THE IMPACT OF PESTICIDE EXPOSURE ON BREAST CANCER INCIDENCE: EVIDENCE FROM COSTA RICA, 1996-2000

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ABSTRACT

The low percentage of breast cancer cases related to reproductive history risk factors and to genetics suggests that the environment may play a role in breast cancer etiology. Pesticide exposure has been hypothesized to have an important effect. This ecological study tests whether breast cancer incidence in Costa Rica is related to pesticide exposure, after controlling for parity, socioeconomic status, age at first full-term pregnancy, and access to health care. Spatial analysis techniques were used to test for spatial autocorrelation and to rule out the heterogeneity of a possible relationship between breast cancer and pesticides. Because of the time-lag between exposure and incidence, migration bias was also mitigated. Results suggest that there is a heterogeneous association between pesticides and breast cancer, which is significant only in some rural agricultural areas of the country. Conclusions about causality can not be drawn from an ecologic approach, like the one taken in this study.

1. INTRODUCTION

High breast cancer incidence rates in developed countries like the United States and countries in Western Europe contrast with the low rates in Japan and most other Asian countries. Although incidence rates differ in developed and developing countries, breast cancer is one of the most frequent cancers affecting women (Bray et al., 2004; Sasco, 2003). In terms of incidence, breast cancer is increasing worldwide, and is the second most common (lung cancer has the highest incidence). Breast cancer is the most prevalent cancer in the world today (Parkin et al., 2001), and is the leading cause of death from cancer in women worldwide (Lacey et al., 2002; Pisani et al., 1999).

Costa Rica has achieved outstanding health standards. Total life expectancy in this Central American country is 79 years, which is comparable to Canada's total life expectancy of 80 years and United States' figure of 78 years (Population Reference Bureau, 2005). As a result of the demographic and epidemiological transition in this country, in recent decades the causes of morbidity and death have shifted from communicable to non-communicable diseases like cardiovascular disease and cancers (Rosero-Bixby, 1991). Breast cancer is the most frequent cancer type in Costa Rica's female population, followed by cervix uteri and stomach cancer. As shown in **Graph 1**, the incidence of breast cancer in U.S. tends to be two to three times higher than in Costa Rica. Part of this difference is presumably related to better screening in the US.

Increased cell division induced by exogenous or endogenous stimulation is the core of pathogenesis of human cancer. For breast cancer, as well as for most cancer types, causes are only partially understood. But as for most health conditions, at least a portion of cases can be explained by socio-economic, environmental, genetic, and behavioral risk factors. Breast cancer incidence is known to be related to reproductive characteristics of women. But those risk factors

account for approximately 30% of the cases (Timander and McLafferty, 1998). It is estimated that the effect of genetic factors account for 10% or less of cases (Rosser, 2000). Although environmental factors may also have an influence on breast cancer, they are not as often considered in etiologic research as individual reproductive factors. The low percentage of cases that are related to reproductive characteristics and genetic endowment suggest that environment may be playing an important role in cancer etiology. Pesticide exposure is one of those environmental factors mentioned in a number of studies (for example Charlier et al., 2003 and Wesseling et al., 1999).

This analysis has the aim of testing whether pesticide exposure across the country may be related to breast cancer incidence. Even though more comprehensive research should ideally include data on genetic factors (like mutations in genes BRCA1 and BRCA2) as well as behavioral risk factors (like physical activity or alcohol consumption among others) those risk factors are not considered in this approach. This paper is focused on trying to provide etiologic clues for breast cancer in Costa Rica based on demographic, socioeconomic and, critically environmental characteristics.

