ORGANOCHLORINE EXPOSURE AND THE RELATIONSHIP TO BREAST CANCER INCIDENCE IN ONTARIO WOMEN

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Abstract

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This thesis is an investigation of the relationship between organochlorine exposure and breast cancer incidence in Ontario women. This paper explores the level and duration of exposure to organochlorines from an amalgamation of information from various sources. Exposure was classified and age cohorts were established to determine if a critical age of exposure to organochlorines exists. Breast cancer incidences were compared for the various critical ages during the different stages of exposure.

The findings of this study confirm that women in Ontario were exposed to several different organochlorines from approximately 1947 to the 1990s. While the study did not prove or disprove a causal relationship, comparing breast cancer incidence for various critical ages at exposure did reveal the potential influence of both age and timing of exposure and shows the necessity for comparing breast cancer incidence during subsequent years. The findings of this thesis also highlight the importance of determining the age of subjects in previous studies investigating the relationship between organochlorines and breast cancer.

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1.1 Introduction:

The Problem:

The use of chemical inputs into agricultural food production follows an extensive history. Early pesticides such as arsenic containing compounds, used since the 16th century, had significant drawbacks. While effective at controlling insect infestations they could result in the death of both the target insects and of the applicator. With the production of modern synthetic chemicals commencing in the early 1930's advances in chemical compounds led to pesticides which appeared to have high insecticidal properties while being less toxic to the applicator.

Since World War II the use of various pesticides in North American agriculture, household and industry has increased dramatically. As a direct result of technical advancements in chemical production during this period, various insecticides, fungicides and fumigants assumed an important role in agriculture and food production (Smith, 1991). One particular group of chemicals, the organochlorines, has had a controversial history of use since the late 1960's. DDT (2,2-bis(p-chlorophenyl)-1,1,1-trichlorethane), the best known example of this group of persistent organic pesticides, was rediscovered in 1939 by Nobel Prize winner Paul Muller. DDT was used extensively during the war to control biting insects transmitting malaria. Since its discovery, DDT has been credited with saving millions of lives in the public health battle against malaria. However, in recent

years, there has been a growing concern about the persistance of DDT, mirex and other organochlorines in the environment, and their potential ability to cause adverse health effects.

The high level of chlorination of these compounds makes them virtually resistant to microbial degradation and able to persist in the environment (WHO, 1989). Residues in soil have been detected decades after their initial application or from "spray drift" during application (Carey et al, 1975 and Martijn et al, 1993). Similarly, contamination of water and sediments has resulted in the bioaccumulation and biomagnification of these chemicals throughout the food web (Government of Canada, 1991). Since most of the chemicals in this class are lipophilic, they are stored in fatty tissue of mammals. Humans for example are known to store DDE, a metabolite of DDT, quite tenaciously in fatty tissue (Hayes, 1993). The major source of organochlorine exposure to humans is thought to be from exposure thorough food sources, and exposure to this group of chemicals has occurred throughout the decades of their use (Smith, 1991). Despite severe restrictions or complete banning in the developed world, many of these substances continue to be used in developing countries. Human exposure is therefore predicted to continue to occur from contaminated food products, and by release from contaminated sites or through atmospheric transportation (Ayotte et al, 1995; and Hargrave et al, 1992).

In 1962, Rachel Carson heightened public concern about chemical contamination of food, water and the environment through her book Silent Spring (Carson, 1962). This

book marked the beginning of an intense investigation into chemical contamination and the link to human health. Interest in this area has remained strong over the decades since the book's first publication. Recent surveys indicate that Canadians perceive chemical residues in food to be of a greater health risk than microbial residues, despite scientific evidence that microbial residues present a more common (albeit qualitatively different) health risk (Slovic et al, 1993). The explanation for this discrepancy in beliefs rests in the knowledge that increased levels of fear are perceived when humans believe they are not in control of the level or source of exposure to unknown agents (Brunk, 1994). Fear is further amplified when exposure is presumed to cause high outrage diseases such as cancer verses short-term irritants such as acute gastroenteritis. Public concern continues to centre around the potential long term health effects from chronic exposure to low level residues in food.

Over the past several decades the public has been exposed to a wide range of controversial opinions regarding the health effects of exposure to chemicals. Publications in the fields of epidemiology, toxicology, and wildlife studies revealed a broad range of effects in various animal species and humans. Detection of chemical residues such as DDT in dwindling populations of various species of birds led to speculation that these chemicals were responsible for egg shell thinning and feminization (Colborn, 1993). Recent investigations of wildlife populations in the Great Lakes area supports the belief that exposure to pesticides and chemicals from agriculture and industry sources may lead to adverse human health effects (Environment Canada, 1991; Government of Canada, 1991). The

continued uncertainty in human exposure combined with controversial media reports of various health effects from chemical exposure further amplifies the public's anxiety toward chemical contamination.

Escalating incidence, in developed countries, of the "hormone responsive diseases" such as breast, testicular, prostate, and cervical cancers and endometriosis have led many researches to investigate the potential relationship between exposure to xenoestrogens (environmental estrogens) and these diseases. Several studies have pointed to a potential link between human exposure to organochlorines specifically, and the development of adverse human health effects. The most intensely investigated of the hypotheses links the relationship between human body burdens of organochlorines and breast cancer (Wolff et al, 1993; Kreiger et al, 1994; Toniolo et al, 1994). However, other adverse health effects such as declining semen quality, feminization, endometriosis, prostate cancer and cryptorchidism have also received attention in recent years (Swain, 1991; Davis et al, 1993; Colborn et al, 1993; Sharpe and Shakkebaek, 1993; Jackson et al, 1986; Auger et al, 1995; Rier et al, 1995). The contradictory outcomes of studies investigating xenoestrogens have necessitated further research into the relationship. To date, the relative impact of these substances in the development of human disease outcomes remains controversial. Several alternative causal pathways related to dietary habits, lifestyle, exposure to phytoestrogens and reproductive history, have also been introduced to account for the increase in these diseases in developed countries (Adlercreutz, 1995, Safe, 1995). Despite the large number of researchers interested in this subject neither a

definitive causal relationship nor a definitive explanation has been established for the increased incidence of hormonal responsive diseases.

More recently, investigations pertaining to the health of agroecosystems have sought to elucidate the link between agricultural health and human health (VanLeeuwen et al, 1997). In this regard, an important component of a healthy agroecosystem is the ability to produce food products free from residues, or contaminants that pose an immediate or long term health risk to humans. Thus one way to assess the ability of an agroecosystem to meet this requirement and hence the degree to which it might be undermining the health of the community which supports it, is to examine the level and effects of contaminants present in food intended for human consumption.

Previously, residue monitoring programs recorded levels of exposure to individual chemicals in food, or at best, documented residues of particular groups of chemicals. More recently risk assessment techniques have been used to estimate risk from certain residues in food consumed by humans; however, in the past, both models have focused on a single chemical residue from a single source or food group and compared these findings to the Acceptable Daily Intakes (ADI), or Maximum Residue Limits (MRL) as recommended by the Health Protection Branch in Canada, Environmental Protection Agency (EPA) in the United States or international agencies such as the World Health Organization (WHO). Market basket studies have also been used to determine the level of human exposure from food, but problems with sampling and study design have limited the

accuracy of this information for various risk groups such as children , or people with particular food consumption patterns (National Research Council, 1993).

Many studies have not taken into full account the typical pattern of human exposure to multiple contaminants on sequential episodes from food, water and the environment, or the potential for synergistic, additive or cumulative effects from exposure to single chemicals or mixtures (Smith et al, 1975; Conacher et al, 1989). They have also failed to account for past exposure and existing human body burdens which may affect the total exposure outcome. Little attention has been given to the potential impact of timing and duration of exposure on the development of adverse health effects. To ignore one or more of these factors or the possibility of combined effects from chemicals with similar outcomes could determine the difference between declaring a substance safe or not. For example, water contamination studies clearly show that chemicals used for agricultural purposes such as food production can be found as residues in water, while environmental studies show that these chemicals can also be detected in our air, lakes, and in soil (Williams, et al., 1978; Martijn, et al., 1993; Government of Canada, 1991). If exposure models only examine individual food sources at specific points in time, the potential significance of exposure from all sources and from previous exposures cannot be fully understood. While certain food groups may be the dominant route of exposure to organic chemicals, air and water have been suggested to contribute approximately 10% and 15% respectively to total human exposure (Government of Canada 1991; Waltner-Toews and McEwen, 1994). It is therefore the total exposure to these chemicals and the timing of

exposure that will determine the potential for adverse human health effects. A complete historical examination of this information will define the nature of exposure for individuals and populations over the entire period of exposure and predict which groups may be at increased risk from exposure to organochlorines.

Data documenting organochlorine use in various sectors, residues in food, water, and the environment and human body burdens such as blood, adipose tissue and breast milk have been collected in Canada since 1967. However, to date a collective examination of these data has not been published. With chronic diseases such as cancer, which have been postulated to require an extensive lag phase ranging upwards of ten years to perhaps as long as thirty years from the time of initial exposure (Fisher et al, 1995), monitoring for the potential development of adverse human health effects demands that a detailed investigation of exposure be determined. An examination of the exposure trends in specific groups and the cancer outcomes in these groups will provide information regarding the plausibility of a relationship between exposure to organochlorines and adverse human health effects.

Birth cohort or age-period-cohort analysis have been used to investigate the relationship between age, time, disease incidence and mortality for such diseases as tuberculosis and cancer (Frost, 1939, Holford, 1991, Tarone and Chu, 1992, Adami et al, 1993). Age-period-cohort analysis can be an extremely effective approach to understanding a disease process. Initial approaches graphically displayed mortality or incidence rates at various

ages for specific birth cohorts, and these graphs were used to examine patterns in disease rates over time (Frost, 1939). Subsequently, statistical analysis using regression models have been designed to quantify the separate effects of age, period, and cohort (Kupper et al, 1985).

Age has been recognized to play a role in the etiology of many diseases, with changes in disease rates as individuals or groups age. Different birth cohorts or groups may also have different levels of exposure to risk factors as they age, which can be expected to result in changes in disease incidence for individuals born at particular times (Holford, 1991). In age-period-cohort analysis the age of the subject at time of diagnosis, the years of diagnosis and the year the subject is born is taken into consideration to establish trends in disease outcomes. These findings can be displayed graphically and can be analysed using regression models.

Adami et al, 1993, examined the effect of birth cohort on cancer incidence in Sweden. The authors found higher incidence rates of cancer in cohorts born during the 1950s compared to those born in 1873 - 82. The findings of this study pointed to increasing incidence of cancer and expressed concern for increasing population exposure to carcinogenic influences. Tarone and Chu, 1992, examined the implication of birth cohorts patterns in breast cancer in the United States. These authors found an increase in breast cancer risk in birth cohorts born from 1900 to 1916 and a decreasing breast cancer risk in birth cohorts born after 1926.

Age-period-cohort analysis do however have some limitations (Kupper et al, 1985). Concerns about problems with nonidentifiability arise, where the true cause of the trends detected may be overlooked for other biologically plausible explanations. Changes in diagnostic procedures, and diagnostic errors can impact the resulting accuracy of time-related patterns (Kupper et, al, 1985) As such, interpretations made on these models requires caution.

In epidemiological studies, a variety of criteria are used to demonstrate causation, none of which are considered to be sufficient by themselves (Martin, et al, 1987, and Evans 1978). Biological plausibility is considered to be important but not necessary; that is it strengthens inferences when present but does not negate arguments on other grounds. There must be evidence that the factor under investigation precedes the disease, and that there is a statistically significant increase in the relative risk or odds ratio for those individuals exposed verses non-exposed. In the case of populations, the population attributable fraction should be greater during the exposure period. Evidence supporting a dose response relationship would give greater credibility that the factor and the outcome are causally related, as would consistency in findings from different studies investigating the factor and the outcome.

The establishment of the type of causal relationship and whether the factor is a direct or indirect cause of breast cancer is extremely important in establishing breast cancer prevention. One should consider whether exposure to organochlorine is a necessary

cause, where the disease cannot occur without exposure to the factor; or a sufficient cause where exposure to organochlorines always produces breast cancer; or a component cause where organochlorines serves as a part of a group of factors that produce sufficient cause. Are there any known intervening variables between organochlorine exposure and breast cancer development? Given the complex nature of breast cancer development, the etiology points towards a whole group of factors that may confer an increased risk of developing breast cancer. In this case, it may not be necessary to identify all causes of breast cancer but rather to determine if exposure to a single factor may be a key component in the development of breast cancer, or if removal of this factor may render the remaining components insufficient or less likely to promote breast cancer.

To establish that organochlorine exposure is causally related to breast cancer, it would be logical to expect that most, if not all, of these criteria should be met. Therefore, in addressing the criteria of causality, for organochlorines and breast cancer to be causally related, there should be evidence that a biologically plausible pathway exists; that a time sequence is present - where the factor precedes the disease or in this case where the incidence of breast cancer in the population (given an undetermined lag phase) is statistically greater following exposure during the peak period of organochlorine use than prior to organochlorine exposure; and that a dose response relationship, if detected, adds further evidence of a causal relationship.

The Hypothesis

The research reported in this thesis is an investigation of the potential relationship between chronic exposure to organochlorines used for agricultural purposes and the occurrence of breast cancer in exposed populations. The specific question being posed in this study is investigated in the context of an exposure model that accounts for the source, timing of exposure, total level, duration and storage of organochlorines. This model is then applied to people residing in Ontario (Figure 1.1). The study period under investigation spans several decades, from the time prior to initial use to the present day. This time frame incorporates a period of non-exposure, peak exposure and declining exposure. Given this model the temporal relationships between exposure patterns to organochlorines and the temporal pattern of breast cancer incidence will be examined. The present study will identify which organochlorines represent the greatest potential for negative effects and which groups or individuals are at highest risk of developing adverse effects from exposure to this group of chemicals.

Given the multiple exposure scenarios for humans, the hypothesis under investigation has several components which could determine the potential development of adverse human health effects. The hypothesis under investigation states that any exposure to sufficient quantity of organochlorines (at this point the actual dose required is unknown) at any age confers some increased risk of developing breast cancer; that a dose response effect exists, which would be manifest as an increased incidence in those people exposed during the period of highest use; that the timing of exposure is influential such that exposure at a

critical age further increases the risk of developing breast cancer; and that the combination of these factors would infer the greatest risk of developing breast cancer. Thus, exposure at a critical age, durning the period of highest use, plus increased duration of exposure infers the greatest risk of developing breast cancer.

The Study Design

Existing sources of information on sales and use of organochlorines in Ontario were used to establish a temporal exposure pattern. Data on organochlorine sales, residue levels in various food groups, market basket studies, water and environment residues, and human body burdens were combined to determine total exposure. Data from Canadian studies were also examined to compare and corroborate the values detected in Ontario. Secondly, exposure routes for both females and males were proposed, and age cohorts, based on biologically plausible risk categories, were created for the females in order to determine exposure trends in specific developmental or life stages. Thirdly, the pattern of reported breast cancer incidence was described overall, and by the cohorts established in step two. Finally, the historical pattern of exposure was related to the pattern of breast cancer incidence, stratified by age cohorts. Predictions have been made regarding future trends in breast cancer incidence if the suggested relationship is indeed causal.

Factors such as lactation, pregnancy, consumption of fish, obesity and vegetarian versus meat eaters were investigated to determine their potential effect on the total exposure, since these variables may influence the total human body burden in individuals and

populations. These factors and other personal lifestyle choices (such as use of birth control pills and hormone replacement therapy) may also influence women's endocrine status or general health status and could thereby confound the relationship between organochlorine exposure and breast cancer. Given the nature of this study, and the lack of precise information available, these variables could not be accounted for in a quantitative fashion. Nevertheless, they have been carefully considered in making inferences from the patterns seen. While this study focuses on the health outcome breast cancer, the exposure portion of this study could also be used to determine if a relationship exists between exposure to organochlorines and other human health outcomes.

1.2 Organochlorines and Health Effects

1.2.1 Introduction

Since the early 1970s, reported escalating rates of reproductive abnormalities in birds, fish, and turtles have stimulated an extensive scientific investigation into the role of organochlorines in the environment and the health of wildlife. A myriad of wildlife studies implicated exposure to organochlorines with decreased fertility, hatching success, abnormalities in thyroid function, alterations in immune function and feminization in fish, birds and other mammals (Elliott and Martin, 1994; Colborn et al, 1993; Environment Canada, 1991). Reports of organochlorine residues in fatty tissue and eggs of herring gulls, fish and eagles have been documented especially in the Great Lakes region (Environment Canada, 1991).

Elliot and Martin (1994) investigated the relationship between chlorinated hydrocarbons and shell thinning in eggs from hawks; they found that egg shell thickness pre - 1947 (prior to DDT use) was significantly greater than in 1986. The authors concluded that, while the banning of these substance, in the early 1970, resulted in rapid declines in contamination levels in eggs and subsequent improvements in breeding success, detectable levels of organochlorines were still present in 1986-1989. These levels were associated with the continued use of chlorinated hydrocarbons in the wintering grounds of these birds. Similar studies on ospreys showed that birds of prey (top predators of the food chain) could accumulate significant levels of DDT and metabolites (Environment Canada, 1991). In these birds, a ten percent egg shell thinning could be associated with a two ppm (wet weight) DDE content, and 20 percent egg shell thinning with a nine ppm DDE content. Reports showed that eggs collected prior to World War II were over twenty percent thicker than those collected between 1960 and 1970. Eggs collected in the early 1990s showed very few eggs with greater then ten percent thinning and most had less than four ppm DDE. The decline in DDE levels has been speculated to give rise to an escalating Osprey population during the last decade (Environment Canada, 1991).

Studies of fish populations have found thyroid enlargement and precocious sexual maturation in male, and feminization which has been suggested to be related to high levels of endocrine-disrupting chemicals in the environment (Colborn et al., 1993). While the entire topic of organochlorine exposure and wildlife health effects remains controversial, the fact that these health effects had not been reported prior to the 1950's and that there

appears to be a recovery in the wildlife populations since restrictions have been put in place suggests that adverse health effects in wildlife were related to organochlorine exposure. Early animal studies showed that DDT and other organochlorines could increase some drug metabolising enzymes. Increases were detected almost immediately after initial exposure and continued for weeks to months after the final dose (Hayes, 1956). Animal studies on rats, mice, dogs and monkeys have been conducted for most of the organochlorines. Mice, in particular, appear to be particularly sensitive to organochlorines and hepatocarcinogenicity in this species shows a dose-response relationship (Smith, 1991). DDT can also be hepatocarcinogenic to rats and hamsters but studies in dogs and monkeys appear to be inconclusive. Organochlorines, are in general, negative in mutagenicity tests; however their ability to promote cancer is suspected to be linked to the induction of microsomal enzymes and other enzymatic processes. Numerous studies have shown that organochlorines can cause disturbances in the function of the immune system, thyroid gland, and the adrenal gland and promote various hormonal changes in animals (Smith, 1991). Speculation that similar effects could result in humans propelled scientific investigation into the potential effects of these substances.

By the early 1960's the general scientific community recognized that organochlorines could have the potential to impact human health and recognized the need to investigate the source, amount and proportions stored in the body. Numerous articles addressed the need to determine the toxicological significance of what was termed the "invasion of the privacy

of our fat" (Durham 1965; Lancet, 1965; Robinson, 1969; Dale and Quinby, 1963). DDT was the most intensely investigated of the compounds and it was recognized very early that the general population had significant exposure to this organochlorine. By 1965 researchers concluded that food was the major route of exposure and that over 90% of DDT was absorbed through food sources in the general population (Durham, 1965).

The potential for adverse human health effects from exposure to organochlorines has been investigated on a world wide basis. Wang et al, 1988 showed that there was a significant correlation between DDT, DDE and beta benzene hexachloride (*B*-BCH) levels in ear wax and mortality rates from liver, colon, rectal and lung cancer in men and colon cancer in women. However numerous studies of occupationally exposed workers did not show an increase in occurrence of tumours (Smith, 1991). Given that hepatomegaly and microsomal enzyme induction can be documented in humans, and are slow to regress, the potential for tumour induction over time does exist. Subsequently, the International Agency for Research on Cancer (IARC) has classified many of the organochlorines as possible human carcinogens (Smith, 1991).

In Canada and the United States, particular attention has been focused on the human populations around the Great Lakes. These areas have been targeted as sites where apparently extensive human exposure to toxic chemicals has occurred, largely from contaminated food products derived from the Lakes (Swain, 1991). Several studies have investigated potential relationships between exposure to organochlorines such as PCBs

and DDT, and various health outcomes such as birth defects, cancer risks, neonatal health, growth, morbidity, duration of lactation, semen quality, estrogen-related cancers and reproductive outcomes (Foran et al 1989; Rogan et al, 1987; Rogan et al, 1986; Rogan et al, 1987; Carlsen et al, 1995; Adami et al, 1995). The relationship between organochlorines and breast cancer, in particular, has been studied extensively in both countries.

1.2.2 Estrogenic Action of Organochlorines and Health Effects

The estrogenic properties of DDT were first noted in 1968, followed by several studies showing the estrogenic activity of o,p 'DDT in various species (Kupfer and Bulger, 1980). Recent investigations have shown that numerous pesticides such as chlordane, hexachlorobenzene, toxaphene, dieldrin, endosulfan, DDT, methoxychlor, kethane, PCBs and atrazine are capable of mimicking estrogenic activity (Soto et al, 1991, 1994; and U.S. Government Printing Office, 1994). In general, the organochlorines are considered to be weak estrogens in vitro compared to natural estrogens such as 17*B* -estradiol and much debate regarding the relative estrogenic potency exists, with some authors concluding that humans are exposed to far greater levels of naturally occurring levels of estrogen compounds in the diet than found in environmental estrogens (Safe, 1995). However, while individual concentrations of these chemicals may occur below levels where overt detrimental hormonal effects arise, evidence shows that each chemical may act additively to produce overt estrogenic effects (Soto, 1994). This, in combination with a prolonged life time exposure, lipophilic nature, accumulation in fatty tissue and

slow metabolism of organochlorines, has led to regarding the estrogenic nature of this substance as a potential etiological pathway.

In laboratory tests and wildlife studies, organochlorines have been shown to mimic the activity of reproductive hormones (Wolff and Toniolo, 1995; Colborn et al, 1993). Studies in monkeys show that exposure to DDT can produce increased uterine weights and vaginal cornification (Smith 1991). Wildlife studies have shown feminization of various species and consequently point to the potential for feminization effects in humans (Colborn et al., 1993). Several of the organochlorines have been shown in vitro to stimulate growth of human breast cells (Soto et al, 1994). These findings suggest a potential pathway for adverse human health effects and have stimulated numerous epidemiological studies examining the relationship between several of the organochlorine pesticides and human breast cancer.

Bradlow et al, (1995) have shown that oestradiol metabolism occurs by hydroxylation at either the C-2 or C-16 alpha sites. The catechol pathway produces a weakly estrogenic and nongenotoxic catechol estrogen 2-hydroxyestrone (2-OHE1), while the other pathway produces a fully potent estrogen which causes increased cell proliferation and is tumorigenic and genotoxic. Materials which increase the ratio of 16 alpha -OHE1/2-OHE1 have been suggested as potential breast cell carcinogens (Bradlow et al, 1995). Using a radiometric assay Bradlow et al, (1995), have shown that estrogen receptor positive human breast cells exposed to organochlorines such as DDT, kepone, HCB,

endosulfan and PCBs produce an increased ratio of 16 alpha-OHE1/2-OHE1 metabolites then compared to cells treated with a known rodent carcinogen and control cells.

Evidence reported to date suggests that the estrogenic potency of these substances is very low in comparison to estradiol or other estrogenic substances. The variability in estrogenicity of the PCBs in particular adds to the complexity, in that several congeners appear to have estrogenic activity while other congeners have antiestrogenic effects or no estrogenic effects (Wolff and Toniolo, 1995). PCBs with low chlorination isomers and shorter half-lives appear to have the greatest estrogenic effect, and have been shown to increase uterine weight and cause precocious puberty in monkeys. While PCBs with zero to one ortho chlorine or coplanar isomers have moderate persistence and have been shown to have antiestrogenic effects (the latter is thought to arise from interaction with the Ah receptor), isomers with greater than 1 ortho chlorine are highly persistent and appear to have weak estrogenic activity. The exposure to PCBs with differing persistence and effects in the body could influence the net estrogenic impact in the exposed individual. Therefore the relationship of PCBs and breast cancer could depend on the relative body burdens of the different congeners (Wolff and Toniolo, 1995). While the estrogenicity of organochlorines may not prove to be significant in the dramatic increase in breast cancer, other plausible mechanisms have been suggested and the possibility of other pathways existing must not be ruled out at this point in time. Most recently, organochlorines have been postulated to promote breast cancer by inhibiting the gap junctional intercellular communication in normal human breast epithelial cells (Kang et al, 1996).

To date, however, those who hypothesize a causal relationship between organochlorines and breast cancer favour an estrogenic mechanism. The biological plausibility for an estrogenic effect of these chemicals is based on the scientific observation that estrogens play at least some role in human cancer development (Guyton, 1989). Estrogenic substances are known to stimulate cell proliferation and may therefore actively promote cancer growth in target organs such as the mammary gland, prostrate, ovaries, uterus and testis (Fisher, 1995). Many of the known risk factors associated with breast cancer have some relation to women's hormonal status. Interestingly, many of these risk factors can also influence the total exposure and body burden of the individual and thus affect the total duration of effect. The documentation of human exposure and storage of organochlorines may therefore be important in investigating both the estrogenic hypothesis and other biologically plausible causes of breast cancer resulting form exposure to this group of chemicals.

1.3 Breast Cancer

1.3.1 Introduction

Breast cancer is a malignant tumour which originates in the breast. There are numerous types of breast tumours and these have been categorized according to the structural unit of the breast tissue they are associated with namely small, medium and large ducts (Fisher et al, 1995). The most common duct tumours are the infiltrating duct carcinomas not otherwise specified (NOS) meaning that no special type of histologic structures are identified. These tumours account for approximately 80 % of breast cancer and carry the

poorest prognosis. On palpation they can be felt as a stoney hard mass. They readily metastasize to the axillary lymph nodes. Medullary carcinomas comprise approximately five to seven percent of all mammary carcinomas (Fisher et al, 1995). These tumours are well circumscribed larger masses that demonstrate low-grade infiltrative properties and have a better prognosis. Mucinous or colloid carcinomas are slow growing tumours, carrying a good prognosis, and comprise 3% of breast cancers. Lobular carcinomas in situ account for approximately 2.5 to 2.8% of all breast tumours. Papillary carcinomas account for approximately 1 % of breast cancer cases and rarely infiltrate the surrounding tissue; survival rates with this form of breast cancer approach 100% when these tumours are successfully excised. Other histological types include adenocystic carcinoma, carcinosarcomas, squamous cell carcinomas and basal cell carcinomas. All have been observed; however these latter tumours are rare (Fisher et al, 1995).

Breast cancers have also been classified according to their estrogen receptor (ER) status. Estrogen receptor positive tumours bind greater than 10 femtomole of titrated estradiol/mg of cytosolic protein while estrogen receptor negative tumours bind less than 10 fmol/mg of cytosolic protein (Houghten and Ritter, 1995). Glass and Hoover (1990), using a population-based tumour registry in the United States from 1960 to 1985, found that the incidence of estrogen positive breast tumours increased 131% in comparison to estrogen negative receptor cancers, which had increased by only 22-27% during the same time period. The growth in ER positive cancers may, in part, reflect the effects of mammographic screening as screening detects small tumours which are more likely to be

ER positive (Waddell, 1998 and Morrison, 1998, personal communication). While estrogen receptor positive breast cancer carries a better prognosis, the requirement to minimize endogenous estrogen levels in these patients means that pregnancy and the use of birth control pills in estrogen receptor positive breast cancer cases is at best risky. As well, the dramatic increase in estrogen receptor positive breast cancer suggest a hormonal influence in the development of this disease in women (Fisher et al, 1995; Glass and Hoover, 1990).

Breast cancer is the second most common cause of cancer in women in Canada and the United States, after non-melanoma skin cancer, and is the second most common cause of cancer related death in women. While the disease is exceedingly rare in women less than 20 years old, the incidence increases substantially between 25 and 50 years of age (Fisher et al, 1995). The overall incidence of this type of cancer has increased dramatically over the last several decades in many developed countries. In the United States the incidence increased by approximately 28% during the time period from 1973-1989 (Helzlsouer, 1995). With the increase in women smokers, mortality from lung cancer has recently overtaken breast cancer as the leading cause of cancer death in women in the United States. However, breast cancer still remains the single leading cause of death in women age 35-54 (Fisher et al, 1995). In the United States 43,746 people died of breast cancer in 1990. By 1992, 180,000 cases of breast cancer were diagnosed, and 46,000 deaths were attributed to breast cancer (Kelsey and Horn-Ross 1993). The annual incidence of breast cancer has been rising by approximately 3% annually between 1980 and 1985 in the

United States. This increase in incidence of breast cancer can not be completely accounted for by increased screening or changes in risk factors (Pollner, 1993; Fisher et al, 1995).

In Canada, the incidence of breast cancer in post menopause women has increased markedly since approximately 1981, while the incidence in pre-menopause women has remained stable throughout the recording of cases. In 1993 there were 16, 300 cases of breast cancer diagnosed in Canada and 5,400 Canadian women lost their lives to breast cancer (Houghton and Ritter, 1995). In 1997, there were 5,100 breast cancer deaths and 18, 400 incident cases (Canadian Cancer Statistics, 1997). While the rate of breast cancer has consistently been increasing the mortality rate of this form of cancer has been falling since the mid 1980s. The latter is most likely due to improved screening techniques early detection by self examinations and improved forms of treatment. Increased public awareness through promotion of monthly self examinations and the need for screening high risk individuals with mammography has contributed to early detection of breast cancer in Canada. The rise in the number of estrogen receptor positive breast cancer cases, which carry a better prognosis, may also account for improved prognosis of breast cancer patients. However, despite improved prognosis, a significant number of women lose their battle with breast cancer. In 1988 for example, one death in twenty resulted from breast cancer for women in Canada (Guadette and Roberts, 1988).

Breast cancer rates have traditionally been higher in North America and Northern Europe

than in other parts of the world. In recent years there has been a steady increase in some Asian and Central European countries (Kelsey and Horn-Ross, 1993). International differences in breast cancer rates have been suggested to be related to the general dietary habits, weights, estrogen levels and reproductive and lactation patterns of women residing in these countries. Studies of immigrants to Canada and the United States show that women from low incidence countries attained higher breast cancer rates than found in their mother country and that second generation immigrants who were exposed to our North American environments at an early age had even greater incidence of breast cancer (Kelsey and Horn-Ross, 1993). These findings suggest a possible link between environmental exposure or dietary habits found in North America that could be resulting in increased breast cancer rates.

Escalating financial costs from an increasing incidence of breast cancer pose a tremendous burden on society from a medical care system perspective and from a psychological perspective. Economic studies in the United States have estimated annual expenditures for medical treatment of breast cancer at $ 6,599 million dollars per year (expressed in 1990 dollars), and the estimated annual aggregate treatment expenditures for 1990 amount to 6.6 million dollars (Brown and Fintor, 1995). Breast cancer remains one of the highest annual medical care expenditures for single site cancers. When one includes both direct costs such as resources for medical care in the prevention, diagnosis and treatment of breast cancer and the indirect cost such as time and output lost by the patient, family friends and other individuals involved with each diagnosed case the cost of breast

cancer is staggering. From an agroecosystem health perspective, this disease compromises socioeconomic and community health adaptability by sequestering large amounts of financial and personal resources in every aspect of diagnosis, treatment, and fatalities.

1.3.2 Risk Factors

The etiology of breast cancer has not been established firmly. Despite years of research into the potential causes of breast cancer, only 30 - 40 % of breast cancer cases can be attributed to known risk factors (Helzlsoeur, 1995). Consequently, intense focus has been given to detecting potential causes of breast cancer. While the etiology is unknown in most cases of breast cancer, there are several risk factors that have been proposed to cause an increased risk of breast cancer. In determining the relationship between breast cancer development and organochlorine exposure, it is important to understand the potential confounding factors, that is, factors related to both the outcome and the hypothesized etiology. Since changes in some of these factors influence the overall exposure to organochlorines, knowledge of the various confounders is important. The following is a brief overview of the known risk factors.

a) Presence of BRCA genes

A family history of breast cancer particularly in first degree relatives (mothers, daughters and sisters) is associated with an elevated risk. However, women with second-degree relatives with breast cancer are not at increased risk (Weber et al, 1994 and Fisher et al,

1995). Approximately 5% of women diagnosed with breast cancer have been found to have a loss of heterozygosity of the chromosome locus 17q21 (Helzlsouer, 1995). Two specific breast cancer genes, BRCA1 located on chromosome 17 and BRCA2 located on chromosome 13 have recently been discovered. Mutations on either gives an inherited susceptibility to breast cancer (Miki et al, 1994). Mutations on BRCA1 are thought to confer a 80-90% lifetime risk to breast cancer and approximately a 40-50% lifetime risk of ovarian cancer (Josefson, 1996). A genetic analysis test has been developed to identify BRCA1 gene mutations in the United States and has received widespread attention. While women with this gene in their chromosomal makeup do have significantly higher risks of breast cancer the absence of this gene does not prevent the development of breast cancer. Only 5 - 10 % of breast cancer is directly related to inherited factors (Fisher et al, 1995).

b) Hormones

Hormones in general play a major role in the normal physiological functioning of all mammals including humans where the development of the genital organs is under hormonal control (Guyton, 1989). Women, especially during their reproductive years, depend extensively on endogenously produced estrogen and progesterone for normal body functioning. The breast tissue of women is particularly sensitive to changes in estrogen levels with high levels stimulating increases in breast tissue. Estrogen has been shown to stimulate stem cell division, growth of intermediate cells and established cancer cells and may therefore lead to an accumulation of genetic errors leading to neoplasia. (Malone et al, 1993; and Helzlsouer, 1995) Women may therefore experience heightened effects

from exposure to intrinsic or external estrogenic substances. Exposure to varying levels of hormones, particularly in the pubescent years, could have a dramatic effect on breast tissue development.

Hormones are presumed to play a significant role in the development of breast cancer since several of the known risk factors can be associated with increased hormone levels in women's bodies (Bernstein and Ross, 1993; and Kelsey et al., 1993). Early age at menarche, late age at first full-term pregnancy, parity, lactation and age at menopause have all been associated with the risk of breast cancer (Kelsey et al, 1993; Helzlsouer, 1995). The connection with hormone levels is further supported by the low level of breast cancer in men and the evidence that the majority of breast cancer cases are estrogen receptor positive. Estrogen, in particular, has been implicated in the etiology of breast cancer.

Early age at menarche is associated with a higher risk of breast cancer. This finding may be related to the fact that these women have an earlier onset of a regular ovulatory menstrual cycle and therefore an earlier exposure to the fluctuating hormones associated with a normal menstrual cycle. Women who experience the onset of menarche prior to 12 years of age have twice the incidence of breast cancer compared to women who's onset of menarche occurred at age 13 or greater (Fisher et al, 1995). Women who experience menarche at an early age have been shown to have higher levels of estrogen for several years and likely throughout their reproductive lives (Kelsey et al., 1993). Similarly, late

age at menopause has been associated with a greater risk of breast cancer, where a five year increase in age at menopause has been associated with a 17% increase in risk (Brinton et al., 1988). Menopause at or prior to 45 years confers a twofold decreased risk of breast cancer development compared to menopause at age 55 or older (Fisher et al, 1995). When bilateral oophorectomy was performed prior to age forty a 50% decrease in lifetime risk of breast cancer could be found in comparison to women who experienced natural menopause. (Kelsey et al., 1993) These finding suggests an important role of hormones in the development of breast cancer and suggests that breast cancer is directly related to the number of regular ovulatory menstrual cycles (Fisher et al, 1995).

Several studies have shown that young age at first full term pregnancy lowers the risk of breast cancer. While the strength of association has been variable, suggesting this risk factor may not be an independent risk factor, the finding of decreased risk has been documented in several countries including the United States and Scandinavian countries (Layde et al, 1989; and Ewertz et al, 1990) Also, women who have their first full term pregnancy after age 30 have a higher risk of breast cancer in comparison to nulliparous women (Kelsey et al, 1993). The latter may be due to a reduced likelihood of tumour initiation during early age at full term pregnancy while late age at full term pregnancy may promote the growth of existing tumour cells.

An increase in the number of pregnancies may have a mild sparing effect on risk of breast cancer; however it appears that the most significant difference is between nulli parity and

parity. Parity and multiparity appears to have a sparing effect in women age 40-50 or greater and not in women diagnosed at younger ages. There has been some controversy regarding an increased risk of breast cancer immediately following pregnancy which may be attributable to growth promotion of cancerous cell from the high levels of hormones that fluctuate through the body during pregnancy and lactation. This could explain why a sparing effect is only detected in women many years post full term pregnancy (Kelsey et al, 1993).

A prospective study conducted by Toniolo et al, (1995) shows that women with high circulating levels of endogenous estrogen are at higher risk of developing breast cancer. The study examined estrone and estradiol blood levels from 130 identified breast cancer case (which had developed during the first five and a half years of study) and 260 controls from a cohort of 14 291 New York City women. The eligibility requirements restricted entry to women with a previous history of hormonal medications of any type and those who had been pregnant in the past six months so that no information could be gained on the impact of previous hormone use such as the pill or previous pregnancy. The authors concluded that women who developed breast cancer tended to have higher levels of estrone, total estradiol, free estradiol and a lower percent of estradiol bound to sex hormone-binding globulin. Obese post menopausal women tended to have higher serum estrogen levels and decreased serum sex hormone binding globulin (SHBG) which may have inferred an increased risk of developing breast cancer (Pike et al, 1993). These studies and others like it support the role of estrogen in the development of breast cancer.

c) Lactation History

Breast feeding has been suggested to have a sparing effect for breast cancer. Conflicting results have been obtained regarding this risk factor. Several case control studies support the hypothesis while other cohort and case control studies have shown little to no effect from lactational status on breast cancer development (Kelsey et al, 1993). Studies of women from China, who tend to breast feed for several years, have shown that long-term breast feeding has a protective effect (Kelsey et al., 1993) Recently, a case-control study in Mexico City, where prolonged lactation has been the norm, found that parous women who had breast-fed had a decreased risk of developing breast cancer. They also discovered that the duration of lactation was inversely related to the rate of breast cancer. This effect was most strongly observed with long lactation duration especially among the first full term birth group of women, with a significant protective effect observed after thirty six or more months of lactation. However, as little as three months of breast feeding appeared to provide a sparing effect (Romieu et al., 1996). The authors of this study speculate that the decreasing trends in lactation by Mexican women may in part be responsible for the increasing rates of breast cancer in that country.

Age at first lactation has also been suggested to influence the chance of developing breast cancer. However, the Mexico City study did not find a significant difference in the rate of breast cancer in the various age categories under study (Romieu et al, 1996). The link between lactation and breast cancer has been postulated to be due to the change in hormonal status of the women during lactation since this process suppresses ovulation and

affects the estrogen levels. Other explanations include structural changes in the mammary gland that may be protective or a mechanical flushing out of carcinogens and other chemicals/toxins such as the organochlorines (McTiernan and Thomas, 1986). The latter concept can be supported by findings from animal studies where mice had more tumours in the non-suckled side than the suckled teats. This is further supported by studies in women who had nursed infants either unilaterally or bilaterally. In individuals who had nursed from one breast only there was a significant increase in risk of cancer in the unsuckled breast (Ing and Petrakis, 1977).

d) Oral Contraceptives

Oral contraceptives and breast cancer have been studied extensively, since a high proportion of women rely on the birth control pill as a means of preventing pregnancy (Malone et al, 1993). The fact that increasing numbers of women of younger ages are using oral contraceptives gives rise to greater concern should a link be established between the contraceptive pill and breast cancer. The combination pill which contains estrogen and progestin was the first oral contraceptive pill to be approved in the United States. To date this type of pill remains the most commonly used. However the early contraceptives contained higher doses of estrogen than the ones currently employed. The newer preparations, which contain less that 50 *u*g of estrogen, or progestrogen only pills, may pose even less risk.

Currently the debate over the relationship between oral contraceptives and breast cancer

continues, with most of the epidemiological studies finding little or no differences between pill users and non users. (Malone et al., 1993) Meta-analyses of women ever having used the pill reveal that there is a relative risk of approximately 1.0 (Thomas, 1991). However, more recently studies have indicated an increased risk for a specific subgroup of women may exist. Women who have begun oral contraceptive use at an early age and or continue to use these substances past 45 years of age may be at increased risk of developing breast cancer (Fisher et al, 1995).

e) Post Menopause Hormone Use

Estrogen replacement therapy has been used by a significant number of North American women to minimize the symptoms of menopause, prevent osteoporosis and heart disease. Estrogen replacement therapy is generally thought to slightly increase the risk of breast cancer (Pike et al, 1993). While scientific findings are inconsistent, a meta-analysis by Fisher et al., (1995) indicates that estrogen replacement therapy has no significant effect on the risk of developing breast cancer. Of greater concern is perhaps the connection between estrogen replacement therapy and endometrial cancer.

f) Dietary Factors

There are numerous dietary factors that have been investigated in relation to breast cancer. Dietary fat, meat or fish consumption, specific micronutrients such as beta-carotene, ascorbic acid and vitamin E, and exposure to phytoestrogens in the diet have all been investigated. Evidence that women in Asian countries have substantially different rates of

breast cancer than women in western countries, and that when Asian women immigrate to Western countries their breast cancer risk increases to the level detected in that country, has given credence to the potential of dietary factors associated with increased risk in breast cancer. The rates of breast cancer in North America and Northern Europe are 5-6 times greater than the rate in Asia and Africa (Fisher et al, 1995).

Dietary fat has been implicated as the greatest difference between western and eastern diets and consequently this factor has been investigated intensely. Some studies have suggested a link between high fat consumption and breast cancer while other studies show no effect (Miller et al, 1994). In the National Breast Screening Study in Canada, which considered the effect of fat calories in relation to all calories, an increased risk was detected (Howe et al, 1991). However in other studies where fat calories are not distinguished from total calories no association was found which suggest an interrelationship between total caloric intake and dietary fat levels may play a significant role.

The relationship to total caloric intake and the approximate proportions of saturated, mono unsaturated or polysaturated fat content of the diet may be the primary factor in the relationship between diet and cancer development (Doll, 1992). Current evidence suggests that an association with dietary fat and breast cancer exists, however, the association is likely not linear and appears to be very complex. In order to gain a beneficial response, substantial reductions in individual western diets would have to be

undertaken to reach the dietary fat content found in Asian diets. This reduction may be extremely difficult to attain on a North American population basis (Fisher et al, 1995).

Increased dietary fat has been suggested to influence the concentration of female sex hormones. Bennet and Ingram (1990) showed that, when women were switched from meat diets to vegetarian diets, their circulating estradiol levels were significantly decreased. High fat consumption was directly associated with increased prolactin levels and SHBG was significantly decreased. Increased levels of meat consumption have been weakly related to elevated risk in breast cancer (Toniolo et al, 1994). In particular, high intakes of red meat have been associated with an increase in breast cancer risk. Whether this relationship is due to increase in dietary animal fat or potentially the contaminants found in meat, has not been established. However, the importance of diet in breast cancer development remains one of the main differences between Asian and Western countries. Breast cancer incidence in Western countries has been found to be four to ten fold greater in comparison to breast cancer incidence in Asian countries, and interestingly populations in the former typically have much higher consumption levels of animal products. Toniolo et al, 1994 also suggest that the diet patterns in the prepubertal, pubertal and teen age period may impact the lifelong endocrine balance and that these factors should be taken into consideration when determining the relative impact of dietary intake and breast cancer development.

