-lactating group had 26 plasma samples and 26 saliva samples. The mean, standard deviations and range were used to describe the variability in the samples.

Tests of normality were performed on the means of all three biological samples, the breast milk, plasma and saliva. The milk variable was normally distributed. (Figure 4) However, the plasma and saliva variables were positively skewed. After verifying the data entries, four plasma chlorpyrifos values and three saliva chlorpyrifos values were identified as greater than three standard deviations from the mean and were therefore deleted (Figures 5 and 6).

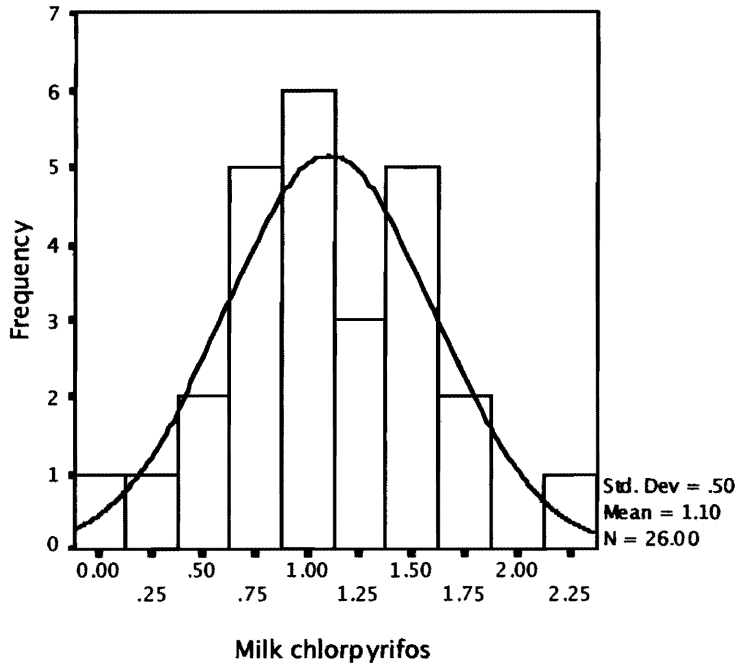


Figure 4. Milk chlorpyrifos concentration in parts per billion (ppb)

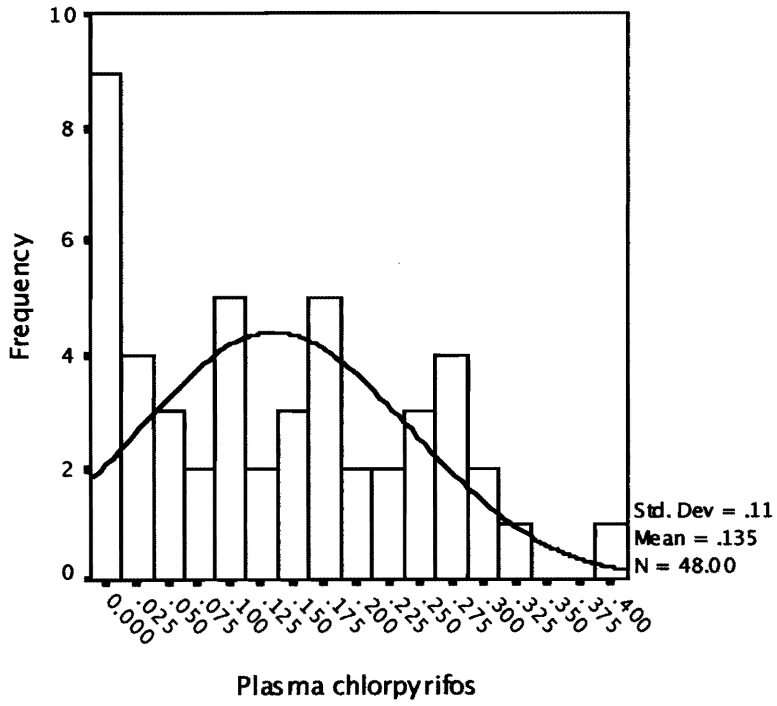


Figure 5. Plasma chlorpyrifos concentration in parts per billion (ppb)

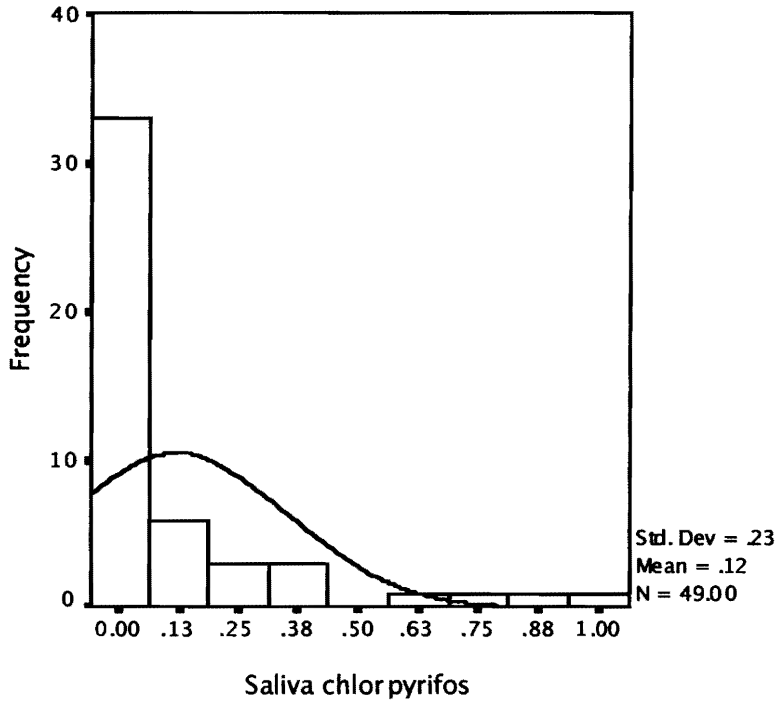


Figure 6. Saliva chlorpyrifos concentration in parts per billion (ppb)

It is important to note that 7 out of 52 plasma and saliva concentrations were extremely high and were therefore deleted. This could be a significant finding in that those seven subjects could have had acute chlorpyrifos exposures resulting in abnormally high levels. This could be a significant finding in and of itself.