2. BACKGROUND

2.1. Breast cancer etiology

2.1.1. Reproductive history and hormones

There is epidemiologic evidence that natural ovarian hormones affect breast cell division rates, acting as promoters of carcinogenesis. Although ovaries produce other hormones, estradiol and progesterone are the major ovarian hormones that play important roles in increasing breast cancer risk (Bernstein and Ross, 1993; Pike et al., 1993). Non-hormonal dependent adult cancers like lung or stomach cancer rise monotonically and rapidly with age. Breast cancer incidence, in contrast, slows down at around age 50, which is the average age at menopause (Parkin et al., 2001; Timander and McLafferty, 1998). This slowing of the rate of increase around age at menopause has been observed in many countries (Gordis, 2004). Data from Costa Rica for the period of this study shows the same pattern, as can be observed in the line component of **Graph 2**. Thus, etiologic elements for breast cancer appear to be present in premenopausal women and to be reduced thereafter.

Most breast cancer cases are diagnosed after age 45. Only about 20% of new cases of breast cancer were diagnosed before age 45 in Costa Rica between 1996 and 2000 (histogram, **Graph 2**). The apparent contradiction of lower percentages of cases but higher incidence rates at older ages is due to the denominator of incidence rates: there are fewer women in the older age groups. Although older women have a higher risk of breast cancer, cases diagnosed at older ages contribute only a small proportion of the total number of cases at all ages.

That breast cancer risk is influenced by endogenous hormones is well established. The general observation is that longer exposure to menstrual activity, and therefore to sex hormones, is associated with risk of breast cancer. Early age at menarche (11 or younger), late age at menopause (55 or older), and late age at first full-term pregnancy (30 or older) are associated with modest elevations in risk for breast cancer (Timander and McLafferty, 1998).

It has been reported that women with early menarche have higher estrogen levels for several years after menarche and probably throughout their reproductive lives. In addition, the increased risk associated with late age at natural menopause is generally not seen until after the age of 65. The increased risks associated with early age at menarche and late age at menopause suggest that longer exposure to sex hormones during the reproductive years is associated with higher risk of breast cancer (Kelsey et al., 1993).

Late age at first full-term pregnancy is generally accepted as a major breast cancer risk factor (Pike et al., 1993). There is a large body of evidence that states that on average, the younger a woman is when she has her first full-term pregnancy, the lower is her risk of breast cancer. A first pregnancy that is not carried out to term does not offer such protective effects. An early full-term pregnancy acts as a protective factor about 10 years after the pregnancy. The immediate effect is an increase in risk due to hormonal changes such as the large increases in estradiol and progesterone. After a period of about 10 years this short term adverse effect is replaced by a long-term beneficial effect (Bernstein and Ross, 1993). This effect is brought about by a decrease in the number of formerly undifferentiated cells that develop into fully differentiated cells, which are less susceptible to genetic damage and to subsequent propagation of damaged cells (Brody and Rudel, 2003). Nulliparity is associated with breast cancer diagnosed after age 40 (Kelsey et al., 1993).

There is a relationship between the use of hormone replacement therapy (HRT) and the risk of breast cancer. The available evidence indicates that the risk is higher while women are using HRT, and it increases with duration of use. Most of the evidence relates to use of HRT containing estrogens alone (Beral et al., 1999). Nevertheless, there is also evidence of increased risk of breast cancer with combined estrogen/progestin HRT (Writing Group for the Women's Health Initiative Investigators, 2002).

Long-term use of oral contraceptives may slightly increase the risk of breast cancer, even though there is not conclusive evidence of this relation (Malone et al., 1993). According to Pike et al. (1993), for those women diagnosed before age 45 there is a small but statistically significant increase in breast cancer risk per each additional year of use of oral contraceptives. Nevertheless, there is evidence that the possible small increase in risk in women taking oral contraceptives diminishes when contraception ceases and, after 10 years, none of the risk remains (Reeves, 1996). A case-control study conducted in Costa Rica between 1982 and 1984 found no elevation in the relative risk of breast cancer in users of oral contraceptives as compared to never users (Lee et al., 1987).