High consumption of phytoestrogens, such as ligans and isoflavones have been suggested

to impart a sparing effect on breast cancer risk. Whole grains, fibre, flax seed and several fruits and vegetables have high level of ligans while isoflavones have been found at high levels in legumes. Asian populations who classically consume a diet high in phytoestrogens have significantly lower rates of breast cancer. However, when Asian women adopt more western diets and lifestyles their incidence of breast cancer has been shown to increase (Barrett, 1996). These findings suggest that high consumption rates of vegetables and fruits and subsequently higher level of phytoestrogens may impart a protective effect.

Phytoestrogens have been demonstrated to inhibit cancer cell growth. In MCF- 7 human breast cancer cells, researchers have shown that the ligan, enterolactone, in the presence of estradiol (an endogenous hormone), inhibits cell proliferations (Barrett, 1996). Phytoestrogens have also been shown to affect sex hormone metabolism, intercellular enzymes, protein synthesis and malignant cell proliferation (Barrett, 1996; and Aldercreutz, 1995). Phytoestrogens stimulate an increased level of SHBG which subsequently lowers the level of free estradiol and it is through this pathway that they most likely reduce the lifetime risk of developing breast cancer. In studies of women consuming various diet types women who consumed macrobiotic diets had the highest levels of ligans compared to lactovegetarian and ominovorous women, the latter having the lowest level (Adlercreutz, 1995). Interestingly, the highest level of these compounds in the diet can be found in regions that have the lowest incidence of hormone dependent cancers (Barrett, 1996).

g) Body Weight

Both obesity and body fat distribution have been associated with increased incidence of breast cancer (Kumar et al, 1995; and Zhang et al, 1995). Increased body fat during the post menopause period in particular has been related to elevated risks in post menopausal breast cancer. North American women, are on average, more obese during their post menopause phase than their Japanese counterparts (Pike et al, 1993). In African American women, who tend to be significantly heavier than white or Hispanic women, high body weight has been associated with elevated risk of breast cancer. This finding holds true despite generally being younger at age of first pregnancy, and having more live births and a greater number of pregnancies (Weiss et al, 1995). The timing of weight gain also appears to play a role in the risk of breast cancer. Kumar et al (1995) found that women who progressively gain weight from puberty to adulthood and, particularly in the post menopause stage, had a higher risk of developing breast cancer.

Obesity has also been related to a poorer prognosis. Researchers have speculated that this may be because masses may be more difficult to detect during breast examinations in obese women or elevated levels of endogenous estrogen would accelerate tumour growth (Zhang et al, 1995; and Pike et al, 1993). Body fat distribution has been related to increased risk with women who have high fat deposition in the abdomen, breast region having higher risk than those who deposit fat in the thigh region (Kumar et al, 1995). However, the relationship to high fat and high calorie intake may be the driving force in the relationship between obesity and breast cancer (Kumar et al, 1995). Similarly body

weight can be confounded by several factors such as age, education, income, alcohol use and genetic predisposition (Kumar et al, 1995). High birth weight and first pregnancies have been associated with higher maternal levels of total estrogen and estradiol and may be associated with increased risk of developing breast cancer (Gammon and John, 1993).

h) Smoking and Alcohol

Smoking is presumed to be the most important single risk factor in causing cancer (Gray, 1995). However, several research papers have shown that breast cancer may occur less frequently in women who smoke (MacMahon et al., 1982). It has been suggested that decrease risk is associated with smoking's effect on a women's hormonal balance and estrogen metabolism. Women who smoke have lower levels of estrogen in their urine than non-smoking women (MacMahon et al.,1982). Research findings suggest that either estradiol metabolism is increased in smokers (Domanski et al., 1977) or the rate of estrogen catabolism is increased in smokers (Berta et al., 1992). There is little evidence to suggest that smoking increases breast cancer risk with most studies finding no or very minimal positive associations (Palmer and Rosenberg, 1993).

Alcohol consumption has been shown to have a positive correlation with breast cancer in the majority of studies (Miller et al, 1994). Increased risk has been associated with alcohol consumption in early adult life and appears to be most strongly associated with higher consumption levels, namely greater that 17 drinks per week or greater than 40 grams per day. The possibility that alcohol consumption may be a marker of other

factors which increase the risk of breast cancer has also been suggested (Miller et al, 1994).

i) Mammography

The large scale introduction of mammographic screening of the breast has been argued by several authors to be responsible for a significant proportion of breast cancer cases diagnosed over the last 15 years (Wun et al, 1995, Miller et al, 1991, and Waddell 1998). Mammographic screening prior to 1982 was used quite minimally. Since the introduction of this screening technique, it has been used with increased frequency for early detection of breast cancer. In the United States, a National Health Interview Survey indicated that approximately 17% of women age 40 and older had received mammographic screening. By 1990, 33% had received mammographic screening within the past year (Wun, 1995). While these models appear to provide a good statistical fit, Wun et al (1995), caution that they are not grounded in firm epidemiologic understanding.

1.3.3 Organochlorine Exposure and Breast Cancer

To date the role of estrogen in breast cancer remains a significant linking factor between known risk factors and the development of breast cancer. The observation that substances such as DDT can stimulate breast cells in vitro and the ability of some of these substances to mimic reproductive hormones in laboratory and wildlife settings have lead to investigation of the plausibility that organochlorines may play some role in the dramatic increase in breast cancer rates in developed countries. Several epidemiological studies

have found contradictory evidence in the relationship between breast cancer and organochlorine residues. The early studies tend to support the finding that a link between exposure to organochlorines and increased risk for the development of breast cancer exists, while the later studies suggest there is no significant difference between organochlorine levels in the blood of those with breast cancer compared to those without.

The first study which documented organochlorine compounds in neoplastic and normal breast tissue was that of Wassermann et al (1976). The authors found that there were increased levels of organochlorine compounds in malignant breast tissue when compared to adjacently normal tissue. This case-control study involved only nine cases and five controls. No mention was made of the women's age, parity, lactation status etc which could significantly bias the results of this study. The authors found that concentrations of the organochlorines, DDT, PCB's, gamma BHC, heptachlor, and dieldrin were increased in neoplastic breast tissue in comparison to normal breast tissue.

In 1984 Unger et al examined adipose tissue taken from 14 women diagnosed with breast cancer, 21 non-cancer patients, 18 deceased breast cancer patients, and 35 cancer free autopsy controls. The authors found no statistical difference in the mean concentration of DDE and PCB between cases and controls; however, when logistic regression models were used, PCB and DDE were statistically associated with cancer mortality after controlling for confounders. The potential confounders taken into consideration included weight, height, occupation and residence. Dietary intake, parity or lactational history were

not examined. The exclusion of these potential confounders may have substantially biased the results of this study. As well, the authors only examined specimens for PCB, DDT and DDE levels and did not preform residue analysis for other organochlorines. The potential significance of chemical interaction and/or the relationship between organochlorines levels in breast tissue and cancer was not fully investigated.

Mussalo-Rauhamaa et al (1990), performed an epidemiological study exploring the relationship between organochlorines and breast cancer. This case-control study used adipose samples taken from 44 Finish women diagnosed with breast cancer. Samples were taken as close to the malignant tissues as possible and these cases were compared to 33 adipose samples taken from accident victims free from any malignancy as determined on post mortem. Information on age, height, weight, smoking, fish eating habits, parity and breast feeding history was determined by questionnaire, and no significant difference was determined between cases and controls for age weight and height. There were 14 nulliparous cases compared to 4 nulliparous controls; however the parity status for two of the cases were unknown, while nine of the controls were of unknown status. The effect of this lack of classification may have biased the results toward or away from the null. If as McTiernan and Thomas (1986) suggests, that lactation results in mechanical flushing of carcinogens, then nulli parity which was identified as a risk factor could have been a surrogate measure for organochlorine levels. The authors found that there was a statistically significant greater level of HCH in cancer patients when compared to controls, while there was no statistically significant difference for the other organochlorines

detected in the adipose samples.

In 1992 Falck et al investigated the levels of organochlorines in biopsies taken from 50 Caucasian women with a palpable breast mass of which 23 had mammary carcinoma and 27 had benign masses. From this initial group forty samples were submitted for residue analysis representing 20 benign controls and 20 malignant cases. The selection criteria for inclusion in these latter groups were not reported. The study did not investigate the parity, lactational history or dietary habits of subjects which could have significantly altered body burdens and influence the final outcome of the study. The research results indicated that there were higher levels of PCBs, DDE and DDT in women diagnosed with malignant breast cancer than in those with benign masses. However, the authors recognized that several of the known risk factors for breast cancer were not considered and pointed to the need for larger more extensive studies to determine the relationship between breast cancer and organochlorine levels.

The investigation by Wolff et al (1993), was the first of a group of studies that explored multiple facets of the relationship between organochlorine and breast cancer. This blinded case-control study examined blood levels of DDE and PCBs in the sera of 58 women diagnosed with breast cancer 1-6 months after entering the study. There were 171 matched controls from the same study population who had not developed breast cancer. In the case samples, there is a question of how the presence of breast cancer would affect the organochlorine samples given the short time period between sampling and breast

cancer diagnosis. The authors did not analyse the samples for residues of other organochlorines normally found in the breast tissue of women and this omission could have impacted their findings. In this study, the investigators considered several of the potential confounding factors namely; lifetime lactation, age at menarche, age at first full term pregnancy, family history of malignant and benign breast cancer, race, history of smoking, and/or alcohol consumption.

The results of this investigation revealed that higher mean levels of DDE and PCBs were found in breast cancer patients, however only DDE levels were found to be statistically significant after adjusting for potential confounders. The effect of lactation when considered by itself proved to be protective and an increase in duration (by months) of lactation showed a continuous sparing effect. Adjusting for lactation history also strengthened the association between DDE levels and breast cancer and the authors pointed out that studies ignoring the potential effect of lactation could significantly bias the outcome. The relative risk of breast cancer and DDE levels showed an approximately log-linear relationship with increasing level of DDE.

Krieger et al (1994), examined the relationship between breast cancer and serum levels of DDE and PCBs. The study design involved 150 case patients and 150 matched control subjects from a cohort of 57 040 women. Residue analysis was performed on frozen blood samples taken in the late 1960s and the study subjects were followed up during this time period until December 1990. A further 50 women diagnosed with breast cancer were

sampled per racial/ethnic group and matched to a cancer free control. The study results indicated that there was no statistically significant difference between the serum DDE or PCB levels of the cases and controls, except when black women were compared. In the latter situation cases had higher levels of DDE compared to controls. White women also showed a difference in the level of PCBs with cases having lower level of PCBs than controls. This raises an important point in that some PCBs may actually be associated with anti-estrogenic effects and the level of these types of PCBs in comparison to the level of estrogenic PCBs and other estrogenic organochlorines detected in individual in the study may significantly affect the authors conclusions regarding the relationship between organochlorines and breast cancer. The Krieger study also found that black and Asian women had significantly higher levels of organochlorines than white women, which may be related to environmental or dietary exposure.

One of main difficulties of this study design was the omission of lactational history for the study subjects. The authors admit that this omission may have a great impact on the findings of the study especially given the finding in the Wolff et al (1991) study where lactation proved to be a significant confounding factor. In this study, and the others, blood values were determined only at one stage in the developmental history of the individuals under study. It would have been extremely interesting to have determined blood levels at the beginning, middle and end of the study period in order to determine if significant changes had occurred in exposure status and to monitor the total body burden of these women. Similarly, samples of adipose tissue over this same time frame could

have provided the authors with an increased data pool from which to make conclusions regarding the relationship between organochlorine and breast cancer. The lack of information on residue levels of other organochlorines in the samples tested give rise to the potential for uncorrected confounding factors especially if the balance of organochlorine residues is towards the estrogenic side versus anti-estrogenic.

Dewailly et al (1994), showed that women with estrogen receptor-positive (ER-positive) breast cancer had high body burdens of organochlorines. This study examined adipose samples taken from 41 women volunteers who had undergone a biopsy for a breast mass at a Quebec City university hospital. Fasting serum samples of organochlorines were also determined. Of the 41 volunteers, 20 case patients diagnosed with infiltrative adenocarcinoma, and 17 control patients diagnosed with benign breast disease were chosen. Adipose and plasma concentrations were higher in case versus control patients; however only differences in HCB levels were statistically significant. The authors found that the mean and median level of DDE and PCB congener 99 in adipose and serum sample of ER-positive case patients were higher than control subjects. The study findings suggest that women with hormone-responsive breast cancer are at greater risk from exposure to organochlorines than women with benign or estrogen receptor negative breast cancer. While the study sample size is small, the authors did examine confounders such as age, weight loss, parity and breast-feeding. The case and control groups had a similar mean age, parity and an average body-weight reduction over the previous year. However, the breast feeding history of the case patients showed that 88.9 % of case

patients verses 76.5% of control patients had never breast fed. This latter situation could account for a lower body burden of organochlorines in those patients that had breast fed and may thus be related to both the diagnoses of breast cancer as well as the detected levels of organochlorines in the patients in the study.

Hunter et al (1997), found that exposure to high levels of DDE and PCBs was nonsignificantly associated with a lower risk of breast cancer development. In this case control study, the authors measured levels of DDE and PCBs in plasma samples from 240 women taken in 1989 and 1990 who subsequently developed breast cancer over the following three years. Blood samples were selected from 32,826 samples voluntarily submitted from over 121,700 women enrolled in the Nurses' Health Study. Controls were matched on menopausal status, month in which blood samples was returned, time of day of sampling fasting status, and postmenopausal hormonal use by postmenopausal women.

There are several factors of concern with this study. Firstly, samples were voluntarily submitted to the authors and the women who submitted samples to this study they were more likely to have a history of familial or benign breast mass. This finding could potential bias the results in that the relationship between other confounding factors and genetic predisposition has not been elucidated. Blood samples were taken in 1989 to 1990 which results in a very large period of time from actual exposure to organochlorines and sample collection. Despite a tendency for slow metabolism of organochlorines (DDT half life has been estimated at 10 years (Wolff et al, 1993), significant fluctuations could

result from sample collected this long after initial exposure.

The relatively short follow up period of three years post sampling could also have significant importance given that false negative controls could exist. This is particularly true in the case of breast cancer where a palpable lump may take several years to detect. Similarly, the presence of cancer has been suggested to increase catabolism which could conceivably lower the levels of organochlorines in case patients, especially given the short time interval between sampling and breast cancer detection in this study.

The authors also failed to fully assess lactational or parity history. This omission is disturbing in that lactation history could have a significant impact on the total detectable body burden. This statement is further supported by the authors own findings in this study that, among the parous women, more women in the lowest third of DDE and PCB levels had breast fed for greater then 6 months duration. Had an effort been made to investigate both the number and duration of lactation periods in both the case and controls the findings of this study would have been more valid. Of interest is the finding that case patients had a higher rate of maternal and sibling history of breast cancer as well as an increased history of benign breast disease. The relationship between these factors and other confounding factors have not been elucidated and perhaps it is of more interest to know how organochlorine body burdens affect populations without genetic predisposition to breast cancer since, if a relationship exists between organochlorines and breast cancer, it may be more evident in populations not genetically predisposed to breast cancer.

The findings of these studies appear to suggest that a link between exposure to organochlorines and the development of breast cancer is possible, and until further studies are carried out which account for all the confounding factors, the verdict remains unclear. The absence of information on the levels of other organochlorines in these studies is disappointing given the fact that other organochlorines such as dieldrin, and HCB can be found in human adipose tissue and breast milk samples and have also been shown to have estrogenic effects. The sum total of exposure to organochlorines may therefore play a more important role in the development of breast cancer than the individual chemicals themselves. The inter relationship between these chemicals and the net balance of estrogenicity is of extreme importance in teasing out the potential relationship between exposure to organochlorines and breast cancer. As such, future studies should take into consideration the total human body burden and the changing trends in exposure thorough diet and metabolism or secretion via breast milk.

The relative risk from exposure to organochlorines in women residing in Ontario, has never been addressed in relation to breast cancer. Exposure history plays a significant role in the body burdens of organochlorines these women carry and consequently the risk of developing breast cancer as a result of organochlorine exposure if a relationship exists between breast cancer and organochlorine exposure. It is therefore important to determine if women in Ontario had significant exposure to organochlorines, the stage of development, duration and the level of exposure that occurred for the various groups of women during the decades of exposure, and to determine if these exposure scenarios are

likely to be associated with an elevated risk for developing breast cancer.

1.4 Exposure Assessment

1.4.1 Introduction

The discovery of the persistence of organochlorines in the environment and in human tissues led to a world wide investigation of these substances in soil (Carey, et al, 1975; and Martijn et al, 1993), rainwater (Pearce et al, 1978), drinking water (Williams et al, 1978), food (Frank et al, 1970; 1975; 1979; 1987; 1989; 1990) wildlife (Muir and Sudar, 1987; Environment Canada, 1991; and Bishop et at, 1992), and human body burdens (Dale and Quinby GE, 1963; Hunter et al, 1963; Polishuk et al, 1977; and Frank et al, 1988). In Canada and the United States, market basket studies and food product sampling have been used to record the exposure to organochlorine and other chemical residues in food. Human body burdens have been recorded extensively by numerous countries such as Canada, United States, Britain, England, Japan, Holland and Sweden (Durham, 1965; Frank et al, 1988; and Mes et al, 1986). Blood, adipose tissue, breast milk and fetal cord samples have been tested to estimate the total body burden of people in these countries. Comparisons of total body burdens between countries have been made over the decades of detection (Durham, 1965; Newsome et al, 1995; Duarte-Davidson et al, 1992; Frank et al, 1988; and Van Hove Holdrinet et al, 1977), with the assumption that lower levels of residues compared to other countries was favourable. The following is a review of the various types of organochlorines in a vast array of substances over the decades of detection.

1.4.2 Exposure Assessment in Food

To date, a large number of studies have investigated organochlorine and other chemical residues in food. Individual residue analysis in specific food products such as bovine meat, bovine milk, fruits and vegetables, eggs and poultry have been carried out by many countries. In Ontario, residue testing of food commodities was carried out extensively and remains to date the most comprehensive data for food residue analysis in Canada (Frank et al, 1979, 1988, 1987, 1989, 1990). Market basket studies have also been employed to measure the general population's exposure to chemicals from composite samples of commonly consumed foods. Results from these studies have been used in the past to compare different countries and their levels of exposure via food sources. In Canada, the results have also been used to assess the relative acute safety of the Canadian food supply (Smith, 1971, 1972; Smith et al, 1973, 1975; Mcleod et al, 1980; Johnson et al, 1979; and Pennington, 1983).

Market basket studies examined the residue levels in found food samples purchased from various sources (such as grocery stores) that have been prepared in a similar fashion to that consumed in the home. For example, foods normally fried, boiled, peeled, trimmed, processed or consumed raw would be prepared and subsequently tested for chemical residues. These studies include food products imported into the region, such as citrus fruits and speciality foods, thereby encompassing residues from food produced both locally and imported into the region. Classically food sampling has been carried out to obtain a representative sample of the average diet of people under study (Smith, 1970;

Pennigton, 1983). Exposure estimates were generally calculated based on average consumption per person on a daily basis times the average residue level found in the food groups tested. In general, the residue information generated by a market basket analysis has been suggested to provide a more accurate indication of human exposure in comparison to direct monitoring programs of food commodities because the effects from cooking and processing, which may alter the level of chemical found, are taken into consideration (Conacher and Mes, 1993). However, variation in consumption trends, composite sampling techniques and analysis methods can result in potential errors when relying on market basket studies alone to provide details about human exposure to organochlorines.

The very first market basket studies were performed in the United States in 1964 (Duggan and McFarland, 1967). Similar studies were also carried out in England and Wales during this time period (Abbott et al., 1969). These studies indicated that the general population in these countries had exposure to chemical contaminants through consumption of food. In Canada, market basket studies have been used since 1969 to record the level of human exposure to organochlorine residues in food products across the country (Smith, 1970). While market basket studies can be used to gain knowledge about the relative exposure of individuals in Ontario and Canada, the information provided by these studies exists only from 1969 to 1993. This leaves a twenty-two year time period, from approximately 1947 to 1969, when organochlorines which were definitely in use in Canada, unaccounted for. Variations in sampling, residue analysis and consumption

patterns also necessitate that exposure trends be confirmed by other forms of residue testing so that we can be relatively confident of the total human exposure picture.

Residue testing of individual food products has been carried out extensively over the decades particularly in Ontario and the United States (Frank et al, 1970, 1979, 1987, 1989). The first indication that organochlorines could be found in food products occurred in 1957 when Clifford (1957) reported pesticide residues in fluid milk in the United States. Since these first accounts, there have been a succession of publications documenting organochlorine residue levels in milk and dairy products in various countries. (Clifford et al., 1959; Duggan, 1967). The first Canadian reports documenting organochlorine residues in food products were published in 1970. Results from the Ontario milk survey programs initiated in 1967 by the Ontario Department of Agriculture and Food determined that several organochlorines could be detected in bovine milk intended for human consumption (Frank et al, 1970). Results indicated that nearly all samples contained DDT as well as its metabolites and dieldrin, with several samples above government tolerance levels. Lindane and heptachlor epoxide were also isolated. The results of this first study led to the subsequent ban on the sale of dieldrin and aldrin in 1969 by the Department of Health of Ontario under the Pesticide Act of 1969, and marked the beginning of a series of publications recording the organochlorine burden in Ontario bovine milk samples from 1967 to 1986 (Frank et al., 1970, 1975, 1979,1985, 1989). Organochlorine residues have been detected in bovine milk samples for decades in most countries.

Residue testing for various chemicals in meat and eggs has been undertaken by many countries. In Ontario, bovine and porcine residue analysis for organochlorine and organophosphorus contamination were undertaken from 1969 to 1981 (Frank et al, 1983), and again in 1986 to 1988 (Frank et al, 1990). Similarly, residue testing of abdominal and egg fats of chickens has been recorded for the time period from 1969 to 1982 (Frank et al, 1985). This form of residue analysis can be used to give an indication of the exposure that animals received to organochlorines through such sources as feed, direct insecticidal sprays and through indirect exposure. In cases of higher than expected levels these studies have been used to track down the source of exposure or to prevent the reoccurrence of high residues in subsequentshipments of food products from the same source.

Residue testing in fruits and vegetables was undertaken as part of the food safety monitoring program initiated in Ontario. Samples were analysed for organochlorines as well as other insecticides, herbicides and fungicides from 1980 to 1988 (Frank et al, 1987, 1990). There does not appear to have been very much interest in residue testing for organochlorines in these individual commodities prior to this time. Since the use of most of the persistent organochlorines had ceased by this point, the usefulness of this information from the organochlorine residue perspective is limited. Therefore exposure to organochlorines from fruit and vegetable need to be approximated prior to 1980. However, information post-1980 can be used to corroborate the results from the market basket analysis. The information provided from residue analysis of specific food products

provides information on which food groups represent the greatest source of organochlorine residues, and, at a population scale, the approximate historical period of peak exposure from these sources. The information for individual food products can also be used to confirm the general trend of exposure to organochlorines in Ontario. Based on the abundance of information on organochlorine residues in food products from various countries, it can be seen that contamination of food products is a worldwide issue.

1.4.3 Exposure Assessment in Humans

Human exposure to organochlorines can be measured in several ways. Samples of adipose tissue and blood serum can indicate the amount of exposure a person has received over an extended period of time or, if measured serially, they can indicate current exposure. Human breast milk samples have been used to indicate past exposure of lactating women and to provide insight into levels consumed by nursing infants (Mes et al, 1993; Newsome et al, 1995). Serum samples taken from the placenta or umbilical cord can be used to determine the amount of placental transfer and give an indication of the initial exposure of the infant and fetus (Rogan et al, 1987). Samples of major organs, urine, hair, or ear wax have also been used to determine human body burden of organochlorines (Smith, 1991).

Human body burdens of organochlorines have been documented in several countries with extensive information available from such countries as Canada, the United States, Japan and Sweden (Durham 1965; Van Hove Holdrinet et al, 1977; Frank et al, 1988). The first study recording DDT residues, in an individual occupationally exposed, was published

in 1948 (Durham, 1965). Subsequent to this finding, a general survey was conducted in the United States in 1950 to determine the average level of DDT exposure in 75 people with no history of occupational exposure. This study determined an average residue level of 5.3 ppm in adipose samples and established that the general population had exposure to, and subsequently stored DDT, in their bodies (Durham, 1965). Prior to the initial production of DDT, human fat samples did not contain residues of DDT. This finding was confirmed by analysis performed on human adipose samples taken from people who died before DDT manufacturing had begun (Hayes, 1958).

Since the first publication of residue analysis of human body burdens, there have been numerous studies worldwide providing evidence that DDT and other organochlorines could be detected in adipose samples from individuals in the general population. While initial studies only recorded DDT contamination, subsequent studies recorded residue levels of other organochlorines. The organochlorines that have consistently been detected in human tissue samples include DDT and its metabolites, dieldrin, PCBs , heptachlor and chlordane. More recently, mirex and HCB have been detected (Frank et al 1985). Several studies have attempted to compare the levels and types of organochlorines detected in humans from developed countries in order to determine the relative quantities of exposure (Durham, 1965). Generally, samples from Canada have compared favourably to those of other industrialized countries and on this basis have been judged to be safe (Mes et al, 1992). Until recently, the prospect of chronic health effects from exposure to and the persistence of organochlorines in human bodies have not been considered.

Unfortunately, human body burdens trends have not been documented as fully as would be required to make an accurate calculation of total exposure. Since samples in general were taken from a cross section of individuals with no repeat sampling for each individual, a true trend in exposure for the individual cannot be determined. Establishing general population exposure trends solely from existing human body burdens information is not possible based on these data. Analysing the average human body burden also becomes complex given that there is a lack of uniformity of exposure, variability in percentage body fat, differences in past history of breast feeding or being breast fed, along with a large variation in life style habits that makes it difficult to determine a consistent exposure pattern for individuals as well as populations. Inaccurate or inconsistent results can also arise from several other sources which include small sample size, variations in analytical techniques and a lack of consistent documentation of various age groups and sexes.

If samples were available on specific groups of individuals documented from birth and followed through to death, and if information on dietary habits, lifestyles, lactation history, occupational status, having been breast fed, weight loss and weight gain history had been recorded, this information would have helped to provide a meaningful history on those factors which can influence exposure and determine the individual's entire exposure history. This information could, in turn, be used to give reasonable estimates of the population's exposure to organochlorines over the entire period of exposure and could have helped to provide meaningful estimates for specific groups of people. Since this extensive documentation is not available, other surrogates of exposure must be combined

with the available body burden analysis to derive the most accurate exposure history for individuals and specific groups in Ontario. Surrogates that can be used include: market basket studies; levels in specific food products; trends in use or sales of organochlorines; and levels detected in water and the environment. The exploration of all the available information in all these areas will provide information that is invaluable to determining the population's true exposure to organochlorines.

1.4.4 Quantities of Organochlorines Used

Several attempts have been made to estimate the global use of organochlorines. Volder and Li (1995) have most recently attempted to record the global use of HCH, lindane, DDT, and toxaphene. They have estimated that approximately 1 500 000 metric tonnes of DDT and 450 000 metric tonnes of toxaphene have been used from approximately 1950 to 1993. The authors of this study have also encountered the tremendous amount of secrecy and unrecorded information on use of this particular group of chemicals - leading them to comment that there are large spatial and temporal gaps and high variability in data quality. Even in developed countries such as Canada where records exist on company and product sales statistics, and sales summaries for each province, only composite information has been divulged and full disclosure of chemical content in composites has not been given to ensure that the competitive position of a producer is not jeopardized (Volder and Li, 1995). Since there is no global registry, and many countries have a similar attitude toward chemical use, factual documentation of sales is excessively difficult to obtain. In general, this may mean that aggregate information from various sources gives a more

accurate picture of exposure for regional populations than apparently more precise estimates at smaller units of concern.

Accurate documentation of quantities and application sites for organochlorines is virtually non-existent for Canada. Prior to 1968 the Dominion Bureau of Statistics recorded pesticide sales for Canada as a whole but individual provincial figures were not recorded. Subsequently, the Pesticide Control Service of the Ontario Ministry of Health required sales documentation of DDT, TDE, aldrin, chlordane, dieldrin, heptachlor, endrin and lindane; however, application rates and uses were not recorded. The information provided from 1947 to 1968 is the best and in some cases the only information available on organochlorine use in Ontario. In 1972, the first Ontario pesticide use survey was undertaken, recording pesticide use on field crops, fruits, vegetables and roadside applications (Roller, 1975). By this time, however, many of the organochlorine compounds had been banned or severely restricted for agricultural use.

Table 1.4.4.1 outlines some of the dates of restriction and banning of organochlorines in Ontario. In Ontario, agricultural application of organochlorines occurred primarily in the southern portion of the province, in areas like Essex, Kent, and Middlesex counties (Frank, 1970). Some rough estimates of organochlorine use have been documented in these regions. In 1968 for example, 30,200 pounds of DDT and DDD as well as 32,000 pounds of aldrin and dieldrin were applied to various crops in southern Ontario. Extensive use of organochlorines also occurred in forests and on park land for control of nuisance

insects such as spruce budworm throughout the entire province (Frank, 1970). The amount of organochlorine used in this fashion has not been documented but probably represents a significant proportion of the total organochlorines used in Ontario (Frank, 1970).

General use of DDT in the United States began in 1942 (Hayes, 1955). U.S. sales of synthetic organic pesticides increased from 279 million pounds in 1954 to 691 million pounds in 1964. That same year about one-third of U.S. pesticide exports went to Canada (Headley, 1969). In 1963 manufacturers in the U.S. shipped a total of 828 million pounds of chlorinated hydrocarbon pesticides valued at $131.4 million (U.S.). By 1967, production had decreased to 679 million pounds valued at $119 million (Agricultural Chemicals 1969a). During this same year $3,754,000 or one third of all foreign-aid financed pesticide purchases were spent on DDT, mainly for international malarial eradication programs (Agricultural Chemicals, 1969b). U.S. data indicate that 88,632 pounds of DDT were used in 1967 by the forest service to control forest pests. However, by 1968, this form of application was dramatically reduced to 81 pounds of DDT as organochlorines were replaced by newer pesticides (Agricultural Chemicals, 1969a) Organochlorines were also used as household insecticides. While the quantities used in household are unknown, a 1969 urban survey found that 89 percent of responding families used pesticides, with 38 percent using pesticide within the home on a weekly basis. A survey of household pesticides use in Guelph in 1992 found that DDT was still on the shelf in at least one household (Ellis, 1993).

1.4.5 Wildlife Exposure to Organochlorines

Particularly in Canada and the United States wildlife samples have been monitored with an emphasis in the Great Lakes region (Environment Canada, 1991; Muir and Sudar, 1987). This area has been a major areas of focus since many different wildlife species inhabit this region (Environment Canada, 1991). A considerable number of studies have been performed recording levels of organochlorines in eggs of various species of birds, in fresh water fish, and in wild game.

While the exposure history of wildlife can give an indication of the relative amount and trends in exposure of this region, it may not be a good indicator of exposure in humans. For example, samples taken from top predators (such as eagles) would be expected to have relatively higher amounts of organochlorines than human inhabitants of the same region, since the bulk of the eagle's diet consists of fish which are known to bioaccumulate organochlorines. However, trends in exposure in wildlife can be used to corroborate information on general patterns of human exposure to organochlorines.

1.4.6. Summary

Given the complex nature of organochlorine exposure assessment and the difficulties of accurately determining the quantities of organochlorines used or found as residues in various samples, it is imperative that all available information be examined and assessed as a unit in order to most accurately assess exposure to organochlorines over the decades. To date, no studies have attempted to aggregate the available residue and sales

information from all sources to determine the total organochlorine exposure from the initial period of organochlorine use to the final banning of these substances. An accurate accounting of organochlorine exposure in humans is extremely valuable in determining the extent of the population's exposure. This information is also critical in determining possible relationships with adverse human health effects from exposure to organochlorines. By combining the residue data from various sources together with sales and pesticide use information, a basic exposure model can be made which may allow us to determine if trends in exposure can be related to trends in human disease outcomes such as breast cancer. While it would have been preferable to look at the relationship between organochlorines and breast cancer by comparing the level of exposure for different regions of Ontario to the level of breast cancer in these regions we were unable to obtain raw data from previous residue monitoring programs. Therefore we have chosen to pursue this topic with the best available information with the intent to shed some light on a very complex issue. In the following section, the chemistry of the organochlorines being considered are reviewed. The purpose of this is to provide further rationale for the selection of chemicals which form the core of this study.

1.5 Persistent Organochlorines

1.5.1 Introduction

The organochlorines or chlorinated hydrocarbons are a large group of chemical compounds used mostly as insecticides. Classically, they have been divided into five groups: DDT and its analogs; benzene hexachloride (BHC); cyclodienes and similar

compounds; toxaphene and related chemicals; and mirex and chlordecone. (Hayes, 1991) They are all either aryl, carbocyclic or heterocyclic compounds ranging in molecular weight from 291 to 545. (Smith, 1991) The chemical structure of these pesticides evokes a relatively persistent action post application. In general, this group of chemicals can be considered to be highly persistent in the environment and substantially resistant to microbial degradation or photolysis, since the carbon-chloride bond present in the organochlorines is not readily hydrolysed (Smith, 1991)

Despite bannings and restrictions on organochlorine use in Canada these chemicals remain an environmental and human health issue, due to their ability to migrate form the original use site to other regions. Climatic conditions influence the transportation of these chemicals such that warmer temperatures result in greater volatization while cooler temperatures favour greater partitioning from the vapour phase to particles suspended in the atmosphere (Ayotte et al, 1995). Subsequently, there is an increased likelihood that these particles will be transported to the earth's surface by rain or snowfall in colder climates while in warmer climates the organochlorines tend to volatize (Iwata et al, 1994). Continued application of organochlorines in third world countries (which employ these pesticides on a regular basis for the control of malaria and other pests) would therefore facilitate the dissipation of organochlorines from these regions to such regions as Canada (Muir et al, 1992).

Mammals in the Canadian arctic in particular have been documented to have

contamination with such chemicals as DDT and dieldrin despite the fact that these chemicals were not applied in this region (Barrie et al, 1992; Muir et al, 1992; Volder and Li, 1995; Ayotte et al, 1995). In colder climates, these chemicals tend to persist longer and can therefore enter the human food chain. Higher than expected levels of organochlorines have been detected in arctic fish and other wildlife species with concentrations of DDT, PCB's, toxaphene and chlordane in adipose tissue of sea mammals ranging from 1 to 5 *ug*/g (Muir et al, 1992). Similarly, Hargrave et al, 1992 detected DDT, DDE, PCBs, dieldrin and other organochlorines in zooplankton, particulate matter and liver tissue samples taken from fish collected from the Arctic Ocean. Inuit living and consuming domestic food sources have been documented to have higher body burdens and subsequently greater levels of PCBs and DDE in breast milk samples than in samples collected from average Canadians (Dewailly et al, 1989). These findings would suggest that this particular group of women would be especially interesting subjects when studying the relationship between organochlorine body burdens and the risk of breast cancer.

Organochlorines tend to be very lipophilic, consequently they can be found almost universally in fatty tissue of humans, mammals, birds and other wildlife. However, their lipophilic tendency varies with some organochlorines being stored more tenaciously then others. In general, highly chlorinated biphenyls are accumulated to a greater extent then the less chlorinated compounds (Smith, 1991). Varying rates in metabolism and excretion of the parent compound and its metabolites results in differing rates of storage. For

example, DDT and its metabolites are metabolized quite slowly in comparison to methoxychlor, and consequently the former is found at higher levels in humans than the latter (Smith, 1991).

In general, most organochlorines have low acute toxicity to humans. Despite their insecticidal efficacy, there have been very few human fatalities due to organochlorines. Table 1.5.1.1 compares the toxicity of some of the organochlorines in various species using LD50 values for comparison. Animals show various signs of toxicity when exposed to organochlorines. In general, neuronal hyperactivity occurs after exposure to high levels of organochlorines. DDT for example, first produces tremors which progress to convulsions, while with dieldrin, lindane and toxaphene convulsions are the first sign of poisoning (Smith, 1991). Fish, in particular, appear to be acutely sensitive to various organochlorines and consequently massive fish kills were associated with DDT spraying in places such as New Brunswick during spruce budworm control programs in the 1950's (McEwen and Stephenson, 1978). Deaths in birds were documented following DDT spray campaigns in Canada and the United States (Agricultural Chemicals, 1969c). Most human fatalities related to organochlorine exposure have been documented to result from accidental exposure to chemical spills during occupational exposure or via intestinal absorption following intentional ingestion of lethal doses (Smith, 1991).

While the acute toxicity of this group of chemicals historically have not been considered to be problematic, effects from chronic exposure has drawn significant attention. Given the

ability of these chemicals to persist in the environment and within the human body, chronic exposure and storage represents a potential pathway for these chemicals to produce negative health effects. Of particular interest in this regard are DDT and its metabolites, dieldrin, aldrin, heptachlor, chlordane, mirex, toxaphene, hexachlorobenzene and PCBs. More recently the dioxins and furans have come under closer scrutiny. However, the bulk of available information regarding human exposure to organochlorines consist of the former group and consequently this investigation has focused on only those organochlorines for which an extensive history of exposure could be determined. While there is a long list of possible candidates for consideration, this review of chemical properties, uses and metabolism will focus on those persistent organochlorines which have been used most widely and thus are of the greatest concern. These include DDT and metabolites, dieldrin, chlordane, lindane, benzene hexachloride, heptachlor and PCBs.

1.5.2 DDT

History:

1,1'-(2,2,2-trichloroethylidene)-bis(4-chlorobenzene) or DDT as this chemical is universally referred to, has been used in Canada since 1947 (Cutten, 1995). The compound was initially synthesized by Zeidler in 1887 and later rediscovered by Paul Muller in 1939. DDT was originally used during the Second World War for controlling biting insects (which acted as vectors for malaria and typhus), and was credited with saving millions of human lives for it's use in this fashion. Prior to 1945 the entire amount of DDT produced in the United States was allocated by the military for medical and

public health applications (Smith, 1991).

DDT was first released for commercial use on August 31, 1945 in the United States for a limited number of applications and in 1947 for commercial use in Canada (Smith, 1991). In Ontario and in Canada DDT was used extensively for agricultural, industrial and household applications for controlling biting insects and other pests (Canadian Department of Agriculture, 1974). This chemical had a broad range of applications in fruit and vegetable production, where it was used to minimize crop losses from various pests such as, tarnished plant bug, spittle bugs, cutworms and flea beetles. Direct application, in the form of spray solutions, was used to control several species of biting insects during the rearing of livestock and horses. As well, the product was employed as a residual surface spray for application on barn walls and in milking parlours (Canadian Department of Agriculture, 1969).

Industrial and household applications included the use of DDT dust and solutions for the control of bedbugs, bats, moths and carpet beetles. DDT was also used extensively for greenhouse and nursery applications and large quantities were used for commercial and private lawn care. Mosquitoes, black flies, and spruce budworm were controlled by ground application and aerial spraying of this chemical in both rural and urban areas (Canadian Department of Agriculture, 1969). In Canada and the United States, DDT spray programs were initiated to control mosquito larvae populations by applying this substance in the form of 1% oil or water spray solutions to pools, ponds and other bodies

of water. The widespread use of DDT and the belief that DDT was essential to maintain production levels in agriculture resulted in the use of large quantities of this substance and consequently resulted in the detection of residues of DDT and metabolites in virtually all tested specimens.

Extensive contamination of agricultural products, animal and human tissue and detection of DDT in the environment lead to restrictions in DDT use in Canada and other countries. Sweden was the first country to ban its use in March 1969, while Canada and the United States implemented restrictions in 1969 and 1970 respectively. The pesticide was banned in 1972 in the United States (Hayes, 1993). Ontario restricted the use of DDT as early as Jan. 1, 1970. (Ontario Ministry of the Environment, 1988), when use was restricted to control of cutworms in tobacco, tarnish plant bugs in apples and control of bats. Further restrictions eliminated the use of DDT against cutworms in 1972 and tarnish plant bugs in 1973. In 1986, Agricultural Canada discontinued the registration of DDT for use in Canada, however, existing stocks were allowed to be depleted. Only in 1988 did the province of Ontario officially ban the use of DDT. Existing stocks at this point were moved to the United States, and as of 1989 the product has been officially regarded as a hazardous waste (Ontario Ministry of Environment, 1988). Most industrialized countries have severe restrictions or bans in place, however, several third world countries continue to use DDT for malaria control and other agricultural purposes. For example, estimates of use in Latin America indicate 16,000 tonnes of DDT were used in 1978 and approximately 11, 000 tonnes in 1988 (Volder and Li, 1995). The use of DDT for malaria control

continues to receive international support from the World Health Organization, as an economical method of vector control (WHO, 1989). DDT will therefore continue to be used extensively in third world countries.

Chemical Structure and Properties

Pure DDT, C14H9CL5, is a white tasteless, relatively odourless crystalline solid while technical DDT is a waxy solid. It has a melting point at 107-109 degrees Celsius, low water solubility and high lipophilicity. DDT and metabolites are relatively resistant to biodegradation and therefore are capable of bioconcentrating in fatty tissue of numerous organisms (Hayes, 1991). Biomagnification can occur in the food chain resulting in higher levels of exposure for top predators. The substance easily volatilizes and disperses to other areas via long-range transport by waterways, ocean currents and air as is evident by its universal detection in a wide range of food products and human tissue samples. It has been well documented that DDT contaminants found in the Canadian Arctic are the direct result of long range transport as this chemical had very few uses in this location (Ayotte, 1995).

DDT and its metabolites have persisted in soil and water for extended periods of time. While initially this was considered a favourable trait, since it increased the duration of effectiveness, detrimental effects such as biomagnification soon became obvious. Martijn et al, (1993, performed residue analysis on samples taken, five years after cessation of DDT, from an agricultural field. A considerable fraction of originally applied DDT could

still be detected. This study determined that the approximate half life of p,p' DDT and o,p' DDT to be 20 and 15-20 years respectively. Model ecosystems have been established to determine the environmental fate of DDT and other persistent organochlorines. These systems have shown that organochlorines can accumulate in the tissue of fish and snails to levels of 250 times for DDT and 125 times for DDE, greater than those found in the water of the model systems (Metcalf et al, 1973).

Metabolism and Storage

There are several properties of DDT that have made this chemical notorious. Firstly, the lipophilic nature of this substance ensures that this chemical is readily absorbed from the gastrointestinal tract especially when dissolved in animal fats. Intestinal absorption can be affected by the level of dietary fibre and fat, and total food intake (Smith, 1991). Other modes of entry include inhalation, dermal absorption, and ocular absorption. These routes do not represent a significant mode of exposure for the average individual but may represent important routes of exposure in occupationally exposed individuals (Smith, 1991).

In humans, DDT is stored in all tissues with the highest concentrations in adipose tissue. Following initial and repeat dosages the chemical is stored in the fat, initially at a rapid rate, then gradually peaks and finally plateaus to a constant level (Smith, 1991). Repeated exposure to moderate doses results in greater storage of the compound in adipose tissue than would result from a large single dose. DDE a metabolite of DDT, and also a

component of technical DDT, is stored more tenaciously in humans than DDT itself (WHO, 1989). After discontinuation of exposure to DDT the levels of DDT stored in the fat slowly decrease while the levels of DDE increase. This occurs as a consequence of an increased rate of metabolism of the parent compound DDT to the metabolite DDE in comparison to the rate of excretion of DDE from the body (Smith, 1991). Morgan and Roan (1977), calculated that it would take 10-20 years post ceasing DDT exposure for body burdens to disappear, while DDE storage would persist for a life time. Given the present state of exposure, residents in Ontario will therefore be expected to continue to store DDT and DDE in their adipose tissues despite declining levels of exposure. This situation can be seen in monitoring trends in human body burdens as outlined in the human exposure section of this thesis. These substances could therefore exert an effect in the body during the entire lifespan of the individual.