The mean age for the lactating group was 28.28 years +/- 5.899 SD (range 19 to 38). The mean weight was 168.53 pounds +/- 40.31 SD (range 132 to 320) and height was 66.12 inches +/- 3.00 SD (range 60 to 72). There was one African American and one Hispanic in the lactating group; the rest were

Caucasian. The mean income was $30,000 to $40,000 +/- 1.41 SD (range <10,000 to >40,000). The majority of the subjects did not work (15 out of 26), 2 worked part time and 9 worked full time, and the majority of them were married (22 out of 26 married, 4 single). The mean number of pregnancies was 2.16 +/- 2.19 SD (range 1 to 12), and the mean number of live births was 1.85 +/- 1.43 SD (range 1 to 8). There were two subjects who smoked, eleven who used prescription medications and six who used herbal medications. Seven of the subjects had cesarean sections, and the rest were delivered vaginally. The mean for the number of weeks postpartum at the time of data collection was 5.16 +/- .845 SD (range 3.6 to 7.0) (Table 1).

Table 1. Demographic statistics for lactating group

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Age	25	19	38	28.28	5.899
Weight	19	132	320	168.53	40.310
Height	25	60.00	72.00	66.1200	3.00444
Pregnancy	25	1	12	2.16	2.192
Live births	26	1	8	1.85	1.434
Marital	26	0	2	1.81	.491
Smoke	26	0	2	1.04	.344
Prescription meds	26	0	25	1.38	4.842
Herbal meds	25	0	1	.24	.436
Weeks postpartum	26	3.6	7.0	5.162	.8457

The mean age for the non-lactating group was 22.84 years +/- 4.35 SD (range 18 to 35). The mean weight was 162.05 pounds +/- 32.39 SD (range 105 to 205) and height was 64.15 inches +/- 3.12 SD (range 58 to 70). There was one African American in the non-lactating group, and the rest were Caucasian. The mean annual income was $10,000 to $20,000 +/- 1.31 SD (range <10,000 to >40,000). The majority of the subjects did not work (19 out of 26), 4 worked part time and 3 worked full time), and the majority of them were married (14 out of 26), 11 were single and 1 divorced. The mean number of pregnancies was 1.96 +/- .841 SD (range 1 to 4), and the mean number of live births was 1.81 +/- .749 SD (range 1 to 3). There were ten subjects who smoked, four who reported current health problems, six who used prescription medications and one who used herbal medications. Six of the subjects had cesarean sections, and the rest were delivered vaginally. The mean for the number of weeks postpartum at the time of data collection was 5.15 +/- .914 SD (range 4.1 to 7.5) (Table 2).

Table 2. Demographic statistics for non-lactating group

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Age	25	18	35	22.84	4.356
Weight	19	105	205	162.05	32.399
Height	26	58.00	70.00	64.1538	3.12016
Pregnancy	25	1	4	1.96	.841
Live births	26	1	3	1.81	.749
Marital	26	1	3	1.62	.571
Smoke	26	1	2	1.38	.496
Prescription meds	26	0	3	.35	.689
Herbal meds	26	0	1	.04	.196
Weeks postpartum	26	4.1	7.5	5.150	.9140

Lactating Group

25 of 26 milk samples were positive for chlorpyrifos (mean 1.10 ppb +/- .503 SD, when detectable the range was .32 to 2.29 ppb). 19 of 26 plasma samples were positive for chlorpyrifos (mean .119 ppb +/- .106 SD, when detectable the range was .019 to .296 ppb) and 10 of 26 saliva samples were positive (mean .186 ppb +/- .299 SD, when detectable the range was .066 to .972 ppb) (Table 3).

Eight of the subjects in the lactating group were positive for chlorpyrifos in all three biological samples. Of these eight, the mean age was 29.37 years, mean weight 188.83 pounds and mean height 67.4 inches. Seven of eight subjects were Caucasian one was African American. The reported income was $20,000 to $40,000 but most of them did not work. The majority of the subjects was married and most reported one live birth. Only one smoked, two used prescription drugs and none of them reported use of herbal medications. The mean for the number of weeks postpartum at the time of data collection was 5.43 weeks.

Three significant correlations were found in the lactation group (Table 4). These correlations were between milk chlorpyrifos concentrations and number of live births (r=. 517, p=. 007, N=26) (Figure 7); between plasma chlorpyrifos concentrations and height (r=. 478, p=. 021, N=23) (Figure 8); and between milk chlorpyrifos and plasma chlorpyrifos concentrations (r=. 444, p=. 030, N=24) (Figure 9).

Table 3. Statistics for biological samples in lactating group

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Milk	26	ND	2.29	1.1015	.50321
Plasma	24	ND	.296	.11975	.106155
Saliva	26	ND	.972	.18623	.299087

Table 4. Spearman Rho Correlation matrix for lactating group

Spearman's Rho Correlations

		Milk	Plasma	Saliva	Height	Live births
Milk	Correlation Coefficient	1.000				
	Sig. (2-tailed)					
	N	26				
Plasma	Correlation Coefficient	.444*	1.000			
	Sig. (2-tailed)	.030				
	N	24	24			
Saliva	Correlation Coefficient	-.037	.363	1.000		
	Sig. (2-tailed)	.857	.081			
	N	26	24	26		
Height	Correlation Coefficient	.013	.478*	.272	1.000	
	Sig. (2-tailed)	.949	.021	.188		
	N	25	23	25	25	
Live births	Correlation Coefficient	.517**	.097	-.342	-.198	1.000
	Sig. (2-tailed)	.007	.652	.088	.344	
	N	26	24	26	25	26

* Correlation is significant at the .05 level (2 – tailed).
** Correlation is significant at the .01 level (2 – tailed)

