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Comparison of Serum Versus Lipid-Adjusted Concentrations of Organochlorine Pesticides in the Evaluation of Associations with Exposure Variables and Health Effects in a Community-based Environmental Health Study

THESIS

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in Environmental Toxicology

by

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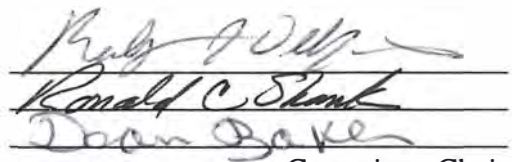

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ABSTRACT OF THE THESIS

Comparison of Serum versus Lipid-Adjusted Concentrations of Organochlorine Pesticides in the Evaluation of Associations with Exposure Variables and Health Effects in a Community-Based Environmental Health Study

By

Richard Campbell

Master of Science in Environmental Toxicology

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Professor Dean B. Baker, Chair

Adipose tissue concentrations of organochlorine pesticides are difficult to measure; therefore, serum concentrations are used as a proxy. Lipid-adjustment of serum concentrations has been proposed as a more accurate indicator of adipose concentrations. This study examined clinical data from 500 participants in the Del Amo/Montrose Community Environmental Health Program to assess correlations between serum and lipid-adjusted concentrations of organochlorine pesticides. The study also analyzed associations between these pesticides and demographic and exposure variables, and with health outcomes to determine whether the patterns of association were affected by lipid-adjustment or were different among the pesticides. General linear models and least squares linear regression were used to analyze exposure variable associations, while logistic regression was used to analyze dichotomous health outcomes.

Adjusting for lipids did not substantially change the patterns of associations for pesticides in the analyses of exposure variables or health outcomes. Pesticide concentrations were

associated with increasing age and higher educational level, but the trends were weaker with lipid adjustment. DDT, hexachlorobenzene, and hexachlorocyclohexane concentrations were associated with prior residence in Mexico or Latin America, but other pesticides were not. Aldrin/dieldrin, chlordane, and DDT were associated with local egg consumption. Heptachlor and hexachlorocyclohexane were associated with fish consumption. Some health outcomes were associated with pesticide concentrations, but in no general pattern. Overall, lipid adjustment did not materially affect associations between organochlorine pesticide concentrations and exposure variables or health outcomes. Associations for some pesticides were consistent with expected routes of exposure and suggest the possibility of excess past environmental exposures.

INTRODUCTION AND PURPOSE

Assessing human health risks from exposure to xenobiotic lipophilic compounds poses a unique set of methodological challenges. One of these challenges is how to best quantify body burden of these compounds. Measurement of body burden is relevant because these compounds are highly persistent in biological systems, so body burden concentrations can be interpreted as an indicator of cumulative exposure to the compounds. The compounds are highly lipophilic, so the best measure of body burden is the pesticides' concentrations in adipose tissue. However, adipose tissue samples are difficult to obtain and biopsy or aspiration of adipose is generally not acceptable to patients in clinical settings or participants in epidemiological studies. Therefore, serum concentrations are often used as a proxy indicator for adipose concentrations, based on the assumption that serum concentrations of these compounds are in equilibrium with adipose concentrations and that the two measurements are correlated.

Various methods of quantifying xenobiotic lipophilic compounds in serum have been utilized, including wet weight (xenobiotic lipophilic compound per unit of serum), lipid weight (xenobiotic lipophilic compound per unit of serum lipids), standardization (conceptually similar to using body mass index to account for adiposity), use of a log-linear model with serum lipids included as a separate term in the regression equation, or two-stage analyses where serum lipids are regressed on serum xenobiotic lipophilic

compound concentrations with the residuals entered as an individual risk factor (Schisterman et al. 2005).

For quite some time, the most common method of reporting the concentration of xenobiotic lipophilic compounds in serum was by wet weight. However, recently it was proposed that the serum concentrations of xenobiotic lipophilic compounds should be adjusted, or corrected, to an individual's serum lipid level. This lipid-adjusted concentration is thought to be the xenobiotic concentration to which pharmacological response is proportional (Brown and Lawton 1984). In a recent study which statistically analyzed non-adjusted (wet weight), standardized, adjusted, and two-stage models, the adjusted model was found to perform quite well under a variety of circumstances, and better overall than the other models analyzed (Schisterman et al. 2005). Because of the equilibrium between adipose tissue and serum, the lipid-adjusted concentration, which is effectively the concentration in the lipid compartment of the serum, is equal to the compounds concentration in adipose tissue.

To examine whether or not adjusting serum concentrations of xenobiotic lipophilic compounds for serum lipids affects (1) associations between possible sources of exposure, such as demographic and neighborhood variables, and serum pesticide concentrations and (2) associations between pesticide serum concentrations and health outcomes, data from the Del Amo/Montrose Community Environmental Health Program was analyzed, comparing equivalent analyses using serum wet weight concentrations versus lipid-adjusted serum concentrations for several organochlorine pesticides.

Chapter 1

BACKGROUND

The Montrose Chemical Plant was located in Los Angeles County, California, near the city of Torrance. From 1947 until 1982 it manufactured, ground, packaged, and distributed the pesticide dichlorodiphenyltrichloroethane (DDT) in technical grade. During this period of manufacturing, hazardous substances entered the environment through various means, such as releases of wastewater directly into the ground, through storm water drainage pathways, aerial dispersion of DDT dust, and disposal of DDT in soil fill. Also released into the environment through similar mechanisms was monochlorobenzene, which is the raw material used in making DDT. Both DDT and monochlorobenzene are dense non-aqueous phase liquids and do not dissolve well in water. However, significant quantities of DDT remain dissolved in the monochlorobenzene in the ground. Also present at the Montrose plant during its operations was the pesticide hexachlorocyclohexane (CDHS 2004). Additional chemicals stored at the site, as shown by an Environmental Protection Agency (EPA) notification, were chloral, corrosives, and chlorobenzene. The Montrose site was capped in 1985 (Baker 1999). The EPA has designated the Montrose Chemical Plant area as a Superfund Site, and is continuing to investigate and remediate the area. (CDHS 2004)

Located adjacent to the Montrose Chemical Plant was the Del Amo Facility which consisted of a styrene plant, a butadiene plant, and a synthetic rubber plant. These plants ceased operation in 1971. A 3.7 acre area on the site, which is located near residential property, was the primary waste disposal area. The primary contaminants in this area are volatile aromatic hydrocarbons such as benzene and ethylbenzene, and polycyclic aromatic hydrocarbons. The site of the Del Amo Facility is also designated as a Superfund Site (Baker 1999).

Based on concerns regarding these sites, the Del Amo/Montrose Community Environmental Health Program was established to "assess the health status of community members living near the Del Amo/Montrose hazardous waste sites in order to provide individual counseling and to identify possible public health interventions (Baker 1999)." One aspect of this multifaceted environmental health program was to establish a local environmental health specialty clinic and offer free medical evaluations to residents of a well defined neighborhood area adjacent to the two Superfund sites. This clinic operated for about three years, during which nearly 600 residents voluntarily participated in the medical evaluations. These evaluations included measurement of serum concentrations of DDT, DDE, and DDD, which were analyzed by a specialty laboratory at the US Centers for Disease Control and Prevention (CDC). The wet-weight serum concentrations of these pesticides were then evaluated in the residents to see if they were associated with a number of variables such as age, gender, location and length of residence in the neighborhood, history of having lived in Mexico or Latin America, or behaviors such time spent participating in activities in the garden or yard that involved

contact with the soil, whether the person ate fish or shellfish caught by them or someone they know from a pier or boat, or consumption of eggs from chickens raised in the neighborhood. The serum concentrations were also examined for associations with a number of health outcome variables. Also measured, but not previously evaluated because they were not part of the original program, were serum concentrations of organochlorine pesticides such as aldrin, dieldrin, endrin, chlordane, heptachlor, mirex, hexachlorobenzene, and hexachlorocyclohexane.

Using data obtained from the Del Amo/Montrose Community Environmental Health Program, this study examined the serum concentrations of certain xenobiotic lipophilic compounds (organochlorine pesticides) to determine if there was a significant difference between wet-weight and lipid-adjusted serum concentration values. This study examined the relationships between these organochlorine pesticides and several of the original exposure variables and health outcomes used in the Del Amo/Montrose Community Environmental Health Program to evaluate whether adjustment for serum lipids changed any associations between the exposure variables or health outcomes studied.

Chapter 2

LITERATURE REVIEW

Common Properties of Organochlorine Pesticides

The organochlorine pesticides are lipophilic xenobiotics that are persistent in the environment and human body. They can be classified into four categories: dichlorodiphenylethanes such as DDT, cyclodienes such as chlordane and dieldrin, chlorinated benzenes such as hexachlorobenzene (HCB), and cyclohexanes such as hexachlorocyclohexane (HCH). When released into the environment, some organochlorine pesticides adhere to soil or particles in the air, while others are volatile. They are adsorbed to sediments in aquatic systems, and then bioaccumulate in fish, shellfish, and marine mammals (CDC 2005). They generally have slow biotransformation and degradation, and therefore tend to bioconcentrate in higher levels of food chains. Their slow biotransformation and degradation is partially due to their complex aromatic ring structures, and additionally due to their chlorine substituents which are difficult to remove by the enzymatic processes available in most tissues. Since these compounds are highly lipophilic and biotransform/degrade slowly, they accumulate in tissues of the body with a high lipid content. They can remain in such tissues for long periods of time, with only small amounts equilibrating with the blood and being degraded and excreted (Klassen 2001). Therefore, measurement of these organochlorines in the

serum may indicate either recent or past exposure, or both. Furthermore, since organochlorines are stored in adipose tissue, loss of this tissue, such as during fasting, will mobilize the stored compound and increase blood levels (ATSDR 2005). Although there is minimal current use of organochlorines in the United States, some other countries continue to use them.

There are a number of ways that humans can be exposed to these organochlorines. They may enter the environment from disposal of contaminated wastes in landfills, direct application and runoff, releases from manufacturing plants that produce them, and emissions from waste incinerators. Therefore, exposures continue from sources such as hazardous waste sites, areas of previous use such as farms, and food. In general, the main source of exposure to organochlorine pesticides is through food. Since these compounds are soluble in fat, they are found in highest concentrations in fatty foods. Therefore, ingestion of fatty foods such as fish, meat, and dairy products, is a common source of exposure for the general population (CDC 2005). Other food products or supplements can also be a source. For example, a recent study showed that thirteen out of thirty (43%) of tested samples of ginseng extract did not comply with the maximum residue limits for hexachlorobenzene, total hexachlorocyclohexane, lindane, and total heptachlor (Durgnat, et al. 2005). Exposure to the fetus may occur through the placenta, or through human breast milk for infants. A study of human breast milk from healthy volunteers in Taiwan showed the predominant organochlorine pesticides were p,p'-DDE, p,p'-DDT, alpha-chlordane, heptachlor epoxide, heptachlor, beta-hexachlorocyclohexane, and gamma-

hexachlorocyclohexane, with median levels of 228, 19, 7.4, 4.0, 2.3, 1.2, and 0.8ng/g lipid, respectively (Chao et al. 2005).

Aldrin and Dieldrin Metabolism and Toxicity

Aldrin and dieldrin are structurally similar compounds that were produced from the 1950's to 1970 as pesticides. They were used extensively on crops such as corn and cotton, and dieldrin was used as a sheep-dip pesticide. All use of aldrin and dieldrin was cancelled by the U.S. Department of Agriculture in 1970, but in 1972 the Environmental Protection Agency approved aldrin and dieldrin for use in killing termites. This use continued until 1987 (ATSDR 2002).

Aldrin and dieldrin are still present in the environment from past use as pesticides. They have also entered the environment from accidental spills or from leaking containers at waste sites. After agricultural application, aldrin either volatilizes or is rapidly converted to dieldrin (CDC 2005). In biologic systems, aldrin is quickly converted to dieldrin through a microsomal oxidation reaction (epoxidation). Dieldrin, however, degrades very slowly. It adheres to soil and may remain there unchanged for many years. The half life in temperate soils is approximately five years, but less than one month in tropical soils. It does not dissolve well in water, but does adhere to sediments at the bottom of bodies of water. In the air, dieldrin changes to photodieldrin within a few days. It can be taken up from the soil and stored by plants. Animals or fish who consume dieldrin-contaminated plants or wildlife can store large amounts of dieldrin in their fatty tissue.

Blood levels of dieldrin are specific for both aldrin and dieldrin due to the rapid conversion in the body of aldrin to dieldrin. As with other organochlorines, the blood level may indicate either recent or past exposure, or both. The mean half-life of dieldrin in the blood has been estimated to be from 266 to 369 days (ATSDR 2002).

Humans can be exposed through inhalation, oral, or dermal routes. Humans can be exposed to aldrin or dieldrin either directly by working with these compounds, by coming into contact with contaminated air, soil, or water, or by consuming dieldrin-contaminated plants, fish, or animals. The highest bioaccumulations seem to be in fish and mollusks. The dietary route of exposure appears to be the most significant, with contributions from plants or animals grown on contaminated land. Foods high in animal fat, such as dairy, meat, or fish, could be significant sources of these compounds. Daily intake of aldrin and dieldrin in the United States was reported to be 40 ng/kg/day and 80 ng/kg/day, respectively, from 1965-1970. In 1988, dieldrin intake had decreased to an estimated 5 ng/kg/day for adults and 11 ng/kg/day for infants. Currently, aldrin is below the level of detection in food. At hazardous waste sites, exposure can occur from breathing volatilized aldrin or dieldrin, or through the skin by touching contaminated soil. Aldrin and dieldrin can be found in areas distant from hazardous waste sites, as these compounds can be redistributed by air currents after volatilizing from the soil. However, a recent study showed that blood dieldrin levels decreased by 1.6 ng/g with each mile of distance from a potential dieldrin source (Gaffney et al. 2005). Due to the persistence of these compounds, humans can also be exposed to them by living in a home that was once treated for termites using either aldrin or dieldrin. However, this route of exposure

should be uncommon because detectable levels are usually found only for about 10 years after the last application, and use of aldrin and dieldrin for this purpose was discontinued in 1987. Dieldrin can cross the placenta. Dieldrin can be mobilized from fat stores during lactation, and infants may be exposed by ingesting contaminated human breast milk. Most dieldrin and its metabolites, such as 9-hydroxydieldrin, leave the body through the feces, although some may be excreted through the urine (ATSDR 2002).

Heavy exposure to aldrin or dieldrin can cause central nervous stimulation and lead to convulsions, and may cause liver or kidney damage. Moderate chronic exposure may lead to headaches, dizziness, irritability, vomiting, or myoclonic jerking. It may also lead to hemolytic anemia. It is unclear whether or not aldrin and dieldrin have adverse reproductive effects (ATSDR 2002). Stimulated by the finding that Parkinson-like symptoms can be caused by the exogenous substance MPTP (an impurity of synthetic heroin that is similar in structure to the herbicide paraquat), and epidemiologic studies that suggest an association between pesticides in general and Parkinson's disease, there has been speculation that pesticides may be associated with the onset of Parkinson's disease (Le Couteur et al. 1999). In fact, when postmortem brain samples from 20 Parkinson's disease patients, 7 Alzheimer's disease patients, and 14 controls were assayed for organochlorine pesticides, dieldrin was detected in 6, 1, and 0 of the brain samples, respectively. The association between Parkinson's disease and dieldrin in this small study was found to be significant (Fleming et al. 1994). One recent review of the literature concluded that "there is mounting evidence that chronic moderate pesticide exposure is neurotoxic and increases risk of Parkinson disease (Kamel, F and Hoppin, JA

2004)." However, another recent review of the literature found no sufficient evidence of a causal association between Parkinson's disease and dieldrin (Li et al. 2005). One group of researchers believe there may be an association between dieldrin exposure and breast cancer (Hoyer et al. 2002) and an association between dieldrin exposure and breast cancer survival (Hoyer et al. 2000). The EPA has determined, based on animal studies, that aldrin and dieldrin are probable human carcinogens. The International Agency for Research on Cancer (IARC) has determined that aldrin and dieldrin are not classifiable as to their carcinogenicity to humans (ATSDR 2002).

Chlordane Metabolism and Toxicity

Technical grade chlordane is mixture of more than 140 closely related chemicals, of which 10 are major components. Most are trace components. It contains sixty to eighty five percent *cis*- and *trans*-chlordane stereoisomers. The two other major components are heptachlor and *cis*-and *trans*-nonachlor. Other components, present in more than minor amounts, are alpha, beta, and gamma-chlordene; 3a,4,5,5a,6-exo-hexachloro-1a,2,3,3a,5a,5b-hexahydro-1,4-methano-1H-cyclobuta[cd]pentalene; and 2,4,4,5,6,6,7,8-octachloro-2,3,3a,4,5,7a-hexahydro-1,4-methano-1H-indene. It was used prior to 1983 as a pesticide for lawns, crops, gardens, and as a fumigating agent. Between 1983 and 1988, it was approved for use only to control termites (ATSDR 1994).

Chlordane is still present in the environment from past use. It attaches strongly to the particles in the upper layers of soil and generally does not enter groundwater. It is not known if chlordane breaks down in soil. It can persist in some types of soil for 20 years or more, and is lost from the soil mainly through evaporation. Chlordane attaches to sediments in water. In the air it can break down by reacting with light and other chemicals, but it can travel long distances before this breakdown occurs. Chlordane bioaccumulates in the fatty tissues of birds, fish, animals, and humans (ATSDR 1994).