DDT and its metabolites have been under close scrutiny since approximately the mid 1960's. Wildlife studies have implicated this organochlorine as the culprit in egg shell thinning and other wildlife reproductive problems (Colborn, 1991, and Environment Canada, 1991). DDT and metabolites have been shown to have some immunosuppressive effects (Smith, 1991). In occupationally exposed individuals a statistically significant increase in mortality from cerebrovascular disease was detected and a non-significant increase in mortality from liver and biliary cancer (Ritter et al, 1995). Kupfer and Bulger (1980) showed that DDT and DDE could exert an estrogenic effect that was suspected to be related to DDT's ability to induce microsomal enzymes. In rodents, DDT

has been shown to support the growth of estrogen sensitive tumours (Robinson et al, 1985). Soto et al, (1994) developed an E-Screen test to screen chemicals for estrogenic effects. The test uses human breast cancer cells which are sensitive to the effects of estrogen. Using this test, the authors determined that DDT and it's metabolites have estrogenic properties. While the relative estrogenic potency is low, several researchers believe that it is through this route that DDT and it's metabolites may cause breast cancer. DDT and DDE in particular have been suggested by numerous researchers to play a role in the development of breast cancer and other human diseases (Wolff et al, 1993 Davis et al, 1993 and Toniolo, 1995). The hypothesis most commonly accepted is that these chemicals act as estrogenic substances in the body and thereby stimulate the proliferation of malignant breast cells (Soto et al, 1991).

1.5.3 Dieldrin

History

Dieldrin and aldrin are considered members of the cyclodiene family of the organochlorines. Since aldrin is readily metabolized to dieldrin in plants and animals and their mode of action appears to be similar, the two compounds are frequently considered together. The insecticidal properties of dieldrin were first discovered in 1949 (Smith, 1991). Both chemicals have been commercially produced since 1950. Their use worldwide includes public health applications, such as tsetse fly control for the prevention of tropical vector-borne diseases, control of soil pests and as seed treatments to minimize production losses. A large proportion of dieldrin was used worldwide principally as a soil

treatment (Smith, 1991). However, large quantities were also used as residual sprays for controlling insects transmitting malaria. In Canada, these products have been registered for use as agricultural pesticides since 1950 (Cutten, 1995). Agricultural applications included control of strawberry root weevil, wireworm and bulb flies. As well, dieldrin and aldrin have been used extensively for the control of structural pests such as termites and for landscaping in the control of grubs, grasshoppers and thrips (Agriculture Canada, 1974). In the United States, the main agricultural applications include control of insects in corn and citrus production Hayes, 1991).

It has been estimated that global production in 1972 was approximately 13, 000 tonnes/year (WHO, 1989). However, manufacturing bans were implemented as early as 1974 in the United states. In Canada the use of these substances, with the exception of use for termite control, was suspended in 1976 (Smith, 1991 and Cutten, 1995). Numerous countries have instituted restrictions or complete bans on both these substances. However, several countries continue to use these chemicals for pest control in agriculture. In Ontario, applications continued to 1990 when the products were restricted to subterranean termite control for exterior soil perimeter treatment only. Complete banning of aldrin and dieldrin did not occur until 1994 and at this point existing stocks were disposed as hazardous waste (Cutten, 1995).

Chemical Structure and Properties

Dieldrin has the structural formula C12H8CL6O with a molecular weight of 380.93. It is

a colourless to light tan solid with a mild odour. Dieldrin is highly persistent and stable in the environment, with a half-life in water of 723 days, the longest of all the organochlorines (WHO, 1989). Aldrin has the structural formula C12H8CL6, with a molecular weight of 364.93. It is an odourless crystalline white solid. Technical aldrin is tan to brown in colour, with a mild odour and contains no less than 90% aldrin. The substance is moderately soluble in halogenated solvents, aromatics and paraffins, and poorly soluble in water. Aldrin is rapidly converted to dieldrin in most organisms and thus cannot be detected in most sampling studies (Smith, 1991). It is for this reason that when assessing dieldrin's impact on human health, we will actually be measuring the influence of both chemicals.

Dieldrin has been detected in soil samples long after its use (Martijn et al, 1993) The average half life of dieldrin in soil has been estimated at five years in temperate soils (WHO, 1989) . Residues have also been determined in drinking water and can be related to trace amounts in the environment (Williams et al, 1978). However, levels detected in drinking water were generally low since these chemicals tend to bind to soil particles (WHO, 1989). Ecological modelling systems have shown that dieldrin has a high biomagnification factor in fish and snails and has a very low biodegradability index (Metcalf et al, 1973).

Metabolism and Storage

Dieldrin can be absorbed into the body by several routes including, inhalation, dermal

absorption, ingestion, and ocular exposure. Dermal exposure of dieldrin results in rapid absorption while absorption from the gastrointestinal tract, via the hepatic portal vein, is relatively slow (Smith, 1991). In human studies, dermal exposure results in absorption of approximately 7-8% of the applied dose (WHO, 1989). The biological half life of dieldrin was 369 days after dosing ceased (Smith, 1991). The ratio of dieldrin adipose, liver, brain and blood is approximately 150:15:3:1. Dieldrin is metabolized in the body and these metabolites are excreted primarily via the bile in faeces (WHO, 1989).

Fetal exposure occurs from maternal transfer via the placenta, but accumulation occurs at a lower level than found in the mother (WHO, 1989). Dieldrin can also be detected in human breast milk samples and provides a route of exposure for infants (Van Holdrinet et al, 1977). For most individuals oral exposure to organochlorines has been the major route of exposure, while dermal exposure is the source of greatest exposure in individuals occupationally exposed. Dieldrin has been detected in a vast number of human and animal adipose tissue residue studies (Frank et al, 1988), but aldrin levels have not been detected. It is assumed that dieldrin levels represent exposure to both aldrin and dieldrin.

Dieldrin and aldrin are highly toxic for humans: 10 mg/kg is the lowest recorded dose that has produced a fatal outcome in humans (WHO, 1989). In poisoning cases aldrin and dieldrin cause signs of nausea, headache, dizziness, vomiting, and muscle twitching progressing to convulsions (Smith, 1991). Several long term mortality studies on workers occupationally exposed to aldrin and dieldrin have not shown an increased risk in cancer

mortality. Studies to date have not specifically investigated dieldrin or the potential synergistic effect this chemical could have with other organochlorines once in the body. However, dieldrin has been shown to have estrogenic properties using the E-screen test (Soto et al, 1994). This, in combination with evidence that this compound can be detected in adipose tissue of the general public and that it can be found in breast milk would emphasize the importance of investigating this compound in determining the relationship between organochlorine exposure and breast cancer.

1.5.4 Chlordane

History

1,2,4,5,6,7,8,8-octachloro-3a,4,7,7atetrahydro-4,7-methanoindan, more commonly called chlordane, has two main isomers. The cis isomer is more abundantly found than the trans isomer (Smith, 1991). Chlordane was used extensively for soil-dwelling pests in agriculture, for landscape and turf pests and for control of subterranean termite control. In Canada the chemical was used for agricultural purposes from approximately 1945 to 1975 (Cutten, 1995). Agricultural soil treatments were employed to control wireworm and corn root worm. Other agricultural applications included soil control of pests affecting strawberries, potatoes, and other vegetable crops (Agriculture Canada Oct. 1983). Household applications were commonly used to control cockroaches, biting insects and wasps (Cutten, 1991).

The discovery of the persistence of this chemical in soil, water and human adipose tissue

lead to the implementation of restrictions on agricultural use worldwide (Hayes, 1993). In 1975 the Canadian federal government placed the first restrictions limiting the use of chlordane to controlling wasps, cockroaches and wood destroying pests. By 1983 the federal government recognized that continued use of chlordane on food crops would adversely affect international trade and proposed revisions to the acceptable uses of chlordane (Agricultural Canada, Oct. 1983). Further restrictions in 1985 limited its use to the control of subterranean termites. In 1990 Ontario restricted use to exterior soil perimeter use in structural control of subterranean termites and by 1994, chlordane was banned for use in Ontario (Cutten, 1995).

Chemical Structure and Properties

The empirical formula of chlordane is C10H6CL8, with a molecular weight of 409.80. Technical chlordane can contain varying amounts of heptachlor, nonachlor, hexachloro-cyclopentadiene and other compounds The chemical is an amber-coloured liquid that is soluble in most organic solvents, including petroleum oils, and is virtually insoluble in water (Smith, 1991). Persistence in soil occurs for years after application. In one study, 15% chlordane, applied over a three year period, was detectable 15 years after the time of application. Organic matter and moisture content of the soil can also affect the volatilization or persistence of chlordane. Increased organic matter causes greater adsorption while increased moisture causes greater volitization. Water levels tend to be low as chlordane generally accumulates in the sediment (WHO, 1984).

Metabolism and Storage

Chlordane is absorbed into the body by dermal contact, inhalation and through gastrointestinal absorption following ingestion of this compound. Chlordane is highly lipophilic and can be detected almost immediately following ingestion in adipose tissue, kidney, liver, blood and muscle tissue. The mean ratio of chlordane in fat and blood is approximately 136 (Smith, 1991). Chlordane is metabolized in the body by the liver to oxychlordane and other metabolites such as trans-nonachlor 1,2-dichloro chlordene, and chlordene chlorohydrin. While most of these metabolites are less toxic then the parent compound, oxychlordane appears to be much more toxic. The dermal route of exposure to this substance shows an extremely rapid rate of absorption and consequent toxic effects. In one occupational exposure accident, where a worker spilled a solution of chlordane and DDT on her thighs and abdominal area, death occurred within an hour of exposure despite attempts to wash the solution off (Smith, 1991). In the United States 13 people showed gastrointestinal and/or neurological signs after drinking water contaminated with chlordane in concentrations of up to 1.2 g/litre. Signs of toxicity to chlordane include ataxia, vomiting, convulsions. However, in most cases, recovery is complete after treatment. In rats oxychlordane has an LD50 of 19.1 mg/kg, while in humans the acute toxicity is 25-50 mg/kg (Smith, 1991).

For most Canadians the major route of exposure to chlordane appears to be chemical residues in food, while the dermal route of exposure appears to be the most significant in occupationally exposed individuals (WHO, 1984). Human body burdens have been

documented for chlordane in Ontario citizens and around the world (Frank, 1988; Durham, 1965). To date this chemical has not been investigated in relationship to breast cancer. However, chlordane has been shown to produce estrogenic effects in laboratory studies (Soto et al, 1994). This in combination with the detection of these compounds in human tissue samples suggests that the exposure history of this organochlorine should be investigated when trying to elucidate the relationship between organochlorines and breast cancer.

1.5.5 Lindane and Benzene Hexachloride:

History

Benzene hexachloride (BHC) was first used in World War 1 as a smoke bomb and by the early 1940's the insecticidal properties of the gamma isomer of this organochlorine were discovered. (Smith, 1991) There are several isomers of BHC of which the gamma isomer, lindane, has been used most extensively in Canada from approximately 1945 to 1983 (Cutten, 1995). The chemical was used predominantly for control of soil dwelling insects and had public health applications for the control of biting insects transmitting malaria and other vector borne diseases, and head lice. Preparations for use in humans included scabicidal shampoos, lotions and creams (Smith, 1991). Direct animal applications included topical sprays to control biting insects and ectoparasites and lindane, in the form of vaporizers, was commonly employed for in barn use. Agricultural applications on a variety of crops minimized crop damage and subsequent storage losses from such pests as aphids and slugs. Lindane and BHC have also been used in combination with fertilizers

and wood preservatives. In the United States agricultural applications accounted for 80% of use, while veterinary applications in treatment of animals accounted for 5%, forestry use accounted for 10% and other uses comprised 5% of total use of this chemical. (Beigel, 1988).

Occasionally the use of BHC and lindane on crops resulted in noticeable foul flavour which became apparent at the time of cooking or shortly thereafter, and this prevented the use of these chemicals in specific food crops. However, the discovery that overheating of lindane in chemical vaporizers resulted in the production of hydrochloric acid and produced significant irritation, resulted in restrictions as early as 1951 (Smith, 1991). The detection of these chemicals in the environment and as residues in animal and human tissue samples resulted in further restrictions in several countries. Despite agricultural restrictions lindane continues to be used as a scabicide for human cases of sarcoptes scabii and head lice, where the compound is usually used in 1% shampoo, cream, ointment of solution (Smith, 1991). It is considered to be highly effective and preferential to other compounds for use as a scabicide except in infants, pregnant women and people with severely excoriated skin.

Chemical Structure and Properties

BHC is an off-white to brownish powder and has an empirical formula of C6H6Cl6 with a molecular weight of 290.85 (Smith, 1991). The chemical is insoluble in water, slightly soluble in fats and oils and moderately soluble in benzene, chlorinated hydrocarbon

solvents and acetone. It has a low melting point and relatively high vapour pressure (Smith, 1991) The compound is extremely stable to light, air, heat and acids. However, in alkalis the chemical undergoes dechlorination. There are four isomers of BHC, alpha, beta, gamma and sigma and they are all relatively persistent in the environment (WHO, 1991).

Metabolism and Storage

BHC is absorbed from the gastrointestinal tract, lungs and the skin, however the different isomers are absorbed at different rates, with an average rate of absorption of 94.9%. Beta BHC is stored at a much greater rate and for longer duration than the other isomers, while the alpha isomer is stored at higher levels in the brain (Smith 1991). All the isomers are preferentially stored in fat and the alpha and beta isomers can be found in high concentrations in liver tissue. Total BHC has been detected in adipose samples from residents of several countries with relatively high levels in Japan, Argentina, France, Italy, and India. (Smith, 1991). In most cases the beta isomer predominates.

Studies in human subjects show that dermal exposure to lindane resulted in 9.3% absorption within five days of exposure, and that washing with soap and water shortly after application results in an increase in the rate of absorption (Smith, 1991). BHC can be transmitted to the fetus via the placenta and can be found in mothers milk in exposed individuals. The secretion of BHC in human milk corresponds to the rate of use of the compound in the country of residence of the mother (Smith, 1991). The beta isomer tends

to persist relatively long especially compared to the gamma isomer. When the latter is found it indicates a recent exposure to BCH, while the former tends to represent an accumulated source in the food chain. Lindane is rapidly metabolised in animals and consequently is unlikely to be found in meat or eggs unless the compound was applied directly on the animal or directly on their food (Smith, 1991).

Absorption of lindane induces liver production of mixed-function oxidase enzymes and the metabolized compounds are excreted in the faeces and urine or stored in fatty tissue. Exposure to phenobarbital can increase the rate of excretion in humans (Smith, 1991). Oral toxicity to lindane ranges from 190 mg/kg in the rat to 200 mg/kg in the rabbit (Smith, 1991). In humans death due to lindane has occurred infrequently. Most cases of poisoning results from children consuming pellets intended for use in vaporizers (Smith, 1991). Signs of toxicity include faintness, dizziness, collapse, convulsions and occasionally foaming at the mouth. In general, this chemical has not been associated with protracted illness. However, lindane has been shown to be positive in dietary hepatocarcinogenicity tests in mice, while not in rats (Smith , 1991). Given these findings and that the chemical can be found in human tissue samples we have considered the exposure to this organochlorine in conjunction with the other organochlorines when determining the relationship to breast cancer in humans.

1.5.6 Polychlorinated Biphenyls:

History:

Polychlorinated biphenols, or PCBs as they are more commonly known, include approximately two hundred and nine different combinations of chlorinated derivatives of biphenyl (WHO, 1993). PCBs have had widespread use since the 1930's, mostly for industrial purposes where they were used as insulation fluids in electrical transformers and capacitors, and as hydraulic fluids. They can also be found in carbonless copy paper, plastics, lubricants insulating tapes, fireproofing materials, inks, paint additives, and various pesticide preparations (Shibamato, 1993).

Vast amounts of PCBs were produced over the decades. In the United States it has been estimated that more than one billion pounds of PCBs were produce by 1977 (Shibamato, 1993). Approximately 450 million pounds of discarded PCBs are estimated to be present in the environment in the United States alone. In the early 1960's PCBs were discovered at relatively high concentrations as contaminants of many analytical samples taken for DDT analysis during that time period (Frank et al, 1988; Van Holdrinet et al, 1977). This lead to an increased determination to document PCB residues in numerous substances, and PCBs were subsequently detected in a wide range of materials including food, human tissue samples and the environment (WHO, 1993).

Chemical Structure and Properties:

PCBs are produced by the chlorination of biphenyl by anhydrous chloride under heated

reaction conditions in the presence of catalysts. Depending on the reaction conditions, various mixtures of different congeners can be obtained. The degree of chlorination varies from three monochlorinated isomers to fully chlorinated decachlorobiphenyl isomers (WHO, 1993). PCBs without ortho substitution are referred to as coplanar while the others are considered noncoplaner (Smith, 1991). In general, PCB's are extremely resistant to heat, acids, bases, water and electrical currents (Shibamato, 1993). These substances have low water soluabililty and therefore can be found as trace contaminants in organic matter, soil, sediments and fatty tissue. Isomers that have increased numbers of chlorine tend to be more lipid soluble and less water soluble, making them more persistent in the environment. These substances are consequently able to bioaccumulate in the food chain. The highest levels of PCB contamination have been found in fish from the Great Lakes (Shibamato, 1993). In the environment, the average half life ranges from 10 days to one and a half years depending on the level of chlorination (Smith, 1991).

Metabolism and Storage:

PCBs can be readily absorbed from the gastrointestinal tract with greater than 90% absorption rates possible after dietary exposure (Shibamato, 1993). Once absorbed into the body, they are extensively stored in adipose tissue. Intermediate levels can be found in the skin, adrenal glands, and blood. Biphenyls derivatives containing higher percentages of chlorine are metabolized and excreted less rapidly then fewer chlorinated biphenyls (Shibamato, 1993). PCBs can be excreted from the body through the faeces, urine and breast milk with the faecal route representing the major route of excretion.

PCB's can be transmitted to the fetus via the placenta and to the infant via contaminated mothers' milk (WHO, 1993).

In general, the PCBs have a low acute toxicity. LD50 levels range from 2 -10 g/kg. The coplanar PCB's are the most notorious of this group of organochlorines and appear to cause the bulk of adverse effects. In monkeys, acute toxic effects can be seen at 250-400 mg/kg. Levels as low as 5.0 ppm in rhesus monkeys have given rise to chloracne and edema. As well, changes in menstrual cycles and difficulties in maintaining pregnancy were observed (Shibamato, 1993). In humans exposure to contaminated rice oil in Japan and Taiwan lead to symptoms of conjunctivitis, hypersecretion of the meibomian glands, pigmentation of the nail and mucous membranes and occasionally fatigue, nausea and vomiting. Hyperpigmentation, and acne formation were also classic following exposures to PCB contaminated food. These symptoms could be detected in children born to up to seven years after maternal exposure with symptoms subsiding at approximately 12 years of age. Poor cognitive development was also found in these children (Smith, 1991). Similarly children born to women consuming high levels of fresh water fish and subsequently higher levels of PCB gave birth to children with poorer short term memory and increased irritability (Swain, 1991, and Rogan et al, 1987).

The main source of human exposure results from food products especially fresh water fish (Swain, 1991). It has been suggested that PCBs promote carcinogenesis by inducing the microsomal enzyme system. In rats, these substances have been shown to be carcinogenic.

IARC has classified these substances as probable human carcinogens (Group 2A) since there is evidence that they can cause cancer in animals and have adverse effects on the immune system. Some of the PCBs have been shown to have estrogenic effects while others have antiestrogenic effects. To date, PCBs have been investigated as a group with no effort to isolate or determine the type or potential estrogenicity. This in combination with the evidence that PCBs can be detected in virtually all human tissue samples suggests the need to determine human exposure to these substances both as a group and individually, in order to fully assess the relationship between organochlorine exposure and human breast cancer.

Figure 1.1: Organochlorine Exposure Pathway

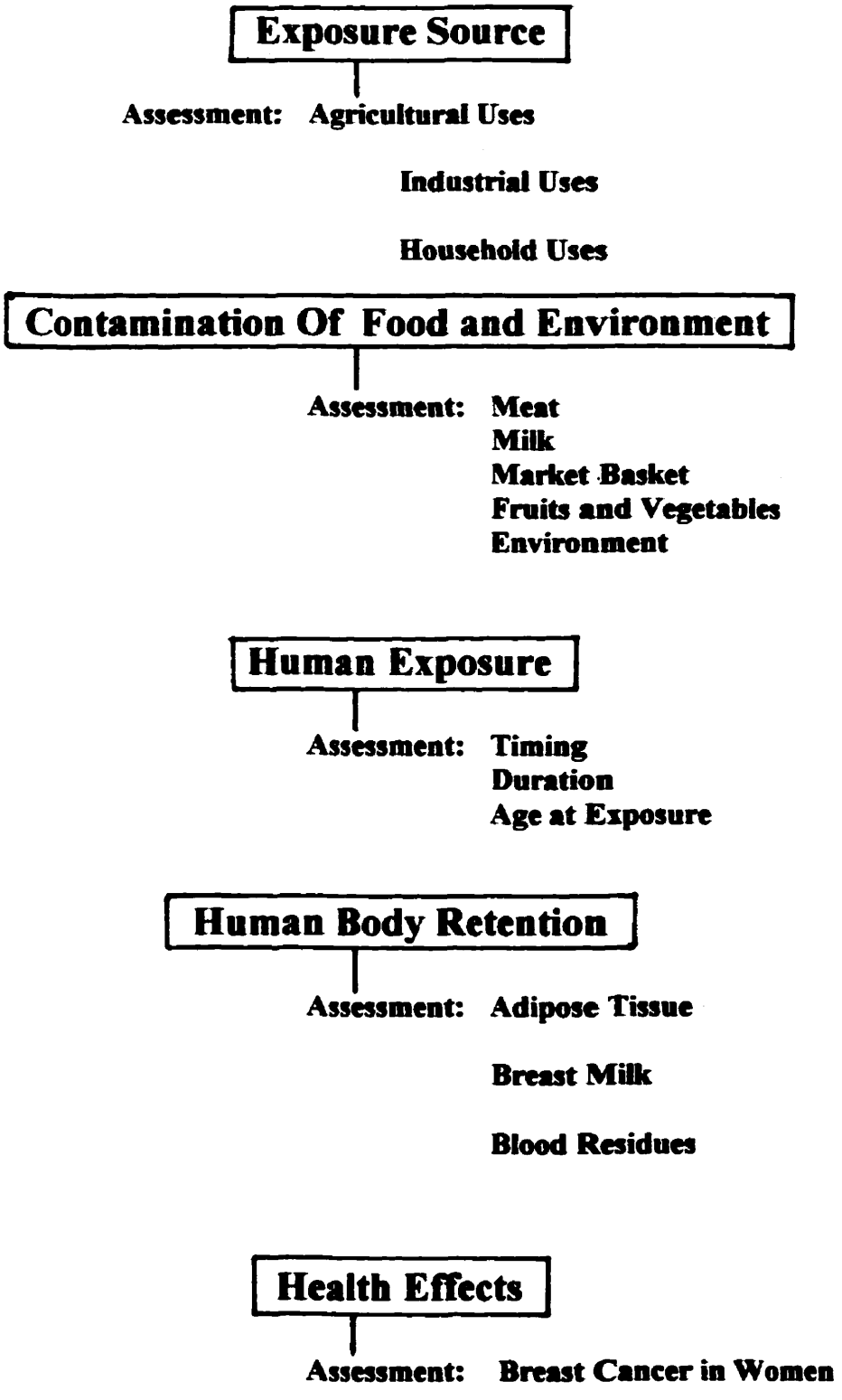

Table 1.4.4.1 Organochlorine Restriction and Banning Dates:

	First Used	Restrictions	Banning
DDT	1947	1970 Restricted agricultural uses in Ontario 1973 All agricultural uses prohibited 1986 Discontinuation of Canadian registration Existing stocks allowed to be used	1972 USA full suspension 1988 Ontario all uses
Chlordane	1940	1975 Restricted use to wasps, cockroaches And wood destroying insects Ontario 1985 Use restricted to sub terrain termites 1990 Ontario restricts use to exterior Sub terrain termite control	1994 Ontario
Dieldrin	1950	1976 Uses suspended in Canada except Sub terrain termite control 1990 Ontario restricted to exterior soil Termite control	1994 Ontario
Chlordecone	1962	1970 Restricted use but existing stocks used For bait in ant traps	1994 Ontario existing stock to other provinces

Table 1.5.1.1: Comparison of LD 50s of Several Organochlorines in Select Species:

	Humans	Rat	Rabbit	Birds
DDT	?	100 mg/kg	-	500mg/kg
Dieldrin	10 mg/kg	37 mg/kg	-	26.6-381mg/kg
Aldrin	83 mg/kg	-	33 mg/kg	6.6-520mg/kg
Chlordane	25-50mk/kg	335 mg/kg	20-300mg/kg	1200 mg/kg
HCB	?	-	-	-
Heptachlor	?	40 mg/kg	80-90 mg/kg	62 mg/kg
Mirex	?	600-3,000 mg/kg	800 mg/kg	1,400-10,000mg/kg
PCB's	-	2-10 g/kg	-	747->5,000 gm/kg
Toxaphene	-	-	365 mg/kg	-

1. Smith , 1991
2. WHO, 1989
3. WHO, 1989
4. WHO, 1984
5. WHO, 1984
6. Smith, 1991
7. WHO, 1984
8. Smith, 1991
9. Smith, 1991

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Chapter 2 Exposure Trends to Organochlorines in Ontario

2.1 Introduction

Exposure to organochlorines in Ontario began approximately in 1947. Despite extensive use of these chemicals, accurate documentation of application sites, quantities used, and subsequent residues in food and animal feeds have not been consistently recorded in Ontario. Sales and pesticide use data can be found in various forms documenting quantities used over the decades, while human body burdens have been documented only since the late 1960's. Limited information is available to ascertain the exposure levels in humans for the twenty-year period of organochlorine use prior to the first recordings of residues in tissue samples of Ontario citizens. A similar situation exists for food residue analysis. The lack of accurate and complete information of use, application sites, and residue levels in food products for the entire period of organochlorine use necessitates that we determine exposure by evaluating the best available information for each time period. Unfortunately, the results generated by this method could be confounded by other chlorinated organic substances such as dioxins and PCBs. However, the information despite these limitations can then be amalgamated to give a general indication of the trend in exposure to organochlorine pesticides in Ontario. The general trend in exposure can then be compared to trends in breast cancer to determine if a relationship exists between breast cancer and exposure to organochlorines at a population level.

From the period of 1947 to approximately 1968 the best available information for organochlorine exposure consists of pesticide sales data. While this information does not

directly imply human exposure, it does give an indication of the quantity of organochlorine used and can show the general trend of organochlorine use during this time period. From 1968 to present exposure information can be obtained from several areas including market basket studies, food residue analysis and human tissue samples. All of these sources of information can then be used to provide a composite picture of exposure to organochlorines for those individuals who were sampled, but, more pertinent to this thesis, for the general population. This information can also be used to predict which population groups received the greatest dose, longest duration and possibly predict which groups would be expected to be at risk of developing adverse health effects following exposure.

While human body burden testing provides the best available data for acute and chronic human exposure, the degree to which those samples are representative of populations is not usually clear. Individual differences in exposure can lead to marked differences in detected body burdens. For example, individuals occupationally exposed could be expected to have significantly higher body burdens than those not exposed occupationally. Human residue testing can however provide a good approximation of the general trends in exposure, but in general they do not provide detailed exposure histories or specific information regarding sources of exposure for the individual tested. Therefore precise information such as which foods contain the highest residues of organochlorines, and subsequently if consumed in high quantities would impart an increased level of exposure, needs to be determined by examining specific food residue levels. The latter can also

provide information for establishing trends in exposure to organochlorines from individual food sources and can be used to estimate trends in exposure for the general population as well as specific risk groups. Since changes in consumption patterns of specific food groups can affect the general populations' exposure to organochlorines residues, an evaluation of food consumption patterns in Ontario was also examined to determine how these factors influence the total exposure picture in Ontario.

The findings in this chapter have been combined to provide an overall general trend in exposure to organochlorines for the population for Ontario. For ease of analysis the entire time frame from 1947 to present has been divided into approximately 10 year periods namely 1947 - 1955, 1955 - 1965, 1965 - 1975, 1975 - 1985 and 1985 - 1995. During these periods several different phases of exposure were identified ranging from a period of no exposure to peak to minimal exposure. The residue and organochlorine use data were investigated separately to determine the average trend and then examined as a whole to determine the overall trend in exposure given all the available exposure information. These findings were further used to determine specific birth cohort groups that would have received exposure during various phases of human development at specific periods of organochlorine exposure. These findings were then used to examine possible relationships between organochlorines and breast cancer.

2.2 Organochlorine Sales Information

2.2.1 Introduction

Historical records of organochlorine pesticide sales can provide information on the approximate amount of organochlorines used during the years of chemical production. This information can then be related to the amount of chemicals accessible to agriculture and the environment, and subsequently available to humans through the food chain. Sales information can be particularly useful to provide a historical perspective of organochlorine exposure when more accurate data such as human body burden information are lacking. While sales records can show the total volumes of organochlorines produced per year, it should be recognized that these records only provide an estimate of the actual amount used each year. Since the amount produced yearly may not be consumed during the year of production, a small over-estimation of the organochlorines used each year arises when sales records are used. This finding can be supported by the fact that stocks of organochlorine pesticides existed for several years after production ceased (Cutten, 1995). Despite this potential for over-estimation of use on a yearly basis, sales data can give a good indication of the overall trend of organochlorine use and can therefore be used as a surrogate of organochlorine exposure. However, sales and use, of non-pesticidal compound such as PCBs and dioxin may not have occurred in the same proportion to sales of organochlorine pesticides and in this regard may confound the findings of organochlorine sales data. Despite this shortcoming, existing sales data for Canada and Ontario have been examined to give an indication of organochlorine exposure in the early stages of use.

The earliest year on record for organochlorine sales in Canada was in 1947. At this point the Dominion Bureau of Statistics began recording sales of organochlorine pesticides produced and used in Canada. The Bureau compiled survey results from all Canadian and United States firms which were registered with the Department of Agriculture to sell pest control products. Surveys were carried out on a yearly basis from 1947 to 1973. However, specific information regarding provincial use patterns were not recorded by the Bureau. It seems reasonable to assume however, that the Canadian trends in sales are representative for Ontario. Given that the province has historically been a highly agricultural and industrial province, Canadian trends should provide a good approximation of provincial trends.

Relatively good consistency exists between the recording style and survey methods between 1947 and 1965, with survey results representing approximately 95% of the total sales of pest control products (Dominion Bureau of Statistics, 1947-1965). However, after 1965, the data become problematic, in that the sales categories change, making comparisons difficult. Shortly thereafter, in 1967, organochlorine production levels became confidential for specific organochlorines, creating an artificial decrease in the overall reported production levels. As such, published production levels post 1965 do not provide an accurate description of organochlorine sales. This finding correlates directly with increasing levels of controversy regarding the use of organochlorine pesticides such as DDT and the subsequent restrictions on agricultural applications. Volder and Li, 1995, in their attempt to record global usage of selected persistent organochlorines, faced

similar problems with obtaining accurate records and faced confidentiality blocks in many of the countries under investigation. Despite these shortcomings, the historical information that is accessible provides the best information attainable from 1947 - 1965 documenting organochlorine exposure in Ontario. This information can then be used to estimate trends in organochlorine use for Ontario during this time period.

Very limited data exist for organochlorine use in Ontario prior to 1973. From 1973 to the present, the Ontario Pesticide Use Survey provides some indication of the amount of organochlorine used. However, by this time the number of organochlorines still utilized in agricultural practice had decreased dramatically. In particular, the persistent organochlorines were virtually eliminated from agricultural use in Ontario. Given that better exposure estimates can be derived from other sources for the 1970s and beyond, sales data have been used only for the period from 1947 to the late 1960s.

2.2.2 Results

The Dominion Bureau of Statistics recorded pesticide sales under the headings of Agriculture, Household and Industrial Insecticides, Disinfectants, Rodenticides and Exports. The agricultural records were subdivided into two sections to document sale of: a) crop and seed treatment; and b) livestock treatments. From an organochlorine perspective the two categories Agriculture, and Household and Industrial Insecticides are the most relevant. Production levels in these areas were examined to determine the trends in organochlorine sales from 1947 - 1965. Unfortunately, in the livestock treatments

section the information is not broken down into chemical components, thus preventing recording of organochlorine sales for this specific section. Since organochlorines such as DDT, lindane and others were definitely applied to livestock, for controlling biting insects, the lack of this information is troublesome.

Figure 2.2.2.1 shows the quantity of total organochlorines sold under the category of Agriculture - crop and seed treatments. From 1947 to 1959 both liquid (imperial gallons) and solids or powder (pounds) forms of the chemicals were recorded. However, by 1960 values were given in pounds only. Examination of the total organochlorine sales in pounds from 1947 - 1965 show an increasing amount of organochlorine sales from 1.39 million pounds in 1947 to 4.33 million pounds in 1950. Sales dropped slightly during 1951/52 and then increased beginning 1953 to a peak sales level in 1954/55 of approximately 4.77 million pounds. Sales levels declined in 1956 and remained relatively constant until 1960 when sales dropped to 1.91 million pounds. In 1961 there was an increased level of sales at 3.39 million pounds. Sales levels declined from this latter level to 1.93 million pounds in 1965.

Examination of liquid (imperial gallons) production peaked at 3.8 million imperial gallons in 1948, and then stayed at approximately 0.06 million imperial gallons in 1949-1959. Examination of the overall agriculture sales data would suggest that sales levels increased from the initial recording in 1947 to a peak level in 1954/55. Levels subsequently decreased to a low in 1965. Production levels after this time period are not available.

Figure 2.2.2.2 shows the production levels of organochlorine chemicals used for household and industrial applications from 1947-1965. The earliest production levels on record show that approximately 84 thousand pounds of organochlorines were sold during 1947. These levels remained relatively constant, fluctuating only several thousand pounds over the years until 1960 when sales of household and industrial insecticides appear to increase rapidly to 310 thousand pounds. A rapid and steady increase in production for household and industrial pesticides occurred from this time to 1964. Data after 1964 become less reliable as reported sales levels became confidential.

Sales levels of fluid (imperial gallons) organochlorines (Figure 2.2.2.2) remained relatively constant over the years at approximately 100 thousand imperial gallons. Overall, the total production of organochlorines for household and industrial applications appeared to remain stable until 1960 when was an apparent dramatic increase in the sales of organochlorines to a peak level in 1963 of 576 804 pounds and 75397 imperial gallons of total organochlorines use for household and industrial insecticides. From this information one can determine an approximate trend in exposure to organochlorines (Figure 2.2.2.3) which can be combined with other sources of information to map out an approximate general exposure scenario for Ontario.

2.2.3 Limitations of the Data

The Canadian sales data are extremely useful for determining the trends in sales of organochlorines but do not provide details on the destination or intended use of these

chemicals beyond the general classification of agriculture or household and industrial insecticides. Despite recognizing the significance of detailed use and application site information for each of the organochlorines, prior to 1973, recording systems documenting the particulars such as application rates and sites simply did not exist. Consequently, the exact levels of organochlorines produced or imported into Canada, and used in Ontario, cannot be determined from the data given. The lack of this specific information creates a situation where assumptions and predictions must be made in order to determine organochlorine use and exposure trends.

There are several assumptions that can reasonably be made when inferring from the trends in sales in Canada to production trends in Ontario. The first assumption is that exposure to organochlorines did not occur prior to 1947 in Ontario. Given that sales of organochlorines prior to 1947 did not occur in Canada exposure to these chemicals would have been virtually nonexistent. Some limited exposure may have resulted from early use of the organochlorines in the United States; however, given that most of the early use was for military purposes in that country, the exposure levels in Ontario would have been virtually null.

Secondly, sales/production levels in Canada would roughly approximate the quantities used in Canada. While this assumption may slightly overestimate the actual yearly quantities used, given that some storage of chemicals post purchasing probably occurred, the actual trends in sales and subsequent use would likely be closely related. It would

seem logical that overall sales trends for Canada would be similar to sales/use trends in Ontario. Assuming this relationship holds true, peak agricultural organochlorine use appears to have occured, based on sales data, during 1954/1955, with levels declining after this point. Similarly, given the large industrial base in Ontario and significant population size of Ontario, one may assume that the trends in household and industrial insecticide use for Canada may equally represent the trends of use in Ontario. The exact levels of sales may not hold true for each of the provinces but it is likely that the overall trends would be similar. In this case peak use of household and industrial organochlorine use appears to have been during the early 1960s.

2.2.3 Conclusions

From the above analysis it can be seen that a combination of sales data, exact production levels and specific records on application sites and rates are essential for a complete picture of organochlorine production and use. Under this ideal scenario one could accurately assess general trends in human exposure to organochlorines based on chemical production and use data. However, given the reality of incomplete record keeping on the sale and use of organochlorines the currently available information can only give a rough approximation of the trends in exposure to these chemicals. Despite problems with the accuracy of this information, the data provide a reasonably good picture of trends in aggregate use of organochlorines, which can be used as a surrogate for human population exposure during the early stages of organochlorine use in Ontario. This information, in combination with other exposure information can be used to determine if similarities exist

between trends in production/sales, exposure, and human body burdens. The information can then give an indication of the best available predictions of exposure and the changes over the decades since initial production of organochlorines.

From the sales data investigation, one can determine that significant amounts of organochlorines were used during the period from 1947 to 1965. Overall, there appears to have been an increase in the use from 1947-1955 with a peak in sales by 1955-1960. These levels then began to decline from 1960 to 1965. After 1965 the results from this study become unreliable. However, given that this stage of use marked the period of increased controversy regarding the safety of organochlorine use and that many of the initial restrictions and bans began to be set in place during the late 1960's it should be fair to assume that organochlorine production and sales would at least have begun declining post 1965.

Figure 2.2.2.3 shows the general trend in sales of organochlorines based on the information attained in this section. The graph has been divided into six stages of approximately ten year periods to provide a consistent format for comparing the other available exposure information. Exposure has been roughly classified as zero, low, medium, and high. From this graph we can see that, prior to 1947, there was no use of organochlorines in Ontario. During the period from 1947 to 1955 there was an increasing level of sales classified as medium to high exposure and from 1955 to 1965 there was a high to decreasing level of organochlorine use, classified as high to medium. After this

period, the level of organochlorine use can be predicted to decrease to virtual no use by the late 1980s. This portion of the graph gives an indication of the overall general trend in exposure to organochlorines from the period of 1947 to 1965.

2.3 Food Residues

2.3.1 Market Basket Studies

Introduction

Market basket studies have been used since the late 1960s to determine the amount and frequency of chemical contamination in food. A market basket study examines the residue levels in food samples purchased from various sources, such as grocery stores, that have been prepared in a similar fashion to that consumed in the home. The advantage of this type of assessment is that it provides residue analysis on a total diet basis rather than specific food components. As well, this type of study includes food products imported into the region, thereby encompassing residues from food produced both locally and imported. The disadvantage of market basket studies is that the residue calculations are based on average consumption patterns. Therefore, differences in food consumption patterns and preferences cannot be addressed.

In Canada, market basket studies have been used to record the level of human exposure to organochlorine residues in food across the country. Survey results exist from 1969-1988 (Smith 1969, Smith et al, 1972, 1973, 1975, McLeod et al, 1980 and Conacher 1989). Market basket studies were not carried out prior to 1969. The Canadian surveys

involved residue testing of food samples incorporating various food groups purchased from several cities across Canada. While sampling locations were not consistent, there are several years where samples were taken from cities in Ontario specifically. The results from these surveys have been reevaluated in order to assess the exposure to the total amount and type of organochlorine residues found in food products in Ontario specifically.

Table 2.3.1.1 summarises the Canadian market basket studies indicating the year, location, seasonal component and the number of samples analysed. In general, samples were purchased from supermarkets and grouped into the following composite samples; milk and dairy products; meat, fish and poultry; cereals; potatoes; leafy vegetables; legumes; root vegetables; garden fruits; fruits; oils and fats; sugars and adjuncts; and finally drinks. These composite samples were then submitted for analysis and the chemical exposure per person per day was calculated based on these findings.

In the 1969-1973 studies, 93 different foods made up the composite samples. Exposure calculations were based on residue levels found in these composite samples times the average consumption per person per day. The latter were determined by using the Dominion Bureau of Statistics annual tables of the disappearance of food in Canada, 1965-66. Since these values are based on average calculations, exposure for specific age groups or dietary habits cannot be determined. In 1976-1978, 120 different foods were sampled, comprising 11 composite samples. The composite group "drinks" were

cancelled during this study and average consumption was calculated on the basis of eating habits for a cross-section of age groups from five cities in Canada. Consumption averages were determined from the Nutrition Canada survey in 1973 (McLeod et al., 1980). Averages for various age groups and sexes were also investigated, which provided greater exposure details. However, total exposure was still calculated as an average exposure per person per day. Exposures for children or specific ethnic groups were not included. By 1985 the market basket studies were completely overhauled to include a larger number of composite samples in order to more accurately represent the consumption patterns of Canadians.

Several changes in analytical techniques occurred over the years of testing which could affect the ability to accurately compare the residue results between years. The earlier studies employed less sophisticated techniques for determining residues in food and can be considered to be less accurate at distinguishing chemical components such as DDT from PCBs. The latter studies relied on extremely sensitive methods of analysis which made detection of extremely low levels of contamination possible, and allowed for accurate distinctions between chemicals (Conacher et al, 1989). By the 1976-78 surveys, techniques had improved to the point of being able to determine more than 98 different pesticides. Table 2.3.1.2 outlines the specific organochlorine residues detected in the individual Canadian surveys. In general, organochlorines were found on a regular basis in all of the market basket studies.

Market Basket Survey Results

Results reported in these market basket studies show that DDT and metabolites, dieldrin, heptachlor, BCH, endosulfan, kelthane, aldrin and endrin could be found in most food composites. As analytical methods improved, PCB's, HCB, toxaphene and chlordane were also detected in food samples. For the purposes of this investigation, organochlorine residues were considered as a total group and the changing trends in exposure over the years were determined. Figure 2.3.1.1 shows the trends in total organochlorine residues detected over the years in the Canadian market basket studies conducted from 1969-1985.

In general, there was a decrease in the overall exposure to total organochlorines during 1969 -1988. The first record of exposure in 1969 indicated that humans, on average, were exposed to 0.025 mg/person/day. This daily exposure translates to 9.125 mg/person/year. In 1970 the average daily exposure to total organochlorines declined to 0.016 mg/person/day or 5.84 mg/person/year. The levels rose slightly in 1971 to 0.018 mg/person/day (6.57 mg/person/year) and decreased to 0.013 mg/person/day (4.7 mg/person/year) in 1973. By 1976-78, organochlorine residues in food had decreased to 0.0064 mg/person/day or 2.3 mg/person/year, approximately less than one quarter of the original values found in 1969. The variation in values observed during these years may be in part due to the change in sampling location, since the samples taken over these years were from different regions of Canada, or due to the changes in analytical technique. Figure 2.3.1.2 shows the level of DDT type organochlorines residues verses dieldrin type or others. It can be seen from this graph that in the initial years of market basket studies

greater levels of DDT type residues were detected than dieldrin or other organochlorines. During the latter years of analysis these levels had declined and were also detected at lower levels than the "other" organochlorine. This decline in food residues can be directly attributable to the severe restriction placed on the agricultural use of many of the organochlorine pesticides during this time period.

In the first series of market basket studies from 1969-1973 average food consumption was based on the disappearance of foods in Canada (Smith, 1969). Using these consumption patterns, an average amount of exposure to organochlorine residues in specific food groups was calculated. These studies show that the major source of exposure to organochlorines in Ontario resulted from the consumption of cereals, meats, dairy, garden fruits, leafy vegetables, and root crops while little to no residues were found in legumes. Beginning in 1976, the average diet was calculated using the nutrition Canada survey of 1973, which was based on eating habits of a cross-section of different age groups in five Canadian cities (McLeod et al, 1980). These latter studies also showed the previously mentioned food groups as a major source of exposure to organochlorines.

Limitations of Data

The market basket residue data have some weaknesses which limit our ability to use them as the sole basis for estimating human exposure to organochlorine residues. Firstly, the variability in location for purchasing food products can by itself account for some of the changing trends in exposure to organochlorines residues. While it would have been ideal

to have sampled food products from the same location over the total time span under study, thereby improving the consistency of the data, it is difficult to assess just how much impact variation in sampling location would have on the trends in exposure. If one examines the levels reported for cities in Ontario it would appear that these levels were somewhat higher than those reported for other cities in Canada. This may in fact be due to an increased agricultural use of organochlorine in Ontario compared to other locations.