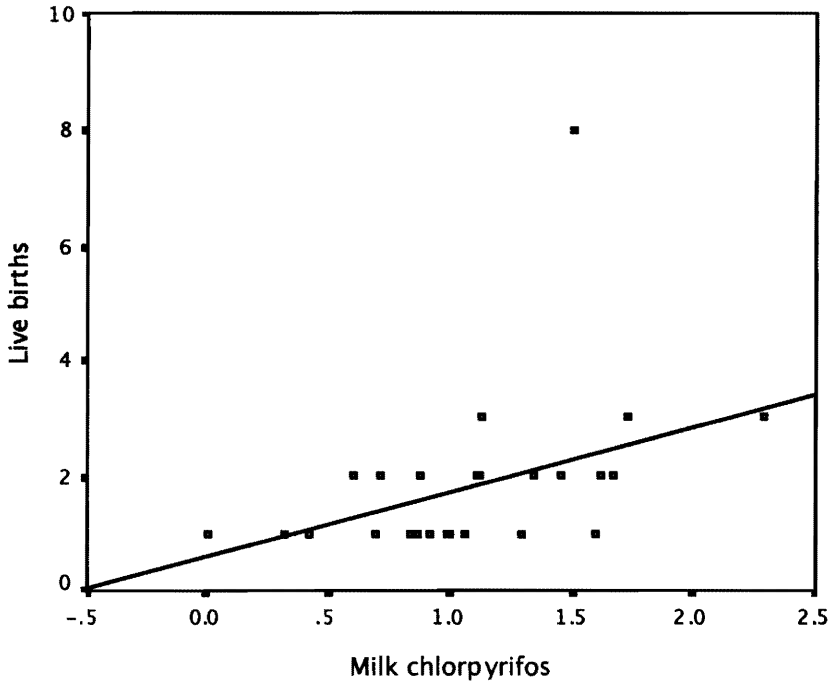


Figure 7. Positive relationship of milk chlorpyrifos concentration in parts per billion (ppb) and live births

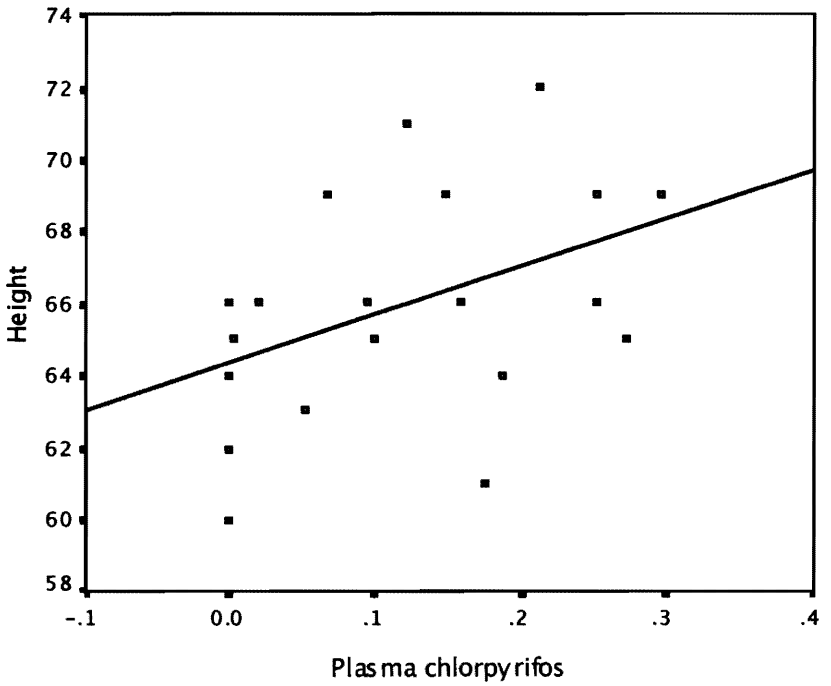


Figure 8. Positive relationship of plasma chlorpyrifos concentration in parts per billion (ppb) and height

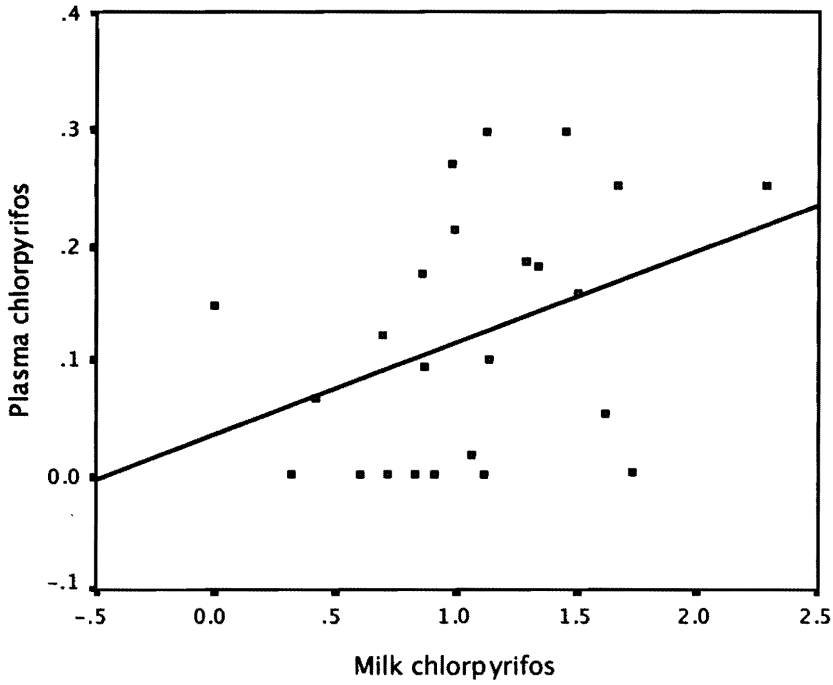


Figure 9. Positive relationship of milk and plasma chlorpyrifos concentration in parts per billion (ppb)

Milk chlorpyrifos concentrations were not correlated with any other demographic variables [age (r=. 167, p=. 424, N=25), weight (r=. 185, p=. 448, N=19), race (r=-.311, p=. 121, N=26), income (r=. 105, p=. 625, N=24), work (R=. 005, p=. 979, N=26), marital status (r=. 371, p=. 062, N=26), use of prescription medications (r=-.057, p=. 783, N=26) or herbal medications (r=-.039, p=. 853, N=25) or with the number of weeks postpartum (r=-.081, p=. 694, N=26)]. Milk chlorpyrifos concentrations were not correlated with salivary chlorpyrifos concentrations (r=-109, p=. 603, N=26).