Essentially all residents of the United States have been exposed to low levels of chlordane. Humans can be exposed through inhalation, oral, or dermal routes. Before its ban in 1988, those who worked in the manufacture, formulation, or application of chlordane may have been exposed to high levels. Today, humans receive the highest exposure from homes that had been treated with chlordane to control termites. It is estimated that at least 50 million persons have lived in chlordane-treated homes. The indoor air levels in these homes have been found to range from 0.00003 to 0.06 milligrams per cubic meter, with even higher levels found in basements and crawl spaces. Another source of exposure is from eating food that is contaminated with chlordane. It was widely used on farmland, where it can persist for decades. Chlordane can also be found in fish and shellfish from contaminated waters. The FDA calculated that daily intake from food was 0.0013 ug/kg body weight for infants and 0.0005-0.0015 ug/kg body weight for teenagers and adults. Metabolism in humans results in a number of oxidation products such as chlordane chlorohydrin; monohydroxylated dihydrochlordene; oxychlordane; 1,2-dichlorochlordene; 1-hydroxy-2-chlorochlordene; 1-hydroxy-2-chloro-

2,3-epoxychlordene; 1,2-hydroxychlordene; heptachlor; heptachlor epoxide; trihydroxydihydrochlordene; and beta-glucuronide-1-hydroxydihydrochlordene. Chlordane and its metabolites are excreted primarily through the bile and to a lesser extent through the urine. Chlordane and its metabolites can also be passed to infants through human breast milk and through the placenta (ATSDR 1994).

Heavy exposure to chlordane may lead to nervous system abnormalities such as convulsions. Lower level exposures can lead to other neurologic effects such as headaches, irritation, confusion, weakness, and vision problems. Exposure can also lead to gastrointestinal complaints such as vomiting, diarrhea, cramps, and jaundice. There may be changes to liver function with chlordane use. The International Agency for Research on Cancer (IARC) has determined that chlordane is not classifiable as to its carcinogenicity in humans. Chlordane or its metabolites may be hepatotoxic and immunotoxic. There is no clear evidence that chlordane causes birth defects or reproductive problems (ATSDR 1994).

DDT Metabolism and Toxicity

Dichlorodiphenyltrichloroethane (DDT) is an organochlorine pesticide which is quite persistent in the environment. Technical-grade DDT, as manufactured at the Montrose site, also contains its metabolites dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD) as contaminants. DDT was used extensively to

control mosquitoes, but its use was banned in the United States by the EPA in 1973. The primary route of exposure is ingestion when a person eats food contaminated with these compounds. Although it is no longer used in the United States, DDT is still used in other countries and therefore food imported from other countries may contain DDT or its metabolites (CDC 2005). Small amounts may also be inhaled and absorbed into the body through the lungs (ATSDR 2002). Very limited amounts of DDT may be absorbed through the skin (Klaassen 2001). Fetuses may be exposed via placental transfer and infants may be exposed by breastfeeding.

Once ingested or absorbed, DDT can undergo reductive dehalogenation by DDT dehydrochlorinase and reduction by glutathione. Both DDT and DDE are very persistent in the human body, with the metabolite DDE being most persistent. Neither of these compounds can be excreted because they are quite insoluble in water and accumulate in the adipose tissues. Therefore, to be excreted, each must be further metabolized. It is thought that this metabolism occurs via the enzyme DDT 2,3-dioxygenase, which may oxidize one of the benzene rings to form 1,1-dichloro-2-(dihydroxy-4'-chlorophenyl)-2-(4-chlorophenyl)ethylene. This metabolite may then be cleaved to form the more water soluble dienoic acid (Shank 2004). The metabolism is slow, as humans only metabolize approximately 1% of the body burden of DDT per year. Therefore, the concentration of DDT or its metabolites in the body is determined by the combination of both current and past exposures.

Toxicity in humans appears to be neurologic, but there have been recent studies suggesting additional toxic effects of DDT, such as increased levels of DDE in the blood may lead to increased risk of having a pre-term baby (Venners et al. 2005) and asthma in young children (Sunyer et al. 2005). Studies in animals have shown that long-term exposure to moderate amounts of DDT may affect the liver in various ways which includes causing liver cancer. Studies in animals have also shown that DDT and its metabolites can have a harmful effect on the adrenal gland and on reproduction. Studies in humans have not demonstrated that DDT or its metabolites lead to increased cancers or deaths, but the US Department of Health and Human Services made a determination that DDT is reasonably anticipated to be a human carcinogen (ATSDR 2002).

Heptachlor/Heptachlor Epoxide Metabolism and Toxicity

Heptachlor is an organochlorine insecticide. In technical-grade it was used on crops, in homes, and in buildings until 1988. Technical-grade heptachlor contains approximantely 65% heptachlor, 22% trans-chlordane, 2% cis-chlordane, and 2% nonachlor (Schimmel et al. 1976). Heptachlor is also a component of the technical grade insecticide, chlordane, which contains about ten percent heptachlor by weight. Heptachlor epoxide is the primary degradation product of heptachlor, but may also be manufactured in its pure form. Although it is no longer used on crops, in homes, or in buildings, it is still approved for use in killing fire ants in power transformers (ATSDR 2005).

Heptachlor is present in the environment from past use as an insecticide. It can also enter the air, soil, groundwater, and surface water from leaks at hazardous waste sites or landfills. Heptachlor and heptachlor epoxide adhere to soil and sediment. Heptachlor and heptachlor epoxide are lipid soluble, with heptachlor dissolving much less easily in water than heptachlor epoxide. Once heptachlor enters the environment, bacteria readily break it down to the more harmful substance heptachlor epoxide, with approximately 20% of heptachlor being broken down within hours. Heptachlor epoxide, however, breaks down very slowly in the environment. In the body, heptachlor is rapidly metabolized mainly to heptachlor epoxide. *In vitro* studies have shown that the primary metabolites of heptachlor are heptachlor epoxide; 1-exo-hydroxychlordene; 1-exo-hydroxy-2,3-exo-epoxychlordene; and 1,2-dihydroxydihydrochlordane (ATSDR 2005).

Humans can be exposed through inhalation, oral, or dermal routes. Both plants and animals can take up and accumulate heptachlor. Humans can therefore be exposed by directly working with these compounds; by coming into contact with contaminated air, soil, or water; or by consuming contaminated plants, fish, or animals. The foods most likely to contain heptachlor or heptachlor epoxide are vegetables, dairy products, meat, fish, and poultry. Contaminated fish and shellfish have been found to contain between 2-750 parts per billion (ppb) heptachlor and 0.1-480 ppb heptachlor epoxide. If milk is contaminated with heptachlor or heptachlor epoxide, children who drink large amounts of milk may have significant exposures. Fetuses can be exposed via placental transfer and infants can be exposed by drinking contaminated breast milk. Human breast milk samples have been found to have levels ranging from 0.13 to 128 ppb. Heptachlor,

heptachlor epoxide, and the other metabolites are excreted for the most part through the feces within a few days after exposure. However, some of the heptachlor and heptachlor epoxide is stored in the adipose tissues for long periods of time (ATSDR 2005).

Animal studies have found that heavy exposure to heptachlor or heptachlor epoxide may be hepatotoxic, cause excitability, and lead to decreased fertility. Chronic exposure in animals may be associated with liver tumors. It is unclear whether or not humans are affected similarly as most studies in which heptachlor and heptachlor epoxide were examined involved exposure to a number of organochlorine pesticides (ATSDR 2005). There have been a few studies examining the health effects of consumption of milk products from cows that were fed heptachlor contaminated feed which show an association between gestational exposure to heptachlor and subtle latent effects on neurobehavioral performance (Baker et al. 2004). In children born during the time period of milk contamination, no indication of higher mortality or cancer incidence was found (Maskarinec 2005). Blood levels of heptachlor or heptachlor epoxide may indicate recent or past exposure to heptachlor, heptachlor epoxide, or chlordane. However, if other chlordane residues such as nonachlor or oxychlordane are not found in the sample, it can reasonably be assumed that the heptachlor epoxide found is not due to exposure to chlordane. There is no conclusive human data linking heptachlor with cancer, but one recent, but small study suggested a possible association between heptachlor epoxide and breast cancer (Cassidy et al. 2005). The EPA classifies heptachlor and heptachlor epoxide as possible human carcinogens, while IARC classifies heptachlor as a possible human carcinogen (ATSDR 2005).

Hexachlorobenzene Metabolism and Toxicity

Hexachlorobenzene is an industrial chemical that is not currently manufactured as an end product in the United States, but it can be produced as a by-product in the manufacture of chlorinated solvents and other chlorinated compounds such as carbon tetrachloride, trichloroethylene, tetrachloroethylene, and various pesticides such as pentachloronitrobenzene, chlorothalonil, dacthal, pentachlorophenol, atrazine, simazine, propazine, and maleic hydrazide. It can also be released into the environment by waste incineration (van Bergelen 1998), or as a by product in waste streams of chlor-alkali and wood-preserving facilities. It is very soluble in organic solvents, fats, and oils. It is essentially insoluble in water. Prior to 1984, it was used as a fungicide on the seeds of onions, sorghum, wheat, and other grains, which accounts for the majority of its current presence in the environment. It was also used in the past to make synthetic rubber, fireworks, and ammunition (ATSDR 2002).

Hexachlorobenzene shares common properties with other organochlorine compounds, such as good chemical stability, persistence in the environment, slow degradation and biotransformation, and bioaccumulation in the adipose tissue and other lipid rich tissues. (Klaassen 2001). Its half-life in soil is 3-6 years, in water is 2.7-5.7 years, in groundwater is 5.3-11.4 years, and 0.63 to 6.28 years in the air. Almost all people who are tested for this industrial chemical have traces of it in their system, most likely due to the consumption of low levels in food. The yearly uptake is estimated to be 68 micrograms for adults, 22 micrograms for toddlers, and 5 micrograms for infants

(ATSDR 2002). In a study of blood serum samples taken from accident victims in Mexico, the mean serum level of hexachlorobenzene was 2.1 ng/ml (Waliszewski 2004). The main route of exposure for hexachlorobenzene is ingestion, but it may also enter the body through the lungs or through the skin. A person can be exposed due to contact with contaminated air and soil. Ambient air samples generally range from 0.1 picograms per square meter, to 1.5 picograms per square meter. Some agricultural soil has been found to have up to 400 ng/gm. Lake sediment has been detected at 15 nanograms per gram. Since hexachlorobenzene is nearly insoluble in water, this is not felt to be a pathway of exposure. Generally, water samples usually have concentrations below 0.1 parts per trillion. Fetuses may be exposed via this chemical crossing the placenta, and infants may be exposed from breastfeeding. The concentration in human breast milk has been found to range from 11-70 nanograms per gram of fat (ATSDR 2002).

The main route of exposure for hexachlorobenzene is ingestion, but it may also enter the body through the lungs or through the skin. Once it enters the body, hexachlorobenzene quickly disperses to many tissues, especially to adipose. A significant amount of hexachlorobenzene can be transferred from breastfeeding mother to child via the breast milk. Some of this chemical may also transfer to the unborn child through the placenta (ATSDR 2002). It is slowly metabolized to pentachlorophenol through the cytochrome P450 system (CYP3A1, CYP3A2, and CYP3A4 isoforms). It is then conjugated with glutathione which forms pentachlorothiophenol, or reductively dechlorinated to form pentachlorobenzene. Additional metabolites can include less chlorinated benzenes, cholorophenols, S-conjugated phenols, and benzenes. The pentachlorophenol is then

converted to tetrachlorohydorquinone. Based on animal models, it appears that most of the hexachlorobenzene leaves the body unchanged via the feces, but a small amount of metabolites do leave the body through the urine (ATSDR 2002). No data on the half-life of hexachlorobenzene in humans is available, but its half-life in rhesus monkeys has been estimated to be 2.5-3 years (van Bergelen 1998).

Hexachlorobenzene can have adverse effects on the liver, skin, bone, thyroid, neurological system, endocrine system, and immunological system in humans. It can cause developmental problems, porphyria, and has been shown to cause death. Organs that are most sensitive to hexachlorobenzene are the central nervous system, liver, and ovary. Animal studies suggest that it may cause liver, kidney, and thyroid cancer, but this has not yet been proven in humans. It has been determined by the U.S. Department of Health and Human Services that hexachlorobenzene may reasonably be anticipated to be a carcinogen in humans (ATSDR 2002).

Hexachlorocyclohexane Metabolism and Toxicity

Hexachlorocyclohexane is an organochlorine with eight isomers, of which only alpha-, beta-, gamma-, and delta-hexachlorocyclohexane are of commercial significance. Technical grade hexachlorocyclohexane is a mixture of isomers, 60-70% alpha, 5-12% beta, 10-15% gamma, 6-10% delta, and 3-4% epsilon. Products that contain greater than 99% of the gamma isomer are referred to as lindane. Most of the insecticidal properties

of hexachlorocyclohexane reside in the gamma isomer. Therefore, both technical grade hexachlorocyclohexane and the gamma isomer were previously used in the United States as insecticides. Gamma-hexachlorocyclohexane has not been produced in the United States since 1976, but it is still sometimes imported and used as an insecticide or used in prescription medication to eradicate scabies or head lice. Technical grade hexachlorocyclohexane has not been produced or used in the United States in over 20 years (ATSDR 2005).

Hexachlorocyclohexane shares common properties with other organochlorine compounds, such as good chemical stability, persistence in the environment, slow degradation and biotransformation, and bioaccumulation in the adipose tissue and other lipid rich tissues (Klaassen 2001). In a study of blood serum samples taken from accident victims in Mexico, the mean serum level of hexachlorocyclohexane was 3.6 ng/ml (Waliszewski 2004).

The main non-medicinal route of exposure in humans is by ingestion of food containing the pesticide. Foods which may contain the pesticide are plants, animals, animal products, and milk. Some exposure may also occur through drinking water. Fetuses may be exposed to this chemical as it can cross the placenta, and infants can be exposed through breastfeeding, ingestion of baby foods, and ingestion of household dust (ATSDR 2005).

Metabolism occurs mainly in the liver by the cytochrome P-450 oxygenase system. The liver microsomes convert hexachlorocyclohexane via dechlorination, dehydrogenation, dehydrochlorination, and hydroxylation. Many metabolites are formed, such as trichlorophenols, dichlorophenols, tetrachlorophenols, pentachlorophenol, and dihydroxychlorobenzenes. These are eliminated primarily in the urine (ATSDR 2005). Limited data on the biologic half-life of hexachlorocyclohexane is available. Assuming a first order kinetic model for excretion, the median half-life for beta-hexachlorocyclohexane was calculated to be 7.2 years in whole blood and 7.6 years in extractable lipids (Jung et al. 1997).

The principal target organ is the central nervous system. The isomers differ qualitatively and quantitatively in biological activity, with the alpha and gamma isomers being central nervous system stimulants and the beta and delta isomers being depressants (Besbelli, 2001). Other affected organs are the heart and the liver. It has been determined by the U.S. Department of Health and Human Services that hexachlorocyclohexane may reasonably be anticipated to be a carcinogen in humans (ATSDR 2005). Additionally, it may also be immunotoxic, as lindane exposure in humans at chronically high levels appears to affect cytokine levels (Seth et. al. 2005).

Table 1 shows a summary of potential exposure sources and effects of these organochlorine pesticides.

Table 1: Summary of Exposure Source and Effects of Selected Organochlorine Pesticides

Pesticide	Exposure Source	Effects
Aldrin/Dieldrin	Workers in manufacture, formulation, or application. Dietary especially fish, mollusks, foods high in animal fat, plants grown on contaminated land. Hazardous waste sites. Less common-home treated for termites.	Heavy exposure-CNS stimulation, convulsions, liver or kidney damage. Moderate chronic exposure-headaches, dizziness, irritability, vomiting, myoclonic jerking, hemolytic anemia, breast cancer, decreased breast cancer survival. EPA-probable human carcinogens.
Chlordane	Workers in manufacture, formulation, or application. Homes treated for termites. Crops grown on contaminated land. Fish and shellfish from contaminated waters.	Heavy exposure-CNS abnormalities such as convulsions. Lower levels-neurologic effects such as headaches, irritation, confusion, weakness, vision problems. Also, gastrointestinal complaints such as vomiting, diarrhea, cramps, jaundice, increases in serum liver enzyme levels. IARC-not classifiable as to carcinogenicity.
DDT, DDE, DDD	Workers in manufacture, formulation, or application. Contaminated crops or animals, often imported.	Neurologic symptoms, increased risk of pre-term baby, asthma in young children. DHHS-reasonably anticipated to be a human carcinogen.
Heptachlor/Heptachlor Epoxide	Workers in manufacture, formulation, or application. Vegetables, dairy products, meat, poultry grown/raised on contaminated land, or contaminated fish and shellfish.	Possibly hepatotoxic. May cause excitability and lead to decreased fertility. Possibly associated with breast cancer. Decreased neurobehavioral performance in children exposed during gestation. IARC-possible human carcinogen.
Hexachlorobenzene	Workers in manufacture, formulation, or application. Transfer through placenta or human breast milk. Crops and seafood from contaminated areas.	Adverse effects on liver, skin, bone, thyroid, neurologic, endocrine, and immunologic systems. Developmental problems. Porphyria. DHHS-reasonably anticipated to be a human carcinogen.
Hexachlorocyclohexane	Workers in manufacture, formulation, or application. Medicinal use (lindane). Ingestion of crops, animals, and seafood from contaminated areas. Drinking water. Transfer through placenta or human breast milk. Household dust.	CNS dysfunction. Alpha and gamma isomers are CNS stimulants, beta and delta are CNS depressants. Heart and liver abnormalities. Possibly immunotoxic. Changes to cytokine levels. DHHS – reasonably anticipated to be a human carcinogen.