The number of samples taken can be considered to be problematic in that too few samples were analysed given the low levels of residues to be detected. The accuracy of the residue results are further compromised by the fact that food samples were analysed in composite samples. This could have a significant dilutional effect on the actual residue present in the individual sample. Mean residue values were used to report the market basket studies findings, without giving an indication of the data distribution, which may be inappropriate if the data were log-normally distributed.

Changing or improved technology would also have a direct impact on the total number of organochlorine residues detected in the market basket analysis. While methods for residue analysis remained constant from 1969- 1973, improvements in residue detection limits and increased capabilities in detecting greater numbers of chemical contaminants in food samples arose in 1976. The significance of this change in technology is that the years after 1976 would have a comparatively higher recovery rate of total organochlorines than before 1976. Therefore, the values observed from the earlier years are probably an

underestimation of the actual level, given that there would have been residues of some organochlorines that would not have been tested for in the earlier studies.. The impact on the observed trend is that the slope (the decrease in residues levels) would probably be greater had a similar technology been available for the entire time frame. However, the general conclusion that a decrease in human exposure to organochlorine occurred since 1969 remains true, despite limitations in the determination of exact residues levels.

While the residue data generated by these market basket studies can give some indication of the average exposure for an average individual during the year of study, the market basket analyses do have several limitations when describing exposure levels for select groups in the population. Because data are generated on the basis of average consumption and exposure is calculated based on a per person per day level the figures given do not provide accurate information on exposure levels for differing age groups (such as children and young adolescents) or varying food preferences. It would be valuable to make approximations for these specific groups since exposure during this stage of development could be extremely significant when addressing the possibility of chronic human health effects. Estimates of exposure for children could be made by determining average or specific intakes of food categories and then multiplying these values by the residue levels found in the various food composites. Changes in societal food consumption patterns such as decreases in the amount of meat consumption could also be incorporated in order to give best estimates of exposure for specific risk groups.

Conclusions

The market basket studies undertaken in Ontario and Canada confirm that organochlorines were historically present in a wide variety of foods consumed by residents of Ontario. This is particularly true for the early stages of analysis. By the mid 1980s, these levels had declined dramatically to virtually nondetectable levels. Despite several limitations regarding sampling and residue analysis of food substances market basket analysis can provide a good indication of the source and type of organochlorine that individuals were exposed to during the history of organochlorine use.

Having estimated exposure during 1947 - 1965 from the pesticide sales figures, exposure estimates form 1965- 1995 were based on market basket studies and corroborated with information from other sources, (Figure 2.2.2.3). From the market basket studies, we can determine that the general trend during period four (1965 - 1975) was high to declining level of organochlorine residues in food products in Ontario. For the period five (1975 - 1985) there was a declining level of exposure to organochlorine in Ontario and during period six (1985 - 1995) there were very minimal level of organochlorine exposure via food in Ontario. These general trends based on market basket studies were corroborated and clarified by examining residue trends for individual food commodities.

2.3.2 Milk Residues

Introduction

Organochlorine residues were first reported to occur in bovine milk by Clifford (1957) in

the United States. Since these first accounts there have been a succession of publications documenting organochlorine residue levels in milk and dairy products in numerous countries. (Bro-Rasmussen et al. 1968, and Duggan, 1967). The first Canadian reports were published in 1970 based on milk survey programs initiated in 1967 by the Ontario Department of Agriculture and Food (Frank et al, 1970).

The source of organochlorine residues in dairy products has generally been assumed to arise from direct exposure of animals. Direct application of lindane and other organochlorines to the hair coat of dairy cattle (for control of biting insects) served as a significant dermal exposure route. Exposure through ingestion of contaminated feed, direct contact from vaporizers, or premise spray used in the barn and milk houses also provided pathways for chemical contamination of bovine milk (Frank et al., 1970). Since most organochlorines are highly lipophilic, these substances bind to milk fat molecules and are thus transferred from the animal's body to the milk excreted by the mammary glands. Human exposure subsequently arises as a direct consequence of consumption of contaminated milk, in the form of fluid milk or other processed dairy products such as butter, cheese, and ice cream. High fat products in particular, can be expected to result in greater levels of organochlorine residues on a similar volume basis and therefore result in greater human body burdens when consumed in large quantities.

Throughout the last several decades, milk and dairy product consumption in Canada has remained relatively high and therefore this food source represents a significant route of

human exposure to organochlorine residues. Young children in particular consume relatively large amounts of dairy products in proportion to their body weight. As such, milk and dairy products represent a high risk food when investigating organochlorine exposure for young children (National Research Council, 1993). Women also typically consume high levels of milk and dairy products during pregnancy and lactation which would result in higher body burdens in both the mother and fetus (NRC, 1993). Exposure during these early stages of development establishes body burdens early on and provides an increased total life of time exposure. Therefore the potential to develop adverse health consequences during a time when these individuals are in a growth phase and possibly at higher risk of cancer promotion, would be theoretically possible (NRC, 1993).

High consumption levels of milk and dairy products in Ontario prompted extensive research by the Ontario Department of Agriculture and Food. The first studies found that nearly all milk samples contained DDT as well as its metabolites and dieldrin, with several samples above administrative tolerances. Lindane and heptachlor epoxide were also isolated. These findings led to the subsequent ban on the sale of dieldrin and aldrin in 1969 by the Department of Health of Ontario under the Pesticide Act, 1969, and marked the beginning of a series of publications recording the organochlorine burden in Ontario bovine milk samples from 1967 to 1986 (Frank et al., 1970, 1975, 1979, 1985, 1989). The findings from these publications will be reevaluated in this section to determine the overall trends in human exposure from bovine milk during this time period.

Human Exposure to Organochlorines via Dairy Products

Trends in human exposure to organochlorine residues in dairy products can be determined by evaluating the trends in organochlorine residues in milk. While specific information on organochlorine residues in dairy products such as butter, ice cream, cheese is not available, general trends in bovine milk residues, though lower in absolute terms than in high fat products, should be relatively indicative of the trends in residues found in all dairy products. In Ontario, residue data for bovine milk exist from 1967 until 1986 (Frank et al, 1970, 1975, 1979, 1984, 1989). These values can be used to determine the relative amount of human exposure resulting from consumption of dairy products for this time period. While information on geographical exposure exists and would have been ideal for an in-depth analysis on a regional basis, the raw data from the Ontario Ministry of Agriculture and Food and Rural Affairs was unavailable for use (personal communication Ripley, 1995). As a result, the only information available to document organochlorine exposure through bovine milk and dairy products are the published results from these studies. While this information is limited, it does provide the best available estimates of the total residue levels in bovine milk during this time period and can be used to determine the general trends in exposure from dairy products in Ontario.

Residue analysis in milk did not occur to any significant extent prior to 1967, and consequently, only inferences about organochlorine exposure, based on pesticide use, can be made from 1947 to 1967. Since organochlorine use in agriculture (topical sprays, applications to crops intended for animal consumption and premise sprays) constitutes a

major source of bovine exposure, one could assume that periods of high organochlorine use would result in relatively high milk residues and low levels of organochlorine use would result in minimal exposure in dairy cattle. Given organochlorine sales data (Dominion Bureau of Statistics, 1947-1966) it is likely that exposure to organochlorines in dairy products increased from minimal levels in 1947 to peak levels by approximately 1955. Exposure to organochlorines could then be expected to have declined from 1955 to 1966 and would be at least similar or greater than found in 1967. While the exact values are unknown, we can assume that significant exposure to organochlorines from dairy products did occur during this time period.

By 1967 residue testing was initiated for Ontario bovine milk samples. A total of 1651 samples were collected from across the province from November 1967 to June 1969 (Frank et al, 1970). The samples were taken from bulk tankers which on average would represent 16 producers' milk. Sampling was purposive with the goal of sampling to include milk samples from all Ontario milk producers. Analysis was carried out on a regional basis subdividing the province into southern, western, central, northern and eastern regions. DDT and metabolites, dieldrin, lindane, and heptachlor epoxide could be found in the samples tested. In 1970 - 71 and 1973 residue testing on 337 and 350 bulk tanker samples respectively were performed in the southern region only to assess the effects of legislative restrictions of organochlorine use in this region (Frank et al, 1975). In 1977, 308 bulk tank samples were collected from 11 southern Ontario counties (Frank et al, 1979). By 1983 sampling was again extended to the other regions of the province

with 359 bulk tankers representing 16 districts in Ontario (Frank et al, 1985).

In 1985 to 1986, 1184 bulk tankers were sampled from all the regions in Ontario, however, by this time, only PCBs and DDE residue levels were determined (Frank and Braun, 1989). Results for residue testing of bovine milk in Ontario were not reported after 1986 and consequently estimates of residue levels after 1985 will have to be made. Because of inconsistencies in sampling sites for each study, only the southern region of the province reported residue values for all the years mentioned. However, there were several sequential years where sampling was undertaken in multiple regions. Given that there were only slightly higher levels in the early studies for the southern regions, one could assume that trends in exposure for the southern regions samples would be relatively representative for the entire province during the years of testing, despite perhaps being a slight over estimation of the actual level of exposure.

Figure 2.3.2.1 shows the trends in exposure to total organochlorine residues in bovine milk over the time period 1967-1986. Exposure in 1967 was relatively high, at 0.244 mg/kg on a milk fat basis. Levels remained approximately constant during 1968-1969 and 1970-1971. By 1973 levels began to drop to 0.182 mg/kg on a milk fat basis. Total organochlorine levels began a steady decline to 0.069 mg/kg in 1977, 0.0574 mg/kg in 1983 and 0.036 mg/kg in 1985-86. Frank et al, (1985), argued that the decrease in organochlorine levels in bovine milk was a direct result of the agricultural restrictions on chemical use put in place in the late 1960's early 1970's (Frank 1985). This would seem

reasonable given the higher levels detected during the earlier years of testing when organochlorines were used extensively.

The type and proportion of organochlorines that make up the total organochlorine burden in bovine milk have changed over time. Figure 2.3.2.2 shows the temporal changes in DDT and metabolites in bovine milk samples, for southern Ontario since 1967. The total level of DDT and metabolite has decreased from 0.193 mg/kg on a milk fat basis in 1967 to 0.0136 mg/kg in 1983 (Frank et al, 1985). The level of DDE in bovine milk samples has also declined dramatically from 0.096 mg/kg in 1970-71 to 0.016 mg/kg in 1985-86. Dieldrin residues in milk have declined from 0.043 mg/kg in 1969 to 0.011 mg/kg in 1979 and finally to 0.0069 mg/kg in 1985.

Discussion of Data Quality

There are several factors that pertain to the quality of the residue findings in this section. These factors can all potentially influence the exact level of organochlorine residues available for human exposure and as such will be discussed in order to identify the impact on the total exposure from this food source. All the samples were composite bulk tank milk samples, derived from multiple producers, thus representing a large volume of milk. As a result, there is a dilution effect which may impart an underestimation of actual residue levels from individual producers. Since milk can be used as fluid milk or for dairy products, and milk or milk products may be consumed locally or by individual farm families there may be slight differences in the subsequent exposure levels for these select

individuals. However, in general, the dilution effect would be consistent for the population as a whole and would not dramatically alter the overall trends in organochlorine exposure on a population basis.

Sample numbers for the studies were generally greater than 280 bulk tank samples and in some cases greater than 1000 bulk tank samples. A lack in consistency in the number of producers each bulk tank sample contained may affect the results in that large composites may effectively be diluted to a greater extent than smaller composites. In general the sampling numbers are small given that very large numbers of samples must be taken to detect infrequent cases of above threshold levels for organochlorine residues in milk. Sampling location in these studies varied tremendously and was not consistent throughout the time period. Based on initial positive results of higher levels of organochlorine in the southern region of the province, subsequent studies tended to focus on this region. While information is lacking for the entire province in several of the surveys throughout this time frame, the data from the southern region tends to represent an upper boundary for exposure for the entire province. This is assuming that the southern region of the province always had a higher residue burden than the other regions in the province. The extent of over estimation of the individual residues can only be speculated and may not actually be very significant on an overall provincial basis.

While these factors can influence the exact level of exposure, the impact on the general trends in exposure should be minimal. One would expect the trends in the southern region

to be similar to trends in the rest of the province. If one examines the surveys where there are both values for Ontario and the southern region the levels found are quite similar and the trends appear approximately the same. This suggests that the southern region's results can be a good surrogate for the entire province when determining the trends in exposure to organochlorines residues in bovine milk.

Conclusions

Direct exposure of animals to topical sprays and premise sprays or indirect exposure through contaminated feeds provides a source of animal exposure to organochlorines. Once the animal is exposed, these substances are excreted in the milk dissolved in fat globules. These contaminated milk products also provide an important source of organochlorine residues when made into other dairy products such as butter, cheese and ice cream. The findings of this section show that bovine milk and, in turn, dairy products represented an important source of human exposure to organochlorine pesticides over the decades under investigation. While the levels prior to 1969 have not been documented, they can be expected to be at least as high as those found in 1969. From 1969 to 1986 the levels of organochlorine residues in milk declined dramatically with several changes in the overall composition of the organochlorine residues. Levels after 1986 have not been recorded but can be expected to be minimal given the fact that many organochlorines were completely restricted for agricultural use in Ontario. During the first three periods (1947 - 1965) there was no information available. Therefore, we must rely on the information generated by the organochlorine sales data. By the fourth period levels of

organochlorine were high and decreasing. By the fifth period the levels were low and declining rapidly to minimal exposures. These findings make up the best available information to generate an overall trend in exposure to organochlorines from dairy foods.

2.3.3 Residues in Meat and Eggs

Introduction

Meat and egg comprise a significant proportion of the Canadian diet. The average annual consumption of red meat increased from 51 kg to 84 kg per person per year in 1920 to 1976, while poultry consumption increased from 3 to 20 kg during the same time period (Caputo and Putman, 1990). In 1967 the average individual consumption of eggs was approximately 250 eggs per year (Danielson and Roberts, 1980). Therefore any contamination of meat and eggs would result in a significant source of exposure to humans consuming these products.

Testing for organochlorine residues in meat has been undertaken for several decades in various animal species. Initially testing was established to monitor the amount of residues in beef following agricultural applications of organochlorine pesticides in livestock production, and the findings of these studies lead to the formation of official monitoring programs (Frank et al, 1983). Organochlorine insecticides such as lindane, methoxychlor and toxaphene were used routinely on livestock for the purposes of controlling biting insects and were found to be contaminants of meat taken from treated animals. Direct contact with these chemicals was presumed to give rise to the residues found in the animal

carcasses. However, residues in animal fats were also discovered to result from dermal exposure, environmental and premise spraying and via absorption from contaminated feeds, water and bedding (Frank et al, 1983). Other organochlorines not used directly in livestock management such as dieldrin and PCB's were also detected, presumably as a result of contamination of animal feed (Van Holderinet et al, 1977). Residues of various organochlorines have subsequently been detected in meat samples taken from numerous species. Sampling programs have been undertaken over the years to determine the extent of human exposure to organochlorines and other chemical through animal sources.

In Ontario, residue testing of bovine, porcine and chicken carcasses, intended for human consumption, began in 1969 with the initiation of a monitoring program established by the province (Frank et al, 1983, 1985, 1990). Residues of DDT and metabolites, dieldrin, heptachlor epoxide, chlordane, gamma BHC, lindane and PCB's were discovered in the initial bovine and porcine samples taken. These studies were repeated throughout the years and, in the latter studies, residue testing was expanded to include meat samples taken from rabbits, sheep and turkeys. The findings of these monitoring programs provide the best available information on organochlorine residues in meat for Ontario, and perhaps Canada, during the period under study. Meat residue analysis was not extensively undertaken prior to 1969. However, given that organochlorines were used, it would be reasonable to assume that exposure from this source would have occurred. Contamination levels would have been at least as high as those found in 1969 and most likely at even greater levels, if the organochlorine sales data is indicative of animal exposure and if one

assumes that organochlorine levels used decreased as restrictions were put in place. The monitoring programs continued until 1988 and the findings of these studies will be evaluated to help determine the trends in organochlorine exposure for humans consuming animal protein.

Findings

a) Beef

Frank et al, (1983) collected renal fat samples from 2483 bovine and 554 porcine carcasses randomly chosen from provincially inspected abattoirs across Ontario during 1969 to 1981. During this time, 2483 bovine samples were batched to give 505 composite samples and subsequently analysed to determine the level of organochlorine residues. Figure 2.3.3.1 shows the general trends in the level of total organochlorine residues in the samples taken. Table 2.3.3.1 shows the number of carcasses sampled and the subsequent number of composite samples analysed.

During the first year of the study, 137 composite samples representing 835 carcases were sampled with an average of 0.557 mg/kg total organochlorines in extractible bovine fat. DDT and metabolites and PCBs represented the greatest proportion of total organochlorine residues during the early years. In the early years of testing, most samples had detectable levels of organochlorines. In 1969-70 the average total level of organochlorine was 0.557 mg/kg. By 1988, 170 bovine samples were taken with only 28% of samples tested having any detectable organochlorine, and, of these, the average

residue level was 0.116 mg/kg total organochlorines. The percentage of levels of DDT detected in the meat samples had also decreased markedly. In 1969 the mean level of total DDT was 0.257 mg/kg while in 1981 the levels had declined to 0.012 mg/kg. The number of samples detected with levels of total DDT higher than 0.101 mg/kg decreased from 56.9% in 1969-70 to 0.5% in 1981 (Frank et al, 1983).

DDE as a percent of total organochlorines has increased since 1969, which indicates that the exposure to DDT decreased over the time frame under investigation. By 1988, DDE remained the only metabolite detectable in bovine meat. While there is a substantial difference between the first and last year of the study with 39.3 and 60.8 percent DDE of the total DDT respectively there does appear to be yearly fluctuation. Most interestingly the decrease in 1981 of percent DDE of the total suggests that exposure to DDT occurred during that year. By 1988, the presence of only DDE indicates that exposure had ceased and body burdens in beef consisted of exposure that occurred in previous years and represents the metabolism of DDT to DDE in the animal's body. The levels of other organochlorines can also be found to reduce both in total level and in the number of positive samples tested during the time period under study.

During 1986 -1988 one hundred and seventy bovine meat samples were tested for organochlorine and organophosphate insecticides (Frank et al, 1990). While the latter group of chemicals were used more extensively in livestock production during this period, residues of these chemicals did not occur in any of the samples tested. Residues of

organochlorines were however consistently detected despite minimal level of use and numerous restrictions on organochlorine applications in agriculture. DDE, dieldrin, lindane and PCB's were found in 48.2% of the samples tested. However, by this point the average level of contamination was significantly reduced in comparison to earlier years. Eighteen percent of samples tested contained DDE residues with a mean level of 0.03 mg/kg in extractable fat. No levels of DDT or TDE were found. PCB levels were detected in 7% of the samples with an average of 0.061 mg/kg. These findings show that organochlorines were present in bovine meat at relatively high levels in the early stages of monitoring and that these levels declined substantially over the decades. This decline corresponds to the decreased use of this group of chemicals in livestock production and in the environment.

b) Pork

The 554 porcine samples were randomly collected during 1969 to 1981. These samples were batched into a total of 122 composite samples and analysed for organochlorine residues (Frank et al, 1983) During 1988 there were 150 porcine samples randomly selected (Frank et al, 1990). Table 2.3.3.2 shows the year and number of porcine samples taken and the actual number of composite samples that were tested. The average total level of organochlorines in 1969 was 0.559 mg/kg in extractible fat and constituted DDT and metabolites, dieldrin, heptachlor epoxide, chlordane, lindane, PCB and gamma-BCH. The average level declined substantially in 1988 to 0.176 mg/kg in extractible fat with only DDE, lindane and PCB detected. Figure 2.3.3.2 shows the trends in

organochlorine residues from 1969 to 1988. In the initial stages relatively high levels of DDT (total) were detected with 100% of samples containing greater than 0.010 mg/kg in porcine fat. By 1981 only 26% of samples tested contained greater than 0.010 mg/kg of DDT and metabolite residues (Frank et al, 1983). Of interest is the general increase in DDE as a percentage of the total DDT from a low in 1969 of 35.4 % to DDE being the only metabolite detected in 1988. This suggests a decrease in actual DDT exposure in the latter years compared to the earlier years of study.

By 1986, only 15% of the samples tested contained residues of DDE and of these all levels were below 0.1 mg/kg. Similarly, only 15% of samples contained PCBs and the highest level detected were 0.3 mg/kg in porcine fat (Frank et al, 1990). There was no evidence of dieldrin, chlordane or BHC in any of the samples taken after 1986. Lindane was still present as a residue in porcine meat, but in only seven of the 150 samples tested and at an average level of 0.032 mg/kg. These findings show that there was both a decline in the total level of organochlorine residues detected in porcine meat and a decline in the number of positive samples from 1969 to 1988.

c) Chicken and Eggs

Between 1969-1982 five hundred and thirty-seven carcasses were collected at random from abattoirs and batched into 137 composite samples for residue testing (Frank et al, 1984). The total amount of organochlorines detected in abdominal fat decreased from 1.374 mg/kg in abdominal fat in 1969-70 to 0.174 mg/kg in abdominal 1981-82. DDT

and metabolites, dieldrin, chlordane, lindane and PCBs were detected in the samples tested. During this same time period the number of samples testing positive for organochlorine residues also declined, indicating an overall decrease trend in exposure from 1969-1982. Total DDT levels declined from 0.391 mg/kg in 1969-70 to 0 .0023 mg/kg in 1981-82 and the number of samples with residue levels greater than 0.010 mg/kg declined from 100% in 1969 to 6% in 1981-82. In the early years 5% of samples exceeded the maximum residue level (MRL) of 1 mg/kg while in the latter years no samples were above the MRL. The relative percentage of DDE increased over this time period indicating a decrease in acute exposure to DDT.

Levels of dieldrin on average declined from 0.028 mg/kg in abdominal fat in 1969-70 to 0.00074 mg/kg in 1981-82. Two samples tested greater then the MRL of 0.200 mg/kg prior to 1972 and 79% of samples exceeded 10 ug/kg in 1969-70 verses none in 1981-82. PCBs, levels fluctuated from 0.946 mg/kg in abdominal fat to 0.054 mg/kg in 1971-72 then increased to 0.391 mg/kg in 1975-76 and declined to 0.0048 mg/kg in 1981-81. In the case of PCBs 30%, 66%, 25%, 64% of samples tested in 1969-70, 1971-72, 1973-74, and 1975-76 respectively were above the MRL of 0.100 mg/kg while samples tested after 1979-80 no samples tested above the MRL.

Residue testing was performed in 118 composite samples representing 419 eggs from flocks ranging in age from 15-36 weeks old chickens from across the province during 1969-1982 (Frank et al, 1985). The total level of organochlorines detected in these

samples declined from 0.6063 mg/kg in extractable fat in 1969-70 to 0.0139 mg/kg in 1981-82. DDT and metabolites, dieldrin, heptachlor epoxide, lindane and PCB were detected in the samples tested. DDT levels decreased from a high in 1969-70 of 0.188 mg/kg to a low of 0.0039 mg/kg in extractable fat in 1981-82. Over the years of testing the average concentration of the metabolite DDE increased from 70% to 94% of the total DDT, indicating a decreasing level of exposure to DDT. Similarly, the number of samples testing above the MRL decreased from 60% of samples tested in 1969-70 to none by 1981-82. Residue levels of dieldrin decreased in a similar fashion over the same time period with a high in 1969-70 of 0.0083 mg/kg less then the detection level of <0.0005 mg/kg in extractable fat. PCB residues were relatively high in 1969 at 0.405 mg/kg in extractable fat and declined to < 0.010 mg/kg in 1981-82. Heptachlor epoxide and chlordane were in most cases less then 0.001 mg/kg , while lindane declined from 0.005 mg/kg in 1969-70 to <0.0001 mg/kg in 1981-82.

Limitations of Data

There are several factors which give concern regarding the accuracy of the carcass residues for determining human exposure to organochlorines from meat. The monitoring programs in Ontario measured residues in the abdominal fat only, rather than levels in muscle tissue; therefore it is not known if the actual residue level detected is close to what humans would truly be exposed to. Since there is no mention of the relative distribution of organochlorines in animal fat verses tissue and since organochlorines are lipophilic, adipose samples would be expected to have higher residues. Therefore, if people eat a

greater proportion of meat tissue and trim off fat, using the findings of these residue studies may result in an overestimation of the actual exposure to organochlorines. Conversely, for people consuming more marbled meat cuts or not trimming off fat, this would result in an under estimation of actual exposure. Changes in meat composition, trimming off fat or consuming lean cuts of meat would also be expected to influence the level of exposure from this source. Documentation of types of cuts and their relative proportion of fat would be important in residue testing of meat products when determining human exposure.

While samples were chosen randomly, they were not consistently taken from the same areas over the years of study and this oversight makes it impossible to determine if residues were consistency higher in specific regions of the province. The number of samples and the sampling location for each year were different and this would also have an effect on the ability to compare results between years. The first year of the study had substantial larger numbers of samples taken, while the subsequent years had significantly fewer numbers, with exception of the year. In the final year, there were greater number of samples, however given the relatively low levels of contamination at this point one would question the ability of this sample size to be able to detect a positive sample.

The fact that all samples were batched into composite samples compounds the problem of small sample size, since batching would result in a dilutional effect. We may therefore have an underestimation of organochlorine residues in some cases due to this factor.

Similarly we could expect an underestimation by sampling too few specimens. Changes in technology would also result in a relative underestimation of organochlorine residues in the early stages of the monitoring program, while improved technology would give more accurate results in the latter stages. Despite inaccuracies in the actual total level of organochlorines detected the general exposure trends should hold true.

Summary

This section shows that contaminated meat and eggs were a continued source of human exposure to organochlorines from the late 1960s to the 1980s. DDT, dieldrin, lindane PCB's, chlordane and heptachlor were the most commonly found residues in meat and egg samples analysed from 1969-1988. During this time period residues declined substantially both in average levels and in the percentage of samples testing positive for organochlorine residues. Residues prior to 1969 can be assumed to be higher or at least similar to those level detected in 1969 and by the late 1980's the level of organochlorine residues in meat can be considered minimal. Maximum exposure to organochlorines therefore occurred prior to 1969. Figure 2.2.3.1 shows the general trends in human exposure to organochlorine in relation to the specific phases of exposure previously established.

The trends in organochlorine residues from meat sources are similar to those trends found in other food sources and points to the diminishing levels of residues once chemical restrictions and bans were put in place. While the exact amount of residues present in meat and egg samples is questionable given the limitations of the residue data, it is the best

information available, and does provide a good indication of the trends in human exposure from this food source. From this perspective, if we take the information generated from this section we can see that there are substantial changes in the levels of exposure during the time periods under study. Period one, two and three are lacking in information, but the approximate trend in exposure could be determined from the organochlorine sales section. Period four shows a high level of exposure initially which rapidly declines to a lower level. By period five we see very low level to minimal levels in meat and eggs.

2.4 Human Body Burdens

Introduction

Human exposure to pesticides can result from various sources such as food, water, and through occupational exposure. Once an individual is exposed, these chemicals can be taken up by the body via several routes. The most common route of exposure for the general public, to organochlorines has been through ingestion of contaminated food products. Dermal absorption and inhalation of chemicals can also occur especially during occupational exposure; however, for the average individual, these pathways represent minimal exposure routes (Hayes, 1993). Within the body, these chemicals can be processed into various metabolites or remain in their original forms. In general, pesticides are detoxified and excreted from the body, via the kidneys and liver, as waste products in the urine and feces, but several of the more persistent agricultural chemicals are actively stored in the body and excretion occurs over prolonged periods of time (Hayes, 1993). Because these compounds are very lipophilic, they tend to accumulate in the body and

remain stored in fatty tissue for years while they are slowly metabolized. Organs and tissues with high fat content contain proportionally higher levels of organochlorine residues. As a result, adipose tissue followed by kidney, and liver contains the highest levels of these chemicals (Smith, 1991). Residues have also been found in other tissues samples including human hair and ear wax.

The extent of human exposure can be determined by performing residue analysis on various specimens such as adipose tissue, blood, breast milk and urine. The concentration of residues found in these samples can give an indication of the degree of human exposure to the specific chemicals on an individual or population basis. Body burdens measure both the past and present exposure, but generally give a better indication of the total exposure that a person has received minus the amount metabolized and excreted. In the case of DDT, levels in blood and fat tend to provide a reasonable indication of the current exposure, while levels of DDE, its metabolite, tend to give a better estimation of the historical exposure to DDT (Hayes, 1991). Tissue sample measurements taken at different time intervals for specific age groups can provide a good indication of both the average exposure and the changing trends in exposure over time. Given that most organochlorines are metabolized slowly in the human body, it can be expected that decreases in human body burdens will show a lag phase when compared to exposure trends. While exposure measurement in specific individuals would provide an excellent means of determining the exact level of storage, excretion and subsequent exposure over the decades, individuals have not been consistently followed. Instead, residue testing has

frequently been carried out by performing analysis on convenience samples with these findings providing the basis to determine the average body burden for the individual and the population. In most cases, samples represent autopsy specimens but some surgical samples have also been examined.

When determining if exposure to organochlorines results in adverse effects, it is not known if daily exposure, total intake, total storage, or duration of exposure and storage are crucial to the likelihood of developing negative effects. The possibility that a threshold level, or a specific length of exposure or a critical age at exposure could impact the outcome has not been examined to any extent. The possible significance of these factors may be enormous since exposure to organochlorines has occurred over many years, at various levels and during different stages of human development. As such, a comprehensive examination of organochlorine exposure is required to give a clearer understanding of the potential significance to human health. This means that both residue values and exposure histories are required to determine any potential health effects from exposure.

2.4.1 Adipose Tissue Samples

The first published reports of organochlorine residues in people were documented in 1948 (Van Holdrinet et al, 1977). DDT storage of seven ppm in adipose tissue was recorded at that time in a man occupationally exposed to organochlorines in the United States. In 1950 the first general survey carried out in the United States recorded DDT residues of a mean 5.3 ppm (range 0-34 ppm) in non-occupationally exposed individuals (Durham,

1965). The first Canadian study documented DDT residues in human adipose samples taken in 1959-60. (Reid and McKinley, 1961) An average of 4.9 ppm of DDT was detected (Reid and McKinley, 1961). The first Ontario study in 1965-67 revealed a mean exposure of 3.8 ppm (Brown, 1969). Several studies exist documenting human body burdens for Canadians from 1969 - 1982 (Ritcey et al., 1973; Mes et al., 1977 and 1982). Extensive testing of adipose samples from Ontario residents has been performed by the Ontario Ministry of Agriculture and Food (OMAF). These OMAF studies recorded residues in adipose samples taken at time of autopsy for a period from 1967 to 1986 (Van Holdrinet et al., 1977; Frank et al., 1988). Other studies have also recorded adipose samples taken from Ontario residents in various regions of the province (Williams et al., 1984, and 1988) This information can be used to provide an indication of the level of exposure during the time from 1969 - 1986 and can provide information on the changing trends in exposure for Ontario citizens. Comparisons of these levels can be made to those found in Canada and other countries.

Analysis of Data

a) Human Adipose Samples (Ontario Findings)

Human body burden data was first recorded in Ontario in 1969 (Van Holdrinet et al., 1977). Prior to 1969 there were no collection systems in place to determine the residues in samples of humans exposed to organochlorines. As such, trends in exposure need to be extrapolated from other sources in order to give some indication of the level of human body burdens during the period from 1947 - 1969. However, after 1969 human body

burdens were measured in various human tissue samples. From 1969 to 1974 four hundred and forty-four human adipose samples were collected for analysis (Van Holdrinet et al, 1977). The majority of these samples came from the southern region of the province. However, 127 of the samples were obtained from the rest of the province. Non- random subcutaneous adipose samples were taken from autopsies at various hospitals. Cause of death was not recorded. No sampling criteria were given and there appears to have been no attempt to gain samples from particular age groups, race, or sex. Residue levels were recorded for the years under study and were classified by sex and age. Twenty-year age intervals were established. In general, relatively few samples were recorded for age groups 0-20 and 21-49. The majority of samples were taken from individuals age forty-one and older. From 1976 to 1984, 570 human adipose samples were taken for residue analysis (Frank et al, 1988). All these samples were taken from a single hospital making direct comparison between regions impossible. Cause of death was recorded but not reported in the research paper. No sampling criteria were given and there appears to have been no attempt to stratify samples by age or sex. Results were stratified by sex and divided into twenty year age groups. Residue analysis was undertaken in a similar fashion as the previous study. The findings of these studies provide the best available information on human body burdens for residents in Ontario. While information on geographical exposure exists and would have been ideal for an in depth analysis on a regional basis, the raw data was unavailable (Ripley, 1995). As a result the only available information is the published results from these studies.

Findings

In Ontario, organochlorine use began in 1947 when DDT was first used (Cutten, 1991). The application of this particular organochlorine and others increased from this initial use to the late 1960's when the level of use decreased dramatically (Frank et al, 1978). Exposure prior to 1947 can therefore be assumed to be zero for the general population in Ontario. This conclusion can be supported by the fact that human samples tested in the United States from individuals who died prior to DDT production did not contain residues of DDT (Hayes, 1958). As such, one can reasonably assume that a similar finding would arise in Ontario. Human exposure to organochlorines subsequently would have increased, peaked and/or plateaued and then declined after the discontinuation of use for most of the organochlorine pesticides in agriculture.

The trends in human body burdens from 1969 - 1984, for Ontario residents, can be seen in Figure 2.4.1.1. The studies clearly show that humans have detectable levels of several organochlorines not just DDT and PCB. The early studies found detectable levels of DDT and metabolites, dieldrin, HCB and PCBs. The 1980 - 1983 study also recorded levels of chlordane, hepatachlor epoxide, and mirex (These components were not listed individually in the first series of analyses). The latter studies also tested for, but did not identify methoxychlor, endrin, endosulfan, aldrin, dicofol, and lindane. The data show that the earliest recorded levels, in 1969, were, on average, 7.1 mg/kg total organochlorines in human adipose tissue for the southern Ontario region. Slightly lower levels of 5.29 mg/kg were obtained for the rest of the province.

The first analysis measured only DDT and metabolites, and dieldrin, while in 1970 and 1971 PCBs were also measured. By 1972 HCBs were being detected and beginning in 1976, other organochlorines such as heptachlor epoxide, chlordane and mirex were also measured. The level of total organochlorines in adipose samples recorded from the southern region for 1970, 1971 and 1972 were slightly higher at 9.46, 8.55 and 8.96 mg/kg respectively, than recorded in the previous two years. These higher values may be in part due to an increase in the number of individual organochlorines detected compared to those detected in 1969, or as a result of changes in sampling numbers or specific variations in exposures for the individual sampled.

By 1973 the levels of organochlorine residues appear to decline to 6.61 mg/kg and by 1974, levels had dropped to 4.88 mg/kg. However, both years of sampling had substantially lower numbers of adipose tissues sampled than the years prior and following. In 1976 adipose samples from the southern region contained 7.49 mg per kg of total organochlorine residues while in 1978 the levels were slightly higher at 8.86 mg/kg. It should be noted that the latter findings are higher because there was an increase the number of different organochlorines tested for and detected. If the first study had investigated the same number of organochlorines, the total level of organochlorines determined from 1969-1974 could be expected to be proportionally higher than actually detected. By 1980 and 1984 the levels appear to be on the decrease to 6.77 and 4.87 mg/kg respectively.

Sampling from other regions in the province occurred only for three years, namely, 1969, 1972 and 1973. Samples were not determined for the other years as the sampling frame focused on just the southern region. The apparent motivation for this strategic sampling frame was based on the observation that sampling from the southern region would give an indication of the highest level of human exposure to organochlorine pesticides (Frank et al., 1988). Levels in the rest of the province were generally lower than those in the south. For example, the average level of total organochlorine residues in human adipose samples was 6.97 mg/kg in 1972 for the rest of the province compared to 8.96 in the southern region. However, in 1973, there was an average of 6.59 mg/kg in the adipose samples from the entire province versus a similar level of 6.61 in the southern region.

The increased levels in the southern region may perhaps be due to the fact that more organochlorines were used for agricultural purposes in the southern region than other regions of the province as discussed in the bovine milk residue analysis (Van Holdrinet, 1976). However, more detailed information on residue levels in human adipose samples from all regions of Ontario would have allowed one to determine if a difference in residue levels was sustained across the province over the entire time period from initial use of organochlorine pesticides to the present day. With the present information it seems reasonable to speculate that the levels of human body burdens in other parts of Ontario have been less than or equal to those of the southern region.

Table 2.4.1.1 gives an indication of the individual components and (percent of total)

making up the total organochlorine residues detected in adipose tissue sampled during the years under investigation for the southern region. From the data, it can be seen that DDT and metabolites made up the bulk of the residues detected in the first three years of study. In all cases DDT and metabolites compose greater than 50% of the residues detected in the adipose samples tested. Of interest is the percentage of DDE which increased from 69.9 % in 1969 to 81.6 % in 1974. This decrease in DDT indicates that there was a decrease in the current level of DDT exposure during this time period. Similar findings arise in the studies conducted from 1976 - 1984 where DDE approximated 95.5 % of the total DDT detected. The latter findings would be expected, given the restrictions in use and outright banning put in place during this same period.

An examination of the age distribution of organochlorine residues shows that, in general, samples taken from individuals age 0 - 20 and 21 - 40 had lower levels of total organochlorine residues than those taken from age 41 - 60, 61 - 80 and age 81 - 100. This would be expected as older people would have been exposed to organochlorines for significantly longer periods than younger individuals and consequently would have increased opportunity to store these chemicals. The finding of difference in age distribution clearly point to the necessity to examine historical exposure to organochlorines and to consider both the age and timing of exposure to organochlorines in order to determine if organochlorines play a role in the development of breast cancer or other diseases.

Once again information on age distribution is only available from 1969 - 1984 and for a limited number of samples in the younger age categories. While it would have been extremely valuable to have adipose residue results from individuals prior to 1969, this information is simply not available. However, examination of the age distribution data for the older groups measured would allow us to determine that organochlorine exposure prior to 1969 was significant and that the greater the number of years of exposure the greater the level of organochlorine residues. For example individuals in the age category of 61 - 80 years in 1969 had 7.87 mg/kg total organochlorine residues which represent an accumulation of organochlorines during an exposure period from 1947 to 1969. These individuals would have been age 39 to age 58 when the exposure took place.

Similarly, individuals age 0 - 20 years during 1969 had an average level of 4.88 mg/kg total organochlorines indicating significant exposure to organochlorines prior to 1969. The level in this latter group can be expected to be lower since, on average, the total number of years of exposure would be less than for the older individuals. For example, all individuals in the 61- 80 years old group can be expected to have 22 years of exposure to organochlorines while individuals in the 0 - 20 year group can have from 0 to 20 years of exposure. Therefore, on average, younger individuals would have lower levels due specifically to the fewer years of lifetime exposure. As the use of organochlorines decline over the years, the level of body burdens in the youngest groups can be expected to decrease significantly in comparison to the older groups since the latter would be retaining body burdens from previous years of exposure. For instance, individuals age 0 - 20 years

old tested in 1983, when the level of exposure to organochlorines was minimal, had significantly lower level of residues than individual age 41 and older.

Other studies have been undertaken in Canada to determine residue levels and can contribute to the information already given. Williams et al, (1984) compared adipose tissue samples between Ottawa and Kingston taken during 1979-1981. The authors did not detect aldrin, endrin, heptachlor or methoxychlor in the samples taken and found that residue levels were not significantly different between cities for the female samples. Only slightly higher residues were found in samples taken from males in Kingston. Mes et al, 1992 made a comparison of chlorinated hydrocarbon residues in human populations from the Great Lakes and other regions of Canada and found that samples taken in 1985-86 did not differ to any great extent between the Great Lakes region and other parts of Canada. This finding suggests that levels determined in Ontario could be considered to be representative of the Canadian values and may therefore describe the exposure for Canadians as a whole. Mes et al, 1977 measured adipose residues in humans taken in 1972 from several regions in Canada the authors found several organochlorine residues including dieldrin, DDT and metabolites, HCB, HCH, oxychlordane, heptachlor epoxide and PCBs. In general, levels in younger ages (0-20 years) were lower than in the older ages (26 -51 year and greater), which further substantiates the higher levels of exposure prior to the late 1960's.

Limitations of Data

One of the major limitations of any human body burden analysis is the tremendous fluctuation in results of average residue levels. This is particularly true when small numbers of samples are used to give an indication of the average exposure. Despite examining residues levels stratified on an age and sex basis, there remains large variation in the levels of organochlorine residues. Consequently, there are large standard deviations for the average values given for each specific time period, age group and/or sex. This implies that the average value of organochlorine residues recorded for the time period from 1969-1985 may not give a completely accurate indication of the actual body burdens in the population. In some cases, the average level could be an overestimate of the total body burden while, in other cases, the average would underestimate the body burden.

The significance of large standard deviations is important if the data are used to determine the exact level of exposure. In this case, given the relatively small sample size of the residue studies the impact of outliers may be more profound. However, if the residue data are used to determine the approximate trends in human body burdens during the period of organochlorine use, then the resulting data could provide a relatively good approximation of the trends despite having large standard deviations. The range in body burden values could also be used to give an indication of the worst and best case scenarios for exposure in the population as a whole when examining the high and low levels for each year. It must be recognized that, ideally, information from a large population of people

would have given optimum results, but the expense of such a residue testing program would have been enormous.

There are several factors affecting human storage of organochlorines that on an individual basis or a specific risk group basis could influence the total level of organochlorines despite a similar intake level. For example, individuals who are pregnant and/or breast feed consequently eliminate significant amounts of organochlorines from their body reserves via maternal transfer to the fetus and through breast milk secretions (Rogan, 1986). Effectively, body burdens of the mother are transferred to the developing infant thereby reducing the mother's total body burden. Despite a similar intake in organochlorine residues women who have had children or have a history of lactation will have lower levels than women with no history of pregnancy or lactation. Similarly, individuals occupationally exposed or those consuming high levels of contaminated foods will have higher levels of total organochlorines in their tissues. For example, high meat consumers can be expected to have higher levels of organochlorine exposure than those consuming lesser quantities. The fact that most human body burden studies have not taken into consideration these factors when sampling, results in less than ideal estimates for population exposure to organochlorines. Despite these short comings the human body burden data is the best and only available information and must therefore be used to give an indication of the general trends in exposure to organochlorines. This information despite its inaccuracies will show the fluctuations in exposure levels.

Summary

The human body burden data despite its limitations shows that individuals in Ontario were exposed to organochlorines at relatively high levels in the 1960s with declining exposure since then. Humans have retained levels of DDT and metabolites, chlordane, dieldrin, heptachlor, HCB, and PCBs. Exposure measurements show that storage of organochlorines occurred over the early decades and that the average level of exposure declined during the most recent decades. Higher levels of organochlorines in older individuals suggest that exposure prior to 1969 was higher than exposure during 1969 - present. This finding substantiates the results from the previous section on organochlorine sales which suggest a peak exposure to organochlorines at approximately 1955, and the food residue studies which show a decline in organochlorine residues after restrictions were put in place on organochlorine use.

When we examine human body burden data from 1947 to present, (Figure 2.2.3.1) we can see that the highest recorded levels were in 1970 and that the body burdens decreased from this point. Whether the body burdens were actually higher in the period preceding (1955 - 1965) is unknown and will have to be determined from other forms of assessment. One can suspect that these levels were at least similar or perhaps higher since the slow rate of metabolism of organochlorines would have ensured that the levels detected in post 1969 would represent some of the exposure prior to 1969. Period one, two and three on the graph will need to have estimated values from the organochlorine sales data. During period four there is a high level of exposure for most of the period and then a decline to

lower levels. The initial phase of period five the body burdens are low and then increase only to decrease once again. By the sixth period we would expect to see body burdens decline further.