To test for differences in chlorpyrifos concentrations between the three biological samples in the lactation group, the t-test for dependent samples was used. A paired samples t-test was performed on milk/plasma, milk/saliva, and plasma/saliva. Analysis showed that there was significantly higher milk chlorpyrifos concentrations than either plasma (t=9.734, df=23, p=. 000) or salivary concentrations (t=7.690, df=25, p=. 000) (Table 5). The mean chlorpyrifos milk concentration was 1.06 ppb +/- .502 SD compared to the means for plasma chlorpyrifos .119 ppb +/- .106 SD and saliva chlorpyrifos .188 ppb +/- .299 SD (Table 6).

Table 5. Significance of difference in milk and plasma/saliva chlorpyrifos

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Milk - Plasma	.94108	.473645	.096682	.74108	1.14109	9.734	23	.000
Milk - Saliva	.91531	.606918	.119026	.67017	1.16045	7.690	23	.000
Saliva - Plasma	.06871	.289686	.059132	- .05362	.19103	1.162	23	.257

Table 6. Statistics for lactating group

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Milk - Plasma	1.0608 .11975	24 24	.50276 .106155	.10262 .021669
Pair 2	Milk - Saliva	1.1015 .18623	26 26	.50321 .299087	.09869 .058656
Pair 3	Saliva - Plasma	.18846 .11975	24 24	.310490 .106155	.063379 .021669

Non-lactating Group

In the non-lactating group, all but three of the plasma samples were positive for chlorpyrifos with a mean of .149 ppb +/- .111 SD (range .020 to .395 ppb). Sixteen of the saliva samples were positive for chlorpyrifos with a mean of .044 ppb +/- .061 SD (range .008 to .230 ppb) (Table 7). Fourteen of the subjects in the non-lactating group were positive for chlorpyrifos in both plasma and salivary samples. The mean age was 22.15 years, mean weight was 157.5 pounds (although five of the subjects did not report a weight) and mean height was 63.78 inches. Thirteen of fourteen subjects were Caucasian and one was African American. Most reported an income of $10,000 to $20,000 and most did not work. Half the subjects were married and most reported two live births. Five subjects smoked, four used prescription drugs and none of them reported use of herbal medications. The mean for the number of weeks postpartum at the time of data collection was 5.19 weeks.

Table 7. Statistics for biological samples in non-lactating group

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Plasma	24	.000	.395	.14992	.111530
Saliva	23	.000	.230	.04478	.061405

Fourteen of the subjects in the non-lactating group were positive for chlorpyrifos in both plasma and salivary samples. The mean age was 22.15 years, mean weight was 157.5 pounds (although five of the subjects did not report a weight) and mean height was 63.78 inches. Thirteen of fourteen subjects were Caucasian and one was African American. Most reported an income of $10,000 to $20,000 and most did not work. Half the subjects were married and most reported two live births. Five subjects smoked, four used prescription drugs and none of them reported use of herbal medications. The mean for the number of weeks postpartum at the time of data collection was 5.19 weeks.

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Two significant correlations were found in the non-lactation group (Table 8). These correlations were between plasma chlorpyrifos concentrations and live births (r=-. 433, p=. 035, N=24) (Figure 10) and between plasma chlorpyrifos concentrations and work status (r=-.518, p=. 009, N=24) (Figure 11). The more live births and the more the subject worked, the lower the plasma chlorpyrifos concentrations.

To test for differences between the plasma and salivary concentrations in the non-lactation group, the t-test for dependent samples was used. A paired samples t-test was performed on plasma and saliva. Analysis showed that there were significantly higher plasma chlorpyrifos concentrations than salivary in the non-lactation group (t=-3.860, df=20, p=. 001) (Table 9). The mean chlorpyrifos plasma concentration was .153 ppb +/- .110 SD compared to the mean chlorpyrifos salivary concentration .043 ppb +/- .062 SD (Table 10).

Table 8. Spearman Rho Correlation matrix for non-lactating group

Spearman's Rho Correlations

		Work	Live births	Plasma
Work				
	Correlation Coefficient	1.000		
	Sig. (2 – tailed)			
	N	26		
Live births				
	Correlation Coefficient	.389*	1.000	
	Sig. (2 – tailed)	.050		
	N	26	26	
Plasma				
	Correlation Coefficient	- .518**	- .433*	1.000
	Sig. (2 – tailed)	.009	.0	
	N	2	35	24
		4	24	

* Correlation is significant at the .05 level (2 – tailed).

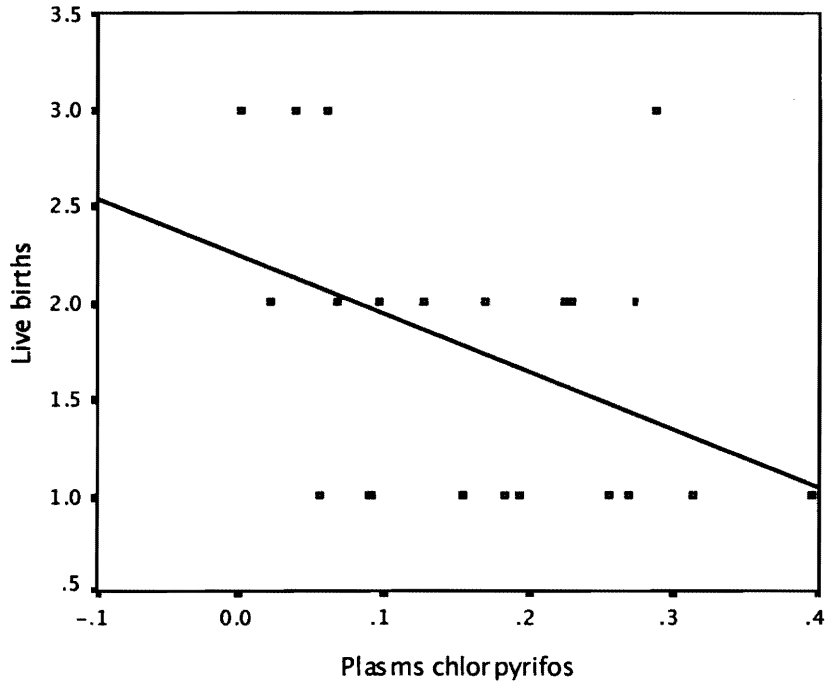


Figure 10. Negative relationship of plasma chlorpyrifos concentration in parts per billion (ppb) and live births