Correcting Serum Concentration of Lipophilic Xenobiotics for Serum Lipids

After ingestion or absorption of xenobiotics such as organochlorine pesticides, these lipophilic substances do not stay in solution in the aqueous portion of the blood. They form bonds with various lipophilic blood components and then partition themselves among various tissues based on their lipid content. The persistent lipophilic xenobiotics accumulate in these tissues, most notably in the adipose tissues of the body. Therefore, their concentration in the adipose tissue is a good indicator of an individual's cumulative exposure and the body burden of the xenobiotic. However, adipose tissue is not easily obtained for analysis, so serum concentrations of lipophilic xenobiotics have been used as a proxy for adipose tissue concentrations. This approach is possible because the persistent lipophilic xenobiotics move between the adipose tissue and serum in a dynamic equilibrium established in large part by the fat content of the serum, thereby allowing the serum to serve as a proxy for adipose tissue in indicating levels of exposure and body burden (Brown and Lawton 1984). Historically, serum organochlorine pesticide levels have been reported on a wet-weight basis. Over the past few years, however, it has become increasingly common for the serum organochlorine concentrations to be reported on a lipid corrected basis.

A major limitation to using wet-weight serum pesticide concentration as a proxy measure of the adipose concentration is that serum lipids fluctuate throughout the day, most notably increasing a short time after a meal is ingested. Triglycerides and total plasma lipid may increase 20 to 30 percent shortly after a fatty meal. As early as the 1930's it

was proposed that the effect of lipophilic substances was related to lipids in the blood. In the early 1980's it was further proposed that since the serum levels of lipophilic xenobiotics were dependent upon serum lipid levels, correcting the serum level by the lipid level would provide a more accurate measurement of exposure to a given lipophilic xenobiotic. Theoretical considerations and experimental data were presented showing that polychlorinated biphenyl partitioning between adipose tissue and serum is related to variations in the lipid content of the serum (Brown and Lawton, 1984: Guo et al. 1987). Additionally, it was found that non-fasting serum levels of chlorinated hydrocarbons (PCB's, hexachlorobenzene, and DDE) had 22 to 29 percent higher mean concentrations than fasting samples. When these concentrations were then corrected by total serum lipids, there was no statistical difference between the fasting and non-fasting samples (Phillips et al. 1989).

Since blood lipid levels fluctuate, and the lipid level determines the blood concentration of the xenobiotic, one method of overcoming this variability is by collecting fasting blood samples. Unfortunately, this approach will only overcome the problem for measurements taken within the same individual. If levels of xenobiotics are to be compared between individuals, then fasting samples will still make those with higher baseline lipid levels appear to have higher concentrations of the xenobiotic. Therefore, correcting for lipids is a reasonable solution to overcome this problem. In so doing, the serum xenobiotic levels would be expressed in concentrations per weight of total lipid, instead of by total weight or volume of serum. The measurement is expressed as a lipid standardized value, which is the concentration of the lipophilic xenobiotic divided by the weight of serum lipids, in

contrast to the "wet-weight basis" which is the concentration of the xenobiotic divided per unit of total weight or volume of serum (Akins et al. 1989).

Although lipid adjustment has been found to be superior to the wet-weight basis, it can be prone to error (Shisterman et al. 2005). Issues such as biologic availability of the xenobiotic and the question of whether the xenobiotic itself exerts effects on the level of lipids need to be addressed when evaluating the health outcomes of xenobiotics (Hennig et al. 2005). Nevertheless, when researchers consider biology, biologic medium such as nonfasting blood samples, laboratory measurement, and other underlying modeling assumptions, a statistical plan can be devised that will minimize bias and give the best assessment of health outcomes in relation to environmental exposures (Shisterman et al. 2005).

When correcting for lipids, the first step is to determine the total lipids in the blood. It is important not only to obtain an accurate measurement, but also to have a method of determining serum lipid concentrations that is reliable and convenient. One such method is the "summation" method, in which the individual lipid components are determined and then summed to give the total lipid content. The enzymatic "summation" method uses automated, enzymatic methods to determine the serum total cholesterol (TC), non-esterified cholesterol (FC), triglycerides (TG), and phospholipids (PL). The total lipids (TL) are then calculated using he equation $TL = 1.677(TC-FC) + FC + TG + PL$. The correction factor is required since the measurement must be corrected for the weight of fatty acids in the cholesterol. To do this the cholesterol component of the cholesterol

ester fraction is given directly by TC – FC. It is then multiplied by a factor that represents the ratio of the mean molecular weight of serum cholesterol esters to the molecular weight of cholesterol, which is 648.25 divided by 386.67, equaling 1.677 (Akins et al. 1989).

This enzymatic method correlates well with measurements determined by gravimetric analysis. There are several advantages in using the enzymatic summation method instead of gravimetric analysis. The enzymatic method is much quicker, which is useful when a large number of samples must to be measured. Additionally, a defined total lipid value is determined, not just the weight of substances extracted by organic solvents. Therefore, the measurements are less likely to include nonlipid species, such as amino acids, sugars, and inorganic salts (Akins et al. 1989).

The CDC toxicology laboratory calculates the total lipids using the formula TL = 62.3 + 2.27(TC + TG). Lipid adjustment is then accomplished using the formula (pesticide) / TL(1000mg lipid / 1 gm lipid)(100 ml serum / 1 dl serum). This calculation has served as a good approximation of the calculation described by Brown and Lawton.

Other approaches of quantifying xenobiotic lipophilic compounds have been reported in the literature, including two-stage analyses in which serum lipids are regressed on serum concentrations with the residuals entered as an individual risk factor, or the use of a log-linear model with serum lipids included as a separate term in the regression equation (Shisterman et al. 2005).

In summary, to obtain a more accurate measurement of the serum concentration of a lipophilic xenobiotic, it has been proposed that the serum concentration should be adjusted for the level of serum lipids. By adjusting blood concentrations of a lipophilic xenobiotic for lipids, blood xenobiotic concentration can more accurately be used to compare an individual over time, concentrations across study populations, and health effects. Furthermore, if an estimate of a person's total body fat is available, this can be used to determine the total body burden of the lipophilic xenobiotic (Brown and Lawton 1984). Therefore, adjusting serum levels of lipophilic xenobiotics for serum lipid levels will presumably give a more accurate reflection of the body burden of that xenobiotic, which in turn would yield the most accurate estimate of its effects on human health and outcomes in relation to environmental exposures.

Chapter 3

MATERIALS AND METHODS

The Del Amo/Montrose Community Environmental Health Program was implemented by the University of California, Irvine (UCI) Center for Occupational and Environmental Health and the Occupational and Environmental Health program at the University of California, Los Angeles (UCLA) School of Medicine, in collaboration with the Agency for Toxic Substances and Disease Registry, the California State Department of Health Services, local political leaders, and community representatives. It was designed to address concerns of the community members residing near the Del Amo and Montrose Superfund sites regarding their possible exposure to toxic substances. The program conducted community outreach and education programs, established and facilitated community-based environmental health committees, and established an environmental health clinic that offered free comprehensive clinical evaluations by environmental health specialists. Participation in the comprehensive medical evaluation was voluntary.

The medical evaluation consisted of completion of an extensive medical and exposure questionnaire, physical examination, medical laboratory tests including complete blood count and liver function tests, and measurement of serum concentrations of organochlorine pesticides. A total of 563 persons completed the medical evaluation and had serum drawn for pesticide analysis (Baker 1999). For the purposes of this study,

only residents of the Del Amo/Montrose communities, excluding those who had been employed by the Montrose facility, were included in the study population. Of the 563 participants, 38 were excluded because they had never lived in the Del Amo/Montrose neighborhoods. These persons were allowed to participate in the original program because they had lived near the area and had significant health concerns, or they were family members of persons who otherwise qualified for the medical evaluation. In addition, this study excluded 13 persons (including 8 residents and 5 non-residents) who reported having worked in the Montrose facility when it operated. These persons were excluded because of their occupational exposures to DDT.

Questionnaire

The questionnaire was adapted from standard medical and environmental questionnaires, with the incorporation of questions developed by the California State Department of Health Services (DHS) to assess possible exposure to DDT and DDE in the Del Amo and Montrose neighborhoods, based on a history of the sites and the DHS and US EPA site evaluation data. It assessed past and current health status, residential history, occupational and environmental history, and activities that may have been associated with exposures in the neighborhood. It was administered in either English or Spanish by the program nurses or community outreach workers.

The questionnaire included demographic variables such as age, gender, and highest grade of education. The questionnaire did not specifically ask about race/ethnicity, but it was

administered in the person's primary language (English or Spanish), which was noted in the chart. For this study, age was stratified into six categories and highest grade of education was stratified into four categories.

A full occupational and residential history was obtained, including all past residences. Two variables were created from this information. One variable is whether the person had ever done paid or unpaid work on a farm. Another variable is whether the person had ever lived for at least a year in Mexico or Latin America. These variables were analyzed as dichotomous variables.

The questionnaire asked about activities in the neighborhood that could have led to pesticide exposure. These variables included whether the person did gardening, yard work or other activities in the neighborhood that involved touching the soil; whether the person ate eggs raised in the neighborhood; whether the person ate fruits or vegetables grown in the neighborhood; and whether the person ate fish or shellfish caught by them or someone they know from a pier or boat. For each positive response to these questions, the questionnaire asked the person to estimate the frequency of these activities. For this study, the responses were dichotomized into yes or no for each activity.

The questionnaire also asked about past and current symptoms, health conditions, and medication use. Symptoms included, for example, frequent or severe headaches, frequent nose bleeds, cough in the morning, shortness of breadth, abdominal pain, upper or lower

back pain, dizziness, and anxiety or depression. For this study, responses were coded as yes or no.

Medical Laboratory Analysis

During the medical evaluation, participants' blood was drawn peripherally by trained phlebotomists using standard blood drawing equipment. Vaccutainer tubes were used to obtain uncoagulated blood for a complete blood count (CBC), serum for multiple chemistry analysis including liver function tests (LFTs), and serum for analysis of organochlorine pesticides. The analyses of CBC and LFTs were done using standard procedures by the Harbor-UCLA Medical Center medical laboratory in Torrance, CA.

For this analysis, the continuous laboratory values were dichotomized into normal and either low (for hematocrit) or high (for the liver function tests) values. The medical laboratory provided criteria to define the normal values, which could vary by the participant's age and gender. The following criteria were used:

- Hematocrit: If (age is less than or equal to 12 and hematocrit is less than 0.36) or (age is 12 or above, the person is male, and hematocrit is less than 0.40) or (age is 12 or above, the person is female, and hematocrit is less than 0.35), then hematocrit was low; otherwise, hematocrit is normal.

- Aspartate aminotransferase (AST): If (age is less than or equal to 65 and AST is above 42 IU/L) or age is above 65 and AST is above 55 IU/L), then AST is high; otherwise, AST is normal.
- Alanine aminotransferase (ALT): If ALT is greater than 48 IU/L, then ALT is high; otherwise ALT is normal.
- Gamma-glutamyltranspeptidase (GGT): If (age is between 3 and 12 years and GGT is above 65 IU/L) or (age is between 13 and 65 years, the person is male, and GGT is above 65 IU/L) or (age is between 13 and 65 years, the person is female, and GGT is above 45 IU/L) or (age is above 65 years and GGT is above 75 IU/L), then GGT is high; otherwise, GGT is normal.
- Liver Function Tests (LFTs): If any of AST, ALT, or GGT are high then the LFT is high; otherwise, LFT is normal.

Serum Pesticide Analysis

The blood was centrifuged to obtain the supernatant serum, which was frozen and stored. Batches of sera were sent on dry ice to the toxicology laboratory, Division of Laboratory Science, CDC, for analysis.

The CDC laboratory analytical methods were similar to those used by the same laboratory for the analysis of organochlorine pesticides in the National Health and Nutrition Examination Survey (NHANES). In summary, as adapted from the laboratory manual, serum specimens (1–1.5 mL) to be analyzed were spiked with $^{13}C_{12}$-labeled

internal standards and the analytes of interest were isolated in hexane using a C18 solid phase extraction (SPE) procedure followed by extraction through neutral silica and Florosil SPE columns. Pesticides were eluted from the Florosil column with hexane and 1:1 dichloromethane /hexane. Each analytical run consisted of nine unknown specimens, one method blank, and two quality control samples. Before quantification, the vials were reconstituted with 10μL ^{13}C-labeled external standard. Sample extracts were then analyzed simultaneously for PCBs and pesticides by HRGC/ID-HRMS where 1 μL was injected, using a GC Pal auto sampler, into a Hewlett-Packard 6890 gas chromatograph operated in the splitless injection mode with a flow of 1 mL/min helium through a DB-5ms capillary column (30 m x 0.25 mm x 0.25 μm film thickness) where analytes were separated prior to entering a Thermo Finnigan MAT95 XP (5 kV) magnetic sector mass spectrometer operated in EI mode at 40 eV, using selected ion monitoring (SIM) at 10,000 resolving power (10% valley). Two ion current responses corresponding to two masses were monitored for each native (^{12}C) compound and its corresponding ^{13}Cinternal standard. The instrumental response factor for each analyte was calculated as the sum of the two ^{12}C isomers divided by the sum of two ^{13}C- isomers. Calibration of mass spectrometer response factor versus concentration was performed using calibration standards containing known concentrations of each ^{12}C compound and its corresponding ^{13}C internal standard. The concentration of each analyte was derived by interpolation from individual linear calibration curves and adjusted for sample weight. The method detection limit (MDL) for each analyte was calculated, correcting for sample weight and recovery. The total lipid content of each specimen was estimated from its total cholesterol and triglycerides values using a "summation" method. Analytical results were

reported on a whole-weight [ng/g or parts-per-billion (ppb)] and lipid-adjusted basis [ng/g or ppb].

The CDC reported serum levels of aldrin, dieldrin, alpha chlordane, gamma chlordane, oxychlordane, trans-nonachlor, o,p-DDE, o,p-DDT, p,p'-DDD, p,p'-DDE, p,p'-DDT, endrin, heptachlor, heptachlor epoxide, hexachlorobenzene, beta hexachlorocyclohexane, gamma hexachlorocyclohexane, and mirex. They also reported serum cholesterol and triglyceride levels. Pesticide concentrations were reported as wet-weight serum concentrations (ng/g or almost equivalently ng/mL) and lipid adjusted concentrations (ng/g). However, the calculations they used for the lipid-adjusted pesticide concentrations were inconsistent during the study period, so for this study lipid-adjusted levels were calculated using the formula: (pesticide) / TL(1000mg lipid / 1 gm lipid)(100 ml serum / 1 dl serum), where TL = 62.3 + 2.27(TC + TG).

Medical Diagnoses

The Del Amo/Montrose Environmental Health Program conducted an aggregate analysis of the clinical evaluation findings. In order to conduct the analysis, the program nurses used a chart abstraction form to record documented clinical histories and diagnoses recorded by the examining physicians in the medical charts. Approximately 2,000 conditions or diagnoses were recorded for 596 persons who participated in the clinical evaluations. The earlier program defined diagnostic categories, so that diagnoses could

be grouped together for the analysis. This approach was used because for some conditions there were only a few persons affected and so it would not be possible to identify any pattern based on the few cases. At the same time, there were some conditions, such as reported skin rashes or dermatitis, for which the doctors could not identify a specific diagnosis. In order to evaluate all possibly relevant health outcomes, the analysis included conditions that were reported by participants from earlier medical evaluations, even if the condition was not present at the time of the examination. These conditions were listed as being diagnosed "by history." The outcomes used in this study were based on the conditions that were recorded as being diagnosed by the program physicians or reported in the medical history as being diagnosed earlier by a physician. All health outcome variables were scored as "yes-no" dichotomous variables.

Data Management and Analysis

Data bases for aggregate analysis were developed at the UCI Center for Occupational and Environmental Health and data were entered by the project staff. Access and dBASE were used for data management. The data were transferred to SAS, which was used for data cleaning and analysis. Standard procedures were followed for quality control and assurance in data management. An external audit on randomly selected charts verified complete accuracy of the data bases (Baker 1999).

For statistical analyses, all demographic variables; neighborhood and exposure variables; and health outcome variables were scored as categorical or dichotomous variables. Age was stratified into six categories, education was stratified into four categories, and all other variables were scored as dichotomous variables. The distributions of demographic, neighborhood, and exposure variables were calculated using the prevalence of the variables. All subsequent analyses were stratified by age into children through 17 years of age and adults 18 years or older.

Serum concentrations of pesticides were reported as continuous variables. Pesticide concentrations below the limit of detection were assigned a value equal to the limit of detection/square root of 2, which was the CDC laboratory's recommended approach. An initial analysis was performed on the organochlorine pesticides to evaluate outliers, which could have influenced the regression analysis. This analysis examined the distribution, mean, standard deviation, median, inter-quartile range (IQR = range from the 25^{th} to 75^{th} percentiles), largest five observations, and smallest five observations. Three children were found to have very high DDT and DDE concentrations – much higher than any other children. All three of these children were from the subgroup of eleven children who had lived in Mexico. This group of three children within an already small group of eleven could significantly bias apparent associations and influence the regression analysis. Because the number of children who had lived in Mexico was so small, it was not possible to stratify or statistically control for "lived in Mexico versus not lived in Mexico." Therefore the eleven children who had lived more than a year in Mexico were not included in the analysis. One adult inexplicably had extremely high

serum concentrations of all pesticides, and therefore was not included as this would have skewed the analyses. Another adult had a dieldrin concentration that was nearly ten times higher than the adult with the next highest concentration. So that the analyses related to dieldrin would not be skewed by this one extreme value, this participant's dieldrin level was set to two IQRs above the adult with the next highest serum dieldrin concentration. Therefore, twelve of the eligible study population of 512 participants were eliminated, leaving 500 participants for the data analysis.

