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DISSERTATION

ORGANOCHLORINE PESTICIDES AND REPRODUCTIVE ENDPOINTS IN HISPANIC WOMEN

Submitted by

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In partial fulfillment of the requirements

for the Degree of Doctor of Philosophy

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY JUDY E. AKKINA ENTITLED ORGANOCHLORINE PESTICIDES AND REPRODUCTIVE ENDPOINTS IN HISPANIC WOMEN BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

ORGANOCHLORINE PESTICIDES AND REPRODUCTIVE ENDPOINTS

IN HISPANIC WOMEN

Organochlorine pesticides (OCPs) and their persistent metabolites are ubiquitous environmental contaminants which bioaccumulate in adipose tissue and are thought to have potential endocrine disrupting effects. This cross-sectional epidemiologic study utilized an existing national data base, the Hispanic Health and Nutrition Examination Survey (HHANES), conducted in 1982-1984, to investigate associations between exposure to seven OCPs/metabolites, ppDDT, DDE, beta-hexachlorocyclohexane (beta-HCH), hexachlorobenzene (HCB), dieldrin, oxychlordane and *trans*-nonachlor, and the female reproductive endpoints spontaneous abortion, stillbirth, low birth weight (< 2500 gm.), birth defects, age at menarche and age at menopause. Farm work was also examined as an exposure variable. Factors associated with detection of the OCPs/metabolites in serum were investigated.

The study sample included 1,599 Hispanic women 12-74 years of age who resided in three regions of the U.S., the southwest (Mexican American), Dade County, Florida (Cuban), and New York City area (Puerto Rican). Concentrations of the OCPs/metabolites were measured in serum. A reproductive history was collected via interview. Demographic and potential confounding variables evaluated were: age and season at the time serum sample was drawn, Hispanic group, serum cholesterol, education, income, country of birth, smoking alcohol consumption, living outside a central city area, Body Mass Index (BMI), parity, oral contraceptive use and diabetes.

A history of spontaneous abortion, low birth weight, having a child with a birth defect and age at menarche were not associated with detection in serum of any of the OCPs/metabolites examined. Logistic regression analysis showed a moderately elevated risk for stillbirth among women who had detectable serum dieldrin; however, the risk estimate was imprecise (adjusted OR=2.24; 95% CI 0.79-6.35). The adjusted risk estimates for stillbirth and low birth weight were elevated among women who had ever done farm work. These effects were modified by parity. The odds ratios for stillbirth and low birth weight and ever having done farm work among women with parity < 3.0 were 3.80 (95% CI 1.34-11.54) and 2.67 (95% CI 1.46-4.86) respectively, and for women with parity > 3.0 were 1.18 (95% CI 0.59-2.93) and 1.47 (95% CI 0.83-2.59), respectively. Ever having done farm work was also associated with ever having a child with a muscle, bone or joint defect (adjusted OR=2.08; 95% CI 0.63-6.83) or a mouth defect (adjusted OR=3.12; 95% CI 0.81-11.97). The sample size for specific types of birth defects was small; therefore, these risk estimates are imprecise.

Exposure to ppDDT, beta-HCH, and *trans*-nonachlor was associated with earlier age at menopause in a dose dependent manner. Analysis of variance showed a significant decrease in mean age at menopause for women in the highest exposure category for ppDDT, beta-HCH and *trans*-nonachlor. Women with serum DDT level > 6.00 parts per billion (ppb), beta-HCH > 4.0 ppb, or *trans*-nonachlor $\geq$ 2.00 ppb, had an adjusted mean age at menopause on average 5.65, 3.35, and 5.18 years earlier, respectively, than women with serum levels of these pesticides below the detection limit. Significant two-way

interactions among the three significant OCPs were found and appeared to represent additive effects. Serum levels of dieldrin, hexachlorobenzene, and oxychlordane, or ever having done farm work, were not associated with age at menopause. Alteration of the timing of menopause may be of clinical importance because of the relationship of menopause to chronic conditions such as osteoporosis and cardiovascular disease.

There are important limitations of this study which necessitate viewing the study findings conservatively. Since one cannot be certain in a cross-sectional study which has occurred first, the exposure or the outcome, the possibility that higher levels of serum OCPs may be a consequence of early menopause must be considered. Lactation is an important pathway for elimination of OCPs from the body, and lack of information on lactation history may have lead to significant exposure missclassification. Women who lactated may have had lower levels of OCPs at the time of exposure measurement than at the time of the adverse reproductive events which occurred prior to lactation, driving the effect estimate downward. OCPs are long lived in adipose tissue, adipose levels may range from 200-400 times greater than whole weight serum levels, and exposure for many OCPs is considered to be continual, but the temporal stability of OCP serum levels over many years is unknown; therefore, misclassification of exposure status may have occurred due to the varying length of time between the reproductive events and measurement of exposure.

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DEDICATION

To my family, for their love and support, and to all of the teachers who have contributed to my education and inspired me to keep learning.

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ABREVIATIONS

ADL	Above the Detection Limit
AOV	Analysis of Variance
BDL	Below the Detection Limit
beta-HCH	Beta-Hexachlorocyclohexane
BMI	Body Mass Index
CI	Confidence Interval
DDE	1,1-dichloro-2,2-di(4-chlorophenyl)ethylene
DDT	1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane
HAA	Hormonally Active Agent
HCB	Hexachlorobenzene
HHANES	Hispanic Health and Nutrition Examination Survey
OCP	Organochlorine Pesticide
OR	Odds Ratio
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion

CHAPTER I

INTRODUCTION

Significance of Research

There is significant concern among the scientific community and the general public that environmental contaminants may interfere with the normal functioning of the endocrine system in both humans and animals (Colborn et al., 1996). Exogenous chemical agents capable of interfering with the synthesis, storage/release, transport, metabolism, binding, action or elimination of natural blood-borne hormones responsible for the regulation of homeostasis, reproduction, development, and/or behavior are termed "endocrine disruptors" (EDs) (Cooper and Kavlock, 1997) or "hormonally active agents" (HAAs) (NRC, 1999). There are a variety of chemical agents in the environment identified in the literature as possessing endocrine disrupting ability. These include natural compounds such as phytoestrogens, as well as synthetic compounds such as organochlorine pesticides,

polychlorinated biphenyl (PCBs) compounds, and monomers and additives used in the plastics industry (eg., phthalates, bisphenol A). HAAs may potentially affect multiple physiologic systems, including the reproductive, neurologic, and immunologic systems (NRC, 1999). HAAs have also been associated with carcinogenic effects (NRC, 1999).

The purpose of this epidemiologic research study is to investigate the possible female reproductive effects of exposure to an important group of "hormonally active agents", the organochlorine pesticides and their persistent metabolites.

Organochlorine Pesticides

Many organochlorine pesticides (OCPs), such as DDT, chlordane, dieldrin and hexachlorobenzene, came into widespread use in the 1940's and 1950's. Today OCPs, along with many other organochlorine compounds such as PCBs, are considered persistent organic pollutants (POPs), due to their resistance to photolytic, biological and chemical degradation (POP Assessment Report, 1995). They are highly lipophilic and therefore bioaccumulate in fatty tissues and biomagnify through the food chain, leading to greater exposures in organisms at the top of the food chain. Another physio-chemical property of OCPs is their semi-volatility, which allows them to move long distances in the atmosphere before deposition occurs (POP Assessment Report, 1995). These various properties of OCPs allowed them to become ubiquitous in the environment, even in places where they were never actually used. Once the undesirable properties of OCPs were fully understood and their potential adverse effects on animals, humans and ecologic systems were

recognized, their use was no longer permitted in many industrialized countries. Monitoring studies of environmental media, wildlife and human tissues, continue to demonstrate the presence of these persistent pollutants, although the levels are declining in industrialized countries (ATSDR, 1991, 1992a, 1992b, 1992c, 1994).

Organochlorine Pesticides and Reproductive Endpoints

The reproductive process is highly complex and orchestrated primarily by the neuro-endocrine system and the gonads (Knobil and Neill, 1988). The multiple steps involved in successful reproduction include the biological processes involved in both male and female fecundity and fertility leading to conception and the biological processes in both the fetus and mother that result in appropriate growth and development of the fetus and a successful delivery. Disruption at any of these steps could lead to varied and multiple effects (Sever, 1988; Savitz and Harlow, 1991). Every component of the reproductive process is susceptible to some exogenous agent, and exposure to multiple toxic agents often Occurs. The extent and type of effect also depends on the timing and extent of the exposure. Therefore, multiple endpoints should be considered in any investigation of the effects of toxic exposure on reproduction.

Animal studies have demonstrated various reproductive effects of OCPs, including reduced fertility, spontaneous abortion, perinatal death, low birth weight, and congenital malformations (ATSDR, 1991, 1992a, 1992b, 1992c, 1994). Although the dose administered in animal studies is generally much higher than apparent human exposure, a

few studies have indicated reproductive system effects at dose levels (low ppm) approximating human exposure levels (Tiemann et al., 1998; Crellin et al., 1999). The effects of chronic, low dose exposure to OCPs, as occurs in humans, has not been well studied in animals.

The human epidemiologic literature on the association between exposure to specific OCPs and reproductive endpoints is limited. A few studies have suggested associations between specific OCPs and decreased fertility, spontaneous abortion, preterm birth, and low birth weight (Saxena et al., 1980; O'Leary et al., 1970a; O'Leary et al., 1970c; Gerhard et al., 1999). Many of these studies were small, often did not control for confounders, and produced inconsistent results. Other studies have suggested associations between occupation in agriculture and stillbirth, low birth weight, and congenital malformations (Arbuckle and Sever, 1998; Engle et al., 1995; Garcia, 1998). This increased risk for adverse reproductive outcomes in agricultural workers is thought to be due in part, to their higher risk of exposure to pesticides compared to the general population.

The accumulated evidence in the literature suggests that some segments of the human population may be exposed to levels of OCPs which may lead to adverse reproductive effects. Epidemiologic studies are needed which incorporate the following into the design: a focus on specific OCPs, adequate exposure or body burden determination at the individual level, the ability to determine exposure to mixtures of OCPs and to evaluate potential interactions, a sufficient sample size to investigate rare reproductive endpoints,

and the ability to control for potential confounders. In addition, age at menarche and age at menopause are important reproductive health parameters which have been neglected in human epidemiologic studies of reproductive effects of OCPs to date.

Hispanic Health and Nutrition Examination Survey (HHANES)

The Hispanic Health and Nutrition Examination Survey (HHANES) was conducted in 1982-1984 by the National Center for Health Statistics (NCHS, 1985). It is a cross-sectional, population based survey of the three largest subgroups of Hispanics (Mexican American, Cuban and Puerto Rican) living in the continental United States with a total sample size of 11,653 men, women, and children. Data collection included an interview in which information on medical history, reproductive history, socio-demographic characteristics, and diet history was obtained. Blood samples were obtained, and serum levels of many persistent OCPs and their major metabolites were determined on a random half sample of the entire HHANES sample. Data from the HHANES has been used extensively to examine the health status and health care needs of Hispanic Americans and a supplement to the American Journal of Public Health (1990;vol.80) was devoted to findings from the HHANES (eg. Guendelman et al., 1990; Marks et al., 1990; Higginbotham JC et al., 1990). The HHANES data base provides a unique opportunity to assess adverse reproductive endpoints potentially associated with women's body burden of multiple persistent OCPs and metabolites.

Hypothesis

This epidemiologic study evaluated the hypothesis that exposure to organochlorine pesticides or their persistent metabolites, is associated with adverse reproductive endpoints in women, including spontaneous abortion, stillbirth, low birth weight, and congenital malformations, and alters age at menarche and age at menopause.

Specific Aims of the Study

Utilizing the existing national HHANES data base described above, for female participants:

1. describe the frequency of detection and serum levels of the following OCPs: dieldrin, DDT, DDE, hexachlorobenzene, beta-hexachlorocyclohexane, oxychlordane and *trans*-nonachlor.
2. determine the socio-demographic and lifestyle factors associated with the detection of dieldrin, DDT, DDE, hexachlorobenzene, beta-hexachlorocyclohexane, oxychlordane and *trans*-nonachlor in serum, and if detection of one OCP of interest in serum is associated with detection of any of the other OCPs of interest in serum.
3. describe the prevalence of the following reproductive endpoints: spontaneous abortion, stillbirth, low birth weight, and congenital malformation. In addition, to describe the distribution of age at menarche and age at menopause in the sample.
4. evaluate the possible association between serum level of dieldrin, DDT, DDE, hexachlorobenzene, beta-hexachlorocyclohexane, oxychlordane, *trans*-nonachlor,

or a history of farm work, and the reproductive endpoints spontaneous abortion, stillbirth, low birth weight, congenital malformation, age at menarche, age at menopause.

CHAPTER II

LITERATURE REVIEW

This review includes an overview of the endocrine system and potential mechanisms by which hormonally active agents (HAAs) may interfere with its normal functioning. The HAAs of primary concern in this study are organochlorine pesticides and their persistent metabolites. The toxicology of five specific organochlorine pesticides (OCPs) is reviewed: chlordane, dieldrin, DDT, hexachlorobenzene and hexachlorocyclohexane. Environmental sources, routes of exposure, body burden, distribution, metabolism, and major health effects in humans for each OCP are reviewed. Finally, reproductive effects or endpoints which may result from the interference of HAAs on the human female reproductive system are discussed.

Overview of the Endocrine System

The endocrine system plays an important integrating and regulatory function in the organism (Hadley, 1988). It carries out this function through the activity of hormones, which act as chemical messengers and travel in the blood in very small concentrations and bind to specific cell sites called receptors in order to cause their effect. Hormones may travel in the blood in a free state or may be bound to a carrier protein. Hormones integrate and regulate development, growth, reproduction, metabolism and many other bodily functions. Hormones are secreted by ductless glands that secrete the hormone directly into the bloodstream and may have target tissues that are quite distant from the gland (Hadley, 1988).

There are three classes of hormones, steroids, peptides, and amines. Steroid hormones are derived from cholesterol, are not stored by cells, and are lipophilic. Steroid hormones are secreted by the gonads, adrenal cortex and the placenta. Examples of steroid hormones secreted by the gonads are estrogen and testosterone. Because of their lipophilicity, steroid hormones are able to pass through the target cell membrane and bind to a nuclear membrane receptor. This activated hormone-receptor complex then binds to DNA and activates specific genes, leading to protein synthesis. (Hadley, 1988)

Peptide hormones are short chains of amino acids and are hydrophilic (Hadley, 1988). Peptide hormones are secreted by the pituitary, parathyroid, heart, stomach, liver and kidneys. Amine hormones are derived from the amino acid tyrosine and are secreted by the

thyroid and the adrenal medulla. Peptide and amine hormones are stored by the producing cell until needed. These nonsteroid hormones do not enter the target cell but bind to cell membrane receptors and influence activity within the cell by transmitting a signal called an intracellular second messenger, to regulate ion channels or enzymes. (Hadley, 1988)

The endocrine system uses negative feedback and cyclicity to regulate hormone levels in the body (Hadley, 1988). For example, gonadal hormones are controlled through the interactions of multiple glands known as the Hypothalamic Pituitary Axis. The hypothalamus in the brain monitors the levels of circulating hormones and produces Gonadotropin Releasing Hormone (GnRH), which in turn stimulates the anterior pituitary gland in the brain to produce gonadotropins, which in turn stimulates the gonads to produce their hormones (Knobil and Neill, 1988). Examples of cyclicity in the endocrine system include the circadian rhythms evident in the secretion of many hormones, such as Growth Hormone, and the monthly cycle of hormones that regulate the menstrual cycle (Knobil and Neill, 1988).

The endocrine system can be adversely affected through multiple mechanisms (Crisp et al., 1998) and through both receptor dependent and receptor independent pathways (NRC, 1999). Alteration of hormone synthesis, storage, and release can affect the amount of hormone available. Alteration of hormone transport and clearance can also affect hormone availability. HAAs can displace endogenous hormones from human steroid-binding proteins, resulting in more unbound endogenous hormone, possibly increasing hormone

delivery to target cells. Interference with hormone receptor recognition and binding will alter hormonal activity. Altered hormone post-receptor activation can also lead to changes in hormonal activity.

A single exogenous chemical can have multiple effects on an organism and can act through multiple mechanisms (NRC, 1999). Various toxicologic mechanisms of action are associated with HAAs. The most well studied mechanism to date is the ability of many HAAs to alter steroid hormone receptor recognition and/or binding (Danzo, 1997). HAAs can act by mimicking the natural steroid hormone ligands and bind to the intracellular steroid receptors. Once bound, the agent may act as an agonist, or by inhibiting binding of the natural ligand, act as an antagonist. Some chemicals have the ability to bind to more than one type of intracellular receptor. Nuclear hormone receptors which have been implicated as targets of HAAs include receptors for estrogens, androgens, thyroid hormones, and adrenal glucocorticoids (NCR, 1999). Examples of OCPs which can bind to the estrogen receptor are DDT, methoxychlor, lindane and chlordecone (NCR, 1999). The fungicide vinclozolin and the DDT metabolite DDE are known to bind to the androgen receptor (Crisp et al., 1998). The extent of the biologic effect of the HAA is dependent upon the target cell type, the concentration of the HAA in the target cell, and the binding affinity of that HAA for the receptor (NCR, 1999).

HAAs may also act through mechanisms which do not involve steroid hormone receptors, though these mechanisms are less well studied (NCR, 1999). Evidence is accumulating

that HAAs may interfere with second messenger pathways which are important for the release and action of many peptide hormones (Crisp et al., 1998). Peptide hormone receptors are located on and in the cell membrane. After binding to their receptor, peptide hormones act through the transduction of a signal across the membrane. This type of cell signaling is mediated by the activation of second-messenger pathways. Examples of second messenger pathways are cAMP, phosphatidylinositol, 4,5 bisphosphate, inositol 1, 4,5-trisphosphate, tyrosine kinase, and Ca^{2+} ion flux. Interference with second messenger pathways can alter the serum levels of many hormones (Crisp et al., 1998). The pesticide dieldrin is reported to activate protein kinase C and decrease Ca^{2+} pump activity across cell membranes (Tomkins et al., 1991; Mehrotra et al., 1989). In addition, dieldrin is reported to alter the phosphatidylinositol signal transduction pathway in the fertilization process of *Bufo arenarum* oocytes (Fonovich de Schroeder and Pechen de D'Angelo, 1995). The organochlorine compound beta-hexachlorocyclohexane induces some estrogen receptor specific responses but does not interact with the estrogen receptor (Steinmetz et al., 1996). This suggests its action may be related to the modulation of signaling pathways, such as the activation of mitogen-activated protein kinase (MAPK), which increases the activity of the estrogen receptor (Cheek et al., 1998).

Toxicology of Organochlorine Pesticides

OCPs have been detected in all environmental media (air, soil, water), foods and biologic tissues, but the concentrations vary greatly (ATSDR 1991, 1992a, 1992b, 1992c 1994). The more volatile OCPs, such as the hexachlorocyclohexanes (HCH), move readily

through the atmosphere from warmer climates and are distilled out onto vegetation, soil, and water in colder climates. The distillation process Occurs to a much lesser degree for less volatile OCPs such as DDT. Therefore, the concentrations of DDT tend to reflect current and past local usage (NRC, 1999). Though use of many of these OCPs is no longer allowed in the United States, they continue to be found in the environment, in animals, and in humans, due to their chemical properties. Monitoring studies indicate their concentrations are declining, however (ATSDR 1991, 1992a, 1992b, 1992c 1994).

Routes of exposure for humans and animals for organochlorine compounds (OCPs) include inhalation, oral, and dermal, however dietary exposure and gastrointestinal absorption are considered the most important routes for vertebrates (NRC, 1999). The absorption of chemicals in the gut can be influenced by many factors including: the composition of the diet, the pH in the gut, the rate of digestion, the residence time of food, and the microflora composition in the gut. Once absorbed, OCPs enter the blood stream, which is the central medium of distribution to organs and cells in the body. OCPs may also be eliminated from the body through metabolism to a more polar compound that is excreted via the urine or other routes. They are also excreted in the milk of mammals (NRC, 1999).

Due to their lipophilic nature, OCPs are not in solution in the aqueous portion of blood, but form weak, nonspecific bonds with hydrophobic sites on blood proteins such as lipoproteins and albumin (Phillips et al., 1989; Gomez-Catalan et al., 1991). OCPs may

also form similar bonds with the cellular component of blood. This partitioning among blood components Occurs quickly and is determined by the lipid content of the various blood components and the lipid solubility of the OCP. Similarly, distribution from the blood to the tissues Occurs and eventually an equilibrium is reached among all tissues, generally according to the tissue lipid content and lipid solubility of the OCP. The concentrations of OCPs are generally much greater in tissues than in blood. Tissue to blood ratios vary by the specific OCP compound and by the specific tissue of interest, with adipose tissue having the highest ratio (NRC, 1999; Needham et al., 1990).

The equilibrium among tissues is a dynamic equilibrium, therefore a change in the lipid content of one tissue would alter the equilibrium. For example, a postprandial increase in serum lipids will proportionately affect serum OCP levels. Phillips et al. (1989) observed that a 20% increase in total human serum lipid levels led to similar size increases in serum PCB, HCB and p,p'-DDE concentrations. These authors also found that when serum OCP levels were expressed on a lipid weight basis, there were no longer significant differences between fasting and nonfasting serum samples.

Mohammed et al. (1990) investigated the distribution of DDT among lipoprotein fractions in human plasma and rat plasma. The authors reported that the highest uptake (52%) for DDT in human plasma was in the albumin-rich bottom fraction, with 15% in each of the Very Low Density Lipoprotein (VLDL) fraction and High Density Lipoprotein (HDL) fraction, and 18% in the Low Density Lipoprotein (LDL) fraction. The results were

different for rat plasma, indicating species-related differences. Noren et al. (1999) reported similar results for DDE and HCB, with 74% and 45% respectively, found in the albumin fraction of human plasma samples.

Gammon et al. (1997) investigated temporal variation of human serum PCBs, p,p'-DDE, and *trans*-nonachlor over a period of one to three months among noncancer patients. Their results indicate that temporal changes in OCP levels during this time period were minimal. Temporal variation in OCP serum levels can, however, be associated with weight change. Weight gain may result in lower serum levels as the OCP is redistributed in a larger body mass. Weight loss may result in higher serum OCP levels as lipolysis releases stored OCPs into the blood, although this phenomenon may be compound specific. In a study of the effects of fasting in mice loaded with o,p'-DDT and beta-HCH, Bigsby et al. (1997) reported that fat stores of beta-HCH, but not o,p'-DDT, were mobilized during fasting. The nutrient components of the diet may also affect OCP distribution and deposition. Tanaka et al. (1980) found that rats fed a high protein diet had a lower retention in organs and a higher biliary excretion of dieldrin than rats fed a low protein diet.

Chlordane

Technical chlordane is an OCP which contains a mixture of many chemicals, with the major components being *trans*-chlordane, *cis*-chlordane, beta-chlordene, heptachlor, and *trans*-nonachlor. Chlordane was used in the U.S. from 1948 to 1988. It was used on

agricultural crops, lawns, and gardens as a fumigating agent from 1948 to 1982, however from 1983 to 1988 its only approved use was to control termites in homes. In 1988, the use of chlordane as a termiticide was canceled by the Environmental Protection Agency and all legal use in the U.S. stopped. (ATSDR, 1992b)

Chlordane and many of its forms are POPs found in food, air, water, and soil. Due to bioaccumulation and biomagnification, they are also found in the fat of fish, birds, mammals, and most humans. Chlordane degrades very slowly and can remain in soils for over 20 years with greater persistence in heavy, clayey, or organic soil compared to sandy soils. The degradation products of chlordane in soil have not been reported. Contamination of water sources Occurs primarily through agricultural and urban run-off into lakes and streams, since chlordane migrates poorly through soil. Chlordane is hydrophobic and in water will attach to sediment and particles. The atmosphere is contaminated with chlordane through volatilization from soils and water, and wind erosion (ATSDR, 1992b).

Data from various monitoring studies conducted from 1969 to 1987 at both rural and urban settings across the U.S. indicate concentrations of chlordane in soils ranging from < 1 ppb to 141 ppm (ATSDR, 1992b). Compared to surface or ground water, the highest concentrations are found in sediments. Sediment concentrations of chlordane in the Great Lakes harbors range from 1.4 to 14 ppb (ATSDR, 1992b). Reported surface water concentration means range from 0.004 ppb to 0.15 ppb (ATSDR, 1992b). Chlordane is

not commonly found in groundwater, but has been detected in groundwater at National Priorities List hazardous waste sites and in New Jersey, Massachusetts, and Idaho. Concentrations of chlordane are higher in indoor air compared to urban or rural air, particularly in homes treated with chlordane for termite control. Reported mean chlordane concentrations ranged from not detectable to 2,200 ng/m^3 in indoor air, 38.4 ng/m^3 in urban air and 1.05 ng/m^3 in rural air (ATSDR, 1992b). There is a seasonal effect for indoor air concentrations, with higher levels in summer than in winter.

Exposure of humans to chlordane occurs primarily through living in chlordane treated houses and through occupational exposure (eg. applicator, manufacturer, farmer). Another source, although less important, is ingestion of contaminated food. Sim et al. (1998) reported a significant positive association between high oxychlordane level in human breast milk and living in housing treated for termite control in Australia. Other factors which were positively associated with high oxychlordane level in milk were age, body mass index, smoking, and home use of insecticides. Areas of the U.S. where homes were commonly treated for termites included the southeast and the southwest with moderate to heavy use extending from Pennsylvania and the lower New England states south and west to the lower portion of Colorado and up to northern California (ATSDR, 1992b). Chlordane vaporizes gradually in treated homes for over 10 years, accumulating in the body through inhalation and dermal contact. Using data from the Second National Health and Nutrition Examination Survey, Stehr-Green (1989) showed that individuals living in the southern or western U.S. had approximately twice the risk of having quantifiable

trans-nonachlor in their serum. Other factors positively associated with quantifiable serum *trans*-nonachlor were age, male gender, nonwhite race, living on a farm, and having serum sample drawn in winter or spring.

Estimates of exposure through food have been made using the Food and Drug Administration's (FDA) Total Diet Study. Results from the 1982-1984 Total Diet Study indicate total daily dietary intake of chlordane per unit body weight was 0.002-0.0027 micrograms/kg/day for teenagers and adults (ATSDR, 1992b). In a survey of rural residents, quantifiable levels of oxychlordane and *trans*-nonachlor were directly associated with average daily servings of eggs from home-raised hens and average daily servings of home-grown root vegetables (Stehr-Green et al., 1988).

Chlordane can enter the human body through the respiratory and gastrointestinal tracts and through dermal absorption. Initial distribution is to the liver and kidneys and then to fat. Due to the lipophilicity of chlordane, redistribution is primarily to fat stores, resulting in higher levels in fat and liver than in blood. Through oxidative metabolism involving hepatic microsomal cytochrome P-450, chlordane is metabolized to the relatively stable oxychlordane. Heptachlor epoxide is another metabolite of chlordane. The *trans*-nonachlor component of chlordane and the chlordane metabolite oxychlordane, are found in fat at higher levels than other chlordane compounds, reflecting their greater stability. Absorption and excretion of chlordane is primarily through passive diffusion.(ATSDR, 1992b)

Chlordane and/or its metabolites have been measured in blood, adipose tissue, brain, liver, kidney, milk, skin lipids, urine, feces and ovarian follicular fluid (ATSDR, 1992b; Jarrell et al., 1993). In a U.S. national population based survey conducted from 1976 to 1980, reported serum concentrations for oxychlordane and trans-nonachlor ranged from not detected to 23 ppb and 17 ppb, respectively. Jerrell et al. (1993), reported finding both *cis*-chlordane (alpha chlordane) and oxychlordane in the ovarian follicular fluid of women in three Canadian cities who were undergoing in-vitro fertilization. Mean follicular fluid concentrations in the three cities for both *cis*-chlordane and oxychlordane ranged from not detected to 0.30 ppb. Data from several studies of Occupational exposure demonstrates a correlation between higher levels of chlordane and/or metabolites in biological samples and higher levels of exposure (ATSDR, 1992b). Taguchi and Yakushiji (1988) reported a strong correlation between the length of exposure to atmospheric chlordane in a termite-treated home and the concentration of chlordane in human milk fat.

The acute toxic effects of exposure to chlordane involve primarily the liver and the neurologic and immunologic systems (ATSDR, 1992b). These effects will depend on dose and duration of exposure. A general mechanism of toxicity is the ability of *cis*- and *trans*-chlordane and their metabolites to bind irreversibly with cellular macromolecules such as protein, RNA, and DNA and therefore cause cellular death or altered function. In the liver, chlordane causes induction of hepatic cytochrome P-450 and other xenobiotic metabolizing enzymes. Neurologic signs of acute toxic exposure in both humans and animals include tremors and convulsions. The exact mechanism for neural toxicity is

unknown, however there are several hypotheses. Chlordane may competitively inhibit gama-aminobutyric acid (GABA), reducing the ability of GABA to inhibit post-synaptic neuronal excitability. Chlordane may also inhibit neural transmission by altering membrane permeability to Ca^{++}, restricting the release of norepinephrine. Acute toxicity of chlordane in rats reportedly increased by approximately 2.3-4.6 times when there was previous exposure to the OCPs aldrin, dieldrin, or DDT (ATSDR, 1992b). Feeding rats a protein deficient diet is also reported to increase chlordane toxicity (ATSDR, 1992b).

Chlordane induces liver cancer in mice, however, most genotoxicity tests for chlordane are negative. Chlordane is thought to act as a tumor promoter. The suggested mechanism for tumor promotion is its inhibition of gap junction intercellular communication, which normally functions to control proliferation of transformed or neoplastic cells. Alteration of gap junction intercellular communication may involve changes in cAMP-dependent protein kinase phosphorylation of hepatocellular gap junction proteins, leading to increased permeability at the gap junctions (ATSDR, 1992b).

Effects of chronic chlordane exposure have been suggested by several epidemiologic studies of humans living in chlordane treated homes or exposed to chlordane during its manufacture or use. These effects include blood dyscrasia, dermatitis, migraine headaches, unspecified skin neoplasms, and unspecified ovarian and uterine disease. Menconi et al. (1988) reported a significant dose-response relationship for the health conditions sinusitis, bronchitis and migraine with indoor level of chlordane. Individuals in

the highest category of exposure had approximately 2.5 to 3 times the risk for migraine, sinusitis, and bronchitis compared to individuals in the low exposure category.

Little information is available about the reproductive effects of chlordane in humans. In rats, the effect of oral exposure to chlordane on fertility was investigated (ATSDR, 1992b). Male and female rats fed chlordane at 16 mg/kg/day exhibited reduced fertility, with a reduction in the number of mated females that delivered litters. In addition, none of the litters survived to weaning. Investigators reported a reduction in the number of females that became pregnant when female mice were given intraperitoneal injections of chlordane (25 mg/kg) and then mated to untreated males (ATSDR, 1992b). Other investigators have reported that exposure to chlordane can affect metabolism and circulating levels of steroid hormones and reduce receptor binding of progesterone in the endometrium (ATSDR, 1992b). In male rats, oral chlordane at 19.5 mg/kg/day for 90 days increased androgen receptor sites in the ventral prostate (ATSDR, 1992b).

DDT and DDE

Technical DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane or dichlorodiphenyl trichlorethane) is an OCP which is a mixture of three forms, *p,p'*-DDT (85%), *o,p'*-DDT (15%), and *o,o'*-DDT (trace amounts) (ATSDR, 1992a). DDT was widely used around the world since the early 1940's to control insects on agricultural crops and to control insects which transmit diseases such as malaria and typhus. Agricultural use of DDT in the

U.S. was primarily for cotton, peanut and soybean crops. DDT is a POP and a suspected carcinogen, therefore the EPA banned all uses of DDT in the U.S. in 1972, except in cases of public health emergencies. In the U.S., monitoring of environmental media generally indicates that levels of DDT have been declining since the ban. DDT continues to be used however, in other countries around the world, providing a continual source of global contamination. (ATSDR, 1992a)

Due to the physical and chemical properties of DDT and its metabolites, they are found in all environmental media. Reports of the half life for DDT biodegradation in soil range from 2 to >15 years (ATSDR, 1992a). DDT binds strongly to soil, but loss of DDT in soil may occur through volatilization, plant absorption, water runoff, and chemical transformation. Slow conversion of DDT to DDE Occurs under aerobic conditions, however under anaerobic conditions, a more rapid conversion to DDD occurs. Data from 1,500 yearly soil samples evaluated in the U.S. National Soils Monitoring Program indicated average levels of p,p'-DDT were 0.18 ppm, 0.02 ppm and 0.13 ppm in 1970, 1972 and 1973 respectively (ATSDR, 1992a). Data in the STORET database on approximately 1,100 samples of sediments from water bodies during 1980-1983 indicate the median level for DDT was 0.1 ppb. Biota samples from selected sites during this time period had much higher levels of DDT, with a median level of 14 ppm. River sediment from a river in northern Alabama which was contaminated by waste water from a DDT manufacturing plant, had DDT levels ranging from 12 to 2,730 ppm.

DDT in the atmosphere is primarily particle bound and is removed from the atmosphere by precipitation or is degraded and transformed by photooxidation and reaction with photochemically produced hydroxyl radicals. The reaction process with hydroxyl radicals produces a half life of DDT in the atmosphere of approximately 2 days. Bidleman, et al. (1986) reported the average concentration of DDT and DDE in the air of Denver, CO in 1980 and Columbia, SC in 1977-1982. In Denver, the concentration of DDT and DDE was 0.043 ng/m^3 and 0.021 ng/m3, respectively. In Columbia, the concentrations of DDT and DDE were 0.048 ng/m^3 and 0.093 ng/m3, respectively. A report by Foreman and Bidleman (1990), indicates that in 1986 the average level of DDT in ambient air in Denver, CO had decreased to 0.018 ng/m^3, however the average DDE concentration had changed little.

There are numerous reports of DDT levels in specific bodies of water throughout the U.S. Data in the STORET database operated by EPA indicates that from 1980-1983, of 3,500 to 5,700 ambient water samples analyzed, 45% of the samples contained either DDT, DDE or DDD, with median concentrations for DDT and DDE of 1 ppt (ATSDR, 1992a). Levels of DDT and DDE in the ppb range, with maximum concentrations of 5 and 20 ppb respectively, were reported from groundwater in California (ATSDR, 1992a).

DDT and DDE in soil are taken up by plants, which are subsequently eaten by animals and people, resulting in bioaccumulation and biomagnification up the food chain. Results from Market basket surveys conducted from October 1980 to March 1982 found detectable

residues of DDT only in leafy vegetables (0.4 ppb) and root vegetables (0.6 ppb) (ATSDR, 1992a). DDE however, was detected in almost all food categories tested, with the exception of the grains, cereal, and beverages categories. The food categories with the highest DDE levels were root vegetables (4.6 ppb), meat, fish and poultry (3.0 ppb), and leafy vegetables (2.0 ppb). From these market basket surveys, the dietary intake of DDT and DDE in 1980 was estimated to be 2.4 micrograms/day per person and in 1981 was 2.2 micrograms/day (ATSDR, 1992a). Levels of DDT in fish have been monitored at 112 locations across the U.S. with the mean concentration of p,p'DDT decreasing from 0.05 to 0.03 ppb from 1976 to 1984 and the mean concentration of p,p'DDE decreasing from 0.26 to 0.19 ppb during the same period (ATSDR, 1992a).

Although DDT and its metabolites are ubiquitous in the environment, the most significant source for general human exposure is believed to be the diet. Exposure through drinking water is thought to be negligible because of the hydrophobic nature of these compounds and the efficiency of standard water treatment methods in eliminating them (ATSDR, 1992a). Exposure via inhalation is also considered negligible due to low atmospheric concentrations.

Once absorbed, DDT and its metabolites distribute to all body tissues. Their lipophilic character leads to storage in tissues in proportion to the organ tissue lipid content. Therefore, the highest concentration is found in adipose tissue. The distribution of oral DDT, DDE, and DDD was investigated in human volunteers (ATSDR, 1992a). The ratio

of concentration of DDT stored in adipose tissue to that present in blood was estimated to be 280:1. The results from this study also indicated that the affinity for storage in adipose tissue was related to the degree of lipophilicity of each compound, with p,p'-DDD being the most lipophilic and p,p'-DDE being the least lipophilic.

DDT is initially metabolized in the liver, where it is converted by different pathways to DDE and DDD. That portion converted to DDD is further degraded and excreted in the urine as 2,2-bis(p-chlorophenyl) acetic acid (DDA). The conversion of DDT to DDE Occurs more slowly than conversion to DDD. In the study of human volunteers mentioned above, less than 20% of DDT was converted to DDE over the course of the three year study. DDE however, undergoes little metabolism and therefore accumulates in the adipose tissue. Excretion of DDT and its metabolites is primarily through urine, and to a lesser extent through feces. DDT and its metabolites are also excreted in human milk, due to the high lipid content of human milk, resulting in significant exposure for breastfed infants.

Monitoring of DDT body burden in the U.S. from 1969 to 1975 was conducted using adipose tissue samples, through the National Human Monitoring Program for Pesticides (ATSDR, 1992a). Combining all age groups, the geometric mean lipid total DDT (including metabolites) levels reported for years 1970-1974 were 7.88, 7.95, 6.88, 5.89, and 5.02 ppm, respectively. The results indicate decreasing body burden over time, however the frequency of occurrence in tissue samples reportedly did not decline over this

time period. The authors reported that increasing age and black race were associated with higher body burdens. Serum levels of DDT and its metabolites were determined in the Second National Health and Nutrition Examination Survey conducted during 1976-1980 (Murphy and Harvey, 1985). DDT was detected in 31% of the samples, with a median quantifiable concentration in these samples of 3.3 ppb. DDE was detected in 99% of the samples, with a median quantifiable concentration of 11.8 ppb. Concentrations of DDT and its metabolites in human milk were determined in the U.S. National Human Milk Study conducted from 1969-1970 (ATSDR, 1992a). The p,p'-isomer of DDT and DDE were detected in 100% of samples, with mean concentrations of 0.19 and 1.9 ppm (lipid-basis), respectively.

Baukloh et al. (1985) reported the presence of DDT and DDE in the ovarian follicular fluid of 47 women in Austria and Germany enrolled in an *in vitro* fertilization and embryo transfer program. The mean concentration of total DDT (DDT + metabolites) in follicular fluid was approximately 5 ppb. Concentrations in the follicular fluid were in the same range as concentrations in serum. The level of DDE in follicular fluid has also been reported for 74 women in Canada undergoing *in vitro* fertilization (Jarrel et al, 1993). In this study, the mean concentration of DDE in follicular fluid was approximately 1 ppb and in serum was approximately 1.4 ppb. Schafer and Zahradnik (1998) examined endometrial tissue from 17 German women undergoing hysterectomy for uterine myoma and found a mean total DDT level of 5.4 ppb. Saxena et al. (1987) compared the level of DDT and DDE in leiomyomatous human uterine tissue to normal uterine tissue from women in

India. Significantly higher levels of total DDT were found in leiomyomatous tissue (mean = 0.845 ppm) versus normal tissue (mean = 0.103 ppm). DDT and DDE levels have been measured in placental tissue, cord blood, amniotic fluid, and vernix caseosa (O'Leary et al., 1970). DDE level was highest in the vernix caseosa of the newborn infants, followed in descending order by the placenta, cord blood and amniotic fluid.

Stehr-Green (1989) investigated demographic and seasonal influences on human serum pesticide residue levels using data from the NHANES II. Quantifiable p,p'-DDT levels were positively associated with age, nonwhite race, household income below the poverty level, living on a farm, residing in the southern or western U.S., and having provided the serum sample in the spring. Factors positively associated with increasing DDE level were similar except the season associated with higher serum levels was winter, and DDE level was associated with male gender. Laden et al. (1999) reported that the most reliable predictors of serum DDE level in a group of 240 women were age and serum cholesterol. In addition, women living in the western U.S. had higher DDE levels. In this study, serum DDE level could not be predicted from consumption of meat, fish, poultry, dairy products, vegetables, fruits, and grains. In contrast, Devoto et al. (1998) found consumption of beef and lamb to be positively correlated with serum level of total DDT in a group of 297 elderly people living in Germany. However, there was no significant difference in the total DDT concentration in serum between self-reported vegetarians and meat eaters.

The major health effects of exposure to DDT include neurotoxicity, hepatotoxicity,

metabolic effects, reproductive effects and cancer (ATSDR, 1992a). The mechanisms for neurotoxicity involve DDT's interference with the movement of ions through neuronal membranes. Specifically, DDT is reported to delay the closing of the sodium ion channel and prevent the full opening of the potassium gates, target a neuronal adenosine triphosphatase (ATPase) which controls the rate of sodium, potassium, and calcium fluxes through the nerve membrane, and inhibit the ability to transport calcium ions in nerves (ATSDR, 1992a). These mechanisms combine to maintain depolarization of the nerve membrane, leading to tremors and convulsions. These effects on membrane ion transport may also lead to changes in neurotransmitter release. A deficiency of brain serotonin and increases in serotonin and norepinephrine metabolites, aspartate, and glutamate have been reported in the brains of rats exposed to DDT. Experimental data in animals indicate that the doses at which neurological effects occurred were lower in the chronic studies than the acute studies (ATSDR, 1992a).

DDT and DDE affect the liver by inducing the production of liver enzymes, particularly the mixed function oxidase enzymes, including cytochrome P-450 microsomal enzymes. This may lead to altered metabolism of drugs, xenobiotics, and steroid hormones. Effects of subchronic and chronic oral exposure in animals are hepatic cell hypertrophy, histopathologic alterations, necrosis, and hyperplasia.(ATSDR, 1992a)

Although it has not been shown in human populations, animal studies indicate that DDT and its metabolites are probable or possible human carcinogens (ATSDR, 1992a). Liver

tumors, pulmonary adenomas and malignant lymphomas have been reported in mice. Liver tumors occurred at doses as low as 0.26 mg DDT/kg/day. A potential mechanism for tumor promotion is interference with gap junctional intercellular communication. Both Zhong-Xiang et al. (1986) and Aylsworth et al. (1989) reported that DDT inhibits gap junctional communication *in vitro*. Aysworth's experiments using chinese hamster V79 cells, also showed a synergistic effect of DDT with 12-0-tetradecanoylphorob-13-acetate (TPA) or oleic acid in inhibition of gap junctional communication.

Genotoxic effects have also been reported. Results of several studies of workers Occupationally exposed to DDT indicate increased chromosomal damage such as sister chromatid exchanges and chromosomal aberrations compared to controls (ATSDR, 1993a). Similar effects have been shown in *in-vitro* experiments.