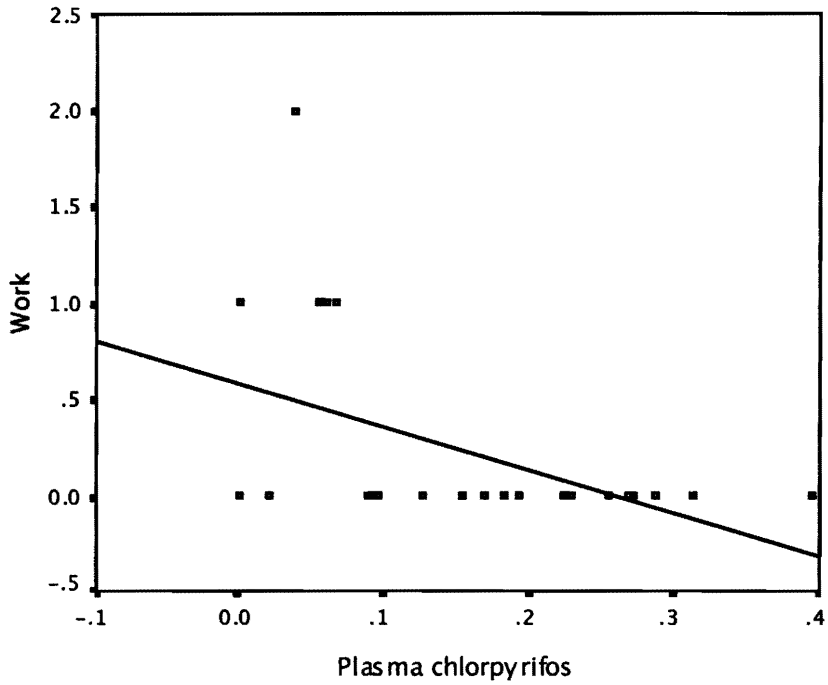


Figure 11. Negative relationship of plasma chlorpyrifos concentration in parts per billion (ppb) and work status

Table 9. Significance of difference in plasma chlorpyrifos

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Saliva - Plasma	-.10962	.130132	.028397	-.16885	-.05038	-3.860	21	.001

Table 10. Statistics for non-lactating group

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Saliva	.04390	21	.062022	.013534
	Plasma	.15352	21	.110290	.024067

To test for differences in plasma and salivary chlorpyrifos concentrations between the lactating and non-lactating group, a t-test for comparison of means for independent samples was used. The mean plasma chlorpyrifos concentration in the lactation group was .119 ppb +/- .106 SD compared to .149 ppb +/- .111 SD in the non-lactation group. However, the mean salivary chlorpyrifos concentration in the lactation group was .186 ppb +/- .299 SD compared to .044 ppb +/- .061 SD in the non-lactation group (Table 11). There was not a significant difference in plasma chlorpyrifos concentrations between the two groups. However, in the salivary chlorpyrifos concentrations, the Levene test did provide sufficient evidence of group difference (F=26.74, p=. 000) so the equal variances not assumed test was used (t=2.356, df=27.36, p=. 026). There appears to be a significant difference in salivary chlorpyrifos concentrations between the two groups and based on the mean, the salivary concentrations in the lactation group are significantly higher than in the non-lactation group (Table 12).

Table 11. Statistics for lactating and non-lactating groups

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2 tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Plasma	Equal variances assumed	.035	.852	- .960	46	.342	- .03017	.031430	- .093432	.033098
	Equal variances not assumed			- .960	45.888	.342	- .03017	.031430	-.093436	.033102
Saliva	Equal variances assumed	26.778	.000	2.224	47	0.31	.14145	.063588	.013526	.269371
	Equal variances not assumed			2.356	27.369	.026	.14145	.060037	.018340	.264556

Table 12. Significance of difference in salivary chlorpyrifos

Group Statistics

	FEED 3	N	Mean	Std. Deviation	Std. Error Mean
Plasma	breast	24	.11975	.106155	.021669
	bottle	24	.14992	.111530	.022766
Saliva	breast	26	.18623	.299087	.058656
	bottle	23	.04478	.061405	.012804

CHAPTER FIVE

DISCUSSION

This study was conducted to determine if an organophosphate insecticide, chlorpyrifos, was present in the breast milk, plasma, and saliva of lactating mothers in comparison to non-lactating mothers. Various pesticides, such as chlordane, DDT, dieldren and heptachlor, have already been documented in human breast milk. Because organophosphates, along with synthetic pyrethroids, comprise 70% of the insecticide market share, it was possible that chlorpyrifos may also be found in women's breast milk. The results of this study have shown that chlorpyrifos is present in breast milk, plasma, and saliva of lactating and non-lactating mothers in measurable concentrations. A discussion of the findings is presented in this chapter, followed by the implications for nursing and recommendations for future studies.

The purpose of this study was to determine the concentration range of chlorpyrifos in human breast milk, plasma and saliva from lactating mothers compared to non-lactating mothers. The samples were chosen from a previously funded research study on stress and lactation. There were a total of 26 participants in two groups: one from women who were exclusively lactating and the other from formula feeders. Overall, for the lactation group, milk chlorpyrifos concentrations were significantly higher than plasma or saliva. Plasma chlorpyrifos concentrations were significantly higher than saliva in the non-lactation group. Comparing the two groups, saliva chlorpyrifos was significantly

higher in the lactation group.