Following the adjustments for outliers, the univariate distributions of the serum and lipid-adjusted pesticide concentrations were summarized by reporting the proportion detected, the mean, and the standard deviation. Several of the individually reported pesticides were isomers of the same parent pesticide, metabolites of the pesticide, or very closely related pesticides. Therefore, the analysis summed the concentrations of these related pesticides to calculate "total" pesticide values for these pesticides. Aldrin and dieldrin were grouped together as aldrin/dieldrin. Alpha-chlordane, gamma-chlordane, oxychlordane, and trans-nonachlor were grouped together as "total chlordane." "Total DDT" was the sum of o,p-DDT; p,p'-DDT' o,p-DDE; p,p'-DDE; and p,p'-DDD. Heptachlor and heptachlor epoxide were summed as "total heptachlor." Concentrations of beta- and gamma-hexachlorocyclohexane were summed as "total hexachloro-cyclohexane." The proportion detected for the "total" pesticide variables was calculated by assuming that the "total" pesticide was detected if any of the component pesticides was detected; otherwise, it was not detected. Because all participants had detected DDT, the analysis reported the proportion of participants with a total DDT above 4.55 ng/mL

serum, which was the median in the total population before the exclusions of non-residents, the one person with pesticide outliers, and the 11 children who had lived in Mexico. Most of the analyses were based on the five "total" pesticides: aldrin/dieldrin, total chlordane, total DDT, total heptachlor, and total hexachlorocyclohexane and the individual pesticide, hexachlorobenzene. Correlations between pesticides were analyzed using Pearson's correlation coefficient.

A general linear model (GLM) analysis was used to examine associations between the serum concentrations of pesticides and the demographic, neighborhood, and exposure variables. The analyses were performed based on the wet-weight serum pesticide concentrations and repeated for the lipid-adjusted pesticide concentrations to see if the patterns of associations were different between the serum and lipid-adjusted values for the same pesticide and among the various pesticides. The GLM analysis allowed for calculation of means of each pesticide for each category of the predictor variable, while adjusting for the effects of all of the other predictor variables. These mean values were reported as the "LSMEANS" in the PROC GLM in SAS.

Because wet-weight serum and lipid-adjusted serum units are different, the pesticide concentrations were standardized to a mean of 10 and standard deviation of 1, so additional comparisons could be made between wet-weight serum and lipid-adjusted values. This approach was used so that the effects of the demographic and exposure variables could be directly compared among the various pesticides. A least-squares linear regression of the standardized pesticide concentrations was performed using the

same demographic, neighborhood, and exposure variables as were used for the general linear model. Because each predictor variable in the regression model was scored as a "1-0" dichotomous variable, the regression coefficients can be interpreted as the difference in the means of the standardized pesticide concentrations – in standard deviation units – between the "1" and "0" response categories for each variable in the model (e.g., female versus male, or ate eggs from neighborhood versus did not eat eggs from neighborhood), while adjusting for all other variables in the model.

In addition to examining associations between the serum pesticide concentrations and the demographic and exposure variables, the analysis explored the associations between the serum pesticides and the reported symptoms and diagnosed health conditions. For these analyses, the health outcome was considered the dependent variable and the serum pesticide concentration was considered the independent variable. Using PROC MEANS, the first analysis examined the means of the pesticide concentrations according to whether the participants did or did not have the health outcome. A second analysis used a logistic regression model (PROC LOGISTIC) to calculate the odds ratio (OR) and p-value associated with the standardized pesticide concentrations. The odds ratio is interpreted as the relative risk (or increase in likelihood) of having the health outcome for a one standard deviation increase in the serum pesticide concentration. The logistic model for adults also included age and whether the person had lived at least a year in Mexico or Latin America as co-variables to adjust for these factors. The model for children included age as a co-variable.

Chapter 4

RESULTS

A total of 563 participants completed the medical evaluation and had serum concentrations of pesticides analyzed by the CDC toxicology laboratory. Of the 563 participants, 38 were not eligible for this study because they had never lived in the Del Amo/Montrose area. An additional 13 (including 8 residents and 5 non-residents) were excluded because they had occupational exposure to DDT from working in the former Montrose facility. One adult was eliminated because of multiple pesticide outliers, while 11 children who had lived at least a year in Mexico were eliminated because the group was too small to analyze as a separate strata on this important variable. The analysis, therefore, was conducted on a total of 500 participants.

Demographic Characteristics

Table 2 shows the demographic characteristics and neighborhood exposure variables for the adults. Age was stratified into six age categories for the analysis: two categories for children (0-9 years, 10-17 years) and four categories for adults (18-31, 32-44, 45-64, and 65 and above years). Participation by persons in the oldest age category was limited (3.3%), but the rest of the age categories were well represented in the study population.

A greater number of females than males participated in the clinical evaluations. A majority of the participants were Hispanic (not shown). For adults, educational level was stratified into four categories: grammar school, middle school or some high school, high school, and some education beyond high school. The percentage of adults attaining each of the first three levels was similar, with a smaller group in the fourth level (some education past high school). The percentage of adults who completed high school or past high school was 45.9%, which is generally consistent with the educational attainment for Hispanics in the surrounding area. The percentage of Hispanic adults in the Los Angeles/ Riverside/Orange County area in the year 2000 who had completed high school was 49.9%. The percentage of all adults in the Los Angeles/Riverside/Orange County area in the year 2000 who had completed high school was 76.6% (USCB 2000).

Nearly half of the adults had lived for at least one year in Mexico or Latin America, which is important in that many of these countries continue to allow the use of pesticides no longer approved for use in the United States, and they often have less stringent requirements for environmental protections. The remainder of the variables in table 2 represent possible routes of exposure related to residence in the Del Amo/Montrose area, such as doing activities in the garden or yard that involved contact with the soil, eating eggs from chickens raised in the neighborhood, eating fruit or vegetables grown in the neighborhood and whether the person ate fish or shellfish caught by them or someone they know from a pier or boat.

Table 2. Demographics and Exposure Variables among Adults

	Number	Percent
Age (years)		
Adult 18-30	111	33.7
Adult 31-44	117	35.6
Adult 45-64	90	27.4
Adult 65+	11	3.3
Gender		
Male	125	38.0
Female	204	62.0
Adult-Highest Educational Level		
Elementary (6^{th} grade or less)	87	26.4
Middle or Some High School	91	27.7
Completed High School	92	28.0
Some Past High School	59	17.9
Lived in Mexico or Latin America > 1 year		
Yes	160	48.6
No	169	51.4
Worked on a Farm		
Yes	67	20.4
No	262	79.6
Activities in Garden or Yard		
Yes	212	64.4
No	117	35.6
Eat Eggs From Neighborhood		
Yes	67	20.4
No	262	79.6
Eat Fruit or Vegetables From Neighborhood		
Yes	227	69.0
No	102	31.0
Eat Fish Caught on Pier or Boat		
Yes	82	24.9
No	247	75.1

Table 3 presents the demographic and neighborhood exposure variables for the children. Again, slightly more females participated than males. About one-half of the children were reported doing activities in the garden or yard, only 15% ate eggs produced in the neighborhood, 18% ate fish caught off a pier or boat, and nearly 58% ate fruit or vegetables grown in the neighborhood.

Table 3. Demographics and Neighborhood Variables among Children

	Number	Percent
Age (years)		
Child 0-9	106	62.0
Child 10-17	65	38.0
Gender		
Male	81	47.4
Female	90	52.6
Activities in Garden or Yard		
Yes	94	55.0
No	77	45.0
Eat Eggs from Neighborhood		
Yes	26	15.2
No	145	84.8
Eat Fruit or Vegetables from Neighborhood		
Yes	99	57.9
No	72	42.1
Eat Fish Caught on Pier or Boat		
Yes	31	18.1
No	140	81.9

Serum Pesticide Concentrations

Participants' serum was sent to the CDC toxicology laboratory where it was tested for concentrations of the organchlorine pesticides. It was also analyzed for cholesterol and triglycerides, so lipid-adjusted pesticide concentrations could be calculated. Table 4 summarizes the prevalence of each of these pesticides that were found above the limit of detection in the serum of adults and children. For adults, the prevalence of detected p,p'-DDE was the highest at 100%, followed by hexachlorobenzene at 87.5%. The pattern was similar for children; p,p'-DDE had the highest detected prevalence at 99.4%, followed by hexachlorobenzene at 69.5% detected.

Table 4. Prevalence of Detected* Pesticides among Adults and Children

Pesticide	Adults - % Detected	Children - % Detected
Aldrin	23.4	26.9
Dieldrin	26.6	16.8
alpha-Chlordane	2.9	0.7
gamma-Chlordane	18.3	19.5
Oxychlordane	29.5	15.8
trans-Nonachlor	32.6	5.3
p,p-DDT	28.2	14.3
o,p-DDT	14.7	5.4
p,p-DDE	100.0	99.4
o,p-DDE	6.2	7.3
p,p-DDD	15.4	7.5
Heptachlor	16.1	10.7
Heptachlor Epoxide	6.6	0.0
Hexachlorobenzene	87.5	69.5
beta-hexachlorocyclohexane	50.0	15.3
gamma-hexachlorocyclohexane	1.0	3.0
Endrin	16.5	9.8
Mirex	10.3	3.2

*Percent of specimens with concentrations above the limit of detection.

Table 5 summarizes the distribution of pesticides among adults and children. Beginning with this table, the concentrations of parent compounds and the related metabolites are summed to provide "total" pesticide concentrations, as described in the methods chapter. These sums were obtained for aldrin/dieldrin, chlordane, DDT, heptachlor, and hexachlorocyclohexane. The concentrations for hexachlorobenzene are reported separately. Endrin and mirex were excluded from further analysis as the prevalence of each was low, making analysis difficult.

Table 5. Distribution of Total Pesticides* among Adults and Children

		Serum		Lipid-Adjusted	
Age Group - Pesticide	Percent Detected	Mean	Standard Deviation	Mean	Standard Deviation
Adults					
Aldrin/Dieldrin	36.8	0.127	0.150	17.2	19.0
Chlordane, Total	48.0	0.316	0.341	42.3	36.5
DDT, Total > median 4.55	66.2	13.9	20.3	1,921	2,616
Heptachlor, Total	21.9	0.123	0.192	17.3	24.0
Hexachlorobenzene	87.5	0.220	0.192	31.4	27.9
Hexachlorocyclohexane, Total	51.6	0.410	0.692	55.4	86.8
Children					
Aldrin/Dieldrin	34.3	0.133	0.253	24.6	55.0
Chlordane, Total	32.2	0.195	0.142	35.4	29.4
DDT, Total > median 4.55	17.7	3.50	4.31	617	778
Heptachlor, Total	10.1	0.105	0.158	18.1	26.0
Hexachlorobenzene	69.5	0.124	0.129	22.0	24.6
Hexachlorocyclohexane, Total	17.3	0.209	0.370	37.7	70.7

* Serum concentration in ng/ml serum; lipid-adjusted concentration in ng/g lipid.

Of note in table 5, when the concentration of aldrin/dieldrin is compared between adults and children, the mean serum concentration was slightly higher for children (mean serum concentration is 105% of the adult mean). With adjustment for lipids, the children had a mean concentration 143% that of the adults. For total heptachlor, the mean serum

concentration in children was 85% that of adults. However, with lipid adjustment, the children had 105% the mean concentration of adults. For the remainder of the pesticides analyzed, the adults had higher serum and lipid-adjusted concentrations than the children. This pattern was expected because these persistent compounds tend to accumulate with age, so the body burden should be higher in adults, and because adults had greater potential for environmental exposures in the past before the use of the pesticides was restricted or banned in the United States.

Table 6 presents the correlations among serum and lipid-adjusted pesticide concentrations for all eligible study participants. It shows that the serum and lipid-adjusted measures for each organochlorine pesticide concentration were highly correlated. The correlation ranged from a low of 89% between serum total chlordane and lipid-adjusted total chlordane concentrations, to a high of 96% correlation between serum total heptachlor and lipid-adjusted total heptachlor and serum total hexachlorocyclohexane and lipid-adjusted total hexachlorocyclohexane. Additionally, aldrin/dieldrin concentration had a relatively strong correlation with total chlordane, while total DDT, hexachlorobenzene, and total hexachlorocyclohexane tended to correlate with one another.

Table 7 shows the correlations between serum and lipid-adjusted pesticide concentrations for adults who lived at least a year in Mexico or Latin America (subsequently referred to as "Mexico," since the vast majority lived in Mexico), while table 8 shows the correlations between the serum and lipid-adjusted pesticide concentrations for adults who never lived in Mexico. Again, serum and lipid-adjusted pesticide concentrations were

highly correlated. However, the patterns of correlations were somewhat different among the two sub-groups of adults. Total heptachlor showed a stronger correlation with all other pesticides for adults who had lived in Mexico than for adults who had not lived in Mexico Total hexachlorocyclohexane also showed a stronger correlation with all other pesticides (except total DDT) for adults who had lived in Mexico than for adults who had not lived in Mexico. DDT serum concentration was strongly correlated with that for chlordane (r=0.55) and hexachlorocyclohexane (r=0.77) among the adults who had not lived in Mexico, but it was not correlated with these pesticides (r=-0.01 and r=0.15, respectively) among adults who had lived in Mexico.

Table 6: Correlations among Serum and Lipid-Adjusted Pesticide Concentrations–Adults and Children

	Serum Concentrations						Lipid-Adjusted Concentrations					
	A/D	**Chlor**	**DDT**	**Hept**	**HCB**	**HCCH**	**A/D**	**Chlor**	**DDT**	**Hept**	**HCB**	**HCCH**
A/D	1.00	0.38	0.05	0.21	0.09	0.17	0.93	0.42	0.01	0.18	0.04	0.11
Chlordane		1.00	0.18	0.28	0.18	0.27	0.24	0.89	0.10	0.17	0.04	0.18
DDT			1.00	0.06	0.37	0.34	-0.01	0.11	0.95	0.02	0.28	0.26
Heptachlor				1.00	0.19	0.44	0.13	0.23	0.04	0.96	0.13	0.37
HCB					1.00	0.32	0.02	0.09	0.34	0.14	0.92	0.28
HCCH						1.00	0.07	0.21	0.28	0.36	0.23	0.96
A/D - L							1.00	0.39	-0.01	0.15	0.04	0.06
Chlor – L								1.00	0.10	0.20	0.06	0.19
DDT – L									1.00	0.02	0.33	0.25
Hept – L										1.00	0.12	0.33
HCB – L											1.00	0.24
HCCH – L												1.00

A/D = aldrin/dieldrin
HCB = hexachlorobenzene
HCCH = total hexachlorocyclohexane
Hept = total heptachlor
-L = lipid adjusted

Table 7: Correlations among Serum and Lipid-Adjusted Pesticide Concentrations– Adults Who Had Lived in Mexico

	Serum Concentrations						Lipid-Adjusted Concentrations					
	A/D	**Chlor**	**DDT**	**Hept**	**HCB**	**HCCH**	**A/D**	**Chlor**	**DDT**	**Hept**	**HCB**	**HCCH**
A/D	1.00	0.46	0.06	0.42	0.11	0.33	0.92	0.37	-0.02	0.35	-0.06	0.23
Chlordane		1.00	-0.01	0.44	0.13	0.31	0.27	0.92	-0.10	0.33	-0.05	0.20
DDT			1.00	0.03	0.32	0.15	0.01	-0.05	0.96	0.03	0.25	0.13
Heptachlor				1.00	0.23	0.63	0.34	0.44	0.00	0.97	0.14	0.57
HCB					1.00	0.30	0.02	0.09	0.29	0.23	0.92	0.28
HCCH						1.00	0.25	0.29	0.09	0.62	0.20	0.97
A/D - L							1.00	0.28	-0.01	0.33	-0.04	0.20
Chlor – L								1.00	-0.08	0.37	0.00	0.24
DDT – L									1.00	0.02	0.31	0.11
Hept – L										1.00	0.19	0.60
HCB – L											1.00	0.24
HCCH – L												1.00

A/D = aldrin/dieldrin
HCB = hexachlorobenzene
HCCH = total hexachlorocyclohexane
Hept = total heptachlor
-L = lipid adjusted

Table 8: Correlations among Serum and Lipid-Adjusted Pesticide Concentrations– Adults Who Had Not Lived in Mexico

	Serum Concentrations						Lipid-Adjusted Concentrations					
	A/D	**Chlor**	**DDT**	**Hept**	**HCB**	**HCCH**	**A/D**	**Chlor**	**DDT**	**Hept**	**HCB**	**HCCH**
A/D	1.00	0.37	0.17	0.01	0.14	0.19	0.97	0.33	0.13	-0.02	0.05	0.12
Chlordane		1.00	0.55	-0.02	0.22	0.23	0.30	0.92	0.44	-0.06	0.11	0.16
DDT			1.00	-0.01	0.16	0.77	0.13	0.48	0.94	-0.06	0.08	0.64
Heptachlor				1.00	0.01	-0.04	0.03	-0.01	-0.01	0.97	0.02	-0.05
HCB					1.00	0.17	0.08	0.16	0.11	-0.04	0.91	0.10
HCCH						1.00	0.15	0.22	0.63	-0.07	0.12	0.94
A/D - L							1.00	0.32	0.12	0.03	0.05	0.12
Chlor – L								1.00	0.45	-0.02	0.16	0.20
DDT – L									1.00	-0.03	0.11	0.57
Hept – L										1.00	0.02	-0.04
HCB – L											1.00	0.11
HCCH – L												1.00

A/D = aldrin/dieldrin
HCB = hexachlorobenzene
HCCH = total hexachlorocyclohexane
Hept = total heptachlor
-L = lipid adjusted

Serum Pesticide Concentrations and Neighborhood and Exposure Variables

Adults. Tables 9 and 10 summarize the mean serum and lipid adjusted pesticide concentrations by demographic and neighborhood variables among adults. The means shown in these tables for each variable were adjusted using a general linear model for the effects of all of the other demographic and exposure variables in the tables. As expected because lipophilic pesticides are located virtually all within the lipid portion of serum, and lipids constitute less than one percent of serum, the lipid-adjusted concentrations were consistently much larger than the serum concentrations. Specifically, the means of the lipid-adjusted concentrations were 149 times greater than the means of the serum pesticide concentrations (indicating that on average the serum contained 0.67% lipid).