Animal studies of DDT have shown a variety of reproductive effects including early onset of estrus, infertility, low birth weight, prematurity, and fetal and perinatal death (ATSDR, 1992a). Alm et al. (1998) exposed bovine oocytes *in vitro* to varying concentrations of DDT and found depression of oocyte maturation occurred in a dose-dependent manner, however there was no significant difference in fertilization rates between control and treated groups. On day 7 to 8 post fertilization, the number of morulae and blastocysts were significantly different between control and treated groups, indicating effects on further embryonic development.

Epidemiologic studies in humans have suggested reproductive effects. O'Leary et al. (1970a) reported higher fetal blood DDE levels in premature infants compared to controls. They did not find increased levels of DDE in women who experienced spontaneous abortion compared to women who had normal pregnancies (O'Leary et al. 1970c). Saxena et al. (1981) explored the relationship between maternal, fetal and placental DDE levels and spontaneous abortion and prematurity. Significantly higher mean DDE levels were found in women with spontaneous abortions and preterm births compared to controls with full-term births. Procianoy et al. (1981) studied 54 maternal-infant pairs, 30 with term births and 24 with pre-term births. He found that cord blood DDT correlated negatively with infants' birth weights. Other researchers have reported negative findings with regard to preterm birth and premature rupture of the membranes and serum DDT or DDE levels (Berkowitz et al., 1996; Ron et al., 1988). In a recent study of female fertility and chlorinated hydrocarbons, immunological infertility and lower conception rates were observed by Gerhard et al. (1999) among women with elevated serum DDT. Rogan et al. (1987) demonstrated an effect of serum DDE on length of lactation, with children of mothers with higher levels of DDE breast feeding for significantly shorter time periods.

A number of biologically plausible pathways for the effects of DDT and DDE on reproductive processes exist. DDT exhibits estrogenic properties in mammals such as binding to the uterine estrogen receptor, competitively inhibiting the binding of 17-beta-estradiol, increasing uterine weight and causing the induction of "induced protein" (Robison et al., 1984 and 1985). DDE is reported to have multiple endocrine effects

including acting as an estrogen, an anti-estrogen, an anti-androgen, an anti-progestin, and an anti-glucocorticoid, depending on the system studied (Crellin et al., 1999; Sohoni and Sumpter, 1998). As mentioned above, DDT stimulates hepatic microsomal enzymes and this could influence the biologic half-life of endogenous steroids involved in reproductive activity such as estrogen and progesterone. In addition, DDT's effects on cell membranes may lead to detrimental effects on cells in the reproductive system.

Fertility could be affected by alteration of follicular growth and hormone biosynthesis or by changing granulosa cell-oocyte interactions (Crellin et al., 1999). Tiemann et al. (1998) used cultured bovine oviductal cells to demonstrate a dose dependent increase in depolarization and decreased oxidative activity in the cells at dosages which were not cytotoxic. Tiemann has also reported that DDT inhibits the ATPase system of oviductal and endometrial membranes and inhibits the proliferation and steroid synthesis of cells from the female reproductive tract in *in vitro* systems. In a study using cultured porcine granulosa cells, DDE at 10 ppb affected granulosa cell response to protein kinase A activators by altering the expression of the P450 gene and thereby increased progesterone synthesis (Crellin et al., 1999).

Dieldrin

Dieldrin and aldrin are structurally similar OC insecticides (ATSDR, 1991). Both were used from the 1950's until the early 1970's on crops such as corn and cotton. The

U.S.D.A. canceled their use on crops in 1970. In 1972 they were approved as termiticides by the EPA and this use was canceled voluntarily by the manufacturer in 1987. Once aldrin enters the environment it rapidly changes to dieldrin. Aldrin is readily converted to dieldrin in the human body as well. Therefore, with respect to chronic human exposure, dieldrin is the compound of interest and will be the focus of this review.

Dieldrin is a POP which is still present at low levels in all environmental media, particularly soil and sediment. Similar to other POPs, dieldrin is hydrophobic, is taken up by plants from the soil and bioaccumulates up the food chain. General human exposure Occurs primarily through contaminated food, especially food products high in animal fat. Exposure from dieldrin treated homes is also significant. Dieldrin is present at many National Priority List (NPL) Hazardous Waste Sites (ATSDR, 1991).

Dieldrin can be slowly lost from soil through volatilization. Small amounts of dieldrin in the soil are degraded by ultraviolet light to form photodieldrin. The half life of dieldrin in soil is estimated to be 868 days (ATSDR, 1991). Several studies illustrate the resistance of dieldrin to biodegradation. Soil in the upper 6 inches near the foundation of a house treated with dieldrin contained 10% of the original dose 21 years after the application (ATSDR, 1991). Dieldrin residues on soil plots treated for 15 years were similar at the time treatment stopped (0.37 ppm) and 4 years later (0.42 ppm) (ATSDR, 1991). Dieldren was detected at mean concentrations ranging from 1 to 49 ppb in soil samples from 24 states in the National Soils Monitoring Program (ATSDR, 1991).

Surface runoff of water leads to contamination of rivers and lakes with dieldrin, with significant levels found in sediments. The EPA monitored dieldrin in U.S. drinking water in 1975 and found that less than 17% of the samples contained dieldrin. The concentrations in 78% of the positive samples ranged between 4 and 10 ng/L (ATSDR, 1991). Data from the STORET database for 1980-1982 indicated a median dieldrin level of 0.001 ppb in 7,609 water samples, 0.8 ppb in 1,812 sediment samples, and 0.03 ppb in 530 biota samples. Dieldrin attaches strongly to soil particles, does not migrate well through the soil, and therefore is not a major groundwater contaminant. However, detection of dieldrin occurred in 0.71% of groundwater samples from NPL sites at a mean concentration of 0.40 ppb in the positive samples (ATSDR, 1991). Dieldrin was also found in 9% of 422 groundwater samples from a sandy, alluvial aquifer in Illinois and from 4 % of groundwater samples taken near an agrichemical dealer facility. The median concentration in the Illinois aquifer samples was 0.01 micrograms/L (ATSDR, 1991). Even though the use of dieldrin was banned in much of the Great Lakes Basin in the early 1970's, levels in sediment samples from Lake Ontario showed an increase from approximately 26 ng/g (26 ppb) in 1970 to 48 ng/g (48 ppb) in 1980 (ATSDR, 1991).

Dieldrin is subject to atmospheric transport and is present in precipitation. In the Great Lakes Basin, during 1986 dieldrin was detected in wet precipitation at mean concentrations ranging from 0.41 to 1.81 ng/L (ATSDR, 1991). At College Station, Texas in 1979-1980, dieldrin was detected in the ambient air at a mean concentration of 0.08 ng/m^3, and in rainfall at a mean concentration of 0.80 ng/L (ATSDR, 1991). Results from

a study of indoor air in homes treated with dieldrin for termites demonstrated dieldrin concentrations ranging from 0.01-0.51 micrograms/m^3 (0.0006-0.03 ppb) in living rooms and bedrooms 1-10 years after treatment (ATSDR, 1991).

Monitoring of OCPs in human serum was conducted as part of the NHANES II survey during 1976-1980. Dieldrin was detected in quantifiable levels in 8.6% of individuals 12-74 years old with a median level of 1.4 ppb. A 1981 study of OCP residues among Florida citrus industry fieldworkers found quantifiable dieldrin in 3.0% of workers with a mean level of 1.8 ppb (Griffith and Duncan, 1985). The Human Adipose Tissue Survey conducted by the EPA on a nationwide basis in 1982 found dieldrin in 14 of 56 samples taken from cadavers and surgical patients with a mean concentration of 458 ppb (wet tissue basis) (ATSDR, 1991). Using data from the FDA Total Diet Study conducted between 1982 and 1984, estimated dieldrin intake from the diet for adults males 25-30 years old was 0.007 micrograms/kg/day, for toddlers was 0.016 micrograms/kg/day, and for infants was 0.010 micrograms/kg/day (ATSDR, 1991). Dieldrin in human breast milk has also been monitored. Savage et al. (1981) reported quantifiable dieldrin in 80.8% of milk samples from 1,436 women across the U.S. with a mean fat-adjusted residue level of 164 ppb. The greatest percentage of positive samples (88.9%) was found in the southeastern U.S. and the lowest percentage in the northeast (63.9%). Dieldrin has also been found in placental and fetal tissues. Polishuk et al. (1977) reported that dieldrin concentrations in extracted lipids of fetal blood (1.22 ppm) and placenta (0.80 ppm) exceeded those in maternal blood (0.53 ppm) and uterine muscle (0.54 ppm). In contrast,

in a study of OCP residues in Kenyan women, dieldrin was found in maternal blood and milk, but not in cord blood (Kanja, L. et al, 1992). Dieldrin was detected in the follicular fluid of 47 women in Germany and Austria at a mean concentration of approximately 0.2 ppb (Baukloh et al., 1985). The mean dieldrin concentration in follicular fluid was similar to the mean concentration in serum.

Demographic characteristics reported to be associated with dieldrin exposure are age and low socioeconomic status (Sim et al., 1998). Murphy and Harvey (1985) reported the percent of the population exposed rose from the youngest to oldest age group, however the median level for quantifiable positive results did not change significantly with age. Devoto et al., (1998) did not find an association between dieldrin exposure and age, but they did report dieldrin level in serum was positively associated with consumption of saltwater fish.

Dieldrin can enter the human body through the respiratory and gastrointestinal tracts and through dermal absorption. Distribution in the body is general initially and then primarily to adipose tissue stores with an average ratio of adipose tissue to blood concentration of 156 (ATSDR, 1991). The estimated half-life of dieldrin in blood is 369 days (ATSDR, 1991). Metabolism of dieldrin has been studied in rats. Two pathways predominate, the first is direct oxidation by cytochrome oxidases resulting in the formation of 9-hydroxydieldrin and the second is the opening of the epoxide ring by epoxide hydrases resulting in the formation of 6,7-trans-dihydroxydihydroaldrin (ATSDR, 1991).

Metabolism and excretion of dieldrin is 3-4 times more rapid in male than in female rats. Male rats are believed to have a greater ability to metabolize dieldrin to its more polar metabolite (9-hydroxydieldrin) than do females. In mice, dogs, and humans given repeated doses of dieldrin, a state of equilibrium is reached between the intake, storage, and excretion of dieldrin (ATSDR, 1991).

The bioconcentration and elimination of dieldrin are related to the total lipid mass of the individual, with the leanest subjects having the highest adipose concentration, but the lowest body burden (ATSDR, 1991). This phenomenon was demonstrated in chickens fed dieldrin contaminated feed. The apparent gender difference in accumulation of dieldrin in fat (male > female) was attributed to a diluting effect related to the female's tendency to increased fat deposition with age compared to the male (Siegel and Latimer, 1981).

A study of the body burden of dieldrin showed there was no increase in dieldrin concentration in whole blood when subjects were under surgical stress or in periods of complete fasting (ATSDR, 1991). A similar finding was noted by Bigsby et al. (1997) in mice for *o,p'*-DDT, where fasting did not increase the uterine weight of mice loaded with *o,p'*-DDT. Mice that had been loaded with beta-HCH however, did demonstrate an increase in uterine weight when fasted.

Health effects of acute, high dose exposure to dieldrin include central nervous system excitation leading to convulsions and death. Low level dieldrin exposure in workers

suggests the following nervous system effects: headaches, dizziness, vomiting, irritability, and uncontrolled muscle movements (ATSDR, 1991). Volunteers who ingested daily doses of dieldrin as high as 0.003 mg/kg/day did not exhibit any effects on central nervous system activity, peripheral nerve activity, or muscle activity (ATSDR, 1991) Hepatic effects have been demonstrated in rats such as increased liver weight, histopathologic changes, and increased microsomal enzyme activity. Immunosuppression by dieldrin has been demonstrated in mice. Carcinogenicity bioassays in mice have shown an increase in hepatocellular adenoma and/or carcinomas with chronic exposure to dieldrin. Dieldrin also significantly decreased the time to tumor development in mice given doses as low as 0.013 mg/kg/day in females and 0.13 mg/kg/day in males (ATSDR, 1991). Evidence for carcinogenicity in humans is lacking, however.

With respect to its neurologic effects, the major site of action of dieldrin is the GABA receptor chloride channel complex. There are two types of chloride currents, one is highly sensitive to dieldrin and the other has a low sensitivity to dieldrin. Neurons possess one or the other type of chloride currents. Initially dieldrin has a stimulatory effect, and subsequently, a suppressive effect. The suppressive effect may be due to desensitization and causes hyperexcitation which leads to massive influx of calcium through the glutamate receptor-channel. This increase in the intracellular calcium concentration could potentially disrupt other enzyme activities in the cell leading to other biochemical effects (Narahashi et al., 1997). The GABA receptor-channel is composed of five subunits. Dieldrin enhancement of GABA was seen only when the gamma2s subunit was present in the

neuron, however dieldrin suppression was observed in multiple combinations of subunits tested (Narahashi et al., 1997).

Another mechanism for neurotoxicity associated with dieldren and other OCPs is the inhibition of ATPase. Mehrotra et al. (1989) reported that dieldrin and aldrin inhibit both brain synaptic plasma membrane and heart sarcoplasmic reticulum Ca^{++} pump activity in vitro in a concentration dependent manner, thus disrupting Ca^{++} membrane transport mechanisms. Dieldrin disrupts the Ca^{++} pump through inhibition of calmodulin, which regulates the Ca^{++} pump. Both the decrease in Ca^{++} pump and ATPase activity lead to decreased Ca^{++} flux and result in the accumulation of Ca^{++} in the cell, which could lead to cell death.

Dieldrin is also believed to alter the functional integrity of the cellular plasma membrane. One factor in this disruption of membrane integrity may be accelerated phospholipid degradation by dieldrin due to alteration in the enzymes responsible for phospholipid metabolism (Fonovich de Schroeder and Pechen de D'Angelo, 2000). This mechanism is hypothesized to be involved in the decrease in the fertilization rate in amphibian oocytes associated with dieldrin.

Another toxic mechanism by which dieldrin may act is disruption of cellular communication through inhibition of gap junctions. Gap junctions are transport channels for low molecular weight cytoplasmic components. Regulation of cell proliferation and

differentiation is believed to occur through gap junction mediated communication between contiguous cells (Wade et al., 1986).

Several alterations in the body's handling of glucose are reportedly associated with dieldrin. Pulido et al. (1990) reported that dieldrin decreased the transport of glucose across rat brain cells *in vitro*. In rhesus monkeys, Mahmood et al. (1981) found dieldren elevated the uptake of glucose from the intestine. Finally, Fox and Virgo (1986) investigated dieldrin induced hyperglycemia in adult and immature rats.

Reproductive effects such as decreased fertility and decreased postnatal survival have been associated with aldrin or dieldrin exposure in animal studies (ATSDR, 1991). Decreased fertility occurred with exposure in both male and female animals. In particular, multiple adverse reproductive effects on the male have been identified including decreased sperm count, degeneration of germ cells, decreased weights of seminal vesicles and prostate and coagulating glands, decreased seminiferous tubule diameter, decreased plasma and testicular testosterone, decreased prostatic fructose content and acid phosphatase activity, and decreased plasma luteinizing hormone (LH) and follicle stimulating hormone (FSH) (ATSDR, 1991). Female rats exposed to feed mixed with 2 ppm dieldrin demonstrated decreased serum FSH and LH levels after the first and second week of exposure and increased levels in the 3rd week. In addition, histopathologic changes were noted with submucosal interstitial hemorrhage in the uterus and severe hemorrhage and lymphocytic infiltration in the graafian follicles (Ateia et al., 1990). Since dieldrin is known to induce

hepatic monooxygenases which also metabolize steroid hormones in addition to xenobiotics, Virgo (1980) investigated whether levels of plasma progesterone would be effected by dieldrin exposure in hemiovariectomized pregnant mice. The results indicated that dieldrin did not reduce plasma progesterone. This failure of dieldrin exposure to reduce progesterone levels could be due to increased progesterone synthesis to compensate for its elevated metabolism, or the induction of hepatic enzymes does not increase progesterone metabolism.

The ability of dieldrin to bind the estrogen receptor and cause estrogenic effects was investigated by Ramamoorthy et al. (1997) in several assays. Dieldrin did not significantly bind to the mouse uterine estrogen receptor in a competitive binding assay and did not increase the uterine weight of the immature mouse uterus. A mixture of toxaphene and dieldrin did increase progesterone levels, whereas this was not observed when the compounds were administered separately. These results indicate that dieldrin is only weakly estrogenic.

In humans, Saxena et al. (1980) reported significantly higher levels of OCPs, including aldrin, in maternal blood and placental tissue of women who experienced abortion between 12 and 32 weeks gestation compared to women who experienced a full term birth. Ron et al. (1988) compared OCP blood levels in women with premature rupture of fetal membranes (PROM) in full term pregnancies to women at full term who did not have PROM. None of the OCPs, including dieldrin, were significantly elevated in the women

with PROM compared to the controls. In another study, mean serum dieldrin concentration was not significantly different among women with either recent or passed missed abortions when compared to women in their second trimester of normal pregnancy (Bercovici et al., 1983).

Hexachlorobenzene

Hexachlorobenzene (HCB) is an OCP used in the U.S. until 1985 when its registration was canceled by the EPA (ATSDR, 1994). As a pesticide, it was used primarily as a fungicide on seed grains. It was also used in the production of fireworks, ammunition and synthetic rubber. Unlike other OCPs previously mentioned (chlordane, DDT, dieldren), in addition to being manufactured for pesticidal use, HCB is produced as a byproduct of the manufacture of chlorinated solvents, in waste streams of chlor-alkali plants and wood preserving plants, in fly ash, and in flue gas effluents from municipal incineration (ATSDR, 1994). HCB is also present as an impurity in several pesticides (primarily herbicides) which are in current use. The level of HCB contamination in these pesticides is now below 0.5%, although it has been as high as 10% in the past (ATSDR, 1994).

HCB is a POP which bioaccumulates in the environment, in animals, and in humans (ATSDR, 1994). Most HCB in the environment exists tightly bound to soil particles. Its half life in soil ranges from three to six years (ATSDR, 1994). Because of HCB's hydrophobicity, it is not found in high concentrations in lake water or ground water.

Direct discharge of HCB into water from chemical manufacturing plants is a source of water contamination, however. HCB has a half life in water of 30-300 days. HCB released to water will attach to soil particles in the water and accumulate in the sediment. For example, the concentration of HCB in water from the Mississippi River near Baton Rouge, Louisiana measured 2 ppb, whereas the soil sediment from the same location contained 167 ppb (ATSDR, 1994).

The occurrence of HCB in agricultural soil is generally associated with its use as a fungicide on seed grains. In a 1976 survey of agricultural sites in 11 states, only 2 of 391 sites contained HCB at concentrations ranging from 10 to 20 ppb (ATSDR, 1994). HCB is found in urban soils as a result of releases during manufacture, and use or disposal of HCB or HCB containing wastes. During the 1970's, levels of HCB in a study of urban soil samples ranged from 10 to 590 ppb. The highest levels of HCB in soil have been found in hazardous waste disposal sites. Disposal sites near Sorrento, California, Crystal City, Texas, contained 62,000 ppb and 20,000 ppb HCB in soil, respectively (ATSDR, 1994).

The majority of HCB released to the air also originates from chemical processing facilities. In addition, HCB can become airborne when pesticides which contain this contaminant are applied to the soil. Volatilization from soil can occur and the rate of volatilization will increase with increasing soil temperature. The half life of HCB in the atmosphere is 90 days (ATSDR, 1994). Levels of HCB in urban air have been estimated to be 0.3 ng/m3. Much higher concentrations have been found in air samples from a chemical waste landfill

(16,000 ng/m3) and inside an industrial plant (150,000 ng/m3) (ATSDR, 1994).

Concentrations of HCB in water are generally lower than in soil or air. High levels have been found however, in industrial waste water (300 ppb) and in containment pond water at a hazardous waste site (8,100,000 ppb) (ATSDR, 1994). The level of HCB in drinking water from three cities near Lake Ontario ranged from 0.00006 to 0.0002 ppb. Water samples from the Great Lakes and the Niagara River contained HCB concentrations of 0.00002-0.017 ppb (ATSDR, 1994).

The lipophilic character of HCB and other POP's lead to their biomagnification up the food chain. HCB has been detected in a wide variety of foods including dairy products, meat, fish, poultry, oils and fats, and vegetables and fruits. For the general population, the primary source of exposure to HCB is believed to be the diet. Estimates of HCB intake based on total diet analyses conducted in 1982-1984 were 0.0020 micrograms/kg body weight per day for 14-16 year old males (ATSDR, 1994). Of particular concern is exposure through eating fish from highly contaminated lakes and rivers. For example, fresh water trout from the Great Lakes region were found to contain concentrations of HCB ranging from 8 to 127 ppb (ATSDR, 1994).

Dietary predictors of serum HCB level have been investigated. Devoto et al. (1998) found consumption of beef and lamb was significantly positively correlated ($r = 0.19$) with HCB level in the serum of an elderly German population. In a rural population, Stehr-Green et

al. (1988) reported an inverse association of serum HCB level with average daily servings of dairy products from home-raised cows.

Monitoring of human populations has detected the presence of HCB in adipose tissue, serum, human milk, fetal cord blood (Bush et al., 1984) and ovarian follicular fluid. Results from EPA's National Human Adipose Tissue Survey conducted yearly from 1974 through 1983 (excluding 1980 and 1982) indicate that 98.8% of the U.S. population had detectable levels of HCB in their adipose tissue at an estimated median concentration level of 0.037 ppm (Robinson et al., 1990). The median concentration level did not change significantly over the 9 year time period. Participants from the western U.S. region had a higher median concentration level, compared to participants in the north central, south or northeast regions. Results from the NHANES II study conducted in 1976-1980 indicated only about 3.3% of the population 12-74 years of age in the Northeast, Midwest and South regions had quantifiable serum HCB levels, with a median level of 1.7 ppb (Murphy and Harvey, 1985). The detection limit for HCB in the NHANES II study was 1 ppb.

A more recent study conducted in 1996 by Stellman et al. (1998), evaluated both serum and adipose concentrations of HCB and other OCPs, in 293 women from Long Island, New York. The HCB detection limit in serum was 0.014 ppb, a level much lower than reported in the NHANES II study. Stellman et al. reported HCB was detected in the serum of 98.8% of the study participants with a mean concentration of 0.22 ppb and a maximum concentration of 2.24 ppb. The ratio of HCB adipose to serum concentration

was 92.2 with a significant correlation coefficient of 0.841.

Baukloh et al. (1985) reported mean HCB concentrations of approximately 2 ppb in the ovarian follicular fluid and approximately 3 ppb in the serum of 12 women from Austria. Jerrell et al. (1993) found lower mean concentrations of HCB in follicular fluid and serum, approximately 0.15 ppb and 0.25 ppb respectively, in 74 women in Canada. In both studies, the serum concentration was significantly higher than the follicular fluid concentration.

HCB preferentially partitions to adipose tissue and tissues of high lipid content. A study in rats indicates that HCB adipose tissue partitioning is increased by a low protein or low energy diet (ATSDR, 1994). In animals, HCB is slowly metabolized to pentachlorophenol by the hepatic cytochrome P-450 system, conjugated with glutathione to yield pentachlorothiophenol, or reductively dechlorinated to form pentachlorobenzene.

Toxicity data in humans and laboratory animals indicate the heme biosynthesis pathway is the major target of HCB toxicity (ATSDR, 1994). HCB induces prophyria by interfering with the enzymes involved in the porphyrin metabolic pathway. Dermal effects known as porphyria cutanea tarda, were demonstrated in humans accidentally consuming flour made from HCB contaminated grain seeds in Turkey in 1955. Simliar to other OCPs, HCB can cause both induction of the hepatic cytochrome P-450 enzymes with liver enlargement, and neurologic effects such as hyperexcitability. Animal studies indicate that other targets

are the lungs, kidneys, and thyroid as evidenced by histopathologic changes in these organs. Endocrine, reproductive, and immunologic effects have also been reported. There is insufficient evidence to conclude that HCB is carcinogenic in humans, however in experimental animal studies there is evidence of carcinogenicity. HCB given orally in animal studies has produced multiple tumor types (hepatomas, hematomas, hemangioendotheliomas, parathyroid and bile duct adenomas, and thyroid neoplasms) in hamster, rats, and mice (ATSDR, 1994).

Some of the endocrine effects of HCB are thought to be due to interference with serum binding proteins and direct effects on the pituitary or hypothalamus mediation (ATSDR, 1994). HCB administered orally to rats lead to altered serum levels of thyroxine, progesterone, parathyroid hormone, and triiodothyronine uptake (ATSDR, 1994). In addition, evidence from animal studies indicates HCB is toxic to the mammalian ovary in a dose dependent manner and may interfere with mechanisms regulating ovarian steroidogenesis (ATSDR, 1994).

Several human populations with elevated exposure to HCB have been studied to determine health effects related to exposure. A cross-sectional study of 1,800 inhabitants of a rural village in Spain was conducted in 1994 (Sala et al., 1999). The inhabitants were exposed to high levels of HCB in the air which originated from an electrochemical factory in the village. Median serum HCB concentration in study participants was 16.5 ppb. Workers at the factory had higher HCB levels than those not working at the factory. In

women, serum level of HCB increased with age, duration of residence in the village, and by consumption of local fish. In men, levels increased with duration of residence in the village, proximity to the electrochemical factory, local fish consumption, and work in the factory. The investigators were unable to identify any specific health effects related to exposure in the general population. For male workers at the factory (very few women worked at the factory), there was an elevated odds ratio for cancer prevalence of 1.9 (95% CI 0.5,7.6). In women, there was no association noted between the reproductive outcomes spontaneous abortion, low birth weight and congenital malformations and HCB exposure. The results of this study are limited by potential selection bias due to a low response rate of less than 50% and possible differential out migration due to poor health.

As mentioned previously, in 1955-57, a population in southeastern Turkey was accidentally exposed to HCB after eating contaminated seed grain. Exposure was high enough to cause porphyria cutanea (PCT) and increased mortality. Jerrell et al. (1998) conducted a retrospective cohort study (40 years post exposure) of reproductive outcomes among 42 women who were exposed as children to the contaminated seed grain and who had clinically confirmed PCT at the time of exposure. These highly exposed women were compared to two controls groups. The first control group consisted of 42 aged matched women from the same geographical location as the exposed women. The second control group consisted of 42 age matched women from a large city, 700 km from where the other two groups resided. Surprisingly, serum HCB levels were not significantly different between the three groups, with the highest frequency of detection of HCB in the

control group from the large urban city. The three groups were then combined to determine if there was an association between serum HCB level and spontaneous abortion. Higher levels of serum HCB were found to be significantly associated with frequency of spontaneous abortion.

Gerhard et al. (1998) investigated blood levels of organochlorine compounds (OCPs) in women with a history of repeated miscarriages who attended the Clinic of Reproductive Endocrinology between 1989 and 1993 in Heidelberg, Germany. The mean blood level of HCB in subjects was 679 ppt. Women with a history of more than three miscarriages had higher blood levels of HCB, PCBs, and gamma-hexachlorocyclohexane. Investigators also found associations between OCP levels and various hormonal and immunologic parameters. Significant correlations were found concerning the thyroid and pituitary axis, and for testosterone levels. Total measured OCP burden was directly associated with FSH and prolactin and inversely correlated to testosterone. These results suggest a specific ovarian and pituitary OCP sensitivity. Regarding immunologic parameters, increasing HCB levels were associated with decreasing T-suppressor cell counts. In contrast to the two studies mentioned above, Leoni et al. (1986) failed to find a difference in blood HCB levels in a group of 120 women experiencing spontaneous abortion compared to a control group of 120 women with full term pregnancy.

Postnatal viability was decreased among children of mothers in Turkey who were exposed to the contaminated seed grain. Many of these children died between the first and second

year of life from respiratory and cardiovascular failure (ATSDR, 1994). Developmental effects studies in the rat, rabbit and mouse indicate various developmental effects in offspring of exposed mothers at doses as low as 2.5 mg/kg including negative geotaxis reflex, olfactory discrimination, and exploratory behavior tests, alteration in liver weights and liver enzymes, reductions in body weight gain and reduced viability and lactation indices (ATSDR, 1994). Evidence for teratogenic effects of HCB in animal studies with similar maternal exposure is conflicting, with one rat study demonstrating no effect, whereas in another study, cleft palate and renal agenesis occurred (ATSDR, 1994).

Hexachlorocyclohexane

Hexachlorocyclohexane (HCH), formerly known as benzene hexachloride (BHC), is a synthetic OCP which exists in four primary isomers known as alpha, beta, delta and gamma HCH (ATSDR, 1992c). Technical grade HCH consists of 64-72% alpha-HCH, 10-12% beta-HCH, 11-13% gamma-HCH, and 9% delta-HCH. Technical grade HCH has not been produced in the U.S. since 1983. The gamma form, commonly called lindane, continues to be used in the U.S. and in other countries as an insecticide on fruit, vegetable, and forest crops, as a seed treatment, and on animals. It is also used in human medicine to treat head and body lice and scabies. Beta-HCH has been found as a contaminant in lindane (Lay et al., 1981). The beta form is the most stable, due to the planar configuration of its chlorine atoms, and therefore is the isomer which accumulates in the environment, animals and humans. (ATSDR, 1992c)

HCH is a POP found in air, surface water, groundwater, sediment, soil, fish and other aquatic organisms, wildlife and other animals, food, and humans (ATSDR, 1992c). Soil HCH can become airborne through wind erosion and via volatilization. Airborne HCH is present as a vapor or attached to small particles of soil or dust and can be washed out of the atmosphere by rain and into surface waters. Gamma-HCH both dissolves in surface water and adsorps to sediment. Gamma-HCH may remain in its original form in air for as long as 17 weeks and in water for approximately 30 days. Bioconcentration of gamma-HCH is greater among aquatic organisms than for plants and terrestrial organisms (ATSDR, 1992c).

Environmental monitoring information is available for the gamma-HCH isomer, however little data is available on the concentration in environmental media of the other isomers. Gamma-HCH has been detected in soil at concentrations ranging from 0.01 ppm in Alabama to 0.15 ppm in Iowa. In sediment samples collected from the Niagara River, the average concentration of gamma-HCH was 2 ppb (ATSDR, 1992c). Creek sediments collected in 1996 near the James River in Virginia contained gamma-HCH in concentrations ranging from 7.3 to 8.5 ppb. Almost 20% of sediment samples from the Gulf of Mexico in 1987 had detectable gamma-HCH at a mean concentration of 0.07 ng/g (ATSDR 1992c).

Data from surface water samples described in the EPA's STORET database indicate that gamma-HCH was detected in 27% of 4,505 samples collected in the U.S. at a median

concentration of 0.020 micrograms per liter (ATSDR, 1992c). In water samples taken in 1980-1981 from the Niagara River, the mean concentration of gamma-HCH was 2.1 ppt (ATSDR 1992c). Gamma-HCH has been detected in drinking water samples from Ohio, South Carolina and Hawaii at mean concentrations of 0.01 ppt, 10 ppt, and 0.1 ppt, respectively (ATSDR, 1992c). Ground water can also be contaminated with HCH. Ground water samples from Chesterfield County, South Carolina and Hampton, South Carolina contained median concentrations of 16 ppt and 163 ppt gamma-HCH (ATSDR, 1992c).

Bidleman, et al. (1986) reported the average concentrations of OCPs in the air of Denver, CO in 1980 and Columbia, SC in 1977-1982. In Denver and Columbia, the concentration of HCH in the air was 0.22 and 0.09 ng/m^3, respectively. In a study of nine U.S. localities representing both urban and rural areas, gamma-HCH was detected only in three urban sites at levels of 0.1-7.0 ng/m^3 (ATSDR, 1992c). The reported concentration of gamma-HCH in the atmosphere of the Mississippi Delta during 1972-1974 was 9.3 ng/m^3 (ATSDR, 1992c).

HCH isomers have been detected in dairy products, meat, fish, poultry, garden fruits, oils and fats, leafy and root vegetables; sugar and adjuncts (ATSDR, 1992c). Gamma-HCH was detected in 6% of foods collected in eight market basket surveys from different region of the U.S during 1982-1984 (ATSDR 1992c). Fat samples from domestic farm animals in Ontario, Canada in 1986-1988, contained mean concentrations of gamma-HCH ranging

from 0.012 to 0.032 mg/kg (ATSDR, 1992c). Gamma-HCH was detected in 80% of oyster samples collected in 1987 from the Gulf of Mexico at a mean concentration of 1.74 ng/g (ATSDR, 1992c). Detection of alpha- and gamma-HCH in a survey of freshwater fish in the U.S. declined from 1976-1981, however the highest concentrations of alpha-HCH (0.03-0.04 ppb) were found in fish from the southwestern and midwestern U.S.(ATSDR, 1992c). Information from the Total Diet Studies of the Food and Drug Administration (FDA) for fiscal year 1981-1982 indicates the daily intake of HCH isomers in adult diets was 0.010 micrograms per kilogram per day (ATSDR, 1992c).

Humans are exposed to HCH isomers through contact with contaminated air, water, soil, and food, and through cutaneous application of lindane to control ectoparasites (ATSDR, 1992c). HCH is lipophilic and accumulates in adipose tissue. The presence of gamma-HCH in human tissue is thought to reflect current exposure and beta-HCH cumulative exposure.

Data from the National Human Adipose Tissue Survey conducted from 1970 through 1983 indicate that 98.7% of the U.S. population had detectable levels of beta-HCH (Robinson, 1990). The estimated national median concentration level of beta-HCH in this survey was 0.14 ppm. Higher beta-HCH level was positively associated with increasing age and residence in the South Census Region. During the period of study, 1970 to 1983, the estimated national median body burden level of beta-HCH decreased from 0.28 ppm to 0.08 ppm. In a more recent study, Stellman et al. (1998) measured beta-HCH in the

adipose tissue of women in Long Island, New York during 1994-1996. Beta-HCH was detected in 91.9% of the 221 participants with a mean concentration of 22.2 ppb. Serum levels of beta-HCH were determined as part of the NHANES II conducted in 1976-1980. Results from this study indicated that 13.9% of the population 12-74 years of age in the Northeast, Midwest, and South regions had detectable (≥ 1 ppb) beta HCH in their serum with a median level of 1.7 ppb (Murphy and Harvey, 1985).

Serum beta-HCH level was also measured in the 1994-1996 study of women in Long Island, New York mentioned above. The proportion of women with detectable (≥0.028) beta-HCH in serum was 97.1% with a mean of 0.82 ppb. The ratio of adipose to serum beta-HCH in this study was 27.0 and the serum vs. adipose correlation coefficient was 0.584 ($p<0.001$). Schafer and Zahradnik (1998) found beta-HCH in the endometrial tissue of 17 women in Germany at a mean concentration of 0.8 ppb. Baukloh et al. (1985) reported similar concentrations of beta- HCH in the follicular fluid and serum in a sample of German and Austrian women, with a mean concentration of approximately 0.5 ppb.

Metabolism of HCH is dependent on hepatic microsomal enzymes, particularly the P-450 oxidative system. Chlorophenols, including pentachlorophenol, were the primary urinary metabolites of gamma-HCH excreted by Occupationally exposed workers (ATSDR, 1992c). The half life of gamma-HCH in humans is about one day (Jung et al. 1997). The half life of beta-HCH is much longer, 7 - 8 years, indicating differing metabolism of the two compounds, probably due to their different chemical structure (Jung et al. 1997).

Unlike the other isomers, all the chlorine substituents of beta-HCH are in the *trans* configuration to each other. Gamma-HCH has a greater ability than beta-HCH to induce hepatic microsomal enzymes and this also accounts for some of the differences in half-life and storage (Srinivasan and Radhakrishnamurty, 1983). In rats, beta-HCH accumulation in all tissues was progressive with duration of treatment, however gamma-HCH levels in tissues other than fat reached an equilibrium after 5 days of treatment. Therefore, with the exception of brain tissue, beta-HCH accumulation is always greater than gamma-HCH (Srinivasan and Radhakrishnamurty, 1983).

Jung et al. (1997) reported that the clearance rate for beta-HCH was dependent on the percentage of body fat of the individual, with a lower clearance rate for fat individuals compared to thin individuals. In studies of the distribution of gamma and beta-HCH in rat tissues, food restriction did not result in significant redistribution of these HCH residues to other organs, as they tended to remain in fat tissue. This is reported to be true for dieldrin also. In contrast, food deprivation reportedly lead to movement of DDT and HCB into the brain and other organs (Srinivasan, 1983).

Exposure to HCH may be associated with hematological, hepatic, renal, immunologic, neurologic, and reproductive effects (ATSDR, 1992c). Various blood disorders have been observed in humans heavily exposed to gamma-HCH by all routes except dermal, including anemia, leukopenia, leukocytosis, granulocytopenia, granulocytosis, eosinophilia, monocytosis, pancytopenia, and thrombocytopenia (ATSDR, 1992c). In rats,

beta-HCH was associated with reduced numbers of red and white blood cells (ATSDR, 1992c).

Animal studies indicate various degrees of liver toxicity associated with alpha, beta, gamma, and technical grade HCH including increased microsomal activity, increased liver weight, mild to moderate liver necrosis and fatty degeneration, and liver cancer (ATSDR, 1992c). Increased liver enzymes were reported in humans Occupationally exposed to technical grade HCH. In rats, gamma-HCH was a more powerful inducer of the mixed function oxygenase enzyme system in the liver than beta-HCH, however liver weights were increased more by beta-HCH (Srinivasan and Radhakrishnamurty, 1983). Renal dysfunction has not been seen in humans, but is seen in rats. Workers exposed to technical grade HCH exhibited a significant increase in the level of IgM, however an increase in immunoglobins has not been reported for animals. Changes in several immune parameters were reported in mice exposed to 300 mg/kg beta-HCH in the diet, including a 25% decrease in T-lymphocyte-mediated cytolysis of tumor targets with a concurrent reduction of 45% in Natural Killer cell activity (Cornacoff et al., 1988).

Ingestion or dermal exposure to large amounts of gamma-HCH has lead to neurologic effects in humans including paresthesia of the face and extremities, headaches, vertigo, abnormal EEG patterns, seizures and convulsions (ATSDR, 1992c). The mechanism of action associated with the neurologic effects of HCH is its interference with the GABA receptor chloride channel complex (Damgaard et al., 1999). Gamma-HCH inhibits the

GABA-induced chloride influx thus blocking GABA mediated neurotransmission. In addition, gamma-HCH acts as a non-competitive antagonist at the GABA-A receptor. Attia et al. (1990) reported that gamma-HCH augmented pineal and serum melatonin levels in rats and stimulates circulating norepinephrine levels. Gamma-HCH but not beta-HCH, was found to decrease the transport of glucose across rat brain cortex cells (Pulido et al., 1990)

There is no evidence regarding carcinogenic effects in humans, however alpha, beta, and gamma isomers have been shown to be liver carcinogens in rats and mice following long-term oral exposure in multiple studies (ATSDR, 1992c). The carcinogenicity of HCH isomers was examined separately and in combination by Ito et al. (1973). The results indicated that by themselves at the dose given, beta, gamma- or delta-HCH did not cause hepatocellular carcinoma. However, when these isomers were given with alpha-HCH, hepatocellular carcinoma occurred, suggesting that alpha-HCH is a more potent carcinogen or the involvement of an interactive effect.

Reproductive effects for HCH isomers have been reported in animals and humans. In rats, a single dose of oral gamma-HCH (33 mg/kg) was associated with increased length of the vaginal cycle and anti-estrogenic properties were seen in female rats given oral gamma-HCH at 20mg/kg/day for 15 weeks. These effects were not seen at the lower dose of 5 mg/kg/day (ATSDR, 1992c). Male rats given oral gamma-HCH at 75 mg/kg/day for 90 days exhibited testicular atrophy, degeneration of seminiferous tubules, and disruption of

spermatogenesis (ATSDR, 1992c). In studies with beta-HCH, rats exposed for 13 weeks to 0.5 mg/kg/day had significantly increased ovary and uterus weights suggesting estrogenic action, however when given 12.5 mg/kg/day, atrophy of the uterus and ovary occurred (Van Velsen et al. 1986). Beta-HCH given to female rats (10 mg/kg/day) during gestation and lactation caused increased fetal deaths within 5 days of birth (ATSDR 1992c). For male rats, degeneration of seminiferous tubules and disruption of spermatogenesis Occurredurred following exposure to 12.5 mg/kg/day beta-HCH.

Inhibitory effects of gamma-HCH on mouse embryo development, evidenced by decreased blastocyst formation and hatching, were reported by Alm et al. (1996). Tiemann et al. (1998) investigated the effects of gamma-HCH on bovine oviductal cells in vitro and did not find that gamma-HCH interfered with oviductal cell viability. Exposure to DDT or methoxychlor at 64 micro molar concentration however, did significantly decrease oviductal cell viability. In another study using bovine oviductal cells, gamma-HCH at 64 micro molar concentration did not inhibit Mg^{++} ATPase (which is associated with oxidative phosphorylation) in the microsomal fraction of bovine oviductal cells, but did inhibit Mg2+ in the bovine endometrial cell fraction (Tiemann and Kuchenmeister, 1999). Alm et al. (1998) investigated bovine oocyte maturation and postfertilization development after exposure to gamma-HCH. Maturation of bovine oocytes was depressed in a dose-dependent manner, however there was no effect on the fertilization rate of exposed oocytes compared to controls. A concentration of 29 ppm resulted in more than 50% degeneration of exposed oocytes. Structural changes in the ovary of the air-breathing

catfish (H. fosselis) were identified after exposure to 1 ppm BHC, including partial disruption of ovarian follicles, vacuolation in the cytoplasm of germinal cells, reduction in the number of matured ovum and secondary oocytes, and loss of interfollicular connective tissue (Hazarika and Das, 1998).

Ewes fed a diet treated with 1 mg/kg/day of gamma-HCH experienced a significant decrease in the pregnancy rate compared to controls (Beard et al. 1999). Also in this study, the thyroid follicle size was significantly increased in exposed ewes. In another study in ewes, gamma-HCH (2.5 mg/kg) was given orally in capsules three times a week for 36 days and then hormone levels were analyzed (Rawlings et al., 1998). Gamma-HCH exposure resulted in a decrease in thyroxine and basal LH concentrations and an increase in insulin and estradiol concentrations. Beard et al. (1999) investigated the developmental effects of gamma-HCH in ram lambs, exposed during gestation and until 28 weeks of age. Effects noted included a decrease in reproductive behavior, decreased serum LH and estradiol concentrations during reproductive development, decreased LH pulse frequency at 27 weeks and decreased testosterone secretion after GnRH treatment.