Traditionally, environmental epidemiologists examine the association between exposures to pesticides and adverse health outcomes through risk assessment. This process involves a quantitative estimation of the environmental exposure and the subsequent entry into the human body. In general, most pesticide studies collect historical information regarding pesticide use and exposure via questionnaires or interviews. Since this study involved the use of secondary analysis of biological specimens from a previous study, this researcher was not able to correlate pesticide exposure with the pesticide concentrations found in the three specimens. Even though chlorpyrifos exposure can be variable and can occur through multiple routes, pattern use of chlorpyrifos applications or other types of information could have been an important supplement to biological monitoring. For instance, an acute exposure might explain the difference in chlorpyrifos concentrations among the different specimens in a particular individual. Also, information such as workplace environment or pesticide use during pregnancy would have been helpful in quantifying higher chlorpyrifos concentrations. Since over 40% of Tennessee's total land area is farmland, with cropland accounting for more than 63% (Tennessee Agriculture, 2004), additional information such as proximity to agricultural fields would have also been important information.

This study used quantitative analytical methodology, such as biological monitoring, to determine whether chlorpyrifos was in breast milk, plasma, and

saliva and in measurable concentrations. Each biological sample was analyzed for chlorpyrifos using an ELISA that measured concentrations in parts per billion. The results validated that chlorpyrifos is present not only in breast milk but also in plasma and saliva as well. This is the first known publicized study of chlorpyrifos analysis in human breast milk in the United States, and it is the first publicized study to use an ELISA specific to chlorpyrifos for the analysis of human biological specimens.

Virtually all laboratory studies utilize gas chromatography to assess low levels of environmental contaminants such as pesticides. Not only is this type of analysis extremely expensive but it also requires the use of skilled analysts. Therefore, there is a growing demand for reliable, cost effective methods for determining pesticide exposure by more efficient techniques. Following an extensive search, an immunoassay specific to chlorpyrifos was discovered for use in water, soil, cereal, and fruit. Even though this particular assay had never been performed with human body fluids, the assay performed appropriately when the samples were spiked with varying amounts of chlorpyrifos concentrations. The breast milk, plasma and saliva concentrations followed an appropriate linear curve based on the amount of chlorpyrifos spiked in the sample.

Pre-analytic activities, such as specimen collection, transport, specimen handling and storage, and identification of patient information are important measures. Because this study used biological samples taken from a previous research study, this researcher was unable to validate those methods employed

by the mother in collection of the breast milk or saliva samples. In addition, this researcher was unable to validate the manipulation or storage of the samples upon arrival to the laboratory.

There are also quality assurance procedures to aid in the reproducibility of the assay guide. This includes accuracy and precision to ensure the validity of the assay results. Standards and controls were incorporated into every assay to ensure appropriate testing performance. In addition, several sample assays were run to determine the ability of the assay to consistently reproduce the same result from the same specimen. The interassay coefficient of variation was 18.56% in the breast milk specimens sampled.

There were several issues with the assay that were out of this researcher's control. For instance, the Chlorpyrifos RaPID Assay, states on the package insert that the test is unable to differentiate between chlorpyrifos and other related compounds that have the same phosphate moiety; however, according to the limit of quantification, the test is at least 8X more sensitive to chlorpyrifos than any of the other compounds. In addition, the limit of detection (LOD) for this particular assay was 0.1 ppb with a linear range of 0.22 to 3.0 ppb. Therefore, it is impossible to determine if those samples with non-detectable concentrations were actually zero or simply below limits of detection of the assay.

Unfortunately, it is not possible to compare the results of this assay to previous studies because all other reported chlorpyrifos studies used gas chromatography as their instrument of analysis. Some researchers analyzed the

parent compound, some chlorpyrifos oxon and other researchers analyzed chlorpyrifos metabolites. For example, in the study from India, the researchers describe the use of gas chromatography as their instrument for organophosphate analysis, but they did not report how chlorpyrifos was analyzed. However, as in this study, the researchers did find 12 out of 12 breast milk samples positive for chlorpyrifos with a mean of .230 ppm +/- 0.024 SD (range .085 to 0.355 ppm). It is important to note the differing concentrations between the studies. Sanghi et al, reported chlorpyrifos concentrations in the parts per million as compared to this and other studies of chlorpyrifos concentrations in the parts per billion. Because chlorpyrifos is used extensively in agriculture, particularly wheat and rice crops, this might explain the discrepancy in high concentration levels (Sanghi, Pillai, Jayalekshmi & Nair, 2003).

Lactating Group

In the lactation group, there were 26 samples each of breast milk, plasma and saliva. In this group, the findings were that 25 out of 26 (96%) breast milk samples were positive for chlorpyrifos, 19 out of 26 (73%) plasma samples were positive, and 10 out of 26 (38%) saliva samples were positive for chlorpyrifos. The findings also show that there was significantly higher chlorpyrifos concentrations in milk than either in plasma or salivary concentrations. In addition, 8 out of 26 subjects (30%) were positive in all three biological samples.

Many chemicals can be transferred from the blood and/or from the body

fat stores into the breast milk of a lactating mother. Lipophilic chemicals, such as chlorpyrifos, can accumulate in maternal fat reserves and then be mobilized via lactation through the process of depuration. This pathway, known as the lactational transfer pathway, depends on a number of factors such as the chemical itself, the mother and the milk.

The penetration of a chemical across the cell membrane is dependent upon several properties of the chemical. These include among others, degree of ionization, molecular size, hydrophobicity, and binding to maternal plasma proteins. Chlorpyrifos is non-ionized and since breast milk is more acidic (pH of 6.5 to 7.2) than plasma (7.5), chlorpyrifos concentrations should be higher in the milk since chlorpyrifos passes readily through the cell membrane. Diffusion into the cell membrane is also dependent upon the molecular size of the chemical in that the smaller the molecular weight, the easier it transfers into the lipid barrier of the cell. The mammary epithelium membrane is semi-permeable in that it allows chemicals of low molecular weight passage through the cell membrane. Chlorpyrifos has a molecular weight of 350.6 and thus is able to penetrate through the cell membrane.

The most significant property associated with chlorpyrifos is its hydrophobicity, better known as its lipid solubility. Chemicals are able to partition into milk according to their lipophilicity or octanol: water partition coefficient. Chlorpyrifos has a high lipid solubility coefficient of 4.7 to 5.3 rendering it capable of concentrating in the milk fat and thus producing higher levels in the milk than

in the plasma. Finally, those chemicals that become bound to proteins in the blood become trapped and unavailable for lactational transfer. Both plasma and milk contain proteins but none of the major proteins in breast milk tend to bind chemicals. Therefore, lipid soluble chemicals, like chlorpyrifos, tend to be in continual exchange with the maternal circulation and the milk.