Breastfeeding is hypothesized to be a protector factor for women contracting breast cancer before age 40. It is unclear if it has any relationship with breast cancer after age 40 (Steingraber, 2001). There is no conclusive evidence that breast cancer risk decreases with increasing duration of breastfeeding. But if such a protective effect exists, it has been postulated that it may be related to the breastfeeding effect of delaying reestablishment of ovulation and hence diminishing the cumulative number of ovulatory cycles. A protective effect of breastfeeding could also be related to hormonal changes, such as increased prolactin and decreased estrogen production during lactation, or to physical changes in cells of mammary ducts or the mechanical flushing out of carcinogens during lactation (Kelsey et al., 1993). A case-control study conducted in Costa Rica found no evidence of association between breastfeeding and breast cancer (Rosero-Bixby et al., 1987).

2.1.2. Genetic factors

Family history of breast cancer also increases the risk, especially if a first degree relative was diagnosed at an early age (Timander and McLafferty, 1998). Three genes conferring dominant susceptibility to breast cancer have been mapped: *BRCA1* on chromosome 17q, *BRCA2* on chromosome 13q, and mutations in the tumor suppressor gene *TP53* (Parkin et al., 2001). It is estimated that around 2 percent of breast cancers are due to *BRCA1* in Western populations, but around 10 percent for women diagnosed before the age of 40 (Ford et al., 1995). Overall, *BRCA1* is estimated to account for perhaps 5 percent of all cases (Davis and Bradlow, 1995).

It has been estimated that overall only 5 to 10 percent of breast cancer cases are inherited (Rosser, 2000). As reported by Easton et al. (1993), Hulka and Stark (1995), and Muir et al. (2004), predisposing genetic factors are believed to account mainly for premenopausal breast cancer cases, which usually are the lowest proportion of cases. Reproductive history accounts for only approximately 30% of the cases (Timander and McLafferty, 1998). Breast cancer incidence has been increasing and the majority of this increase can not be explained by the rate of utilization of mammograms (Steingraber, 2000). Thus, we still appear to be missing a critical piece of the breast cancer puzzle.

Lichtenstein and colleagues (2000) conducted analyses of cohorts of twins from Sweden, Denmark, and Finland in order to study the environmental and genetic factors in cancer causation. They concluded that the environment has the principal role in cancer etiology. According to Parkin et al. (2001) most of the international differences in incidence of breast cancer are the consequence of differing environmental exposures. It has been hypothesized that breast cancer may be caused by environmental factors like pesticides, electromagnetic fields and ionizing radiation (Brown et al., 2001). Results from such studies have thus far been inconclusive, but not entirely negative.

That genes and the environment interact is well established. Mutations in the tumor suppressor gene *TP53* are found at a high frequency in breast tumors. Some of these mutations are thought to be associated with exposure to organochlorine pesticides (Høyer et al. 2002). In general, pesticides may damage DNA. Long-term accumulation of DNA damage increases the risk of cancer. Results from a case-control study conducted in Costa Rica showed a significant increase in DNA damage in women who were occupationally exposed to pesticides after working from 5 to 15 years in banana farms (Ramírez and Cuenca, 2002). The role of pesticide exposure in breast cancer causation is discussed with more detail below.

2.1.3. Pesticide exposure

Cancer may be caused by endogenous as well as exogenous factors. Nevertheless, most epidemiological literature mainly emphasizes how breast cancer is influenced by endogenous hormones and pays less attention to exogenous agents that may act similarly and trigger the disease. Rising incidence and poor prediction of individual risk factors have prompted a search for additional modifiable risk factors (Brody and Rudel, 2003). During the last decades, some scientists have focused their attention on investigating potential environmental causation of

breast cancer, often concentrating on endocrine disrupters –chemicals that affect the endocrine system (Brown et al., 2001; Pollner, 1993).

A number of pesticides are endocrine disrupters and some hypotheses have been advanced in relation to a risk for breast cancer in women (Arnold et al., 1996). Evidence from both animal and epidemiologic studies suggests that there may be vulnerable periods, perhaps during gestation or adolescence or between menarche and birth of a first child, when exposure is most important (Brody and Rudel, 2003). Even though existing studies do not yet allow researchers to sort out exactly which pesticides are linked to which cancers (U.S. Department of Health and Human Services, 2003), it has been found that some groups of pesticides can mimic endogenous estrogen and might be associated with breast cancer via their estrogenic activities.