To allow for easier comparison between the serum and lipid-adjusted concentrations, the pesticide concentration variables were standardized to a mean of 10 and a standard deviation of 1. This transformation does not change the shapes of the pesticide distributions or their associations with the demographic or exposure variables, but it rescales the variables so the units can be interpreted as changes in standard deviation units. Table 11 summarizes the regression of standardized serum pesticide concentrations on demographic and neighborhood variables among adults with associated p-values. Table 12 is similar except that it is a summary of the lipid-adjusted values. For the regression analysis, age of 18-30 years, male gender, education – grammar school only, and "no" for the dichotomous variables were defined as the referent levels. The comparisons of associations between the pesticide concentrations and demographic and

exposure variables can be examined by looking at tables 9 and 10 to see the differences in the adjusted means, or by looking at tables 11 and 12 to see the regression coefficients (equivalent to the difference of an adjusted mean) for the standardized coefficients. The patterns of associations and p-values are the same between the tables because standardization does not affect these patterns.

In comparing the patterns of associations for the serum and lipid-adjusted pesticides, all of the serum pesticide concentrations were associated with increasing age. The lipid-adjusted pesticide concentrations were also positively associated with age, but the trends were weaker and fewer of the differences in mean concentrations (or regression coefficients) by age category were significant at the $p<0.05$ level. Most of the serum pesticide concentrations were inversely associated with educational level, although the trends by educational level were weaker than for the effect of age. The inverse trends by educational level were weaker in the lipid-adjusted pesticides. Each of the pesticide concentrations were weakly associated with eating eggs from chickens raised in the neighborhood, except for total chlordane which showed a strong association for both serum and lipid-adjusted concentrations (0.558 standard deviation [SD] increase in concentration; $p = 0.001$, 0.435 SD increase, $p=0.008$, respectively). Again the associations for eating eggs were somewhat weaker for the lipid-adjusted concentrations than for the serum pesticide concentrations.

The patterns of associations for the serum concentrations compared to the lipid-adjusted concentrations were not meaningfully different for the other demographic, neighborhood

and exposure variables. There were no clear differences comparing the associations for serum versus lipid-adjustment concentrations for working on a farm, prior residence in Mexico or Latin America, doing activities in the garden, or generally for eating fish. However, for total HCCH and fish consumption, the standardized regression coefficient was somewhat stronger for the lipid-adjusted concentration (0.282 SD increase in adjusted pesticide concentration, p=0.02) compared to the coefficient for the serum concentration (0.244 SD increase, p=0.06).

In comparing the patterns of associations across the pesticides, it was again observed that all of the pesticides were positively associated with age and inversely associated with educational level. All of the pesticides except for hexachlorobenzene were positively associated with eating eggs, but only the association for total chlordane and egg consumption was statistically significant. Besides these general associations, the serum pesticide concentrations were not consistently associated with other demographic or exposure variables. The serum concentrations of total DDT, hexachlorobenzene, and hexachlorocyclohexane were all significantly higher in adults who had lived in Mexico or Latin America than among the adults who had not lived in Mexico or Latin America; however aldrin/dieldrin, total chlordane, and total heptachlor were not associated with prior residence in Mexico or Latin America. Total chlordane was significantly associated with eating eggs from the neighborhood. Chlordane concentration was not significantly associated with any variables other than age and egg consumption, and egg consumption was not significantly associated with other serum pesticide concentrations. The concentration of hexachlorocyclohexane was significantly associated with several

variables that were not associated with any of the other pesticides: the concentration was higher in females compared to males, lower among persons who did activities in the garden or yard, and higher in persons who ate fish from a pier or boat.

Table 9: Adjusted Mean Serum Pesticide Concentration* by Demographic & Neighborhood Variables among Adults

		Aldrin/Dieldrin	Total Chlordane	Total DDT	Total Heptachlor	HCB	Total HCCH
Age –	18-30 years	0.109	0.284	13.4	0.110	0.152	0.364
	31-44 years	0.146	0.333	15.6	0.119	0.187	0.367
	45-64 years	0.165*	0.496+	23.6+	0.150	0.249+	0.609+
	65 + years	0.203	0.579+	38.5+	0.282+	0.325+	2.03+
Gender –	Male	0.158	0.448	21.3	0.169	0.233	0.765
	Female	0.153	0.398	24.2	0.161	0.224	0.922**
Education – Grammar		0.202	0.493	26.0	0.217	0.243	0.844
	Middle/some high school	0.135**	0.421	22.5	0.163	0.221	0.891
	High school	0.138**	0.393	22.8	0.125**	0.239	0.836
	Past High School	0.147	0.384	19.8	0.156	0.210	0.803
Lived in Mexico / Central America	No	0.163	0.424	18.6	0.156	0.182	0.637
	Yes	0.149	0.422	27.0+	0.175	0.275+	1.05+
Worked on a farm -	No	0.164	0.404	21.4	0.182	0.231	0.827
	Yes	0.147	0.442	24.2	0.148	0.226	0.860
Activities in garden or yard -	No	0.157	0.423	25.0	0.172	0.233	0.921
	Yes	0.154	0.423	20.6	0.158	0.223	0.766**
Eat eggs from neighborhood -	No	0.136	0.341	20.5	0.146	0.248	0.785
	Yes	0.175	0.505+	25.1	0.185	0.209	0.902
Eat vegetables from neighborhood	No	0.167	0.407	24.7	0.162	0.216	0.822
	Yes	0.144	0.439	20.9	0.168	0.240	0.865
Eat fish caught on pier or boat -	No	0.151	0.437	21.0	0.157	0.239	0.769
	Yes	0.160	0.409	24.5	0.173	0.218	0.918

* Serum concentration in ng/ml serum. ** $p < 0.05$ + $p < 0.01$

Table 10: Adjusted Mean Lipid-Adjusted Pesticide Concentration by Demographic & Neighborhood Variables among Adults

		Aldrin/Dieldrin	Total Chlordane	Total DDT	Total Heptachlor	HCB	Total HCCH
Age –	**18-30 years**	15.3	38.2	1,873	17.5	25.5	54.1
	31-44 years	19.6	42.7	2,170	17.4	27.3	50.4
	45-64 years	19.9	58.1+	2,955+	19.2	33.1	77.1**
	65 + years	24.5	69.5**	4,231+	36.7**	42.7	245.7+
Gender –	**Male**	19.8	53.1	2,550	23.4	31.2	96.1
	Female	19.9	51.2	3,066	21.9	33.1	117.5**
Education – Grammar		24.0	56.0	3,085	27.8	30.9	98.5
	Middle/some high school	17.4	52.6	2,795	22.9	31.1	114.7
	High school	17.6	48.9	2,854	17.7**	34.7	109.1
	Past High School	20.3	51.0	2,496	22.4	31.9	104.9
Lived in Mexico or Latin America	**No**	20.7	52.7	2,147	21.8	24.7	76.8
	Yes	18.9	51.5	3,468+	23.6	39.6+	136.8+
Worked on a farm -	**No**	21.8	51.8	2,658	24.5	31.6	104.2
	Yes	17.8	52.5	2,957	20.9	32.6	109.4
Do activities garden or yard -	**No**	20.5	52.9	3,073	24.2	33.8	118.2
	Yes	19.1	51.4	2,542	21.2	30.5	95.4**
Eat eggs from neighborhood -	**No**	17.9	44.7	2,607	20.8	34.7	101.7
	Yes	21.7	59.6+	3,008	24.5	29.6	112.0
Eat vegetables from neighborhood	**No**	21.7	50.9	3052	23.1	30.7	102.9
	Yes	17.9	53.4	2563	22.3	33.6	110.7
Eat fish caught on pier or boat -	**No**	19.3	53.8	2548	21.7	34.1	95.2
	Yes	20.3	50.5	3067	23.7	30.2	118.4**

* Lipid-adjusted concentration in ng/g lipid. ** $p < 0.05$ + $p < 0.01$

Table 11: Regression of Standardized* Serum Pesticide Concentrations on Demographic and Neighborhood Variables among Adults – Regression Coefficient (SD units)

	Aldrin/Dieldrin		Total Chlordane		Total DDT		Total Heptachlor		HCB		Total HCCH	
	Serum	p-value	Serum	p-value	Serum	p-value	Serum	p-value	Serum	p-value	Serum	p-value
Intercept	10.20	0.0001	10.18	0.0001	9.79	0.0001	10.22	0.0001	9.84	0.0001	9.16	0.0001
Age+ 31-44	0.193	0.09	0.168	0.30	0.125	0.40	0.055	0.72	0.194	0.20	0.004	0.97
45-64	0.293	0.02	0.721	0.0001	0.585	0.0003	0.222	0.18	0.544	0.0009	0.402	0.006
65+	0.494	0.06	1.004	0.008	1.444	0.0001	0.954	0.008	0.969	0.006	2.740	0.0001
Gender - Female	-0.029	0.77	-0.169	0.23	0.167	0.20	-0.044	0.74	-0.047	0.72	0.257	0.03
Educ+ mid/some HS	-0.349	0.01	-0.243	0.22	-0.198	0.28	-0.295	0.12	-0.118	0.53	0.077	0.65
High school	-0.331	0.03	-0.338	0.12	-0.182	0.37	-0.507	0.02	-0.019	0.92	-0.012	0.95
Past high school	-0.285	0.09	-0.369	0.12	-0.358	0.10	-0.333	0.14	-0.180	0.42	-0.067	0.73
Lived in Mexico	-0.072	0.53	-0.008	0.96	0.482	0.002	0.107	0.50	0.518	0.0009	0.677	0.0001
Worked on Farm	-0.087	0.49	0.129	0.47	0.164	0.31	-0.187	0.27	-0.032	0.85	0.053	0.72
Activities in Garden	-0.015	0.89	-0.001	0.99	-0.251	0.07	-0.080	0.58	-0.057	0.69	-0.254	0.04
Eat Eggs	0.202	0.10	0.558	0.001	0.262	0.09	0.217	0.19	-0.216	0.18	0.193	0.17
Eat Vegetables	-0.118	0.28	0.110	0.47	-0.219	0.12	0.033	0.82	0.135	0.35	0.071	0.58
Eat Fish from pier/boat	0.044	0.68	-0.094	0.53	0.201	0.16	0.090	0.53	-0.119	0.40	0.244	0.06

* Pesticide concentrations standardized to mean = 10 and SD = 1, so coefficients mean change in SD units.

\+ Reference categories: Age = 18-31 years; Education = elementary school .

Table 12: Regression of Standardized* Lipid-Adjusted Pesticide Concentrations on Demographic and Neighborhood Variables among Adults – Regression Coefficient (SD units)

	Aldrin/Dieldrin		Total Chlordane		Total DDT		Total Heptachlor		HCB		Total HCCH	
	Lipid	p-value	Lipid	p-value	Lipid	p-value	Lipid	p-value	Lipid	p-value	Lipid	p-value
Intercept	10.08	0.0001	9.97	0.0001	9.26	0.0001	10.29	0.0001	9.64	0.0001	9.06	0.0001
Age+ 31-44	0.118	0.13	0.131	0.39	0.132	0.38	-0.006	0.97	0.067	0.65	-0.045	0.72
45-64	0.129	0.13	0.577	0.0005	0.479	0.003	0.069	0.65	0.278	0.08	0.280	0.04
65+	0.255	0.16	0.911	0.01	1.044	0.003	0.777	0.02	0.635	0.07	2.336	0.0001
Gender - Female	0.003	0.97	-0.056	0.67	0.229	0.08	-0.061	0.62	0.068	0.60	0.261	0.02
Educ+ mid/some HS	-0.085	0.06	-0.097	0.61	-0.129	0.49	-0.201	0.26	0.007	0.97	0.197	0.21
High school	-0.179	0.09	-0.205	0.32	-0.102	0.62	-0.411	0.03	0.138	0.49	0.129	0.46
Past high school	-0.103	0.37	-0.144	0.52	-0.261	0.24	-0.220	0.30	0.037	0.87	0.077	0.68
Lived in Mexico	-0.049	0.54	-0.035	0.82	0.585	0.0002	0.076	0.60	0.546	0.0004	0.731	0.0001
Worked on Farm	-0.110	0.20	0.021	0.90	0.133	0.41	-0.149	0.35	0.037	0.82	0.063	0.65
Activities in Garden	-0.041	0.57	-0.043	0.76	-0.235	0.09	-0.119	0.37	-0.124	0.37	-0.277	0.02
Eat Eggs	0.104	0.21	0.435	0.008	0.178	0.25	0.150	0.32	-0.191	0.23	0.126	0.34
Eat Vegetables	-0.106	0.15	0.073	0.61	-0.216	0.12	-0.032	0.81	0.104	0.46	0.094	0.43
Eat Fish from pier/boat	0.027	0.71	-0.096	0.50	0.230	0.11	0.079	0.56	-0.143	0.30	0.282	0.02

* Pesticide concentrations standardized to mean = 10 and SD = 1, so coefficients mean change in SD units.

\+ Reference categories: Age = 18-31 years; Education = elementary school .

Children. Tables 13 and 14 show the adjusted mean serum and lipid-adjusted concentrations by demographic and neighborhood variables among children. Table 15 summarizes the regression of standardized serum pesticide concentrations on demographic and neighborhood variables and table 16 shows the regression of the standardized lipid-adjusted concentrations among the children. For these regression analyses, age 0-9 years, male gender, and "no" for the dichotomous variables were defined as the referent levels.

All pesticides showed lower mean concentrations in the older children (ages 10-17 years compared to children 0-9 years), with total chlordane and total DDT showing statistically significant differences. A possible explanation for this finding is that a major source of exposure for children can be breastfeeding as infants. Breastfeeding increases the body burden and, thus, serum concentrations of the organochlorine pesticides. As the children get older, they stop breastfeeding and their exposure substantially decreases. As the children grow, their mass of adipose tissue increases, so the pesticide concentration per gram of adipose decreases, resulting in lower serum concentrations in the older children compare to those in the younger children.

Lipid adjustment also affected the strength of the pesticide-age associations in children, but among the children, the inverse age associations were stronger with lipid-adjustment (i.e., more negative standardized regression coefficients a stronger inverse association), while in adults the positive age associations were weaker with lipid-adjustment. The explanation for children is that older children had higher lipid levels (specifically

triglycerides) compared to younger children (162 mg/mL compared to 129 mg/mL triglycerides, respectively), so the inverse age-pesticides associations would be somewhat strengthened with lipid-adjustment. The differences in lipid levels by age among children were not great, so the effects of lipid-adjustment on the age association were modest and virtually none of the p-values changed substantially with lipid-adjustment.

Other than the association with age, the serum pesticide concentrations in children did not have any consistent associations with other demographic or exposure variables. There were also no substantial differences in the patterns of associations between the serum pesticides and the lipid-adjusted pesticides. There were no significant associations between the serum pesticide concentrations and the predictor variables for aldrin/dieldrin, total chlordane, total DDT, or total hexachlorocyclohexane. Total heptachlor was significantly increased among children who ate fish caught from on a pier or boat, but this difference was not significant for the lipid-adjusted pesticide concentration. The concentrations of hexachlorobenzene were significantly lower in children who ate eggs from chickens raised in the neighborhood. There is no clear explanation for the egg consumption to be associated with a lower serum pesticide concentration, so this apparently significant association may have occurred by chance given the multiple comparisons.

For these children, eating fruit or vegetables grown in the neighborhood was associated with an increase in hexachlorobenzene concentration ($p=0.08$).

Table 13: Adjusted Mean Serum Pesticide Concentration* by Demographic and Neighborhood Variables among Children

		Aldrin/ Dieldrin	Total Chlordane	Total DDT	Total Heptachlor	HCB	Total HCCH
Age	**0-9 years**	0.174	0.227	3.88	0.149	0.115	0.234
	10-17 years	0.093	0.170**	2.50**	0.116	0.096	0.168
Gender	**Male**	0.146	0.201	2.90	0.145	0.098	0.226
	Female	0.121	0.197	3.48	0.120	0.112	0.176
Do activities in garden or yard	**No**	0.142	0.187	3.44	0.132	0.095	0.178
	Yes	0.125	0.211	2.94	0.133	0.116	0.223
Eat eggs from neighborhood	**No**	0.119	0.191	3.25	0.145	0.134	0.186
	Yes	0.148	0.206	3.13	0.150	0.076**	0.215
Eat vegetables from neighborhood	**No**	0.115	0.200	2.89	0.133	0.085	0.229
	Yes	0.151	0.197	3.50	0.133	0.125	0.173
Eat fish caught on pier or boat	**No**	0.128	0.187	3.30	0.100	0.090	0.223
	Yes	0.139	0.210	3.08	0.165**	0.121	0.179

* Serum concentration in ng/ml serum. ** $p < 0.05$ + $p < 0.01$

Table 14: Adjusted Mean Lipid-Adjusted Pesticide Concentrations* by Demographic and Neighborhood Variables among Children

		Aldrin/Dieldrin	Total Chlordane	Total DDT	Total Heptachlor	HCB	Total HCCH
Age	**0-9 years**	30.3	39.2	652	25.0	19.4	39.9
	10-17 years	13.1	26.7**	387**	19.6	15.6	28.0
Gender	**Male**	26.6	34.4	476	25.2	17.0	39.1
	Female	16.8	31.5	563	19.4	18.1	28.9
Do activities in garden or yard	**No**	23.0	31.9	586	23.5	15.9	31.2
	Yes	20.4	34.0	454	21.1	19.1	36.8
Eat eggs from neighborhood	**No**	21.8	33.8	552	19.4	23.5	33.0
	Yes	21.6	32.1	488	25.2	11.6**	35.0
Eat vegetables from neighborhood	**No**	17.9	33.0	460	22.9	13.7	39.5
	Yes	25.5	32.9	580	21.7	21.4	28.5
Eat fish caught on pier or boat	**No**	22.3	33.3	580	18.2	15.8	40.1
	Yes	21.1	32.6	460	26.4	19.2	27.9

* Lipid-adjusted concentration in ng/g lipid. * $p < 0.05$ + $p < 0.01$

Table 15: Regression of Standardized* Serum Pesticide Concentrations on Demographic and Neighborhood Variables among Children – Regression Coefficients (SD units)

	Aldrin/Dieldrin		Total Chlordane		Total DDT		Total Heptachlor		HCB		Total HCCH	
	Serum	p-value	Serum	p-value	Serum	p-value	Serum	p-value	Serum	p-value	Serum	p-value
Intercept	10.70	0.0001	9.95	0.0001	9.66	0.0001	10.30	0.0001	9.49	0.0001	10.08	0.0001
Age 10-17 +	-0.420	0.07	-0.192	0.02	-0.079	0.05	-0.187	0.22	-0.106	0.39	-0.109	0.27
Gender - Female	-0.135	0.54	-0.013	0.87	0.034	0.39	-0.142	0.33	0.080	0.50	-0.082	0.40
Activities in Garden	-0.087	0.70	0.082	0.32	-0.029	0.47	0.005	0.98	0.121	0.32	0.074	0.45
Eat Eggs	0.149	0.62	0.051	0.65	-0.006	0.91	0.197	0.33	-0.326	0.05	0.048	0.73
Eat Vegetables	0.188	0.43	-0.012	0.89	0.035	0.40	0.000	0.99	0.224	0.08	-0.092	0.37
Eat Fish from pier/boat	0.053	0.85	0.078	0.43	-0.013	0.80	0.358	0.05	0.173	0.24	-0.072	0.56

* Pesticide concentrations standardized to mean = 10 and SD = 1, so coefficients mean change in SD units.