In humans, effects of gamma- and beta-HCH on several reproductive outcomes have been investigated in epidemiologic studies. Ron et al. (1988) reported significantly elevated levels of gamma-HCH in the maternal and cord blood of a group of 20 women who experienced premature rupture of membranes (PROM) at term, compared to 15 control women with normal deliveries at term. In a case-control study in India of 10 women with

spontaneous abortion, 15 women with preterm labor and 25 with normal delivery, Saxena et al. (1981) reported higher maternal and placental blood levels of both beta- and gamma-HCH in women with spontaneous abortion and preterm labor. In contrast, Bercovici et al. (1983) conducted a case control study in Israel with 17 women with current spontaneous abortions, 7 women with a past history of spontaneous abortion, and 7 women with normal pregnancy, and did not find a significant difference in serum gamma-HCH level between the groups. Gerhard et al. (1998) examined blood OCP levels in 89 women with a history of at least two miscarriages, and reported blood gamma-HCH, PCB and HCB levels were higher in women with a history of more than three miscarriages than in women with two miscarriages. In addition, blood OCP levels were associated with various immunological and hormonal parameters in these women: an inverse correlation between thyroid stimulating hormone and beta-HCH, gamma-HCH, PCB and DDE, an inverse correlation between testosterone levels and beta-HCH, gamma-HCH and PCP and the OCP score (an index for total OCP); and increasing gamma-HCH levels associated with higher folicular estradiol concentrations.

A cross-sectional epidemiologic study was conducted in Germany by Gerhard et al. (1999) to investigate blood CHC levels in 489 infertile women. Results of univariate analyses indicated higher beta-HCH levels were associated with a history of previous miscarriage and with the presence of antinuclear antibodies. For alpha-HCH, univariate analyses indicated associations with thyroid antibodies and uterine fibroids. However, in multivariate logistic regression analysis which included potential confounders such as age,

these associations were no longer significant.

Female Reproductive Endpoints

The following female reproductive endpoints and their association with exposure to xenobiotics (HAAs and pesticides in particular) will be reviewed: spontaneous abortion, stillbirth, congenital malformation, low birth weight, age at menarche, and age at menopause.

Spontaneous Abortion

Spontaneous abortion has been used as an endpoint in numerous environmental and Occupational toxicant exposure studies. Both spontaneous abortion and miscarriage are terms used interchangeably to refer to embryological and early fetal deaths. In the general population approximately 10 to 20% of recognized pregnancies will terminate in spontaneous abortion before the 28th week of gestation. Approximately 30 to 50% of spontaneous abortions are chromosomally abnormal and 50% to 70% are chromosomally normal (Stein and Hatch, 1986). Toxic agents can lead to spontaneous abortion by causing direct toxicity, lethal chromosomal or developmental abnormalities, or by affecting the normal maternal-fetal biologic processes necessary for a successful pregnancy. One difficulty in using spontaneous abortion as a marker of toxic effect is that approximately 20% of pregnancies end in fetal loss before clinical evidence of pregnancy and therefore may not be reported by study subjects (Wilcox et al., 1988).

Spontaneous abortion is associated with many toxic agents including: ethylene oxide, alkylating agents, folate antagonists, anesthetic gases, organic solvents, lead, and PCBs (Stein and Hatch, 1986). In the previous discussion of OCP's, evidence from human epidemiologic studies is conflicting, with several studies suggesting associations between spontaneous abortion and exposure to DDE, HCB, and beta- and gamma-HCH, and other studies suggesting no effect. Small sample sizes and failure to control for confounding in many of these studies may partially explain these inconsistencies. Exposure to pentachlorophenol (which is used as a wood preservative and also is a metabolic product of HCB and beta-HCH) was linked to miscarriages in a small cohort study and also in a case series of women (Arbuckle and Sever, 1998).

A number of studies have linked various agricultural pesticides to increased risk for spontaneous abortion. In an extensive review of pesticide exposures and fetal death, Arbuckle and Sever (1998) found evidence in the literature that exposure to *specific* pesticides including methyl isocyanate, organophosphates, carbamates, 1,2-dibromo-3-chloropropane (DBCP), and thiram may be associated with spontaneous abortion, with the estimates of risk (odds ratio) ranging from 1.2 to 2.0. Arbuckle and Sever (1998) also concluded that "most of the published reports of exposure to *unspecified* pesticides have found positive associations with the risk for spontaneous abortion." The odds ratios in these studies, where the names of the pesticides were not specified and exposure was often to multiple pesticides, ranged from 1.5 to 5.1. In several large cohort studies, women working in agricultural occupations and other occupations where exposure to

pesticides is common, were found to be at higher risk for spontaneous abortion. In a large study of Finnish women hospitalized for spontaneous abortion, the occupational category of working in agriculture had the highest spontaneous abortion rate (Arbuckle and Sever. 1998).

Demographic, lifestyle, and reproductive history variables have been associated with spontaneous abortions. Increased risk is most consistently associated with increasing maternal age, history of prior spontaneous abortion (Wilcox et al., 1990), nonwhite race (Mills et al., 1988), lower socioeconomic status, smoking , alcohol (Windham et al., 1997) and caffeine consumption (Chatenoud et al., 1998). Diabetes has been investigated as a medical risk factor for spontaneous abortion. Mills et al. (1988) concluded that diabetic women with good metabolic control are not at increased risk for spontaneous abortion, however diabetic women with elevated blood glucose and glycosylated hemoglobin levels in the first trimester are at significantly increased risk. In a large case-control study in three New York hospitals, use of oral contraceptives within the three months prior to conception was protective against spontaneous abortion of a chromosomally normal conceptus (Sackoff et al., 1994). Use of oral contraceptive after conception Occurs is associated with increased risk for spontaneous abortion.

Stillbirth

Stillbirth refers to late fetal death and is usually defined as death of the fetus at a gestational age of 28 weeks or greater, however, this cutoff is an administrative decision

and varies between countries from 28 completed weeks to 16 weeks (Arbuckle and Sever, 1998). The case definition varies in medical research as well, with stillbirths often being combined with either spontaneous abortions to create one outcome of fetal death or combined with neonatal deaths to create one outcome of perinatal deaths (Pastore et al., 1997) Since the case definition varies between studies, it is difficult to make comparisons of study results.

Stillbirth has a low prevalence of about 0.5-1% of pregnancies in the general population. The 1989 U.S. fetal death rate for 20 weeks gestation or more was 7.5 per 1000 live births (Pastore et al., 1997). In 1980, the fetal mortality rate for 28 weeks of gestation or more was 9.1 per 1000 live births (Guyer et al. 1999). Since stillbirth is a rare outcome, relatively large sample sizes are needed to identify increases in risk for environmental exposures.

The etiologies of stillbirths are heterogeneous, ranging from direct toxicity to the mother or fetus, to maternal complications of pregnancy such as diabetes or toxemia. Chromosomal abnormalities cause approximately 6% of stillbirths, a lower proportion than for spontaneous abortions (30-50%) (Stein and Hatch, 1986). In a study using stillbirths identified from death certificates in non-urban counties of California during 1982-1984, the cause of death was distributed as follows: congenital anomalies (13%), complications of the placenta, cord, or membranes (31%), other maternal causes of perinatal morbidity and mortality (15%), other conditions originating in the perinatal period (41%), and other

(1%). Demographic and lifestyle risk factors for stillbirth cited in the literature include advanced maternal age, non-white race, smoking, alcohol use, and lower socioeconomic class (Pastore et al., 1997). Having had a previous fetal loss is also an important risk factor.

No relevant studies on exposures to *specific* OCPs and stillbirths were identified, however several studies investigated exposure to specific pesticides or groups of pesticides. A large study of stillbirth (defined as 28 or more weeks gestation) and parental occupational exposures, used data from the 1980 U.S. National Natality Survey and the National Fetal Mortality Survey (Savitz et al., 1989a). In this study, exposure to specific agents was assigned based on the mother's occupation. One exposure category of organic compounds was alicyclic halogens, which included chlordane, insecticides, and pesticides. The adjusted odds ratio for maternal exposure to alicyclic halogens was 1.4, with a 95% confidence interval (CI) of 0.7-2.9 (Savitz et al., 1989a). Additional analyses were conducted with this data set using different exposure criteria. In these analyses, exposure was determined based on the subjects response to a questionnaire eliciting information on exposure to pesticides, herbicides, or fungicides at work or at home, instead of probability of exposure to specific chemicals based on occupational category. In this analysis, the odds ratios for stillbirth were 1.5 (95% CI, 1.3-1.7) and 1.6 (95% CI, 1.3-2.1) for maternal exposure to pesticides at home or work, respectively (Savitz et al., 1989b).

Rita et al. (1987) conducted a small case control study of couples working in grape

gardens in India. The twelve couples in the case group were exposed to nine different pesticides, including OCPs and organophosphates. Increased incidence of stillbirths among the case group compared to the 15 couple control group was identified, however the numbers were small.

Exposure to malathion (an organophosphate) and its relationship to stillbirth was explored in a case-control study conducted in the San Francisco Bay area of California, where aerial spraying of malathion was used to control the Mediterranean fruit fly in 1981-1982 (Thomas et al., 1992). The odds ratio for malathion and stillbirth was elevated (OR=1.51), however the 95% confidence interval was wide and indicated a lack of statistical significance (95% CI, 0.21-11.3).

Arbuckle and Sever (1998) reviewed a number of studies which investigated the Occurrence of stillbirth and exposure to *unspecified* types of pesticides. Most studies reported a positive association with odds ratios ranging from 1.4 to 3.6. Using birth certificate records from Washington State, Engle et al. (1995) reported the adjusted relative risk for late fetal loss in women working in agriculture was 2.4 (95% CI 1.5-4.0). In contrast, Schwartz et al. (1986), using birth records from a major hospital in Imperial County, California, did not identify a difference in the rate of stillbirth between agricultural workers and nonagricultural workers.

In a recent case-control study of stillbirth and environmental exposures (Pastore et al.,

1997), the investigators divided stillbirths into two cause of death groups (congenital anomalies and complications of the placenta, cord, and membranes) and differentiated between occupational and residential exposure. Exposure was analyzed using time specific exposure windows throughout the pregnancy. Stillbirth was defined as fetal deaths after 20 weeks of gestation and infant deaths within 24 hours of birth. A positive association was identified between occupational exposure to pesticides during the first two months of gestation and stillbirths due to congenital anomalies (adjusted OR=2.4, 95% CI 1.0-5.9). Occupational exposure to pesticides during the first and second trimester was positively associated with stillbirths due to all causes of death (adjusted OR 1.3-1.4, 95% CI 1.0-1.7) and also to stillbirths due to complications of the placenta, cord, and membranes (adjusted RR 1.6-1.7, 95% CI 1.1-2.3). For home pesticide exposure, there was also a positive association with stillbirths due to congenital anomalies (adjusted OR=1.7, 95% CI 1.0-2.9).

Congenital Malformation

Congenital malformations or birth defects occur in approximately 3-7% of live births (Savitz and Harlow, 1991). Environmental agents known to cause congenital malformations include ionizing radiation, lead, organic mercury compounds, organic solvents, PCBs and pesticides. The various etiologic causes of malformations include single major mutant genes (7.5%), chromosomal aberrations (6%), interactions between hereditary tendencies and usually undefined nongenetic factors (20%), identified environmental factors (6.5%), and the remaining 60% of malformations have no identified

cause (Taskinen, 1990). Malformations can result from genetic damage to germ cells before conception or damage directly to the embryo or fetus. Maternal exposure leading to teratogenesis is usually related to exposure post conception during the organogenesis phase of pregnancy (the first three months or first trimester). Certain anomalies which may affect the musculoskeletal, nervous, external urogenital or cardiovascular systems can occur after this critical organogenesis period (Garcia, 1998). An "acute exposure" model has been the focus of most studies of congenital malformations. Garcia (1998) emphasizes the importance of considering a "chronic exposure" model, because the absorption, distribution, kinetics and metabolism of many chemicals are not well known and certain chemicals (eg. POPs) can be stored in the body and released over time.

Demographic, lifestyle, reproductive and medical history factors have been associated with the Occurrence of congenital malformations. Maternal age greater than 35 years is associated with increased risk for central nervous system defects, oral clefts, circulatory malformations, and chromosomal defects including Down's syndrome and other trisomies. Lower socioeconomic status, smoking and alcohol consumption are also associated with congenital malformations. Heavy alcohol consumption is the cause of fetal alcohol syndrome, which is characterized by low birthweight, microcephaly, reduction of the width of palpegral fissure, and maxillary hypoplasia (Taskinen, 1990). Congenital malformations are three times more common in diabetic mothers compared to nondiabetics.

Garcia (1998) conducted a review of epidemiological studies dealing with Occupational exposure to pesticides and congenital malformations. Few studies characterized exposure by identifying a specific chemical class or specific pesticide. In most studies, exposure was assigned based on belonging to an Occupational group involved in agriculture or pesticide exposure. Outcome classification also varied among studies. All malformations are grouped together in some analyses, and in others, malformations are grouped by body system affected (cardiovascular, neurologic, musculoskeletal, etc.). Other studies investigated specific defects such as, orofacial clefts, limb reduction defects, and neural tube defects. The estimates for association between agricultural work and/or pesticide exposure in the studies reviewed by Garcia (1998) were modest, generally below 2.0. Elevated risks were identified for all malformations grouped together, and for specific types of defects such as central nervous system defects, orofacial clefts, and limb defects. Specific examples of epidemiologic studies of congenital malformations and pesticide exposure are provided below.

A large case-control study was conducted in 29 hospitals in Shanghai, China to investigate the association between several pregnancy outcomes and exposure to chemicals, pesticides or radiation just prior to or during pregnancy (Zhang et al., 1992). When all congenital malformations were grouped together, the odds ratio for exposure to pesticides during pregnancy was 1.8 (95% CI: 0.3-10.5). When malformations were categorized by body system, malformations of the central nervous system were much more common than expected among pesticide exposed mothers.

Shaw et al. (1999) examined the relation between four specific congenital malformations (orofacial clefts, neural tube defects, conotruncal heart defects and limb defects) and exposure to pesticides in a case-control study in California. The odds ratios were not elevated for any of the malformations studied and self-reported Occupationally related pesticide exposure. For use of pesticide products for household gardening by mothers or by professional applicators, however, the estimate of association with most of the studied malformations was ≥ 1.5. In addition, women who reported that a professional applied pesticides products for the control of pests in or around homes had increased risks for neural tube defects (OR=1.6, 95% CI 1.1-2.5) and limb defects (OR=1.6, 95% CI 1.0-2.7). Living proximal (within 0.25 miles) to an agricultural crop was also associated with increased risk for neural tube defects (OR=1.5, 95% CI 1.1-2.1).

In the Netherlands, a case-control study was conducted specifically to explore the risk of spina bifida associated with maternal Occupational exposures (Blatter, BM, et al., 1996). The 470 cases were identified from nine hospitals between 1980 and 1992 and the 456 controls were children born healthy in the hospitals during the same time period and also included children from the general population of the Netherlands. Data on Occupation and exposure to hazardous chemicals was collected through mailed questionnaires and personal interviews. The results indicated an increased risk for women working in agricultural Occupations (OR=3.4, 95% CI: 1.3-9.0) and for cleaning women (OR=1.7, CI:0.9-3.4). Further analyses classifying exposure by specific chemical or physical agents were unsuccessful in explaining the increased risk for women in agriculture and cleaning

occupations.

The relationship between limb reduction defects and parental involvement in an agricultural occupation or residence in an agricultural community has been explored in several studies. Schwartz et al. (1986) reviewed hospital birth records in a major agricultural region of California, examining all singleton births occurring in a four year period in one hospital. Offspring of agricultural working parents (one or both parents) were similar to offspring of non-agricultural working parents with regard to sex ratios, prevalence of low birth weight, stillbirth, and minor and major malformations, and neonatal deaths. The prevalence of limb reduction defects, however, was higher among offspring of agricultural workers (5.05 per 1000 total births versus 2.19 per 1000 total births).

To further explore the association between agricultural occupation and limb reduction defects, Schwarz and LoGerfo (1988) conducted a case-control study using California State birth records from 1982-1984. A total of 237 cases of limb reduction defects were identified and compared to 475 randomly selected controls. After adjusting for potential confounders, no association was identified between limb reduction defects and parental (either or both) work in agriculture. However, when the cases were limited to children with limb defects who also had at least one other anomaly, the odds ratio for parental involvement in agriculture was 1.6 (95% CI: 0.7-3.6). The authors state that this finding is compatible with the possible occurrence of a malformation syndrome induced by a specific

exposure related to agriculture. In addition, the authors classified exposure based on maternal residence in a county of high agricultural productivity versus minimal agricultural productivity. An elevated risk was identified for all limb reduction defects and limb reduction defects with at least one additional anomaly and maternal residence in a county of high agricultural productivity, with odds ratios of 1.7 (95% CI: 1.1-2.7) and 2.4 (95% CI: 1.2-4.7), respectively.

Lin et al. (1994) conducted a similar case-control study in New York using State Congenital Malformation Registry data. These authors also found a positive association for limb reduction defects with an additional defect and parental occupation pesticide exposure, but no association when all limb defects were combined. They did not find, however, an association with any type of limb reduction defect and residence in a farming or high pesticide use county.

Several design characteristics of these studies of limb reduction defects may help to explain the mixed findings. Inaccurate assessment of exposure was potentially generated by using birth certificate data to identify parental occupation and by combining maternal and paternal occupational exposure (Lin et al. 1994). Another problem with combining maternal and paternal exposure is that different etiologic mechanisms of teratogenesis operate in each parent. These problems generate non-differential misclassification of exposure, which bias the estimate of association toward the null. Two more common limitations of many studies of congenital malformations are: small sample sizes, due to the

rarity of the outcome, which leads to low power to find an effect; and inaccurate measurement of outcome, which also may lead to missclassification.

Low Birth Weight

Birth weight is an important predictor of infant health and has been evaluated as an endpoint in many studies of environmental contamination. Low birth weight can result from growth retardation in utero, preterm birth, or both. Low birth weight is defined as a birth weight less than 2500 grams (5 lbs. 8 oz.). Preterm birth is defined as birth before 37 completed weeks of gestation. An infant whose birth weight is below the 10^{th} percentile for gestational age is termed small-for-gestational-age (SGA). Over the past 14 years, the percent of low birth weight births for all races in the U.S. has increased steadily from 6.7% in 1984 to 7.6% in 1998 (Guyer et al. 1999). The percent low birth weight births by race in 1998 in the U.S. for non-hispanic white, black and hispanic was 6.6%, 13%, and 6.4% respectively.

Birth weight fluctuates in response to a variety of exogenous influences and the etiology and risk factors for preterm birth and intrauterine growth retardation are likely to be different, although some overlap should be expected (Berkowitz and Papeirnik, 1993). In their review of the epidemiology of preterm birth, Berkowitz and Papiernik (1993) considered the following as established risk factors: black race, single marital status, low socioeconomic status, previous low birth weight or preterm delivery, multiple second trimester spontaneous abortions, in vitro fertilization pregnancy, placental abnormalities,

gestational bleeding cervical and uterine anomalies, in utero diethylstilbestrol exposure, multiple gestations and cigarette smoking. They considered probable risk factors to be urogenital infections, cocaine use, no or inadequate prenatal care, and seasonality. Maternal weight gain during pregnancy and short interpregnancy interval are factors generally considered associated with low birth weight, however these factors were considered to be weakly or not associated with preterm birth by Berkowitz and Papiernik (1993).

Brook et al. (1989) conducted a prospective cohort study of approximately 1500 pregnant women in London, England to determine effects on birth weight (corrected for gestational age and adjusted for maternal height, parity, and baby's sex) of smoking, alcohol, caffeine, socioeconomic factors, and psychosocial stress. The results of the study indicated maternal smoking was the most important single factor in reducing birth weight. After controlling for the effect of smoking in the analysis, the effect of caffeine consumption became non-significant and the effect of alcohol consumption was significant only for the women who smoked. Other investigators have found that heavy alcohol consumption during pregnancy is consistently a risk factor for low birth weight (Faden and Braubard, 1994), although Berkowitz and Papiernik (1993)considered alcohol consumption as weakly or not associated with preterm birth.

Studies of the relation between employment related physical activity and low birth weight and preterm birth have produced mixed results. Several investigators have reported

increased risks for SGA or preterm birth for long working hours (>50 hours/week) (Tuntiseranee et al., 1998); , prolonged standing (Fortier et al., 1995; Ceron-Pereles P et al., 1996), and heavy lifting (Saurel-Cubizolles et al., 1985). Other investigators have failed to find associations between long working hours and other working conditions and SGA or preterm birth (Klebanoffet al., 1990; Berkowitz et al. 1983). Possible explanations for these inconsistent findings are variations with respect to study populations, outcome measures, exposure assessment, adjustment for confounding, biased recall, and insufficient power for detecting an effect.

Several studies have suggested that there exists protective sociocultural factors for low birth weight among Mexican-American women who have maintained a traditional cultural orientation (Scribner and Dwyer, 1989; Balcazar and Krull, 1999). This cultural orientation has been measured using descriptive cultural traits such as place of birth, generational status, and language use and is often termed acculturation status. Dwyer and Scribner (1989) found a one point increase in their acculturation scale (more U.S. orientation) was associated with a 1.31 increase in risk of low birth weight, after adjusting for age at interview, wealth, city size, years of education and smoking.

Epidemiologic studies have been conducted to examine associations between specific pesticides (OCPs, organophosphates), agricultural occupation, and residence in agricultural area and low birth weight and preterm birth. O'Leary et al. (1970) and Saxena et al. (1981) both reported a positive association between maternal blood DDE levels and

preterm birth. Procianoy et al. (1981) reported DDT levels in cord blood (but not maternal blood) were negatively correlated with birth weight. These studies were small and did not control for potential confounders. In a more recent study, Berkowitz et al. (1996) did not find any substantial difference in maternal serum DDE or PCB levels between cases with preterm birth and matched controls with term births. This study was also small (20 cases and 20 controls), however several potential confounders were controlled for in the study design through matching (maternal age, race, and pre-pregnancy body mass). One of the above studies (Saxena, et al. 1981) also measured blood beta-HCH and lindane concentrations and demonstrated higher blood levels of these OCPs in women with preterm birth compared to controls.

Two prospective studies (Fenster and Coye, 1990; Willis et al., 1993) examined maternal work in agriculture and birth weight in primarily Hispanic women in California. Neither study found an association between birth weight and work in agricultural. Both were studies of prenatal clinic populations and used self-reported work history during pregnancy to determine exposure status. The study by Willis et al. (1993) was unique in that, in addition to self-reported work history, plasma cholinesterase acitivity was measured and used for exposure classification to indicate exposure to organophosphate pesticides. It should be noted that in both of these study populations, the incidence of low birth weight was below the U.S. average.

Le Bacq and Charimari (1993) compared birth weight patterns in three different socio-

economic areas of Kenya, a commercial farming area, a rural area and an urban area. Mean birth weight and low birth weight proportions were not significantly different in the three areas. Preterm birth however, was significantly increased in the commercial farming area compared to the rural area.

In a large case-control study of occupational hazards and pregnancy outcomes in China, Zhang et al. (1992) found an increased risk (adjusted OR 2.9, 95% CI: 0.96-8.6) for SGA in women who had been exposed to pesticides during pregnancy. In addition, women who had been exposed to chemicals (other than pesticides) during pregnancy had an elevated risk for preterm birth (adjusted OR 1.4, 95% CI: 0.99-2.1). Savitz et al. (1989) reported an increased risk for SGA (adjusted OR 1.5, 95% CI: 1.1-2.1) among women exposed to pesticides at home, but not with exposure at work. In this study, preterm birth was not associated with pesticide exposure.

Exposure through occupation and through consumption of contaminated fish to the organochlorine compounds polychlorinated biphenyls (PCBs) has been associated with low birth weight and preterm birth. Fein et al. (1984) investigated the relationship between infant cord blood PCB concentrations, maternal consumption of Lake Michigan fish, and low birth weight and preterm birth. Both maternal fish consumption and cord serum PCB levels were associated with lower birth weight and earlier gestational age. Taylor et al. (1994) reported similar results from their study of women in New York with occupational exposure.

Age at Menarche

A woman's first menstruation is termed menarche, and the age at which this occurs generally varies from nine to fifteen years of age in developed countries. Age at menarche is an important marker of female growth and development. In developed countries, the age at menarche has decreased over the last century, though this trend has stabilized during the last several decades (Dann and Roberts, 1993). In some developing countries, the trend toward earlier menarche has occurred more recently and is still apparent. A recent study of women in China has demonstrated a decrease in the age at menarche of 2.8 years over the last 40 years, from 16.5 to 13.7 years of age (Graham et al., 1999). This trend is thought to partly be due to improved nutrition and socio-economic conditions in China.

Various factors known to be associated with age at menarche include nutrition, genetics, body height, body weight, physical activity, social class, altitude of residence, sleep patterns, family size and health status (Koprowski et al., 1999). The dietary and physical determinants of menarche have been the most studied (Meyer et al., 1990; Koprowski et al., 1999; Rao et al., 1998; St. George et al., 1994). In a large multiethnic cohort of school girls in California, Koprowski et al. (1999) found that taller girls experienced menarche earlier than shorter girls. In addition, girls with a higher body mass index (BMI) experienced menarche earlier than girls with a lower BMI. Meyer et al. (1990) reported a significant inverse relationship between body weight and menarche, but not for body fat. Analyses of dietary intake have not demonstrated a relationship between specific nutrients (protein, fat, carbohydrate) and age at menarche (Koprowski et al., 1999; Meyer et. al.,

1990). Some studies demonstrated an association between higher total energy intake and earlier age at menarche and others have not (Koprowski et al., 1999). Rao et al. (1998) prospectively compared girls in a low socio-economic group (LSE) to girls in a high socioeconomic group (HSE) in India and found the LSE girls had a significantly higher age at menarche (15.4), and lower attained weight, height, skinfold thickness at triceps and body fat. These authors also found that the time between peak height velocity and onset of menarche was similar (1.5 yr.) in both socio-economic groups, as was the mean weight at menarche.

Malina et at. (1977) compared age at menarche in six different areas of Mexico. The median ages of menarche ranged from 12.55 in the urban Tampico area to 14.27 in the rural Oaxaca region. The girls from the four urban areas studied attained menarche at an earlier age than the girls from the two rural areas. These differences were thought to be related to the poorer socio-economic and nutritional circumstances in the rural regions. A similar urban versus rural difference in age at menarche was noted by researchers in China (Graham et al., 1999).

Information on pesticide exposure or and age at menarche is very limited. One study conducted in China did examine pesticide exposure and its relationship to age at menarche. This large study was based on a community survey of about 13,000 women administered in 1993 (Graham et al., 1999). The following factors were all found to be associated with age at menarche: year of birth, literacy status, county of residence, amount

of physical labor, general health status, pesticide exposure before age at menarche, and drinking water source. Pesticide exposure was categorized as either exposure to an endocrine-disruptive pesticide, a non-endocrine-disruptive pesticide, 'other' type of pesticide, or no exposure. Specific names of pesticides were not provided in the article. After adjustment for potential confounders, exposure to endocrine-disruptive pesticides had no significant effect on the age at menarche, however, exposure to non-endocrine-disruptive pesticides was associated with higher age at menarche compared to women with no pesticide exposure. The authors state that because 33% of exposed women reported 'other' as a type of pesticide, these results are inconclusive. Use of pond water was associated with a higher age of menarche than use of tap or well water. The authors suggest this reflects socio-economic status, as pond water users are mostly rural residents. However, it may also be related to pollutants in pond water from farming activities.

Blank et al. (2000) investigated age at menarche among girls exposed *in utero* and postnatally to polybrominated biphenyl (PBB), an organohalogen compound. The authors found that breastfed girls exposed to high levels of PBB in utero ($\geq$ 7 ppb) had an earlier age at menarche when compared to breastfed girls exposed to lower levels of PBB *in utero* or girls who were not breastfed. A possible mechanism for the effect of PBB on age at menarche suggested by the authors was disruption of endocrine function.

Wilcox et al. (1995) investigated age at menarche among 52 daughters of daughters whose mothers' had taken diethylstilbestrol during pregnancy (third generation effects).

Third generation daughters who were not exposed in utero were used for comparison. Using a life-table analysis which estimated the chance of menarche within each year of age, the estimated mean age at menarche was identical for the exposed and unexposed (12.7 years) third generation daughters.

Age at Menopause

Menopause is defined by the World Health Organization as the permanent cessation of menstruation following loss of follicular activity (Sowers and La Pietra, 1995). The age of menopause is assigned by convention retrospectively, after 12 months of amenorrhea not due to other factors such as pregnancy or lactation. The average age at natural menopause in U.S. and European populations ranges from 48 to 52 years. The age at natural menopause in developing countries is approximately four years earlier than for developed countries (Gonzales et al., 1997). In a large prospective cohort study of 2570 U.S. women, the median age at menopause was estimated to be 51 years with a range of 35 to 58 years (McKinlay et al., 1992). There was a deceleration in age at menopause from 45.5 years in 1840 to about 48 years in 1940, however little research has been conducted on the secular trend in age at menopause since that time (Flint, 1997). The perimenopause is defined as the period (2-8 years) prior to the menopause, and the subsequent 1 year of amenorrhea following menopause.

Natural menopause is thought to be due to the depletion of ovarian follicles responsive to the gonadotropins and the consequent decrease of estrogen and progesterone

concentrations (Sowers and La Pietra, 1995). Another view is that age-related changes in the central nervous system triggers the menopausal transition (Wise et al., 1996). The human ovaries contain at birth about 3,000,000 primordial follicles (germ cells surrounded by pregranulosa cells), which falls to about 300,000 at puberty (Vermeulen, 1993). During a woman's reproductive life, the primordial follicles are recruited to grow, differentiate and synthesize estradiol and many will undergo atresia. Little is known about the factors which regulate proliferation and that govern the organization of cells into discrete follicles. The time to depletion of all follicles depends on the initial number at birth and on the factors that determine the rate of loss. There is a doubling of the rate of loss of follicles in women around the age of 35, when about 25,000 primordial follicles remain (Wise et al., 1996). Hormonal changes that occur during the menopausal transition include high estrogen levels relative to progesterone, progesterone levels drop, FSH levels increase, LH becomes nonresponsive to FSH, and inhibin levels decrease (Sowers and La Pietra, 1995).

Some investigators hypothesize that the hypothalamus is the 'pacemaker' that initiates the changes leading to menopause (Wise, et al., 1996). In this view, acceleration of follicular loss reflects a change in the pattern of neuroendocrine messages that govern the dynamics of follicular recruitment, growth and differentiation. These changes in neuroendocrine messages may be due to deterioration in communication among the neurotransmitters that regulate Gonadotropin Releasing Hormone secretion. The deterioration of the 'biological clock' (neurotransmitter rhythms) with aging may be related to these changes.

It has been suggested that the age at natural menopause is a biological marker of health and aging (Snowdon et al., 1989). Snowden et al. (1989) investigated the relation between age at natural menopause and all-cause mortality in a sample of 5,287 Seventh-day Adventist women in California. The age-specific results of this study indicated that the odds ratio of death (adjusted for potential confounders) decreased with increasing age at natural menopause until age 55. Women with natural menopause before age 40 were three times more likely to have died from any cause, compared to women with natural menopause at ages 50 to 54 (the referent group). Several specific causes of death were examined for associations with early menopause. Women experiencing menopause before age 40 were at increased risk for coronary heart disease and stroke, and at decreased risk for cancer. Estrogen is thought to protect against coronary heart disease in premenopausal women (Gorodeski, 1994). Osteoporosis is another condition associated with menopause and loss of the protective effects of estrogen (Torgerson et al., 1994). Breast and endometrial cancer are associated with a later age at menopause and ovarian cancer is associated with earlier age at menopause.

Various studies have identified many factors related to either early or late age at menopause, but often the results have been inconsistent. The difference in mean age at menopause for most of the factors is one to two years. The genetic, socio-demographic, reproductive, and lifestyle factors found to be associated with early age at menopause include: maternal early menopause, African-American ethnicity, poor nutrition, lower socio-economic status, rural residence, women living in southern or north central U.S.,

living at high altitude, left-handedness, cancer chemotherapy, autoimmune disorders, low BMI, nulliparity, early age at menarche or late age at menarche, shorter menstrual cycles, high interval between births, never using oral contraceptives, lower meat, cholesterol, fat, and coffee consumption, higher consumption of calcium and soy products, weight reduction dieting, no alcohol consumption, and smoking (Leidy, 1990; Torgerson et al., 1994; Gonzales et al., 1997; Nagata et al., 1998; Do et al., 1998; Bromberger et al., 1997; Greendale and Sowers, 1997). Several genetic chromosomal abnormalities including Edward's syndrome, Down's syndrome, and Turner's syndrome are associated with early ovarian failure and early menopause (Flint, 1997).

The factor most consistently associated with earlier age at menopause is smoking (Flint, 1997). Multiple studies have documented a deceleration of 1.5 to 2 years in age of menopause for smokers compared to non-smokers. The biologic mechanism for this association of smoking and earlier menopause is thought to be a toxic effect on the ovary of the aromatic hydrocarbons in cigarette smoke, leading to accelerated aging of oocytes (Flint, 1997). Other possible explanations are that nicotine affects estrogen production and catabolism and estrogen receptor binding is decreased by smoking (Greendale and Sowers, 1997)

Inconsistency among factors associated with a later age at menopause is also evident in the literature. Many factors are the converse of factors associated with early menopause. Factors described in the literature as associated with a later menopause are: higher socio-

economic status, later birth cohort, lower BMI, higher BMI, higher parity, history of irregular menses, early menarche, and use of oral contraceptives (Flint, 1997; Bromberger et al., 1997; Greendale and Sowers, 1997; Van Noord et al., 1997)

Snieder et al. (1998) conducted a large twin study (275 monozygotic and 353 dizygotic twin pairs) in the United Kingdom to assess the genetic contribution to individual differences in age at menopause and age at menarche. The investigators found that both early and late menopause were significantly influenced by genetic factors. Using quantitative genetic modeling, the estimated heritability for age at menopause and age at menarche (adjusted for confounders) was 63% and 45% respectively. This means that 63% of the variation in age at menopause can be explained by genetic factors. The authors did not observe any correlation between age at menarche and age at menopause. In addition, they determined the influence of dominance genetic effects (as opposed to additive effects) was greater on age at menarche than for age at menopause. Consistent with this finding, Van Noord et al. 1997 found age at menarche and age at menopause were not related in their large cohort study.

Although no epidemiologic studies were identified in the literature which specifically address OCPs or pesticides and age at menopause, there are two studies which document the presence of OCPs in human ovarian follicular fluid (Jarrel et al., 1993; Baukloh et al., 1985). In their study of women attending reproductive endocrinology clinic, Gerhard et al. (1998) reported that total OCP burden was associated with increased blood levels of FSH

and prolactin and decreased levels of testosterone. The authors concluded that the ovaries and pituitary are sensitive to OCPs.

Additional studies in animals suggest the potential for toxic ovarian effects. DDT and gamma-HCH have been shown to depress bovine oocyte maturation in vitro (Alm et al. 1998). Dieldrin exposed female rats exhibited decreased serum FSH and LH levels after the first and second week of exposure and increased levels in the third week. In addition, severe hemorrhage and lymphocytic infiltration in the graafian follicles occurred (Ateia et al. 1990). Evidence from animal studies indicates HCB is toxic to the mammalian ovary and may interfere with mechanisms regulating ovarian steroidogenesis (ATSDR, 1994). HCB is also thought to have direct effects on the pituitary or hypothalamus mediation of the endocrine system (ATSDR, 1994). Exposure of the air breathing catfish to 1 ppm of BHC resulted in partial disruption of ovarian follicles, vacuolation in the cytoplasm of germinal cells, reduction in the number of matured ovum and secondary oocytes, and loss of interfollicular connective tissue (Hazarika and Das, 1998).

CHAPTER III

METHODOLOGY

Study Design

This cross-sectional epidemiologic study used an existing national data base (HHANES) to investigate associations between exposure to specific organochlorine pesticides or their metabolites, and reproductive endpoints of interest. Information on many known confounders of reproductive endpoints such as smoking and alcohol consumption were available in the data base. This data base offered the opportunity to assess the proposed relationships in a large sample with good statistical power.

The HHANES was conducted by the National Center for Health Statistics in 1982-1984.

The goals of the survey were: to conduct a study of the health and nutritional status of the Hispanic population; to produce information useful for public health planners and researchers; and to stimulate further research and investigations on Hispanic health issues (Delgado, HHANES: Methodological Considerations). HHANES was designed to study major chronic disease conditions, prevalence of immunizations, alcohol consumption, cigarette consumption, drug abuse, nutritional status, food consumption patterns, and environmental exposure to lead and selected pesticides.

HHANES was not designed as a national Hispanic survey but was a survey of three Hispanic subgroups of the population in selected areas of the U.S. The three Hispanic subgroups and the geographic areas where they were sampled are: Mexican Americans residing in Arizona, California, Colorado, New Mexico and Texas; Cuban Americans residing in Dade county, Florida; and Puerto Ricans residing in the New York City metropolitan area including parts of New York, New Jersey, and Connecticut. The total survey universe included approximately 76% of the 1980 Hispanic-origin population in the U.S.

HHANES utilized a complex, multistage, stratified sampling process. From the three Hispanic universes, primary sampling units (PSU's) were identified. The PSU's were stratified to group similar counties and a PSU was selected from each stratum, then segments and households were selected from each PSU. Households which had at least one Hispanic member were eligible to participate. Sample persons, aged 6 months to 74

years, were selected from family units after screening selected households for eligible Hispanic families. Therefore, some non-Hispanic persons were selected for study because they were members of an Hispanic family unit.

The data collection methods utilized in the HHANES included: interviews, direct physical examination, anthropometry, diagnostic testing, and laboratory analyses. Interviews were administered both in the household and within a mobile examination center, and all examinations and testing were conducted in the mobile examination center located in the community.

The total number of people sampled was 15,924. Of this total, 11,653 people completed both the interview and examination portions of the survey. This resulted in the following response rates by Hispanic group: Mexican American 75.4%, Cuban 60.5%, and Puerto Rican 74.9%. Of the 11,653 people completing the survey, 2,010 were children aged 6 months to 11 years, and the remaining 9643 were adolescents and adults aged 12 to 74 years.

The pesticide analyses were conducted with the half sample of adults (20-74 years of age) who were not chosen for the glucose tolerance test and a half-sample of adolescents aged 12-19 years. This selection process resulted in 3,956 individuals chosen for the pesticide analyses. Of these 3,956 individuals, 861 (22%) were missing pesticide data, leaving 3,095.

For the purposes of the present study, persons of non-Hispanic origin and non-white race were excluded. Of the individuals with pesticide data, there were 166 non-Hispanics, 126 Hispanic but non-white race, and 11 were missing data on either race or Hispanic origin, therefore 303 people were excluded. This resulted in a total sample for pesticide analyses of 2,792 individuals, of which 1,599 were females.

Exposure Assessment

The concentrations of OCPs in serum are highly correlated with their concentrations in adipose tissue; therefore, serum assays are useful measures of human body burden of OCPs in epidemiologic studies (Stellman et al., 1998). Non-fasting blood samples were obtained from subjects at the examination center. All serum OCP analyses were conducted at the Environmental Protection Agency's Pesticide Monitoring Laboratory in Bay St. Louis, Mississippi. The analytical methodology used involved extracting the OCP from serum with hexane (no clean up step was included), and analyzing by packed column gas chromatography (GC) with an electron capture detector (EPA document, NHEERL). A Quality Assurance Project Plan was incorporated into the specific SOPs written by the EPA Bay St. Louis Pesticide Monitoring Laboratory. At least 15% of the serum samples showing detectable residues were confirmed using a Hall Detector, to assure that the electron capture detector response was coming from a chlorinated organic compound. In addition, 5% of serum samples having detectable residues were confirmed using a Finnegan GC/mass spectrometry system. When interference from other compounds was suspected, additional cleanup was performed.

The EPA laboratory looked for a total of eighteen organochlorine pesticides and metabolites. These specific pesticides/metabolites, along with their limits of quantitation in parts per billion (ppb) in parentheses, are as follows: *trans*-nonachlor (1), heptachlor epoxide (1), oxychlordane (1), heptachlor (2), alpha-HCH (1), beta-HCH (1), gamma-HCH (1), delta-HCH (1), aldrin (2), endrin (2), dieldrin (1), o,p'-DDT (2), p,p'-DDT (2), o,p'-DDE (1), p,p'DDE (1), o,p'-DDD (2), p,p'-DDD (2), and hexachlorobenzene (1). Only seven of the above mentioned pesticides were detected in more than 1% of the HHANES serum samples: p,p'-DDT, p,p'-DDE, dieldrin, oxychlordane, beta-HCH, hexachlorobenzene, and trans-nonachlor. Since these seven organochlorine pesticides/metabolites are identified in the toxicologic literature (see literature review) as potential HAA's and were detected in an adequate proportion of the sample for statistical analysis, they were chosen as the exposures of interest for this epidemiologic study.

Assessment of Reproductive Endpoints

A reproductive history for each female participant aged 12-74 years was obtained as part of a detailed medical interview at the time of examination. Therefore, the reproductive endpoints of interest were self-reported and not verified by medical records.

The woman was taken to be the unit of analysis, not pregnancies, as this would have violated the assumption of independent outcomes in the analysis, when multiple babies are born to the same mother. The reproductive endpoints spontaneous abortion, stillbirth, low-birth weight ($\leq$ 2,500 grams or 5.5 lbs), and birth defects, were categorized as either

present (ever) or absent (never) for each woman's entire reproductive history. Birth defects for all pregnancies were further categorized by body part or system involved as follows: heart, eyes, ears, mouth or throat, stomach or intestines, kidneys or urinary system, muscles, bones, or joints, brain or nervous system, as this was the manner in which the information was collected from the mothers.

Women were asked how old they were when their menstrual cycle started and how old they were when their menstrual cycle stopped entirely (not counting during pregnancy). The responses to these questions were used to determine age at menarche and age at menopause.

Information on parity and use of oral contraceptives was also ascertained, since these variables were considered potential confounders. Diabetes is a medical risk factor for many adverse reproductive outcomes, therefore history of diabetes was also ascertained. It was not possible however, to distinguish between Insulin Dependent Diabetes Mellitus (IDDM), Non-Insulin Dependent Diabetes Mellitus (NIDDM), or gestational diabetes, using the information from the questionnaire.