The endocrine disruption for cancer is not a new subject. It was explicitly mentioned by Rachel Carson in her classic book *Silent Spring* published in 1962 (Steingraber, 1998). Among endocrine disruptors, the xenoestrogens are seen as potential factors in breast cancer. Xenoestrogens are chemicals foreign to the body that act like estrogens. They may be present in pesticides, in plastics and detergents (Aschengrau et al., 1998; Davis and Bradlow, 1995). The cells of the breast contain large numbers of estrogen receptors. According to the "xenoestrogen hypothesis", xenoestrogens slip from blood serum into the interior of breast cells, and by tinkering with particular genes, trigger uncontrolled cell division and therefore breast cancer (Steingraber, 2000).

Xenoestrogens are not easily metabolized and excreted from the body. They tend to persist for decades and can accumulate to high levels in breast adipose tissue. They are also known to be present in breast milk (DeBruin and Josephy, 2002; Snedeker, 2001; Davis and Bradlow, 1995). Dichloro-diphenyl-trichloroethane (DDT) is a very persistent pesticide, and so is DDE (its major metabolite). There is evidence of accumulation of DDT and DDE in the milk of nursing mothers in a large number of countries, although they are thought to be higher in developing countries (Albert, 1981). A study conducted in Costa Rica revealed high concentrations of the metabolite DDE in all of the 51 analyzed samples of milk. The highest concentrations of DDE were observed in the provinces where crops were intensively sprayed with DDT since the 1950s until the 1980s (Umaña and Constenla, 1984)

Organochlorine pesticides and polychlorinated biphenyls (PCBs) have raised concern about their relation to breast cancer (Hulka and Stark, 1995). Among those chemicals are: DDT, lindane, cyanazine, aldicarb and atrazine (Muir et al., 2004). Most organochlorine pesticides were restricted in the early 1970s in developed countries, but they were restricted in Central America until the 1980s. Costa Rica first restricted the traditional organochlorines in 1980 and banned them between 1988 and 1990. After 1983 there was no importation of DDT for agricultural use, but it was used for malaria control purposes between 1983 and 1985 (Castillo et al., 1997). Although DDT was banned, it is known to persist in the environment for more than 50 years (Davis and Bradlow, 1995). After other organochlorines were banned, the use of lindane increased in Costa Rica until it was restricted in 1988. According to volume of importation, aldicarb and atrazine are among the major pesticides currently in use in Central America (Castillo et al., 1997).

Pest resistance and pesticide use appear to be escalating fast in Costa Rica. According to Programa Estado de la Nación (2004), even though crop extension has not significantly changed since 1997 in Costa Rica, pesticide importations as well as their use per hectare have been

steadily increasing. As shown in **Graph 3**, since the mid-1980s, there has been an increase in the importation of pesticides classified as carcinogenic (categories A, B1 and B2) according to U.S.A. Environmental Protection Agency (EPA).

Even though several previous studies have analyzed this possible link in different countries, results typically have been inconclusive when trying to establish etiological links between cancer and the environment. Some studies have found evidence of a relation between pesticide exposure and breast cancer. I will briefly refer to some of them. Falck et al. (1992) measured levels of pesticide residues in mammary adipose tissue of women with breast cancer in Connecticut. They concluded that environmentally derived carcinogens were likely to have a role in the occurrence of breast cancer. Kettles and colleagues (1997) created a summary index of triazine pesticide exposure in Kentucky counties. After controlling for several risk factors, they found that exposure to triazine was related to breast cancer. The odds ratio (OR) associated to high levels of triazine exposure was 1.20 and the OR associated to medium levels of exposure was 1.14

Høyer and colleagues (1998, 2000) investigated blood serum concentrations of xenoestrogens in women of Denmark. They concluded that exposure to xenoestrogens – specifically organochlorines- may increase the risk of breast cancer. Petralia et al. (1998) estimated the risk of breast cancer by occupational exposures in Shanghai, China. Based on a small number of cases, after controlling for risk factors, they found elevated standardized incidence ratios of breast cancer in women exposed to pesticides. Wesseling et al. (1999) found evidence that excess risks of hormone-related cancers could be associated with occupational or environmental exposures to pesticides in Costa Rica.