2.4 .2 Human Breast Milk Residues

Introduction

The first studies documenting organochlorine residues in human milk samples from the general population were reported in the United States (Quinley et al., 1965). Since this time several other countries have published human milk sample surveys documenting residues in human milk samples around the world (Miller and Fox, 1973; Currie et al, 1979). Organochlorine residues in breast milk samples have been documented for Canadians residing in various regions throughout the country from 1967 to the present (Ritcey et al., 1972; Mes and Davies, 1979; Mes et al., 1986; Ryan et al., 1993; and Newsome et al., 1995). Van Holdrinet et al., 1977 reported the first of a series of analysis of breast milk samples from women in Ontario. This study and a subsequent one by Frank et al., 1983 recorded the levels of organochlorine residues found in breast milk samples from 1969 - 1985 for Ontario residents. These studies confirm previous studies that found chemical residues in human breast milk samples. Chemicals found in breast milk samples tend to be similar to those found in adipose samples.

The type and level of organochlorines excreted in breast milk can give a good indication of the current and past exposure of the lactating women. Women with high body burdens

(high exposure) will tend to excrete proportionally higher levels of organochlorine in their milk than those with low levels of organochlorine storage (low exposure), given the same milk fat content (Mes et al, 1993). However, women with high milk fat content will tend to have higher concentrations of these chemicals in the breast milk they produce. Nursing infants would be subjected to residue levels dependant on the concentration in the total volume of milk, and the total volume of milk consumed. Milk residue values should be given on a percent milk fat basis to give an indication of the average amount of residue present. Of interest is the current finding that women who have breast fed have a decreased risk of developing breast cancer and that this sparing effect is particularly significant for the first breast feeding but declined in significance after multiple breast-feeding terms (Fisher, 1995; and Romieu et al, 1995). This may perhaps be the result of diminishing body burdens as a direct result of unloading persistent chemicals via the breast milk. Documentation of the changes that can be expected in total human body burdens from breast feeding over time and parity remains an important area to investigate, particularly as chronic and acute exposure patterns change for the population as a whole. When residue levels in human breast milk samples were compared to bovine milk samples in 1967, to give an indication of the level of exposure infants breast fed versus bottle fed with cows' milk and/or formula, infants ingesting human milk were found to have higher exposure levels than bottle fed individuals or than the general population's exposure to organochlorines from bovine milk (Ritcey et al, 1972).

The breast-feeding history of a woman can alter the total body burden and is an important

way for the excretion of lipophilic chemicals (Rogan et al, 1986). Women who have breast-fed multiple times or for long durations can be expected to have lower body burdens than similarly exposed women who have not breast fed (Mes, 1993). Breast feeding is consequently also a significant source of exposure for the breast-fed infant (Rogan et al, 1986). The amount of exposure in breast fed individuals will depend both on the mother's past exposure and breast-feeding history (Mes et al, 1993; and Rogan et al, 1986). In a period of declining organochlorine use breast feeding will increasingly become the most important route of exposure to infants who otherwise would have minimal life time exposure from other sources. However, as human body burdens decline this transfer of contamination will also begin to diminish. On a population basis one could expect over time to have very minimal levels of organochlorines excreted in breast milk as women, who were born during a time of minimal to no exposure to organochlorines, begin to breast feed their children.

The importance of determining the changes in human breast milk residues is several fold. Firstly, breast milk samples can be used to support the findings of human body burden trends in women during the time period under study. Secondly, they provide information regarding the level of exposure to the nursing infant over time and can be used to determine the potential body burdens and infant exposure when other sources of exposure would be minimal for the population in general. Thirdly, they provide information about which organochlorine chemicals are excreted in the breast milk and which chemicals may influence the development of breast cancer. The results of the Ontario and Canadian

studies were investigated to determine the trends in human breast milk contamination over the decades of organochlorine use.

Analysis of Data

The two studies reporting organochlorine milk residues for women residing in Ontario determined that several organochlorines could be detected on a routine basis in breast milk samples over the years of testing (Van Holdrinet et al, 1977, Frank et al, 1983). During the first study, only DDT and metabolites, dieldrin and PCB's were evaluated. In 1969-70 forty eight samples with an average of 3% milk fat were tested. The average age of the donor was 26 years old. Thirty-four samples were collected in 1971-72 while 19 samples were collected in 1973-74, from an average age of 28 and 24 year old women respectively. The average milk fat was 3.0% in 1971-71 and 2.7% in 1973-74. No information was provided as to lactational history, parity or previous exposures. During this time period the total level of organochlorines tested declined from a level of 4.57 ppm in milk fat to 2.73 ppm in milk fat. The level of DDT declining markedly from a high in 1969-70 of 3.48 ppm to 1.39 ppm in 1973-74. The metabolite composition of DDT in the breast milk samples during this time period also changed with an increase in the proportion of DDE compared to DDT and TDE. This change could be expected if the donor was acutely exposed to decreasing levels of DDT as a result of diminishing use as the first restrictions were put in place. Levels of dieldrin decreased from 1969 to 1974. However, the levels of PCBs during this same period remained stable. Testing for HCB became routine starting in 1973 when 14 samples were analysed and revealed 0.10 ppm in

milk fat (Holdrinet et al., 1977).

In 1976 chemical residue testing techniques improved allowing the determination of more organochlorine pesticides, at increasingly smaller levels. Despite this technical improvement, the total number of chemicals found in breast milk did not increase substantially. Substances tested for but not found at greater levels than the detection limit of 0.15 ug/ L included endosulfan, endrin, chlordane, dicofol, lindane and mirex (Frank et al., 1988). Three hundred and forty-eight human breast milk samples were collected from 1975-1985 with detectable levels of DDT and metabolites, dieldrin, heptachlor epoxide HCB and PCB found routinely. Samples were collected at local health units or doctors offices. No sampling criteria were given and there was no mention of past history of exposure or previous lactation history for the individuals tested. The total level of organochlorines, consisting of DDT and metabolites, dieldrin, heptachlor epoxide, HCB and PCB, fluctuated slightly from 50.14 ug/kg whole milk in 1975 to 49.7, 59.1, 42.7 and 45.5 ug/kg in whole milk in 1979, 1980-81, 1983-84 and 1985 respectively. No statistical difference could be detected between those samples taken from urban or rural residents and there were no regional differences despite higher agricultural use of chemicals in the south (Frank et al., 1988). These findings may be an indication of the relative uniform nature of the main source of organochlorine chemicals, namely food.

In comparing the results of the milk sample analysis over time several problems arise, the most important of which is the inconsistency of standard residue reporting. For the period

from 1969-1974 residues were determined on a milk fat basis while from 1976 - 1985 residues were determined on a whole milk basis. Since organochlorines are highly lipophilic, they tend to accumulate in the milk fat and consequently if analysis occurs on a whole milk basis there would be an observed decrease in the reported levels. One would need a conversion factor based on the percent milk fat present to allow an accurate comparison of the residues present on a milk fat. Ritcey et al., 1970 made a conversion of residues determined on a fat basis to whole milk by multiplying the fat residue value by the % milk fat; therefore if we take the values determined in the previous studies and used a similar method we would find the trends in organochlorines to decline as shown in figure 2.4.2.1.

Limitations of Data

Several factors can affect the amount of residues present in the human breast milk samples. For example, number and duration of breast feeding affects the level of organochlorine residues in that women who have breast fed more than once tended to have lower body burdens and subsequently lower levels of organochlorine residues in their breast milk samples. Similarly, average age of the sampled individuals can affect the outcome since older individuals tend to have increased levels of organochlorine residues due to the fact that they have been exposed for greater periods of time or were exposed during peak periods. This fact will become increasingly significant as human body burdens decline, exposure decreases and as a result younger females would be expected to have substantially lower levels of organochlorine residues in their milk. The nutritional

preferences of nursing mothers will also impact the residues detected. For example, meat and fish eaters would be expected to have higher level of residues than non-eaters. When these factors are not taken into consideration, average levels detected could be biased with higher or lower levels detected than would actually be found in the population.

The Ontario studies did not take into consideration or identify any individual exposure factors, age, or reproductive/lactation histories that could influence the level of organochlorine residues detected in the samples taken. This, in combination with small sample numbers, makes the accuracy of the actual residues detected poor. However, given that similar discrepancies occur throughout the years of evaluation one could expect that the trends in breast milk contamination would be approximately correct despite that the actual level of organochlorine residues may be higher or lower than found. In future, determining the age of the donor, breast-feeding history, breast milk fat content, and nutritional history and the timing of exposure in relation to organochlorine use would be appropriate in order to determine the average level of organochlorines in breast milk samples of Ontario residents. These confounding factors would also be important in predicting which women could be expected to have high residues and in turn which children could be expected to receive high levels of organochlorine exposure through breast milk.

A greater emphasis on study design could have provided more information about the actual effects of parity and lactation histories and the impact on organochlorine residues in

breast milk for women in Ontario. It would have been extremely interesting to follow a large group of women over the decades of exposure, during multiple pregnancies and lactations to determine the exact amount of organochlorine excreted in their breast milk and compare these findings to their actual body burdens. This information would have provided details regarding the impact of current and past exposure, the effect of each subsequent pregnancy and lactation period to both the level detected in breast milk and the level in the individuals adipose tissue.

Summary

Despite the severe limitations of the data the findings in this section show that there are several organochlorines excreted in human breast milk. Over the time period under consideration for this study DDT, and metabolites, dieldrin, and PCBs and HCB were all detected at one time or another. In general, the levels decline from high levels in the early stages of organochlorine use from 4.5 mg/kg on a milk fat basis to 0.4 mg/kg on a whole milk basis in 1985.

The findings of this section also point to the importance of breast milk as a source of exposure to organochlorines in the nursing infant particularly during high exposure periods when the maternal exposure is high. Breast feeding is also a significant method to unload the body of organochlorines and this factor alone could contribute significantly to the total and lifetime exposure to organochlorines in both the mother and breast-fed infants. The frequency and duration of breast feeding can play a great role in the actual

amount of organochlorines available to act in the body and if a relationship exists between organochlorine use and breast cancer development these factors should be determined in studies designed to assess the relationship.

2.5 Summary

The findings from the previous sections on sales data, food sources and human tissue samples all clearly indicate that human exposure to numerous organochlorines occurred from 1947 to the late 1980s. Prior to 1947 human exposure to organochlorines did not occur. The combined information shows that from 1947 to 1955 there was an increasing period of exposure to organochlorines, peak human exposure occurred during 1955 to 1965 and from 1965- 1975 there were decreasing levels of human exposure. After 1975 the level of human exposure to organochlorine could be considered to be minimal in Ontario. Despite limitations in the data quality, namely that the exact quantity of individual chemicals are unknown, the aggregate data presented here may give a more accurate picture of the general trend in human exposure to organochlorine over the decades of use than that given by more detailed, but selective, individual-level studies.

The information generated by this investigation was used to determine the type and duration of exposure that individuals or populations would have historically received. This information was then used to determine if a causal relationship is plausible between organochlorine exposure and the development of breast cancer.

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Figure 2.2.2.1 Sales of Organochlorines for Agricultural Use from 1947 to 1965

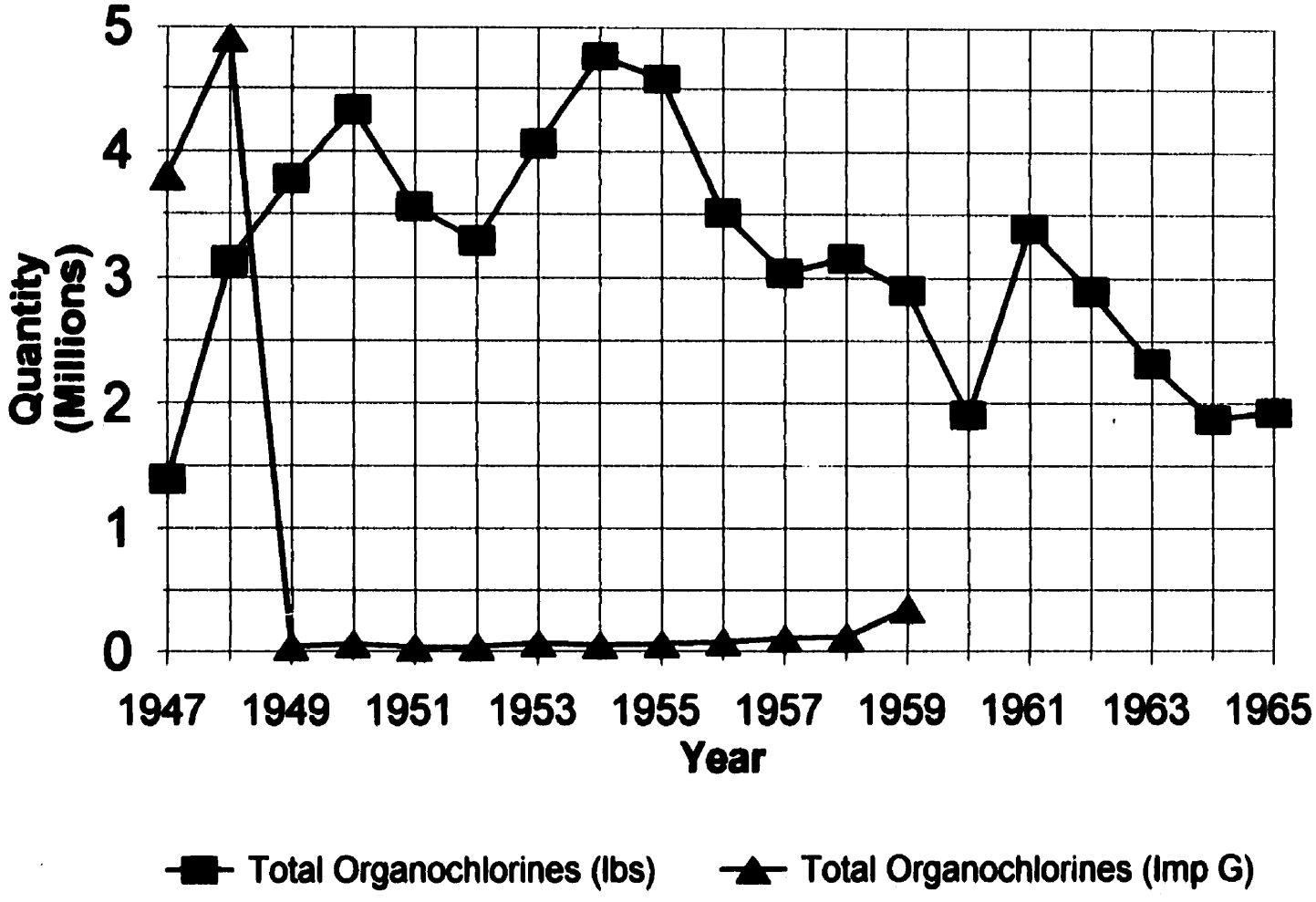

Figure 2.2.2.2 Sales of Organochlorines for Household and Industrial Use From 1947 to 1965

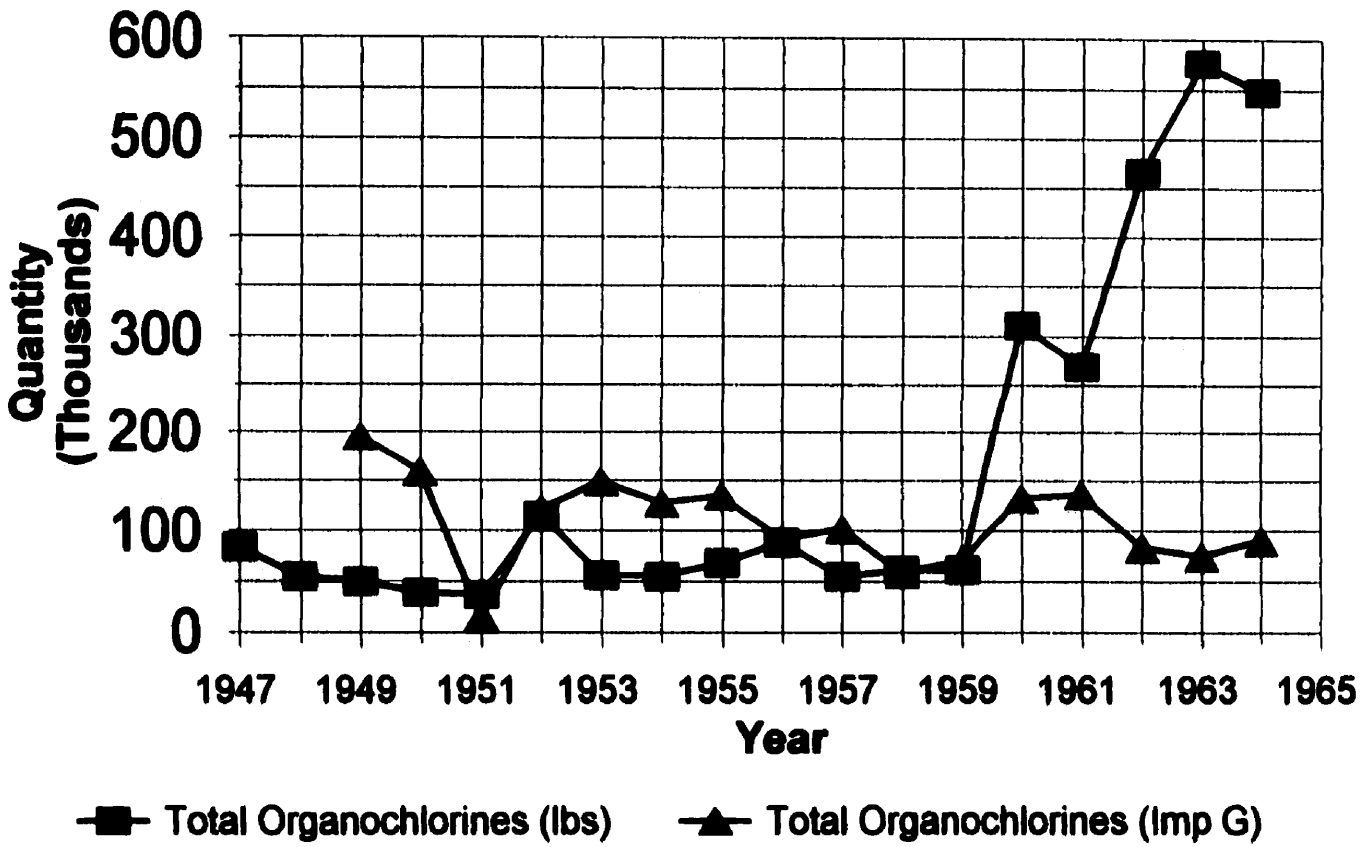

Figure 2.2.2.3 General trends in exposure to organochlorines

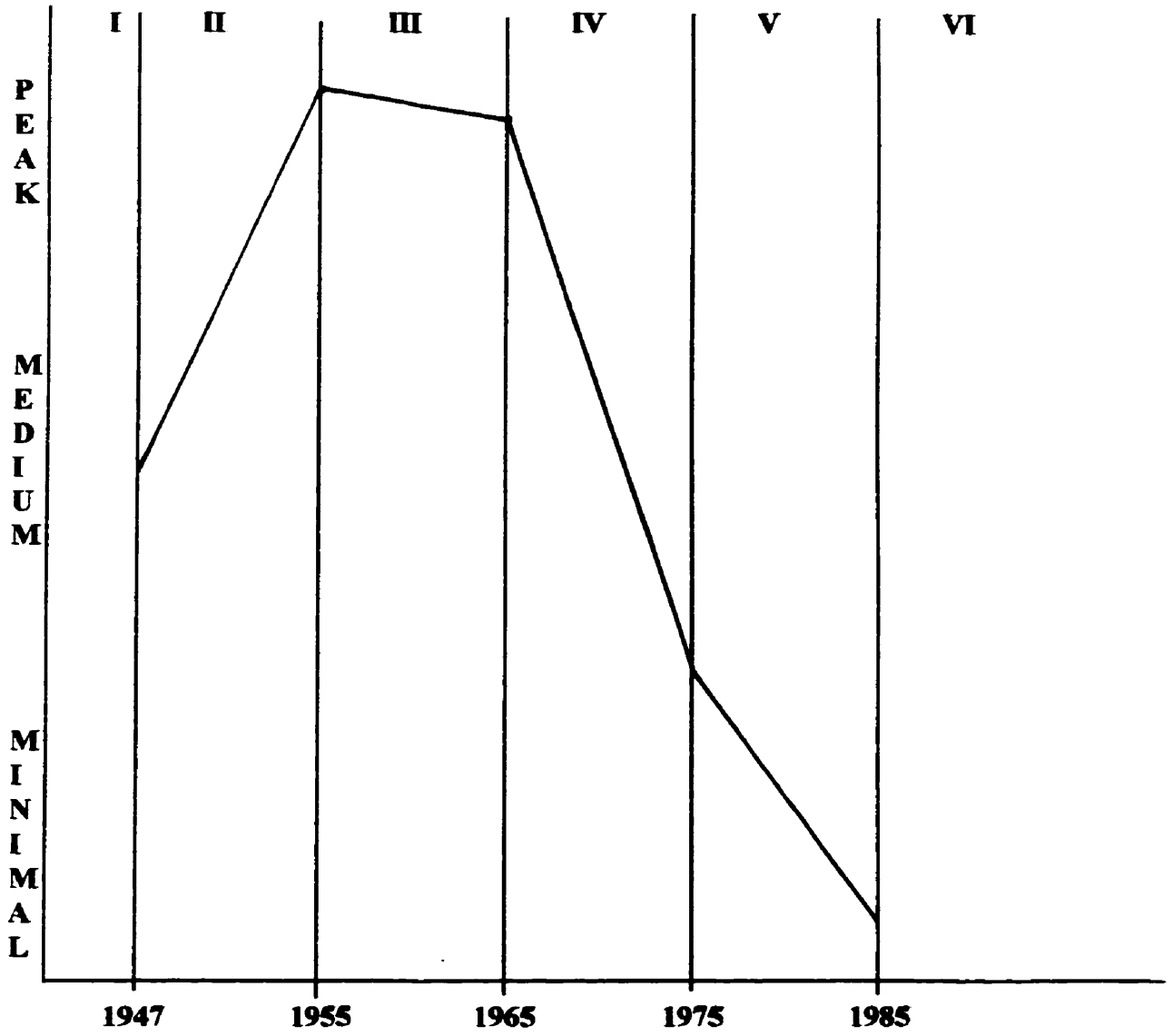

Table 2.3.1.1 Canadian market basket studies for determination of chemical residues in food

Year	Location	Season	Sample Numbers	Consumption
1969	Ottawa	4 Seasons	12 Composites per season	Disappearance of Foods
1970	Vancouver	4 Seasons	12 Composites per season	Disappearance of Foods
1971	Halifax	4 Seasons	12 Composites per season	Disappearance of Foods
1972	Winnipeg	4 Seasons	12 Composites	Disappearance of Foods
1973	Toronto	4 Seasons	12 Composite	Disappearance of Foods
1976-78	Toronto Halifax Montreal Winnipeg Vancouver	Summer and Winter	11 Composites	24 Hour Recall Food Consumption Survey
1985	Ottawa	Summer and Winter	121 Composites	Average Canadian Daily Intake

Table 2.3.1.2 **Year of residue testing for market basket surveys in Canada, and organochlorines detected**

Year	Organochlorines Detected
1969	DDT and metabolites, Dieldrin, Heptachlor, BHC, Aldrin, Endosulfan, Kelthane
1970	DDT and metabolites, Dieldrin, Heptachlor, BHC, Aldrin Endosulfan, Kelthane, Lindane
1972	DDT and metabolites, Dieldrin, Heptachlor, BHC, Aldrin Endosulfan, Kelthane, Endrin
1972-73	DDT and metabolites, dieldrin, Heptachlor, BHC, Endosulfan Kelthane, HCB
1976-78	DDT and metabolites, Dieldrin, Heptachlor, BHC, Endrin, Endosulfan, Kelthane, HCB, Chlordane, PCB'S, Toxaphene

Figure 2.3.1.1 Market Basket Residue Analysis From 1969 to 1978:
Average Total Organochlorine Exposure in Ug Per Person Per Day

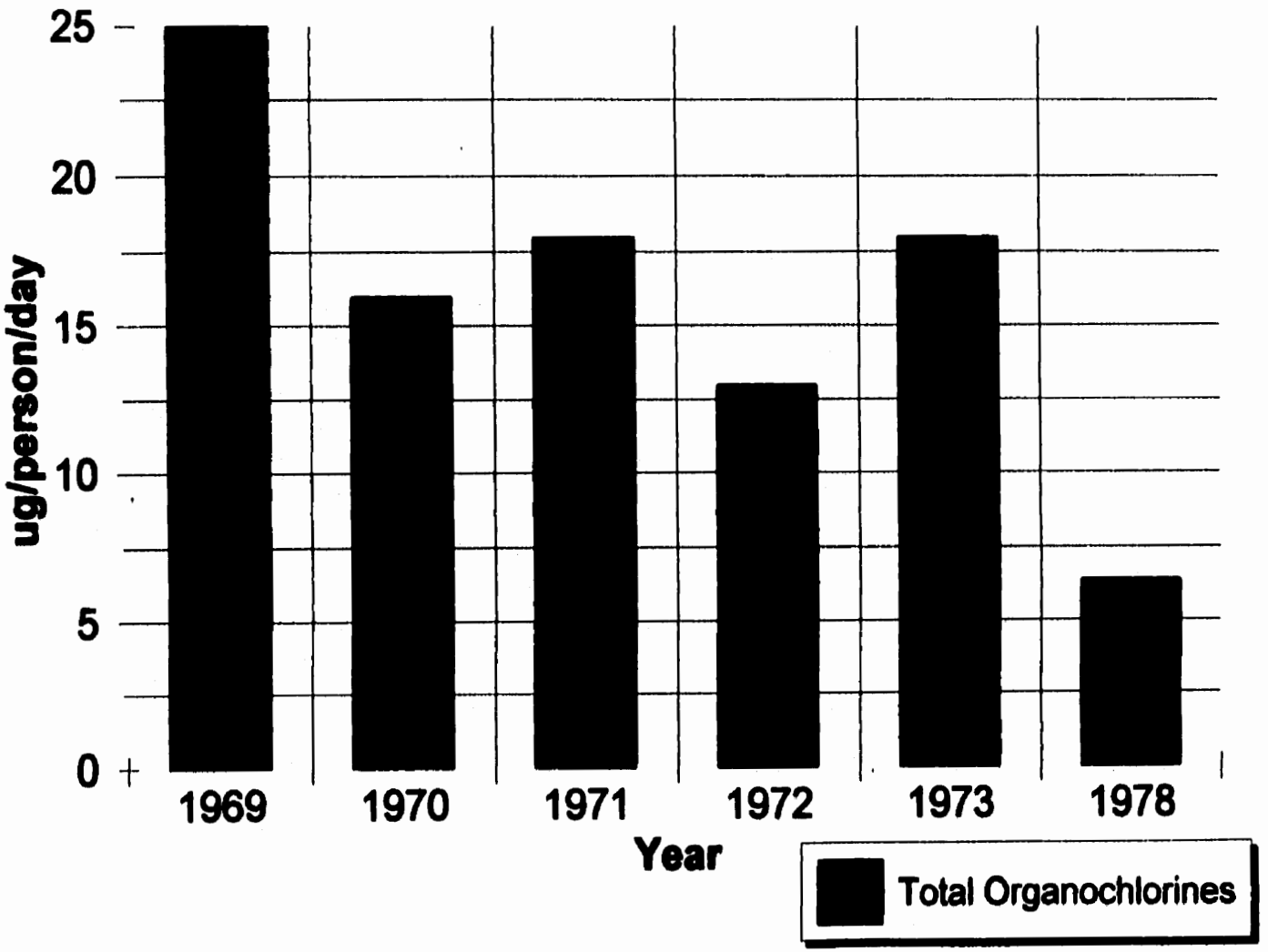

Figure 2.3.1.2 Market Basket Residue Analysis From 1969-1978
Mean Organochlorine Exposure Ug/Person/Day

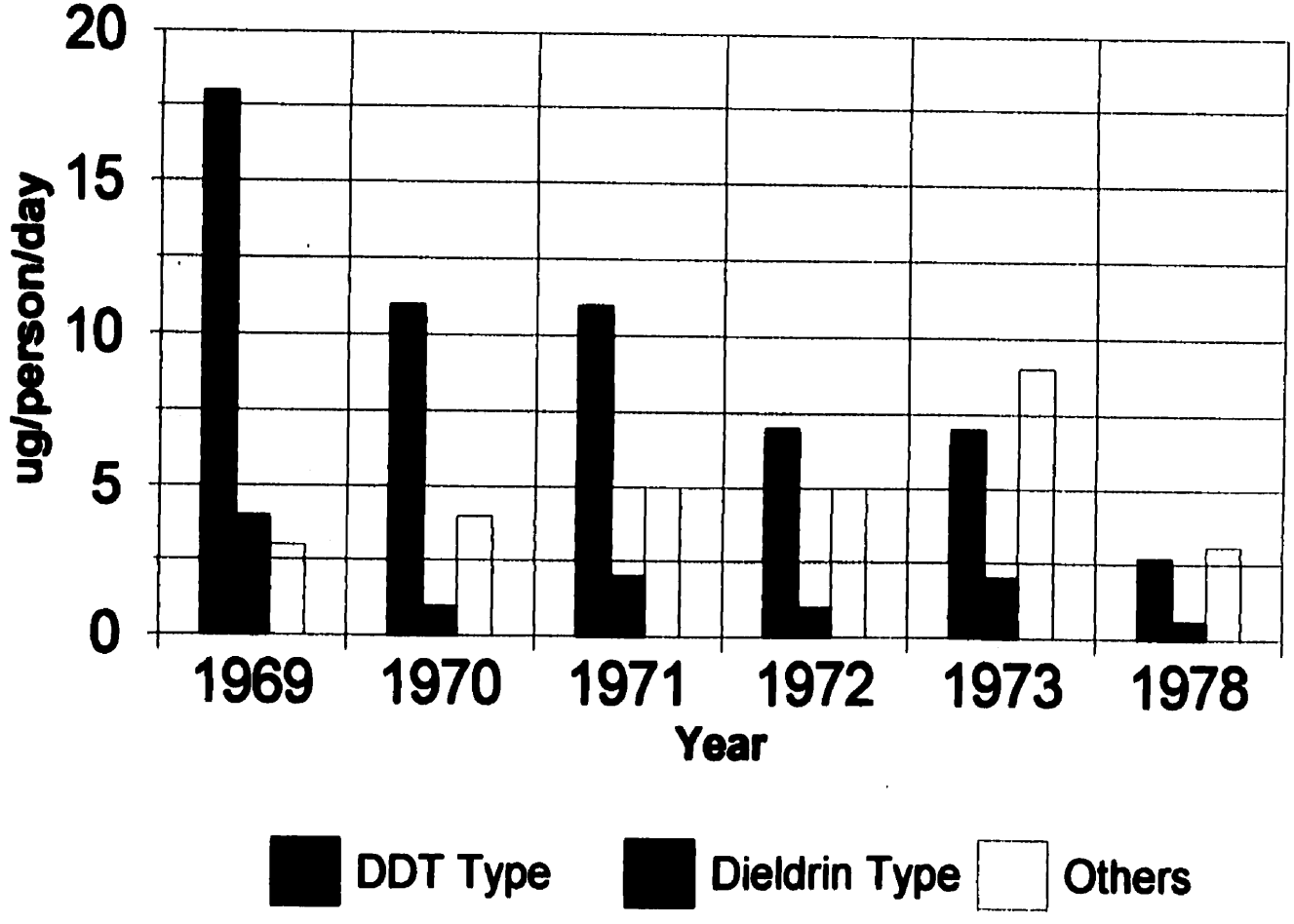

Figure 2.3.2.1 Average Organochlorine Residues Found in Bovine Milk Samples Taken From 1967 to 1986 in Ontario and Southern Ontario

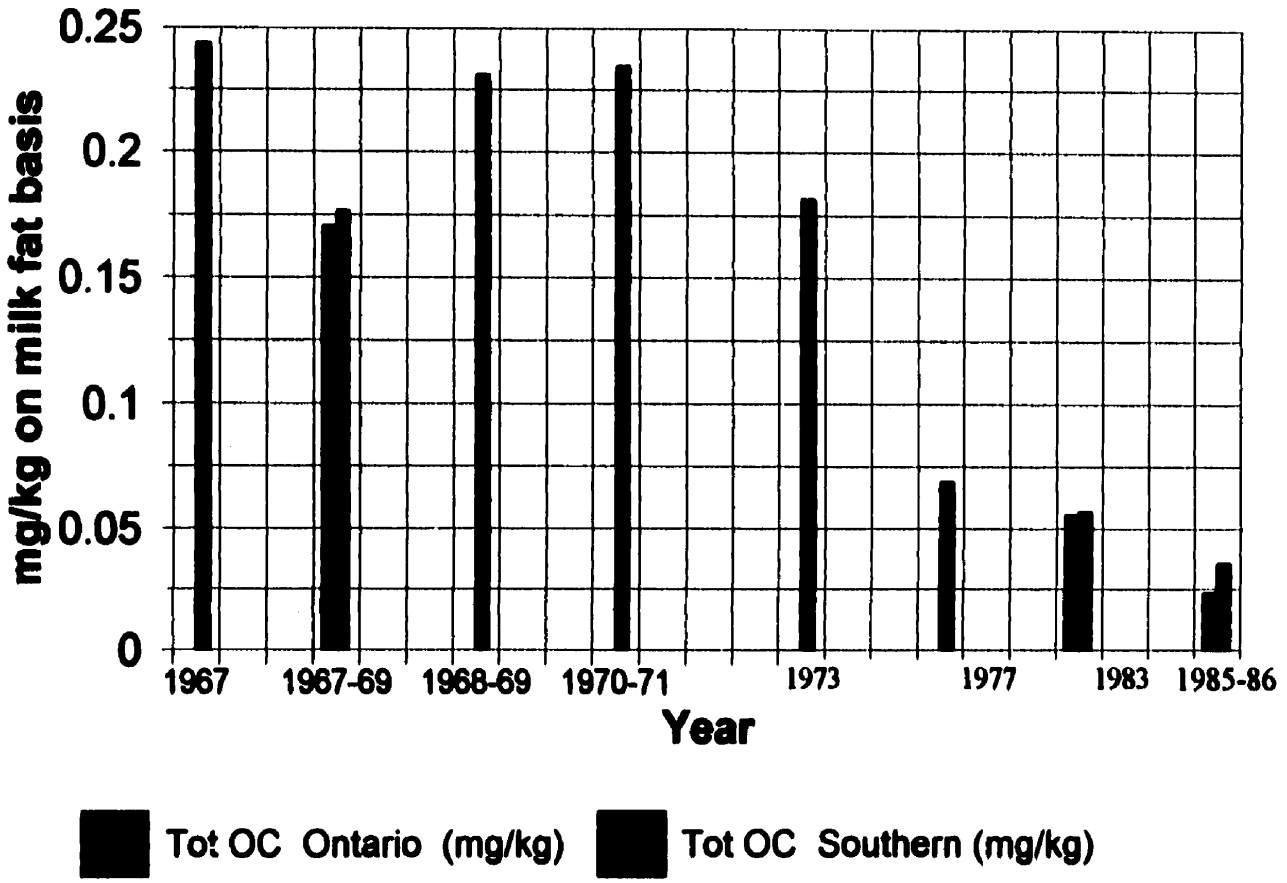

Figure 2.3.2.2 Average Organochlorine Residues Found In Bovine Milk From 1967 to 1986

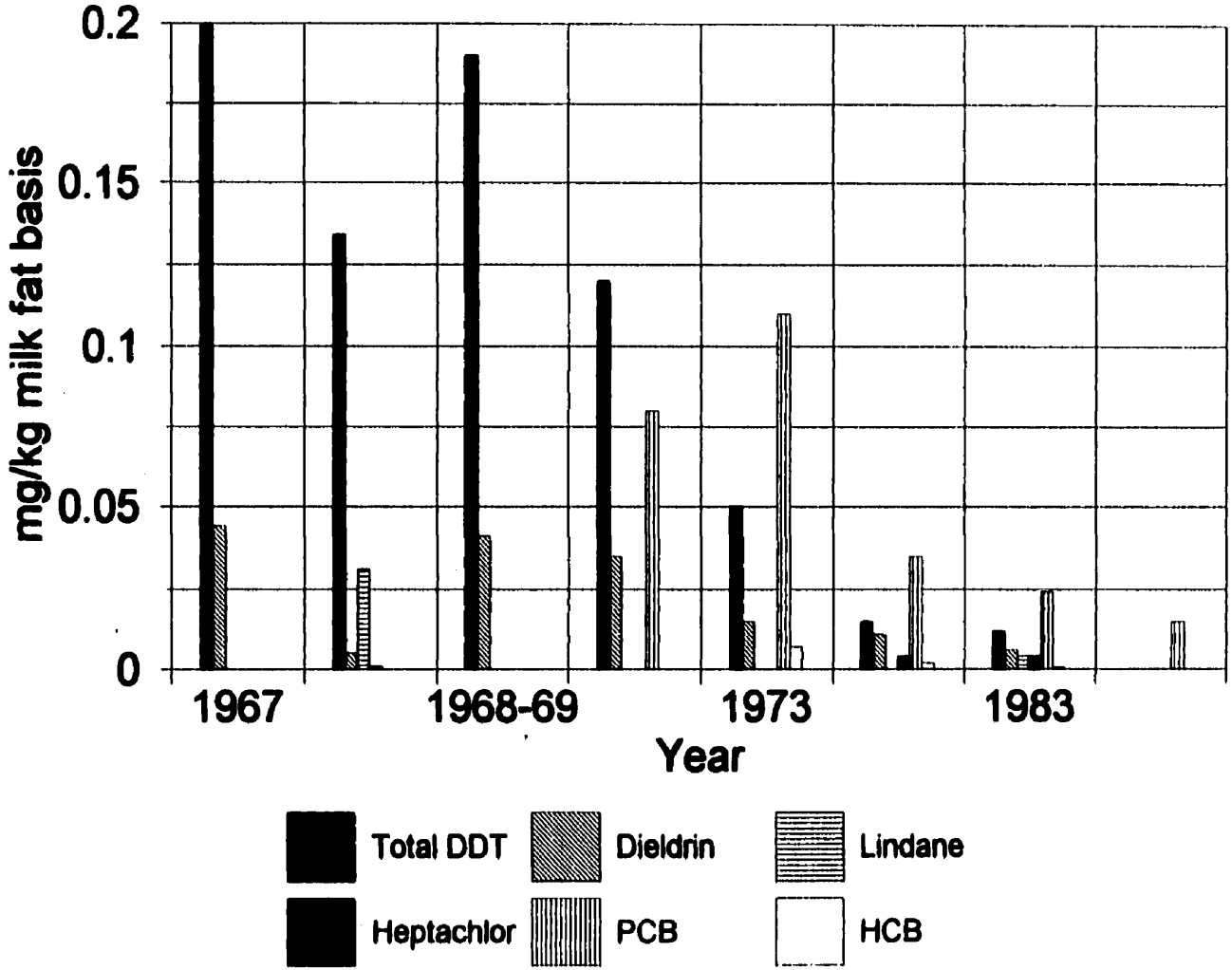

Table 2.3.3.1 **Comparison of composite samples and number of carcasses in the years of Residue testing of Bovine Meat**

Year of Study	Number of Carcasses	Composite Samples
1969-70	835	137
1971-72	143	34
1973-73	198	33
1975-76	53	21
1977-78	50	10
1979	213	73
1981	990	197
1986-88	170	?

Figure 2.3.3.1 Mean Organochlorine Residues in Bovine Carcasses: Ontario Findings From 1969 to 1988.

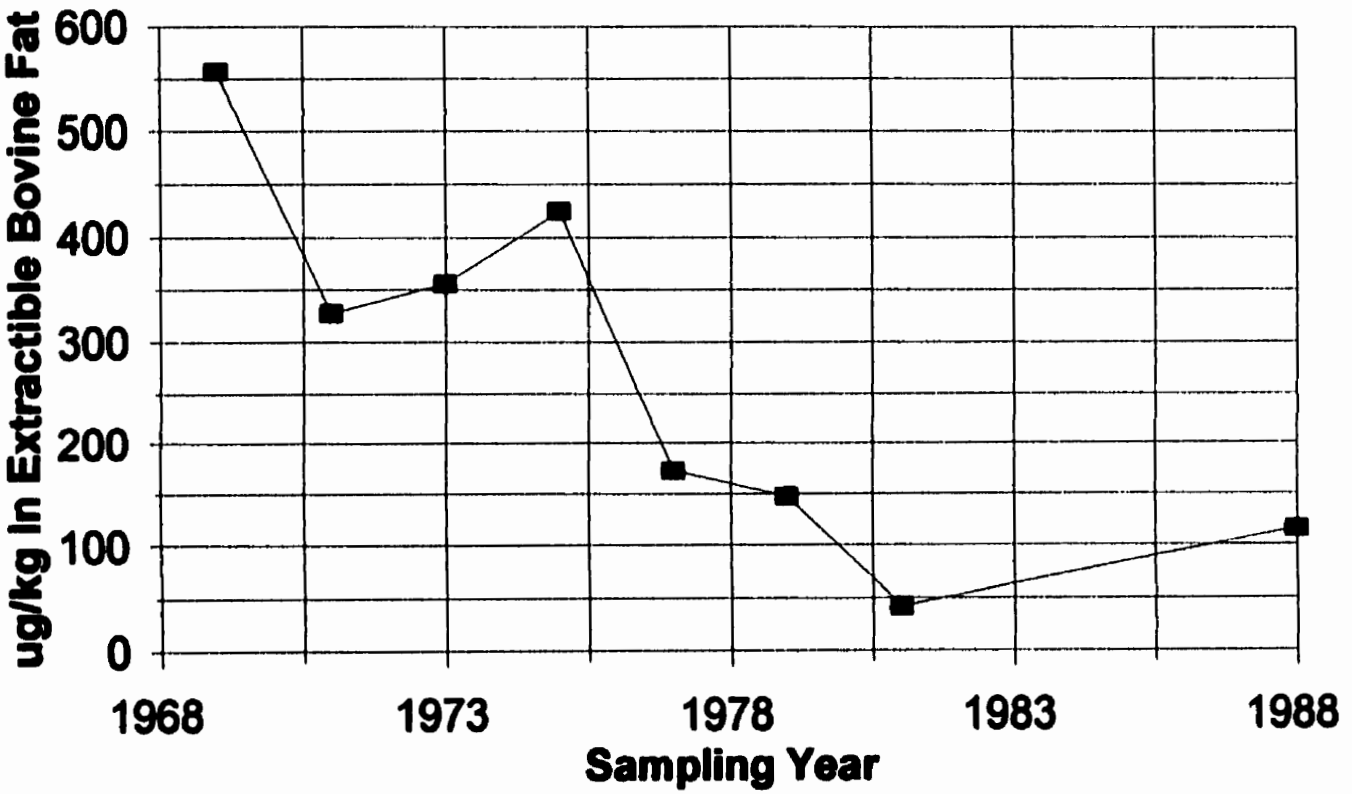

Table 2.3.3.2 Comparison of composite samples and number of carcasses in the years of Residue Testing in Pork:

Year of Study	Number of Carcasses	Composite Samples
1969-70	170	35
1971-72	101	24
1973-73	55	16
1975-78	18	5
1979	20	4
1981	190	38
1986-88	150	?

Figure 2.3.3.2 Mean Organochlorine Residues in Porcine Carcasses: Ontario Findings From 1969 to 1981.