Assessment of Demographic and Lifestyle Variables

The following demographic and lifestyle variables potentially associated in the literature with either body burden of OCPs of interest or a reproductive endpoint of interest, were ascertained from the interview questionnaire: age at the time of examination, Hispanic

group (Mexican American, Puerto Rican, Cuban), marital status (ever or never), education (> 8th grade, ≤ 8th grade), family income (≥ $20,000/yr, < $20,000/yr), cigarette smoking (ever or never at least 100 cigarettes in entire life), alcohol consumption (ever or never), born in the U.S. (yes or no), current residence in central city area of a Standard Metropolitan Statistical Area (SMSA) (yes or no), farm work either paid or unpaid (ever or never), season of examination (winter = December, January, February; spring = March, April May; summer = June, July, August; fall = September, October, November). Alcohol consumption was determined from the food frequency questionnaire. Two physiologic variables potentially associated with body burden of OCPs were also ascertained: serum cholesterol level, which was the only available proxy measure for blood lipid content, and Body Mass Index (BMI), which is calculated as weight in kilograms divided by the square of the height in meters.

Statistical Methods

The Statistical Analysis System (SAS) software package for the mainframe computer was used to conduct all statistical analysis. In order to meet the objectives of the study, the statistical analysis was divided into four phases: 1) descriptive statistics were determined for all study variables, 2) a series of logistic regression models were fit to determine the socio-demographic factors, lifestyle factors, and other serum OCP variables associated with detection of each OCP in serum and a multiple regression model was fit for the continuous serum DDE variable, 3) a series of logistic regression models were fit to determine if detection of OCPs in serum was associated with each reproductive endpoint,

and 4) analysis of variance models were fit to compare mean age at menarche and menopause by serum OCP level.

Descriptive Statistics

To describe the demographic and lifestyle characteristics of the sample and the prevalence of reproductive endpoints, frequencies and proportions by Hispanic group, for all categorical demographic, lifestyle or reproductive endpoint variables, were determined. For continuous variables such as age, serum cholesterol level, BMI, serum OCP levels, age at menarche and age at menopause, the mean, standard deviation, and range were determined. Statistical tests to determine whether the continuous variables were normally distributed were also conducted. The chi-square statistic (for dichotomous variables) and analysis of variance (for continuous variables) were used to test for significant differences among the three Hispanic groups.

Logistic Regression Model Development General Strategy

The logistic regression modeling strategy described below was employed to determine 1) the socio-demographic factors, lifestyle factors, and other serum OCP variables associated with detection of each OCP in serum, and 2) whether an association exists between detection in serum of the OCPs or a history of farm work, and the reproductive endpoints of interest. *The major difference in the modeling strategy for these two objectives was in the designation of the dependent variable.* To determine if there were demographic, lifestyle, or other serum OCP variables associated with the detection of an

individual OCP in serum, models were fit with each dichotomous (above the detection limit [ADL]or below the detection limit [BDL]) serum OCP variable as the dependent variable. To determine if there were associations between serum OCPs, or farm work and reproductive endpoints, models were fit with the dichotomous reproductive endpoints as the dependent variable. The farm work variable was considered an exposure variable in the reproductive endpoint models.

For both of the objectives, the modeling strategy involved three steps. First, bivariate screening was conducted. Second, individual models were fit for each exposure variable (the seven OCP variables and the farm work variable). Third, important exposures were considered simultaneously in a single model.

Logistic Model Development to Determine Factors Associated with Detectable OCPs in Serum

Step 1. Bivariate Screen

Bivariate screening was conducted with the goal of reducing the number of independent variables with a minimum loss of prognostic information. All independent variables were screened twice. First, the dependent dichotomous variable (either an OCP variable or a reproductive endpoint variable) was cross-tabulated with each independent variable to determine if there were sufficient observations in each cell of the table for statistical analysis. If there were fewer than three observations in a cell, and categories could not be combined, the independent variable was excluded from further analyses. Second, those

remaining independent variables were screened using a logistic regression model which also included the continuous variable age at examination. Those independent variables with a $p \leq 0.25$ were selected for further analyses.

Step 2a

All potential explanatory (independent) variables with a $p \leq 0.25$ (excluding OCP and farm work variables, which are addressed in Step 2b) identified in the screening analyses were included in a multivariate logistic regression model. The purpose of this model was to determine which explanatory variables are associated with the dichotomous dependent OCP variable while adjusting for the other explanatory variables. Those explanatory variables with a $p \leq 0.15$ were considered to be important explanatory variables to be included in Step 2b.

Step 2b

For each *independent* OCP variable and the farm work variable (ie. the exposure variables), found in screening to be associated with the *dependent* OCP of interest at $p \leq 0.25$, a separate model was run. Each separate model also included the same set of significant ($p \leq 0.15$) independent variables determined in Step 2a. Separate models for each *independent* OCP variable were fit because of colinearity among many of the OCP variables, which might mask important individual effects. Independent variables having a $p \leq 0.05$, or important confounders (i.e., any model variable that resulted in a change in odds ratio of greater than 10%), were retained in the model. The Hosmer and Lemeshow Goodness-of-Fit (GOF) test was used to assess model fit.

Step 3

After separate models for each *independent* OCP variable were completed as described above, a single multivariate logistic model was fit that included all important independent OCP variables ($p \leq 0.15$) and explanatory variables ($p < 0.05$) as identified in Steps 2a and 2b. The purpose of this analysis was to account for associations among the serum OCP variables. A final model was created including all explanatory variables meeting the selection criteria of $p \leq 0.05$.

Multiple Regression Model Development to Determine the Factors Associated with Level of DDE in Serum

Serum DDE level (cholesterol adjusted and natural log transformed) was analyzed as a continuous variable since greater than 95% of the sample had detectable levels of DDE in their serum. Therefore, multiple regression was used to determine the factors associated with the level of DDE in serum. Multiple regression model development for serum DDE proceeded in a similar fashion as described for logistic regression above and included the same bivariate screening, followed by Step 2a, Step 2b, and Step 3.

Logistic Regression Model Development to Determine Associations Between Reproductive Endpoints and OCPs in Serum and Farm Work

Step 1 Bivariate Screen

Bivariate screening was conducted as described above with the reproductive endpoints as the dependent variables. Then model development proceeded as follows.

Step 2a

All independent variables with a $p \leq 0.25$ (excluding OCP and farm work variables, which are addressed in Step 2b) identified in the screening analyses were included in a multivariate logistic regression model. The purpose of this model was to determine which independent variables are associated with the dependent reproductive endpoint variable while adjusting for the other independent variables. Those independent variables with a $p \leq 0.15$ were considered to be important explanatory variables to be included in Step 2b. It was decided *a priori* to force the Hispanic group variables into all models due to the importance of adjusting for important unmeasured differences between the Hispanic groups which may act as confounders. Age at examination was expected to be associated with both serum OCP detection and reproductive endpoints and therefore was also considered a critical potential confounder and was forced into all models. Serum DDE level (cholesterol adjusted and then natural log transformed) was included in all analyses as a continuous variable.

Step 2b

For each independent OCP variable and the farm work variable (exposure variables) found in bivariate screening to be associated with the dependent reproductive endpoint variable of interest at $p \leq 0.25$ (or if the odds ratio was ≥ 2.00), a separate model was fit. Each separate model also included the same set of significant ($p \leq 0.15$) explanatory variables determined in Step 2a, and age at examination and Hispanic group variables. Separate models for each significant exposure variable were fit because of colinearity among many

of the OCP variables, which might mask important individual effects. Independent variables having a $p \leq 0.10$, important confounders (i.e., any model variable that resulted in a change in odds ratio for the OCP variable of greater than 10%), OCP variables with an odds ratio ≥ 2.00 (even if the p-value was above 0.10), and age at examination and Hispanic group variables, were retained in the final models. Interaction (effect modification) between significant exposure variables and significant explanatory variables was also assessed. Interaction terms with a $p \leq 0.10$ were retained in the final model. The Hosmer and Lemeshow Goodness-of-Fit (GOF) test was used to assess model fit.

Step 3

After separate models for each exposure variable were completed as described above, a single multivariate logistic model was fit that included all confounders (as defined in Step 2a), and significant ($p \leq 0.10$) exposure variables and interaction terms as identified in Step 2b, and interaction terms for multiple significant exposures. The purposes of this analysis were to account for associations among the exposure variables and to investigate interactions among the important exposure variables. A final model was fit including all exposure variables, explanatory variables, and interaction terms meeting the selection criteria of $p < 0.10$. This selection criteria was less stringent than that used in the logistic regression models to determine factors associated with detection of the serum OCPs, so that important exposures would not be missed.

Analysis of Variance Models

Analysis of variance (AOV) was employed to compare mean age at menarche and mean age at menopause among women by serum OCP level, and among women who had ever or never done farm work. To explore differences in mean age at menarche by level of serum OCPs, serum OCP levels were categorized in several ways including above and below the detection limit, a median split above the detection limit, and by whole number ppb categories (eg. 1.00-1.99 ppb). The whole number ppb categories were included to evaluate higher exposure ranges than the median split provided. For beta-HCH and DDT there was an adequate sample size to evaluate dose response using quartiles (ADL), and for DDE, quintiles (ADL). In all exposure categorization schemes, the referent group was women with serum OCP level below the detection limit. First, a single factor AOV model which included age at examination, was fit separately for each exposure variable. All other dichotomous independent variables were also screened using AOV models to identify potential confounders. Continuous independent variables such as BMI and serum DDE were screened using a multiple regression model with age at menarche and age at menopause as the dependent variable. Independent variables (excluding the exposure variables) identified in screening with $p \leq 0.25$ were then included together in a multiple factor AOV model. Those independent variables with $p < 0.10$ were considered to be important potential confounders to be included in the adjusted models for each separate exposure variable. Interaction with other OCPs was investigated for exposure variables (OCPs and farm work) which were significantly associated with age at menarche or menopause.

CHAPTER IV

RESULTS

DESCRIPTIVE ANALYSIS RESULTS

Demographic and Lifestyle Variables

There were significant differences among the three Hispanic groups for all demographic and lifestyle variables examined, except for smoking status (Table 4.1). Comparison of age at examination across age groups and Hispanic groups, reveals a significant difference with the Cuban group containing fewer persons in the 12-20 years category and more persons in the over 50 years category. Almost 60% of the total sample had attained at least a 9th grade education; however, 71% had a household income less than $20,000/yr in 1982-84. Three fourths of the total sample had ever been married. Roughly half of the Mexican and Cuban groups resided outside of a SMSA-central city area, whereas only

12% of the Puerto Rican group did. This is not surprising, since the Puerto Rican sample was from the greater New York City area. For the total sample, approximately 50% were born in the U.S.; however, only 8% of the Cuban sample was born in the U.S. Significantly more Mexican Americans had ever done farm work (21%), compared to Cubans (6%) or Puerto Ricans (5%). Slightly less than a third of the sample had ever smoked more than 100 cigarettes in their life and slightly more than a third ever consumed alcohol. The Mexican American sample was well distributed among the four seasons of examination (winter, spring, summer, fall); however, there were no Cubans examined in the summer or fall and Puerto Ricans were examined predominantly in the summer and fall.

Serum cholesterol level and Body Mass Index (BMI) (physiologic variables which may be related to serum OCP level) were significantly different among the three Hispanic groups (Table 4.2). Serum cholesterol values were not normally distributed and required natural log transformation for parametric analysis. The BMI values were normally distributed. The Cuban group had a lower serum cholesterol and lower BMI compared to the others. In contrast, there was no significant difference among the Hispanic groups for body weight. BMI is an index of a person's weight in relation to height. A BMI greater than 25.0 has been associated with excess morbidity and mortality from coronary heart disease and diabetes in women under 65 years of age (Willet et al., 1999). A BMI greater than 30.0 in women indicates obesity. (DeBruyne and Rolfes, 1989). The mean BMI for the total sample was 24.61, indicating that the sample on average was heavier than ideal (BMI of

22.4), but not overweight.

OCP Variables

DDE was detected in the serum of 97.5% of the total sample (Table 4.3) with concentrations ranging from 1.02 - 228.22 ppb. OCPs partition among various tissues depending on their lipid content. Blood lipids increase following a fatty meal, leading to a rise in serum OCP levels. Phillips et al. (1989) reported that when serum OCP levels were expressed on a lipid weight basis, there was not a significant difference between fasting and nonfasting serum samples. Since the HHANES serum samples were not fasting samples, it was decided to adjust DDE levels for the lipid content of the serum for further analyses. The only blood lipid component measured in the HHANES was serum cholesterol. Therefore, serum cholesterol values were used as a surrogate for the lipid content, thus providing a partial adjustment. The formula used to adjust serum DDE level (micrograms/liter) for serum cholesterol level (milligrams/deciliter) was the following: ((DDE value*0.1)/serum cholesterol value)*1000 = normalized DDE level (micrograms DDE/gram of cholesterol). Results of the normal probability plot indicated the cholesterol adjusted serum DDE values were not normally distributed. A natural log transformation was used to produce a normal distribution for cholesterol adjusted serum DDE. Those few with serum DDE BDL, were assigned a value of one half the detection limit (0.5 ppb).

The proportion of detectable serum values for the other OCPs was considerably less than for DDE (Table 4.3), ranging from 18.2% for beta-HCH to 2.8% for dieldrin. The mean

serum concentration for those with detectable levels of these OCPs was low, ranging from 1.66 ppb for oxychlordane to 5.19 ppb for DDT (Table 4.3). The low number of detectable values resulted in a skewed distribution unsuitable for parametric analysis. Therefore, these OCP variables were dichotomized for further analyses as above the detection limit (ADL) or below the detection limit (BDL).

Reproductive Endpoint Variables

Mean gravidity (number of pregnancies) and parity (number of live births) were significantly different among the Hispanic groups (Table 4.4), with the Cuban group having on average less pregnancies and live births than the Mexican or Puerto Rican groups. The frequencies and proportions of women ever or never experiencing the selected reproductive endpoints of interest by Hispanic group are provided in Table 4.5. Approximately one third of the total sample which had ever been pregnant, had ever experienced a spontaneous abortion, and this proportion was similar across the Hispanic groups. The proportion of women who had ever been pregnant and ever experienced a stillbirth ranged from 1% to 6% across the Hispanic groups, but the actual number of Cuban and Puerto Rican women who experienced a stillbirth was quite small. Among women who had ever had a live birth, the proportion experiencing a low birth weight birth was similar among the Hispanic groups and was 13% for the total sample. The proportion of parous women who had ever had a child born with a birth defect was 8% for all Hispanic groups combined, and was not significantly different among the Hispanic groups. The proportion of women who had ever used oral contraceptives differed significantly

across the Hispanic groups, with a lower proportion of Cuban women (28%) ever using oral contraceptives, compared to 43% of Mexican women and 34% of Puerto Rican women.

To compare the prevalence of the adverse reproductive endpoints of interest in the sample to the prevalence of these endpoints in the literature, the total number of spontaneous abortions and stillbirths were determined and divided by the total number of pregnancies. Likewise, the total number of low birth weight births and birth defect births were determined and divided by the total number of live births. The prevalence of the selected adverse reproductive endpoints by Hispanic group are provided in Table 4.6. With the Hispanic groups combined, the prevalence of spontaneous abortion, stillbirth, low birth weight and birth defect births among all pregnancies and live births was 14.14%, 1.40%, 4.79% and 2.53%, respectively.

Of the 1,599 women aged 12 to 74 years, 40 reported that their menstrual cycles had not started yet, and there was missing information for 13 women. There was one woman who reported age at menarche of 40 years, and this response was considered an outlier and excluded from analysis. For the remaining 1,545 women, the reported age at menarche ranged from 7 to 18 years, with a mean of 12.56 years, and a normal distribution (Table 4.4). The mean age at menarche was significantly different among the Hispanic groups, with the Puerto Rican group having the youngest mean age at menarche of 12.30 years. The objective for this study was to investigate any change in the average age at menarche,

either earlier or later, associated with exposure to OCPs or farm work. Therefore, quartiles and examination of the data distribution were used to assist in categorizing age at menarche into early and late groups (approximately the first and fourth quartiles) with the middle two quartiles serving as the referent group. This resulted in an 'early menarche' category of $\leq$ 11 years, a 'late menarche' category of $\geq$ 15 years, and a referent category of 12-14 years (Table 4.5). This categorization of age at menarche was used in logistic regression analyses, with early and late menarche as separate dichotomous outcome variables.

Of the 393 women reporting that they had experienced menopause, 173 were excluded from analysis due to a history of surgical menopause (hysterectomy, oophorectomy or both). There were 23 women who reported age at menopause of less than 35 years, and these responses were considered outliers and were excluded from analysis. For the remaining 219 women, the reported age at menopause ranged from 35 to 57 years, with a mean of 48.17, and a normal distribution (Table 4.5). The mean age at menopause was significantly different among the Hispanic groups, with the Mexican group having the youngest mean age at menopause of 47.30. The objective for this study was to investigate any change in the average age at menopause, either earlier or later, associated with exposure to OCPs or farm work. Therefore, quartiles and examination of the data distribution were used to assist in categorizing age at menopause into early and late groups (approximately the first and forth quartiles) with the middle two quartiles serving as the referent group. This resulted in an 'early menopause' category of $<$ 45 years, a 'late

menopause' category of > 50 years, and a referent category of 45-50 years (Table 4.5). This categorization of age at menopause was used in logistic regression analyses, with early and late menopause as separate dichotomous outcome variables.

LOGISTIC REGRESSION RESULTS - FACTORS ASSOCIATED WITH DETECTION OF ORGANOCHLORINE COMPOUNDS IN SERUM

Beta-HCH

Bivariate Screen

Screening identified the following variables (adjusted for age at examination and shown with their crude odds ratio and 95% confidence interval) as meeting the criteria for further analysis ($p < 0.25$): age at examination (1.07; 1.06-1.08), Cuban compared to Mexican (0.54; 0.35-0.83), Puerto Rican compared to Mexican (0.15; 0.09-0.26), serum cholesterol (2.16; 1.06-4.42), BMI (1.02; 0.99-1.05), $\leq 8^{th}$ grade education (1.69; 1.26-2.27), ever married (3.01; 1.64-5.52), diabetes (1.37; 0.84-2.25), alcohol drinker (0.68; 0.50-0.92), spring compared to winter (0.76; 0.52-1.12), summer compared to winter (0.50; 0.33-0.76), fall compared to winter (0.58; 0.39-0.85), ever farm work (1.62; 1.15-2.27), DDT ADL (5.50; 3.95-7.65), dieldrin ADL (8.95; 4.19-19.15), hexachlorobenzene ADL (2.96; 1.62-5.42), *trans*-nonachlor ADL (6.23; 3.86-10.21), DDE (3.07; 2.48-3.80).

Multivariate Logistic Regression Model

In Step 2a, all independent variables selected for further analysis in screening (excluding the OCP and farm work variables) were entered into a multivariate logistic regression

model. Those explanatory variables remaining in the model ($p \leq 0.15$) are presented in Table 4.7. In Step 2b, a separate model was created for each OCP variable and farm work identified in screening, and each model also included the significant explanatory variables identified in Step 2a. The results of these models are provided in Table 4.8. In Step 3, a single model was fit which included all important OCPs and explanatory variables, and the results of this model are provided in Table 4.9.

The results of the multivariate logistic regression models indicate that in this sample, serum beta-HCH ADL was associated with increasing age, Mexican Hispanic group, increasing serum cholesterol, education $\leq 8^{th}$ grade, never drinking alcohol, and having the examination (serum sample drawn) during the winter compared to summer or fall. All of the OCPs in serum were associated with beta-HCH in the individual multivariate adjusted models, with oxychlordane having the strongest measure of association (OR=7.93). Ever having done farm work was not associated with the presence of beta-HCH in serum. Adjusting in the final model for all the significant OCPs simultaneously led to attenuation of the odds ratios for all OCPs, and hexachlorbenzene and education were no longer significantly associated with beta-HCH.

p,pDDT

Bivariate Screen

Screening identified the following variables (adjusted for age at examination and shown with their crude odds ratio and 95% confidence interval) as meeting the criteria for further

analysis ($p < 0.25$): age at examination (1.05;1.04-1.06), Cuban compared to Mexican (0.56;0.36-0.87), Puerto Rican compared to Mexican (0.05;0.02-0.13), serum cholesterol (2.39; 1.14-5.01), BMI (1.08;1.05-1.12), education $\leq 8^{th}$ grade (2.43; 1.78-3.31), ever married (2.12; 1.55-2.91), diabetes (1.97; 1.21-3.23), not U.S. born (2.12; 1.55-2.91), alcohol drinker (0.64; 0.47-0.87), spring compared to winter (0.76; 0.50-1.16), summer compared to winter (0.92; 0.60-1.34), fall compared to winter (0.90; 0.60-1.34), ever farm work (1.63; (1.15-2.31), beta-HCH ADL (5.37; 3.86-7.47), dieldrin ADL (8.84; 4.44-17.62), hexachlorobenzene ADL (2.62; 1.45-4.74), oxychlordane ADL (4.68; 2.59-8.48), *trans*-nonachlor ADL (5.35; 3.39-8.43), DDE (15.40; 10.49-22.58).

Multivariate Logistic Regression Model

In Step 2a, all independent variables selected for further analysis in screening (excluding the OCP and farm work variables) were entered into a multivariate logistic regression model. Those explanatory variables remaining in the model ($p \leq 0.15$) are presented in Table 4.10. In Step 2b, a separate model was fit for each OCP variable (and farm work) identified in screening, and each model also included the significant explanatory variables identified in Step 2a. The results of these models are provided in Table 4.11. In Step 3, a single model was fit which included all important OCPs and explanatory variables and the results of this model are provided in Table 4.12.

The results of the multivariate logistic regression model fit in Step 2a indicate that in this sample, serum DDT is associated with increasing age, Mexican Hispanic group, increasing

serum cholesterol, increasing BMI, $\leq 8^{th}$ grade education, and having been born outside the U.S. All of the OCPs in serum were associated with serum DDT in the individual OCP multivariate adjusted models, with DDE having the largest measure of association (OR=22.81), as would be expected since DDE is a metabolite of DDT. Ever having done farm work was not associated with the presence of DDT in serum. Adjusting for all the significant OCPs simultaneously led to attenuation of the odds ratios for all OCPs, and hexachlorbenzene and oxychlordane were no longer significantly associated with DDT.

Hexachlorobenzene

Bivariate Screen

In the first phase of bivariate screening, the dummy variables for Hispanic group had insufficient cell counts, therefore the Cuban and Puerto Rican variables were collapsed into one variable and became the referent group. Screening identified the following variables (adjusted for age at examination and shown with their crude odds ratio and 95% confidence interval) as meeting the criteria for further analysis ($p< 0.25$): age at examination (1.03; 1.02-1.05), serum cholesterol (5.15; 1.38-19.30), BMI (0.95; 0.90-1.02), education $\leq 8^{th}$ grade (0.51; 0.29-0.92), income < $20,000/yr (0.64; 0.37-1.12), ever married (0.61; 0.29-1.28), not residing in SMSA-central city (1.97; 1.16-3.35), spring compared to winter (1.80; 0.83-3.92), summer compared to winter (1.29; 0.58-2.90), fall compared to winter (1.29; 0.58-2.90), ever farm work (1.25; 0.66-2.37), DDT ADL (2.63; 1.46-4.72), beta-HCH ADL (2.91; 1.60-5.30), dieldrin ADL (2.81; 1.11-7.15), oxychlordane ADL (4.01; 1.79-8.96), *trans*-nonachlor ADL (1.85; 0.85-4.03),

DDE (1.98; 1.38-2.86).

Multivariate Model

In Step 2a, all independent variables selected for further analysis in screening (excluding the OCP and farm work variables) were entered into a multivariate logistic regression model. Those explanatory variables remaining in the model ($p \leq 0.15$) are presented in Table 4.13. In Step 2b, a separate model was fit for each OCP variable and farm work identified in screening, and each model also included the significant explanatory variables identified in Step 2a. The results of these models are provided in Table 4.14. In Step 3, a single model was fit which included all important OCPs and explanatory variables and the results of this model are provided in Table 4.15.

The results of the multivariate logistic regression model fit in Step 2a indicate that in this sample, serum hexachlorobenzene ADL is associated with increasing age at examination, increasing serum cholesterol, Mexican Hispanic group, $\leq 8^{th}$ grade education, residing outside of a central city SMSA, and never being married. After adjustment for confounders in the individual models for each OCP, dieldrin, *trans*-nonachlor and farm work were no longer significantly associated with hexachlorobenzene. When adjusting in the final model for all the significant OCPs simultaneously, only DDE remained significantly associated with hexachlorobenzene.

Dieldrin

Bivariate Screen

In the first phase of bivariate screening, the dummy variables for Hispanic group had insufficient cell counts and could not be combined to yield greater than three in a cell. The marital status variable also had an insufficient cell count. Therefore these variables were excluded from further analyses. Screening identified the following variables (adjusted for age at examination and shown with their crude odds ratio and 95% confidence interval) as meeting the criteria for further analysis ($p \leq 0.25$): age at examination (1.07; 1.04-1.08), serum cholesterol (11.05; 2.69-45.29), BMI (1.14; 1.08-1.20), education $\leq 8^{th}$ grade (2.80; 1.32-5.95), income < $20,000/yr (2.02; 0.83-4.89), not U.S. born (0.52; 0.28-0.97), not residing in SMSA-central city (2.71; 1.42-5.16), diabetes (3.71; 1.77-7.75), alcohol drinker (0.40; 0.18-0.89), spring compared to winter (0.14; 0.05-0.41), summer compared to winter (0.32; 0.13-0.78), fall compared to winter (0.41; 0.19-0.86), ever farm work (2.13; 1.11-4.10), DDT ADL (9.20; 4.58-18.48), beta-HCH ADL (9.61; 4.41-20.95), hexachlorobenzene ADL (2.69; 1.04-6.98), oxychlordane ADL (6.97; 3.22-15.09), *trans*-nonachlor ADL (4.44; 2.20-8.97), DDE (2.61; 1.70-4.01).

Multivariate Logistic Regression Model

In Step 2a, all independent variables selected for further analysis in screening (excluding the OCP and farm work variables) were entered into a multivariate logistic regression model. Those explanatory variables remaining in the model ($p \leq 0.15$) are presented in Table 4.16. In Step 2b, a separate model was fit for each OCP variable (and farm work)

identified in screening, and each model also included the significant explanatory variables identified in Step 2a. The results of these models are provided in Table 4.17. In Step 3, a single model was fit which included all significant OCPs and explanatory variables and the results of this model are provided in Table 4.18.

The results of the multivariate logistic regression model (Step 2a) indicates that in this sample, serum dieldrin is associated with increasing serum cholesterol, increasing BMI, $\leq$ 8th grade education, residing outside of a central city SMSA, having diabetes, and undergoing the examination in winter compared to spring or summer. After adjusting for the explanatory variables in the individual models for each OCP, hexachlorobenzene, *trans*-nonachlor and farm work were no longer significantly associated with dieldrin. When adjusting in the final model for all the significant OCPs simultaneously, of the OCPs, only beta-HCH and DDT remained significantly associated with dieldrin.

Oxychlordane

Bivariate Screen

In the first phase of bivariate screening, the dummy variables for Hispanic group had insufficient cell counts, therefore the Cuban and Puerto Rican variables were collapsed into one variable and became the referent group. Screening identified the following variables (adjusted for age at examination and shown with their crude odds ratio and 95% confidence interval) as meeting the criteria for further analysis ($p< 0.25$): age at examination (1.06; 1.04-1.08), serum cholesterol (9.90; 2.64-37.15), BMI (0.95; 0.89-

1.02), income < $20,000/yr (0.50;0.27-0.92), ever married (0.61; 0.29-1.28), not residing in SMSA-central city (2.52; 1.41-4.52), diabetes (3.66; 1.84-7.30), spring compared to winter (0.70; 0.02-0.24), summer compared to winter (0.43; 0.22-0.86), fall compared to winter (0.18; 0.08-0.41), ever farm work (2.36; 1.30-4.29), DDT ADL (4.77; 2.63-8.66), beta-HCH ADL (10.40; 5.09-21.25), dieldrin ADL (7.04; 3.27-15.16), hexachlorobenzene ADL (3.93; 1.74-8.92), *trans*-nonachlor ADL (28.35; 14.62-55.97), DDE (3.30; 2.18-4.98).

Multivariate Logistic Regression Model

In Step 2a, all independent variables selected for further analysis in screening (excluding the OCP and farm work variables) were entered into a multivariate logistic regression model. Those explanatory variables remaining in the model ($p \leq 0.15$) are presented in Table 4.19. In Step 2b, a separate model was fit for each OCP variable and farm work identified in screening, and each model also included the significant explanatory variables identified in Step 2a. The results of these models are provided in Table 4.20. In Step 3, a single model was fit which included all important OCPs and explanatory variables and the results of this model are provided in Table 4.21.

The results of the multivariate logistic regression model fit in Step 2a indicate that in this sample, serum oxychordane ADL is associated with increasing age at examination, increasing serum cholesterol, diabetes, and having the examination in the winter compared to spring, summer or fall. All of the OCPs in serum were associated with oxychlordane in

the individual multivariate adjusted models, with *trans*-nonachlor having the strongest measure of association (OR=21.06), as would be expected since oxychlordane is a metabolite of chlordane and trans-nonachlor is a component of technical chlordane. When adjusting in the final model for all the significant OCPs simultaneously, dieldrin, hexachlorobenzene and farm work were no longer associated with oxychlordane.

trans-Nonachlor

Bivariate Screen

In the first phase of bivariate screening, the dummy variables for Hispanic group had insufficient cell counts, therefore the Cuban and Puerto Rican variables were collapsed into one variable and became the referent group. The marital status variable also had an insufficient cell count and was excluded from further analysis. Screening identified the following variables (adjusted for age at examination and shown with their crude odds ratio and 95% confidence interval) as meeting the criteria for further analysis ($p < 0.25$): age at examination (1.07; 1.05-1.08), Mexican Hispanic group (2.54; 1.49-4.33), serum cholesterol (3.12; 1.08-9.00), BMI (0.97; 0.92-1.02), income < $20,000/yr (0.74; 0.46-1.20), not residing in SMSA-central city (1.71; 1.11-2.63), not U.S. born (0.42; 0.27-0.66), diabetes (1.60; 0.85-3.00), spring compared to winter (0.16; 0.08-0.31), summer compared to winter (0.25; 0.13-0.46), fall compared to winter (0.22; 0.12-0.40), ever farm work (1.83; 1.13-2.95), DDT ADL (5.48; 3.47; 8.64), beta-HCH ADL (6.42; 3.94-10.46), dieldrin ADL (4.57; 2.27-9.19), hexachlorobenzene ADL (1.70; 0.81-3.97), oxychlordane ADL (28.61; 14.84-55.15), DDE (2.92; 2.13-3.99).

Multivariate Logistic Regression Model

In Step 2a, all independent variables selected for further analysis in screening (excluding the OCP and farm work variables) were entered into a multivariate logistic regression model. Those independent variables remaining in the model ($p \leq 0.15$) are presented in Table 4.22. In Step 2b, a separate model was fit for each OCP variable and farm work identified in screening, and each model also included the significant explanatory variables identified in Step 2a. The results of these models are provided in Table 4.23. In Step 3, a single model was fit which included all important OCPs and explanatory variables and the results of this model are provided in Table 4.24.

The results of the multivariate logistic regression model fit in Step 2a indicate that in this sample, serum *trans*-nonachlor ADL is associated with increasing age at examination, Mexican Hispanic group, increasing serum cholesterol, and having the examination in winter compared to spring, summer or fall. After adjusting for the explanatory variables in the individual models for each OCP, hexachlorobenzene and farm work were no longer associated with *trans*-nonachlor. Oxychlordane had the strongest measure of association (OR=20.56) with *trans*-nonachlor, as would be expected since oxychlordane is a metabolite of chlordane and *trans*-nonachlor is a component of technical chlordane. When adjusting in the final model for all the significant OCPs simultaneously, only beta-HCH, DDT, and oxychlordane remained significant. In addition, age at examination and season of examination were dropped from the model.

pp'DDE (Multiple Regression Model)

Bivariate Screen

Screening identified the following variables (adjusted for age at examination and shown with their coefficient and p-value) as meeting the criteria for further analysis ($p \leq 0.25$): age at examination (0.02;<0.01), Cuban compared to Mexican (-0.36; <0.01), Puerto Rican compared to Mexican (-0.92; <0.01), $\leq 8^{th}$ grade education (0.35, <0.01), ever married (0.30; <0.01), not residing in SMSA-central city (0.17; <0.01), not U.S. born (0.06; 0.22), diabetes (0.14; 0.17), alcohol drinker (-0.07; 0.16), spring compared to winter (-0.04; 0.54), summer compared to winter (-0.12; 0.10), fall compared to winter (-0.30; <0.01), ever farm work (0.25; <0.01), DDT ADL (1.11; <0.01), beta-HCH ADL (0.67; <0.01), dieldrin ADL (0.58; <0.01), hexachlorobenzene ADL (0.45; <0.01), oxychlordane ADL (0.69; <0.01), *trans*-nonachlor (0.63; <0.01). Since the serum DDE variable was already adjusted for cholesterol, cholesterol was not included in this analysis as an explanatory variable.

Multivariate Model

In Step 2a, all independent variables selected for further analysis in screening (excluding the OCP and farm work variables) were entered into a multivariate regression model. Those explanatory variables remaining in the model ($p \leq 0.15$) are presented in Table 4.25. In Step 2b, a separate model was fit for each OCP variable (and farm work) identified in screening, and each model also included the significant explanatory variables identified in Step 2a. The results of these models are provided in Table 4.26. In Step 3, a single model

was fit which included all important OCPs and explanatory variables and the results of this model are provided in Table 4.27.

The results of the multivariate logistic regression model fit in Step 2a indicate that in this sample, serum DDE was positively associated with increasing age at examination and not being U.S. born. Serum DDE was negatively associated with both Cuban and Puerto Rican Hispanic group compared to Mexican, and having the examination in the fall compared to winter. After adjusting for the explanatory variables in the individual models for each OCP, all OCPs except oxychlordane were significantly positively associated with serum DDE. Ever having done farm work was not associated with serum DDE. When adjusting in the final model for all the significant OCPs simultaneously, of the OCPs, only beta-HCH and DDT remained significantly associated with serum DDE.

LOGISTIC REGRESSION RESULTS - REPRODUCTIVE ENDPOINTS

Spontaneous Abortion

Bivariate Screen

In the first phase of bivariate screening, all independent variables were determined to have sufficient observations in each cell for statistical analysis. In the second phase of bivariate screening (adjusted for age at examination), the following variables (shown with their odds ratio and 95% confidence interval) were selected for further analyses ($p \leq 0.25$): age at examination (1.02; 1.01-1.02), Cuban (0.70; 0.47-1.04), Puerto Rican (0.72; 0.51-1.00), parity (1.08; 1.03-1.15), < 8th grade education (1.37; 1.05-1.80), ever married (1.98; 1.12-

3.50), ever smoked (1.33; 1.03-1.72), ever used oral contraceptives (1.30; 0.97-1.74). No exposure variables met the $p \leq 0.25$ level of significance.

Multivariate Model

The results of the final logistic regression model used to estimate adjusted risks for spontaneous abortion are provided in Table 4.28. The variables parity and ever used birth oral contraceptives did not meet the criteria to remain in the model. In this sample, an elevated risk for spontaneous abortion was found for women with less than a 9th grade education (OR=1.38; 95% CI 1.04-1.82), for women who had ever been married (OR=2.11; 95% CI 1.17-3.82), and for women who had ever smoked more than 100 cigarettes in their life (OR=1.37; 95% CI 1.05-1.77).

Summary of Exposure Variable Results

The screening analysis p-value was above the 0.25 cut point for all exposure variables (shown with their screening OR and 95% CI); therefore, they were not evaluated further: beta-HCH (1.02; 0.75-1.39), DDT (0.92; 0.66-1.27), dieldrin (0.89; 0.45-1.73), HCB (0.85; 0.46-1.58), oxychlordane (0.95; 0.52-1.74), *trans*-nonachlor (1.13; 0.72-1.78), DDE, cholesterol adjusted and log transformed, (1.00; 0.99-1.01), and farm work (1.15; 0.84-1.57). No exposure variables were found to be associated with spontaneous abortion.

Stillbirth

Bivariate Screen

In the first phase of bivariate screening, the independent variable Cuban Hispanic group had insufficient observations in a cell for statistical analysis and therefore the Hispanic group dummy variables were excluded from further analysis. In the second phase of bivariate screening (adjusted for age at examination), the following variables (shown with their odds ratio and 95% confidence interval) were selected for further analysis ($p \leq 0.25$): age at examination (1.05; 1.02-1.07), BMI (1.04; 0.98-1.10), income < $20,000 (1.89; 0.87-4.12), residence outside central city SMSA (0.65; 0.35-1.15), alcohol drinker (1.69; 0.94-3.04), Parity > 3 (2.63; 1.38-5.02), ever used birth control pills (0.41; 0.19-0.87), ever farm worker (2.12; 1.17-3.87), DDT body burden (1.66; 0.90-3.08), dieldrin ADL (2.01; 0.74-5.50), DDE level (1.01; 0.99-1.04).

Multivariate Model

Exposure variables identified in screening with $p \leq 0.25$ (DDE, DDT, dieldrin, farm work) were modeled with the following independent variables determined to be significant potential confounders in Step 2a, age at examination, parity >3, ever used oral contraceptives, and alcohol drinker. Results from the separate exposure models indicated that there was no association between stillbirth and serum DDT or DDE. Results of the separate exposure models for dieldrin and farm work are provided in Tables 4.29 and 4.31.

Summary of Exposure Variable Results

The screening analysis p-value was above the 0.25 cut point for the following exposure variables (shown with their screening OR and 95% CI); therefore, they were not evaluated further: beta-HCH (1.43; 0.77-2.65), HCB (0.94; 0.27-3.20), oxychlordane (1.14; 0.38-3.38), *trans*-nonachlor and (1.13; 0.48-2.65). When adjusted for age at examination, parity, oral contraceptive use, and alcohol consumption in the multivariate model, DDT (OR=1.10, 95% CI=0.54-2.20) and DDE, cholesterol adjusted and log transformed, (OR=1.00, 95% CI=0.97-1.03) were not significantly associated with stillbirth. Results from the multivariate model indicated that women with detectable serum dieldrin were at increased risk for stillbirth (OR=2.24, 95% CI 0.79-6.35, p-value=0.14); however, the risk estimate was imprecise. An elevated risk was also found for women who had ever done farm work (OR=4.11, 95% CI = 1.41-11.97). The risk was modified by parity however, with the increased risk primarily in the women with a parity ≤ 3 (OR=3.80, 95% CI = 1.34-11.54) compared to women with a parity > 3 (OR=1.18, 95% CI = 0.59-2.93) (Table 4.30). Considering both exposures together in a single model did not change the risk estimates appreciably and there was no significant interaction between the two exposure variables, farm work and dieldrin (Table 4.32). Dieldrin and farm work were the only exposure variables found to be associated with stillbirth.

Low Birth Weight

Bivariate Screen

In the first phase of bivariate screening, the exposure variable hexachlorobenzene had insufficient observations in a cell for statistical analysis and therefore was excluded from further analysis. In the second phase of bivariate screening (adjusted for age at examination), the following variables (shown with their odds ratio and 95% confidence interval) were selected for further analysis ($p \leq 0.25$): age at examination (1.015; 1.002-1.027), Cuban (0.46; 0.23-0.91), ever married (2.14; 0.75-6.05), not U.S. born (0.65; 0.45-0.92), diabetes or hypertension (1.54; 0.84-2.81), smoker (1.26; 0.88-1.81), parity > 3 (2.56; 1.72-3.82), farmwork (2.17; 1.47-3.19), dieldrin ADL (1.74; 0.80-3.80).

Multivariate Model

Exposure variables identified in screening with $p \leq 0.25$ (farm work, dieldrin) were modeled with the following independent variables determined to be significant potential confounders in Step 2a : Cuban, Puerto Rican, parity, and country of birth. Age at examination was not significant, but was forced into the multivariate model based on its *a priori* determined importance.

Summary of Exposure Variable Results

Hexachlorobenzene could not be evaluated due to insufficient cell numbers. The screening analysis p-value was above the 0.25 cut point for the following exposure variables (shown with their screening OR and 95% CI); therefore, they were not evaluated further: beta-

HCH (1.00; 0.65-1.54), DDT (0.95; 0.60-1.49), oxychlordane (0.97; 0.42-2.23), *trans*-nonachlor (1.30; 0.72-2.34), and DDE, cholesterol adjusted and log transformed, (0.99; 0.98-1.01). When adjusted for age at examination, parity, Hispanic group, and country of mother's birth in the multivariate model, dieldrin (OR=1.59; 95% CI=0.71-3.57) was not significantly associated with low birth weight. Farm work however, was associated with low birth weight (OR=2.70, 95% CI=1.50-4.86) in the multivariate model. Interactions between farm work and the significant explanatory variables were investigated. The only significant interaction identified was between farm work and parity. The results of the final multivariate logistic regression model for low birth weight are provided in Table 4.33. Although the p-value for the interaction term (farm work*parity) was greater than 0.05, this interaction term was retained in the final model because its inclusion resulted in a change in the odds ratio for farm work which was greater than 10%. The elevated risk for low birth weight among women who had ever done farm work was modified by parity, with the increased risk primarily among women with parity ≤ 3 (OR=2.67, 95%CI=1.46-4.86) compared to women with parity > 3 (OR=1.47, 95% CI=0.83-2.59) (Table 4.34). Farm work was the only exposure variable associated with low birth weight.

Birth Defects

First, all birth defect types were combined together to create a dichotomous variable with women having at least one live birth who had one child or more born with any physical or mental problem or defect compared to women with at least one live birth who never had a

child born with any physical or mental problem or defect. There were 86 women who reported having at least one child born with a defect. Of these 86 women, 79 reported only one child with a birth defect, five women reported having two children with a birth defect, and 2 women reported having 3 children with a birth defect. Subsequently, birth defects were categorized by body system affected and separate logistic regression models were created for each birth defect category.