\+ Reference category: Age = 0-9 years .

Table 16: Regression of Standardized* Lipid-Adjusted Pesticide Concentrations on Demographic and Neighborhood Variables among Children – Regression Coefficients (SD units)

	Aldrin/Dieldrin		Total Chlordane		Total DDT		Total Heptachlor		HCB		Total HCCH	
	Lipid	p-value	Lipid	p-value	Lipid	p-value	Lipid	p-value	Lipid	p-value	Lipid	p-value
Intercept	11.13	0.0001	10.48	0.0001	9.74	0.0001	10.66	0.0001	9.71	0.0001	10.31	0.0001
Age 10-17 +	-0.480	0.07	-0.365	0.01	-0.117	0.04	-0.221	0.23	-0.138	0.37	-0.145	0.30
Gender - Female	-0.0272	0.29	-0.085	0.55	0.039	0.47	-0.237	0.18	0.039	0.79	-0.124	0.36
Activities in Garden	-0.072	0.78	-0.063	0.67	-0.058	0.29	-0.095	0.60	0.119	0.44	0.068	0.62
Eat Eggs	-0.005	0.99	-0.049	0.80	-0.029	0.71	0.236	0.33	-0.440	0.03	0.024	0.90
Eat Vegetables	0.213	0.44	-0.002	0.99	0.053	0.36	-0.048	0.80	0.283	0.08	-0.135	0.35
Eat Fish from pier/boat	-0.032	0.92	-0.022	0.90	-0.053	0.44	0.332	0.13	0.125	0.50	-0.149	0.40

* Pesticide concentrations standardized to mean = 10 and SD = 1, so coefficients mean change in SD units.

\+ Reference category: Age = 0-9 years .

Serum Pesticide Concentrations and Health Outcomes

Table 17 summarizes the prevalence of certain symptoms and diagnoses among the adults and children. Symptoms were taken from the questionnaire filled out by each participant, while diagnoses were abstracted from the participants' chart by a project nurse.

Tables 18 and 19 summarize the mean serum and lipid-adjusted pesticide concentrations by symptom and diagnosis variables among adults. For participants who had hypertension, the mean serum and lipid-adjusted concentration of all pesticides were higher than the means for participants who did not have hypertension. For participants who had an "ENT or sinus/nasal condition," the mean serum and lipid-adjusted concentration of all pesticides except total heptachlor were higher than the means for participants without "ENT or sinus/nasal condition." For participants with a diagnosis of asthma, the mean serum and lipid-adjusted concentration of all pesticides except hexachlorobenzene were lower than the means for participants who did not have asthma. No other clear patterns emerged.

Table 17: Prevalence of Symptoms and Diagnoses among Adults and Children

Pesticide	Adults - percent	Children – percent
Symptoms (from questionnaire)		
Sinus problems	46.3	32.5
Frequent nose bleeds	12.7	24.0
Cough in morning	33.1	28.7
Shortness of breadth	52.7	25.3
Pins and needles	45.2	9.8
Frequent numbness	33.2	3.0
Laboratory finding		
Low hematocrit	5.5	18.7
AST > reference	8.0	4.9
ALT > reference	16.1	2.5
GGT > reference	14.0	0.7
Diagnosis (from chart)		
Allergies	10.9	9.9
ENT or sinus conditions	20.7	19.3
Asthma	5.2	10.5
Skin rash or dermatitis	18.8	25.7
Hypertension	17.9	0
Hepatitis	3.0	0
Headaches	27.1	13.5
Mood – depression, fatigue	11.3	1.2
Neurological diagnosis	1.2	1.2
Cancer or tumor	1.8	0
Total number	**329**	**171**

Table 18: Mean Serum Pesticide Concentrations* by Symptoms and Diagnoses among Adults

		Aldrin/Dieldrin	Total Chlordane	Total DDT	Total Heptachlor	HCB	Total HCCH
Sinus problems	**No**	0.107	0.307	14.7	0.131	0.226	0.441
	Yes	0.145	0.333	13.7	0.122	0.210	0.399
Cough in the morning	**No**	0.123	0.315	14.9	0.133	0.224	0.436
	Yes	0.123	0.326	13.0	0.115	0.209	0.394
Shortness of breath	**No**	0.119	0.327	14.8	0.139	0.218	0.446
	Yes	0.127	0.310	13.8	0.177	0.220	0.399
Pins and needles	**No**	0.116	0.321	13.9	0.133	0.205	0.458
	Yes	0.132	0.314	14.6	0.118	0.239	0.378
Frequent numbness	**No**	0.123	0.296	13.9	0.136	0.217	0.390
	Yes	0.123	0.368	15.0	0.107	0.225	0.487
Elevated LFTs	**No**	0.120	0.309	13.8	0.128	0.213	0.427
	Yes	0.138	0.356	15.9	0.122	0.245	0.401
ENT or sinus/nasal condition	**No**	0.121	0.302	12.9	0.128	0.215	0.415
	Yes	0.132	0.385	19.4	0.122	0.235	0.447
Asthma	**No**	0.125	0.320	14.3	0.129	0.216	0.425
	Yes	0.092	0.282	13.1	0.087	0.278	0.367
Skin condition, dermatitis	**No**	0.125	0.322	14.0	0.126	0.214	0.439
	Yes	0.117	0.303	15.3	0.132	0.237	0.348
Hypertension	**No**	0.119	0.288	13.1	0.120	0.206	0.369
	Yes	0.141	0.461	19.4	0.163	0.281	0.650
Headaches	**No**	0.119	0.326	15.8	0.124	0.223	0.416
	Yes	0.135	0.296	10.0	0.134	0.209	0.437
CNS or PNS condition	**No**	0.119	0.318	14.7	0.132	0.213	0.418
	Yes	0.153	0.318	11.3	0.090	0.261	0.444

* Serum concentration in ng/ml serum.

Table 19: Mean Lipid-Adjusted Pesticide Concentrations by Symptoms and Diagnoses among Adults

		Aldrin/Dieldrin	Total Chlordane	Total DDT	Total Heptachlor	HCB	Total HCCH
Sinus problems	**No**	14.7	40.6	2120	18.1	33.1	61.9
	Yes	20.0	45.7	1803	17.6	29.4	51.5
Cough in the morning	**No**	16.9	41.6	2097	18.9	32.4	59.1
	Yes	17.2	45.2	1742	15.7	29.8	53.3
Shortness of breath	**No**	16.1	43.3	2012	19.3	30.9	59.5
	Yes	17.8	42.4	1939	16.5	32.1	54.9
Pins and needles	**No**	16.5	43.1	1932	19.1	29.8	62.6
	Yes	17.7	42.4	2028	16.2	33.9	50.4
Frequent numbness	**No**	17.4	39.9	1932	19.5	31.6	52.7
	Yes	16.0	49.3	2063	14.3	31.2	66.3
Elevated LFTs	**No**	16.6	41.8	1950	18.2	30.7	58.4
	Yes	18.6	46.8	2072	16.5	34.7	52.0
ENT or sinus/nasal condition	**No**	16.8	41.0	1799	18.3	30.9	56.5
	Yes	17.7	50.2	2652	16.0	33.9	59.8
Asthma	**No**	17.2	43.0	1996	18.2	31.4	57.7
	Yes	12.9	38.2	1607	11.3	34.9	46.9
Skin condition, dermatitis	**No**	17.1	43.8	1929	17.8	30.9	59.9
	Yes	16.6	38.8	2169	18.1	33.8	45.3
Hypertension	**No**	16.9	40.3	1891	17.4	30.3	51.3
	Yes	17.3	54.8	2334	20.0	37.5	82.3
Headaches	**No**	16.2	43.3	2167	17.6	31.9	55.8
	Yes	19.1	41.4	1458	18.6	30.6	60.8
CNS or PNS condition	**No**	16.7	43.2	2053	18.6	30.8	56.8
	Yes	19.0	40.4	1471	12.8	36.6	59.1

* Lipid-adjusted concentration in ng/g lipid.

Table 20 summarizes the odds ratios obtained by logistic regression of health outcomes on standardized pesticide concentrations among adults. Table 21 is similar except that it is a summary of the lipid-adjusted values. The odds ratios for hypertension were all above 1, except for lipid-adjusted total DDT and total heptachlor, which both had odds ratios of 1.00. Serum hexachlorobenzene had an odds ratio of 1.37 with a confidence interval that did not cross 1.0, and a p-value of 0.03. For the health outcome "ENT or sinus/nasal condition," all confidence intervals crossed 1.0 and all p-values for the odds ratios were greater than 0.05. Only total DDT stood out in that the odds ratio for the standardized serum concentration was 1.23 with a confidence interval of 0.99 to 1.53, and a p-value of 0.06. The standardized lipid-adjusted concentration had an odds ratio of 1.20 with a confidence interval 0.97 to 1.50, and a p-value of 0.10. The odds ratios for asthma were all less than 1.0 (except for hexachlorobenzene), but the p-values were all quite high, indicating that the associations were not significant. For "sinus problems," the odds ratio of the standardized serum and lipid-adjusted concentrations for aldrin/dieldrin were 1.40 and 1.73, respectively. Neither confidence interval crossed 1.0 (1.01-1.94 and 1.05-2.85), and p-values were 0.04 and 0.03. For headaches, the odds ratio of the standardized serum and lipid-adjusted concentrations for total DDT were 0.60 and 0.66 respectively. The confidence interval for the serum value did not cross 1.0. However, the confidence interval for the lipid-adjusted value did cross 1.0. The p-values were 0.02 and 0.02.

Table 20: Logistic Regression of Health Outcomes on Standardized* Serum Pesticide Concentrations among Adults+ - Odds Ratio per Change in 1 SD of Pesticide

	Aldrin/Dieldrin		Total Chlordane		Total DDT		Total Heptachlor		HCB		Total HCCH	
	Serum	p-value	Serum	p-value	Serum	p-value	Serum	p-value	Serum	p-value	Serum	p-value
Sinus problems	1.40	0.04	1.12	0.33	1.06	0.60	1.01	0.96	1.01	0.98	1.02	0.84
Cough in morning	1.06	0.73	1.08	0.51	1.02	0.89	0.96	0.75	0.99	0.97	1.07	0.57
Shortness of breath	1.06	0.73	0.88	0.26	0.90	0.35	0.86	0.21	0.98	0.85	0.86	0.25
Pins and needles	1.00	0.99	0.92	0.47	1.08	0.02	0.96	0.72	1.22	0.12	0.98	0.88
Frequent numbness	0.86	0.45	1.02	0.90	0.97	0.77	0.85	0.28	0.96	0.77	1.16	0.26
Elevated LFTs	1.26	0.18	1.14	0.26	1.09	0.56	0.97	0.83	1.22	0.13	1.03	0.87
ENT or sinus/nasal	1.17	0.37	1.21	0.11	1.23	0.06	0.94	0.66	1.09	0.54	1.01	0.95
Asthma	0.45	0.22	0.75	0.43	0.93	0.17	0.62	0.49	1.27	0.33	0.91	0.71
Skin condition, dermatitis	0.94	0.75	0.95	0.72	1.06	0.61	1.01	0.93	1.25	0.09	0.87	0.45
Hypertension	1.27	0.22	1.26	0.07	1.09	0.51	1.07	0.63	1.37	0.03	1.11	0.45
Headaches	1.23	0.22	0.99	0.93	0.60	0.02	1.06	0.63	0.84	0.26	1.08	0.59
CNS or PNS condition	1.27	0.22	0.92	0.61	0.71	0.15	0.76	0.31	1.09	0.60	0.96	0.81

* Pesticide concentrations standardized to mean = 10 and SD = 1, so OR mean relative risk by SD change in pesticide.

\+ Models include Age and Lived Mexico/South America as co-variates.

Table 21: Logistic Regression of Health Outcomes on Standardized* Lipid-Adjusted Pesticide Concentrations among Adults+ - Odds Ratio per Change in 1 SD of Pesticide

	Aldrin/Dieldrin		Total Chlordane		Total DDT		Total Heptachlor		HCB		Total HCCH	
	Lipid	p-value	Lipid	p-value	Lipid	p-value	Lipid	p-value	Lipid	p-value	Lipid	p-value
Sinus problems	1.73	0.03	1.21	0.003	1.01	0.92	1.02	0.88	0.97	0.79	0.97	0.82
Cough in morning	1.13	0.64	1.16	0.23	0.98	0.88	0.90	0.50	0.99	0.93	1.07	0.59
Shortness of breath	1.22	0.41	0.92	0.51	0.94	0.54	0.87	0.29	1.05	0.69	0.87	0.35
Pins and needles	0.94	0.78	0.93	0.55	1.10	0.40	0.93	0.59	1.24	0.10	0.96	0.80
Frequent numbness	0.76	0.33	1.09	0.46	0.99	0.91	0.79	0.20	0.98	0.87	1.22	0.15
Elevated LFTs	1.20	0.48	1.09	0.51	1.00	1.0	0.91	0.62	1.12	0.41	0.92	0.70
ENT or sinus/nasal	1.09	0.74	1.22	0.12	1.20	0.10	0.86	0.44	1.05	0.76	0.98	0.88
Asthma	0.33	0.24	0.79	0.49	0.88	0.68	0.43	0.44	1.17	0.57	0.89	0.71
Skin condition, dermatitis	0.87	0.65	0.82	0.23	1.06	0.61	0.99	0.97	1.20	0.19	0.76	0.24
Hypertension	1.14	0.68	1.14	0.35	1.00	0.97	1.00	1.0	1.23	0.19	1.09	0.49
Headaches	1.41	0.17	1.05	0.70	0.66	0.02	1.05	0.75	0.85	0.28	1.10	0.53
CNS or PNS condition	1.22	0.51	0.85	0.40	0.66	0.08	0.72	0.28	1.08	0.62	0.96	0.81

* Pesticide concentrations standardized to mean = 10 and SD = 1, so OR mean relative risk by SD change in pesticide.

\+ Models include Age and Lived Mexico/South America as co-variates.

Tables 22 and 23 summarize the mean serum and lipid-adjusted pesticide concentrations by symptom and diagnosis variables among children. Among children, the mean serum and lipid-adjusted concentration of all pesticides were lower for children with asthma than the means for children who did not have asthma. The mean serum and lipid-adjusted concentrations of aldrin/dieldrin and total chlordane were substantially increased in children with "low hematocrit," while total DDT and total hexachlorocyclohexane showed small increases compared to children who did not have low hematocrit.

Table 24 summarizes the odds ratios obtained by logistic regression of health outcomes on standardized pesticide concentrations among children. Table 25 16 is similar except that it is a summary of the lipid-adjusted values. For asthma, the odds ratios for all of the standardized serum and lipid-adjusted pesticides were less than 1.0. However, all the associated confidence intervals crossed 1.0 and p-values were all greater than 0.10. For children with a low hematocrit, the odds ratios of the standardized serum and lipid adjusted concentrations were 1.56 and 1.74 for aldrin/dieldrin, and 1.87 and 1.76 for total chlordane, respectively. The confidence intervals of the odds ratios for aldrin/dieldrin did not cross 1.0, and the p-values for the odds ratios were 0.02 and 0.02. The confidence interval for the odds ratio of serum total chlordane crossed 1.0, while it did not for the lipid-adjusted total chlordane concentration. The p-values were 0.10 and 0.02, respectively.

Table 22: Mean Serum Pesticide Concentrations* by Symptoms and Diagnoses among Children

		Aldrin/Dieldrin	Total Chlordane	Total DDT	Total Heptachlor	HCB	Total HCCH
Sinus problems	**No**	0.159	0.212	3.85	0.121	0.114	0.249
	Yes	0.091	0.175	3.20	0.081	0.130	0.148
Nose Bleeds	**No**	0.124	0.190	3.79	0.112	0.122	0.243
	Yes	0.179	0.232	3.22	0.097	0.112	0.139
Cough in the morning	**No**	0.133	0.197	3.89	0.119	0.123	0.227
	Yes	0.151	0.212	3.02	0.079	0.108	0.188
Shortness of breath	**No**	0.156	0.207	3.79	0.120	0.117	0.212
	Yes	0.082	0.182	3.18	0.072	0.127	0.228
Low Hematocrit	**No**	0.098	0.184	3.62	0.112	0.127	0.215
	Yes	0.334	0.285	3.77	0.088	0.082	0.220
ENT or sinus/nasal condition	**No**	0.122	0.187	3.51	0.104	0.112	0.232
	Yes	0.201	0.256	4.14	0.127	0.147	0.155
Asthma	**No**	0.143	0.208	3.79	0.113	0.124	0.233
	Yes	0.088	0.139	2.45	0.071	0.082	0.165
Skin condition, dermatitis	**No**	0.155	0.206	3.33	0.118	0.120	0.184
	Yes	0.083	0.184	4.59	0.080	0.117	0.314
Headaches	**No**	0.122	0.192	3.60	0.109	0.115	0.222
	Yes	0.251	0.261	3.90	0.102	0.149	0.177

* Serum concentration in ng/ml serum.