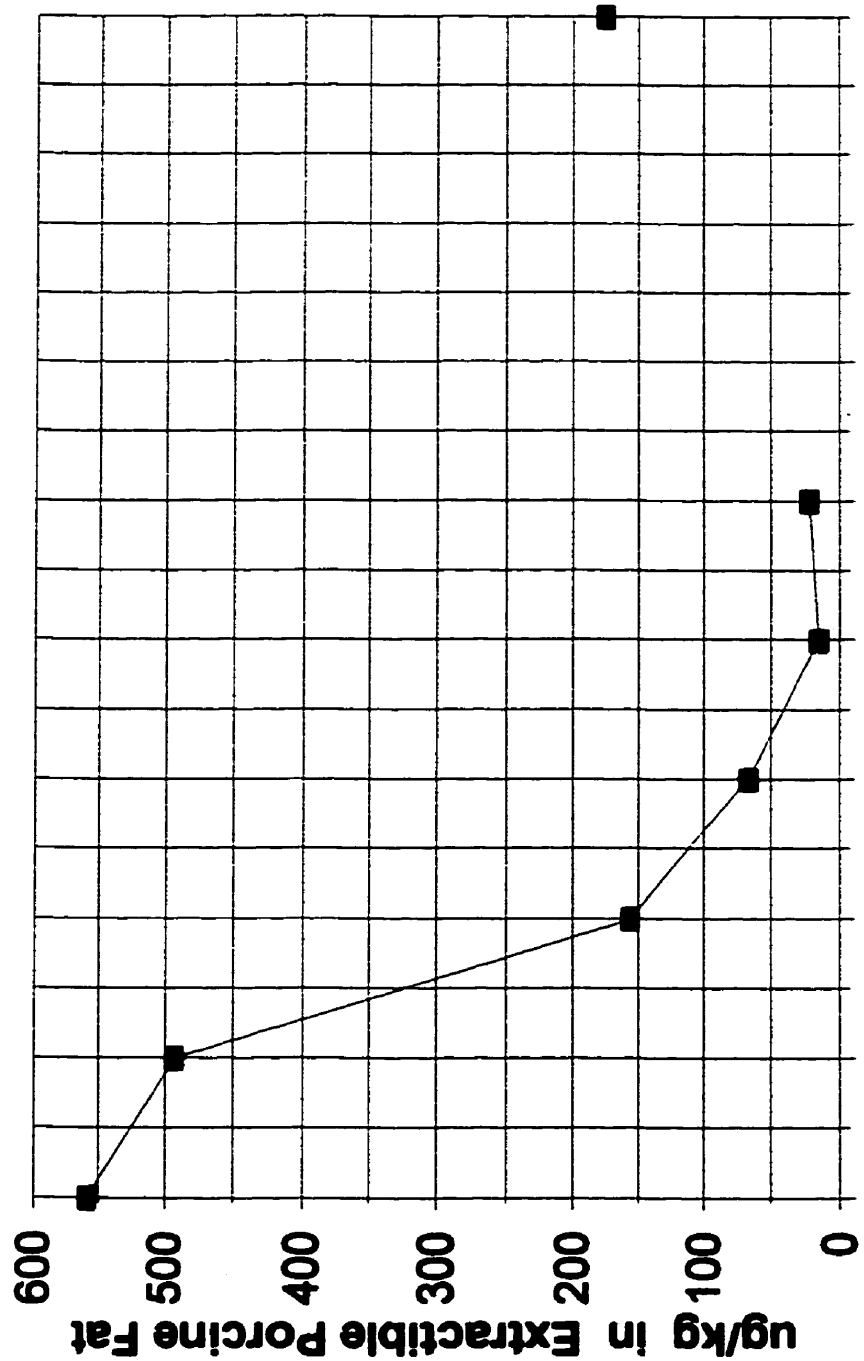

Figure 2.4.1.1 Mean Organochlorine Residues in Human Adipose Samples: Ontario Findings From 1969 to 1984

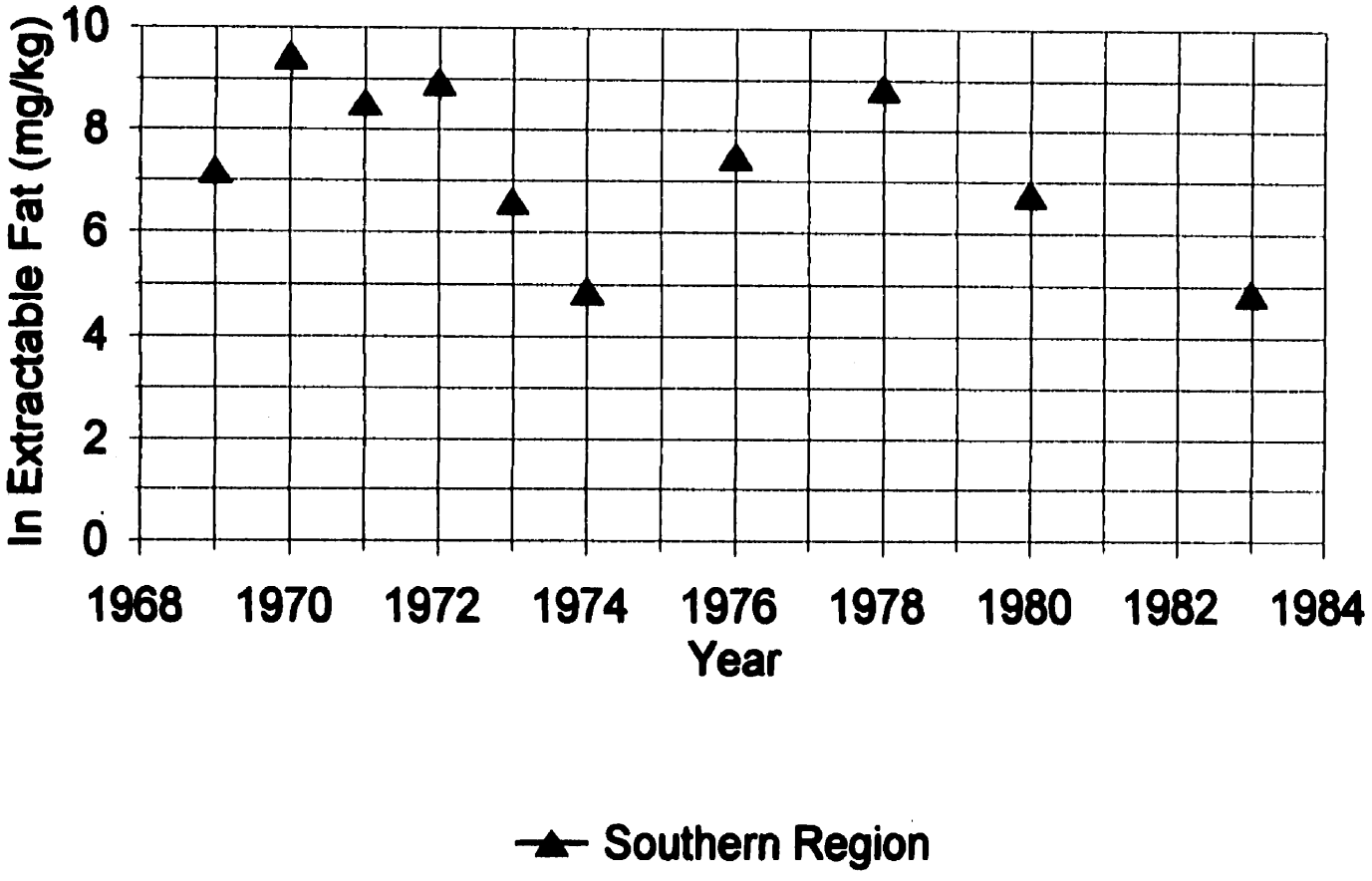

Table 2.4.1.1: Individual Components of Total Organochlorine Residues In Adipose Samples Year and Percent of Total Organochlorine Residue

	Percent of Total Organochlorine Residues									
Year	**1969**	**1970**	**1971**	**1972**	**1973**	**1974**	**1976**	**1978**	**1980**	**1983**
Total DDT	98.7	85.2	78.2	62.1	59.6	63.9	68.5	71.1	67.9	54.0
Dieldrin	1.25	2.1	1.9	1.1	2.6	1.4	0.5	1.1	1.6	0.6
PCB's	0	12.7	19.9	35.7	36.3	32.8	29.4	26.0	28.1	43.1
HCB's	0	0	0	1.1	1.5	1.9	0.4	0.3	0.3	0.2
Heptachlor	0	0	0	0	0	0	0.7	0.7	1.2	1.7
Chlordane	0	0	0	0	0	0	0.5	0.8	0.3	0.3
Mirex/HCH	0	0	0	0	0	0	0	0	0.6	0.1

1. Values given represent total organochlorine found in human adipose samples from the southern region of Ontario.
2. Total DDT = DDT, DDE, and TDE

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Chapter 3 Factors that Influence Human Exposure to Organochlorines in Ontario

The previous chapters have shown that, in Ontario, exposure to organochlorines occurred over several decades. The bulk of exposure occurred from approximately 1947 to 1977, with fluctuations in exposure during this period. The average person was exposed not only to DDT and PCB but several of the other organochlorines such as dieldrin, chlordane, heptachlor, and lindane. The exposure information emphasizes the importance of both the year of exposure and source of exposure when determining exposure histories, since exposure in the early to mid years of organochlorine use could be expected to be significantly higher than exposure in the later years. The findings also suggest the importance of examining both timing of exposure in relation to the age of the individual and the specific food consumption patterns during the decades. These factors can all influence the overall lifetime exposure scenario when ascertaining the relationship between organochlorines and human health effects. In order to assess the effect of timing of exposure, an examination of birth cohorts exposed during different periods is necessary. As well, an examination of trends in food consumption during the different exposure periods is necessary, to determine the impact on the total life time exposure in the general population. The evaluation of this information in totality will provide the most accurate picture of organochlorine exposure in Ontario which will influence the ability to detect any possible relationships between organochlorine exposure and breast cancer.

The question of how organochlorines might produce cancer should also be considered at this point. Carcinogens often progress through several stages including initiation, promotion and progression (Weinstein et al, 1995). Initiation of cancer frequently occurs by the agent damaging the cellular DNA, while an agent which promotes cancer, usually has very weak or no carcinogenic activity, but enhance tumour yield following a low or suboptimal dose of a carcinogen. The progression stage of carcinogenesis can occur during the entire lifespan of the individual (Weinstein et al, 1995). Since organochlorines in general are not known to be strong mutagens they are likely to act as promoters in the carcinogenic process. Investigating exposure during different life stages will help to determine if the latter assumption is plausible.

To investigate the importance of timing of exposure, we need to determine the stage of exposure for the individual or group. From the residue information we have established several phases of exposure which include: a non exposure phase prior to approximately 1947; an increasing exposure phase from 1947-1955; a peak exposure phase from approximately 1956-1965; a decreasing exposure phase from 1966-1974; and a minimal exposure phase from approximately 1975 to present. Within each of these periods, individuals of different ages or generations would have been exposed. The relative amount of exposure and the duration of exposure would be variable for each of these groups based on the age or time at first exposure. It would seem logical therefore that subsequent health effects from exposure to organochlorines could also express variability and that this may correspond with the phase, timing and duration of exposure. With this

in mind, it would be extremely important to consider both the age of the individual/group and the years during which these individuals received the bulk of their exposure. From this perspective, we have established several birth cohorts that would allow us to evaluate the potential significance of organochlorine exposure in the population. With this information we can determine which groups may be considered high risk or which groups could be considered not at risk from exposure to organochlorines. We have also investigated several factors which influence the total body burdens of the cohorts during the years of organochlorine exposure. These include trends in food consumption, parity, breast feeding, and other confounding factors related to breast cancer.

3.1 Age and Time of Exposure to Organochlorines

To study the effect of birth cohorts and exposure phases we have established five age categories that allow comparisons of exposure during different human developmental phases. The first exposure group represents individuals from age zero to nine years of age. This stage marks a phase of rapid growth and development. The second group consists of individuals age nine to nineteen years, accounting for the prepubescent to sexual phase of maturation. Group three includes individuals from age twenty to forty-nine years, namely the premenopause phase, while group four consists of individuals age fifty to greater than eighty years. The latter group represent women in the post menopause phase. An examination of exposure during different exposure phases for each of the age groups will allow us to determine, firstly which birth cohort group at each age category received the greatest exposure (exposure during the peak exposure phase), and

secondly which birth cohorts have the highest rates of breast cancer. It will also allow us to investigate the possibility of organochlorines acting as initiators or promoters.

3.1.1 Exposure During Age Zero to Nine Years

There are several key elements that make this stage of human development a special case when assessing the potential impact of organochlorine exposure. The rapid growth and maturation of bodily organs makes this age group particularly sensitive to external influences. The very early stages of human development are critical for the future health and proper maturation of the infant (Guyton, 1991). Exposure to organochlorines during this stage can occur via maternal transfer, placental transfer, via breast milk or through food consumption. Exposure to high levels of contaminants in mother's milk, formula or in vitro could significantly affect the early body burdens of these individuals or groups and this would be particularly true for those individuals born to mothers exposed during peak organochlorine use. Exposure in early childhood can also strongly influence an individual's health in adulthood (National Research Council, 1993). Children may therefore represent a specific high risk group. Several scientific arguments have been put forward that cells initiated in childhood have a much higher probability of being promoted over the expected life of the individual and thus have a greater chance of progressing to cancer (National Research Council, 1993; Fisher, 1995). This may simply be related to the fact that, if these cells are initiated at this early stage of development, there are more years for the promotion of cancerous cells to occur. However, increased sensitivity of cells at this stage is also of concern. Since cancer has been postulated to take

approximately 10-15 years or greater to express itself, exposure during childhood to cancer causing or promoting substances may take years to show its impact (Fisher, 1995).

Exposure via food sources has also been shown to be proportionately different for children than for adults. Studies of consumption patterns in children show that during their early years they have a tendency to consume relatively large amounts of a small number of food groups (National Research Council, 1995). Similarly, children tend to consume higher levels of food per body mass as compared to adults, thus receiving greater dietary exposure on a milligram per kilogram of body weight to pesticide residues. Children age 1-5 years ingest approximately six times more fruit, five times as much milk, three and a half times as many grain products and approximately twice as much meat and vegetables per body weight as adult women ages 22-30 (National Research Council, 1995). The high rate of consumption of specific products such as milk may result in high exposure to contaminants from these sources. This situation is even more relevant when this age at exposure occurred during peak or increasing periods of organochlorine use or, in the case of breast feeding, when the mother had experienced high levels of exposure leading to high personal body burdens. Therefore exposure during the years from 1947 to approximately 1965 in particular may impart an increased risk of developing adverse health effects if exposure to organochlorines during the infant and early childhood phase is critical.

If exposure during early childhood is critical and organochlorine exposure is a risk factor in breast cancer development, then it would be reasonable to assume that birth cohorts exposed during the infancy to nine years of age stage have a higher risk of developing breast cancer than those not exposed during this phase of development. Similarly, if negative health effects from exposure to organochlorines are dose related individuals from this exposure group, born during peak exposure, could be expected to be at greatest risk in comparison to those individuals born during non exposure or minimal exposure periods. Table 3.1.1.1 shows that, if the critical age of exposure to organochlorines was from age zero to nine years of age, then the birth cohorts at most risk of developing breast cancer are those exposure from 1955 to 1965. This represents any individual born from 1946 to 1965. This birth cohort represents a large group of individuals and one must appreciate that, from these birth cohorts, the individuals at most risk would be those exposed for the greatest number of years during this stage of development during the peak phase of organochlorine use. Table 3.1.1.1 also shows the age of individuals in each birth cohort group during subsequent years, which is of interest in the breast cancer situation, where detection of disease does not generally occur until late pre menopause or post menopause. The latter may be simply related to an inability to detect small slow growing masses.

3.1.2 Exposure During Age Ten to Nineteen Years Old

This stage of human development may represent another critical age of exposure but for different reasons. The teenage years represent a time of rapid growth, development and sexual maturation. During the initial portion of this phase, there is rapid growth and as the

body approaches puberty it may be particularly sensitive to external influences. Early exposure to xenoestrogens such as zeranol for example has been postulated to result in early age of menarche. It seems plausible that exposure to estrogenic pesticides at this stage of development may actually increase the rate of maturation and lead to an earlier age of menarche. The breast tissue, which is undergoing development during this stage may be acutely sensitive to increased estrogen levels or environmental contaminants. Studies investigating radiation point to a high risk phase during this particular maturation stage and would suggest that similar findings may be found for organochlorine exposure.[1] Exposure at this stage of development may also result in the promotion cancerous cells and if promoted, these cells have a greater number of years for the progression of cancer, compared to cells promoted during the adult stage of life.

Food consumption during this stage of development is also high since the body requires increased levels of nutrients for growth (National Research Council, 1995). High consumption of animal proteins, fats and other food products, especially during the years of peak organochlorine use, would have resulted in relatively high exposure rates for this group. Table 3.1.2.1 shows the birth cohorts that were exposed during the different phases of exposure and the various ages of these birth groups during subsequent years. If exposure to organochlorines at this stage is critical, the cohorts born between 1936-1955 would have received peak exposure during this phase of human development and be at

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[1] Morrison H (1996) Laboratory Centre for Disease Control Department of National Health and Welfare, Ottawa, Ontario, Canada. Personal communication

greatest risk. Of this group, the birth cohort members most at risk would be those birth cohorts which received the greatest number of years of exposure during the peak use. The birth cohorts born from 1947 to 1955 would therefore be at greatest risk. Of interest is the finding that these birth cohorts would have also experienced exposure in all stages of human development since those born from 1947 to 1955 in particular would have experience exposure throughout their entire life from birth to adulthood and during several phases of exposure. They would also be the group that has the greatest number of years of lifetime exposure. Therefore, this group in particular may be the group at most risk of developing adverse human health effects.

If there were no critical age of exposure but rather, total exposure or duration of exposure was the driving factor in the relationship between organochlorine use and the development of adverse human health effects, the birth cohorts born from 1947 - 1955 would be at most risk. If a dose response relationship was present, we would expect to find different rates of disease for the various phases of exposure. If, however, the duration of exposure is the most critical, those individuals exposed for the greatest number of years should be at greatest risk of developing breast cancer. These findings should hold true in particular when comparing breast cancer incidence rates between non exposed and exposed birth cohorts.

3.1.3 Exposure During Age Twenty to Forty-nine Years

This stage of life represents the reproductive phase of development for females and is

therefore greatly influenced by several hormones, of which estrogen plays a significant role. The breast tissue of women has fully developed by this point (except structural changes which occur during pregnancy), but is still subject to changes in physical structure as hormones fluctuate during the menstrual cycle (Fisher, 1995). Exposure to environmental chemicals may promote or initiate cancerous cell growth which may be expressed as a palpable lump years later. The exact time required to show clinical signs of breast cancer, after cells have been initiated and promoted, has not been determined. However, scientists have postulated that a typical lag phase of 10-15 years or more may be expected (Fisher, 1995). This means that exposure during the reproductive years may occur during a time when there is cancerous cell growth without clinical manifestation, and that clinical signs from exposure in this phase may actually become apparent in a later stage of development, such as the post menopause stage. Elevated rates of breast cancer in the post-menopause phase may therefore simply be due to the lack of recognition of masses in the pre-menopause or earlier stages of human development. Increased incidence rates in older women may be solely related to an increased ability to recognize masses as they attain a palpable size over time.

Exposure during age twenty to forty-nine is also unique in that it is during this phase that the majority of women naturally undergo tremendous changes in hormone levels. For example, estrogen levels can be expected to fluctuate routinely during the menstrual cycle, but can also rise dramatically during pregnancy (Guyton, 1991). Factors such as lactation, parity and abortions would also impart a dramatic change in the hormonal balance.

Table 3.1.3.1 shows the birth cohorts exposed at age 20 - 49 years of age during the various phases of exposure.

If exposure to organochlorines between age twenty and forty-nine is important, then several cohorts would be at greater risk of developing breast cancer in comparison to cohorts not exposed during this phase. The birth cohort group exposed during the peak phase of organochlorine use would be those individuals born during the years 1906-1945. If this age at exposure is critical, this group could be expected to be at increased risk particularly in comparison to those born prior to 1898. This " at risk group" would be age 35-74 in 1980. This approximate age range is consistent with the group of women expected to develop breast cancer in the 1980's. However, exposure at this critical age may be only one of the factors in the role of organochlorine exposure and human health, and may not be the determining factor.

3.1.4 Exposure During Age Fifty and Greater

Exposure during the post-menopause phase may be considered a critical phase of exposure, in that women of this age may already harbor cells that have been previously initiated and therefore primed for stimulation. In most types of cancer, increasing age by itself infers an increased risk of developing the disease (Fisher, 1995). If organochlorine exposure does indeed promote breast cancer development, exposure at this stage of life could accelerate tumour growth leading to elevated levels of detection in subsequent years. The overall impact may be minimal if this effect is a minor factor however the effect may be substantial if the tumour growth rate is rapid following exposure to

organochlorines.

If one examines the exposure of the various birth cohorts as outlined in Table 3.1.4.1 it is evident that birth cohorts born prior to 1867 will not have experienced any exposure to organochlorines during this stage of development. Birth cohorts from 1875-1915 would have received maximum exposure to organochlorines, as these individuals were exposed during the peak organochlorine use. These individuals would have been age 65 or greater in the 1980s when the first increases in breast cancer rates became evident in the post-menopause women. Similarly, birth cohorts exposed during the late 1960s early 1970, when exposure was still high but declining, would be greater than 55 years old in the 1980s and this group may also be influencing the increased incidence of breast cancer in post menopause women. While the exposure to organochlorine may not be the only factor, the potential for organochlorine exposure to promote breast cancer at this stage of human development must be investigated.

While exposure during a particular critical age may impart a significantly higher risk of breast cancer, it is conceivable that more than one or perhaps any age at exposure confers an increased risk of developing breast cancer over the lifetime of women. The fact that the true relationship between organochlorines and breast cancer remains unknown at this point necessitates a full evaluation of all the possible age at exposure effects. Conversely, if exposure at any age is not related to the development of breast cancer, we would expect to find no difference in the incidence of breast cancer for any of the birth cohort

groups compared to those individuals not exposed to organochlorines. If however, a relationship does exist between exposure to organochlorines and the development of breast cancer, it would be important to determine which groups of women in Ontario are at greatest risk of developing breast cancer. This information would also provide some indication of the time period during which we could expect the greatest rates of breast cancer and conversely when we could expect incidence rates to decline to normal levels.

3.2 Exposure Factors

There are several factors that can influence the total lifetime exposure to organochlorines in individuals and groups of various ages. These elements need to be identified in order to determine how they will impact the overall exposure picture during the time frame under study. While individual preferences or changes will impact the individual's body burden, general fluctuations in societal preferences will influence the population's exposure to organochlorines. Of particular significance to the organochlorine issue are changes in the quantity and type of food consumption, lifestyle changes such as parity, age at first pregnancy, lactational history, and body weight, since increasing or decreasing trends in these confounding factors will significantly affect the total amount of exposure found in both individuals and groups in Ontario. Monitoring these changes will indicate which direction the factor will impact the relationship between organochlorine exposure and adverse health effects.

3.2.1 Breast Feeding and Parity

The presence or absence of breast feeding and parity number can have a significant impact on a woman's lifetime exposure to organochlorines. Women who breast feed experience a reduction in their body burdens as a direct result of secreting organochlorines into the breast milk (Jensen and Slorach, 1990). Levels of organochlorines secreted in breast milk of first time mothers are significantly higher than that found in mothers of higher parities (Mes et al, 1993). Mes et al (1993), found that multiparous mothers had lower levels of DDE, beta-HCH, and PCB's then mothers breast feeding their first child. The number of pregnancies and the duration of breast feeding also has a significant impact on the net level and duration of exposure for these women. For example, a woman who had multiple full term pregnancies at an early age and has breast-fed these infants would have substantially lower levels of organochlorines than a nulliparous women of a similar age and exposure history (Romieu et al, 1995). The former woman's body burdens would be both significantly lower and have lower levels during her accumulation period.

Placental transfer of organochlorines from the mother to the fetus has also been recorded (Rogan et al, 1987). Organochlorines can be detected from blood samples taken from umbilical cords and may thus represent a method to decrease the mother's body burdens of these chemicals. Women who consumed high levels of fresh water fish were found to have greater levels of organochlorines in umbilical cord samples taken at time of delivery (Rogan et al, 1987). First born children to mothers with high body burdens can thus expect to have a greater level of placental transfer of organochlorines and if breast fed will have greater levels of organochlorines than their counterparts who were not breast fed or

were second, third or later parity (Romieu et al, 1995). While this would increase the exposure of the child it would decrease the exposure of the breastfeeding women, and this would cause significant confounding in this study and in other studies where breastfeeding have not been taken into consideration.

The timing of organochlorine exposure for both the mother and infant can have a significant impact on both individuals' body burdens. For example, children born after 1975 (when exposure from food sources where at a minimal level) could receive substantial exposure if breast fed by mothers that accumulated their body burdens during the peak use of organochlorine insecticides. An infant born in 1985 to a thirty-year old mother would receive breast milk containing organochlorines residues from an exposure period for the mother during 1955 - 1985. While the amount transferred depends on several factors such as the mother's diet, previous breast-feeding history, parity etc. the outcome of a short period of exposure during the history of organochlorine use can thus be found to have an impact for many generations. The transfer of body burdens may therefore result in a continuation of detectable human body burdens for several generations despite declining exposure, the significance of which has yet to be determined.

The diet of the mother can have a direct effect on the level of organochlorines secreted in the breast milk. For example, studies have shown that women consuming freshwater fish in the Michigan area have infants which have higher body burdens than those whose mothers consume few to no freshwater fish (Swain, 1991). Schwartz et al (1983), showed

a positive correlation between PCB levels in breast milk and the consumption of contaminated freshwater fish, while Mes et al (1993) showed no such relationship with consumption of fish. However in the latter study there were a low percentage of freshwater fish eaters which could have biased the results towards the null. Similarly, increased exposure to fatty food such as milk and animal products could result in higher body burdens which could subsequently result in higher levels of residues in breast milk. Exposure during this critical phase of development may have a significant impact on the long-term health of the infant receiving contaminated breast milk.

If breast feeding significantly decreases a woman's body burden then women who breast feed should be at decreased risk of developing adverse health effects, as a result of lower body burdens and shorter lifetime exposure to organochlorines, than women who do not have a history of breast feeding. The greater number of pregnancies and frequency or extended duration of breast feeding should subsequently result in a further reduction in the maternal body burdens and substantially decrease adverse human health risks from exposure to organochlorines.

Very little information exists on breast feeding trends in Ontario or Canada. Prior to 1965, little attention was given recording breast feeding trends. Myeres (1979) published one of the only retrospective examinations of infant feeding practices. The study examined breast feeding practices of women in Canada on a provincial basis from 1965 to 1978. The study found that 4% of mothers breast feed exclusively, while 29% used breast

feeding supplemented with bottle feeding during 1965-1971. The duration of breast feeding tended to be short with approximately 75% of women giving up breast feeding by three months. Of interest is the finding that the proportion of breastfeeding mothers was three to four times greater in the highest income group than the lower income groups. Breast feeding trends increased only slightly until 1978 but the duration still remained short (Myeres, 1979). A longitudinal study following 403 women in 1977 to 1979 showed that 71% of the subjects preferred breast feeding but the medium duration of breast feeding was only 3.5 months (Yeung et al, 1981). In the United States, the proportion of breast feeding increased from 25 % in 1973 to 35% in 1975 while the duration remained approximately the same with only 4% of infants breast fed for greater then 3 months (Hendershot, 1981).

Several societal trends such as industrialization, urbanization, decreasing early childhood deaths, increasing participation of women in the labour force and advancements in contraceptive methods have influenced the fertility rate in Canada (Chui, 1996). In Ontario, the number of full term pregnancies per women of child bearing age has decreased dramatically over the decades. Canadian vital statistics indicate that the birth rate per 1,000 populations in Ontario steadily increased from 19.1 in 1947 to 27.0 in 1954 (Dominion Bureau of Statistics, 1954). By 1959, the peak of the baby boom, the average number of children born per woman of childbearing age was 3.94 and declined rapidly from this peak to 1.58 by 1987. By 1990 the average number of children per woman of child bearing age had increased slightly to 1.71 however by 1993 the number had

decreased to 1.66 (Chui, 1996).

The timing of pregnancies has also changed over the decades. The general trend in Canada is for women to give birth to their first child at a much later age than previously. For example the proportion of women age 30-34 giving birth to their first child increased from 12% in 1951 to 31% in 1993 (Statistics Canada, 1995). The number of childless married women has also increased over the decades and these trends are expected to continue over the succeeding years. This change in age at first pregnancy could mean that a greater number of women would harbour their body burdens for longer periods of time.

Another source of exposure to exogenous hormones is the birth control pill. The birth control pill was first used in Ontario starting in 1960. There have been marked changes in the estrogen content over the years, with early oral contraceptives containing greater levels of estrogens than found in the more modern types of birth control pills (Health Canada, 1995). Sequential oral contraceptives were used early in the pills development. These tablets contained high levels of estrogen in the first part of the cycle with progestin added in the latter part of the cycle. In the early 1970s continuos low-dose progestin pills were introduced which did not contain estrogen. Triphasic and biphasic oral contraceptives were added to the market and with these pills the dose of estrogen and progestin varies throughout the cycle. Since 1985 the vast majority of oral contraceptives contain between 30 and 35 mg of ethinyl estradiol. These newer pills have been regarded as safer than their predecessors and resulted in the removal of an upper age limit for oral

contraceptive use (Health Canada, 1995).

An extensive search of the literature on trends in oral contraceptive use showed that there is a great gap in recording both the number of women taking oral contraceptives and the duration. While numerous papers could be found on recommendations for prescribing oral contraceptives, historical use patterns were not found. The lack of this information is disappointing since these forms of exogenous hormones would have been used by a substantial number of women over the decades since their development, and these substances have been considered to be a risk factor in the development of breast cancer (Fisher, 1991).

3.2.2 Food Consumption Habits

Food consumption patterns can also play a definite role in the total level of exposure and resulting body burdens in both the individual and population. Meat eaters and people who have a history of consumption of high levels of animal products tend to have elevated levels of organochlorines, compared to individuals who are vegetarians (Swain, 1991). This would be particularly true for those individuals consuming large quantities of red meat on a regular basis. Changing societal trends in meat and milk consumption over the time period under study could have a subsequent influence on the population's total body burden of organochlorines. These factors could therefore have a significant impact on the risk of developing adverse human health effects from exposure to organochlorines for both the individual and the population.

Canadian diets have changed significantly over the decades with a steady increase in the average annual red meat (beef, pork, mutton and veal) consumption from approximately 51 kg in the 1920s to 84 kg per person in 1976 (Caputo and Poutanen, 1990). Similarly, poultry (chicken, turkey, and other fowl) consumption rates escalated from 3 kg per person to 20 kg per person annually. During the late 1970s and the 1980s red meat consumption decreased while poultry consumption increased, as Canadians responded to educational campaigns encouraging healthy eating (Caputo and Poutanen, 1990). By 1988 the average annual consumption rate of red meat had dropped to 71 kg per person, with most of the decrease resulting from declines in beef consumption. Poultry consumption increased to an average annual amount of 29 kg per person with most of the increase resulting from an increased chicken intake (Caputo and Poutanen, 1990).

Milk and milk product consumption has also changed dramatically since the 1960s with a trend for decreasing consumption of higher fat levels. In 1967, sixty-six litres of 3.25 % milk was consumed per person per year compared to 3.3 litres of skim milk per person per year. While the consumption of skim milk has remained stable over the years, a steady increase in the consumption of 2% verses 3.25% milk has been documented. By 1988 Canadians consumed 65 litres of 2% milk (up from 21 litres in 1967) verses 28 litres of 3.25% milk. These changes have significantly decreased the average fat intake from milk. However, during this same time period the average consumption of cheese products has increased dramatically from 2.8 kg to 6.2 kg per person per year from 1967 to 1988 (Danielson and Robbins, 1986). The total consumption of fats and oils has increased

slightly from 1967 to 1988 but the type of fat consumption has changed significantly with decreased levels of butter and increased levels of margarine.

Mean egg consumption has also decreased from 250 eggs per person per year in 1967 to 205 eggs in 1988. Fish consumption, which has been suggested as an important source of organochlorines exposure, has increased from the late 1960s to the late 1980s. In 1967, average annual consumption of fresh and frozen fish and shellfish increased from 2.5 kg to 4.9 kg in 1988 (Danielson and Robbins, 1986). Body burden samples taken from native Indians have shown they tend to have greater body burdens than the average Ontario resident (Ayotte et al, 1995). These individuals could make an important "at risk group" for studying the health effects from exposure to relatively high levels of organochlorines.

Consumption of fresh fruits and vegetables have increased from an average annual rate of 54 kg fruit and 34 kg vegetables in 1967 to 62 kg fruit in 1987 and 57 kg of vegetables in 1988. However, in 1988, fresh fruit consumption had decreased dramatically to the levels consumed in the late 1960s of 54 kg per person per year. Cereal consumption has also increased over the years and the consumption of refined sugars has decreased over a similar time period (Danielson and Robbins, 1986). The implications of these changes on the relative exposure to organochlorines will be discussed in the analysis section of this paper where their influence can be determined for the various age cohorts under investigation.

3.2.3 Obesity

Increased body weight has been proposed to increase the risk of developing cancer (Peto, 1996). In relation to organochlorines, obese individuals on average can be considered to have higher rates of exposure simply due to the increased levels of food consumed. If increased consumption of food containing peak levels of organochlorines are ingested, it would be logical to assume that these individuals would have the highest exposure rates.

3.3 Summary

The over all examination of the various birth cohorts shows that a 28 year exposure period to organochlorines, from roughly 1947 to 1975, impacted several generations or birth cohorts. One could creditably argue that knowledge of the amount and precise timing of exposure and sorting out which critical age during which phase of exposure, may be pivotal in determining the influence of organochlorine exposure to human health. When exploring human health effects from exposure to organochlorines, the birth cohorts and confounding exposure factors must be taken into consideration as these elements can have significant impact on the duration and extent of life time human exposure. Given the changing trends in Canadian lifestyles and food consumption patterns, and the fact that many of these changes directly affect the level of exposure to organochlorines, the previous section clearly demonstrates the need to investigate organochlorine exposure from both a time period perspective and a birth cohort perspective. Once exposure has been determined on this basis, high risk groups can be established and specific groups can be investigated to determine how societal trends would influence their lifetime body

burdens. The combined information can then be used in the analysis of the cancer data when trying to distinguish if a relationship exists between a organochlorine use/exposure and breast cancer incidence.

The information generated in this section suggests that several birth cohort groups may be at greater risk of developing adverse health effects if exposed during various critical ages of exposure given a causal relationship between organochlorine exposure and breast cancer development. This does not mean that a critical age of exposure is an absolute criterion for the development of adverse effects. Nor does it prove that exposure to organochlorines does indeed result in breast cancer development. A critical age of exposure may modify the relationship, and should be explored to more fully understand the relationship between organochlorines and breast cancer development. Since the potential relationship between organochlorines and human health has not been definitely established investigations of likely scenarios could cast a great deal of light on the potential relationship and help to direct further avenues of research.

Table 3.1.1.1 Birth Cohorts Exposed During Infancy to nine years During the Different Phases of Exposure:

Exposure Phase	Birth Cohorts	Age at 1980	Age at 1990
No Exposure (prior to 1947)	<1938	> 42 years	> 52 years
Increasing Exposure (1947-1955)	1938-1955	25-42	35-52
Peak Exposure (1955-1965)	1946-1965	15-34	25-44
Decreasing Exposure (1965-1975)	1956-1975	5-24	15-34
Minimal Exposure (1975-1985)	1966-1985	unborn-14	5-24

Table 3.1.2.1 Birth Cohorts and Exposure Stages for Age 10 to 19 Years

Exposure Phase	Birth Cohort	Age at 1980	Age at 1990
No Exposure (prior to 1947)	<1928	>52 years	> 62 years
Increasing Exposure (1947-1955)	1928-1945	35-52	45-62
Peak Exposure (1955-1965)	1936-1955	25-44	35-54
Decreasing Exposure (1965-1975)	1946-1965	15-34	25-44
Minimal Exposure (1975-1985)	1956-1975	0-24	15-34

Table 3.1.3.1 Birth Cohorts During Various Exposure Phases at Age 20 to 49 Years

Exposure Phase	Birth Cohort	Age at 1980	Age at 1990
No Exposure (prior to 1947)	<1898	>82 years	>92 years
Increasing Exposure (1947-1955)	1898-1935	45-82	55-92
Peak Exposure (1955-1965)	1906-1945	35-74	45-84
Decreasing Exposure (1965-1975)	1916-1955	25-64	35-74
Minimal Exposure (1975-1985)	1926-1965	15-54	25-64

Table 3.1.4.1 Birth Cohorts During Various Exposure Phases at Age 50-80 Years

Exposure Phase	Birth Cohort	Age at 1980	Age at 1990
No Exposure (prior to 1947)	<1867	>113 years	>123 years
Increasing Exposure (1947-1955)	1867-1905	75-113	85-123
Peak Exposure (1955-1965)	1875-1915	65-105	75-115
Decreasing Exposure (1965-1975)	1885-1925	55-95	65-105
Minimal Exposure (1975-1985)	1895-1935	45-85	55-95

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Chapter 4

The Occurrence of Breast Cancer and Its Relationship to Timing and Duration of Exposure to Organochlorines in Ontario

4.1 Background

To determine if a relationship exists between exposure to organochlorines and breast cancer, several factors were taken into consideration. The source, timing (both year of exposure and age at exposure), and duration of exposure, which have been established in the previous sections, were evaluated to determine the relative impact of these factors. There are several hypothesis tested by this study and they are as follows: that any exposure to sufficient (suggesting a potential minimal or threshold dose, the level of which has yet to be established) organochlorines at any age confers an increased risk of developing breast cancer; that the occurrence of outcomes is dose related, such that exposure during peak periods of organochlorine use confers greater risk, while exposure to lower levels confers less risk; the dose response effect may also be related to the total dose over a lifetime, with those individuals receiving the highest dose for the longest duration, being at most risk of developing breast cancer; that the timing of exposure is influential, so that exposure at a critical age further increases the risk of developing breast cancer; that the combination of these factors would confer the greatest risk of developing breast cancer and subsequently exposure at a critical age, plus exposure during peak organochlorine use, plus increased duration of exposure infers the greatest risk of developing breast cancer.

The exposure assessment section of this investigation established that several phases of exposure to organochlorines exist, specifically a period of no exposure (<1947), increasing exposure (1947-1955), peak exposure (1955-1965), decreasing exposure (1965-75), and minimal exposure (1975-1985). These different exposure periods allow one to compare cancer incidences between different "doses" of organochlorine exposure. If a relationship exists between exposure to organochlorines and breast cancer, one would expect that the incidence of breast cancer in the non exposed group would be lower that the incidence of breast cancer in the exposed groups. If a dose response situation exists then one could expect to find a higher incidence of cancer in those groups exposed during the increasing peak and decreasing phases of organochlorine use, with the greatest incidence of cancer in individuals exposed during peak organochlorine use. Individuals exposed during minimal use and those born after 1985 would be expected to have a lower incidence of breast cancer, possibly as low as those individuals who never had exposure to organochlorines at any age, if organochlorine exposure is a driving factor in breast cancer development.

If a critical age of exposure to organochlorines plays a role in the risk of developing breast cancer, it would be logical that those individuals who received the most exposure during the critical age of human development would be at greater risk and subsequently have a higher incidence of breast cancer. Because previous investigations have not established a critical phase of exposure, we have investigated several potential critical ages for exposure (as outlined in Chapter 3), which may impact the relationship between organochlorine

exposure and the development of breast cancer. Non exposed birth cohorts were compared to specific birth cohorts exposed at critical ages 0 -9 years, 10 - 19 years, 20 - 49 years and 50 - 80 years. Previous arguments for choosing these critical ages of exposure have been established in Chapter 3.

Logically, if a critical age of exposure exists, one would expect to find a significant difference in breast cancer incidence between the critical age and the other birth cohorts. If the impact is dose-related, one would expect to see a higher incidence in those individuals exposed to peak levels of organochlorines during the critical age of exposure compared to those exposed to lower levels. If critical age, exposure period and duration (lifetime exposure) plays a significant role, we would expect to see the highest incidence of breast cancer in those birth cohorts that received exposure at the critical age during the peak phase of organochlorine use and for the longest duration. However, if exposure at any age imparts an increased risk of breast cancer one would expect to see a greater incidence of breast cancer in any of the exposed groups in comparison to the non exposed birth cohorts assuming an ability to account for other confounding factors.

4.2 Method

Several data sources were used to evaluate the relationship between organochlorine exposure and the incidence of breast cancer. In our first attempt to evaluate the situation we tried to access raw data from previously published reports, by the Ontario Ministry of Agriculture and Food, regarding human body burdens and food residue levels in order to

determine if geographical variations in exposure to organochlorine and breast cancer existed in Ontario. However, access to these raw data was unavailable (Ripley, 1995). The published data remained the best source of information available. Breast cancer and population census data were derived without difficulty, and the following paragraphs outline the steps taken to evaluate the different data sets in order to determine the type of relationship that exists between organochlorine exposure and breast cancer development.

a) Breast Cancer Data

Breast cancer data were derived from The Ontario Cancer Treatment and Research Foundation, Division of Epidemiology and Statistics (Feringer, 1995). The data represent breast cancer cases diagnosed from 1964 to 1993. The data set contains information regarding the age, sex, site and year of diagnosis. Since this investigation was restricted to determining the relationship between organochlorines and breast cancer in women; data regarding male breast cancer cases were not utilized. From the initial data set, a new data set (female) was created using SAS statistical analysis to provide information on female breast cancer cases only. The new data set subsequently contained the variables age, year of diagnosis, and frequency of cases for each year and age. For example, in the year of diagnosis of 1964, when the variable age equals 68, the variable frequency equals 49 so that in 1964 there were 49 cases of breast cancer diagnosed in sixty-eight year old women. This new data set provides the basis for calculating breast cancer incidence rates and trends in breast cancer for women in Ontario. Mortality due to breast cancer was not investigated since the main focus of this research project was to determine if

organochlorines play a role in the development of breast cancer.

The original breast cancer data set did not contain information regarding the number of females at risk of developing breast cancer for each year of diagnosis. Also, not included in the data was information regarding the number of females in Ontario for the various age groups diagnosed with breast cancer in each of the given years. The lack of information regarding the number of individuals at risk of developing breast cancer (denominator) for each age and year of diagnosis necessitated deriving this information from other sources in order to generate incidence rates. Once this information was determined, the two data sets could be merged to provide a combined data set which would enable us to evaluate the relationship between breast cancer and organochlorine exposure.

b) Denominator Data

Population census data were acquired for women in Ontario by accessing published records from Statistics Canada's Population Census taken during the years from 1961 to 1991 (Statistics Canada, 1961-1991). Individual censuses were available on a five-year basis namely during census years; 1961, 1966, 1971, 1976, 1981, 1986, 1991. Since population numbers were required on an individual age basis and census data were not available for each year and age, these denominators had to be estimated through interpolation.

After being transferred into SAS files, the data were grouped into diagnostic year,

minimum age (minage), maximum age (maxage) and group denominator. Estimates for non census years were obtained by assuming linear growth or decline over the five-year period between census years, for each age group. For example, population values for 1967 assumed the value of 1966 plus one fifth of the difference between the 1966 and 1971 census population numbers. The variables minage and maxage were created to give five year age groups, namely; 0 - 4, 5 - 9, etc. up to 90 - 100 for each diagnostic year and correspond with the age groups recorded by the individual censuses. For each minage/maxage a group denominator was derived. This group value represents the total number of females for the five-year age group and which was then divided by five to give a value for each individual age. The diagnostic year was used as the year from which to calculate the denominator for each particular age group. For example, if a woman was diagnosed in 1964 with breast cancer and she was 48 years old at time of diagnosis the denominator used to calculate the incidence for this age group would be the value given for the min age/max age group of 45 - 49 from the 1964 diagnostic year.