Any Type of Birth Defect

Bivariate Screen

In the first phase of bivariate screening, all independent variables were determined to have sufficient observations in each cell for statistical analysis. In the second phase of bivariate screening (adjusted for age at examination), the following variables (shown with their odds ratio and 95% confidence interval) were selected for further analyses ($p \leq 0.25$): age at examination (1.02; 1.01-1.04); , Cuban (0.67;0.32-1.4), Puerto Rican (0.56; 0.29-1.09), parity > 3 (1.62; 1.0-2.64), ever married (0.55; 0.24-1.28), education $\leq 8^{th}$ grade (0.62; 0.38-1.02), residence in SMSA-central city (1.36; 0.87-2.12), mother not U.S. born (0.53; 0.36-0.82), ever smoked (1.49; 0.96-2.33), ever alcohol drinker (1.53; 0.97-2.41), ever used oral contraceptives (2.01;1.17-3.48), farm work (1.35; 0.81-2.25). Farm work was the only exposure variable which met the $p \leq 0.25$ level of significance criteria for inclusion into the model.

Multivariate Model

The Hispanic group dummy variables were forced into the final model, although they did not meet the $p \leq 0.10$ criteria to remain in the model. The results of the final multitivariate logistic regression model used to estimate adjusted risks for all birth defects combined are provided in Table 4.35. In this sample, having a child with any type of birth defect was positively associated with increasing age at examination, parity > 3, and ever having used oral contraceptives.

Summary of Exposure Variable Results

The screening analysis p-value was above the 0.25 cut point for all OCP exposure variables (shown with their screening OR and 95% CI); therefore, they were not evaluated further: beta-HCH (0.80; 0.46-1.38), DDT (0.91; 0.52-1.60), dieldrin (0.74; 0.22-2.48), HCB (1.29; 0.49-3.40), oxychlordane (0.84; 0.29-2.43), trans-nonachlor (0.92; 0.42-2.02), and DDE, cholesterol adjusted and log transformed, (1.00; 0.98-2.25). In the multivariate model, farm work was not associated with birth defects (OR=1.15; 95% CI=0.67-1.96). No exposure variables were found to be associated with having a child with a birth defect, when all birth defects were aggregated together.

Birth Defects by Body System Affected

Birth defects are rare outcomes, therefore an adequate sample size for multivariate analysis is an issue with regard to study power. The questionnaire requested the mother to specify which of the following body systems were involved in her child(ren)'s birth

defect: heart; eyes; ears; mouth or throat; stomach or intestines; kidneys or urinary system; muscles, bones, or joints; brain or nervous system. The numbers of women who reported one or more children born with an ear defect (N=4), stomach or intestinal defect (N=6), or a kidney or urinary system defect (N=2) were too small for meaningful analysis. Nine women reported multiple birth defects for a single child. In addition to their inclusion in the analysis by specific body system, these nine women were grouped into a "multiple defects" category. The purpose of the multiple defects category analysis was to investigate the potential association of a birth defect "syndrome" with the exposure variables.

Heart Defect

Bivariate Screen

Those variables excluded from analysis due to insufficient observations in a cell (<3) were: Hispanic group variables, diabetes, marital status, oxychlordane, dieldrin, and hexachlorobenzene. In the second phase of bivariate screening (adjusted for age at examination), the following variables (shown with their odds ratio and 95% confidence interval) were selected for further analysis ($p \leq 0.25$): parity > 3 (2.48; 0.88-6.96), education $\leq 8^{th}$ grade (0.19; 0.05-0.69), not U.S. born (0.24; 0.09-0.68); *trans*-nonachlor ADL (2.16; 0.59-7.98), DDE level (continuous variable, adjusted for cholesterol, log transformed) (0.96; 0.90-1.0).

Multivariate Model

In Step 2a, parity, education, and country of birth remained as important potential confounders and were included in the exposure modeling. The results of the final model are presented in Table 4.36. In this sample, having a child with a heart defect was positively associated with increasing age at examination and parity >3, and negatively associated with education level and mother not being U.S. born.

Summary of Exposure Variable Results

Dieldrin, hexachlorobenzene, and oxychlordane could not be evaluated due to insufficient cell numbers. The screening analysis p-value was above the 0.25 cut point for the following exposure variables (shown with their screening OR and 95% CI); therefore, they were not evaluated further: beta-HCH (0.81; 0.25-2.63), and DDT (1.07; 0.34-3.36). Neither serum *trans*-nonachlor (OR=1.64; 95% CI=0.43-6.18) or DDE level, cholesterol adjusted and log transformed (OR=0.96; 95% CI=0.90-1.03) were associated with a birth defect of the heart, after adjustment for confounding in the multivariate model. No exposure variables were found to be associated with having a child with a birth defect of the heart.

Eye Defect

Bivariate Screen

Those variables excluded from analysis due to insufficient observations in a cell (<3) were: Hispanic group variables, diabetes, marital status, oxychlordane, dieldrin, and

hexachlorobenzene. In the second phase of bivariate screening (adjusted for age at examination), the following variables (shown with their odds ratio and 95% confidence interval) were selected for further analysis ($p \leq 0.25$): age at examination (1.03; 0.99-1.07), parity > 3 (5.67; 1.40-23.02), BMI (1.07; 0.97-1.19), residence in SMSA-central city (0.41; 0.11-1.50), ever used birth control pills (2.63; 0.66-10.42), beta-HCH ADL (0.37; 0.08-1.79).

Multivariate Model

In Step 2a, parity was the only important potential confounder and was included with age at examination in the exposure modeling. The results of the final model are presented in Table 4.37.

Summary of Exposure Variable Results

Dieldrin, hexachlorobenzene, and oxychlordane could not be evaluated due to insufficient cell numbers. The screening analysis p-value was above the 0.25 cut point for the following exposure variables (shown with their screening OR and 95% CI); therefore, they were not evaluated further: DDT (0.95; 0.25-3.61), *trans*-nonachlor (0.66; 0.08-5.35), and farm work (1.16; 0.31-4.26). After adjustment for confounding in the multivariate model, beta-HCH (OR=0.35; 95% CI=0.13-1.69) and DDE, cholesterol adjusted and log transformed, (OR=0.96; 95% CI=0.90-1.03) were not associated with a birth defect of the eye. No exposure variables were found to be associated with having a child with a birth defect of the eye.

Muscle, Bone or Joint Defect

Bivariate Screen

Those variables excluded from analysis due to insufficient observations in a cell (<3) were: Hispanic group variables, diabetes, marital status, oxychlordane, dieldrin, transnonachlor, beta-HCH, and DDT. In the second phase of bivariate screening (adjusted for age at examination), the following variables (shown with their odds ratio and 95% confidence interval) were selected for further analysis ($p \leq 0.25$): age at examination (1.02; 0.98-1.06), $\leq 8^{th}$ grade education (0.43; 0.12-1.59), not U.S. born (0.21; 0.06-0.78), ever alcohol drinker (2.36; 0.74-7.58), ever farm work (2.82; 0.88-9.01).

Multivariate Model

In Step 2a, country of mother's birth was the only important potential confounder identified (Table 4.38). Age at examination and parity were forced into the exposure model because of their *a priori* determined importance. The final model was also run without the variables age at examination and parity, since they were not significantly associated with muscle, bone, or joint defect. The results of this more parsimonious model were similar to the larger model.

Summary of Exposure Variable Results

Beta-HCH, DDT, dieldrin, oxychlordane and *trans*-nonachlor could not be evaluated due to insufficient cell numbers. The screening analysis p-value was above the 0.25 cut point for the following exposure variables (shown with their screening OR and 95% CI);

therefore, they were not evaluated further: HCB (1.86; 0.23-15.14) and DDE, cholesterol adjusted and log transformed, (0.99; 0.94-1.05). After adjustment for confounding in the multivariate model, the risk estimate for the farm work exposure variable decreased from 2.76 to 2.08 (95% CI=0.63-6.83) and the p-value increased from 0.09 to 0.23. However, since the risk estimate for ever having done farm work and muscle, bone or joint defect remained above 2.0 it was kept in the final model (Table 4.38). Farm work was the only exposure variable found to be associated with having a child with a birth defect of the muscle, bone or joints.

Mouth Defect

Bivariate Screen

Those variables excluded from analysis due to insufficient observations in a cell (<3) were: Hispanic group variables, diabetes, marital status, smoker, alcohol drinker, residing in SMSA-central city, oxychlordane, dieldrin, hexachlorobenzene, *trans*-nonachlor, beta-HCH, and DDT. In the second phase of bivariate screening (adjusted for age at examination), the following variables (shown with their odds ratio and 95% confidence interval) were selected for further analysis ($p \leq 0.25$): age at examination (1.03; 0.99-1.08), income < $20,000/yr (0.36; 0.09-1.48), ever did farm work (3.08; 0.82-11.63).

Multivariate Model

In Step 2a, no important confounders were identified. Age at examination and parity were forced into the exposure model with farm work. The final model was also run without the

variables age at examination and parity, since they were not significantly associated with mouth defects. The results of this more parsimonious model were similar to the larger model. The results of this final model are presented in Table 4.39.

Summary of Exposure Variable Results

Beta-HCH, DDT, dieldrin, oxychlordane and *trans*-nonachlor could not be evaluated due to insufficient cell numbers. The screening analysis p-value was above the 0.25 cut point for the following exposure variables (shown with their screening OR and 95% CI); therefore, they were not evaluated further: HCB (2.41; 0.28-19.92), and DDE, cholesterol adjusted and log transformed, (1.00; 0.94-1.06). Since the OR for HCB was above 2.00, it was evaluated in a multivariate model, however when adjusted for potential confounders, the OR and p-value remained similar to the screening results. Farm work was the only exposure variable associated with having a child with a mouth defect (OR=3.12, 95% CI=0.81-11.97). The risk estimate was imprecise however, as the sample size was quite small.

Nervous System Defect

Bivariate Screen

Those variables excluded from analysis due to insufficient observations in a cell (<3) were: Hispanic group variables, diabetes, marital status, income, oxychlordane, dieldrin, hexachlorobenzene, *trans*-nonachlor. In the second phase of bivariate screening (adjusted for age at examination), the following variables (shown with their odds ratio and 95%

confidence interval) were selected for further analysis ($p \leq 0.25$): parity > 3 (3.03; 1.03-8.95), smoker (1.77; 0.6904.54), ever alcohol drinker (2.62; 0.99-6.95). No exposure variables met the criteria ($p \leq 0.25$) for entry into the multivariate model.

Multivariate Model

The results of the final model are presented in Table 4.40. In this sample having a child with a nervous system birth defect was associated with increasing age at examination, parity >3 and ever drinking alcohol. Women with a child with a nervous system birth defect were 2.76 (95% CI=1.04-7.33) times more likely to have ever consumed alcohol.

Summary of Exposure Variable Results

Dieldrin, hexachlorobenzene, oxychlordane and *trans*-nonachlor could not be evaluated due to insufficient cell numbers. The screening analysis p-value was above the 0.25 cut point for the following exposure variables (shown with their screening OR and 95% CI); therefore, they were not evaluated further: beta-HCH (0.83; 0.29-2.40), DDT (0.98; 0.69-1.38), DDE, cholesterol adjusted and log transformed, (1.00; 0.96-1.04), and farm work (1.06; 0.29-2.40). No exposure variables were associated with having a child with a nervous system defect.

Child with Multiple Defects

Bivariate Screen

Those variables excluded from analysis due to insufficient observations in a cell (<3) were: Hispanic group variables, diabetes, marital status, income, residence in SMSA central city, smoker, beta-HCH, oxychlordane, dieldrin, hexachlorobenzene, *trans*-nonachlor, farm work. In the second phase of bivariate screening (adjusted for age at examination), the following variables (shown with their odds ratio and 95% confidence interval) were selected for further analysis ($p \leq 0.25$): BMI (1.07; 0.95-1.21) and mother not U.S. born (0.30; 0.07-1.20). No exposure variables met the screening criteria ($p \leq 0.25$ or OR >2.0) for entry into the multivariate model.

Multivariate Model

The results of the final model are presented in Table 4.41. Having a child with multiple birth defects was positively associated with increasing age at examination and parity, and negatively associated with the mother not being U.S. born.

Summary of Exposure Variable Results

Beta-HCH, dieldrin, hexachlorobenzene, oxychlordane, *trans*-nonachlor, and farm work could not be evaluated due to insufficient cell numbers. The screening analysis p-value was above the 0.25 cut point for the following exposure variables (shown with their screening OR and 95% CI); therefore, they were not evaluated further: DDT (1.49; 0.35-6.33), and DDE, cholesterol adjusted and log transformed (0.98; 0.91-1.05). No exposure variables

were found to be associated with having a child with multiple birth defects.

Early Menarche

Bivariate Screen

In the first phase of bivariate screening, all independent variables were determined to have sufficient observations in each cell for statistical analysis. In the second phase of bivariate screening (adjusted for age at examination), the following variables (shown with their odds ratio and 95% confidence interval) were selected for further analysis ($p \leq 0.25$): Cuban (1.67; 1.17-2.39), Puerto Rican (1.67; 1.25-2.24), BMI (1.04; 1.02-1.07); ever married (1.71; 1.25-2.35), diabetes (1.59; 0.92-2.74), DDT ADL (0.74; 0.50-1.07), and oxychlordane ADL (0.46; 0.19-1.10).

Multivariate Model

Exposure variables identified in screening with $p \leq 0.25$ (DDT and oxychlordane) were modeled separately with the following independent variables determined to be significant potential confounders in Step 2a: age at examination, Cuban, Puerto Rican, BMI, and ever married. Results of the final model are presented in Table 4.42. Early menarche was associated with decreasing age at examination, Cuban or Puerto Rican Hispanic group, increasing BMI, and ever having been married.

Summary of Exposure Variable Results

The screening analysis p-value was above the 0.25 cut point for the following exposure variables (shown with their screening OR and 95% CI); therefore, they were not evaluated further: beta-HCH (0.88; 0.62-1.25), dieldrin (0.99; 0.47-2.07), HCB (0.76; 0.37-1.54), *trans*-nonachlor (0.82; 0.46-1.44), DDE, cholesterol adjusted and log transformed, (0.99; 0.98-1.01), and farm work (0.89; 0.63-1.26). After adjustment for confounders in the multivariate model, neither oxychlordane (OR=0.58; 95% CI=0.24-1.44) or DDT (OR=0.78; 95% CI=0.52-1.19) were associated with early menarche. No exposure variables were found to be associated with early menarche.

Late Menarche

Bivariate Screen

In the first phase of bivariate screening, all independent variables were determined to have sufficient observations in each cell for statistical analysis. In the second phase of bivariate screening (adjusted for age at examination), the following variables (shown with their odds ratio and 95% confidence interval) were selected for further analysis ($p \leq 0.25$): age at examination (1.03; 1.02-1.04), Cuban (0.57; 0.31-1.04), $\leq 8^{th}$ grade education (0.78; 0.55-1.12), not U.S. born (1.40, 0.99-1.97), ever did farm work (1.56; 1.06-2.30), beta-HCH ADL (0.73; 0.47-1.12), dieldrin ADL (0.34; 0.10-1.12), hexachlorobenzene ADL (1.63; 0.83-3.20),

Multivariate Model

Exposure variables identified in screening with $p \leq 0.25$ (farm work, beta-HCH, dieldrin, hexachlorobenzene) were modeled separately with the following independent variables determined to be significant potential confounders in Step 2a: age at examination, Hispanic group, education and country of birth. The results of the separate multivariate logistic regression models for dieldrin and farmwork are provided in Table 4.43. Women with late menarche were less likely to have serum dieldrin ADL (OR=0.35; 95% CI=0.105-1.19) and were more likely to have ever done farm work (OR=1.76, 95% CI=1.16-2.68). Finally, dieldrin and farmwork were considered simultaneously in a single multivariate logistic regression model which included the important confounders age at examination, Hispanic group, education, and country of birth. The results of this model are provided in Table 4.44. The adjusted odds ratios for farm work and dieldrin did not change appreciably when considered simultaneously in a single model. Interaction between dieldrin and farm work was investigated and was not significant.

Summary of Exposure Variable Results

The screening analysis p-value was above the 0.25 cut point for the following exposure variables (shown with their screening OR and 95% CI); therefore, they were not evaluated further: DDT (0.80; 0.51-1.26), oxychlordane (0.78; 0.34-1.79), *trans*-nonachlor (0.87; 0.47-1.61), and DDE, cholesterol adjusted and log transformed, (0.95; 0.78-1.15). After adjustment for confounders in the multivariate model, neither beta-HCH (OR=0.79; 95% CI=0.51-1.23) or HCB (OR=1.71; 95% CI=0.85-3.42) were significantly associated with

late menarche. Dieldrin was found to be protective against a late menarche in the multivariate model (OR=0.35; 95% CI=0.11-1.19) and farm work was found to be associated with an increased risk for late menarche (OR=1.76; 95% CI=1.16-2.68). Dieldrin and farm work were the only exposure variables found to be associated with late menarche.

Early Menopause

Bivariate Screen

In the first phase of bivariate screening, the independent variables marital status and diabetes were determined to have insufficient observations in a cell for statistical analysis and were excluded from further analyses. In the second phase of bivariate screening, the following variables (adjusted for age at examination shown with their odds ratio and 95% confidence interval) were selected for further analysis ($p \leq 0.25$): Cuban (0.22; 0.06-0.79), Puerto Rican (0.45; 0.16-1.32), $\leq 8^{th}$ grade education, ever alcohol drinker (0.45; 0.17-1.23), parity (1.13; 1.01-1.26), ever farm work (3.17; 1.29-7.81), beta-HCH ADL (3.88; 1.75-8.63), DDT ADL (2.79; 0.89-8.71), dieldrin ADL (2.79; 0.89-8.71), hexachlorobenzene ADL (2.28; 0.60-8.68), oxychlordane ADL (3.54; 0.95-13.18), transnonachlor ADL (2.13; 0.84-5.38), DDE level (1.60; 0.97-2.62).

Multivariate Model

The dummy variables for Hispanic group were the only significant independent variables associated with early menopause identified in Step 2a. Due to small numbers in the Cuban

and Puerto Rican categories and the similarity of their effect estimates, the Hispanic group dummy variables were combined into one variable, with Cuban + Puerto Rican as the referent group. Exposure variables identified in screening with $p \leq 0.25$ (farm work, beta-HCH, DDT, dieldrin, hexachlorobenzene, oxychlordane, transnonachlor and DDE) were modeled separately with this Hispanic group variable and age at examination (considered an important potential confounder *a priori*). Interaction between the Hispanic group variable and each exposure variable was investigated and was not significant. The results of these separate logistic regression models are provided in Table 4.45. Elevated risks for early menopause with odds ratios ranging from 2.48 to 3.57 were found for detectable serum beta-HCH, DDT, oxychlordane, and farm work.

In Step 3, the exposure variables which were associated with early menopause at $p \leq 0.15$ in the individual models (beta-HCH, DDT, oxychlordane and farm work), and the control variables (Hispanic group and age at examination), were considered simultaneously in a single multivariate logistic regression model (Table 4.46). After adjustment for the other exposures, oxychlordane was no longer significant ($p=0.75$) and, because its odds ratio dropped below 2.00, was dropped from further analyses. The odds ratios for the other exposure variables were attenuated, but remained above 2.00. Next, all possible interactions between the remaining exposure variables were examined. The interaction term for beta-HCH with DDT was the only significant ($p \leq 0.10$) interaction term, indicating the presence of effect modification. Results of the reduced final model, which includes the interaction term for beta-HCH and DDT, are provided in Table 4.47. Effect

estimates for early menopause and detectable DDT in serum in the presence and absence of detectable beta-HCH in serum, and for early menopause and detectable BHC in serum in the presence and absence of detectable DDT in serum are provided in Table 4.48.

Summary of Exposure Variable Results

All exposure variables met the screening analysis cut point of ≤ 0.25. After adjustment for confounding in the multivariate model, HCB (OR=1.86; 95% CI=0.47-7.33), *trans*-nonachlor (OR=1.56; 95% CI=0.59-4.09), and DDE, cholesterol adjusted and log transformed, (OR=1.31; 95% CI=0.78-2.22) were no longer associated with early menopause. Beta-HCH (OR=2.98; 95% CI=1.24-7.15), DDT (OR=3.57; 95% CI=1.54-8.28), oxychlordane (OR=2.96; 95% CI=0.77-11.37), dieldrin (OR=2.01, 95% CI=0.63-6.51), and farm work (OR=2.48; 95% CI=0.98-6.28) remained associated with early menopause in the individual exposure models after adjusting for confounding. The 95% confidence intervals for oxychlordane, dieldrin and farm work were imprecise and included 1.00, but because their odds ratios were > 2.00, they were considered further. After adjusting for all important exposures and confounders simultaneously, DDT (OR=2.89; 95% CI=1.18-7.10) was the only exposure variable which remained significantly ($p \leq 0.05$) associated with early menopause, although the odds ratios for beta-HCH (OR=2.05; 95% CI=0.79-5.31) and farm work (OR=2.10; 95% CI=0.77-5.68) remained above 2.00. The significant interaction found between serum DDT and serum beta-HCH indicated that a substantial increase in risk for early menopause was associated with having both serum DDT and beta-HCH ADL, and not with having only one of them ADL. The adjusted odds

ratio for women who had serum DDT ADL in addition to beta-HCH ADL was 7.65 (95% CI=1.85-31.72), and for women who had serum beta-HCH ADL in addition to DDT ADL, the odds ratio was 5.64 (95% CI=1.30-24.44).

Late Menopause

Bivariate Screen

In the first phase of bivariate screening, the independent variable marital status was determined to have insufficient observations in a cell for statistical analysis and was excluded from further analysis. In the second phase of bivariate screening, the following variables (adjusted for age at examination and shown with their odds ratio and 95% confidence interval) were selected for further analysis ($p \leq 0.25$): age at examination (1.05; 1.00-1.09), BMI (1.05; 0.99-1.12), beta-HCH ADL (1.83; 0.97-3.43), and ever farm work (2.33; 1.07-5.11).

Multivariate Model

In Step 2a, BMI was the only significant potential confounder identified. The exposure variables identified in screening with $p \leq 0.25$ (beta-HCH, farm work) were modeled separately with BMI, age at examination and Hispanic group. Results from the separate exposure models indicated, after adjustment for confounders, both beta-HCH (OR=2.09, 95% CI=1.00-4.37) and farm work (OR=2.44, 95% CI=1.00-5.92) were associated with late menopause (Table 4.49). Next, beta-HCH and farm work, were considered simultaneously in a model which also included age at examination, Hispanic group and

BMI. The odds ratios for beta-HCH and farm work did not change appreciably when adjusted for each other. Interaction between beta-HCH and farm work was examined and was not significant. Results of the final model are provided in Table 4.50.

Summary of Exposure Variable Results

The screening analysis p-values were above the 0.25 cut point for additional analysis for the following exposure variables (shown with their screening OR and 95% CI); therefore, they were not examined further: DDT (0.96; 0.49-1.90), dieldrin (1.12; 0.34-3.62), HCB (0.97; 0.25-3.73), oxychlordane (1.85; 0.51-6.71), *trans*-nonachlor (1.29; 0.56-2.96), and DDE, cholesterol adjusted and log transformed, (0.95; 0.67-1.35). Multivariate analysis revealed beta-HCH (OR=2.09, 95% CI=1.00-4.37) and farm work (OR=2.44; 95% CI=1.00-5.92) were the only exposure variables associated with late menopause.

RESULTS OF ANALYSIS OF VARIANCE

Age at Menarche

The results of the single factor analysis of variance (including age at examination) by the exposure dose categories are provided in Table 4.51. Hexachlorobenzene was associated with a slightly higher mean age at menarche in the ADL/BDL comparison and when exposure was categorized by a median split ADL, but it was not significant in the whole number ppb categories. No other OCP exposure variables were significantly associated with age at menarche in the unadjusted analysis. Women who had ever done farm work

had an adjusted mean age at menarche (12.83 yrs.) significantly ($p<0.01$) later than women who had never done farm work (12.51 yrs.), but the difference was slight (0.32 yrs).

The independent variables were also screened and a significant difference ($p \leq 0.15$) in the mean age at menarche was found for the following variables: age at examination, BMI, Hispanic group, ever married, and country of birth. The results of the multiple factor (adjusted) analysis of variance are provided in Table 5.52.

There was no significant association identified for any of the OCP exposure variables with mean age at menarche in the adjusted analysis. There was a suggestion ($p=0.09$) of a small increase in mean age at menarche (0.49 yrs.) for hexachlorobenzene for the highest median split category (> 1.33 ppb,), but when serum HCB level was categorized by whole number ppb, there was no increase in mean age at menarche for the highest dose category ($\geq$ 2.00). There was also a suggestion ($p=0.08$) of a small decrease in mean age at menarche (0.62 yrs.) for women with serum *trans*-nonachlor $\geq$ 2.00 ppb. As in the unadjusted analysis, women who had ever done farm work had an adjusted mean age at menarche (12.79 yrs.) significantly ($p=0.01$) later than women who had never done farm work (12.51 yrs.), but the difference was slight (0.28 yrs).

The results of the multiple regression model (including confounders), which was fit for the continuous variable serum DDE concentration (cholesterol adjusted and natural log

transformed) with age at menarche as the dependent variable, indicated there was not a significant linear relationship between serum DDE and age at menarche (coefficient=-0.03, p=0.57).

Age at Menopause

The results of the single factor analysis of variance (including age at examination) for the exposure variables is provided in Table 4.53. A significant decrease in mean age at menopause was found for the highest exposure, both in the median split and ppb categories, for DDT, beta-HCH and *trans*-nonachlor (Tables 4.53). Serum levels of dieldrin, hexachlorobenzene, and oxychlordane, or ever having done farm work, were not associated with age at menopause.

The results of the multiple factor AOV which included all important covariates (age at examination, Hispanic group, education, and age at menarche) were similar to the unadjusted model. A significant decrease in mean age at menopause was found in the highest exposure category for DDT, beta-HCH and *trans*-nonachlor (Tables 4.54). Women with serum DDT level > 6.00 ppb had an adjusted mean age at menopause on average 5.65 years earlier than women with serum DDT below the detection limit. Women with serum beta-HCH $\geq$ 4.0 ppb had an adjusted mean age at menopause on average 3.35 years earlier than women with serum beta-HCH below the detection limit. Women with serum *trans*-nonachlor $\geq$ 2.00 ppb had an adjusted mean age at menopause on average 5.18 years earlier than women with serum *trans*-nonachlor below the detection limit.

A multiple regression model was fit for the continuous variable serum DDE concentration (cholesterol adjusted and natural log transformed) with age at menopause as the dependent variable. This model included the potential confounding variables (age at examination, Hispanic group, education, age at menarche) and the results of the model indicated there was not a significant linear relationship between serum DDE and age at menopause (coefficient = -0.52, p=0.20).

When interaction terms which represented the combined effect of having two OCPs above the detection limit were added to the AOV models, a number of significant interactions were identified (Tables 4.55, 4.56). Results of unadjusted and adjusted analyses were similar. Women with DDT ADL plus either beta-HCH, oxychlordane, or *trans*-nonachlor ADL, had a mean age at menopause approximately three to five years earlier than women who did not have these combinations of OCPs detected in their serum. Women with both serum beta-HCH ADL and oxychlordane ADL had a mean age at menopause approximately four years earlier than women without this combination. The interaction terms for farm work with *trans*-nonachlor was also significant. Individually, the magnitude of the effect of decreasing mean age at menopause for the ADL/BDL comparison (Table 4.55) was approximately one year for beta-HCH, and two years each for DDT and *trans*-nonachlor. Therefore, when compared to the magnitude of the effect for the significant interactions of three to five years (Table 4.56), the interactive effect is consistent with additivity.

Table 4.1 Socio-demographic and lifestyle characteristics of the study sample by Hispanic group, HHANES, 1982-84

Characteristics	Mexican American (n=1096)	Cuban (n=190)	Puerto Rican (n=313)	Total (n=1599)	Chi Square p-value
Age at examination (yrs)					
12-20	298 (27%)	30 (16%)	104 (33%)	432 (27%)	<0.01
21-35	376 (34%)	45 (24%)	76 (24%)	497 (31%)	
36-50	226 (21%)	51 (27%)	75 (24%)	352 (22%)	
>50	196 (18%)	64 (34%)	58 (19%)	318 (20%)	
Education (n=1579)					
$\leq 8^{th}$ grade	483 (45%)	68 (36%)	118 (38%)	669 (42%)	0.03
$> 8^{th}$ grade	601 (55%)	120 (64%)	189 (62%)	910 (58%)	
Income (n=1550)					
< $20,000/yr	745 (70%)	102 (55%)	256 (83%)	1103 (71%)	<0.01
≥ $20,000/yr	314 (30%)	82 (45%)	51 (17%)	447 (29%)	
Marital status					
Ever married	838 (77%)	158 (83%)	208 (66%)	1204 (75%)	<0.01
Never married	254 (23%)	32 (17%)	105 (34%)	391 (25%)	
Residence					
Not SMSA-central city	545 (50%)	117 (62%)	37 (12%)	699 (44%)	<0.01
SMSA-central city	551 (50%)	73 (38%)	276 (88%)	900 (56%)	
Country of birth					
Not U.S.	411 (38%)	174 (92%)	222 (71%)	807 (51%)	<0.01
U.S.	681 (62%)	16 (8%)	89 (29%)	786 (49%)	
Occupation					
Ever farm work	226 (21%)	12 (6%)	15 (5%)	253 (16%)	<0.01
Never farm work	870 (79%)	178 (94%)	298 (95%)	1346 (84%)	
Smoker (more than 100 cigarettes in life)					
Ever	228 (31%)	55 (29%)	107 (34%)	500 (31%)	0.41
Never	757 (69%)	135 (71%)	206 (66%)	1098 (69%)	
Alcohol drinker					
Ever	406 (37%)	93 (49%)	98 (32%)	597 (38%)	< 0.01
Never	681 (63%)	96 (51%)	207 (68%)	984 (62%)	
Season of examination					
Winter (Dec-Feb)	223 (20%)	90 (47%)	28 (9%)	341 (21%)	<0.01
Spring (Mar-May)	254 (23%)	100 (53%)	37 (12%)	391 (24%)	
Summer (Jun-Aug)	265 (24%)	0	131 (42%)	396 (25%)	
Fall (Sep-Nov)	354 (32%)	0	117 (37%)	471 (29%)	

Table 4.2 Univariate Statistics for Serum Cholesterol, Body Weight (lbs.), and Body Mass Index by Hispanic Group, HHANES, 1982-84

Variable	Mexican American	Cuban	Puerto Rican	Total	Analysis of variance p-value*
Serum cholesterol**					
Mean	205.88	204.17	206.94	206	0.03
Standard Deviation	47.44	45.83	47.64	47	
Minimum-Maximum	104-630	125-458	108-412	104-630	
N=1190					
Body weight (lbs.)					
Mean	136.88	135.76	134.48	136.26	0.29
Standard Deviation	28.92	22.77	30.46	28.56	
Minimum-Maximum	60-250	90-235	73-260	60-260	
N=1532					
Body Mass Index					
Mean	24.67	24.22	24.62	24.61	<0.01
Standard Deviation	4.96	3.74	5.72	4.99	
Minimum-Maximum	11.55-44.35	15.48-40.42	14.29-46.90	11.55-46.90	
N=1456					

*adjusted for age at examination
**409 missing; log transformed for AOV analysis

Table 4.3 Distribution and univariate statistics for OCPs in serum for women by Hispanic group, HHANES 1982-84,

OCP	N	Detection limit (ppb)	N (%) above detection limit	Minimum-Maximum* ppb)	Median*/Mean (ppb)
Beta-HCH					
all Groups	1599	1.00	291 (18.2)	1.00-13.68	1.90/2.29
Mex-Am	1096		234 (21.4)	1.00-13.68	1.85/2.25
Cuban	190		41 (21.6)	1.14-9.37	2.31/2.53
Puerto Rican	313		16 (5.11)	1.06-5.51	1.90/2.34
ppDDE					
all Groups	1599	1.00	1559 (97.5)	1.02-228.22	14.06/21.92
Mex- Am	1096		1095 (99.9)	1.20-228.22	16.06/24.56
Cuban	190		183 (96.3)	1.02-169.31	14.56/21.79
Puerto Rican	313		281 (89.8)	1.02-100.07	6.88/11.74
ppDDT					
all Groups	1599	2.00	238 (14.9)	1.60-88.62	3.36/5.19
Mex-Am	1096		202 (18.4)	1.90-88.62	3.36/5.39
Cuban	190		32 (16.8)	1.60-13.06	3.34/4.09
Puerto Rican	313		4 (1.3)	2.45-5.23	3.39/3.61
Dieldrin					
all Groups	1599	1.00	44 (2.8)	1.00-9.00	1.70/2.06
Mex-Am	1096		42 (3.8)	1.00-9.00	1.70/2.02
Cuban	190		2 (1.1)	1.55-3.90	
Puerto Rican	313		1 (0.3)		
HCB					
all Groups	1599		59 (3.7)	1.00-5.67	1.32/1.83
Mex-Am	1096	1.00	50 (4.6)	1.00-3.80	1.31/1.64
Cuban	190		9 (4.7)	1.16-5.67	1.99/2.87
Puerto Rican	313		0		
Oxychlordane					
all Groups	1599	1.00	53 (3.3)	1.00-5.53	1.40/1.66
Mex-Am	1096		40 (3.7)	1.00-5.53	1.22/1.55
Cuban	190		12 (6.3)	1.00-3.97	1.81/2.00
Puerto Rican	313		1 (0.3)	2.18	
***Trans*-Nonachlor**					
all Groups	1599	1.00	97 (6.1)	1.00-5.40	1.50/1.76
Mex-Am	1096		78 (7.1)	1.00-4.60	1.50/1.80
Cuban	190		18 (9.5)	1.03-5.40	1.31/1.62
Puerto Rican	313		1 (0.3)	1.4	

* includes only values above detection limit

Table 4.4 Univariate statistics for selected reproductive characteristics, HHANES 1982-84

Characteristic	Mexican American	Cuban	Puerto Rican	Total	Analysis of Variance p-value*
Gravidity					
Mean	4.28	3.23	4.23	4.14	<0.01
Standard Deviation	3.08	2.15	2.84	2.96	
Median	3	3	4	3	
Minimum-Maximum	1-20	1-15	1-20	1-20	
N=1136					
Parity					
Mean	3.68	2.26	3.32	3.44	<0.01
Standard Deviation	2.65	1.02	2.17	2.46	
Median	3	2	3	3	
Minimum-Maximum	1-15	1-6	1-12	1-15	
N=1089					
Age at menarche					
Mean	12.64	12.43	12.30	12.56	<0.01
Standard Deviation	1.49	1.78	1.66	1.57	
Median	13	12	12	12	
Minimum-Maximum	8-18	7-18	8-18	7-18	
N=1545					
Age at menopause					
Mean	47.44	49.58	48.60	48.17	0.01
Standard Deviation	5.14	4.00	4.02	4.75	
Median	48	50	49	49	
Minimum-Maximum	35-55	37-57	40-56	35-57	
N=219					

*adjusted for age at examination

Table 4.5 Reproductive characteristics of the study sample by Hispanic group, HHANES 1982-84

Characteristics	Mexican American N (%)	Cuban N (%)	Puerto Rican N (%)	Total N (%)	Chi Square p-value
Age at menarche (yrs)					
≤ 11	235 (22)	58 (31)	93 (31)	386 (25)	<0.01
12-14	706 (67)	115 (61)	167 (56)	988 (64)	
≥ 15	119 (11)	14 (8)	38 (13)	171 (11)	
Age at menopause (yrs)					
< 45	28 (22)	3 (6)	5 (12)	36 (16)	0.06
45-50	61 (48)	30 (58)	26 (62)	117 (53)	
>50	37 (29)	19 (36)	11 (26)	67 (31)	
Ever Pregnant					
Yes	781 (73)	140 (75)	216 (14)	1137 (73)	0.82
No	288 (279)	47 (25)	83 (28)	418 (27)	
Gravidity					
1-3	603 (62)	94 (68)	106 (50)	603 (53)	<0.01
≥ 4	378 (38)	45 (32)	110 (50)	533 (47)	
Parity					
1-3	452 (60)	115 (89)	134 (65)	701 (64)	<0.01
≥4	302 (40)	14 (11)	72 (35)	388 (36)	
Miscarriage (among gravidity > 0)					
Ever	268 (34)	41 (29.5)	60 (28)	369 (32)	0.14
Never	514 (66)	98 (70.5)	156 (72)	768 (68)	
Stillbirth (among gravidity >0)					
Ever	43 (6)	2 (1)	7 (3)	52 (5)	0.06
Never	739 (94)	138 (99)	208 (97)	1085 (95)	
Low birth weight (among parity >0)					
Ever	103 (14)	10 (8)	30 (15)	143 (13)	0.14
Never	640 (86)	119 (92)	176 (85)	935 (87)	
Birth Defect (among parity > 0)					
Ever	66 (8.7)	9 (7.0)	11 (5.3)	86 (7.9)	0.25
Never	689 (91.3)	120 (93.0)	195 (94.7)	1004 (92.1)	
Oral contraceptive use					
Ever	460 (43)	53 (28)	101 (34)	614 (40)	<0.01
Never	610 (91.3)	134 (72)	198 (66)	942 (60)	

Table 4.6 Prevalence of selected adverse reproductive endpoints by Hispanic group, HHANES 1982-84

Reproductive Endpoints	Mexican American % (N)	Cuban % (N)	Puerto Rican % (N)	Total % (N)	Analysis of Variance p-value*
Spontaneous abortion (Proportion of total pregnancies**)	12.71% (425)	16.70% (75)	12.80% (117)	14.14% (617)	0.07
Stillbirth (Proportion of total pregnancies**)	2.02% (56)	0.69% (2)	1.17% (8)	1.40% (66)	0.81
Low birth weight (Proportion of total live births***)	4.76% (132)	4.80% (14)	4.97% (34)	4.79% (180)	0.26
Birth defect (Proportion of total live births***)	2.58% (72)	3.78% (11)	1.75% (12)	2.53% (95)	0.57

* adjusted for age at examination
** total pregnancies = 4703
***total live births = 3752

Table 4.7 Adjusted odds ratios and 95% confidence intervals for explanatory factors associated with **beta-hexachlorocyclohexane** in serum, HHANES 1982-84

Explanatory Factor	Beta-HCH ADL	Beta-HCH BDL	Mulitivariate Adjusted Odds Ratio	95% CI
Age at examination*	291	1308	1.05	1.04-1.06
Cuban	41	149	0.52	0.32-0.82
Mexican American	250	1159		
Puerto Rican	16	297	0.12	0.06-0.23
Mexican American	275	1011		
Serum cholesterol†	285	905	2.53	1.18-5.41
Education				
$\leq 8^{th}$ grade	181	488	1.63	1.17-2.26
$> 8^{th}$ grade	107	803		
Alcohol drinker				
Ever	87	510	0.70	0.51-0.97
Never	197	787		
Season of examination				
spring	83	308	0.70	0.56-1.25
winter	86	255		
summer	51	345	0.56	0.35-0.90
winter	86	255		
fall	71	400	0.56	0.36-0.87
winter	86	255		

† continuous variable natural log transformed

* continuous variable, OR represents increase in risk for increase in one unit of age

Table 4.8 Adjusted odds ratios and 95% confidence intervals for OCPs and farm work associated with serum **beta-hexachlorocyclohexane**, *individual models for each OCP, farm work*, HHANES 1982-84

Explanatory Factor	Beta-HCH ADL	Beta-HCH BDL	Multivariate Adjusted* Odds Ratio	95% CI
DDT				
ADL	129	109	3.67	2.57-5.26
BDL	162	1199		
Dieldrin				
ADL	34	10	4.74	2.18-10.31
BDL	257	1298		
Hexachlorobenzene				
ADL	27	32	2.54	1.34-4.82
BDL	265	1276		
Oxychlordane				
ADL	41	12	7.93	3.67-17.13
BDL	250	1296		
trans-nonachlor				
ADL	66	31	4.53	2.68-7.64
BDL	225	1277		
DDE†	291	1308	2.55	2.03-3.22
Farm work				
Ever	74	179	1.05	0.72-1.52
Never	217	1129		

*adjusted for age at examination, Hispanic group, cholesterol, education, marital status, alcohol consumption, season of examination

† continuous variable cholesterol adjusted then natural log transformed, OR represents increase in risk for increase in one unit of variable

Table 4.9 Adjusted odds ratios and 95% confidence intervals for OCPs and farm work associated with serum **beta-hexachlorocyclohexane**, *OCPs entered simultaneously into single model*, HHANES 1982-84

Explanatory Factor	Beta-HCH ADL	Beta-HCH BDL	Multivariate Adjusted* Odds Ratio	95% CI
DDT				
ADL	129	109	1.61	1.06-2.46
BDL	162	1199		
Dieldrin				
ADL	34	10	3.16	1.35-7.44
BDL	257	1298		
Oxychlordane				
ADL	41	12	3.45	1.49-7.95
BDL	250	1296		
trans-nonachlor				
ADL	66	31	2.27	1.27-2.85
BDL	225	1277		
DDE†	291	1308	2.18	1.67-2.85

*adjusted for age at examination, Hispanic group, cholesterol, alcohol consumption, season of examination
† continuous variable cholesterol adjusted then natural log transformed, OR represents increase in risk for increase in one unit of variable

Table 4.10 Adjusted odds ratios and 95% confidence intervals for explanatory factors associated with serum **DDT**, HHANES 1982-84

Explanatory Factor	**DDT ADL**	**DDT BDL**	**Multivariate Adjusted Odds Ratio**	**95% CI**
Age at examination*	238	1361	1.02	(1.01-1.04)
Cuban	32	158	0.40	0.23-0.67
Mexican American	206	1203		
Puerto Rican	4	309	0.03	0.01-0.09
Mexican American	234	1052		
Serum cholesterol[r]	230	960	3.07	1.26-7.47
BMI*	193	1263	1.09	1.06-1.13
Education				
$\leq 8^{th}$ grade	161	508	2.08	1.43-3.04
$> 8^{th}$ grade	77	833		
Country of birth				
not U.S.	170	637	3.79	2.51-5.73
U.S.	68	718		

* continuous variable, OR represents increase in risk for increase in one unit of variable

[r] continuous variable natural log transformed

Table 4.11 Adjusted odds ratios and 95% confidence intervals for OCPs and farm work associated with serum **DDT**, *individual models for each OCP, farm work*, HHANES 1982-84

Explanatory Factor	DDT ADL	DDT BDL	Multivariate Adjusted* Odds Ratio	95% CI
beta-HCH				
ADL	129	162	3.67	2.48-5.43
BDL	109	1199		
Dieldrin				
ADL	31	13	3.85	1.76-8.44
BDL	207	1348		
Hexachlorobenzene				
ADL	22	37	2.35	1.13-4.86
BDL	216	1324		
Oxychlordane				
ADL	30	23	3.82	1.88-7.75
BDL	208	1338		
trans-nonachlor				
ADL	55	42	6.15	3.53-10.71
BDL	183	1319		
DDE†	230	960	22.81	13.69-38.00
Farm work				
Ever	61	192	1.09	0.71-1.67
Never	177	1169		