Table 23: Mean Lipid Adjusted Pesticide Concentrations by Symptoms and Diagnoses among Children

		Aldrin/Dieldrin	Total Chlordane	Total DDT	Total Heptachlor	HCB	Total HCCH
Sinus problems	**No**	30.2	39.3	697	21.2	20.0	45.3
	Yes	15.1	30.6	534	13.5	23.1	25.7
Nose Bleeds	**No**	22.0	34.5	681	19.7	21.4	44.2
	Yes	35.5	42.7	540	15.9	19.6	24.0
Cough in the morning	**No**	23.2	34.9	682	20.2	21.3	40.8
	Yes	31.5	41.1	550	14.6	19.9	34.5
Shortness of breath	**No**	28.9	37.6	663	20.6	20.1	38.3
	Yes	14.4	33.2	588	13.1	23.6	41.1
Low Hematocrit	**No**	16.6	31.8	620	18.7	22.0	37.9
	Yes	68.9	60.2	771	18.8	15.8	44.5
ENT or sinus/nasal condition	**No**	21.7	33.7	629	18.1	19.7	42.1
	Yes	40.6	48.1	705	21.5	26.1	26.7
Asthma	**No**	26.7	38.0	675	19.5	21.8	40.4
	Yes	14.4	24.7	398	12.5	13.7	28.0
Skin condition, dermatitis	**No**	28.6	37.1	579	20.2	20.9	32.7
	Yes	15.3	34.8	846	14.5	21.1	58.3
Headaches	**No**	21.5	34.5	633	18.9	20.0	40.0
	Yes	52.5	50.7	723	17.6	27.9	32.0

* Lipid-adjusted concentration in ng/g lipid.

Table 24: Logistic Regression of Health Outcomes on Standardized* Serum Pesticide Concentrations among Children+ - Odds Ratio per Change in 1 SD of Pesticide

	Aldrin/Dieldrin		Total Chlordane		Total DDT		Total Heptachlor		HCB		Total HCCH	
	Serum	p-value	Serum	p-value	Serum	p-value	Serum	p-value	Serum	p-value	Serum	p-value
Sinus problems	0.76	0.26	0.65	0.35	0.64	0.58	0.66	0.31	1.16	0.56	0.36	0.19
Nose Bleeds	1.17	0.24	1.87	0.09	0.71	0.68	0.92	0.75	0.86	0.61	0.19	0.18
Cough in the Morning	1.04	0.75	1.18	0.66	0.38	0.30	0.57	0.31	0.99	0.98	0.81	0.55
Shortness of Breath	0.67	0.24	0.83	0.68	0.68	0.66	0.10	0.42	1.03	0.92	1.11	0.71
Low Hematocrit	1.56	0.02	1.87	0.10	0.50	0.46	0.65	0.35	0.65	0.21	0.87	0.67
ENT or sinus/nasal	1.20	0.16	2.30	0.03	1.73	0.43	1.15	0.49	1.19	0.52	0.47	0.36
Asthma	0.78	0.51	0.02	0.22	0.06	0.24	<0.001	0.78	0.41	0.13	0.64	0.59
Skin condition, dermatitis	0.61	0.16	0.61	0.28	2.20	0.23	0.56	0.31	0.95	0.86	1.53	0.16
Headaches	1.22	0.15	1.75	0.17	1.28	0.77	0.91	0.78	1.30	0.37	0.67	0.56

* Pesticide concentrations standardized to mean = 10 and SD = 1, so OR mean relative risk by SD change in pesticide.

\+ Models include Age and Lived Mexico/South America as co-variates.

Table 25: Logistic Regression of Health Outcomes on Standardized* Lipid-Adjusted Pesticide Concentrations among Children+- Odds Ratio per Change in 1 SD of Pesticide

	Aldrin/Dieldrin		Total Chlordane		Total DDT		Total Heptachlor		HCB		Total HCCH	
	Lipid	p-value	Lipid	p-value	Lipid	p-value	Lipid	p-value	Lipid	p-value	Lipid	p-value
Sinus problems	0.70	0.23	0.73	0.29	0.55	0.39	0.56	0.29	1.11	0.61	0.43	0.17
Nose Bleeds	1.15	0.23	1.38	0.12	0.62	0.50	0.86	0.55	0.86	0.54	0.17	0.12
Cough in the Morning	1.08	0.50	1.21	0.34	0.55	0.37	0.69	0.34	1.09	0.73	0.89	0.64
Shortness of Breath	0.64	0.25	0.91	0.73	0.86	0.81	0.27	0.29	1.06	0.78	1.07	0.73
Low Hematocrit	1.74	0.02	1.76	0.02	0.93	0.90	0.91	0.67	0.86	0.56	0.98	0.94
ENT or sinus/nasal	1.18	0.15	1.63	0.03	1.35	0.55	1.12	0.50	1.14	0.53	0.51	0.34
Asthma	0.70	0.48	0.20	0.16	0.04	0.15	0.10	0.29	0.43	0.11	0.62	0.54
Skin condition, dermatitis	0.64	0.19	0.83	0.47	1.91	0.19	0.61	0.32	0.97	0.88	1.38	0.16
Headaches	1.19	0.14	1.38	0.15	1.22	0.74	0.89	0.70	1.21	0.40	0.74	0.57

* Pesticide concentrations standardized to mean = 10 and SD = 1, so OR mean relative risk by SD change in pesticide.

\+ Models include Age and Lived Mexico/South America as co-variates.

Chapter 5

DISCUSSION

Correlations between Serum and Lipid-Adjusted Pesticide Concentrations

Serum and lipid-adjusted concentrations of the individual organochlorine pesticides were highly correlated in this group of study participants, with a range of 89 to 96%. When the adults were stratified into subgroups based on whether they had either lived more than a year in Mexico or Latin America or not, the serum and lipid-adjusted concentrations within the subgroups remained highly correlated. For the adults who had lived in Mexico or Latin America, the correlations ranged from 92 to 97%. For the adults who had not lived in these regions, the correlations ranged from 91 to 97%. These findings are consistent with a recent study that showed 86 to 90% correlation between serum and lipid-adjusted concentrations of DDE, hexachlorobenzene, and gamma-hexachlorocyclohexane in children (Karmaus et al. 2005).

Additionally, aldrin/dieldrin concentration had a relatively strong correlation with total chlordane, while total DDT, hexachlorobenzene, and total hexachlorocyclohexane tended to correlate with one another. The overall pattern of correlations indicates that the serum-serum concentrations among pesticides were higher than the lipid-adjusted–lipid-adjusted concentrations. The likely explanation is that the correlations among the serum-serum

pesticides is a function of both the underlying correlation in the pesticide body burdens and correlation due to the total serum lipids, which would cause all of the pesticides to increase or decrease together in an individual based on that person's cholesterol and triglyceride levels.

As previously mentioned, the patterns of correlations were somewhat different among the two sub-groups of adults. Total heptachlor showed a stronger correlation with all other pesticides for adults who had lived in Mexico than for adults who had not lived in Mexico. This pattern suggests that there may have been common environmental sources of pesticide exposure in Mexico, so that people were exposed to multiple pesticides in the same setting. Total hexachlorocyclohexane also showed a stronger correlation with all other pesticides (except total DDT) for adults who had lived in Mexico than for adults who had not lived in Mexico. DDT serum concentration was strongly correlated with that for chlordane and hexachlorocyclohexane among the adults who had not lived in Mexico, but it was not correlated with these pesticides among adults who had lived in Mexico. An explanation for the correlation between DDT and chlordane serum concentrations is not clear; however, it is relevant that both DDT and hexachlorocyclohexane were present in the Montrose facility, so the correlation between these two pesticides may reflect residents' exposure to both pesticides in the Del Amo/Montrose neighborhood.

Demographic and Exposure Variable Patterns for Serum and Lipid-Adjusted Pesticides

All of the serum and lipid-adjusted pesticide concentrations were positively associated with increasing age. However, for the lipid-adjusted concentrations the trends were weaker and fewer of the differences in mean concentrations and regression coefficients by age category were statistically significant. Most of the serum pesticide concentrations were inversely associated with educational level, although the trends by educational level were weaker than for the effect of age. The inverse trends by educational level were also weaker in the lipid-adjusted pesticides. This is consistent with much of the current literature which describes an inverse relationship between educational level and body burden of pesticides.

Both serum and lipid-adjusted pesticide concentrations of total chlordane showed a strong association with eating eggs from chickens raised in the neighborhood. The remainder of the pesticide concentrations were all weakly associated with consumption of these eggs. As with age and educational level, a similar pattern emerged in that the associations for eating eggs were somewhat weaker for the lipid-adjusted concentrations than for the serum concentrations. These patterns of differences in the associations between the serum and lipid-adjusted pesticides can be explained by the differences in serum lipids associated with these demographic and exposure variables. Specifically, total serum lipids increased with age and egg consumption, and decreased with higher educational level (which is an indicator of social class) (data, not shown).

No significant patterns in the associations for the serum concentrations compared to the lipid-adjusted concentrations were found for the other demographic, neighborhood and exposure variables. Of note, for total HCCH and fish consumption, the standardized regression coefficient was somewhat stronger for the lipid-adjusted concentration. It is unclear as to why a stronger association was seen in this case with the lipid-adjusted concentration.

Aldrin/dieldrin, total chlordane, and total heptachlor were not associated with prior residence in Mexico or Latin America, as opposed to serum concentrations of total DDT, hexachlorobenzene, and hexachlorocyclohexane which were all significantly higher in adults who had lived in Mexico or Latin America.

Total chlordane concentration was strongly associated in adults with eating eggs from chickens raised in the neighborhood, but total chlordane concentration was not significantly associated with any other variables except age. Aldrin/dieldrin and total DDT also showed an association with eating these eggs, but the finding was not statistically significant. The strong association between total chlordane concentration and the egg consumption could have occurred by chance, but it does suggest the possibility that eggs in the neighborhood were contaminated with chlordane. It is unclear why these three particular pesticides, and not the others, were associated with eating eggs from neighborhood chickens. Knowing that DDT was produced at the Montrose Chemical Plant, it could be speculated that the soil in the neighborhood that these chickens were raised on had higher DDT, DDE, and/or DDD concentrations than the soil that

commercially raised chickens are raised on, leading to increased exposure. However, aldrin/dieldrin and chlordane are not known to be specific contaminants from the Del Amo and Montrose facilities, therefore this can not be used as an explanation for either of these two pesticides. No statistically significant associations were seen between any of the pesticides and the children who ate the neighborhood eggs, which is unusual in light of the strong association seen between total chlordane concentrations and the egg consumption. Also of interest is that total chlordane concentrations were not associated with eating fruit or vegetables grown in neighborhood gardens or with activities in the garden or yard. If the soil was contaminated with chlordane, then one might expect to see associations with these potential sources of exposure. Additionally, arguing against there being contaminated soil in this community in general was that no statistically significant increases in any pesticide concentrations were associated with eating fruits or vegetables grown in neighborhood gardens or activities in the garden or yard. The only association seen was with lower serum and lipid-adjusted hexachlorocyclohexane concentrations and activities in the garden or yard.

The concentration of hexachlorocyclohexane was significantly associated with several variables that were not associated with any of the other pesticides: the concentration was higher in females compared to males, lower among persons who did activities in the garden or yard, and higher in persons who ate fish from a pier or boat. It is not clear why the pattern of associations for this pesticide was different from those for the other pesticides.

Age and Lipid Adjustment

Adjusting for lipids did not substantially change the patterns of associations between the pesticides and the demographic, neighborhood, or exposure variables, or the associations between pesticides and the health outcomes in most instances. Of note, when the mean concentrations of the pesticides were compared between children (0-17 years old) and adults (18+ years old), lipid-adjustment increased the relative concentrations for the children. For example, children had 62 percent of the mean serum concentration of chlordane that adults had. However, when adjusted for lipids, they had 84 percent. For aldrin/dieldrin, children had higher mean serum and lipid-adjusted concentrations than adults. With lipid-adjustment, again the relative concentration increased, with the children going from 105 percent of the mean serum concentration of adults to 143 percent of the mean lipid-adjusted concentration of adults. For total heptachlor, the children started with a mean serum concentration 85 percent that of the adults, but with adjustment for lipids they had a higher concentration (105 percent) than the adults. For the remainder of the pesticides analyzed, the children had lower serum and lipid-adjusted concentrations, but the concentration in children relative to that of adults increased with each pesticide. Therefore, it appears that adjusting for lipids increases the relative organochlorine concentration in children. This result is most likely due to children having lower serum lipid concentrations than adults. Since adults generally have higher total serum lipid concentrations and greater range of lipid values than children, lipid adjusting will have a greater impact on the adult organochlorine pesticide concentrations.

When the "children" group was stratified into ages 0-9 years and 10-17 years, the 0-9 year old group had higher serum and lipid-adjusted pesticide concentrations than the 10-17 year old group for all of the pesticides evaluated. When adults were then stratified into the subgroups of ages 18-30, 31-44, 45-64, and 65+ years, the 0-9 year old group had higher lipid-adjusted concentrations than many of the adult groups. No adult group had a higher concentration of aldrin/dieldrin than the 0-9 age group. For total heptachlor, only the 65+ group has a higher concentration. For total chlordane, only the 31-44, 46-64, and 65+ groups had higher concentrations. For total DDT, hexachlorobenzene, and total hexachlorocyclohexane, the age group 18-30 years and all groups above had higher concentrations than the 0-9 year old group. In general, the concentrations of all the pesticides decreased with age until the 18-30 year old group, leveled off, then increased progressively for the 18-30, 31-44, 45-64, and 65+ age groups. For total heptachlor, the concentration appeared to level off in the 18-30 and 31-44 year old groups, then started rising in the 45-64 year old group. Therefore, assuming that the lipid-adjusted concentrations of the xenobiotic lipophilic compounds are in equilibrium with and a good proxy for adipose concentrations, then the children in this study who were 0-9 years old had higher levels of aldrin/dieldrin and heptachlor than the adults.

The regression analysis of standardized pesticide concentrations on demographic and neighborhood variables showed a similar pattern. Both serum and lipid-adjusted pesticide concentrations decreased in children 10-17 years old compared with their referent group 0-9 years old. The older age groups in general had higher concentrations

than the referent group of 18-30 year olds. These increases were statistically significant for most part, with statistical significance decreasing with lipid adjustment.

The most likely explanation for the relatively high concentrations in the youngest age group is that the fetus can be exposed through the placenta and infants can be exposed as these lipophilic organochlorine pesticides are mobilized from adipose stores during breastfeeding and passed through the milk. This route of exposure is a plausible explanation of the high organochlorine concentration in the youngest group as this pattern was for all of the pesticides. An alternative, but unlikely explanation might be that all of these pesticides were present in household dust or yard soil, and the infants and toddlers received relatively higher exposure as they often ingest the dirt and dust from their hands and toys. It could also be possible that the youngest group were all exposed to some food product more than older children or the adults, but this is unlikely as the food product would need to contain all of the pesticides evaluated and the same item would need to be consumed by many of the children and in higher relative quantities than the adults. Alternatively, children may be less efficient at excreting these compounds.

Gender

Gender did not appear to be associated with higher pesticide concentrations, except for total hexachlorocyclohexane, which showed a significant positive association with adult females ($p = 0.03$). Although the mean serum concentration of total

hexachlorocyclohexane was higher in the female participants than in the males, the mean concentration for females of 0.410 ng/mL was relatively low compared with the mean of 3.6 ng/mL found in a study of serum samples from accident victims in Mexico (Waliszewski 2004).

Educational Level Achieved

As noted for educational level achieved, the overall trend was for decreased serum concentration of pesticides with increased education educational level achieved, although most pair wise differences across the education categories were not statistically significant. Lipid adjustment did not change the overall trends. Regression analysis showed in general that when compared with the referent age group, increasing levels of education achieved was inversely related to the pesticide levels. This generalization did not hold true for hexachlorobenzene and total hexachlorocyclohexane lipid-adjusted concentrations, where it appeared that increasing levels of education were associated with higher concentrations of the pesticides when compared with the referent age group.

Other Exposure Variables and Pesticide Concentrations

Eating fish caught by you or someone you know from a pier or a boat increased the concentrations of total DDT, total heptachlor, and total hexachlorocyclohexane in adults.

However, for the "eating fish" variable, the $p<0.05$ significance level was reached only with total hexachlorocyclohexane, and only with the lipid-adjusted concentration (serum concentration $p=0.06$). For the children, eating the fish did not lead to an increase in total hexachlorocyclohexane, but the serum concentration of total heptachlor did have a p-value equal to 0.05 (lipid-adjusted concentration $p=0.13$). Curiously, it may be that the group of children who ate these fish for some reason had higher lipids than the group that did not eat these fish. Again, the results between the adults and children were inconsistent, making conclusions difficult.

Health Outcome Variables and Pesticide Concentration

The symptom and diagnosis variables were also analyzed by mean serum and lipid-adjusted pesticide concentrations among adults and children. For adult participants, the mean serum and lipid-adjusted concentration of all pesticides was higher for persons with hypertension than the means for participants who did not have hypertension. The odds ratios were all above 1.00, except for lipid-adjusted total DDT and total heptachlor, which both had odds ratios of 1.00. For the hypertension variable, the only pesticide that met the criteria of odds ratio greater than 1.00, with a confidence interval that did not cross 1.00, and a $p<0.05$ for the odds ratio was serum hexachlorobenzene (OR=1.37, 95% CI=1.03–1.83, $p=0.03$). However, this association was not statistically significant when the concentration was adjusted for lipids (OR=1.23, 95% CI=0.90–1.67, $p=0.19$). This was one of the few instances when lipid-adjustment changed the outcome from what

appeared to be a statistically significant association to one that was not statistically significant. There is scant literature regarding organochlorine pesticides and hypertension, although one study 26 years ago found an association between high serum pesticide organochlorine levels and the subsequent appearance of hypertension (Morgan et al. 1980).