While individual age denominators would have been more accurate, a lack of availability of this data necessitated that an estimation for each age group be generated. Therefore, we have elected to use the denominator from the five year age groups to represent the individual ages for each group to give the best estimate for the denominator data. Given the small number of cases (numerator), verses the large numbers of individuals at risk (denominator) this estimate should not have a significant influence on the relationship between breast cancer and organochlorine exposure. If there is any impact from using

these estimates, we would expect to have a similar impact on all the birth cohort groups and we could expect this decision to drive the hypothesis toward the null.

c) Merging the Data Sets

The female cancer data set was merged with the denominator census data in SAS, in order to determine breast cancer incidence rates and to allow for comparison of cancer incidences over the time frame of available data. The two data sets were first merged by matching data on diagnostic year and age group and subsequently assigning the appropriate denominator for each diagnostic year and age. The information from this procedure was then used to sum the number of cancer cases for each individual age, and for each diagnostic year. These values were used to calculate the incidence of breast cancer for each diagnostic year and age. For example, if the diagnostic year was 1964 there were 24 individuals who were 34 years old during this time who were diagnosed with breast cancer; the denominator for that age was 44, 545. Therefore the incidence of breast cancer for women age 34 in 1964 was 0.00054. Similarly, for the diagnostic year 1964 there were 49 women diagnosed with breast cancer at age 68 with a denominator of 20, 264 for that age. The incidence of breast cancer for sixty-eight years old women in 1964 would be 0.002418. The resulting merged data set provided detailed records of the year of diagnosis, age at diagnosis and the incidence of breast cancer for the diagnostic years at various ages. The combined data were then used to determine breast cancer incidences from 1964-1991. While breast cancer data were available for 1992 and 1993, population census data were not available. Therefore we have restricted the period under

investigation from 1964 to 1991 recognizing that the same methods could be used in subsequent years as the data becomes available.

d) Grouping Data into Birth Cohorts

The merged data set, providing incidence rates for female breast cancer, was further grouped into specific birth cohorts in order to assess the impact of exposure at various critical ages. Table 4.2.1 outlines the birth cohorts established for each critical age of exposure and during the various phases of exposure. The values in the body of the table represent the birth cohorts. Birth cohorts represent up to five years of exposure during each phase for each critical age group. These birth cohorts were calculated by taking the middle birth cohorts from each exposure phase for each critical age group.

A new data set was established for each critical age of exposure which provided incidence rates for each birth cohort group on a yearly basis from 1964 to 1991. This information was then used to create graphs in Quattro Pro 6.0 which allowed for comparison of breast cancer incidences in the birth cohorts, representing different phases of exposure during the specific critical age of exposure. Similar graphs were created to document changes in breast cancer incidences in the non exposed verses exposed at any age, and non exposed verses exposed at combined age groups.

4.3 Results

For ease of interpretation the results have been subdivided into several sections so that

each critical age and exposure component may be evaluated individually for the contribution they make to the relationship between organochlorines and breast cancer. A general overview of descriptive statistics was also undertaken to provide information about the specific characteristics of the two data sets, namely the breast cancer and population data.

4.3.1 Descriptive Statistics for Breast Cancer and Population Data

There were a total of 117,552 cases of breast cancer cases recorded during the twenty-nine years of record keeping in Ontario. 112, 598 cases, or 95.78% of the total cases represent women who were diagnosed with breast cancer during this period. This figure emphasises the female predominance of breast cancer in Ontario relative to men. Figure 4.3.1.1 shows the total number of cases of breast cancer diagnosed during the period under question. In 1964 there were 2353 cases of breast cancer diagnosed in Ontario. Ten years later in 1974 there were 3439 cases of breast cancer detected in Ontario women. By 1994 the numbers had increased to 4245, and in 1993 there were 5829 cases diagnosed. This shows that, over the twenty-nine years of recording there has been a substantial increase in the total number of breast cancer cases diagnosed on a yearly basis. This increase in the total number of breast cancer cases diagnosed yearly in Ontario represents a significant increase in the social and economic burden of this disease.

The increase in total number of cases diagnosed is not simply a result of an increase in population numbers or that of an aging population, but rather reflects a total increased

incidence of breast cancer diagnosis. As evident from Figure 4.3.1.2 there is an overall trend in increased incidence rates of breast cancer over the entire period of study. However, individual years do show some fluctuation. In 1964, there was an incidence rate of 0.0007, while in 1980, the incidence was 0.00085 and climbed to a high in 1993 of 0.00113 or 85 cases per 100, 000 women in 1980 to 113 cases per 100, 000 women in 1993. Figure 4.3.1.3 shows the mean age of breast cancer over the years of diagnosis has remained relatively stable, with only a slight increase in the mean age at diagnosis. In 1964, the average age of breast cancer at diagnosis was 58.5 years while the average age of diagnosis was 61.5 years in 1993. When the data were divided into average age at diagnosis for pre-menopause and post-menopause women, the average age at diagnosis was 42 and 66 years old respectively. In both the pre menopause and post menopause phase the average age at diagnosis remained relatively constant from 1964 to 1993 as shown in Figure 4.3.1.4.

Examination of the Canadian Census data for Ontario shows that there has been a relatively steady increase in the total number of women in the province. In 1964 there were approximately 3 300 000 females in Ontario see figure 4.3.1.5. By 1990 the total number of females had increased to 5 250 000. Figure 4.3.1.6 shows the population distribution for females, classified into twenty year age groups. This graph reveals that the majority of females during 1964 to 1991 were in the age category twenty to forty-nine years old. During this time period there was a steady increase in the total number of females in this age group. Similarly, there was a steady increase in the 50 - 79 year olds

during the same time frame. The increasing number of individuals in this age group, which represents the post-menopause phase where higher rates of breast cancer can be found could be driving the slight increase in the average age at diagnosis, despite an earlier age at diagnosis as a result of improving diagnostic techniques. The number of women in Ontario greater than eighty years of age has remained relatively constant while the number of women less than 19 years of age has decreased.

4.3.2 Missing Values

The breast cancer data were examined to determine the number of missing values. Missing values were defined as a case of breast cancer which was diagnosed, but for which the age at time of diagnosis was unknown. Over the years of investigation there were very limited numbers of missing values, (Figure 4.3.2.1). On average there were 0.6 percent missing values for each year, with a maximum number of missing values of 1.25 percent which occurring during 1967. The number of missing values decreased from the maximum level in 1967 to 0.38 percent in 1990. This number of missing values can be considered to be acceptable and, given the relative consistency over the time period under study, should not impact the results of this study significantly.

4.3.3 Exposure During Critical Ages

4.3.3.1 Exposure During Critical Age = 0 - 9 Years

To evaluate if a relationship could be found between organochlorine exposure and the development of breast cancer during the human developmental phase of zero to nine years,

five birth cohort groups were established. Group 1= birth cohorts born during 1934-1938, group 2= birth cohorts born during 1945-1949, group 3= birth cohorts born during 1954 -1958, group 4 = birth cohorts born during 1965 - 1969, and group 5 = birth cohorts born during 1974 - 1978. Each group represents individuals that experienced exposure to organochlorines of different intensities ranging from non exposure, increasing exposure, peak exposure, decreasing exposure and minimal exposure respectively. By establishing these birth cohorts, the data could be examined to determine if significant differences in incidence rates of breast cancer exists between the different exposure groups, when exposed specifically during the age of zero to nine years old.

Data generated by SAS was imported into Quattro Pro 6.02 to produce graphic images of the results. In order to assess the relationship between organochlorines and breast cancer, when exposure occurs during zero to nine years of age, the average incidences of breast cancer for each of the five birth cohort groups were plotted against average age at diagnosis for each group. This graph visually allows one to determine if a difference in breast cancer trends existed for the different exposure groups over the entire age range. Similarly, the graph could show if a difference in the incidence of breast cancer at specific individual ages could be found. The information in this graph could potentially suggest a specific risk period for developing breast cancer, or a similar age at diagnosis.

Figure 4.3.3.1.1 shows that, when exposure occurs during the age of 0 - 9 years there appears to be similar trends in breast cancer rates for the five birth cohort groups, when

evaluating breast cancer incidence rates at an average age of diagnosis of 15 to age 35. At age 30 and 35 breast cancer incidence was not statistically different for the non exposed cohorts (group 1) and the increasing exposure cohorts (group 2), p= 0.2171 and p= 0.9756 respectively. Similarly, at age 35 there was no statistically significant difference in breast cancer incidence found between group 1 and group 3 (peak exposure), p=0.7747. However, at approximately age 40 to 46 years, the graph appears to show an increase in the average incidence of breast cancer in birth group 2 (increasing exposure) compared to group 1 (no exposure). For example, at age of 45 years birth cohort group 1, with no exposure to organochlorines at 0-9 years of age, has an average incidence of breast cancer of 0.0012 while the group exposed to organochlorines in the increasing phase of organochlorine use, had an incidence of breast cancer of 0.0017. The difference in breast cancer at age 45 for these two groups was found to be statistically significant (p=.0035). Therefore there are approximately 50 more cases of breast cancer diagnosed per 100, 000 women in those women exposed to organochlorines than those women not exposed during this critical age (1.4 times more likely to be diagnosed with breast cancer). Similarly, at age 41 through to age 46 there were statistically significant differences in beast cancer rates between the non-exposed and the increasing exposure cohorts.

What is also evident from this graph is that the average incidence of breast cancer for women exposed to organochlorines during age zero to nine years can not yet be properly assessed. This is because, past age 26 there are insufficient data for each group to allow one to compare the breast cancer incidences. The lack of information post age 26, 37 and

46 for groups 2, 3, and 4 respectively are simply due to the fact that these birth cohorts had not reached these ages by 1991, the last year under investigation. Since the greatest proportion of breast cancer occurs during the post-menopause phase of life the inability to compare results after age 46 is disturbing, if exposure to organochlorines during this early stage of human development is significant to the development of breast cancer. To resolve this problem, further breast cancer comparisons must be carried out after the mid 2050's, in order to precisely determine the role that organochlorines play in the development of breast cancer for individuals exposed during this early stage of development.

The slopes of the breast cancer incidence curves change over time with each critical age of exposure. Therefore the comparison of slopes (or changes in breast cancer incidence for each critical age) would require having data from the same age groups to compare. In the case of the critical age of exposure of zero to nine years, comparison past age thirty five is not possible for peak exposure verses non exposure or increasing exposure. However using SAS to compare slopes between the groups a statistically significant difference was found between group 1 and groups 3, 4, and 5 where $p=0.039$, $p=0.008$ and $p=0.001$ respectively.

If exposure to organochlorines is most significant at this early age, there is a possibility that an increasing trend in breast cancer incidence, as individuals born during high exposure periods attain the post-menopause phase, may continue from now until the mid

2050s. If this were the case, it would suggest that those individuals exposed to organochlorines would have relatively higher rates of breast cancer than those not exposed during this developmental phase and would also suggest that we are currently seeing only the initial stages of this increase in breast cancer cases. If exposure during age 0 - 9 is critical, or at least important, in promoting breast cancer in post- menopausal women we could expect to see the current increase incidences in breast cancer continuing for several more decades. Increases may be expected until at least the year 2040 and may in fact continue until 2050 or greater. If a dose response relationship exists, we should see the greatest increase in breast cancer for those birth cohorts exposed at the critical age of 0-9 years during the peak use of organochlorines with declines in breast cancer as subsequent birth cohorts attain the post menopause phase.

4.3.3.2 Exposure During Critical Age = 10 - 19 Years

To evaluate if a relationship exists between organochlorine exposure and breast cancer when women were exposed to organochlorines during their sexual maturation phase (age ten to nineteen years) five birth cohort groups were established. Group 1 = birth cohorts 1924-1928, group 2 = birth cohorts 1934 - 1938, group 3 = birth cohorts 1944 - 1948, group 4 = birth cohorts 1954 - 1958 and group 5 = 1964 - 1968. These birth cohort groups represent five different phases of exposure during the critical age of 10 - 19 years at the time of exposure. The first group represents those cohorts not exposed to organochlorines, in that exposure for these cohorts began at age 20 years or older. Groups 2, 3, 4 and 5 represent the cohorts that received increasing, peak, decreasing and

minimal exposure respectively to organochlorines during the critical age of 10 - 19 years. The birth cohort groups represent approximately five years of exposure and were established to give the best possible likelihood of finding a potential relationship between organochlorine exposure and breast cancer development if this age at exposure is a critical factor.

Average incidence of breast cancer for the year and age at diagnosis for each birth cohort were derived from the data using SAS. The data were then graphically examined by plotting average incidence of breast cancer for each groups against the age at diagnosis. The resulting graph figure 4.3.3.2.1 visually shows that there were no significant differences between average breast cancer incidences for all five groups between the age of 15 to approximately 35 years. From age 35 to 40 no apparent trend can be established. However, from age 40 to 46, group 3 (peak exposure) appears to have a slightly higher rate of breast cancer than either group 1 or 2. For example, at age 45, the peak exposure cohorts (group 3) had an average incidence of breast cancer of 0.0016 while the non exposure and increasing exposure both have an average incidence of breast cancer of approximately 0.0014. This represents a difference of 20 cases per 100, 000 women at risk. The difference in breast cancer rates between the non exposed cohort and the peak exposed cohorts at age 45 was found to be statistically significant $p=0.0001$. Similarly, the difference in breast cancer incidence between the increasing exposure and peak exposed cohorts was also found to be statistically significant $p=0.0011$. At age 45 there was no statistically significant incidence in breast cancer between the non exposed and the

increasing exposure cohorts.

At age 46 and age 47 there was a statistically significant difference in breast cancer incidence between the peak exposure, increasing and non exposure, p=0.0026 and p=0.0001 respectively. However, at age 47 the non exposed group appears to have a higher incidence of breast cancer. Further comparisons between the peak exposure group and the other exposure groups cannot be made since the birth cohorts from the peak exposure group had not attained an age greater then 47 years by 1991. This is disappointing because it would be extremely important to know future trends in breast cancer for these groups.

Comparisons between group 1 and 2 can be made until 57 years of age. From the age at diagnosis of 40 to 49 years old, there does not appear to be a consistent pattern between the birth cohorts group 2 (increasing exposure) and group 1 (no exposure). During this phase there are periods where the non exposed group has similar, lower and higher incidence of breast cancer in comparison to the increasing exposure group. However, by approximately age fifty to age fifty-seven there appears to be an increased average incidence in breast cancer in group 2 than can be detected for group 1. For example, at age 50 there is a statistically significant difference between breast cancer in the two groups with the increasing exposure group having a higher incidence of breast cancer p=0.0107. At age 55 to 57 the difference visually seen on the graph is not statistically significant and greater than age fifty-seven no comparison could be made, since group 2 birth cohorts were not older than 57 years by 1991. Comparisons of the entire slopes of each birth

cohort were found to be statistically significant with the rate of change also statistically significant. However, more years of actual data is required to establish true trends in breast cancer incidence.

If however, the increasing incidences in average breast cancer trends follow a similar fashion as seen in the tail end of these curves, it would point to a significant increase in breast cancer incidences in the groups exposed to increasing organochlorine use compared to those not exposed during this critical age. This suggests a significant number of Ontario women could be at risk, if exposure during age 10 - 19 years of age was significant.

Figure 4.3.3.2.1 shows that we cannot evaluate the significance of exposure to organochlorines during age 10 - 19 years for birth cohort groups 4 and 5 since these individuals were less than 35 years old and would therefore have very limited numbers of breast cancer cases diagnosed. Studies would have to be undertaken after approximately the year 2048 in order to fully compare these birth cohort groups to those birth cohorts not exposed during age ten to nineteen. Interestingly, Figure 4.3.3.2.1 shows a similar finding as figure 4.3.3.1.1 (For exposure during the critical age 0 - 9 years) in that comparisons between the most important breast cancer risk period (age 45 to >80 years) cannot be determined, at this point in time, since most of the birth cohorts have not attained this age by 1991.

Table 4.3.3.2.1 shows the diagnostic year when the various birth cohorts can be expected to reach a particular age at time of breast cancer diagnosis. Subsequently, in order to compare the first three birth cohort groups (non exposure, increasing and peak exposure) between women age 30 to 80 years old, studies would have to be undertaken after the year 2028. If however, the detection of a dose response were desired, investigations of all five age cohorts would to be necessary and therefore analysis would have to be done after the year 2048. If the age at exposure and the exposure dose were important, any studies done prior to approximately the year 2000 would not be expected to detect a significance difference in breast cancer rates since the majority of women born during the peak exposure phase at this critical age would not have even attained the start of their post-menopause phase. If for example, we wanted to compare the average incidence of breast cancer of women age 50 - 80 years old between non exposure and peak exposure during age 10 - 19 years at time of exposure, we would have to undertake studies after the year 2030. The impact of this finding is significant in that if exposure to organochlorines at an early age significantly affects the outcome of breast cancer, then the studies carried out to date have been undertaken too early to consistently detect a significant association between breast cancer incidence and historical organochlorine exposure.

4.3.3.3 Exposure During Critical Age 20 - 49 Years

In order to investigate if a relationship exists between organochlorine exposure during age 20 - 49 years of age (pre-menopause phase) and breast cancer, five birth cohorts were established to account for the five phases of exposure. Group 1 = birth cohort 1894 -

1898, group 2 = birth cohort 1915 - 1919, group 3 = birth cohort 1924 - 1928, group 4 = birth cohort 1934 - 1938 and group 5 = birth cohort 1944-1948. These birth cohort groups represent women whose exposure ranges from none, increasing, peak, decreasing and minimal during the time that they were age 20 to 49 years old. The birth cohorts represent individuals who would have had approximately five years of exposure during their respective exposure phase and could be expected to show the greatest difference in breast cancer incidence, if exposure during the pre-menopause stage of maturation is significant.

When the data, obtained from SAS (as previously described), were graphically represented by plotting average incidence of breast cancer verses age at diagnosis several interesting observations could to be made. Figure 4.3.3.3.1 shows that there is virtually no difference between the incidence of breast cancer at each individual average age at diagnosis for groups 2, 3, 4, and five during the first forty-five years of age. While the average incidence of breast cancer for groups 2, 3 and 4 initially seem similar, past age 52 the birth cohorts exposed to decreasing levels of organochlorines appear to have a higher incidence of breast cancer than those exposed to peak or increasing levels.

At the age of 55 years at diagnosis, group 4 (decreasing exposure) has an average incidence of breast cancer of 0.0025 while group 3 (peak) has an average incidence of 0.002. Group 2 (increasing) has an average incidence of 0.0017. These differences in average incidence represents a difference of fifty cases per 100,000 women between group

3 and 4 and a difference of 80 cases per 100,000 between group 2 and group 4. These differences are statistically significant p=0.0001.

On further examination of Figure 4.3.3.3.1 one can see that a complete comparison between birth cohorts exposed during age 20 - 49 years and those not exposed, cannot readily be accomplished. Since the birth cohorts (group 1) representing the non exposed phases are greater than 66 years of age at the start of the study, the maximum age of the peak exposure group, and by 1991 group 1 has aged to more than ninety years old, no comparisons can to be made between these two birth cohort groups past an average age at diagnosis of 67 years. Comparisons can be made between three of the birth cohort groups for average age at diagnosis of 66 - 67 years of age and in this case there appears to be a substantial difference in the average incidence of breast cancer among the three groups. At age 67 the final year to compare the non exposed, increasing and peak exposure groups, there is a higher rate of breast cancer in the peak exposure group compared to the increasing and to the non exposed cohorts and the differences between these groups was found to be statistically significant, where p=0.0001 and p=0.0504 respectively.

Unfortunately after the average age of diagnosis of 67 years only comparisons can be made between two groups and, in this case, the increasing exposure groups continue to have higher rates of breast cancer then the non exposed groups from age 68 to 76 years old. For example at age 70 there is a statistically significant difference between the non

exposed and the increasing exposed cohorts with the latter having a higher incidence of breast cancer, p=0.0001. At age 75, there is also a statistically significant difference p=0.0213 between the incidence of breast cancer in the increasing exposed cohorts and the non exposed cohorts with the increasing exposure group having approximately 150 cases per 100,000 women higher incidence of breast cancer. If the trends shown in this graph continue over time it would suggest that exposure to peak and decreasing levels of organochlorines would result in greater incidence in breast cancer compared to cohorts not exposed. Alternatively, if one examines the actual birth cohort dates, those born during the start of organochlorine use would have higher rates of breast cancer than those not exposed, and this may also suggest that exposure at an earlier age may be more important.

4.3.3.4 Exposure During 50 - >80 Years

To evaluate if a relationship exists between organochlorine exposure and breast cancer, when women are exposed during the post-menopause period, five birth cohort groups were established. Group 1 = birth cohorts 1863-1867, group 2= 1884-1888, group 3= 1893-1897, group 4 =1903-1907 and group 5=1913-1917. These groups represent the various birth cohorts which experience exposure during age 50 - >80 years old during a period of non exposure, increasing, peak, decreasing and minimal exposure, respectively. The evaluation of this group in particular would also allow us to determine if exposure to organochlorines has effects with shorter lag times. For example, if organochlorines were able to rapidly stimulate preexisting cancerous cells, we would expect to see a higher

incidence of breast cancer in women exposed organochlorines during this stage of human maturation.

If a dose-response relationship exists then we could also expect to see variations in breast cancer incidence according to the degree of exposure. Similarly, if only exposure to organochlorines in the post menopause phase was significant, we would be able to determine approximately when women in Ontario would cease to be at risk of developing breast cancer. For example, women age 50 - > 80 years old would be exposed to very minimal levels of organochlorines after the year 1980 and we could therefore assume that the incidence of breast cancer in this group of women would decrease compared women of a similar age in the year 1960, if recent exposure to organochlorines was a significant factor in the relationship between organochlorine exposure and breast cancer development.

The data set was evaluated using SAS as previously described. This information was then transferred to Quattro pro to generate a visual picture. Graph 4.3.3.4.1 shows several interesting results. Firstly, for this particular critical age of exposure, we cannot make reliable comparisons between all five birth cohort groups. There are very limited data for the non exposure group because, in order to experience no exposure, the cohort members would have been age fifty and greater than eighty years old prior to 1947 (the first year of exposure). Therefore, their birth cohort group would be < 1867. In 1964 (the first year of our study) these individuals would have already attained 97 years of age and by 1964

many individuals from the non exposure birth cohort group would have been deceased.

A similar problem occurs for group 2 who received exposure during an increasing phase of organochlorine use during this critical age. Information for this group is available from age 76 to 100 years old but no information is available for the earlier ages. Again this results because in 1964 the first year of study these birth cohort members would have been 76 - 80 years of age and would therefore have been greater then 100 years old by the end of the study. The earliest age available to compare group 3 birth cohort members (which represent exposure during the peak phase if exposure occurred during age 50->80 years) is 67 years of age and greater. Lack of information at the later years of age at diagnosis occur for Group 5 (birth cohorts representing minimal exposure) whose maximum age at the end of the study period is 78 years and group 4 (birth cohorts exposed during decreasing phase) who are 88 years at the end of the study.

Despite these limitations there can be some comparisons made between the various birth cohorts groups representing peak, decreasing and minimal exposure during critical age of exposure of 50 and older. If one examines the difference between the birth cohort groups for the ages where information is available then there appears to be a greater average incidence of breast cancer in the minimal exposure group compared to the decreasing exposure (group 4) from approximately age 56 to age 77 at diagnosis. At age 67 for example there is a higher incidence of breast cancer in both group 4 and group 5 when compared to the peak exposed cohorts (group 3) and this difference was found to be

statistically significant p=0.0001 for both comparisons. At age 75, there is a statistically significant increase in breast cancer incidence between the minimal and peak exposed groups p=0.0002 but there is no statistically significant difference between group 4 and group 3 despite the visual suggestion of the graph, p=0.4954.

This graph would suggest that age fifty to greater than eighty years of age at time of exposure is not significant in development of breast cancer since both the minimal and decreasing birth cohorts have higher average incidences of breast cancer for the various ages at diagnosis that can be compared. Alternatively, the findings of this graph could suggest that an earlier age at exposure is critical to the development of breast cancer, or that an increase in the total number of years of exposure is critical to the development of breast cancer. In the latter case the birth cohorts from the group 5 would have started their exposure to organochlorines at approximately 30 years of age, while the cohorts from group 4, 3, 2 and 1 would have started exposure at approximately 40, 50, 60, and greater than 80 years. Therefore, groups 4 and 5 would have had significantly longer periods of lifetime exposure as well as exposure in earlier stages of human development.

Table 4.3.3.4.1 shows the actual age that each birth cohort represents during that various years of exposure to organochlorines. We can see that group 5 represents birth cohorts that began exposure at age 30 while group 4 began exposure at age 40, group 3 at age 50 and group 2 at age 59 and group 1 at age 80. Thus group 5 represents birth cohorts that would have experienced the earliest age of exposure and the greatest number of years of

exposure to organochlorines from the initial year of use in 1947 to present. This would suggest that the total number of years of exposure to organochlorines has some effect on the development of breast cancer.

Exposure Verses No Exposure

There are several possibilities for comparing non exposure to exposure at any age. In the most complete sense we would examine those cohorts who did not receive any exposure to organochlorine at any stage of their life. This examination would examine the breast cancer incidence for women born prior to 1867, if the cut off age was 80 years, or prior to 1847 if the women were included up to 100 years of age. As seen in the critical age category for 49->80 this would not allow us to compare the different breast cancer incidences because in 1964, the first year of this investigation, most of the women in the non exposure group were nearing the end of their lifespan. Therefore results after 1964 would be relatively meaningless.

Table 4.2.1 Birth Cohorts for Critical Age of Exposure during various phases of Exposure

	Phase of Exposure				
Critical Age	**No Exposure (< 1947)**	**Increasing (1947 - 1955)**	**Peak (1955-1965)**	**Decreasing (1965-75)**	**Minimal (1975-85)**
Age = 0 - 9 Years	1934-38	1945-49	1954-58	1965-69	1974-78
Age = 10 - 19 Years	1924-28	1934-38	1944-48	1954-58	1964-68
Age = 20 - 49 Years	1894-98	1915-19	1924-28	1934-38	1944-48
Age = 49 - 80 Years	1863-67	1884-88	1893-97	1903-07	1913-17

Figure 4.3.1.1 Total Number of Breast Cancer Cases For Ontario Women: 1964 to 1991

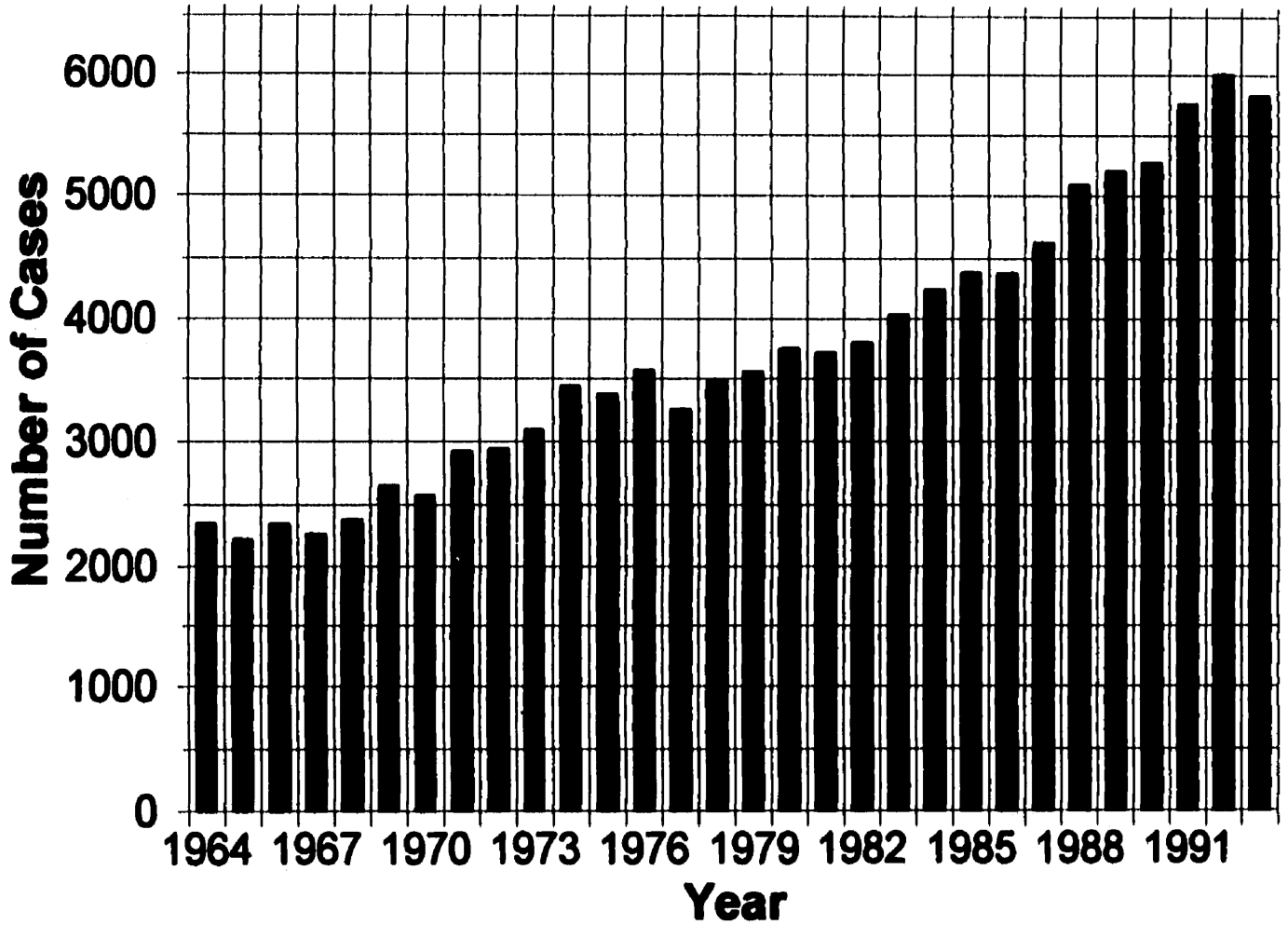

Figure 4.3.1.2 Breast Cancer Incidence in Ontario Women: 1964 to 1991

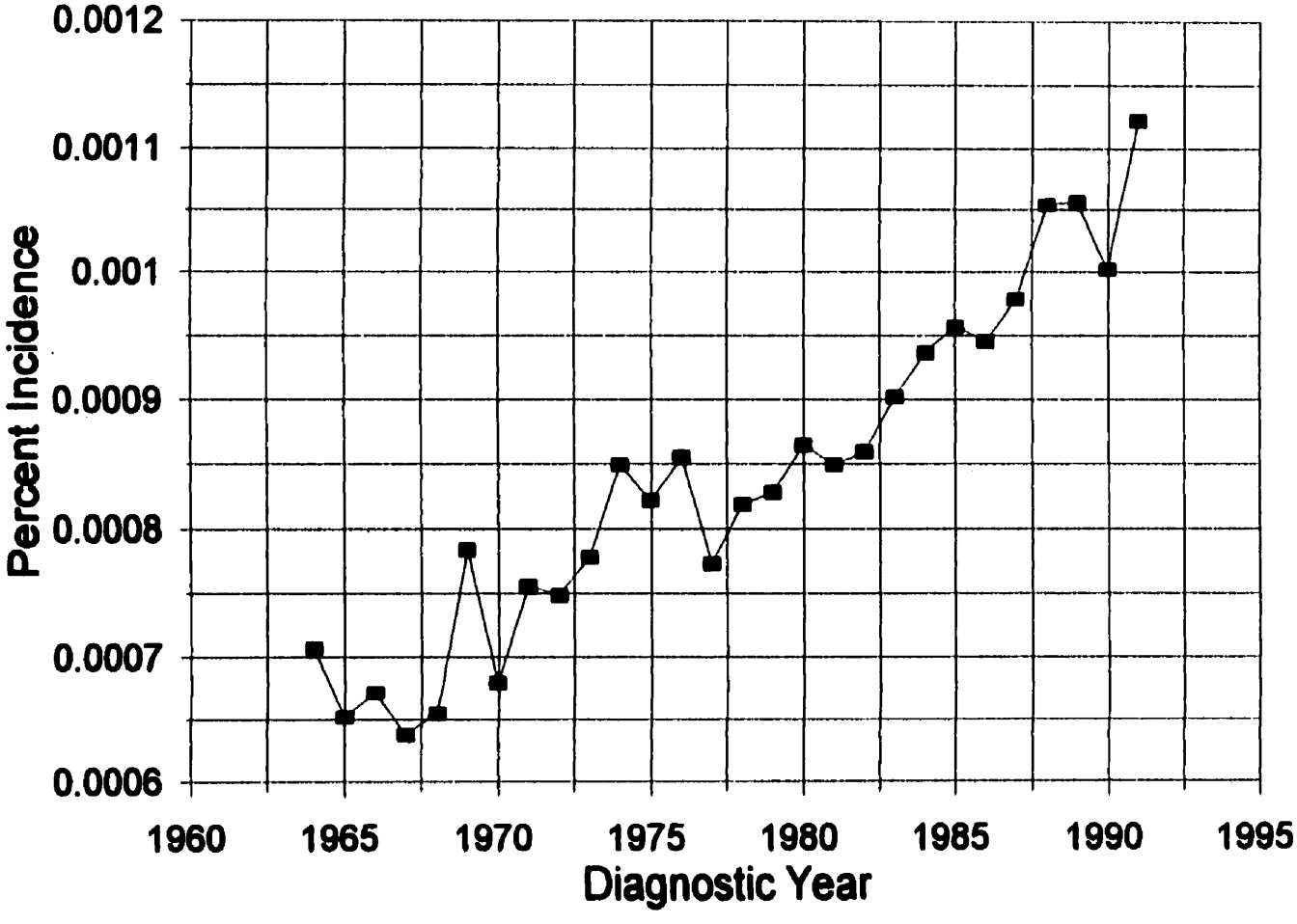

Figure 4.3.1.3 Mean Age of Breast Cancer Diagnosis For Ontario Women From 1964 to 1991

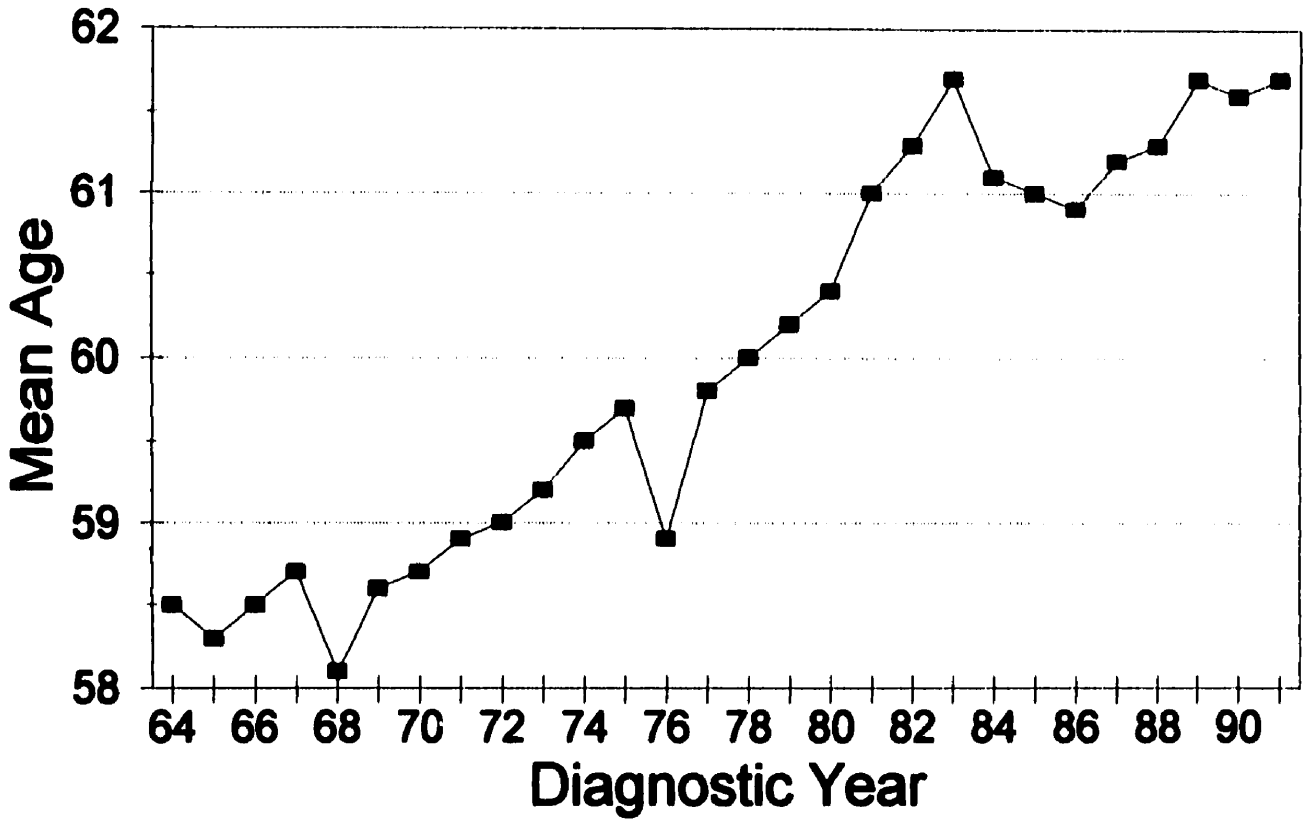

Figure 4.3.1.4 Average Age at Diagnosis of Breast Cancer for Premenopause Verses Post Menopause Women