*adjusted for age at examination, Hispanic group, cholesterol, education, BMI, country of birth
† continuous variable cholesterol adjusted then natural log transformed, OR represents increase in risk for increase in one log unit of variable

Table 4.12 Adjusted odds ratios and 95% confidence intervals for OCPs and farm work associated with serum **DDT**, *OCPs entered simultaneously into single model*, HHANES 1982-84

Explanatory Factor	DDT ADL	DDT BDL	Multivariate Adjusted* Odds Ratio	95% CI
beta-BHC				
ADL	129	162	1.83	1.11-3.03
BDL	109	1199		
Dieldrin				
ADL	31	13	2.64	1.01-6.91
BDL	207	1348		
trans-nonachlor				
ADL	55	42	3.25	1.63-6.50
BDL	183	1319		
DDE†	230	960	20.94	12.34-35.55

*adjusted for age at examination, Hispanic group, cholesterol, education, BMI, country of birth
† continuous variable cholesterol adjusted then natural log transformed, OR represents increase in risk for increase in one log unit of variable

Table 4.13 Adjusted odds ratios and 95% confidence intervals for explanatory factors associated with serum **hexachlorobenzene,** HHANES 1982-84

Explanatory Factor	**Hexachloro-benzene ADL**	**Hexachloro-benzene BDL**	**Multivariate Adjusted Odds Ratio**	**95% CI**
Age at examination*	59	1540	1.04	1.02-1.07
Mexican American	50	1046	2.49	1.18-1.07
Cuban + Puerto Rican	9	494		
Serum cholesterol†	52	1138	4.41	1.16-16.74
Education				
$\leq 8^{th}$ grade	21	648	0.47	0.25-0.91
$> 8^{th}$ grade	37	873		
Residence				
not central city-SMSA	35	664	1.92	1.06-3.46
central city-SMSA	24	876		
Marital status				
ever	47	1157	0.41	0.17-0.99
never	12	379		

* continuous variable, OR represents increase in risk for increase in one unit of variable

† continuous variable natural log transformed, OR represents increase in risk for increase in one log unit of variable

Table 4.14 Adjusted odds ratios and 95% confidence intervals for other OCPs and farm work associated with serum **hexachlorobenzene**, *individual models for each OCP, farm work*, HHANES 1982-84

Explanatory Factor	Hexachloro-benzene ADL	Hexachloro-benzene BDL	Multivariate Adjusted* Odds Ratio	95% CI
DDT				
ADL	22	216	2.85	1.47-5.52
BDL	37	1324		
beta-HCH				
ADL	27	264	2.80	1.44-5.44
BDL	32	1276		
Dieldrin				
ADL	6	38	2.16	0.79-5.89
BDL	53	1502		
Oxychlordane				
ADL	9	44	3.22	1.38-7.56
BDL	50	1496		
Transnonachlor				
ADL	9	88	1.64	0.73-3.66
BDL	50	1452		
DDE†	52	1138	2.24	1.48-3.37
Farm work				
Ever	13	240	1.13	0.56-2.29
Never	46	1300		

*adjusted for age at examination, Hispanic group, cholesterol, education, current residence, marital status
† continuous variable cholesterol adjusted then natural log transformed, OR represents increase in risk for increase in one log unit of variable

Table 4.15 Adjusted odds ratios and 95% confidence intervals for OCPs associated with serum hexachlorobenzene, *OCPs entered simultaneously into single model*, HHANES 1982-84

Explanatory Factor	Hexachloro-benzene ADL	Hexachloro-benzene BDL	Multivariate Adjusted* Odds Ratio	95% CI
DDE†	52	1138	2.24	1.48-3.37

*adjusted for age at examination, Hispanic group, cholesterol, education, current residence, marital status
† continuous variable cholesterol adjusted then natural log transformed, OR represents increase in risk for increase in one log unit of variable

Table 4.16 Adjusted odds ratios and 95% confidence intervals for explanatory factors associated with serum **dieldrin**, HHANES 1982-84

Explanatory Factor	Dieldrin ADL	Dieldrin BDL	Odds Ratio	95% CI
Serum cholesterol†	44	1146	41.61	8.04-215.50
BMI*	35	1421	1.16	1.09-1.24
Education				
$\leq 8^{th}$ grade	34	635	3.26	1.47-7.26
$> 8^{th}$ grade	10	900		
Residence				
not central city-SMSA	29	670	2.26	1.05-4.88
central city-SMSA	15	885		
Diabetes				
Yes	13	72	2.77	1.18-6.50
No	31	1483		
Season of Examination				
spring	4	387	0.14	0.04-0.55
winter	22	319		
summer	7	389	0.32	0.10-0.96
winter	22	319		
fall	11	460	0.54	0.23-1.27
winter	22	319		

† continuous variable log transformed, OR represents increase in risk for increase in one log unit of variable
* continuous variable, OR represents increase in risk for increase in one unit of variable

Table 4.17 Adjusted odds ratios and 95% confidence intervals for other OCPs and farm work associated with serum **dieldrin**, *individual models for each OCP, farm work*, HHANES 1982-84

Explanatory Factor	Dieldrin ADL	Dieldrin BDL	Multivariate Adjusted* Odds Ratio	95% CI
beta-HCH				
ADL	34	257	7.13	2.93-17.35
BDL	10	1298		
DDT				
ADL	31	207	4.55	2.07-10.02
BDL	13	1348		
Hexachlorobenzene				
ADL	6	53	3.33	0.87-12.68
BDL	38	1502		
Oxychlordane				
ADL	12	41	4.36	1.47-12.92
BDL	32	1514		
trans-nonachlor				
ADL	15	82	2.25	0.89-5.72
BDL	29	1473		
DDE†	44	1146	2.31	1.31-4.10
Farm work				
Ever	15	238	1.09	0.48-2.47
Never	29	1317		

*adjusted for cholesterol, BMI, education, current residence, season of examination, diabetes
† continuous variable cholesterol adjusted and log transformed, OR represents increase in risk for increase in one log unit of variable

Table 4.18 Adjusted* odds ratios and 95% confidence intervals for OCPs associated with serum **dieldrin**, *OCPs entered simultaneously into single model*, HHANES 1982-84

Explanatory Factor	Dieldrin ADL	Dieldrin BDL	Multivariate Adjusted Odds Ratio	95% CI
beta-HCH				
ADL	34	257	5.08	2.05-12.57
BDL	10	1298		
DDT				
ADL	31	207	2.86	1.25-6.53
BDL	13	1348		

*adjusted for cholesterol, BMI, education, current residence, season of examination, diabetes

Table 4.19 Adjusted odds ratios and 95% confidence intervals for explanatory factors associated with serum **oxychlordane**, HHANES 1982-84

Explanatory Factor	Oxychlordane ADL	Oxychlordane BDL	Multivariate Adjusted Odds Ratio	95% CI
Age at examination*	53	1546	1.04	1.01-1.06
Serum cholesterol[†]	52	1138	11.35	2.78-46.38
Diabetes				
Yes	15	70	3.00	1.44-6.28
No	38	1476		
Season of Examination				
spring	3	388	0.08	0.02-0.26
winter	30	311		
summer	13	383	0.44	0.22-0.90
winter	30	311		
fall	7	464	0.17	0.07-0.40
winter	30	311		

* continuous variable, OR represents increase in risk for increase in one unit of variable

[†] continuous variable natural log transformed, OR represents increase in risk for increase in one log unit of variable

Table 4.20 Adjusted odds ratios and 95% confidence intervals for other OCPs and farm work associated with serum **oxychlordane**, *individual models for each OCP, farm work*, HHANES 1982-84

OCP, Farm work	Oxychlordane ADL	Oxychlordane BDL	Multivariate Adjusted* Odds Ratio	95% CI
beta-HCH				
ADL	41	250	8.68	4.20-18.08
BDL	12	1296		
DDT				
ADL	30	208	4.02	2.15-7.52
BDL	23	1338		
Dieldrin				
ADL	12	32	3.60	1.56-8.28
BDL	41	1514		
Hexachlorobenzene				
ADL	9	50	3.65	1.42-9.38
BDL	44	1496		
trans-nonachlor				
ADL	35	62	21.06	10.21-43.42
BDL	18	1484		
DDE†	52	1138	3.31	2.11-5.18
Farm work				
Ever	19	234	1.96	1.02-3.76
Never	34	1312		

*adjusted for age at examination, cholesterol, diabetes, season of examination

† continuous variable cholesterol adjusted then natural log transformed, OR represents increase in risk for increase in one log unit of variable

Table 4.21 Adjusted* odds ratios and 95% confidence intervals for OCPs associated with serum **oxychlordane**, *OCPs entered simultaneously into single model*, HHANES 1982-84

OCP	Oxychlordane ADL	Oxychlordane BDL	Multivariate Adjusted Odds Ratio	95% CI
beta-HCH				
ADL	41	250	2.90	1.23-6.86
BDL	12	1296		
Hexachlorbenzene				
ADL	9	50	2.95	0.95-9.20
BDL	44	1496		
trans-nonachlor				
ADL	35	62	11.26	5.14-24.63
BDL	18	1484		
DDE†	52	1138	1.77	1.04-3.01

*adjusted for age at examination, cholesterol, diabetes, season of examination
† continuous variable cholesterol adjusted and log transformed, OR represents increase in risk for increase in one log unit of variable

Table 4.22 Adjusted odds ratios and 95% confidence intervals for explanatory factors associated with serum ***trans*-nonachlor**, HHANES 1982-84

Explanatory Factor	*trans*-nonachlor ADL	*trans*-nonachlor BDL	Multivariate Adjusted Odds Ratio	95% CI
Age at examination*	97	1502	1.06	1.04-1.07
Mexican American	78	1018	2.67	1.53-4.66
Cuban + Puerto Rican	19	484		
Serum cholesterol†	95	1095	3.69	1.20-11.33
Season of Examination				
spring	13	378	0.16	0.08-0.32
winter	52	289		
summer	15	381	0.23	0.12-0.44
winter	52	289		
fall	17	454	0.18	0.10-0.33
winter	52	289		

* continuous variable, OR represents increase in risk for increase in one unit of variable
† continuous variable natural log transformed, OR represents increase in risk for increase in one log unit of variable

Table 4.23 Adjusted odds ratios and 95% confidence intervals for other OCPs and farm work associated with serum ***trans*-nonachlor**, *individual models for each OCP, farm work*, HHANES 1982-84

OCP, Farm work	*trans*-nonachlor ADL	*trans*-nonachlor BDL	Multivariate Adjusted* Odds Ratio	95% CI
beta-HCH				
ADL	66	225	5.03	2.99-8.47
BDL	31	1277		
DDT				
ADL	55	183	4.71	2.89-7.69
BDL	42	1319		
Dieldrin				
ADL	15	29	2.32	1.10-4.89
BDL	82	1473		
Hexachlorobenzene				
ADL	9	50	1.67	0.71-3.92
BDL	88	1452		
Oxychlordane				
ADL	35	18	20.56	10.09-41.87
BDL	62	1484		
DDE†	95	1095	2.87	2.01-4.09
Farm work				
Ever	29	224	1.39	0.82-2.34
Never	68	1278		

*adjusted for age at examination, Hispanic group, cholesterol, season of examination
† continuous variable cholesterol adjusted then natural log transformed, OR represents increase in risk for increase in one log unit of variable

Table 4.24 Adjusted odds ratios and 95% confidence intervals for OCPs associated with serum ***trans*-nonachlor**, *OCPs entered simultaneously into single model*, HHANES 1982-84

OCP	*trans*-nonachlor ADL	*trans*-nonachlor BDL	Multivariate Adjusted* Odds Ratio	95% CI
beta-HCH				
ADL	66	225	2.95	1.66-5.22
BDL	31	1277		
DDT				
ADL	55	183	3.32	1.92-5.75
BDL	42	1319		
Oxychlordane				
ADL	35	18	13.03	6.31-26.93
BDL	62	1484		

*adjusted for age at examination, season of examination

Table 4.25 Multivariate regression model for explanatory factors associated with serum **DDE**, HHANES 1982-84

Explanatory Factor	**Coefficient**	**Standard Error**	**p-value**
Age at examination	0.01	<0.01	<0.01
Cuban Mexican American	-0.62	0.08	<0.01
Puerto Rican Mexican American	-1.05	0.66	<0.01
Education $\leq 8^{th}$ grade $> 8^{th}$ grade	0.20	0.05	<0.01
Country of birth not U.S. U.S.	0.36	0.05	<0.01
Season of Examination spring winter	-0.09	0.07	0.16
summer winter	-0.09	0.07	0.24
fall winter	-0.28	0.07	<0.01

Table 4.26 Multivariate regression model for OCPs and farm work associated with serum **DDE**, *individual models for each OCP, farm work*, HHANES 1982-84

OCP, Farm work	**Coefficient**	**Standard Error**	**p-value**
beta-HCH	0.45	0.06	<0.01
DDT	0.87	0.06	<0.01
Dieldrin	0.29	0.12	<0.02
Hexachlorobenzene	0.31	0.11	<0.01
Oxychlordane	0.51	0.11	0.27
Trans-nonachlor	0.48	0.09	<0.01
Farm work	0.09	0.06	0.15

*adjusted for age at examination, Hispanic group,education, country of birth, season of examination

Table 4.27 Multivariate regression model for OCPs associated with serum **DDE** , *OCPs entered simultaneously into single model*, HHANES 1982-84

OCP	**Coefficient**	**Standard Error**	**p-value**
beta-HCH	0.24	0.06	<0.01
DDT	0.80	0.06	<0.01

*adjusted for age at examination, Hispanic group, education, country of birth, season of examination

Table 4.28 Adjusted odds ratios and 95% confidence intervals for **spontaneous abortion**, HHANES 1982-84

Risk Factor	**Spontaneous Abortion**	**No Spontaneous Abortion**	**Multivariate Adjusted Odds Ratio**	**95% CI**
Age at examination*	369	768	1.01	1.00-1.02
Cuban	41	98	0.73	0.49-1.10
Mexican American	268	514		
Puerto Rican	60	156	0.77	0.54-1.08
Mexican American	268	514		
Education				
$\leq 8^{th}$ grade	183	298	1.38	1.04-1.82
$> 8^{th}$ grade	181	463		
Marital status				
Ever	352	690	2.11	1.17-3.82
Never	16	76		
Smoker				
Ever	156	276	1.37	1.05-1.77
Never	213	492		

* continuous variable, OR represents increase in risk for increase in one unit of variable

Table 4.29 Adjusted odds ratios and 95% confidence intervals for **stillbirth** and farm work,HHANES 1982-84

Risk Factor	Stillbirth	No Stillbirth	Multivariate Adjusted Odds Ratio	95% CI
Age at examination*	52	1085	1.03	1.00-1.05
Parity				
> 3	33	355	3.81	1.66-8.73
≤ 3	16	684		
Oral contraceptive use				
Ever	11	565	0.46	0.21-1.00
Never	41	520		
Alcohol drinker				
Ever	24	429	1.88	1.02-3.48
Never	25	642		
Farm work				
Ever	18	203	4.11	1.41-11.97
Never	34	882		
Farm work x parity	49	1039	0.31	0.08-1.18

* continuous variable, OR represents increase in risk for increase in one unit of variable

Table 4.30 Adjusted odds ratios and 95% confidence intervals for **stillbirth** and farm work by the effect modifying variable parity, HHANES 1982-84

Effect Modifier	Risk Factor	Stillbirth	No Stillbirth	Multivariate Adjusted* OR (95% CI)
	Farm work			
Parity >3	Ever	11	95	1.18 (0.59-2.93)
	Never	22	260	
	Farm work			
Parity ≤ 3	Ever	7	108	3.80 (1.34-11.54)
	Never	12	621	

*adjusted for age at examination, oral contraceptive use, and alcohol consumption

Table 4.31 Adjusted odds ratios and 95% confidence limits for **stillbirth** and dieldrin, HHANES 1982-84

Risk Factor	Stillbirth	No Stillbirth	Multivariate Adjusted Odds Ratio	95% CI
Age at examination	52	1085	1.02	1.00-1.05
Parity				
> 3	33	355	2.78	1.41-5.47
≤ 3	19	729		
Oral contraceptive use				
Ever	11	565	0.47	0.21-1.02
Never	41	520		
Alcohol drinker				
Ever	24	429	1.89	1.02-3.51
Never	25	642		
Dieldrin				
ADL	5	36	2.24	0.79-6.35
BDL	47	1049		

Table 4.32 Adjusted odds ratios and 95% confidence limits for **stillbirth** and farm work and dieldrin, *exposures considered simultaneously in a single model*, HHANES 1982-84

Risk Factor	Stillbirth	No Stillbirth	Multivariate Adjusted* Odds Ratio	95% CI
Farm work				
Ever	18	203	4.05	1.39-11.81
Never	34	882		
Dieldrin				
ADL	5	36	2.12	0.74-6.06
BDL	47	1049		
Farm work x parity	49	1039	0.30	0.08-1.14

*adjusted for age at examination, parity, contraceptive use and alcohol consumption

Table 4.33 Adjusted odds ratios and 95% confidence intervals for **low birth weight** and farm work, HHANES 1982-84

Risk Factor	Low Birth Weight	No Low Birth Weight	Multivariate Adjusted OR	95% CI
Age at examination*	143	935	1.00	0.99-1.02
Cuban	10	119	1.08	0.50-2.34
Mexican American	103	640		
Puerto Rican	30	176	1.60	0.96-2.65
Mexican American	103	640		
Parity				
> 3	79	301	2.85	1.75-4.64
1 - 3	64	634		
Country of mother's birth				
Not U.S.	71	551	0.65	0.43-0.99
U.S.	71	381		
Farm work				
Ever	47	169	2.70	1.50-4.86
Never	96	766		
Farm work x parity	142	932	0.54	0.25-1.19

*continuous variable, OR represents increase in risk for increase in one unit of age

Table 4.34 Adjusted odds ratios and 95% confidence intervals for **low birth weight** and farm work by the effect modifying variable parity, HHANES 1982-84

Effect Modifier	Risk Factor	Low Birth Weight	No Low Birth Weight	Multivariate Adjusted* OR (95% CI)*
	Farm work			
Parity >3	Ever	27	77	1.47 (0.83-2.59)
	Never	52	224	
	Farm work			
Parity 1 - 3	Ever	20	92	2.67 (1.46-4.86)
	Never	44	542	

*adjusted for age at examination, country of mother's birth, and Hispanic group

Table 4.35 Adjusted odds ratios and 95% confidence intervals for having a child (one or more) with **any type of birth defect**, HHANES 1982-84

Risk Factor	Child with Birth Defect	No Child with Birth Defect	Multivariate Adjusted Odds Ratio	95% CI
Age at examination*	86	1004	1.03	1.01-1.05
Cuban	9	120	0.87	0.40-1.88
Mexican American	66	689		
Puerto Rican	11	195	0.61	0.32-1.19
Mexican American	66	689		
Parity				
> 3	43	345	1.53	0.91-2.55
1 - 3	43	658		
Oral contraceptive use				
Ever	47	504	1.90	1.10-3.29
Never	39	500		

*continuous variable, OR represents increase in risk for increase in one unit of age

Table 4.36 Adjusted odds ratios and 95% confidence intervals for having a child (one or more) with a **heart defect**, HHANES 1982-84

Risk Factor	Heart Defect	No Heart Defect	Multivariate Adjusted Odds Ratio	95% CI
Age at examination*	19	1004	1.01	0.97-1.06
Parity				
> 3	10	345	2.89	1.0-8.36
1 - 3	9	658		
Education				
$\leq 8^{th}$ grade	3	441	0.18	0.05-0.67
$> 8^{th}$ grade	16	553		
Country of birth (mother)				
Not U.S.	5	590	0.30	0.10-0.87
U.S.	14	410		

*continuous variable, OR represents increase in risk for increase in one unit of age

Table 4.37 Adjusted odds ratios and 95% confidence intervals for having a child (one or more) with an **eye defect**, HHANES 1982-84

Risk Factor	Eye Defect	No Eye Defect	Multivariate Adjusted Odds Ratio	95% CI
Age at examination*	13	1003	1.01	0.97-1.06
Parity				
> 3	10	345	5.67	1.40-23.02
1 - 3	3	658		

*continuous variable, OR represents increase in risk for increase in one unit of age

Table 4.38 Adjusted odds ratios and 95% confidence intervals for having a child (one or more) with a **muscle, bone or joint defect**, HHANES 1982-84

Risk Factor	Muscle, Bone or Joint Defect	No Muscle, Bone or Joint Defect	Multivariate Adjusted Odds Ratio	95% CI
Age at examination*	12	1004	1.02	0.98-1.07
Parity				
> 3	6	345	1.26	0.36-4.50
1 - 3	6	658		
Country of birth				
Not U.S.	3	590	0.24	0.06-0.92
U.S.	9	410		
Farm work				
Ever	5	197	2.08	0.63-6.83
Never	7	807		

*continuous variable, OR represents increase in risk for increase in one unit of age

Table 4.39 Adjusted odds ratios and 95% confidence intervals for having a child (one or more) with a **mouth defect,** HHANES 1982-84

Risk Factor	Mouth Defect	No Mouth Defect	Multivariate Adjusted Odds Ratio	95% CI
Age at examination*	9	1004	1.03	0.99-1.08
Parity				
> 3	4	345	0.90	0.22-3.73
1 - 3	5	658		
Farm work				
Ever	4	197	3.12	0.81-11.97
Never	5	807		

*continuous variable, OR represents increase in risk for increase in one unit of age

Table 4.40 Adjusted odds ratios and 95% confidence intervals for having a child (one or more) with a **nervous system defect,** HHANES 1982-84

Risk Factor	Nervous System Defect	No Nervous System Defect	Multivariate Adjusted Odds Ratio	95% CI
Age at examination*	18	1004	1.06	1.02-1.10
Parity				
> 3	13	345	3.23	1.09-9.51
1 - 3	5	658		
Alcohol drinker				
Ever	10	388	2.76	1.04-7.33
Never	8	602		

*continuous variable, OR represents increase in risk for increase in one unit of age

Table 4.41 Adjusted odds ratios and 95% confidence intervals for having a child (one or more) with **multiple birth defects**, HHANES 1982-84

Risk Factor	Multiple Birth Defects	No Birth Defect	Multivariate Adjusted Odds Ratio	95% CI
Age at examination*	9	1004	1.04	0.99-1.09
Parity				
> 3	6	345	2.43	0.54-10.86
1 - 3	3	658		
Country of birth (mother)				
Not U.S.	3	590	0.31	0.08-1.28
U.S.	6	410		

*continuous variable, OR represents increase in risk for increase in one unit of age

Table 4.42 Adjusted odds ratios and 95% confidence intervals for **early menarche,** HHANES 1982-84

Risk Factor	Early Menarch (≤ 11 yrs)	Referent Group (12-14 yrs)	Multivariate Adjusted Odds Ratio	95% CI
Age at examination*	386	988	0.98	0.97-0.99
Cuban	58	115	1.88	1.29-2.73
Mexican American	235	706		
Puerto Rican	93	167	1.82	1.33-2.48
Mexican American	235	706		
BMI	356	902	1.04	1.01-1.07
Marital Status				
Ever	298	720	1.83	1.31-2.57
Never	88	266		

*continuous variable, OR represents increase in risk for increase in one unit of age

Table 4.43 Adjusted odds ratios and 95% confidence intervals for **late menarche**, *individual models for each exposure variable*, HHANES 1982-84

Exposure Variable	Late Menarch (≥ 15yrs)	Referent Group (12-14 yrs)	Multivariate Adjusted* Odds Ratio	95% CI
Dieldrin				
ADL	3	40	0.35	0.11-1.19
BDL	168	1219		
Farm work				
Ever	42	191	1.76	1.16-2.68
Never	129	1068		

*adjusted for age at examination, Hispanic group, country of birth

Table 4.44 Adjusted odds ratios and 95% confidence intervals for **late menarche**, *exposures considered simultaneously in single model*, HHANES 1982-84

Risk Factor	Late Menarch (≥ 15 yrs)	Referent Group (12-14 yrs)	Multivariate Adjusted Odds Ratio	95% CI
Age at examination*	167	978	1.03	1.02-1.04
Mexican American	119	883	1.17	0.77-1.77
Cuban or Puerto Rican	52	376		
Education				
≤ 8th grade	71	508	0.66	0.45-0.96
> 8th grade	96	738		
Country of birth				
Not U.S.	107	630	1.64	1.11-2.42
U.S.	63	625		
Dieldrin				
ADL	3	40	0.35	0.10-1.20
BDL	168	1219		
Farm work				
Ever	42	191	1.80	1.18-2.73
Never	129	1068		

*continuous variable, OR represents increase in risk for increase in one unit of age

Table 4.45 Adjusted odds ratios and 95% confidence interval for **early menopause** and serum OCPs and farm work, *individual models for each exposure variable*, HHANES 1982-84, HHANES 1982-84

OCP, Farm work	**Early Menopause (35-44 yrs)**	**Referent Group (45-49 yrs)**	**Multivariate Adjusted* Odds Ratio**	**95% CI**
Beta-HCH				
ADL	22	35	2.98	1.24-7.15
BDL	13	82		
DDT				
ADL	22	32	3.57	1.54-8.28
BDL	13	85		
Oxychlordane				
ADL	5	5	2.96	0.77- 11.37
BDL	30	112		
Farm work				
Ever	11	15	2.48	0.98-6.28
Never	24	101		

*adjusted for age at examination, Hispanic group

Table 4.46 Adjusted* odds ratios and 95% confidence limits for **early menopause**, serum OCPs, and farm work, *exposures considered simultaneously in single model*, HHANES 1982-84

OCP, Farm work	**Early Menopause (35-44 yrs)**	**Referent Group (45-49 yrs)**	**Multivariate Adjusted Odds Ratio**	**95% CI**
beta-HCH				
ADL	22	35	2.05	0.79-5.31
BDL	13	82		
DDT				
ADL	22	32	2.89	1.18-7.10
BDL	13	85		
Oxychlordane				
ADL	5	5	1.27	0.30-5.32
BDL	30	112		
Farm work				
Ever	11	15	2.10	0.77-5.68
Never	24	101		

* adjusted for age at examination, Hispanic group

Table 4.47 Adjusted* odds ratios and 95% confidence limits for **early menopause** and serum OCPs, *combining exposures into single model with significant interaction term,* HHANES 1982-84

OCP, Farm work	Early Menopause (35–44 yrs)	Referent Group (45–49 yrs)	Multivariate Adjusted Odds Ratio	95% CI
beta-HCH				
ADL	22	35	0.79	0.19-3.35
BDL	13	82		
DDT				
ADL	22	32	1.01	0.25-4.20
BDL	13	85		
Farm work				
Ever	11	15	2.06	0.74-5.77
Never	24	102		
DDT*BHC			7.92	1.09-57.53

*adjusted for age at examination and Hispanic group

Table 4.48 Crude and adjusted* odds ratios and 95% confidence limits for **early menopause** and detectable DDT in serum by presence or absence of detectable beta-HCH in serum, and for early menopause and detectable beta-HCH in serum by presence or absence of detectable DDT in serum, HHANES 1982-84

Effect Modifier	Exposure Variable	Early Menopause (35–44 yrs)	Referent Group (45–49 yrs)	Crude OR with 95% CI	Adjusted OR with 95% CI
Beta-HCH	DDT				
ADL	ADL	19	15	8.44 (2.10-33.89)	7.65 (1.85-31.72)
	BDL	3	20		
Beta-HCH	DDT				
BDL	ADL	3	17	1.15 (0.28-4.63)	1.08 (0.25-4.62)
	BDL	10	65		
DDT	Beta-HCH				
ADL	ADL	19	15	7.18 (1.77-29.16)	5.64 (1.30-24.44)
	BDL	3	17		
DDT	Beta-HCH				
BDL	ADL	3	20	0.98 (0.24-3.89)	0.85 (0.19-3.76)
	BDL	10	65		

*adjusted for age at examination and Hispanic group
ADL – above detection limit, BDL – below detection limit

Table 4.49 Adjusted odds ratios and 95% confidence interval for **late menopause** and serum OCP and farm work, *individual models for each exposure variable,* HHANES 1982-84

OCP, Farm work	**Late Menopause (> 50 yrs)**	**Referent Group (45-49 yrs)**	**Multivariate Adjusted* Odds Ratio**	**95% CI**
Beta-BHC				
ADL	30	35	2.09	1.00-4.37
BDL	37	82		
Farm work				
Ever	17	15	2.44	1.00-5.92
Never	50	102		

*adjusted for age at examination, Hispanic group, BMI

Table 4.50 Adjusted odds ratios and 95% confidence interval for **late menopause** and serum OCPs and farm work, *considering exposures simultaneously in a single model,* HHANES 1982-84

OCP, Farm work	**Late Menopause (>50 yrs)**	**Referent Group (45-49 yrs)**	**Multivariate Adjusted* Odds Ratio**	**95% CI**
Beta-HCH				
ADL	30	35	2.20	1.04-6.38
BDL	37	82		
Farm work				
Ever	17	15	2.58	1.05-6.38
Never	50	102		

*adjusted for age at examination, Hispanic group,BMI

Table 4.51 Analysis of variance for **age at menarche** and exposure variables, adjusted for age at examination, individual models for each exposure variable

Exposure variables	ppb	N	Mean age at menarche	Standard error	P-value
Beta-HCH					
BDL	< 1.00	1261	12.56	0.04	ref
ADL	≥ 1.00	284	12.59	0.10	0.77
median split	<1.00	1261	12.56	0.04	ref
	1-1.88	140	12.69	0.13	0.34
	>1.88	144	12.48	0.14	0.60
quartile	<1.00	1261	12.56	0.04	ref
	1.00-1.39	63	12.74	0.20	0.37
	1.40-1.88	77	12.65	0.18	0.60
	1.89-2.59	70	12.45	0.19	0.58
	>2.59	74	12.51	0.19	0.81
ppb	<1.00	1261	12.56	0.04	ref
	1.00-1.99	149	12.73	0.13	0.20
	2.00-2.99	88	12.33	0.17	0.20
	3.00-3.99	22	12.61	0.33	0.89
	≥ 4	25	12.57	0.31	0.96
DDT					
BDL	<2.00	1314	12.56	0.04	ref
ADL	≥ 2.00	231	12.60	0.11	0.68
median split	<2.00	1314	12.56	0.04	ref
	1.00-3.43	114	12.47	0.15	0.59
	>3.43	117	12.73	0.15	0.25
quartile	<2.00	1314	12.56	0.04	ref
	2.00-2.40	58	12.49	0.20	0.77
	2.41-3.43	56	12.45	0.21	0.63
	3.44-4.75	58	12.88	0.20	0.12
	>4.75	59	12.58	0.20	0.90
ppb	<2.00	1314	12.56	0.04	ref
	2.00-2.99	98	12.54	0.16	0.91
	3.00-3.99	75	12.71	0.18	0.39
	4.00-5.99	32	12.54	0.27	0.97
	>6	26	12.60	0.30	0.89

Exposure variables	ppb	N	Mean age at menarche	Standard error	P-value
Dieldrin					
BDL	<1.00	1501	12.57	0.04	ref
ADL	≥ 1.00	44	12.31	0.24	0.27
median split	<1.00	1501	12.57	0.04	ref
	1-1.30	21	12.13	0.34	0.20
	>1.31	23	12.47	0.32	0.76
ppb	<1.00	1501	12.57	0.04	ref
	1.00	27	12.00	0.30	0.06
	≥ 2.00	17	12.80	0.37	0.54
Hexachlorobenzene					
BDL	<1.00	1487	12.54	0.04	ref
ADL	≥1.00	58	13.01	0.20	0.02
median split	<1.00	1487	12.54	0.04	ref
	1.00-1.30	27	12.94	0.30	0.19
	>1.31	31	13.07	0.30	0.06
ppb	<1.00	1487	12.55	0.04	ref
	1.00-1.99	43	13.15	0.24	0.01
	≥2.00	15	12.62	0.40	0.85
Oxychlordane					
BDL	<1.00	1494	12.56	0.04	ref
ADL	≥1.00	51	12.76	0.22	0.35
median split	<1.00	1494	12.56	0.04	ref
	1.00-1.29	24	13.02	0.32	0.15
	>1.30	27	12.53	0.30	0.94
ppb	<1.00	1494	12.56	0.04	ref
	1.00-1.99	40	12.82	0.25	0.30
	≥ 2.00	11	12.57	0.46	0.98
trans-nonachlor					
BDL	<1.00	1452	12.55	0.04	ref
ADL	≥1.00	93	12.68	0.16	0.46
median split	<1.00	1452	12.55	0.04	ref
	1.00-1.47	44	12.92	0.23	0.12
	>1.47	49	12.46	0.22	0.68
ppb	<1.00	1452	12.55	0.04	ref
	1.00-1.99	69	12.88	0.19	0.09
	≥ 2.00	24	12.11	0.32	0.16

Exposure variables	ppb	N	Mean age at menarche	Standard error	P-value
DDE					
quintile	> 4.30	236	12.71	0.11	ref
	4.30-7.36	235	12.54	0.10	0.25
	7.37-11.55	235	12.79	0.10	0.58
	11.56-18.60	236	12.71	0.10	0.96
	>18.60	236	12.77	0.11	0.70
Farm work					
Never		246	12.51	0.04	ref
Ever		1299	12.83	0.10	<0.01

ppb - parts per billion
BDL - below the detection limit
ADL - above the detection limit
ref - reference category

Table 4.52. Analysis of variance for **age at menarche** and exposure variables, adjusted for age at examination, BMI, Hispanic group, ever married, and country of birth (individual models for each exposure variable)

Exposure variables	ppb	N	Mean age at menarche	Standard error	P-value
Beta-HCH					
BDL	<1.00	1151	12.55	0.04	0.90
ADL	≥1.00	252	12.54	0.11	
median split	<1.00	1151	12.55	0.05	ref
	1.00-2.09	126	12.67	0.14	0.45
	>2.09	126	12.40	0.14	0.32
quartile	<1.00	1151	12.55	0.04	ref
	1.00-1.40	59	12.71	0.20	0.45
	1.41-2.09	67	12.63	0.19	0.71
	2.10-2.73	63	12.39	0.20	0.41
	≥2.74	63	12.41	0.20	0.51
ppb	<1.00	1151	12.55	0.05	ref
	1.00-1.99	134	12.72	0.14	0.27
	2.00-2.99	80	12.25	0.18	0.11
	3.00-3.99	19	12.48	0.35	0.83
	≥4.00	19	12.46	0.35	0.79
DDT					
BDL	<2.00	1215	12.56	0.04	ref
ADL	≥2.00	188	12.46	0.12	0.42
median split	<2.00	1215	12.56	0.04	ref
	2.00-3.43	101	12.42	0.16	0.39
	>3.43	87	12.51	0.17	0.75
quartile	<2.00	1215	12.56	0.04	ref
	2.00-2.40	52	12.51	0.22	0.80
	2.41-3.43	49	12.33	0.22	0.30
	3.44-4.75	47	12.70	0.23	0.56
	>4.75	40	12.28	0.25	0.25
ppb	<2.00	1215	12.56	0.04	ref
	2.00-2.99	86	12.50	0.17	0.74
	3.00-3.99	62	12.51	0.20	0.81
	4.00-5.99	22	12.27	0.33	0.37
	≥6.00	18	12.30	0.36	0.46

Exposure variables	ppb	N	Mean age at menarche	Standard error	P-value
Dieldrin					
BDL	<1.00	1368	12.56	0.04	ref
ADL	≥1.00	35	12.30	0.26	0.33
median split	<1.00	1368	12.56	0.04	ref
	1.00-1.30	17	12.18	0.37	0.32
	>1.30	18	12.40	0.36	0.68
ppb	<1.00	1368	12.56	0.04	ref
	1.00-1.99	22	11.99	0.33	0.09
	≥ 2.00	13	12.80	0.43	0.56
Hexachlorobenzene					
BDL	<1.00	1353	12.54	0.04	ref
ADL	≥1.00	50	12.88	0.22	0.12
median split	<1.00	1353	12.54	0.04	ref
	1.00-1.32	21	12.67	0.33	0.69
	>1.33	29	13.03	0.28	0.09
ppb	<1.00	1353	12.54	0.04	ref
	1.00-1.99	36	13.01	0.26	0.06
	≥ 2.00	14	12.53	0.40	0.99
Oxychlordane					
BDL	<1.00	1363	12.55	0.04	ref
ADL	≥1.00	40	12.64	0.24	0.70
median split	<1.00	1363	12.55	0.04	ref
	1.00-1.29	17	12.99	0.37	0.24
	>1.30	23	12.39	0.32	0.62
ppb	<1.00	1363	12.55	0.04	ref
	1.00-1.99	31	12.66	0.28	0.69
	≥ 2.00	9	12.58	0.51	0.95
trans-nonachlor					
BDL	<1.00	1323	12.55	0.04	ref
ADL	≥1.00	80	12.58	0.18	0.85
median split	<1.00	1323	12.55	0.04	ref
	1.00-1.50	41	12.91	0.24	0.15
	>1.50	39	12.24	0.25	0.23
ppb	<1.00	1323	12.55	0.04	ref
	1.00-1.99	60	12.80	0.20	0.22
	≥ 2.00	20	11.93	0.34	0.08

Exposure variables	ppb	N	Mean age at menarche	Standard error	P-value
DDE					
quintile	> 4.30	221	12.76	0.11	ref
	4.30-7.36	224	12.61	0.11	0.31
	7.37-11.55	220	12.81	0.11	0.77
	11.56-18.60	215	12.67	0.11	0.55
	>18.60	196	12.55	0.12	0.22
Farm work					
Never		1182	12.51	0.04	ref
Ever		221	12.79	0.11	0.01

ppb - parts per billion
BDL - below the detection limit
ADL - above the detection limit
ref - reference category

Table 4.53. Analysis of variance for **age at menopause** and exposure variables, adjusted for age at examination (individual models for each exposure variable)

Exposure Variables	ppb	N	Mean age at menopause	Standard error	P-value
Beta-HCH					
BDL	<1.00	132	48.70	0.41	ref
ADL	≥1.00	87	47.43	0.51	0.07
median split	<1.00	132	48.66	0.41	ref
	1.00-2.09	42	48.33	0.73	0.69
	>2.09	45	46.56	0.71	0.01
quartile	<1.00	132	48.66	0.41	ref
	1.00-1.40	20	47.91	1.05	0.51
	1.41-2.09	22	48.71	1.01	0.96
	2.10-2.73	22	46.08	1.01	0.02
	≥2.74	23	47.06	1.01	0.15
ppb	<1.00	132	48.66	0.41	ref
	1.00-1.99	40	48.09	0.74	0.50
	2.00-2.99	27	46.20	0.90	0.01
	3.00-3.99	10	49.77	1.50	0.47
	≥4.00	10	45.74	1.51	0.07
DDT					
BDL	<2.00	146	48.97	0.38	ref
ADL	≥2.00	73	46.57	0.55	<0.01
median split	<2.00	146	48.97	0.38	ref
	2.00-3.43	36	47.33	0.77	0.06
	>3.43	37	45.81	0.77	<0.01
quartile	<2.00	146	48.99	0.37	ref
	2.00-2.40	18	46.78	1.06	0.05
	2.41-3.43	18	47.87	1.06	0.32
	3.44-4.75	18	48.45	1.06	0.63
	>4.75	19	43.20	1.06	<0.01
ppb	<2.00	146	48.98	0.38	ref
	2.00-2.99	30	47.34	0.83	0.07
	3.00-3.99	17	48.23	1.10	0.52
	4.00-5.99	13	46.38	1.28	0.05
	≥6.00	13	42.72	1.26	<0.01

Exposure Variables	ppb	N	Mean age at menopause	Standard error	P-value
Dieldrin					
BDL	<1.00	200	48.35	0.33	ref
ADL	≥1.00	19	46.30	1.08	0.07
median split	<1.00	200	48.35	0.33	ref
	1.00-1.30	9	47.29	1.58	0.52
	>1.30	10	45.41	1.50	0.06
ppb	<1.00	200	48.35	0.33	ref
	1.00-1.99	12	46.18	1.37	0.13
	≥ 2.00	7	46.50	1.79	0.31
Hexachlorobenzene					
BDL	<1.00	205	48.23	0.33	ref
ADL	≥1.00	14	47.23	1.28	0.45
median split	<1.00	205	48.23	0.33	ref
	1.00-1.32	6	46.41	1.95	0.36
	>1.33	8	47.84	1.69	0.82
ppb	<1.00	205	48.23	0.33	ref
	1.00-1.99	9	46.63	1.62	0.34
	≥ 2.00	5	48.25	2.14	0.99
Oxychlordane					
BDL	<1.00	204	48.29	0.33	ref
ADL	≥1.00	15	46.50	1.23	0.16
median split	<1.00	204	48.29	0.33	ref
	1.00-1.13	6	46.26	1.94	0.30
	>1.13	9	46.67	1.59	0.32
trans-nonachlor					
BDL	<1.00	182	48.39	0.35	ref
ADL	≥1.00	37	47.09	0.78	0.13
median split	<1.00	182	48.39	0.35	ref
	1.00-1.50	18	47.86	1.12	0.65
	>1.50	19	46.36	1.09	0.08
ppb	<1.00	182	48.39	0.35	ref
	1.00-1.99	27	48.22	0.91	0.86
	≥ 2.00	10	44.06	1.48	<0.01

Exposure Variables	ppb	N	Mean age at menopause	Standard error	P-value
DDE					
quintile	< 5.46	43	48.90	0.71	ref
	5.46-10.43	44	48.75	0.70	0.88
	10.44-16.09	44	49.36	0.70	0.64
	16.10-23.60	44	47.75	0.70	0.25
	>23.60	44	46.11	0.71	<0.01
Farm work					
Never		176	48.18	0.36	ref
Ever		43	48.12	0.73	0.94

ppb - parts per billion
BDL - below the detection limit
ADL - above the detection limit
ref - reference category

Table 4.54. Analysis of variance for **age at menopause** and exposure variables, adjusted for age at examination, Hispanic group, education, and age at menarche (individual models for each exposure variable)