Several maternal factors influence the risk of chemical concentration in the breast milk: the route of exposure, maternal age and weight, number of births, and duration of lactation. Because chlorpyrifos exposure can occur through all the various exposure scenarios (oral, dermal, inhalation, and take-home pathways), the assumption was made that all women are exposed to and accumulate this chemical over a lifetime. Based on this assumption, it makes sense that the amount of the chemical concentration would increase with age. However, there was no correlation between maternal age and chlorpyrifos concentration in any of the three samples. This finding might be explained in part because of the young age in this population. The average age was 28.28 years, which suggests that these mothers might not have had as much chlorpyrifos exposure in their lifespan.

Weight was not correlated with chlorpyrifos concentrations either. Unfortunately there are virtually no studies regarding the absorption, metabolism, and excretion of organophosphate pesticides in pregnant women. For instance, does post pregnancy weight loss impact the amount of chemical excretion? Since body fat contributes to the concentration of chemicals in the breast milk, it

could be argued that any abrupt weight loss could create a physiological excretion response, not just to the breast milk but to all other secretory pathways as well (i.e. hair, tears, sweat, fingernails, and saliva). Another possibility is that with sudden weight loss, the chemicals can be released back into circulation and thus into the fat of the mammary gland. Since seven of the subjects did not report a weight and another six subjects reported a weight greater than 180 pounds, it is possible that sudden weight loss had not occurred by the time of data collection.

There was a significant positive correlation between plasma chlorpyrifos concentrations and height, despite the finding that there was not a correlation between plasma or milk and body mass index. Because seven subjects in each of the two groups did not report their weight, an explanation is that there might have been a correlation had that information been included. Also, since the subjects reported their own height and weight and the average weeks postpartum at the time of data collection was approximately five weeks, those weight measurements could have been taken following discharge from the hospital (four to five weeks earlier) or at their four week postpartum check (one week earlier), suggesting that their current weight might not have been truly reflected at the time of milk collection.

Another maternal factor that can influence the chemical concentration in breast milk is parity. It has been established that lipophilic chemicals are stored in body fat over time and then mobilized into the milk during lactation. Other

chemical studies have shown that the concentration decreases with the number of children that are breast-fed. There was a significant positive correlation between milk chlorpyrfios concentrations and the number of live births. Because this study was dependent upon the demographic information attained from the original study, this researcher is not sure if the mothers were including their recently born infant as a live birth since the average number of live births was 1.8. In addition, there was not any information regarding feeding type with any previous children so it is impossible to know whether this particular infant was the first to be breast-fed or whether this factor might influence the results of this study.

Factors that influence chlorpyrifos in breast milk include milk composition and milk to plasma ratio. The composition of milk changes according to the gestational age of the infant. Just as chlorpyrifos concentration decreases with the number of nursed children, so does the amount of chlorpyrifos over the course of lactation. As a mother breast-feeds, the concentrations of chemicals in breast milk decline over the weeks and months of lactation.

Changes in the fat content of breast milk can occur during a feeding, from feeding to feeding, and over time. Since lipophilic chemicals pass into the milk, the fat concentration is probably the most crucial and variable of all factors. Lactogenesis II begins about 40 hours after birth and lasts approximately five days. The fat content during this stage is approximately 2.9%. The next stage of milk development is the transitional milk and it generally lasts from day six to day

ten of lactation. During this stage, immunoglobulin and protein content starts to fall while fat begins to rise so that by full lactation (galactopoiesis), the fat content is approximately 4%. In addition, hind milk can have an overall higher fat concentration (4 to 6%) compared to foremilk (1 to 2%).

Studies have shown that lactation is a functional excretion pathway for lipophilic chemicals and by four to six weeks, and in this study the measurable concentrations of chlorpyrifos has diminished. The average number of weeks postpartum at the time of data collection was approximately five weeks, and there did not appear to be a correlation between milk chlorpyrifos values and the number of weeks of lactation. The subjects were carefully scrutinized by the researchers as to their exclusivity to ensure that mothers were strictly bottle feeding or breast feeding and not supplementing with other sources of nutrition. In addition, lactating mothers were given an information sheet instructing them to collect their milk from the breast when finished nursing either by hand expression or breast pumping. The samples were to be collected in the morning between 7:00 a.m. and 12:00 noon and then immediately placed in the refrigerator. However, since the information packets were sent out well in advance of data collection, it is impossible to know not only what time of day the milk samples were taken but also during what part of the feeding cycle milk samples were taken. In addition the variation in sampling time (i.e. three to four weeks postpartum compared to five to seven weeks postpartum) could have played a significant role in the amount of milk chlorpyrifos concentrations.

The milk to plasma (M/P) ratio is an estimation of a chemical concentration in milk compared to its concentration in maternal plasma. Pharmacologists often use this ratio to estimate the amount of drug delivered via breast milk to the nursing infant. Those chemicals with a high M/P ratio are thought to have higher concentrations in the milk than in plasma, and those with lower M/P ratios are thought to be lower in milk and higher in plasma. Most chemical profiles that exhibit higher M/P ratios are very fat-soluble, have weak bases and small ionization potential. Since chlorpyrifos has the appropriate characteristics associated with a higher M/P ratio, this would explain the positive correlation between milk and plasma chlorpyrifos concentrations as well as the statistically higher milk chlorpyrifos values compared to plasma.

Non-lactating Group

There were 26 samples each of plasma and saliva in the non-lactation group. In this group, the findings show that 23 out of 26 (88%) plasma samples were positive for chlorpyrifos and 16 out of 26 (61%) saliva samples were positive. In addition, 14 out of 26 subjects were positive for both. The findings also showed that there were significantly higher plasma chlorpyrifos concentrations in this group.