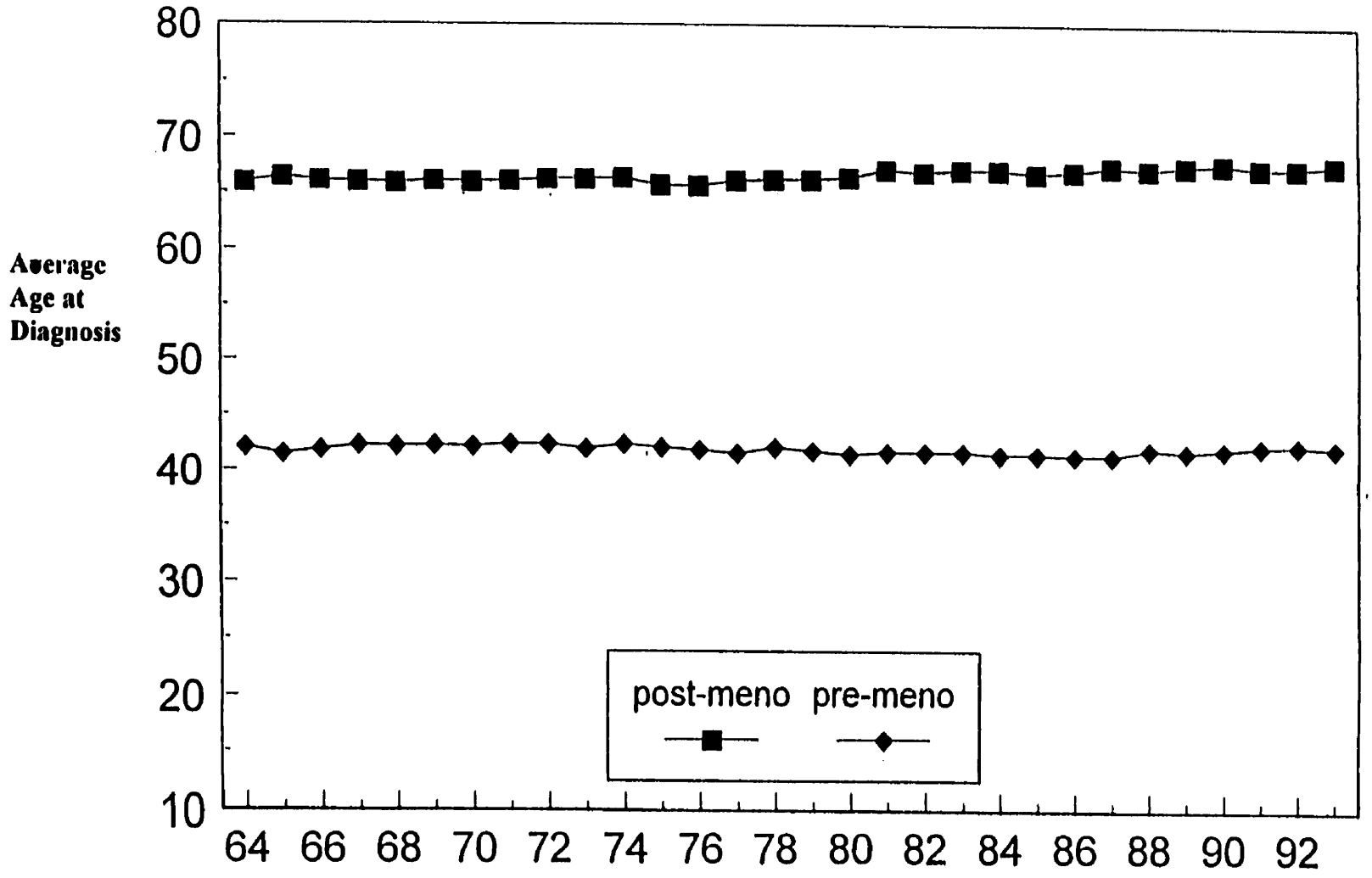

Figure 4.3.1.5 Female Population in Ontario From 1960 to 1995

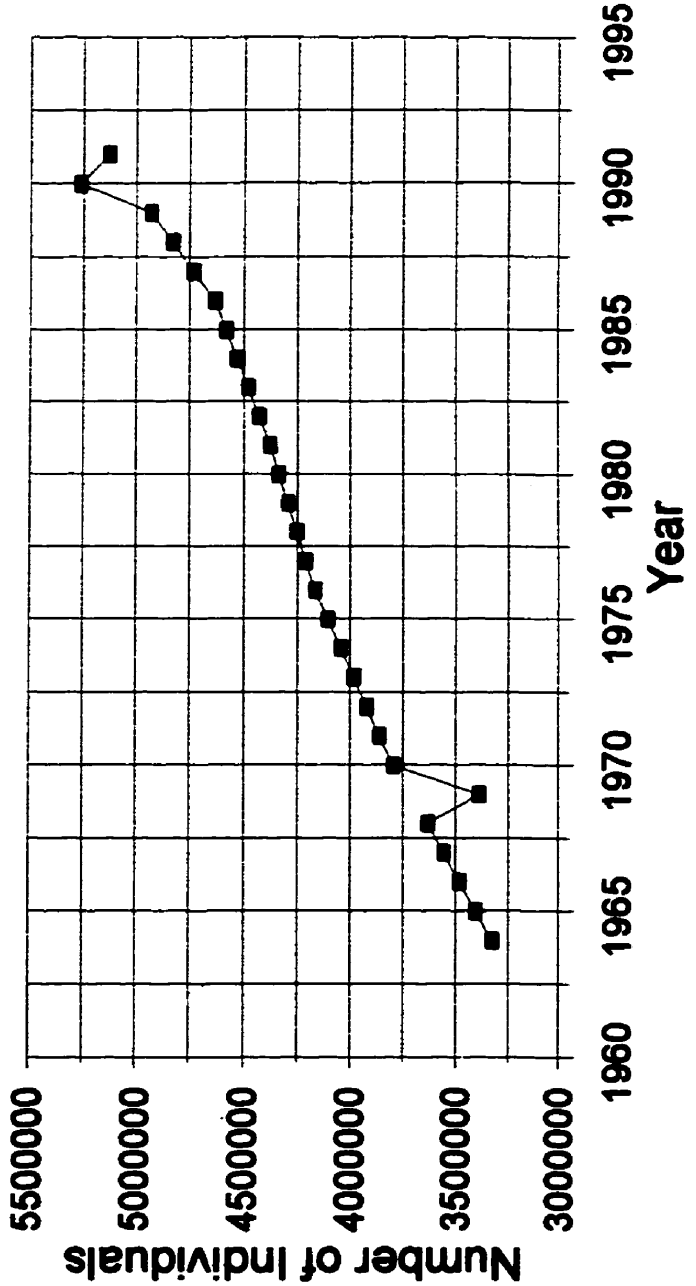

Figure 4.3.1.6 Female Population Distribution In Ontario From 1964 to 1991

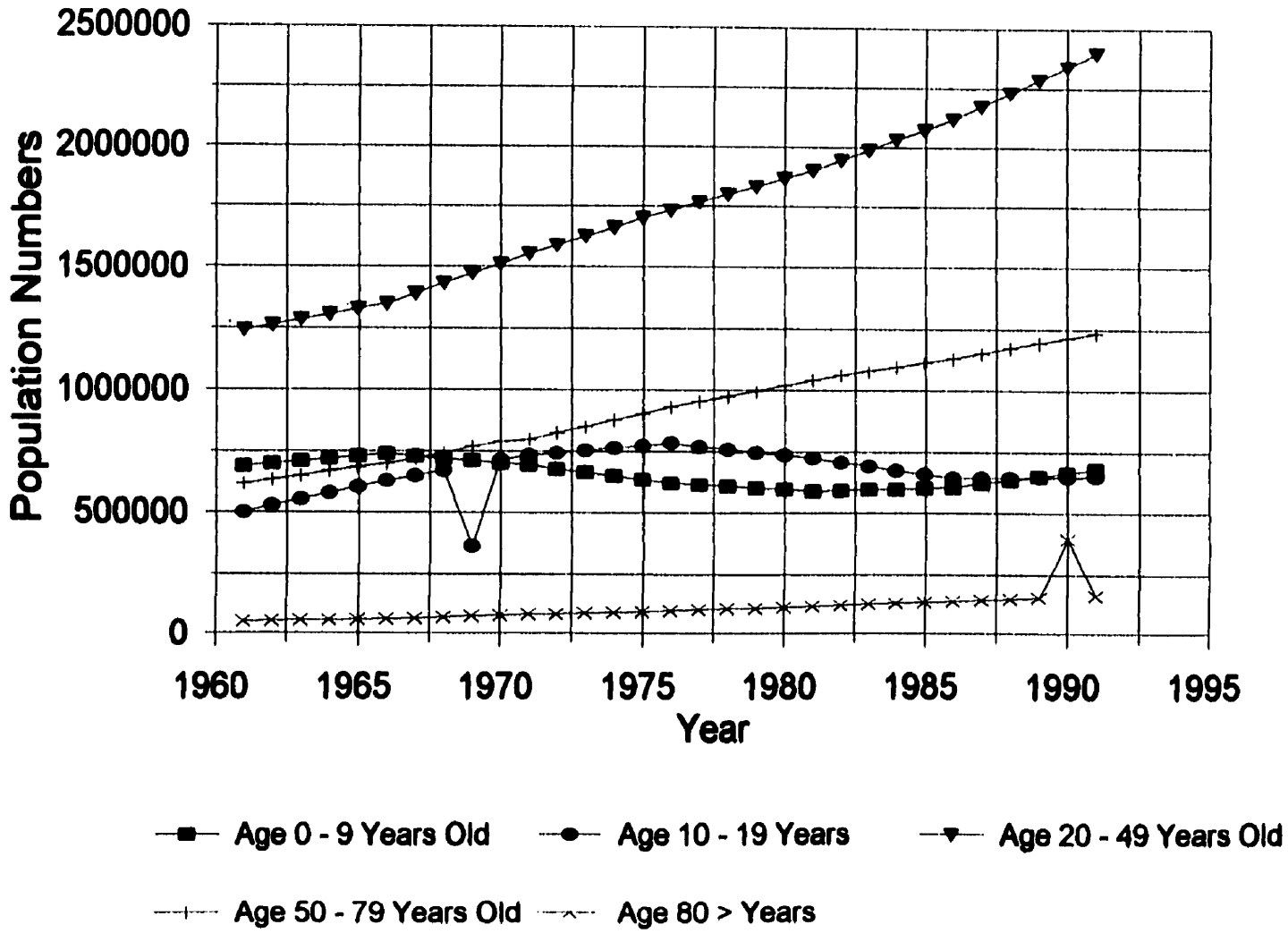

Figure 4.3.2.1 Percent Missing Values For Each Year Of Diagnosis

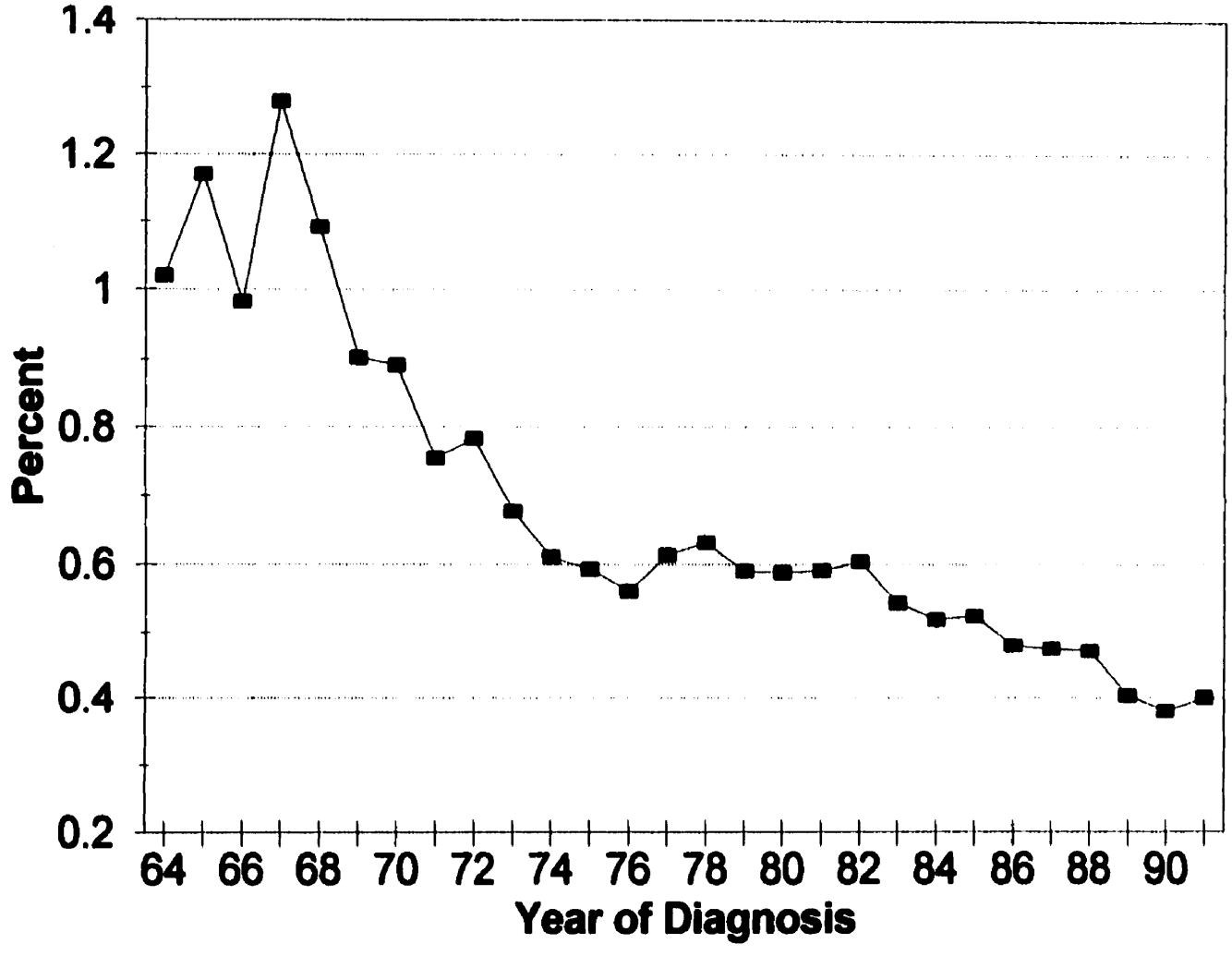

Figure 4.3.3.1.1 Breast Cancer Incidence in Birth Cohorts Exposed to Organochlorines During the Critical Age of 0 - 9 Years

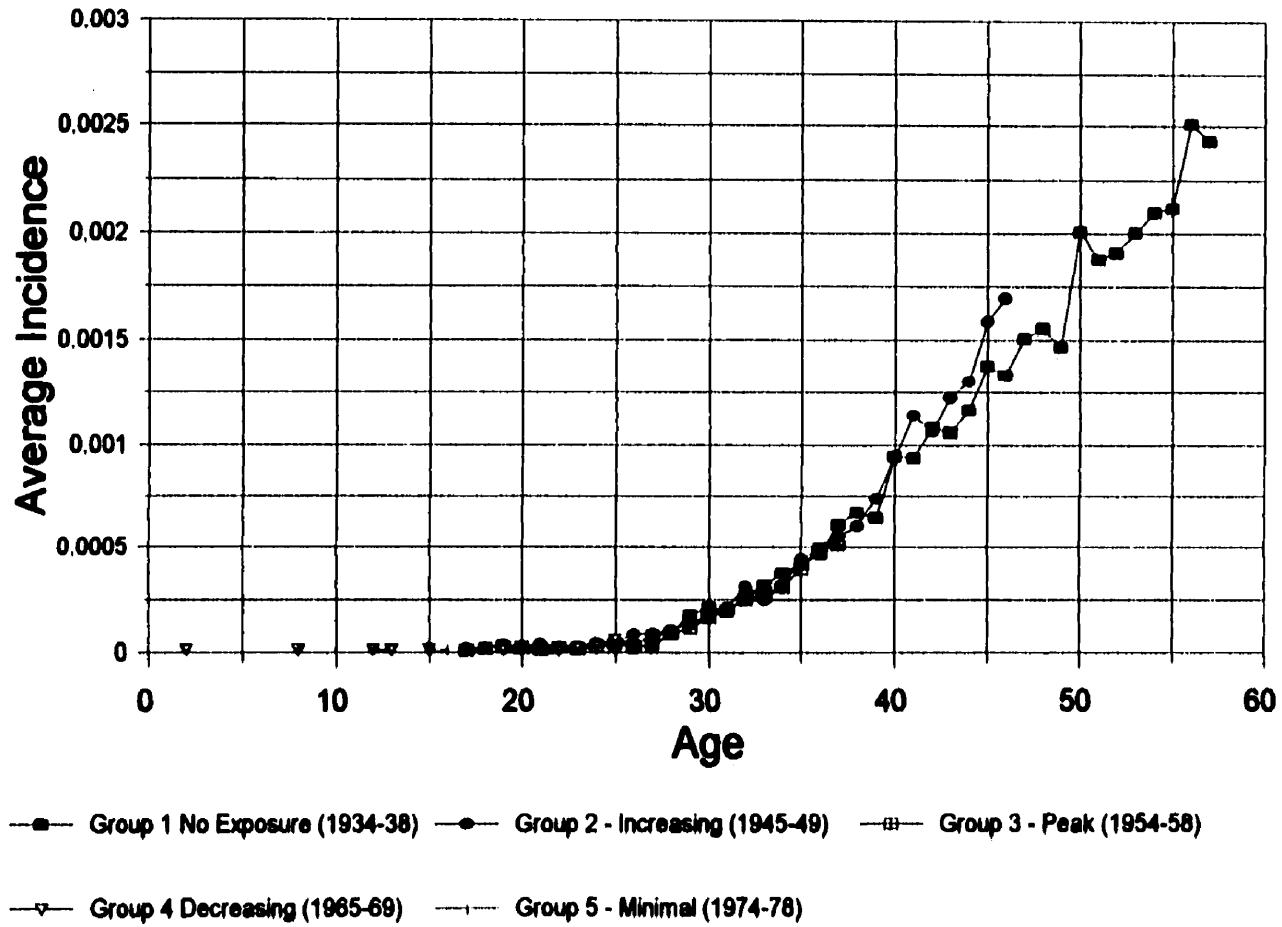

Figure 4.3.3.2.1 Breast Cancer Incidence in Birth Cohorts Exposed to Organochlorines During the Critical Age of 10 - 19 Years

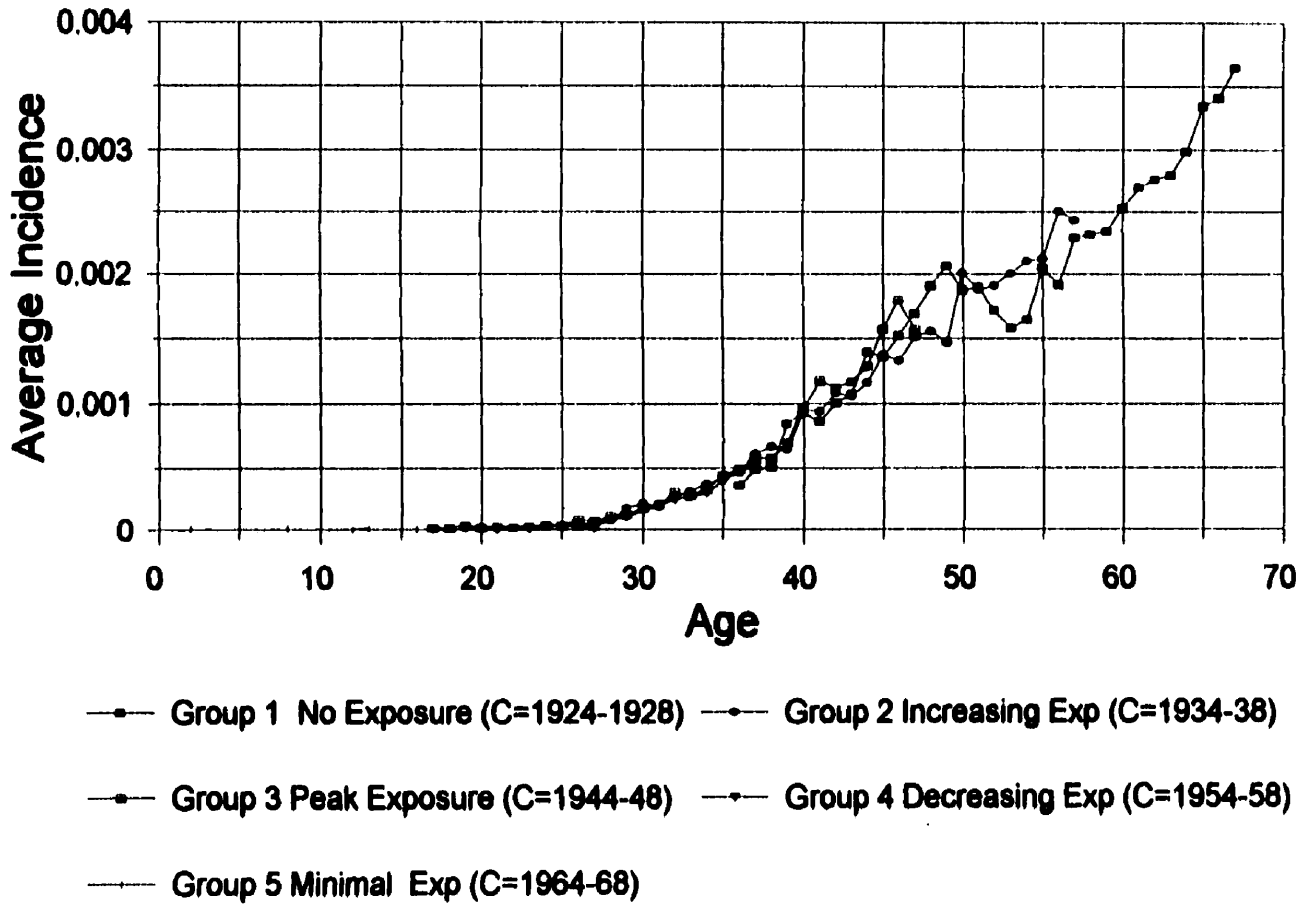

Figure 4.3.3.3.1 Breast Cancer Incidence in Birth Cohorts Exposed to Organochlorines During the Critical Age of 20 - 49 Years

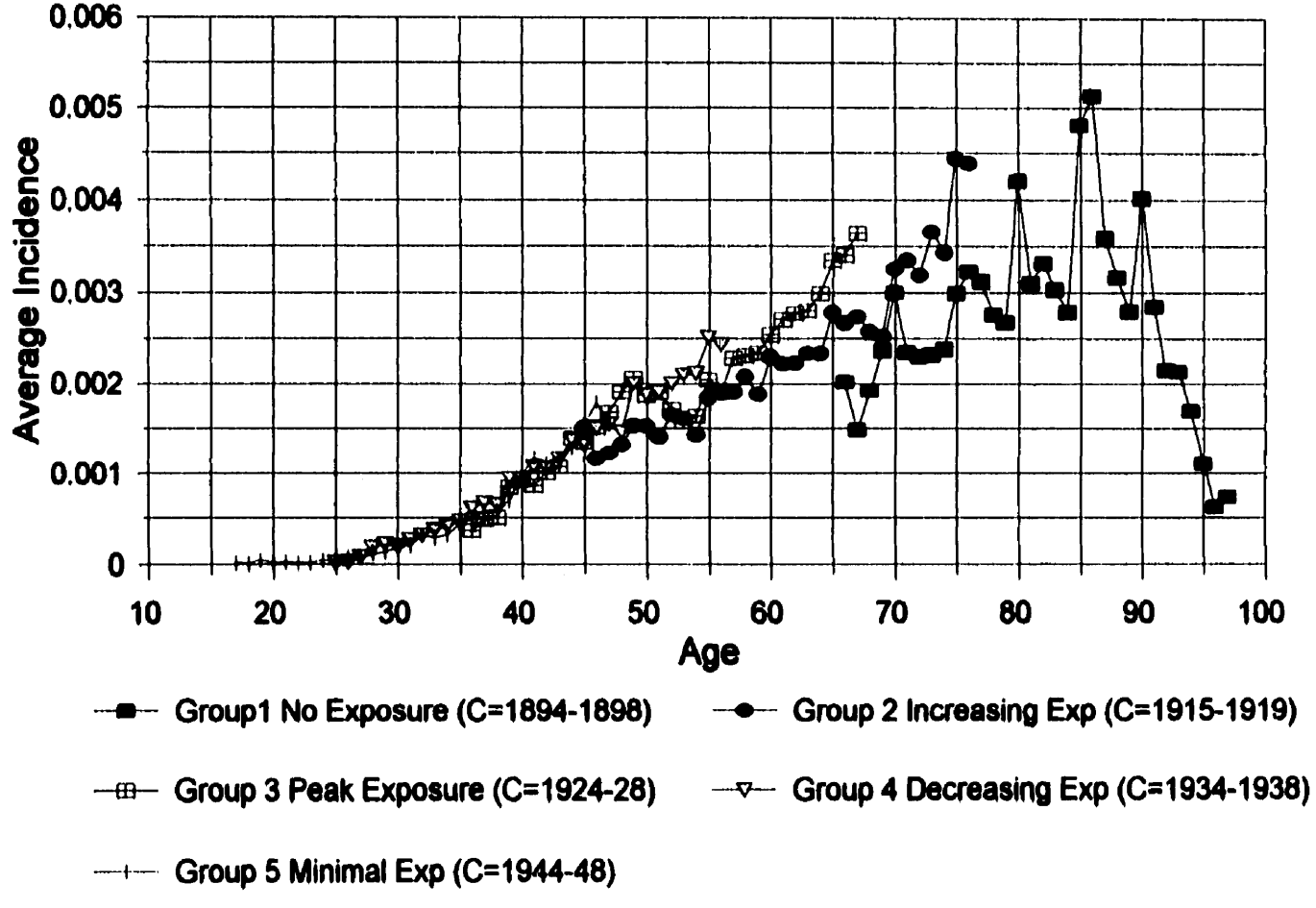

Figure 4.3.3.4.1 Breast Cancer Incidence in Birth Cohorts Exposed to Organochlorines During the Critical Age of 50 Years and Older

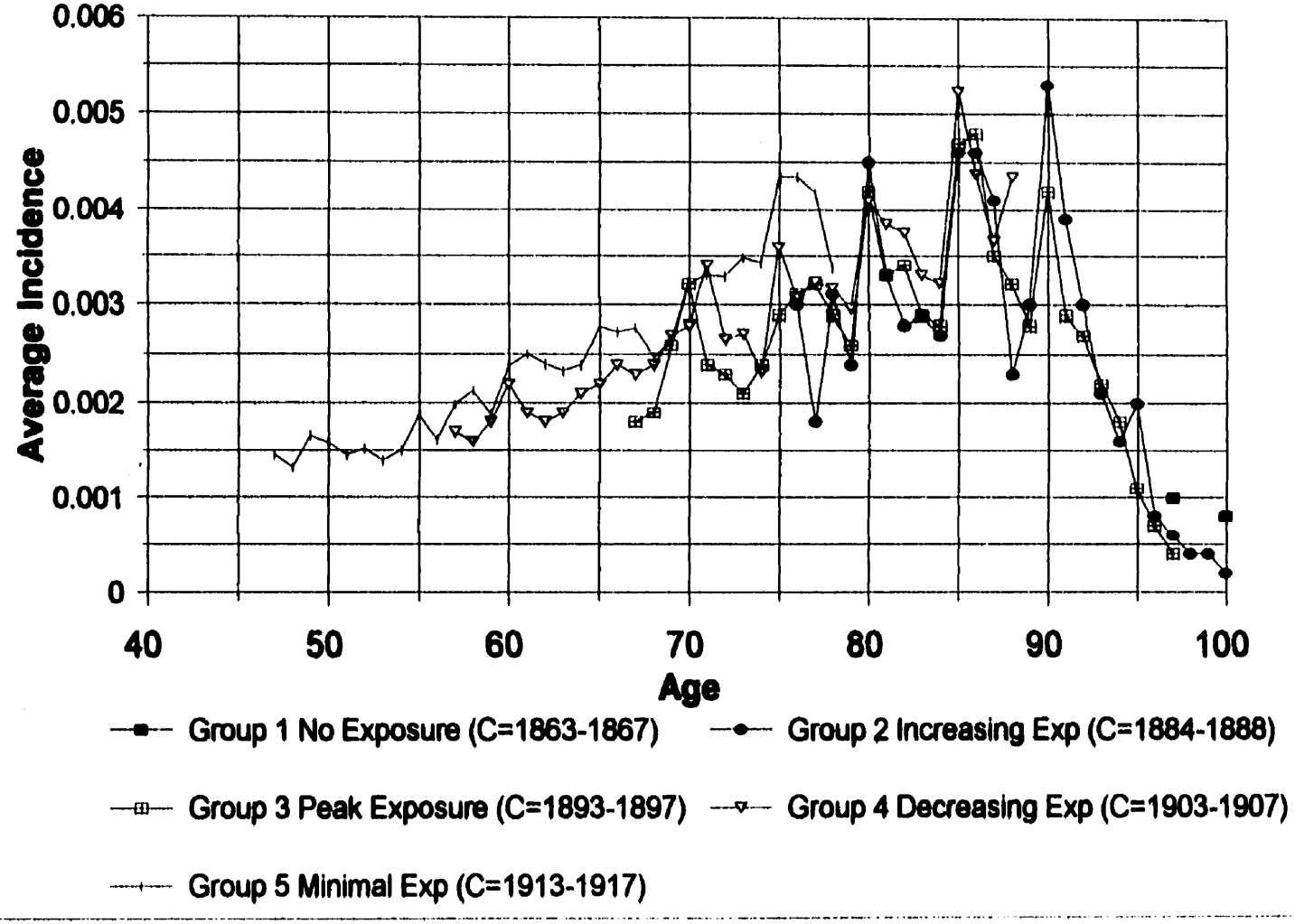

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Table 4.3.3.4.1 Age at exposure for various birth cohorts during the Years of Study

		Exposure Years				
	Birth Cohort	**1947**	**1960**	**1970**	**1980**	**1990**
Group 5	(1913-1917)	30-34	43-47	53-57	63-67	73-77
Group 4	(1903-1907)	40-44	53-57	63-67	73-77	83-87
Group 3	(1893-1897)	50-54	63-67	73-77	83-87	93-97
Group 2	(1884-1888)	59-63	72-76	82-86	92-96	102-106
Group 1	(1863-1867)	80-84	93-97	103-107	113-117	124-127

Chapter 5 Summary and Conclusions

The results generated by plotting average incidence of breast cancer over the lifespan of women of various birth cohorts has further emphasized the complex nature of detecting an increased risk from exposure to an environmental substance. While this type of analysis does not prove or disprove a causal link between organochlorines and breast cancer, the main conclusions from this evaluation do provide some important information that must be taken into consideration when devising future studies to determine the link between organochlorines and breast cancer. Of particular interest is the discovery that, while the bulk of exposure to these substances occurred historically, and for a relatively short period (approximately 1947-1975), the potential consequences from this exposure will take years of observation, to determine the full extent of any relationship. At a minimum an examination of breast cancer trends should be followed for forty more years to determine if the incidence of breast cancer declines as individuals who would not have had any exposure to organochlorines enter menopause.

The critical age of exposure graphs all show a similar finding: that average breast cancer incidence appears to be very similar for all the birth cohort groups from an average age at diagnosis of 15 to approximately 40 years old, despite varying degrees of exposure. In comparison, there appears to be marked difference in the average incidence of breast cancer when the average age at diagnosis increases. This finding supports the suggestion that breast cancer may be a substantially different disease in the pre and post menopause phase of life (Fisher, 1995). It also supports the hypothesis that a difference in breast

cancer incidence may be due to a delayed effect from exposure to a substance requiring numerous years to show its effect (NRC, 1991). Alternatively, it may simply be the direct result of the increased amount of time required, post exposure, before a mass is detectable by palpation or other means of detection. Therefore women in the pre-menopause phase may indeed have breast cancer, which could be influenced by numerous factors, but the recognition of this cancer is difficult during the early stages of the disease.

When looking at the overall results, from the various critical ages at exposure, one can see that the group who received both the longest duration of exposure, and exposure during all the different stages of development, would be those individuals born around the year 1947. These individuals would have received exposure to organochlorines in their early infant and childhood stage as well as exposure to organochlorines at 10 - 19 years, and 20 - 49 years of age. This group of individuals would have also received peak exposure during their late childhood and into the sexual maturation phase of life, and would have experienced both an increasing and declining phases of organochlorine use. Therefore, if organochlorine exposure is a risk factor in the development of breast cancer, this group could be considered to be the highest risk group, from a duration of exposure perspective alone (recognizing that other confounding factors such as lifestyle issue may also play a role in the development of breast cancer). Interestingly, this group of women represents members of the so-called baby boomers and subsequently if they are indeed the highest risk group this could represent an increase in the total numbers of breast cancer developing as these women attain their senior years.

From the examination at critical age of exposure of 10-19 years old and greater, it would appear that this group may indeed be at greatest risk of developing breast cancer. Of particular concern is the fact that women born in 1947 would only start to become 50 years old in 1997, and thus, if exposure to organochlorines at any age or dose has an effect on the development of breast cancer, one could expect to see an even more dramatic increase in breast cancer rates as these women age to 80 years by 2027. If exposure at age 10 - 19 years is the most important period of exposure, then we are currently seeing only the start of a breast cancer epidemic that could continue for several decades. The decline in these rates would therefore only occur as these birth cohorts age and die off. The period of increased incidences as a consequence could therefore be considered to be minimally from 1997 to 2027. However, given that women born several years pre or post 1947 could still be considered to be part of this high risk group, the number of years that we may witness increased breast cancer incidences could be much longer. For example, if we consider women born from 1945 to 1950 to be at greatest risk then the period of increased incidences of breast cancer could be extended from 1995 - 2030. If, however, breast cancer trends can be explained by increased level of screening we would expect to see a levelling off of breast cancer incidence during this same time period.

The lack of ability to compare the five birth cohort groups completely for all the critical ages of exposure, because of insufficient time post exposure, emphasizes the need to undertake further studies in order to be able to detect a relationship. If, for example, the

critical age of exposure is zero to nine years, studies done prior to 1989 would not be able to detect an increased incidence in breast cancer rates in pre-menopausal women. Correspondingly, studies undertaken prior to the year 2013 would not be able to detect an increase in breast cancer rates in post-menopause women if the critical age of exposure was zero to nine years.

Table 5.1 shows the year of diagnosis that would correspond with the various ages at diagnosis for women, given a critical age of 0 - 9 years, during the specific phases of exposure for the birth cohort groups. This table demonstrates that for comparisons to be made between the non exposure and peak exposure phases (where the greatest difference in incidence of breast cancer could logically be found) studies would have to be undertaken after 2040, such that comparisons could be made between the two birth cohort groups from age 30 to age 80. The latter would be essential, given that the majority of breast cancer cases in Ontario are detected between age 30 - 80. Studies done after 2060 would be even more telling if a relationship exists in a dose response fashion. In this case, one would expect to see reduced breast cancer incidences in the latter birth cohort groups.

The findings of this thesis point to the potential influence of age and timing of exposure and suggest the necessity for determining the ages of women investigated in previous studies examining the relationship between breast cancer and organochlorines. For example, in studies prior to 1990, if the age of women in the case group were greater

than 43 years old, it would suggest that these women had no exposure to organochlorines during this potentially critical phase of maturation and consequently these studies could be expected to reveal little or no relationship between exposure and breast cancer if this stage of exposure is critical. If, any age at exposure imparts an increased risk of breast cancer, we would expect these studies to show some relationship between organochlorine exposure and breast cancer. Given this scenario it would be logical to expect that birth cohorts exposed for the entire period, ie: age zero to age eighty, would have higher incidences of breast cancer than those only exposed after say age 45.

The finding that breast cancer incidence in post menopause women increased markedly starting in 1981 would indicate exposure to something in these women's past history could be causing these dramatic findings. If one assumes that, in 1981, the first of the birth cohorts turned 50 - 55 (women born in the birth cohort of 1926 -1931) and subsequently resulted in the increased incidence, these women would have received exposure to organochlorines starting at age 16 to 21 years of age in the increasing phase of organochlorine exposure and would have had a minimum of 34 years of exposure to organochlorines. This marks an exposure period during the increasing, peak, decreasing and minimal phase of organochlorine use. If exposure to organochlorines is a significant risk factor then one would expect to see escalating incidence rates of breast cancer as the high risk groups attain the post menopause phase of life.

If there is indeed a critical age of exposure, and this occurs between 10 - 19 years old as

the results suggest from the findings to date, then studies determining the relationship between organochlorine and breast cancer would have to consider focusing on women exposed during this stage of development. Comparisons of total body burden levels between controls and cases from these birth cohorts would be critical to determine if women with breast cancer have increased levels of organochlorines. As well, one would have to determine if women exposed during peak phases of organochlorine use have increased levels of breast cancer when compared to women exposed during other phases. Subsequently, it would be important to match cases and controls based on age at diagnosis and also to establish specifically groups from different exposure phases. For example, for every age at diagnosis there should be individuals from each critical age of exposure and from the different phases of exposure.

With these factors in mind it would be important to determine the birth cohorts of the women previously investigated to determine when the majority or average women already studied experienced exposure to organochlorines, and during what phase of human development. Examination of age only, with no consideration of the exposure stage, would not provide adequate control for variation in exposure, duration of exposure or the age of individual at time of exposure to organochlorines. For example, if a study uses samples taken in 1960 when the women were on average 45 years old this would represent on average birth cohorts from 1915 and these women would not have experienced exposure to organochlorines in the critical age period of zero to nine years. If however, studies were done in 1995 on women of average age of 45 years, these women

would represent the average birth cohort of 1950 and these women would have experienced exposure during the critical age period from zero to nine years. If age is an important factor, the need to determine which birth cohorts the women from previous studies belong to become immediately visible.

Table 5.2 shows the year of study, average subjects age, and average birth cohort of women under investigation in the previous studies on breast cancer and organochlorine exposure. From this table we can see that the while most of the studies were undertaken at approximately the same time there is a great variability in the average age and therefore of the birth cohort of the women being studied. For example, in the Wolff study undertaken during 1985-1991, the average age of the women studied was 50.7 years of age. Therefore during the six years of study the average birth cohort would have been women who were born during the years 1934 to 1940. The range in age for this study was from 35 - 65 years of age which would represent women born during the years 1920 - 1956. This means that the average women (or older than average) in the study would not have been exposed to organochlorines at the start of organochlorine use during the critical age of 0 - 9 old, but would have been exposed during the critical age of 10 - 19 years and older. While exposure for some women in the Wolff study would have occurred during the critical age of 0 - 9 years old, the average age of exposure during the peak use of organochlorine use would have been 15 to 21 years of age. In comparison, the average age of women in the Krieger study was 45.5 years old. Since the Krieger study was undertaken from the period of 1964 - 1971, this would represent women who were born

during the years 1919 - 1926. Therefore the average women in the Krieger study would not have received exposure to organochlorines during the critical ages of 0 - 9 years or 10 - 19 years. This is because the women of average and older would have been 21 years of age or older starting in 1947 and by the peak period of organochlorine use these women would have been 29 years or older. Interestingly, the Wolff study found a relationship between organochlorine use while the Krieger study found no relationship. If exposure to organochlorines is related to the development of breast cancer, only when exposure occurs during the critical age of 10 - 19 years, we would expect to find a significant relationship in the Wolff study and not in the Kreiger study, simply due to the average age of the study subjects.

In the Hunter study, the average age of participants in the study was 59 years old with a range from 43 to 69 years of age. Therefore the average woman in this study would have been born in 1930, and the women older than average in the study, would have been born between 1920 and 1930. These women would therefore have not experience exposure to organochlorine during the early stages of development and would have only started to be exposed to organochlorines when they were greater then 17 years of age. Thus, if the critical age of exposure was during the 0 - 9 years of age these women would not be expected to show increased incidence of breast cancer. If the critical age of exposure is 10 - 19 years of age, only the women of average age and younger would have experienced any exposure during this critical age of exposure. However, the younger women in this study would have been born from 1930 - 1946. Therefore some of the

very youngest women in this study could have been expected to have been exposed during their early childhood and early teens. If exposure during age 0-9 years or 10-19 years is important to the relationship between organochlorines and breast cancer then the Hunter study could be expected not to show a significance difference between the cases and control.

In the Hunter study, samples were collected many years post exposure and this alone could have significant impact in the actual relationship between body burdens and disease outcome. The follow up period of three years was very short and women diagnosed during this time period may have had increased catabolism rates due to the presence of cancer and subsequently had decreased body burdens. The short follow up period can also be expected to result in false negative controls which could also influence the outcome of this study.

If exposure to organochlorines is dependent on the total lifetime exposure as well as the stage of exposure, we would expect to find a significant relationship in the Wolff study which represents women of the birth cohorts ranging from 1920 to 1956. These women would have received significant exposure from a very early age and during all the years of organochlorine use. In comparison, the average women in the Kreiger would have significantly fewer years of lifetime exposure since exposure would have started at an older age. The first year of exposure would have started at 21 years of age for the average age women in the Kreiger study and this finding alone could have a significant

impact on the ability to detect a causal relationship between organochlorine exposure and breast cancer if the total amount or lifetime exposure to organochlorines was the driving factor in the relationship. The younger women in the Hunter study could be expected to show a difference in breast cancer rates if lifetime exposure is the driving factor in the organochlorine and breast cancer relationship since these women would have received exposure during their entire life. It would therefore be interesting to know the age distribution of women in this study to determine the plausibility that one could expect the studies findings to point towards the null.

Wasserman et al, (1976) found a positive relationship between organochlorine levels and breast cancer. However, the authors did not specify the average age at time of diagnosis or examination. Therefore, we were unable to relate this to the average birth cohort for the women in this study. Mussala-Rauhamaa et al, 1990 reported a significant relationship between increased levels of beta-HCH and breast cancer. The average age of the women in this study was 58 years old with a range in age from 35-86 years. This would mean that the average woman in this study represents women who were born during the year 1927 with the birth year ranging from 1903 - 1950. Interestingly the average women in this study would have received significant lifetime exposure to organochlorines with the bulk of exposure beginning when the average women were approximately 20 years old. However, given the range in the age of the women under study, exposure could have been in any of the critical age groups. For example, women age 35 at the time of this study would have been of the birth cohort of 1950 and these women would have received

peak exposure to organochlorines during the critical age of 0 - 9 years of age. All the women studied in this investigation would have been exposed to organochlorines for the entire period of organochlorine use.

Unger et al, 1984 investigated two groups of women. The first group contained samples taken from deceased patients while the second group of women contained samples from incident biopsy patients. In the first group the average age of the women studied was 61 years of age with a range of 43 - 82 years of age. This would represent women who were born during the years 1900 to 1939. In the second group the women were born during the birth cohort years of 1928 - 1957.

Falck et al, 1992, investigated the relationship between organochlorines and breast cancer in 1987. In this case the average age of the women investigated was 63 years old which would represent women of the birth cohort of 1924. The range in age of the women under study was from 36 to 86 years of age which would represent a range in birth cohort years from 1901 - 1951. Interestingly the authors of this study found a significant relationship between DDE, DDT and PCB and breast cancer. As can be seen from the average age of the women under study and the subsequent birth year the average women in this study would have been exposed to organochlorines for the first time when they were on average 23 years old. The range in age of first time exposure would be from age zero to forty-six years of age. Thus the women in this study would have been exposed to organochlorine during various stages of human development and the women who were 63

years or less would have received exposure to organochlorines for a significant proportion of their lifetime.

Dewailly et al, 1994 studied the relationship between organochlorine body burden and estrogen receptor-positive breast cancer cases. The authors found that women with estrogen receptor-positive breast cancer had a statistically significant higher mean level of DDE and PCB congener 99 in fat samples when compared to control subjects. They concluded that the results of their findings support the role of organochlorines in breast cancer development in hormone-responsive breast cancer. In this study, the mean age of the cases and control were 54.1 and 51.2 years of age with a range of 40 - 69 years old. Given that the study was undertaken during the years of 1991 - 1992, this would mean that the average birth cohort year was approximately 1934 and 1940 for the cases and controls respectively. The range in the birth cohorts would therefore have been between 1922 - 1952. This would suggest that the bulk of women in this study received exposure to peak organochlorine residues at the critical age of 10 to 19 years of age. However, they would have also received exposure to organochlorines during all the stages of human development and would have been exposed to organochlorines for a substantial number of years.

Legitimate arguments can be made that specific birth cohorts are at increased risk of developing breast cancer not from exposure to organochlorines but from a tendency to have increased proportions of other risk factors for developing breast cancer. The type

and number of risk factors or combination could be endless and may play a significant role in the development of breast cancer. The fact that other unknown risk factors may be present should not be ignored. When evaluating the potential relationship between organochlorines and breast cancer development, the major confounding factors are diet, lactation, parity, obesity, and the use of exogenous hormones. The lack of historical information on these confounders makes it extremely difficult to determine their potential impact for the population as a whole. Lactation history (duration and frequency) for example, can significantly decrease both the total and the life time exposure of an individual woman. In studies with limited numbers of subjects, lactation history could have a tremendous impact on the outcome of the study. For example, if cases have either a greater frequency or duration of lactation (or both), the case body burdens could be expected to be lower than controls. This confounder would then drive the findings of the study towards the null or perhaps imply a false protective factor since case subjects would have lower body burdens of organochlorines at the time of the study but perhaps higher prior to breast feeding. While on a population level breast feeding frequency and duration has been low, the examination of this confounder in breast cancer studies is important. This example also demonstrates the need for serial blood sample determinations to conclusively determine the impact of organochlorine body burdens in the life time of the individual. Future studies should be able to shed more light on the influence of these confounding factors and should take these factors into consideration.

The findings of this study suggest that the age at first exposure and duration of exposure

may be critical variables in interpreting observational studies investigating relationships between cancer and environmental chemicals. Furthermore, these variables have not been adequately accounted for in previous studies investing the relationship between breast cancer and organochlorines. Moreover, it is not yet possible to test several hypothesis related to breast cancer development in women born after 1947, so that "evidence of absence" in this group of women may indeed be "absence of evidence".

While this study did not prove that there is a causal relationship between organochlorines and breast cancer, there is however a suggestion that a relationship may exist. In order to fully assess this matter, studies must be undertaken with a complete understanding of the potential impact of: the age at exposure; timing; duration; type of organochlorines; and the overall lifetime exposure for both the individual and the population. The findings of this research project point to the need to develop standard research protocols, so that individual studies would have comparable subjects. Studies need to take into consideration the age and exposure status of the women under investigation and consider taking serial blood samples which account for changes in body burdens and confounding factors.

The ideal research project would evaluate women born during the various phases of exposure for each of the critical ages at exposure. All potential confounders, particularly breast feeding and food consumption patterns, should be determined to assess their impact on the relationship. Ideally, base-line blood or tissue samples should be taken at

the initiation of the study to record the level of exposure at that point in time, and to give an indication of the past exposure history of these women. The values obtained should be related to the age at time of sampling and to the various phases of exposure the individual has received during their life cycles. The women in these new studies should be followed prospectively to detect the development of breast cancer with greater number of years follow up then has been performed to date. Samples of blood or adipose could then be assessed at various time intervals to determine both the changes in organochlorine content and to compare the actual values. While a study of this nature would be costly and perhaps difficult to carry out, the importance of comparing women of different ages and exposure scenarios cannot be under estimated particularly if we wish to detect which group of women are at risk of developing breast cancer.

In summary, if critical ages are important in defining the relationship then studies must be undertaken after 2060 for critical age of exposure during 0 - 9 years old, 2050 for critical age 10 - 19, or 2030 for critical age 20 - 49 years of age. Minimally, studies should state which birth cohorts they are investigating in order to determine what stage of development or phase of exposure the women under study have experienced and how these findings impact the potential relationship between organochlorine exposure and the development of breast cancer. It will be interesting to evaluate the trends in breast cancer over the subsequent years as the various women, from birth cohorts exposed to high levels of organochlorines during at specific critical ages, attain the post menopause phase of life. While we may be unable to change the past history of exposure for these women, we will

be able to determine historically, over the next decades, if these women were indeed at increased risk of developing breast cancer from exposure to organochlorines.

Similarly, we may be able to further evaluate what factors place these women at most risk of developing breast cancer and how we may alter these risk factors. These findings may be important in developing countries where there is a continued use of organochlorines for malaria control. As well, it would be important for countries, such as Canada, where organochlorines can continue to be found as a result of volatilization and long distance aerial transport from developing countries. It would also be important to determine if a relationship does indeed exist between organochlorine exposure and breast cancer, should future outbreaks of diseases such as malaria drive the demand for inexpensive organochlorine use to control biting insects. It is hoped that the findings of this project will contribute to a better understanding of the exposure dynamics of organochlorines in the individual and the population and will help to find explanations for the increasing rates in breast cancer in women all over the world.

Table 5.1

Critical Age of Exposure and Year Of Diagnosis:

Critical Age of Exposure = 0 - 9 Years

Exposure Period	Cohort	Age at Diagnosis and Year at Diagnosis for Age 30	40	50	60	70	80
No Exposure (< 1947)	1934-38	1964-68	1974-78	1984-1988	1994-98	2004-08	2014-18
Increasing (1947-55)	1945-49	1975-79	1985-89	1995-1999	2005-09	2015-19	2025-29
Peak (1955-65)	1954-58	1984-88	1994-98	2004-08	2014-19	2024-29	2034-39
Decreasing (1965-75)	1965-69	1995-99	2005-09	2015-19	2025-29	2035-39	2045-49
Minimal (1975-85)	1974-78	2004-08	2014-18	2024-28	2034-38	2044-48	2054-58

Table 5.2 Comparison of Breast Cancer Studies - Average Age and Birth Cohorts

Principle Author	Relationship Found	Year of Study	Average Age of Case	Resulting Average Birth Cohort
Wasserman		1976	NA	NA
Mussala-Rauhamaa	+ Beta-HCH	1985-86	58(35-82)	1927 (1903-1950)
Flack	+ DDE, DDT + PCB	1987	63(36-86)	1924 (1901-1951)
Unger	a) b)	1982*	a) 61 (43-82) b) 40 (25-54)	a) 1921 (1900-1939) b) 1942 (1928-1957)
Wolff	+ DDE	1985 - 1991	50.7 (35-65)**	1934 (1920 - 1950) 1940 (1926-1956)
Dewailly	+DDE	1991-1992	40 - 59	1932 - 1951
Kreiger	None	1964 - 1971	45.2***	1919-1926
Hunter	None	1989-1990	59(43-69)	1930 (1920 - 1946)

* Approximate Year of Sampling
** Median age
*** Mean age at examination

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