Exposure variables	ppb	N	Mean age at menopause	Standard error	P-value
Beta-HCH					
detection limit	BDL	128	48.46	0.43	ref
	ADL	84	47.72	0.54	0.31
median split	<1.00	128	48.45	0.43	ref
	1.00-2.09	41	48.67	0.76	0.80
	>2.09	43	46.83	0.74	0.07
quartile	<1.00	128	48.46	0.43	ref
	1.00-1.40	19	48.30	1.11	0.90
	1.41-2.09	22	48.95	1.01	0.65
	2.10-2.73	22	46.52	1.01	0.09
	≥2.74	21	47.16	1.06	0.26
ppb	<1.00	128	48.47	0.42	ref
	1.00-1.99	39	48.43	0.78	0.97
	2.00-2.99	27	46.54	0.90	0.06
	3.00-3.99	10	50.11	1.50	0.28
	≥4.00	8	45.12	1.68	0.06

Exposure variables	ppb	N	Mean age at menopause	Standard error	P-value
DDT					
detection limit	BDL	142	48.80	0.40	ref
	ADL	70	46.87	0.58	0.01
median split	<2.00	142	48.80	0.40	ref
	2.00-3.43	34	47.76	0.82	0.27
	>3.43	36	46.04	0.79	<0.01
quartile	<2.00	142	48.83	0.39	ref
	2.00-2.40	17	47.28	1.08	0.18
	2.41-3.43	16	48.21	1.15	0.61
	3.44-4.75	18	48.52	1.09	0.79
	>4.75	19	43.65	1.07	<0.01
ppb	<2.00	142	48.81	0.40	ref
	2.00-2.99	28	47.67	0.87	0.24
	3.00-3.99	16	48.54	1.14	0.82
	4.00-5.99	13	46.70	1.29	0.12
	≥6.00	13	43.16	1.27	<0.01
Dieldrin					
detection limit	BDL	193	48.24	0.34	ref
	ADL	19	47.41	1.12	0.48
median split	<1.00	193	48.24	0.34	ref
	1.00-1.30	9	48.28	1.59	0.98
	>1.30	10	48.61	1.52	0.30
ppb	<1.00	193	48.24	0.34	ref
	1.00-1.99	12	47.32	1.39	0.52
	≥ 2.00	7	47.56	1.80	0.71
Hexachlorobenzene					
detection limit	BDL	198	47.14	0.33	ref
	ADL	14	48.24	1.28	0.41
median split	<1.00	198	48.24	0.33	ref
	1.00-1.32	6	46.35	1.96	0.34
	>1.33	8	47.71	1.66	0.75
ppb	<1.00	198	48.24	0.33	ref
	1.00-1.99	9	46.96	1.63	0.45
	≥ 2.00	5	47.44	2.11	0.71

Exposure variables	ppb	N	Mean age at menopause	Standard error	P-value
Oxychlordane					
detection limit	BDL	199	48.28	0.33	ref
	ADL	13	46.39	1.30	0.16
median split	<1.00	199	48.28	0.33	ref
	1.00-1.13	4	44.86	2.33	0.15
	>1.13	9	47.08	1.57	0.45
trans-nonachlor					
detection limit	BDL	177	48.34	0.35	ref
	ADL	35	47.28	0.80	0.23
median split	<1.00	177	48.34	0.35	ref
	1.00-1.50	18	48.08	1.10	0.82
	>1.50	17	46.42	1.15	0.11
ppb	<1.00	177	48.34	0.35	ref
	1.00-1.99	27	48.48	0.89	0.88
	≥ 2.00	8	43.16	1.64	<0.01
DDE					
quintile	< 5.46	41	48.19	0.77	ref
	5.46-10.43	43	48.78	0.70	0.57
	10.44-16.09	43	49.35	0.71	0.27
	16.10-23.60	42	47.99	0.72	0.85
	>23.60	43	46.51	0.73	0.13
Farm work					
Never		170	48.02	0.36	ref
Ever		42	48.74	0.74	0.39

ppb - parts per billion
BDL - below the detection limit
ADL - above the detection limit
ref - reference category

Table 4.55 Analysis of variance for **age at menopause** and OCPs in serum, adjusted for age at examination, *individual models for each two way interaction term, results shown only for significant (≤ 0.05) interaction terms in models*

OCP†		N	Mean age at menopause	Standard error	P-value
beta-HCH	DDT				
0	0	104	48.67	0.45	ref
0	1	28	48.68	0.86	0.99
1	0	42	49.73	0.70	0.20
1	1	45	45.24	0.68	<0.01
beta-HCH	Oxychlordane				
0	0	129	48.56	0.41	ref
0	1	3	53.23	2.70	0.09
1	0	75	47.84	0.54	0.29
1	1	12	44.76	1.36	0.01
DDT	oxychlordane				
0	0	142	48.89	0.39	ref
0	1	4	51.81	2.30	0.21
1	0	62	46.93	0.59	<0.01
1	1	11	44.49	1.40	<0.01
DDT*	*trans*-nonachlor				
0	0	135	48.84	0.40	ref
0	1	11	50.66	1.39	0.21
1	0	47	47.13	0.67	0.03
1	1	26	45.52	0.92	0.01
Farm work	*trans*-nonachlor				
0	0	148	48.21	0.39	ref
0	1	28	48.01	0.89	0.84
1	0	43	49.15	0.81	0.30
1	1	9	44.24	1.57	0.01

† 0 = below detection limit, 1 = above detection limit

Table 4.56 Multiple factor analysis of variance for **age at menopause** and OCPs in serum, adjusted for age at examination, Hispanic group, education and age at menarche, *individual models for each two way interaction term, results shown only for significant (≤ 0.05) interaction terms in models*

OCP†		N	Mean age at menopause	Standard error	P-value
beta-HCH	DDT				
0	0	100	48.42	0.48	ref
0	1	28	48.93	0.85	0.60
1	0	42	49.78	0.70	0.12
1	1	42	45.44	0.75	<0.01
beta-HCH	Oxychlordane				
0	0	125	48.38	0.42	ref
0	1	3	52.87	2.66	0.09
1	0	74	48.13	0.56	0.73
1	1	10	44.31	1.49	0.01
DDT	oxychlordane				
0	0	138	48.70	0.40	ref
0	1	4	52.13	2.27	0.14
1	0	61	47.36	0.61	0.08
1	1	9	43.62	1.52	<0.01
DDT*	*trans*-nonachlor				
0	0	131	48.69	0.41	ref
0	1	11	50.43	1.40	0.23
1	0	46	47.45	0.69	0.13
1	1	24	45.65	0.97	<0.01
Farm work	*trans*-nonachlor				
0	0	143	48.02	0.39	ref
0	1	27	48.12	0.89	0.92
1	0	34	49.65	0.80	0.07
1	1	8	44.60	1.67	0.04

† 0 = below detection limit, 1 = above detection limit

CHAPTER V

DISCUSSION

SERUM LEVELS OF ORGANOCHLORINE PESTICIDES

Overall, the serum concentrations found in the present study are similar to concentrations found in other studies conducted during a similar time period, the late 1970's to mid 1980's (Table 5.1). Comparing the present study with the U.S. National Health and Nutrition Examination Study conducted in 1976-80 reveals a slightly higher proportion of serum OCP levels above the detection limit among the women in the present study for beta-HCH, oxychlordane and *trans*-nonachlor and a slightly lower proportion above the detection limit for DDT and dieldrin. The proportion above the detection limit for DDE and hexachlorobenzene in the two studies was similar. Since the majority of women in the present study lived in southern regions of the U.S., higher detections of oxychlordane and

trans-nonachlor, compounds which are associated with the use of termiticides used more commonly in the south, would be expected. (ATSDR. 1992).

The OCPs investigated in this study, as described previously in the literature review, are stable lipophilic compounds which accumulate in the fatty tissues of the body. Therefore, serum levels of OCPs reflect the cumulative body burden of the compounds (Lopez-Carillo et al., 1999). As would be expected, six of the seven OCPs were found to be positively associated with the age of the study subjects at the time of the examination when the serum sample was drawn. The exception was dieldrin, which was not associated with age at examination in the logistic regression model. Other investigators (Murphy and Harvey, 1985; Devoto et al., 1998) also failed to find an association of serum dieldrin level with age. The lack of association of dieldrin with age suggests a difference in pharmacokinetic properties for dieldrin.

Hispanic group (Mexican, Cuban or Puerto Rican) was associated with the frequency of detection of these OCPs in serum. This probably reflects regional differences in environmental contamination of the compounds, as the Mexican group from the southwestern U.S. and the Cuban group from the southeastern U.S., had higher frequencies of detection for all of the compounds compared to the Puerto Rican group from the north eastern U.S. Various human monitoring studies have documented a higher percentage of positive samples for many of these compounds in the southeastern and southwestern U.S. compared to northern regions of the country (Savage et al., 1981;

Stehr-Green, 1989). Logistic regression analysis revealed that those subjects born outside the U.S. were at increased risk for detection of serum DDT and also higher serum concentration of DDE. This finding may reflect a higher level and longer period of use of DDT, and therefore higher environmental and dietary contamination, in Mexico, Cuba and Puerto Rico. In addition, those born outside the U.S. may visit their country of birth more frequently than those who were born in the U.S., and therefore have more opportunity for higher exposures.

All of the OCP compounds investigated were positively associated with serum cholesterol, reflecting the lipophilicity of the compounds. Only dieldrin and DDT were found to be positively associated with BMI. Although the dietary route of exposure is considered the most important for many of these OCP compounds, it may be the composition of the diet (proportion of animal fat containing foods, or frequency of eating contaminated fish for example) and not the total calories that determines exposure. Pharmacodynamics may also play a role, with individuals having a higher percentage of body fat in which to store their body burden of OCPs, leading to a diluting effect and lower serum concentrations (ATSDR, 1992). Beta-HCH, dieldrin, oxychlordane, *trans*-nonachlor and DDE were all associated with having the serum sample drawn in the winter season. Stehr-Green (1998) also reported that in the NHANES II, serum DDE was associated with serum sampling conducted in the winter season.

Detection of beta-HCH, DDT and dieldrin and concentration of DDE in serum was

associated with lower educational level ($\leq 8^{th}$ grade). In contrast, detection of hexachlorobenzene in serum was associated with a higher educational level ($> 8^{th}$ grade). Hexachlorobenzene and dieldrin were the only OCPs associated with currently residing in a rural or suburban area (outside of a central city area). Oxychlordane was the only OCP significantly associated with ever having worked on a farm. Study subjects who had ever worked on a farm had an increased risk (OR=1.96, 95% CI 1.02-3.76) of having detectable oxychlordane in their serum compared to those who had never worked on a farm. However, after adjusting for the presence of other OCPs in the serum, farm work was no longer significantly associated with serum oxychlordane. The lack of an association between serum OCPs and farm work supports the belief that exposure to these OCPs is mainly through the dietary route. Most of these OCP's were banned from use in agriculture in the U.S. during the 1970's, therefore agricultural related exposure since then would result from residual soil contamination and not current application. Other sources of exposure are not agricultural related, for example, a significant source of exposure to *trans*-nonachlor, oxychlordane and dieldrin is living in a house treated with chlordane or dieldrin to kill termites (Taguchi and Yakushiji, 1988).

As expected, the metabolite serum DDE was significantly positively associated with detection of the parent compound DDT, and detection of serum oxychlordane was strongly associated with detection of another chlordane related compound, *trans*-nonachlor. In general, detection in serum of any one of the seven OCPs indicated an increased risk for detection of one or more of the other OCPs. Excluding DDE which was

detected in nearly the entire sample, of the 456 study subjects in which one or more of the remaining six OCPs were detected, 218 (48%) had only one OCP detected in their serum and 238 (52%) had two or more OCPs detected in their serum. These results demonstrate that exposure to multiple OCPs is common among those exposed to at least one OCP. Therefore, additive or synergistic effects of mixtures of these compounds should be considered in studies of adverse health effects related to exposure.

Although not a focus of this study, women with detectable serum oxychlordane or dieldrin were found to be at increased risk for diabetes. Diabetes is an endocrine disorder characterized by high levels of blood glucose resulting from defects in insulin secretion, insulin action, or both. Dioxin, another important OCP, was recently reported to be associated with diabetes mellitus, impaired glucose metabolism, and insulin production in humans (Henriksen et al., 1997). Though the biologic mechanism for this finding is unclear, there are several possible clues in the literature. Diabetes is partly an autoimmune disease, with destruction of the pancreatic islet cells that produce insulin by autoantibodies. Various immunologic effects have been attributed to OCPs (NRC, 1999). In a small study of humans exposed to chlordane aerosols for several months, increased titers of autoantibodies were found in 11 of the 12 subjects tested (NRC, 1999). An effect on glucose uptake from the intestines was found in rhesus monkeys experimentally exposed to dieldrin (Mahmood et al., 1981). Dieldrin elevated the uptake of glucose and the activities of brush border sucrase, lactase, maltase and alkaline phosphatase in the intestine.

REPRODUCTIVE ENDPOINTS

Spontaneous Abortion

In the general population approximately 10 to 20% of recognized pregnancies will terminate in spontaneous abortion. The prevalence of recognized spontaneous abortion for this sample of women was 14.4% and falls in the expected range. The socio-demographic and lifestyle risk factors found in this study to be associated with ever experiencing a spontaneous abortion were age at examination, education level, smoking, marital status, and Mexican Hispanic group. The increased risk for lower education level (OR=1.38, 95% CI 1.04-1.82) and ever smoking (OR=1.37, 95% CI 1.05-1.77) were modest. These risk factors have been consistently found in various studies of spontaneous abortion reported in the literature. The maternal age at the time of the spontaneous abortion was not available in this study; however, the association of spontaneous abortion with age at examination probably reflects that older women had more time in which to have had more pregnancies at risk for spontaneous abortion. No exposure variables (serum OCPs and farm work) were associated with spontaneous abortion in this study. Misclassification of outcome status can easily occur in studies of recognized spontaneous abortion because 20% of pregnancies end in fetal loss before clinical evidence of pregnancy. A possible effect on early spontaneous abortion (before clinical recognition) would not be detected with this study design, which relied on maternal knowledge of the pregnancy outcome.

Stillbirth

Stillbirth is a rare outcome and has a low prevalence of about 0.5-1% in the general population. The prevalence of stillbirth (proportion of total live births) in this sample was 1.40%, which is in the expected range. The significant risk factors identified for stillbirth in this study were older age at examination, parity ≥ 3, never use of oral contraceptives, ever drinking alcohol, ever doing farm work, and serum dieldrin above the detection limit. The increased risk for older age at examination and higher parity reflect that older women and women who have had more pregnancies, have had more opportunity to experience stillbirth. Although no studies in the literature were identified which linked stillbirth to use of oral contraceptives, use of oral contraceptives within the three months prior to conception was protective against spontaneous abortion of a chromosomally normal conceptus in a large case-control study (Sackoff, et al., 1994). The association between alcohol use and stillbirth is found in the literature (Pastore et al., 1997).

In this study, the risk of stillbirth for women who had ever done farm work was 4.11 times higher than for women who had never done farm work. This effect of increased risk for ever doing farm work was modified by parity. The increased risk for stillbirth among women doing farm work was restricted primarily to women with a parity ≤ 3. There is no obvious explanation for the interaction between parity and farmwork, especially since the exposure(s) related to farm work which is responsible for the association with stillbirth is unknown. Having had a previous fetal loss is an important risk factor for subsequent fetal loss; therefore, women who have had several pregnancies without experiencing a fetal loss

are less at risk based on their prior experience. After adjustment for farm work and the other associated factors, the risk of stillbirth for women with serum dieldrin above the detection limit was twice the risk for women without serum dieldrin above the detection limit. This risk estimate lacked precision; however, as the confidence interval was wide and included one (OR=2.12, 95% CI, 0.74-11.81). Arbuckle and Sever (1998), in their large review of studies of stillbirth and unspecified pesticides, reported that a positive association was found in many studies with odds ratios ranging from 1.4 to 3.6. In a study using birth certificate records from Washington State, Engle, et al. (1995) reported the adjusted relative risk for late fetal loss for women working in agriculture was 2.4 (95% CI, 1.5-4.0). Savitz, et al. (1989a) reported an adjusted odds ratio for maternal exposure to alicyclic halogens (including chlordane) and stillbirth equal to 1.4 (95% CI, 0.7-2.9).

Low Birth Weight

The prevalence of low birth weight among all live births in this sample was 4.79% . This prevalence is comparable to the proportion of low birth weight births (5.3%) found in a cross-sectional study of Mexican Americans using national vital records in 1981 (Ventura and Taffel, 1985). Low birth weight births comprise both preterm births and births which are small-for-gestational-age (SGA). Risk factors for low birth weight identified in the present study were higher parity, being U.S. born, and ever having done farm work. It has been reported previously in the literature that second-generation Mexican-American women are almost twice as likely to experience a low birth weight outcome than first-generation Mexican-Americans (Jones and Bond, 1999). This is hypothesized to be related

to behavioral factors associated with a traditional Mexican cultural orientation such as lower rates of smoking and alcohol use, fewer premarital births and better nutrition (Scribner and Dwyer, 1989).

None of the serum OCPs investigated were found to be associated with low birth weight. Women in this study who had ever done farm work, however, were 2.7 times more likely to have experienced a low birth weight birth than women who had never done farm work. The effect was modified by parity, with the effect of farm work predominately among women with a lower parity. There is no obvious explanation for the interaction between parity and farmwork, especially since the exposure(s) related to farm work which is responsible for the association with low birth weight is unknown. In general, nulliparous women are at higher risk for low birth weight than multiparous women, which could explain why the risk for farm work is among the women with lower parity (Brooke et al., 1989). In addition, having had a previous low birth weight birth is an important risk factor for subsequent low birth weight births, therefore, women who have had several births without experiencing a low birth weight birth are less at risk based on their prior experience. Low birth weight has been associated with long working hours, prolonged standing and heavy lifting (Tuntiserancee et al., 1998; Fortier et al., 1995; Saurel-Cubizolles et al., 1985). These working conditions may reasonably be expected to Occurur during farm work and could account for the increased risk. Although serum OCPs were not associated with low birth weight, exposure to other classes of pesticides not measured in this study (e.g., organophosphates) might be another explanation for the increased risk

associated with farm work. Zhang et al. (1992) found an increased risk (adjusted OR 2.9, 95% CI 0.96-8.6) for small-for-gestational-age (SGA) births in a large sample of women in China who had been exposed to unspecified pesticides during pregnancy.

Birth Defects

Congenital malformations present at birth, also known as birth defects, Occurur in approximately 3-7% of live births. In this sample of Hispanic women the prevalence of birth defects was 2.53%. None of the serum OCPs explored in this study were associated with birth defects in this sample. It was not possible to evaluate the association between birth defects of specific body systems and many of the OCPs (oxychlordane, hexachlorobenzene, dieldrin) because of the small numbers of women with birth defects. Farm work however, was associated with defects of the muscles, bones or joints and defects of the mouth. The risk for having a child with a muscle, bone or joint defect for women who had ever done farm work was double the risk for women who had never done farm work (OR=2.08, 95% CI 0.63-6.83). Due to a small sample size, the risk estimate was imprecise and the confidence interval included 1.0. This category of muscle, bone or joint defect would include limb reduction defects and an elevated risk for limb reduction defects similar in magnitude to that found in this study, for women involved in agriculture or living in an agricultural region, has been well documented (Schwartz et al., 1986; Schwarz and LoGerfo, 1988). Previous investigators have identified an increased risk for orofacial clefts, which would be included in the mouth defect category, and agricultural work (Garcia, 1998). In the present study, women who had ever done farm work were

3.12 (95% CI 0.81-11.29) times more likely to have had a child with a mouth defect. The risk estimate was imprecise and included 1.0 due to the small sample size.

Nervous system defects in this study were found to be significantly associated with ever drinking alcohol. The odds ratio for ever drinking alcohol and having a child with a nervous system defect was 2.76 (95% CI 1.04-7.33). Fetal Alcohol Syndrome is well characterized in the literature and may explain the association between drinking alcohol and nervous system defects. Fetal damage caused by heavy maternal alcohol consumption includes impaired development of the central nervous system, facial malformations and retarded growth (DeBruyne and Folfes, 1989).

Age at Menarche

The age at menarche generally varies from nine to fifteen years of age in developed countries. In developing countries, and particularly in rural areas, the mean age at menarche is often higher (Malina et al., 1977). The mean age at menarche in this sample was 12.56 years and the median age at menarche was 12 years. The median age at menarche of 12 years found in this study compares well with the median age of menarche of 12.55 years in an urban area of Mexico reported by Malina et al. in 1977. In this study, early menarche ($\leq$ 11 yrs.) was positively associated with lower age at examination, Cuban or Puerto Rican Hispanic group, higher BMI and ever having been married.

In the logistic regression model, no serum OCPs were associated with early menarche.

Late age at menarche ($\geq$ 15 yrs.) was positively associated with late age at examination, Mexican Hispanic group, higher education, and being born outside of the U.S. Women with serum dieldrin above the detection limit were less likely to have had a late menarche (protective effect). Women who had ever done farm work were 1.80 times more likely to have had a late menarche than women who had never done farm work. An association between residing in rural areas and later age at menarche has been noted in previous studies (Malina et al., 1977; Graham et al. 1999)

No OCP exposure variables were associated with age at menarche in the AOV models. Ever having done farm work was associated with a small but significant increase in age at menarche (0.28 yrs.). Malina et al. 1977 reported that girls from rural areas of Mexico had a mean age at menarche 0.5 years later than girls from urban areas. This could explain the later age at menarche found for women who had ever done farm work in this study, as women who had done farm work are likely to have resided in rural areas.

Age at Menopause

The average age at natural menopause in the U.S. and European populations ranges from 48 to 52 years. In developing countries the natural age at menopause is approximately four years earlier than for developed countries (Gonzales et al., 1997). For this sample of Hispanic women, the mean age at menopause was 48.09 years, which is at the low end of the range for the general U.S. population. In logistic regression analysis, early menopause was significantly associated with detectable serum DDT (OR=2.89, 95% CI 1.18-7.10)

and having both detectable serum DDT and detectable serum beta-HCH (OR=5.64, 95% CI 1.30-24.44), suggesting an interactive effect.

The results of analysis of variance were generally consistent with the logistic regression results. Serum levels of DDT, beta-HCH and *trans*-nonachlor were associated with an earlier age at menopause in a dose dependent manner. The magnitude of the decelerating effect on age at menopause of these individual OCPs was dose dependent and ranged from one to five years. Interactive effects were associated with having two-way combinations of DDT plus either beta-HCH, trans-nonachlor, or oxychlordane and for beta-HCH plus oxychlordane. These interactive effects appeared to be additive. The following factors known to be associated with early menopause were evaluated and were included in the analysis as necessary to control for confounding: socio-economic status, BMI, parity, age at menarche, smoking and alcohol consumption (Torgerson et al., 1994). Information on several risk factors for early menopause such as cancer chemotherapy and various autoimmune disorders was not available; therefore, these risk factors were not controlled for in the analysis.

One biologically plausible explanation for the effect of DDT, beta-HCH and *trans*-nonachlor on age at menopause is ovarian damage potentially caused by endocrine disruption or toxic effects due to chronic lifetime exposure to these compounds which are stored in adipose tissue and are also found in ovarian follicular fluid (Jarrel et al., 1993; Baukloh et al., 1985). In utero exposure is unlikely, because these women who had

experienced menopause by 1982-84 were born prior to 1940 when these OCPs began to be manufactured. Natural menopause is thought to be due to the depletion of ovarian follicles responsive to the gonadotropins (Sowers and La Pietra, 1995). Toxicity causing damage to the ovarian follicles or oocytes, or interference with their normal development may lead to an early menopause (Sowers and La Pietra, 1995). It is also hypothesized that the hypothalamus and pituitary gland and changes in the pattern of their neuroendocrine messages may also play an important role in the timing of menopause (Wise et al., 1996). Therefore, an effect on the hypothalamus or pituitary may alter the age at menopause.

Evidence from *in vitro* studies suggest potential ovarian effects for DDT. *In vitro* studies have demonstrated a depressive effect of DDT and gamma-HCH on oocyte maturation and an effect of decreased bovine oviductal cell viability for DDT (Alm et al., 1998; Tiemann et al. 1998). DDT and chlordane are reported to affect ion transport across cell membranes, including inhibition of calcium transport which may affect the action of peptide hormones (ATSDR, 1992) (Table 5.2). These OCPs are also reported to inhibit gap-junction intercellular communication, which may affect growth and maturation of oocytes (Goodenough et al., 1999; Li and Mather, 1997; Anderson and Albertini, 1976; Gilula et al., 1978)(Table 5.2).

There is some evidence in the literature that OCPs may affect central neural endocrine targets. Lipophilic OCPs have been measured in the brain and are able to pass through the

blood brain barrier. The parts of the brain that control reproductive function, such as the median eminence and other circumventricular organs, are outside the blood-brain barrier (NRC, 1999). The effects of OCPs on membrane ion transport (Table 5.2) may also lead to changes in neurotransmitter release. Experimental data in animals indicates that the doses at which neurological effects occurred were lower in the chronic studies than in the acute studies (ATSDR, 1992). Chlordane compounds are also neural toxins, though the exact mechanism is unknown, but may involve inhibition of gama-aminobutyric acid (GABA), and alteration of membrane permeability to Ca^{--} (ATSDR, 1992). In regard to interactive effects, acute toxicity of chlordane in rats was increased by 2.3-4.6 times when there was previous exposure to aldrin, dieldrin, or DDT (ATSDR, 1992).

Additional evidence that OCPs may affect central endocrine targets is provided in a study of OCPs and repeated miscarriages, Gerhard et al. (1998) reported correlations between OCPs with markers of thyroid and pituitary function. They found inverse associations between Thyroid Stimulating Hormone and beta-HCH, gamma-HCH, PCBs and DDE. In addition, they found positive correlations between Follicle Stimulating Hormone and prolactin with total OCP body burden.

Other potential endocrine disrupting mechanisms of action of OCPs may involve changes in metabolism, circulating levels of sterioid hormones, or estrogenic or anti-estrogenic activity. ppDDT can bind to cellular estrogen receptors (Danzo, 1997)(Table 5.2). Beta-HCH does not bind to the estrogen receptor, but has been found to have estrogenic effects

through an influence on the estrogen receptor (Steinmetz et al., 1996). Chlordane can affect metabolism and circulating levels of steroid hormones (ATSDR, 1992b). The effect of an earlier age at menopause seen in this study would be consistent with a possible anti-estrogenic effect.

STUDY LIMITATIONS

There are important limitations of this study which necessitate viewing the study findings conservatively. Limitations related to the temporal relationship between exposure measurement and outcome, the temporal stability of OCPs in serum over time, lack of information on important confounders, and potential recall bias and missclassification are discussed below.

A limitation inherent in a cross-sectional study is that the temporal relationship between the exposure and outcome cannot be established, and therefore cause and effect cannot be determined. Since one cannot be certain in a cross-sectional study which has occurred first, the exposure or the outcome, the possibility of the exposure being a consequence of the outcome must be considered. A reasonable alternative explanation which might explain the association of the serum OCPs with early menopause is that changes in fat metabolism and serum lipids resulting from menopause itself, may cause an increase in either body burden of OCPs or circulating levels of serum OCPs which is more apparent in women with an earlier menopause (Roberts and Silbergeld, 1995). Blood cholesterol levels increase with menopause (Roberts and Silbergeld, 1995). To assess whether serum

cholesterol may be acting as a confounder, a multiple regression model was fit with age at menopause as the dependent variable and age at examination and log transformed serum cholesterol concentration as the independent variables. The results of the model indicated that serum cholesterol concentration was not associated with age at menopause ($p=0.55$), and therefore was not acting as a confounder. There may however, be other measures of lipids or fat metabolism acting as confounders, which were unable to be controlled for in this study.

Another important limitation is that the OCP exposures measured at the time of sampling may not represent the level of exposure at the time of the reproductive events. Exposure was measured for approximately 60% of the sample during their reproductively active period, from 16 to 45 years of age. The age difference between age at examination and age at menopause ranged from 0 to 37 years, with a mean of 10.57 years, median of 8.00 years, and a mode of 2.00 years. The age difference between age at examination and age at menarche was much broader, ranging from 0 to 63 years, with a mean of 22.14 years, a median of 19.00 years and a mode of 3.00 years. These statistics indicate that the distributions were skewed somewhat to the left, towards the lower end of the range.

OCPs have long half-lives (eg., the median half-life of beta-HCH in serum is 7.2 years [Jung et al., 1997]) and accumulate over time in adipose tissue due to continual exposure; therefore, the relative concentrations of these compounds in various groups over time should be constant. The exposure measurements represent a physiological balance

between past exposure, storage in adipose tissue, release into the bloodstream and current exposure. Partitioning of OCPs in the body is based on the lipid content of the tissues, therefore the concentration in serum is much lower than the concentration in adipose tissue (Needham et al., 1990). The adipose (lipid weight) to serum (whole weight) ratio for the OCPs in this study ranges from approximately 200-400:1 (Needham et al., 1990). Therefore, a whole weight serum concentration of 1.00 ppb corresponds to a concentration of 200-400 ppb in adipose tissue. For many reproductive outcomes such as birth defects, the time window of exposure to cause an effect during the development of the embryo or fetus is very specific (Garcia 1998). An effect on age at menopause such as accelerated oocyte depletion (Sowers and La Pietra, 1995) however, might result from chronic exposure over time.

Short term temporal variability of serum OCPs in healthy women was investigated by Gammon et al.(1997). These authors reported that temporal changes in OCP levels within a 1-3 month period were minimal. Longnecker et al. (1999) measured serial levels of serum OCPs (DDE and polychlorinated biphenyls) during pregnancy and postpartum. Blood samples were drawn during each trimester and within 35-48 hours postpartum prior to breast-feeding. The Pearson correlation coefficient among lipid adjusted OCP levels in the first trimester and postpartum was 0.87, indicating a very strong correlation and minimal change in serum OCP levels throughout pregnancy. There were no studies found which examined temporal variability of serum OCPs over longer periods of time (years).

Lactation is an important pathway for elimination of OCPs from the body (Roberts and Silbergeld, 1995). The prevalence of breastfeeding initiated in-hospital in the United States from 1965 to 1984 varied from a low of approximately 25% in 1970 to a high of approximately 60% in 1984 (Ryan, 1997). The inability to adjust for number and duration of lactations in this study, due to the lack of lactation history information, could have introduced significant missclassification of exposure. The direction of the missclassification may likely be toward lower levels of OCPs at the time of the examination compared to the time of reproductive events taking place prior to any lactation (eg., menarche, spontaneous abortion), and would bias the risk estimates downward. With respect to age at menopause however, missclassification of exposure due to lactation would not be an important factor. All women in the analysis of age at menopause and serum OCPs were menopausal at the time of exposure measurement and pregnancy and lactation do not occur after menopause, therefore the time interval between menopause and the exposure measurement did not include pregnancy or lactation.

Inability to control for potential confounding by type or composition of oral contraceptives used (estrogen only versus estrogen and progesterone) and by use of hormone replacement therapy may led to inadequate control of confounding. Although ever/never use of oral contraceptives was evaluated as a potential confounder, if an effect was related to the hormonal composition of the contraceptives, then this effect was not controlled for by a single category of contraceptive use. Information was also lacking in this study on exposure to cancer chemotherapy, which has been associated with an earlier

age at menopause (Torgerson et al., 1994).

The data for many variables, such as for the reproductive effects, have been collected retrospectively and may suffer inaccuracy due to their dependence on the participant's memory. This could result in misclassification of outcome variables. However, misclassification from this source is likely to be non-differential with respect to the exposures of interest (serum OCPs), since the participant was unaware of their exposure status. If there was a systematic recall bias however, a spurious association could result. To evaluate recall bias on age menarche and menopause related to the age at examination, the mean ages at menarche and menopause were determined by stratum of age at examination. The mean age at examination for the menopausal women was 56 years, and there was no significant difference in the mean age at menopause among women older or younger than 56 years at the time of the examination (Table 5.3). There was a small but significant decrease in mean age at menarche for each stratum of age at examination (Table 5.4). This difference in mean age at menarche by age at examination could be explained by a cohort effect, since the age at examination spans three generations, illustrating the secular decrease in age at menarche which has occurred over the last century (Dann and Roberts, 1993). This difference in age at menarche by age at examination could also represent a tendency for women as they age to systematically recall a later age at menarche, but the total difference in mean age at menarche from youngest to the oldest group was only 0.86 years. Investigations of the accuracy of recall of age at menarche have demonstrated moderate-to-high correlations between actual recorded

menarche and recalled menarche in adolescents and adults up to 19 years after the event (Blanck et al., 2000).

This study was limited by a lack of power to find associations for specific types of birth defects and OCPs. Most of the OCPs examined in this study could not be evaluated with regard to their association with specific types of birth defects because the numbers of woman with detectable levels of these OCPs who also had a child with a birth defect of a specific body system, were too small for analysis. In addition, even within body system categories of defects such as heart defects, there are different types of defects which may have different etiologies and mechanisms of effect. When these defects are aggregated together as in this study, missclassification will occur, driving the estimate towards the null.

Misclassification of exposure could also occur due to the limitations of laboratory testing methods, but these methods are relatively sensitive and specific given the technology used by EPA in their laboratory. The HHANES has a good response rate of 71%, but the possibility of some selection bias must be considered.

The confirmation in this study of many well established associations provides some assurance regarding the general sensitivity of the data base; however, the validity of the results for each measure of association must be evaluated separately. The frequency of detection and level of the OCPs in serum found in this study were similar to other studies

conducted around the same time period (Table 5.1). The serum OCPs were positively associated with age and serum cholesterol, as has been described in many previous studies. A number of the risk factors for specific reproductive outcomes found in this study are consistent with what has been reported previously in the literature. Some examples are: association of spontaneous abortion with smoking (Windham et al., 1997); association of stillbirth, low birth weight and muscle, bone and joint defects with farm work (Arbuckle and Sever, 1998; Garcia, 1998; Zhang et al., 1992); and the association of nervous system defects with alcohol consumption.

Many comparisons have been made in this study and some associations could have occurred by chance. The p-values determined in this study were not adjusted for multiple comparisons because, "as descriptors of the patterns in the data, measures of effect such as risk ratios, confidence intervals on those measures, and even p-values need not be adjusted" (Savitz and Olshan, 1998). Adjustment of p-values for multiple comparisons may lead to the dismissal of meaningful results (Savitz and Olshan, 1998; Savitz and Olshan, 1995). Each comparison should be considered based on the available biological and epidemiologic evidence which informed the decision to make the comparison (Goodman 1998). The comparisons made in this study were informed by a body of evidence described in the literature review.

FUTURE RESEARCH

The association of exposure to specific OCPs with age at menopause has not been reported previously. Future studies are needed to validate this finding. A prospective cohort design would address many of the important limitations of this study, since exposure could be measured prior to the outcome measures. Future studies should utilize more sensitive methods of OCP determination in serum with a lower limit of detection in the parts per trillion range (Stellman et al., 1998; Gerhard et al., 1999) and should be able to adjust for total lipid content of the serum (Lopez-Carrillo et al., 1999). A lower limit of detection would enable quantitation of the OCPs in the serum of most women, allowing a more detailed evaluation of the dose response relationship. This is especially important because some *in vivo* investigations of HAAs suggest a nonmonotonic dose-response relationship can occur, including both U-shaped and inverted U-shaped curves. In a study of methoxychlor (a compound similar to DDT) and reproductive development in rats, a U-shaped dose-response curve was reported, with the onset of cyclicity in female offspring accelerated in the low-dose group, normal in the two mid-dose groups, and delayed in the high dose group (NRC, 1999). The basic assumption that there is a threshold concentration below which no adverse effect will occur has also been challenged for HAAs (NRC, 1999).

Toxicologic research is needed to explore the biologic mechanism(s) of effect which may be involved in association of DDT, beta-HCH and *trans*-nonachlor with earlier age at menopause, such as toxic ovarian effects and effects on the hypothalamic-pituitary axis. A

better understanding of the pharmakokinetics of OCPs during menopause and differences relative to premenopausal women is needed to assist in the evaluation of alternative explanations for the association of the three OCPs with early menopause in this study. Future studies should investigate interactive effects of exposure to multiple OCPs. As demonstrated by this study, exposure to multiple OCPs occurs, and therefore it is difficult to attribute effects to a single compound. In addition, because HAAs can have multiple mechanisms of action at multiple target tissues sites, there is the opportunity for synergistic effects.

The results of this study also indicate an association of detectable serum dieldrin and oxychlordane with diabetes. In light of recent investigations conducted by the U.S. Air Force (Henriksen et al., 1997), which identified a positive association between diabetes and dioxin, another OCP not included in the present study, further exploration of this association is warranted.

PUBLIC HEALTH IMPLICATIONS

The finding of an elevated risk for stillbirth, low birth weight and birth defects of the muscle, bones and joints associated with farm work, indicates that farm work is a hazardous occupation for pregnant women. This conclusion is supported by significant evidence in the literature (Savitz et al. 1989; Schwarz and LoGerfo, 1988; Arbuckle and Sever, 1998). These adverse reproductive outcomes have a significant public health impact, and their association with farm work suggests that pregnant farm workers should

be targeted for preventive measures.

The association of OCPs with age at menopause may be of public health concern. An earlier menopause may particularly impact women today who often postpone childbearing. An early menopause may also impact two important diseases associated with loss of the protective effects of estrogen. Women experiencing menopause before age 40 were found to be at increased risk for coronary heart disease and stroke (Snowden et al., 1989). Early menopause may also increase a woman's risk for osteoporosis (Roberts and Silbergeld, 1995). An early menopause would be protective however, against breast and endometrial cancer. Age at natural menopause may be a biological marker of health and aging, as was suggested by the findings of Snowden et al. (1989), which indicated that women with natural menopause before age 40 were three times more likely to have died from any cause, compared to women with natural menopause at ages 50 to 54.

Table 5.1 Published Measurements of Organochlorine Compounds in Serum, Compared with Measurements Obtained in the Present Study

Study location and description	United States Occupational Exposure to Pesticides in 14 Comunities Across the US (Warnick SL, 1972)		United States NHANES II national population based sample (Murphy R, 1985)	United States Florida Citrus Workers (Griffith J, 1985)	**United States HHANES (present study) population based sample of Hispanics**	United States Rural Population (Stehr-Green PA, 1988)	United States Effects of Fasting and Feeding Atlanta, GA (Phillips DL, 1989)	
Study size and composition	Exposure group* N=505 Adult males	Control group** N=217 Adult males	N=4,538-6,732 ages 12-74 Males and females	N=567 Adult citrus field workers	**N=2792 ages 12-74 Males and females**	N=85 mean age=36.9 white dairy farm families	N=20 Adult volunteers who worked at CDC	
Time frame of sampling	1969-70	1969-70	1976-80	1981	**1982-84**	1986	late 1980's	
Organochlorine measurements	ppb in serum (non-fasting)	ppb in serum (non-fasting)	ppb in serum (non-fasting)	ppb in serum (non-fasting)	**ppb in serum (non-fasting)**	ppb in serum (non-fasting)	ppb serum wt (non-fasting)	ppb serum wt (fasting)
Beta HCH	mean=2.3	mean=1.3	median=1.7 range=1-28 %ADL=13.9	mean=2.0 %ADL=28.1	**mean=1.87 median=2.23 range=1-13.68 %ADL=16.9**	mean=0.87 %ADL=36.5	NA	NA
ppDDT	mean=9.2	mean=3.6	median=3.3 range=2-58 %ADL=31	mean=4.6 %ADL=24.5	**mean=4.83 median=3.22 range=1.22-88.62 %ADL=13.2**	mean=3.50 %ADL=12.9	NA	NA

ppDDE	mean=29.3	mean=19.6	median=11.8 range=1-378 %ADL=99	mean=26.9 %ADL=95.6	**mean=22.05 median=13.90 range=1.01-228.22 %ADL=97.8**	mean=13.54 %ADL=100	mean=6.77	mean=5.68
Dieldrin	NA	NA	median=1.4 range=1-16 %ADL=8.6	mean=1.8 %ADL=3.0	**mean=1.91 median=1.60 range=1.00-9.00 %ADL=2.7**	NA	NA	NA
HCB	NA	NA	median=1.7 range=1-17 %ADL=3.3	mean=1.2 %ADL=4.8 (n=62)	**mean=1.79 median=1.41 range=1.00-5.67 %ADL=3.9**	mean=0.27 %ADL=100	mean=0.163	mean=0.138
Oxychlordane	NA		median=1.4 range=1-23 %ADL=2.5	mean=2.0 %ADL=5.5	**mean=1.40 median=1.70 range=1.00-6.60 %ADL=3.9**	mean=0.49 %ADL=70.6	NA	NA
Trans-nonachlor	NA		median=1.4 range=1-17 %ADL=4.4	mean=2.7 %ADL=22.8	**mean=2.0 median=1.50 range=1.00-17.60 %ADL=7.3**	mean=1.27 %ADL=84.7	NA	NA

NA=Not Available

* exposed group composed of pesticide formulators and packagers, farmers, spray-plane pilots, mosquito abatement workers, commercial applicators and pest control operators

** control group composed of workers in jobs of similar physical activity but without pesticide exposure and matched on race, age, and weight

Table 5.2 Examples of hormone receptor dependent and receptor independent modes of action of OCPs

OCP	Receptor Binding	Reference	Receptor Binding Independent (interference with second messenger pathways)	Reference
beta-HCH	does not bind estrogen receptor	Steinmetz, et al. 1996	produces estrogenic like responses (cell division and growth) through signaling pathways that influence the estrogen receptor without binding to it activates tyrosine kinase a "second messenger"	Steinmetz, et al. 1996 Hatakeyama and Matsumura, 1999
ppDDT	binds estrogen receptor	Danzo, 1997	reduction in the activity of Mg2+/Ca2+ dependent ATPases (impairs membrane function) Gap-junction intercellular communication inhibitor	Tiemann and Kuchenmeister, 1999 Tiemann, et al.,1996
DDE	binds androgen receptor (antagonist) binds estrogen receptor	Cooper and Kavlock, 1997	no information on effect of DDE on membranes and second messenger systems was found	
Dieldrin	weakly estrogenic in the E-screen assay but ? if binds estrogen receptor	Sonnenschein and Soto, 1998	disrupts cell membrane Ca $^{2+}$ transport mechanisms interferes with GABA gated chloride channels Gap-junction intercellular communication inhibitor	Mehrotra et al. 1989 Mehrotra et al. 1989 Zhong-Xiang,et al. 1986
Chlordane	Chlordane not estrogenic in E-Screen assay (in vitro cell proliferation assay)	Sonnenschein and Soto, 1998	disrupts cell membrane Ca $^{2+}$ transport mechanisms interferes with GABA gated chloride channels Gap-junction intercellular communication inhibitor	ATSDR, 1992 ATSDR, 1992 ATSDR, 1992

Table 5.3 Mean age at menopause by age group at examination

Age group at examination (yrs)	N	Mean age at menopause	Standard Error	P-value
≤ 56	103	47.63	0.47	Referent
> 56	116	48.65	0.44	0.11

Table 5.4 Mean age at menarche by age group at examination

Age group at examination (yrs)	N	Mean age at menarche	Standard Error	P-value
12-20	391	12.10	0.08	Referent
21-35	493	12.51	0.07	<0.01
36-50	351	12.79	0.08	<0.01
>50	310	12.96	0.09	< 0.01

REFERENCES

Alm H, Tiemann U, Torner H. Influence of organochlorine pesticides on development of mouse embryos in vitro. Reproductive Toxicology 1996;10:321-6.