For "ENT or sinus/nasal condition," the mean serum and lipid-adjusted concentration of all pesticides except total heptachlor were higher for persons with this condition than the means for participants without "ENT or sinus/nasal condition." The confidence intervals of all odds ratios crossed 1.0 except for serum total chlordane concentration with an odds ratio of 2.30 (95% CI=1.10–4.79, p=0.03) and lipid-adjusted total chlordane with an odds ratio of 1.63 (95% CI=1.06–2.50, p=0.03). This showed an association between "ENT or sinus/nasal condition" and elevated chlordane levels in this study group. There is some literature to support this finding, such as and chronic respiratory disease (OR=1.34; 95% CI=1.00–1.81) symptoms in farmers using pesticides (Faria et al. 2005) Although the finding for the other pesticides did not reach the level of statistical significance, there does appear to be a pattern that would benefit from further investigation.

For adults with asthma, the mean serum and lipid-adjusted concentrations of all pesticides except hexachlorobenzene were lower than for adults who did not have asthma. The child participants showed a similar pattern, except that all pesticides showed a decrease in serum and lipid-adjusted concentrations. Again, even though these findings did not reach the level of statistical significance, there appears to be a pattern that would

benefit from further investigation. This finding contradicts much of the literature, such as the finding of higher odds ratios for both asthma (OR=1.51; 95% CI= 1.07–2.14) symptoms in farmers using pesticides (Faria et al. 2005), prenatal DDE exposure associated with asthma (Sunyer et al. 2005), increased self-reported asthma in pesticide applicators (Beard et al. 2003), and exposure to DDE resulting in a significantly higher odds ratio for asthma (odds ratio = 3.71; 95% CI=1.10–12.6) (Karmaus 2001).

In children with low hematocrit, the mean serum and lipid-adjusted concentrations of aldrin/dieldrin and total chlordane were substantially increased, while total DDT and total hexachlorocyclohexane showed small increases compared to participants who did not have low hematocrit. For low hematocrit, elevated aldrin/dieldrin serum concentration showed an odds ratio of 1.56 (95% CI=1.08–2.27, p=0.02). For lipid-adjusted aldrin/dieldrin concentration, the odds ratio was 1.74 (95% CI=1.10–2.74, p=0.02). For serum concentration of total chlordane, the odds ratio was 1.87, however, the 95% CI crossed 1.0. For the lipid-adjusted concentration of total chlordane, the odds ratio was 1.76 (95% CI=1.10–2.79, p=0.02). Therefore, among the children in this study, there was an association between low hematocrit and elevated aldrin/dieldrin serum and lipid-adjusted concentrations, and also with lipid-adjusted total chlordane. The association with aldrin/dieldrin is consistent with the literature as this organochlorine pesticide has been found to cause hemolytic anemia (ATSDR 2002), (Hamilton et al. 1978).

Although the primary target for organochlorine pesticides is the neurologic system, no significant associations were seen between neurologic symptoms such as frequent

numbness, pins and needles, or diagnoses such as headaches or diagnoses related to the central or peripheral nervous systems, and increases in the organochlorine pesticides evaluated. In some cases, the mean concentration of a particular pesticide was higher for participants with these symptoms than in participants without, but none of these associations were statistically significant.

Another target of organochlorine pesticides is the liver. Although mean lipid-adjusted concentrations of four of the six pesticides were higher for participants with elevated liver enzymes, none of the associations was significant. For example, in those participants with elevated liver enzymes, the mean lipid-adjusted concentration of aldrin/dieldrin was 12% higher. The odds ratio was 1.20, but the 95% CI crossed 1.00 and the associated p-value was 0.48.

Study Strengths

Some strengths of this study are that it was a large study with both adult and children participants who had comprehensive medical evaluations and serum pesticides measured. The serum and pesticides were measured by the CDC toxicology laboratory, which is a nationally recognized, high-quality toxicology laboratory. Additionally, neighborhood environmental assessments were performed by the State DHS and EPA and were used in developing the questionnaires that gathered information about the possible routes of exposure and health effects. Also, the study used extensive analyses that examined a

large range of demographic and neighborhood predictor variables and health outcome variables.

Study Limitations

Some limitations of the study are that participation in the Del Amo/Montrose Community Environmental Health Program was voluntary, so the population studied was not necessarily representative of the general population. However, analysis of correlations between serum and lipid-adjusted serum pesticide concentrations should not be biased as these comparisons were based on two measures in the same individuals. An additional limitation is that the questionnaires were limited in scope to the primary routes of exposure for DDT within the Del Amo/Montrose community, so there was little information on other potential sources and routes of exposures to the various pesticides. The questionnaire was designed to assess potential exposures to DDT in the specific neighborhood. Therefore, it was not possible to assess other sources of exposure outside of the neighborhood other than working on a farm, eating fish, or having lived in Mexico or Latin America. It is possible that a more extensive environmental exposure questionnaire might have identified other potential sources of exposures to the pesticides. Another limitation is that the Del Amo/Montrose Environmental Health Program did not conduct environmental monitoring of participants' homes, so there was no objective data on exposures for the specific participants.

Assessment of health outcomes was also limited because the data were collected as part of clinical evaluations, rather than as part of a research protocol. The identification of health outcomes was based on project nurses abstracting information from the clinical charts. The clinical evaluation protocol was not highly standardized, so the number of medical tests and the amount of information recorded in the chart could vary. In general, studies based on secondary analysis of existing data that was not collected specifically for a research study are limited by the quality of the original data and data recording. Another limitation is that the demographic and neighborhood exposure variables and health outcome variables were all coded as categorical or dichotomous variables. This approach simplified the analysis and interpretation of the patterns of associations with the serum pesticide concentrations. Furthermore, this approach was used to make the structure of the variables, and thus the analytical approach, consistent across the variables. However, reducing continuous variables, such as age or amount of time per week spent in the garden, to categorical variables loses information, resulting in some non-differential misclassification bias. This type of bias would bias apparent associations towards the "null" of no association and reduce statistical power to observe statistically significant findings.

Chapter 5

CONCLUSIONS

Among these participants in the Del Amo/Montrose Community Environmental Health Program, adjusting for serum lipids did not substantially change the outcomes of the analysis of demographic, neighborhood, or exposure variables, or the analysis of health outcomes. Lipid adjustment tended to decrease differences observed for age and educational level, but altered the outcome of analysis in only a few instances. Lipid-adjustment tended to increase the relative concentration of pesticides in the youngest versus older age groups. Interestingly, a few associations were only seen with lipid adjustments, while others were only seen using the serum concentration (wet-weight). For the variable "eating fish from a pier or boat," a statistically significant association was seen in adults with lipid-adjusted total hexachlorocyclohexane concentration, and not with serum concentration. Conversely, with the children, a significant association was seen between eating the fish and total serum heptachlor concentration, but it was not seen with the lipid-adjusted concentration.

The youngest age category (0-9 years) was found to have higher aldrin/dieldrin and heptachlor concentrations than most of the adult categories. The 0-9 year old age group was found to have higher concentrations of all pesticides than the 10-17 year old age

group. This is likely due to transfer of the organochlorine pesticides through the placenta and/or breast milk.

Having lived more than a year in Mexico or Latin America was associated with increased concentrations of DDT, hexachlorobenzene, and total hexachlorocyclohexane. Another exposure source that showed a statistically significant association was eating eggs from neighborhood chickens and increased total chlordane concentrations. Weaker associations were also seen between eating eggs and higher aldrin/dieldrin and total DDT concentrations. Eating fish caught on a pier or boat was significantly associated in adults with the lipid-adjusted concentration of total hexachlorocyclohexane, while a significant association in children was only seen with the serum concentration of total heptachlor. Working on a farm and eating fruit or vegetables grown in neighborhood gardens were associated with increased total hexachlorocyclohexane concentration. There appeared to be an association between hypertension and increased pesticide concentrations, most notably with hexachlorobenzene concentration. An association between ENT or sinus condition and elevated chlordane levels was found, in addition to an apparent association with other pesticides. Oddly, both children and adults appeared to show an inverse relationship between pesticide concentrations and asthma, although statistical significance was not achieved. Children showed an association between low hematocrit and elevated aldrin/dieldrin and chlordane concentrations.

Summarizing the data differently, demographic, exposure, or health outcome variables associated with increased pesticide levels were:

Aldrin/dieldrin

- youngest age group
- low hematocrit in children

Total Chlordane

- eating eggs from neighborhood chickens
- ENT or sinus condition
- low hematocrit in children

Total DDT

- 45-64 and 65+ age groups compared with the reference group (18-31 years)
- lived more than a year in Mexico or Latin America

Total Heptachlor

- oldest age group
- eating fish caught by you or someone you know from a pier or boat, children only ($p=0.05$)

Hexachlorobenzene

- 45-64 and 65+ age groups compared with the reference group (18-31 years)
- lived more than a year in Mexico or Latin America
- neighborhood egg consumption in children ($p=0.05$)
- hypertension in adults

Total Hexachlorocyclohexane

- 45-64 and 65+ age groups compared with the reference group (18-31 years)

- lived more than a year in Mexico or Latin America
- activities in the garden or yard that involved contact with the soil
- eating fish caught by you or someone you know from a pier or boat (lipid-adjusted only)

Although this data was collected due to DDT production and contamination at the site of the Montrose Chemical Plant, the only exposure variable other than having lived more than a year in Mexico or Latin America that was associated with DDT concentration was eating eggs from neighborhood chickens. Hexachlorobenzene was also present at the Montrose site, but the only exposure variable associated with elevated hexachlorobenzene concentration was having lived more than a year in Mexico or Latin America. Hypertension was the only health outcome variable associated with elevated hexachlorobenzene concentrations. Unexpectedly, no significant associations were found between neurological conditions or liver abnormalities and elevated organochlorine pesticide concentrations. These findings suggest future directions for research into organochlorine pesticide exposures and related health outcomes.

REFERENCES

Akins, JR, Waldrep, K, & Bernert JT, J. (1989). The estimation of total serum lipids by a completely enzymatic 'summation' method. Clinica Chimica Acta, 184(3), 219-26.

ATSDR (2002). Toxicological Profile for Aldrin/Dieldrin, U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry.

ATSDR (2002). Toxicological Profile for Alpha-, Beta-, Gamma-, and Delta Hexachlorobenzene, U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry.

ATSDR (1994). Toxicological Profile for Chlordane, U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry.

ATSDR (2002). Toxicological Profile for DDT, DDE, and DDD, U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry.

ATSDR (2005). Toxicological Profile for Heptachlor/Heptachlor Epoxide Draft, U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry.

ATSDR (2005). Toxicological Profile for Hexachlorocyclohexane, U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry.

Baker, D and Yang, H (1999). The Del Amo/Montrose Community Environmental Health Program, Center for Occupational and Environmental Health, University of California, Irvine.

Baker, D, Luderer U, Yang H, et al. (2004). Latent Effects of Gestational Exposure to Heptachlor. Epidemiology, 15(4), S119-.

Beard, J, Sladden, T, Morgan, G, et al. (2003). Health impacts of pesticide exposure in a cohort of outdoor workers. Environmental Health Perspectives, 111(5), 724-30.

Besbelli, N (2001). Hexachlorocyclobenzene (Mixed Isomers), Poisons Information Monograph 257, International Programme on Chemical Safety.

Brown JF, J, & Lawton, RW. (1984). Polychlorinated biphenyl (PCB) partitioning between adipose tissue and serum. Bulletin of Environmental Contamination and Toxicology, 33(3), 277-80.

Cassidy, RA, Natarajan, S, & Vaughan, GM. (2005). The link between the insecticide heptachlor epoxide, estradiol, and breast cancer. Breast Cancer Research and Treatment, 90(1), 55-64.

CDC (2005). Third National Report on Human Exposure to Environmental Chemicals, National Center for Environmental Health, Division of Laboratory Sciences, Department of Health and Human Services, Centers for Disease Control and Prevention.

CDHS (2004). Del Amo Superfund Site Near Torrance, Los Angeles County, California, Public Health Assessment, Californian Department of Health Services.

Chao, HR, Wang, SL, Lin, TC, et al. (2005). Levels of organochlorine pesticides in human milk from central Taiwan. Chemosphere (In Press).

Durgnat, JM, Heuser, J, Andrey, D, et al. (2005). Quality and safety assessment of ginseng extracts by determination of the contents of pesticides and metals. Food Additives and Contaminants, 22(12), 1224-30.

Faria, NM, Facchini, LA, Fassa, AG, et al. (2005). Pesticides and respiratory symptoms among farmers. 39(6), 973-81.

Fleming, L, Mann, JB, Bean, J, et al. (1994). Parkinson's disease and brain levels of organochlorine pesticides. Annals of Neurology, 36(1), 100-3.

Guo, YL, Emmett, EA, Pellizzari, ED, et al. (1987). Influence of serum cholesterol and albumin on partitioning of PCB congeners between human serum and adipose tissue. Toxicology and Applied Pharmacology, 87(1), 48-56.

Gaffney, SH, Curriero, FC, Strickland, PT, et al. (2005). Influence of geographic location in modeling blood pesticide levels in a community surrounding a U.S. Environmental protection agency superfund site. Environmental Health Perspectives, 113(12), 1712-6.

Hamilton, HE, Morgan, DP, & Simmons, A. (1978). A pesticide (dieldrin)-induced immunohemolytic anemia. Environmental Research, 17(2), 155-64.

Hennig, B, Reiterer, G, Toborek, M, et al. (2005). Dietary fat interacts with PCBs to induce changes in lipid metabolism in mice deficient in low-density lipoprotein receptor. Environmental Health Perspectives, 113(1), 83-7.

Hoyer, AP, Gerdes, AM, Jorgensen, T, et al. (2002). Organochlorines, p53 mutations in relation to breast cancer risk and survival. A Danish cohort-nested case-controls study. Breast Cancer Research and Treatment, 71(1), 59-65.

Hoyer, AP, Jorgensen, T, Brock, JW, et al. (2000). Organochlorine exposure and breast cancer survival. Journal of Clinical Epidemiology, 53(3), 323-30.

Jung, D, Becher, H, Edler, L, et al. (1997). Elimination of beta-hexachlorocyclohexane in occupationally exposed persons. Journal of Toxicology and Environmental Health, 51(1), 23-34.

Kamel, F, & Hoppin, JA. (2004). Association of pesticide exposure with neurologic dysfunction and disease. Environmental Health Perspectives, 112(9), 950-8.

Klaassen, Curtis D, Casarett & Doull's Toxicology The Basic Science of Poisons, 6th ed., New York, The McGraw-Hill Companies, Inc., 2001.

Karmaus, W. (2001). Infections and atopic disorders in childhood and organochlorine exposure. Archives of Environmental Health, 56(6), 485-.

Le Couteur, DG, McLean, AJ, Taylor, MC, et al. (1999). Pesticides and Parkinson's disease. Biomedicine & Pharmacotherapy, 53(3), 122-30.

Li, AA, Mink, PJ, McIntosh, LJ, et al. (2005). Evaluation of epidemiologic and animal data associating pesticides with Parkinson's disease. Journal of Occupational and Environmental Medicine, 47(10), 1059-87.

Maskarinec, G. (2005). Mortality and cancer incidence among children and adolescents in Hawaii 20 years after a heptachlor contamination episode. Journal of Environmental Pathology, Toxicology, and Oncology, 24(4), 235-49.

Morgan, DP, Lin, LI, & Saikaly, HH. (1980). Morbidity and mortality in workers occupationally exposed to pesticides. Archives of Environmental Contamination and Toxicology, 9(3), 349-82.

OSHA (2006). Sampling and Analytic Methods; Chlordane

Phillips, DL, Pirkle, JL, Burse, VW, et al. (1989). Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. Archives of Environmental Contamination and Toxicology, 18(4), 495-500.

Schisterman, EF, Whitcomb, BW, Louis, GM, et al. (2005). Lipid adjustment in the analysis of environmental contaminants and human health risks. Environmental Health Perspectives, 113(7), 853-7.

Seth, V, Ahmad, RS, Suke, SG, et al. (2005). Lindane-induced immunological alterations in human poisoning cases. Clinical Biochemistry, 38(7), 678-80.

Schimmel, SC, Patrick JM, J, & Forester, J. (1976). Heptachlor: toxicity to and uptake by several estuarine organisms. Journal of Toxicology and Environmental Health, 1(6), 955-65.

Shank, Ronald, Environmental Toxicology Lecture 16, University of California, Irvine, 2004.

Sunyer, J, Torrent, M, Munoz Ortiz, L, et al. (2005). Prenatal dichlorodiphenyldichloroethylene (DDE) and asthma in children. Environmental Health Perspectives, 113(12), 1787-90.

USCB (2000). Educational Attainment in the United States. Current Population Survey 2000. United States Census Bureau.

van Birgelen, AP. (1998). Hexachlorobenzene as a possible major contributor to the dioxin activity of human milk. Environmental Health Perspectives, 106(11),683-8.

Venners, SA, Korrick, S, Xu, X, et al. (2005). Preconception serum DDT and pregnancy loss: a prospective study using a biomarker of pregnancy. American Journal of Epidemiology, 162(8), 709-16.

Waliszewski, S. (2004). Organochlorine pesticide levels in blood serum samples taken at autopsy from auto accident victims in Veracruz, Mexico. Archives of Environmental Health, 59(9), 441-8.