During pregnancy, many physiological changes occur in the mother as a result of the rapid growth of the fetus and reproductive organs. These changes directly influence the toxicokinetics (absorption, distribution, metabolism, and elimination) of lipophilic chemicals. For instance, cardiac output increases by

50%, resulting in an increased blood volume followed by increased chemical distribution. In addition, the amount of chemicals bound to plasma proteins also increases considerably. Therefore, fat body stores would be expected to mobilize with subsequent release into the circulatory system. This would explain the high plasma chlorpyrifos concentrations. It is theorized that the chemical in the adipose storage depot is in relative equilibrium with the concentration in the blood or plasma so that as the chemical is released into the blood, it is also being eliminated from the blood to maintain the equilibrium. According to Rozman and Klaassen (2001),

> The compartment where a chemical is concentrated can be thought of as a storage depot. Toxicants in these depots are always in equilibrium with the free fraction in plasma. As a chemical is biotransformed or excreted from the body, more is released from the storage site. As a result, the biological half-life of stored compounds can be very long.

Because the pharmacokinetics for plasma and saliva are comparable to each other, it would seem logical that chlorpyrifos would behave similarly in both compartments. However, before any chemical circulating in the plasma can be excreted into the salivary duct, it must contain certain physicochemical properties that allow it passage into the lipophilic layer of the salivary epithelial cell membrane, such as lipophilicity and molecular size. Those chemicals with high lipid: water partition coefficients, and low molecular weights can influence passive diffusion from the plasma to the saliva. This is accomplished by

permeating cell membranes through the aqueous pores and therefore expending no energy (Rozman & Klaassen, 2001). In a study of rats exposed to diazinon (another organophosphate with a similar chemical structure as chlorpyrifos), concentrations in saliva were consistently lower than those in arterial plasma. The authors suggest that this difference is most likely due to protein binding of diazinon in the plasma (Fenske, Lu, & Showlund-Irish, 2005). Since chlorpyrifos and diazinon are so chemically similar, this could explain the resultant concentrations in the two compartments.

There were two significant correlations in the non-lactation group between plasma chlorpyrifos concentrations, live births and work status. The more live births reported the lower the plasma concentrations and those that worked part-time had lower concentrations as well. It could be argued that fat stores were mobilized into the circulatory system and potentially excreted (kidneys, saliva, etc.) with each subsequent pregnancy and birth. In addition, those that worked part-time to full-time spent less time at home with subsequently less chlorpyrifos exposure. Again, since the subjects were younger in age, it is possible that their work environments were safer than home in that some may still have chlorpyrifos containing products in their garages or under their sinks.

Analysis between the lactation group and the non-lactation group revealed significantly higher salivary chlorpyrifos concentrations in the lactation group. Since the lactation group had significantly higher milk chlorpyrifos concentrations as well, it makes sense that both of these excretion pathways have been

saturated with chlorpyrifos. In addition, the fact that chlorpyrifos is so lipophilic and the fat in milk is higher than in saliva, would explain why the milk would contain higher concentrations than the saliva.

Nursing Implications

Based upon the findings of this study, it can be concluded that chlorpyrifos is present in breast milk in measurable amounts. If chlorpyrifos is present, then what other environmental chemicals are also present in breast milk? This begs the question, "What are these chemicals doing to our precious children?" Those questions can only be answered through future studies.

The nursing profession has a unique opportunity to take the lead in addressing the health issues associated with environmental contaminants. All nurses need to be more educated with toxicology concepts and public health issues as this affects nurses at every level. Nurses need to become more involved in research endeavors so that we are armed with scientific evidence of health effects of chemical contamination and exposure. The results of this study alone could be a pivotal beginning in spurring bigger laboratories to test not only this chemical but also other chemicals like it.

Breast is always best; however, mothers need to be aware of the associated dangers of this and other pesticides. Not only pregnant women and nursing mothers but also women everywhere need to be better informed of the potential effects of pesticide exposure throughout the lifespan. Of course, the first and most important concern is the health of our children and the disastrous

effects these chemicals could be having on their developing brains. However, another concern, which this paper did not address, is women's health issues, such as breast cancer, autoimmune diseases, and osteoporosis. Is there an increased propensity for women to develop breast cancer as a result of a lifetime of toxic poisons stored in their breast tissue? What about the women who do not breast-feed? Are these toxins getting excreted in other ways or are they stored continuously in the adipose tissue? Education is paramount, and unfortunately we can no longer leave the education role in the hands of the Environmental Protection Agency or in the hands of the professional exterminator. Now is the time to better educate our communities on the dangers of chlorpyrifos, diazinon or any other toxic chemical. We cannot wait until there is sufficient evidence to ban the chemical from use because as we now know, that is too late as it has already saturated itself into the soil, the water, and our ecological system. In summary, nursing can protect the health and well being of women, mothers and their infants by generating new knowledge via scientific findings, communicating the associated health implications and implementing this knowledge into nursing practice.

Future Studies

The future of a healthy tomorrow depends on the science of yesterday. We are walking petri dishes full of scientific evidence of environmental exposures. All one has to do is test. We cannot rely on animal testing to determine the biopartition of any chemical in the environment or in the body. All

of these chemicals need to be tested on animals, retested on animals, and then tested again on animals until the scientific community is absolutely confident that a chemical does not pose a threat to our health. Even this study could be validated using gas chromatography so that more chemical assays could be developed to allow for easier and cost effective monitoring.

Participation in a comprehensive breast milk-monitoring program is key to an informed public. This would allow for the development of standardized protocols for specimen collection and analysis worldwide. Dr. LaKind (2001) and associates have agreed unanimously to develop such a program so that women from various socioeconomic backgrounds and geographic locations can be tested under similar conditions. This testing would provide scientifically based information to breast-feeding women on the risks of various chemicals.

It is imperative that studies be done on our children. In a study by Perea (2003), chlorpyrifos concentrations were strongly associated with decreased birth weight and body length in babies of African-American women living in New York City. Additional studies, not just those involving chlorpyrifos, need to be prospectively performed to assess cognitive motor deficits, IQ and developmental delays.

Summary

There is mounting evidence of pesticide contamination in the breast milk of lactating women. This study has contributed to this knowledge by documenting chlorpyrifos concentrations in breast milk, plasma and saliva of lactating and non-

lactating women. Bio monitoring of human breast milk provides an easy way to determine the body burden of lipophilic chemicals such as chlorpyrifos. Our children's health depends on it.

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VITA

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