Alm H, Torner H, Tiemann U, Kanitz W. Influence of Organochlorine Pesticides on Maturation and Postfertilization Development of bovine Oocytes In Vitro. Reproductive Toxicology 1998;12:559-63.

Anderson E, Albertini DF. Gap junctions between the oocyte and companion follicle cells in the mammalian ovary. Journal of Cell Biology 1976;71:680-6.

Arbuckle T, Sever L. Pesticide exposures and fetal death: a review of the epidemiologic literature. Critical Reviews in Toxicology 1998;28:229-70.

Ateia M, Zaki A, Korayem W. Toxic effect of dieldrin on gonadotrophin levels (FSH and LH) in serum of mature female albino rats. Archives of Experimental Veterinary Medicine 1990;44:357-60.

ATSDR (Agency for Toxic Substances and Disease Registry). Toxicological Profile for Aldrin/Dieldrin. 1991. Atlanta, GA, USDHHS, Public Health Service.

ATSDR (Agency for Toxic Substances and Disease Registry). Toxicological Profile for 4,4'-DDT, 4,4'-DDE, 4,4'-DDD. 1992a. Atlanta, GA, USDHHS, Public Health Service .

ATSDR (Agency for Toxic Substances and Disease Registry). Toxicological Profile for Chlordane. 1992b. Atlanta, GA, USDHHS, Public Health Service.

ATSDR (Agency for Toxic Substances and Disease Registry). Toxicological Profile for Hexachlorocyclohexane. 1992c. Atlanta, GA, USDHHS, Public Health Service .

ATSDR (Agency for Toxic Substances and Disease Registry). Toxicological Profile for Hexachlorobenzene. 1994. Atlanta, GA, USDHHS, Public Health Service.

Aylsworth C, Trosko J, Chang C, et al. Synergistic inhibition of metabolic cooperation by oleic acid or 12-0-tetradecanoylphorbol-13-acetate and dichlorodiphenyltrichlorethane (DDT) in chinese hamster V79 cells: implications of a role for protein kinase C in the regulation of gap junctional intercellular communication. Cell Biology and Toxicology 1989;5:27-37.

Balcazar H, Krull J. Determinants of birthweight outcomes among Mexican-American women: examining conflicting results about acculturation. Ethnicity and Disease 1999;9:410-22.

Baukloh V, Bohnet H, Trapp M, et al. Biocides in human follicular fluid. Annals of the New York Academy of Sciences 1985;442:240-50.

Beard AP, Bartlewski PM, Chandolia RK, et al. Reproductive and Endocrine Function in Rams Exposed to the Organochlorine Pesticides Lindane and Pentachlorophenol from Conception. Journal of Reproduction and Fertility 1999b;115:303-14.

Beard AP, Bartlewski PM, Rawlings NC. Endocrine and Reproductive Function in Ewes Exposed to the Organochlorine Pestices Lindane or Pentachlorophenol. Journal of Toxicology and Environmental Health 1999a;56:23-46.

Bercovici B, Wassermann M, Cucos S, et al. Serum levels of polychlorinated biphenyls and some organochlorine insecticides in women with recent and former missed abortions. Environmental Research 1983;30:169-74.

Berkowitz G, Kelsey J, Holford T, et al. Physical activity and the risk of spontaneous preterm delivery. Journal of Reproductive Medicine 1983;28:581-8.

Berkowitz G, Lapinski R, Wolff M. The role of DDE and polychlorinated biphenyl levels in preterm birth. Archives of Environmental Contamination and Toxicology 1996;30:139-41.

Berkowitz G, Papeirnik E. Epidemiology of preterm birth. Epidemiologic Reviews 1993;15:414-43.

Bidleman T, Billings W, Foreman WT. Vapor-particle partitioning of semivolatile organic compounds: estimates from field collections. Environmental Science and Technology 1986;20:1038-43.

Bigsby R, Caperell-Grant A, Madhukar B. Xenobiotics released from fat during fasting produce estrogenic effects in ovariectomized mice. Cancer Research 1997;57:865-9.

Blanck HM, Marcus M, Tolbert, et al. Age at menarche and Tanner Stage in girls exposed *in utero* and postnatally to polybrominated biphenyl. Epidemiology 2000;11:641-7.

Blatter B, Roeleveld N, Zielhuis G, et al. Maternal Occupational exposure during pregnancy and the risk of spina bifida. Occupational and Environmental Medicine 1996;53:80-6.

Bromberger JT, Matthews KA, Kuller LH, et al. Prospective study of the determinants of age at menopause. American Journal of Epidemiology 1997;145:124-33.

Brooke O, Anderson H, Bland JM, et al. Effects on birth weight of smoking, alcohol, caffeine, socioeconomic factors, and psychosocial stress. British Medical Journal 1989;298:795-801.

Bush B, Snow J, Koblintz R. Polychlorobiphenyl (PCB) congeners, p,p'-DDE, and hexachlorobenzene in maternal and fetal cord blood from mothers in upstate New York. Archives of Environmental Contamination and Toxicology 1984;13:517-27.

Ceron-Pereles P, Mireles P, Harlow S, Carrillo S. The risk of prematurity and small-for - gestational age birth in Mexico City: the effects of working conditions and antenatal leave. American Journal of Public Health 1996;86:825-31.

Chatenoud L, Parazzini F, Di Cintio E, et al. Paternal and maternal smoking habits before conception and during the first trimester: relation to spontaneous abortion. Annals of Epidemiology 1998;8:520-6.

Cheek A, Vonier P, Oberdorster E, et al. Environmental signaling: a biological context for endocrine disruption. Environmental Health Perspectives 1998;106:5-10.

Colborn T, Dumanoski D, Myers J. Our Stolen Future: Are We Threatening Our Fertility, Intelligence, and Survival? New York: :Plume/Penquin, 1996.

Cooper R, Kavlock R. Endocrine disruptors and reproductive development: a weight-of-evidence overview. Journal of Endocrinology 1997;152:159-66.

Cornacoff J, Lauer L, House RV, et al. Evaluation of the Immunotoxicity of Beta-Hexachlorocyclohexane. Fundamental and Applied Toxicology 1988;11:293-9.

Crellin NK et al. Dichlorodiphenyldichloroethylene Potentiates the Effect of Protein Kinase A Pathway Activators on Progesterone Synthesis in Cultured Porcine Granulosa Cells. Biology of Reproduction 1999;61:1099-103. 1999.

Crisp TM, Clegg ED, Cooper RL, et al. Environmental Endocrine Disruption: An Effects Assessment and Analysis. Environmental Health Perspectives 1998;106[suppl 1]:11-56.

Cunningham MJ, Clifton DK, Steiner RA. Leptin's actions on the reproductive axis: perspectives and mechanisms. Biology of Reproduction 1999;60:216-22.

Damgaard I, Nyitrai G, Kovacs H, et al. Possible involvement of $GABA_A$ and $GABA_B$ receptors in the inhibitory action of lindane on transmitter release from cerebellar granule neurons. Neurochemical Research 1999;24:1189-93.

Dann TC and Roberts DF. Menarcheal age in University of Warwick young women. Journal of Biosocial Science 1993;25:531-8.

Danzo BJ. Environmental Xenobiotics May Disrupt Normal Endocrine Function by Interfering with the Binding of Physiological Ligands to Steroid Receptors and Binding Proteins. Environmental Health Perspectives 1997;105:294-301.

De Bruyne LRS. Life Cycle Nutrition: Conception through Adolescence. St. Paul, MN: West Publishing Company, 1989.

Devoto E, Kohlmeier L, Heeschen W. Some Dietary Predictors of Plasma Organochlorine Concentrations in an Elderly German Population. Archives of Environmental Health 1998;53:147-155.

Do K, Treloar S, Pandeya N, et al. Predictive factors of age at menopause in a large Australian twin study. Human Biology 1998;70:1073-91.

Engel L, O'Meara E, Schwartz S. Maternal Occupation in agriculture and risk of adverse birth outcomes in Washington State, 1980-1991. American Journal of Epidemiology 1995;141:S73 (abstract).

Faden V, Graubard B. Alcohol consumption during pregnancy and infant birth weight. Annals of Epidemiology 1994;4:279-84.

Fein G, Jacobson J, Jacobson S, et al. Prenatal exposure to PCBs:effects on birth size and gestational age. The Journal of Pediatrics 1984;105:315-20.

Fenster L, Coye M. Birthweight of infants born to Hispanic women employed in agriculture. Archives of Environmental Health 1990;45:46-52.

Flint M. Secular trends in menopause age. Journal of Psychosomatic Obstetrics and Gynecology 1997;18:65-72.

Fonovich de Schroeder T, Pechen de D'Angelo A. The turnover of phospholipid fatty acyl chains is activated by the insecticide dieldrin in *Bufo arenarum* oocytes. Journal of Biochemistry and Molecular Toxicology 2000;13:82-7.

Fonovich de Schroeder T, Pechen de D'Angelo A. Dieldrin modifies the hydrolysis of PIP_2 and decreses the fertiliation rate in *Bufo arenarum* oocytes. Comp Biochem Physiol 1995;112C:61-7.

Foreman W, Bidleman T. Semivolatile organic compounds in the ambient air of Denver, Colorado. Atmospheric Environment 1990;24A:2405-16.

Fortier I, Marcoux S, Brisson J. Maternal work during pregnancy and the risks of delivering a small-for-gestational-age or preterm infant. Scandinavian Journal of Work and Environmental Health 1995;21:412-8.

Fox G, Virgo B. Relevance of hyperglycemia to dieldrin toxicity in suckling and adult rats. Toxicology 1986;38:315-26.

Gammon M, Wolff M, Neugut A, et al. Temporal variation in chlorinated hydrocarbons in healthy women. Cancer Epidemiology, Biomarkers and Prevention 1997;6:327-32.

Garcia A. Occupational exposure to pesticides and congenital malformations: a review of mechanisms, methods, and results. American Journal of Industrial Medicine 1998;33:232-40.

Gerhard I, Daniel V, Link S, et al. Chlorinated hydrocarbons in women with repeated miscarriages. Environmental Health Perspectives 1998;106:675-81.

Gerhard I, Monga B, Krahe J, Runnebaum B. Chlorinated hydrocarbons in infertile women. Environmental Research Section A 1999;80:299-310.

Bilula NB, Epstein ML, Beers WH. Cell-to-cell communication and ovulation. A study of the cumulu-oocyte complex. Journal of Cell Biology 1979;78:58-75.

Gomez-Catalan J, To-Figueras J, Rodamilans M, Corbella J. Transport of organochlorine residues in the rat and human blood. Archives of Environmental Contamination and Toxicology 1991;20:61-6.

Gonzales G, Villena A, De La Cruz D. Age of natural menopause among women in Lima City, Peru. International Journal of Gynecology and Obstetrics 1997;57:69-72.

Goodenough DA, Simon AM, Paul DL. Gap junctional intercellular communication in the mouse ovarian follicle. Novartis Foundation Symposium 1999;219:226-35.

Goodman SN. Multiple comparisons, explained. American Journal of Epidemiology 1998; 147:807-12.

Gorodeski G. Impact of the menopause on the epidemiology and risk factors of coronary artery heart disease in women. Experimental Gerontology 1994;29:357-75.

Graham M, Larsen U, Xu X. Secular trend in age at menarche in china: a case study of two rural counties in Anhui Province. Journal of Biosocial Science 1999;31:257-67.

Greendale G, Sowers M. The menopause transition. Endocrinology and Metabolism Clinics of North America 1997;26:261-77.

Griffith J, Duncan RC. Serum Organochlorine Residues in Florida Citrus Workers Compared to the National Health and Nutrition Examination Survey Sample. Bulletin of Environmental Contamination and Toxicology 1985;35: 411-7.

Guendelman S, Gould JB, Hudes M, Eskenazi B. Generational differences in perinatal health among the Mexican American population: findings from HHANES 1982-1984. American Journal of Public Health 1990;80(suppl):61-65.

Guyer B, Hoyert D, Martin JA, et al. Annual summary of vital statistics 1998. Pediatics 1999;104:1229-46.

Hadley ME. Endocrinology. 2nd Ed. Englewood Cliffs, N.J:Prentice-Hall, 1988.

Hazarika R, Das M. Toxicological Impact of BHC on the Ovary of the Air-Breathing Catfish Heteropneustes fossilis (Bloch). Bulletin of Environmental Contamination and Toxicology 1998;60:16-21.

Hatakeyama M, Matsumura F. Correlation between the activation of Neu tyrosine kinase and promotion of foci formation induced by selected organochlorine compounds in the MCF-7 model system. Journal of Biochemical and Molecular Toxicology. 1999;13:296-302.

Higginbotham JC, Trevino FM, Ray LA. Utilization of curanderos by Mexican Americans: prevalence and predictors, findings from HHANES 1982-84. American Journal of Public Health 1990;80(suppl):32-5.

Jarrel JF, Villeneuve D, Franklin C, et al. Contamination of Human Ovarian Follicular Fluid and Serum by Chlorinated Organic Compounds in Three Canadian Cities. Canadian Medical Association Journal 1992;148:1321-7.

Jarrell J, Gocmen A, Foster W, et al. Evaluation of Reproductive Outcomes in Women Inadvertently Exposed to Hexachlorobenzene in Southeastern Turkey in the 1950s. Reproductive Toxicology 1998;12:469-76.

Jones M, Bond M. Predictors of birth outcome among Hispanic immigrant women. Journal of Nursing Care and Quality 1999;14:56-62.

Jung D, Becher H, Edler L, et al. Elimination of beta-hexachlorocyclohexane in Occupationally exposed persons. Journal of Toxicology and Environmental Health 1997;51:23-34.

Kanja L, Skaare J, Ojwang S, Maitai C. A comparsion of organochlorine pesticide residues in maternal adipose tissue, maternal blood, cord blood, and human milk from mother/infant pairs. Archives of Environmental Contamination and Toxicology 1992;22:21-4.

Klebanoff M, Shiono P, Carey J. The effect of physical activity during pregnancy on preterm delivery and birth weight. American Journal of Obstetrics and Gynecology 1990;163:1450-6.

Knobil E and Neill JD. The Physiology of Reproduction. New York, NY: Raven Press, 1988.

Koprowski C, Ross R, Mack W, et al. Diet, body size and menarche in a multiethnic cohort. British Journal of Cancer 1999;79:1907-11.

Laden F, Neas LM, Spiegelman D, et al. Predictors of Plasma Concentrations of DDE and PCBs in a Group of U.S. Women. Environmental Health Perspectives 1999;107:75-81.

Lay J, Klein W, Korte F. Metabolism of beta-hexachlorocyclohexane in rats following low dosing in the daily diet. Journal of Environmental Science and Health 1981;3:227-38.

Le Bacq F, Charimari L. Birth weight patterns in a commercial farming area, rural area, and urban area of Karoi District, Zimbabwe. Journal of Tropical Pediatrics 1993;39:238-42.

Leidy L. Early age at menopause among left-handed women. Obstetrics and Gynecology 1990;76:1111-4.

Leoni V, Fabiani L, Marinelli G. Spontaneous abortion in relation to the presence of hexachlorobenzene in the Italian environment. International Agency for Research on Cancer 1986;143-6.

Li R, Mather JP. Lindane, an inhibitor of gap junction formation, abolishes oocyte directed follicle organizing activity in vitro. Endocrinology 1997;138:4477-80.

Lin S, Marshall E, Davidson G. Potential parental exposure to pesticides and limb reduction defects. Scandinavian Journal of Work and Environmental Health 1994;20:166-79.

Longnecker MP, Klebanoff MA, Gladen BC, Berendes HW. Serial levels of serum organochlorines during pregnancy and postpartum. Archives of Environmental Health 1999;54:110-14.

Lopez-Carrillo L, Torres-Sanchez L, Lopez-Cervantes M, et al. The adipose tissue to serum dichlorodiphenyldichloroethane (DDE) rati: some methodological considerations. Environmental Research Section A 1999;81:142-5.

Mahmood A, Agarwal N, Sanyal S, et al. Acute dieldrin toxicity: effect on the uptake of glucose and leucine and on brush border enzymes in mondey intestine. Chemical-Biological Interactions 1981;37:165-70.

Malina R, Chumlea C, Stepick C, Guteirrez-Lopez F Age of menarche in Oaxaca, Mexico, schoolgirls, with comparative data for other areas of Mexico. Annals of Human Biology 1977;4:551-8.

Marks G, Garcia M, Solis JM. Health risk behaviors of Hispanics in the United States: findings from HHANES, 1982-1984. American Journal of Public Health 1990; 80(suppl):20-5.

McKinlay S, Branbilla D, Posner J. The normal menopause transition. Maturitas 1992;14:103-15.

Mehrotra B, Bansal S, Desaiah D. Comparative effects of structurally related cyclodiene pesticides on ATPases. Journal of Applied Toxicology 1982;2:278-83.

Mehrotra B, Moorthy K, Reddy S, Desaiah D. Effects of cyclodiene compounds on calcium pump activity in rat brain and heart. Toxicology 1989;54:17-29.

Menconi S, Clark JM, Langenberg P, Hryhorczuk D. A Preliminary Study of Potential Human Health Effects in Private Residences Following Chlordane Applications for Termite Control. Archives of Environmental Health 1988;43:349-52.

Meyer F, Moisan J, Marcoux D, Bouchard C. Dietary and physical determinants of menarche. Epidemiology 1990;1:377-81.

Mills J, Simpson J, Driscoll S, et al. Incidence of spontaneous abortion among normal women and insulin-dependent diabetic women whose pregnancies were identified within 21 days of conception. The New England Journal of Medicine 1988;319:1617-23.

Mohammed A, Eklundm A, Ostlund-Lindqvist A, Slanina P. Distribution of toxaphene, DDT, and PCB among lipoprotein fractions in rat and human plasma. Archives of Toxicology 1990;64:567-71.

Murphy R, Harvey C. Residues and Metabolites of Selected Persistent halogenated Hydrocarbons in Blood Specimens from a General Population Survey. Environmental Health Perspectives 1985;60:115-20.

Nagata C, Takatsuka N, Inaba S, et al. Association of diet and other lifestyle with onset of menopause in Japanese women. Maturitas 1998;29:105-13.

Narahashi T, Ginsburg K, Nagata K, et al. Ion channels as targets for insecticides. NeuroToxicology 1998;19:581-90.

NCHS (National Center for Health Statistics). . Plan and Operation of the Hispanic Health and Nutrition Examination Survey, 1982-1984. 1985. Washington, D.C., USDHHS, Public Health Service . Programs and Collection Procedures.

Needham LL, Burse VW, Head SL, et al. Adipose tissue/serum partitioning of chlorinated hydrocarbon pesticides in humans. Chemosphere 1990;20:975-980.

Noren K, Weistrand C, Karpe F. Distribution of PCB congeners, DDE, hexachlorobenzene, and methylsulfonyl metabolites of PCB and DDE among various fractions of human blood plasma. Archives of Environmental Contamination and Toxicology 1999;37:408-14.

NRC (National Research Council). Hormonally Active Agents in the Environment. Washington, D.C.: National Academy Press, 1999.

O'Leary J, Davies J, Edmundson W, Feldman M. Correlation of prematurity and DDE levels in fetal whole blood. American Journal of Obstetrics and Gynecology 1970a;106:939.

O'Leary J, Davies J, Edmundson W, Reich G Transplacental passage of pesticides. American Journal of Obstetrics and Gynecology 1970b;107:65-8.

O'Leary J, Davies J, Feldman M. Spontaneous abortion and human pesticide residues of DDT and DDE. American Journal of Obstetrics and Gynecology 1970c;108:1291-2.

Pastore L, Hertz-Picciotto I, Beaumont J. Risk of stillbirth from Occupational and residential exposures. Occupational and Environmental Medicine 1997;54:511-8.

Phillips DL, Pirkle JL, Burse VW, et al. Chlorinated Hydrocarbon Levels in Human Serum: Effects of Fasting and Feeding. Archives of Environonmental Contamination and Toxicology 1989;18:495-500.

Polishuk Z, Wasserman D, Wasserman M, et al. Organochlorine compounds in mother and fetus during labor. Environmental Research 1977;13:278-84.

POP Assessment Report. December 1995. United Nations Environmental Protection Agency.

Procianoy R, Schvartsman S. Blood pesticide concentration in mothers and their neworn infants: relation to prematurity. Acta Paediatrica Scandinavia 1981;70:925-8.

Pulido J, del Hoyo N, Perez-Albarsanz M. Effects of lindane on the glucose metabolism in rat brain cortex cells. Life Sciences 1990;47:1099-107.

Ramamoorthy K, Wang F, Chen I, et al. Estrogenic activity of a dieldrin/toxaphene mixture in the mouse uterus, MCF-7 human breast cancer cells, and yeast-based estrogen receptor assays: no apparent synergism. Endocrinology 1997;137:1520-7.

Rao S, Joshi S, Kanade A. Height velocity, body fat and menarcheal age of Indian girls. Indian Pediatrics 1998;35:619-28.

Rita P, Reddy P, Reddy S. Monitoring of workers Occupationally exposed to pesticides in grape gardens of Andhra Pradesh. Environmental Research 1987;44:1-5.

Roberts JS, Silbergeld EK. Pregnancy, lactation, and menopause: how physiology and gender affect the toxicity of chemicals. The Mount Sinai Journal of Medicine 1995; 62:343-55.

Robinson PE, Mack GA, Remmers J, et al. Trends of PCB, Hexachlorobenzene, and Beta Benzene Hexachloride Levels in the Adipose Tissue of the U.S. Population. Environmental Research 1990;53: 175-92.

Robison AK, Mukku VR, Spalding DM, Stancel GM. The Estrogenic Activity of DDT: The in vitro Induction of an Estrogen-Inducible Protein by o,p'DDT. Toxicology and Applied Pharmacology 1984;76: 537-43.

Robison A, Schmidt W, Stancel G. Estrogenic activity of DDT: estrogen-receptor profilesand the responses of individual uterine cell types following op'--DDT administration. Journal of Toxicology and Environmental Health 1985;16:493-508.

Rogan W, Gladen B, McKinney J, et al. Polychlorinated biphenyls (PCBs) and Dichlorodiphenyl Dichloroethane (DDE) in human milk: effects on growth, morbidity, and duration of lactation. American Journal of Public Health 1987;77:1294-7.

Ron M, Cucos B, Rosenn B, et al. Maternal and fetal serum levels of organochlorine compounds in cases of premature rupture of membranes. Acta Obstet Gynecol Scand 1988;67:695-7.

Ryan AS. The resurgence of breastfeeding in the United States. Pediatrics 1997;99:E12-6

Sackoff J, Kline J, Susser M. Previous use of oral contraceptives and spontaneous abortion. Epidemiology 1994;5:422-8.

Sala M, Sunyer J, Otero R, et al. Health Effects of Chronic High Exposure to Hexachlorobenzene in a General Population Sample. Archives of Environmental Health 1999;54:102-9.

Saurel-Cubizolles M, Kaminski M, Llado-Arkhipoff J, et al. Pregnancy and its outcome among hospital personnel according to Occupation and working habits. Journal of Epidemiology and Community Health 1985;39:129-34.

Savage EP, Keefe JJ, Tessari JD, et al. National Study of Chlorinated Hydrocarbon Insecticide Residues in Human Milk, USA. American Journal of Epidemiology 1981;113:413-22.

Savitz D, Harlow S. Selection of reproductive health end points for environmental risk assessment. Environmental Health Perspectives 1991;90:159-64.

Savitz D, Whelan E, Kleckner R. Effect of parents' Occupational exposures on risk of stillbirth, preterm delivery, and small-for-gestational-age infants. American Journal of Epidemiology 1989;129:1201-18.

Savitz D, Whelan E, Kleckner R. Self-reported exposure to pesticides and radiation related to pregnancy outcome - results from National Natality and Fetal Mortality Surveys. Public Health Reports 1989;104:473-7.

Savitz DA, Olshan AF. Describing data requires no adjustment for multiple comparisons: a reply from Savitz and Olshan. American Journal of Epidemiology 1998; 147:813-14.

Savitz DA, Olshan AF. Multiple comparisons and related issues in the interpretation of epidemiologic data. American Journal of Epidemiology 1995;142:904-8.

Saxena M, Siddiqui M, Bhargava A, et al. Role of chlorinated hydrocarbon pesticides in abortions and premature labour. Toxicology 1980;17:323-31.

Saxena M et al. Organochlorine pesticides in specimens from women undergoing spontaneous abortion, premature or full-term delivery. Journal of Analytical Toxicology 1981;5:6-9.

Saxena M, Siddiqui M, Seth T, et al. DDT and its metabolites in leiomyomatous and normal human uterine tissue. Archives of Toxicology 1987;59:453-5.

Schafer WR, Zahradnik HP. Organochlorine Compounds and Xenoestrogens in Human Endometrium. Reproductive Toxicology 1998;444:5-8.

Schwartz D, Newsum L, Markowitz Heifetz R. Parental Occupation and birth outcome in an agricultural community. Scandinavian Journal of Work and Environmental Health 1986;12:51-4.

Schwarz D, LoGerfo J. Congenital limb reduction defects in the agricultural setting. American Journal of Public Health 1988;78:654-9.

Scribner R, Dwyer J. Acculturation and low birthweight among Latinos in the Hispanic HANES. American Journal of Public Health 1989;79:1263-7.

Sever L. The state of the art and current issues regarding reproductive outcomes potentially associated with environmental exposures: reduced fertility, reproductive wastage, congenital malformation, and birth weight. U.S. Public Health Service 1988.

Shaw G, Wasserman C, O'Malley C, et al. Maternal pesticide exposure from multiple sources and selected congenital anomalies. Epidemiology 1999;10:60-6.

Siegel H, Latimer J. Breed effects on dieldrin accumulation in the fat of growing chickens. Poultry Science 1981;60:2218-20.

Siegel H, Latimer J. Effect of sex hormones on the retention of dieldrin in the fat of growing chickens. Poultry Science 1981;60:2210-7.

Sim M, Forbes A, McNeil J., Roberts G. Termite control and Other Determinants of High Body Burdens of Cyclodiene Insecticides. Archives of Environmental Health 1998;53:114-21.

Snieder H, MacGregor A, Spector T. Genes control the cessation of a woman's reproductive life: a twin study of hysterectomy and age at menopause. Journal of Clinical Endocrinology and Metabolism 1998;83:1875-80.

Snowdon D, Kane R, Beeson W, et al. Is early natural menopause a biologic marker of health and aging? American Journal of Public Health 1989;79:709-14.

Sohoni P, Sumpter J. Several environmental oestrogens are also anti-androgens. Journal of Endocrinology 1998;158:327-39.

Sowers M, La Pietra M. Menopause: its epidemiology and potential association with chronic diseases. Epidemiologic Reviews 1995;17:287-302.

Srinivasan K, Radhadrishnamurty R. Induction of liver mixed function oxygenase system by beta and gamma hexachlorocyclohexane . Indian Journal of Biochemistry and Biophysics 1983;20:84-91.

Srinivasan K, Radhakrishnamurty R. Studies on the distribution of beta and gamma isomers of hexachlorocyclohexane in rat tissues. Journal of Environmental Science and Health 1983;B18:401-18.

St. George I, Williams S, Silva P. Body size and the menarche: the Dunedin study. Journal of Adolescent Health 1994;15:573-6.

Stehr-Green PA. Demographic and Seasonal Influences on Human Serum Pesticide Residue Levels. Journal of Toxicology and Environmental Health 1989;27:405-21.

Stehr-Green PA, Farrar JA, Burse VW, et al. A Survey of Measured Levels and Dietary Sources of Selected Organochlorine Pesticide Residues and Metabolites in Human Sera from a Rural Population. American Journal of Public Health 1988;78:828-30.

Stein Z, Hatch M. Reproductive problems in the workplace. Occupational Medicine State of the Art Reviews 1986;1:361-539.

Steinmetz R, Young PCMC-GA, et al. Novel Estrogenic Action of the Pesticide Residue Beta-Hexachlorocyclohexane in Human Breast Cancer Cells. Cancer Research 1996;56: 5403-9.

Stellman SD, Djordjevic MV, Muscat JE, et al. Relative Abundance of Organochlorine Pesticides and Polychlorinated Biphenyls in Adipose Tissue and Serum of Women in Long Island, New York. Cancer Epidemiology, Biomarkers and Prevention 1998;7:489-96.

Tanaka R, Susumu F, Nakai K. Study on the absorption and protein binding of carbaryl, dieldrin and paraquat in rats fed on protein diet. The Journal of Toxicological Sciences 1981;6:1-11.

Taskinen H. Effects of parental Occupational exposures on spontaneous abortion and congenital malformation. Scandinavian Journal of Work and Environmental Health 1990;16:297-314.

Taylor P, Lawrence C, Hwang H, Paulson A. Polychlorinated biphenyls: influence on birthweight and gestation. American Journal of Public Health 1984;74:1153-4.

Thomas D, Pettiti D, Goldhaber M, et al. Reproductive outcomes in relation to malathion spraying in the San Francisco Bay area, 1981-1982. Epidemiology 1992;3:32-9.

Tiemann U, Kuchenmeister U. Influence of Organochlorine pesticides on ATPase Activities of Microsomal Fractions of Bovine Oviductal and Endometrial Cells. Toxicology Letters 1999:104:75-81.

Tiemann UT, Pohland R, Kuchenmeister U, Viergutz T. Influence of Organochlorine Pesticides on Transmembrane Potential, Oxidative Activity, and ATP-Induced Calcium Release in cultured Bovine Oviductal Cells. Reproductive Toxicology 1998;12:551-7.

Tomkins P, Molloy S, Finnegan M. Inhibition of superoxide dismutase is associated with reduced gap junctional intercellular communication in an in vitro system. Biochemical society Transactions 1991;20:64S.

Torgerson D, Avenell A, Russell I, Reid D. Factors associated with onset of menopause in women aged 45-49. Maturitas 1994;19:83-92.

Tuntiseranee P, Geater A, Chongsuvivatwong V, Kor-anantakul O. The effect of heavy maternal workload on fetal growth retardation and preterm delivery. Journal of Environmental Medicine 1998;40:1013-21.

van Noord P, Dubas J, Dorland M, et al. Age at natural menopause in a population-based screening cohort: the role of menarche, fecundity, and lifestyle factors. Fertility and Sterility 1997;68:95-102.

Van Velsen F, Danse L, Van Leeuwen F, et al. The subchronic oral toxicity of the beta isomer of hexachlorocyclohexane in rats. Fundamental and Applied Toxicology 1986;6:697-712.

Ventura S, Taffel S. Childbearing characteristics of U.S. and foreign-born Hispanic mothers. Public Health Reports 1985;100:647-52.

Vermeulen A. Environment, human reproduction, menopause, and andropause. Environmental Health Perspectives 1993;101:91-100.

Virgo B. Unilaterally ovariectomized pregnant mice: dieldrin induction of the hepatic monooxygenases and plasma progesterone levels. Canadian Journal of Physiology and Pharmacology 1980;58:638-42.

Wade M, Trosko J, Schindler M. A fluorescence photobleaching assay of gap junction-mediated communication between human cells. Science 1986;232:525-8.

Warnick SL, Carter JE. Some Findings in a Study of Workers Occupationally Exposed to Pesticides. Archives of Environmental Health 1972;25: 265-70.

Wilcox A, Unbach D, Hornsby P, Herbst A. Age at menarche among diethylstilbestrol granddaughters. American Journal of Obstetrics and Gynecology 1995;173835-6.

Wilcox A, Weinberg C, Baird D. Risk factors for early pregnancy loss. Epidemiology 1990;1:382-5.

Willis W, de Peyster A, Molgaard C, et al. Pregnancy outcome among women exposed ton pesticides through work or residence in an agricultural area. Journal of Occupational Medicine 1993;35:943-9.

Willet WC, Dietz WH, Colditz GA. Guidelines for healthy weight. New England Journal of Medicine 1999;341:427-34.

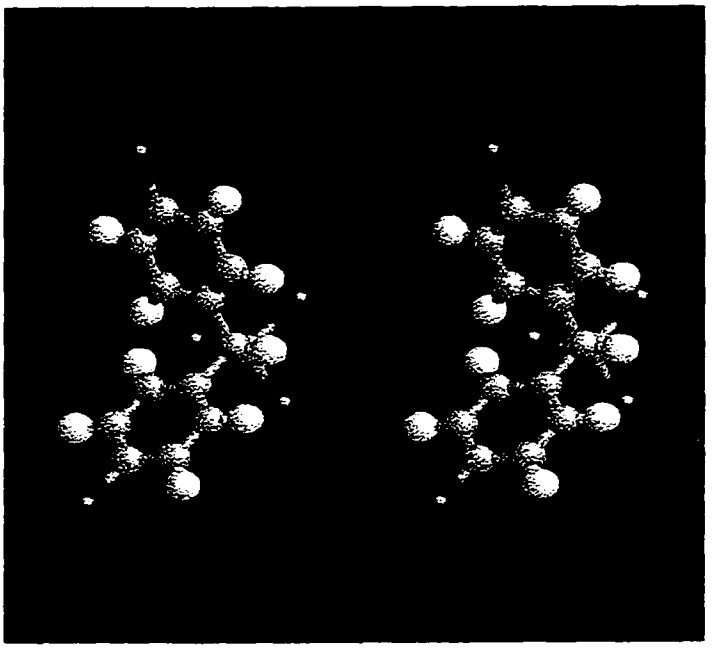
Windham G, Von Behren J, Fenster L, et al. Moderate maternal alcohol consumption and risk of spontaneous abortion. Epidemiology 1997;9:583-4.

Wise P, Krajnak K, Kashon M. Menopause: the aging of multiple pacemakers. Science 1996;273:67-70.

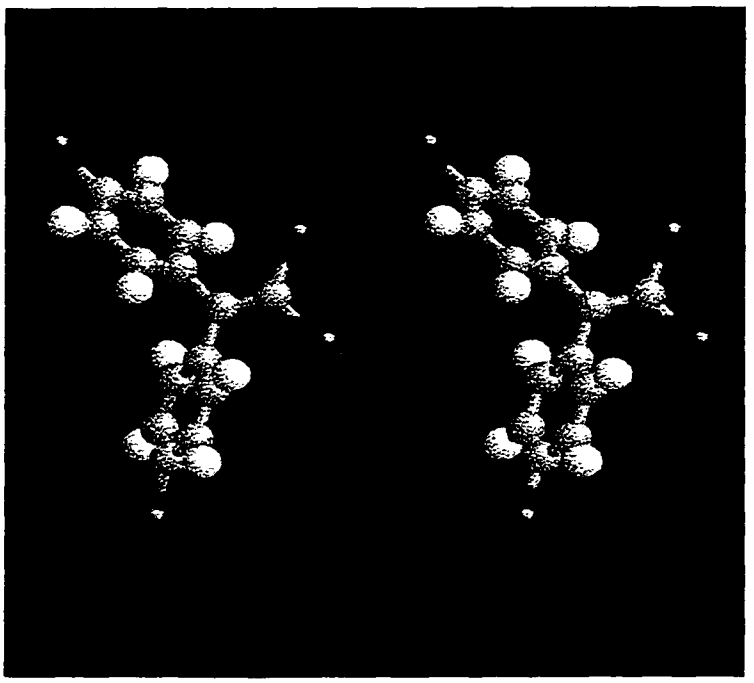
Zhang J, Wen-wei C, Lee D. Occupational hazards and pregnancy outcomes. American Journal of Industrial Medicine 1992;21:397-408.

Zhong-Xiang L, Davanagh T, Trosko J, Chang C. Inhibition of gap junctional intercellular communication in human teratocarcinoma cells by organochlorine pesticides. Toxicology and Applied Pharmacology 1986;83:10-9.

APPENDIX



DDT ($C_{14}H_9Cl_5$)



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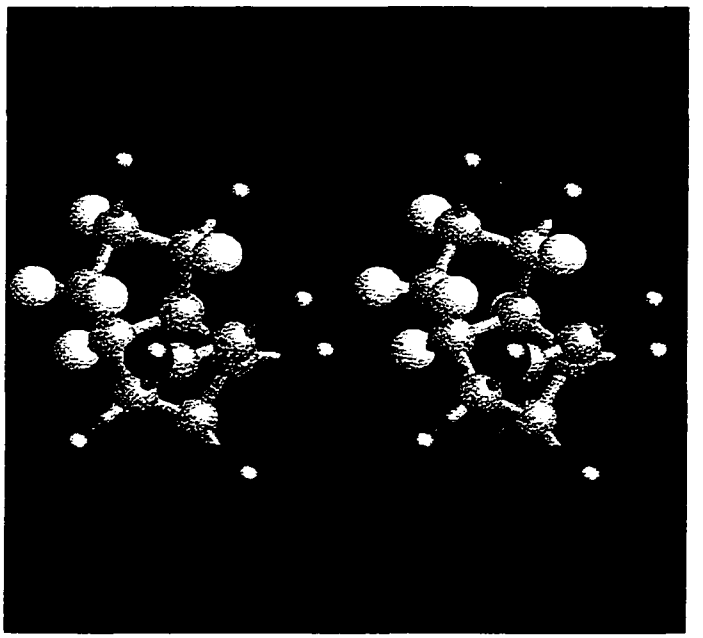
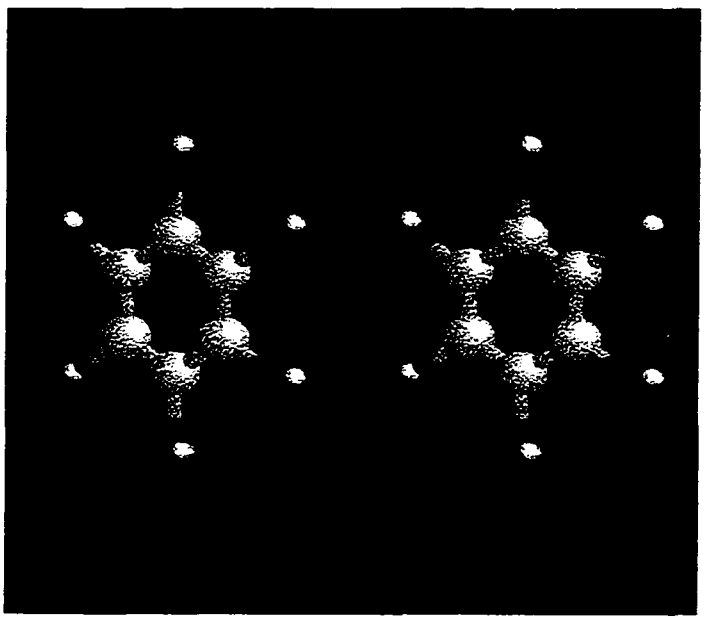
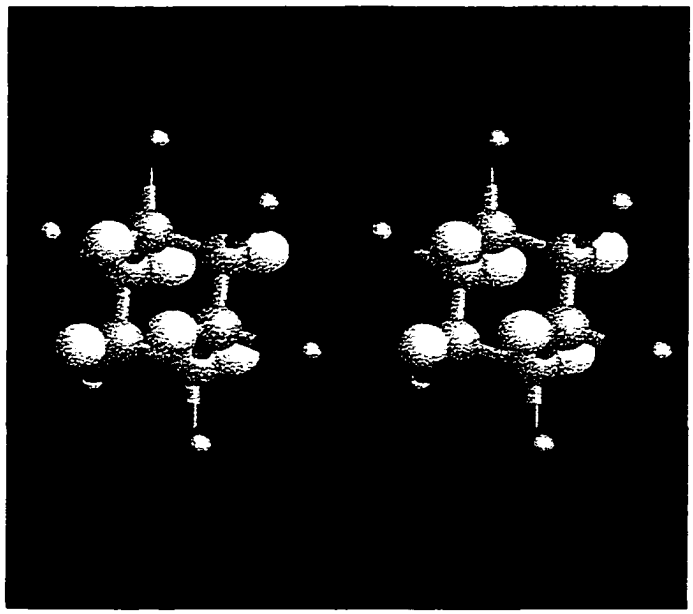


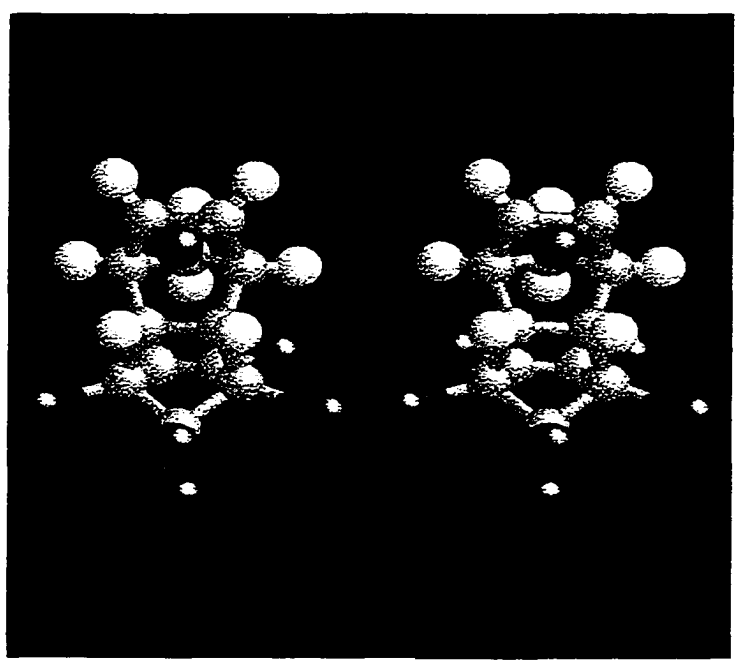
Chlordane ($C_{10}H_6Cl_8$)



Hexachlorobenzene (C_6Cl_6)



Hexachlorocyclohexane ($C_6H_6Cl_6$)



Dieldrin ($C_{12}H_8Cl_6O$)