Band et al. (2000) conducted a case-control study in Canada and found that women occupationally exposed to pesticides had an excess breast cancer risk. Dolapsakis and colleagues (2001) reported the results of a case-control study in Greece that used mammography screening to test the impact of occupational exposure to pesticides in greenhouses. Their preliminary results indicated that exposed women had higher risks of incidence of breast lesions, which are risk markers for subsequent breast cancers.

A Belgian case-control epidemiologic study (Charlier et al., 2003) measured blood levels of pesticide residuals in breast cancer patients and controls. They found that women with breast cancer were more likely to have pesticides in their blood than were women without breast cancer. Finally, a spatial analysis conducted in England showed some evidence that pesticides could be related to breast cancer at least in the rural areas of one the two studied counties (Muir et al., 2004).

Nevertheless, some other studies have found no evidence of relationship between breast cancer and pesticide exposure. I will briefly mention some of them. Dorgan and colleagues (1999) conducted a case-control study nested in a cohort study in Columbia, Missouri. They analyzed blood samples to determine if pesticide residues were associated with breast cancer. Their results did not support a role for organochlorine pesticides in breast cancer etiology. Also in 1999, Zheng et al. published results of a case-control study in which breast tissue levels of DDE and DDT were measured. They did not find an association between tissue levels of DDE and DDT and breast cancer risk.

Laden et al. (2001a) combined the cases and controls of five large studies of women conducted mainly in the north-eastern US in 1993. These studies evaluated the association of levels of DDE and PCBs in blood plasma or serum with breast cancer. They concluded that the combined evidence of those five studies did not support the association. Laden and colleagues (2001b) conducted another case-control study nested in a cohort study. This study also measured plasma levels of DDE and PCBs. Overall, their results did not support the hypothesis that exposure to DDE and PCBs increases the risk of breast cancer.

Gammon et al. (2002) conducted a case-control study in Long Island, New York to determine whether breast cancer risk is increased with exposure to organochlorines. Their findings did not support the hypothesis of a relationship. Hopenhayn-Rich and colleagues (2002) developed indices of environmental exposure to the pesticide atrazine in Kentucky. They analyzed the data by county and by area development districts, and found no association between exposure to atrazine and breast cancer.

Reynolds et al. (2004) found no evidence of elevated breast cancer incidence in areas of recent agricultural pesticide use in California. Brody et al. (2004) conducted a case-control study in Cape Cod, Massachusetts. They controlled for risk factors and allowed a latency period of five years between pesticide exposure and breast cancer diagnosis. No pattern of association between pesticide use and breast cancer was found.

Finally, López-Cervantes and colleagues (2004) performed a meta-analysis of 22 articles that studied the relationship between DDE and DDT and breast cancer. They found no evidence of publication bias. Overall, they found strong evidence to discard the relationship between DDE and breast cancer. Nevertheless, they pointed out that some aspects are not yet accounted for in the studies performed so far. The exposure to DDT during critical periods of human development (from conception to adolescence) may be related to breast cancer. Furthermore, individual variations in metabolizing enzymes of DDT and its derivates are likely to modify the consequences of exposure to DDT. They concluded that these aspects needed further research.

In general, much of the previous research that supports as well as that does not support the hypotheses of association between pesticides and breast cancer has important limitations. The inconsistencies among them may be related to study designs, or lack of information on other contributing and vulnerability factors. Although past research offers important insights into the effects of environmental contaminants on breast cancer, much of that research has failed to control for known risk factors, so significant findings may be spurious. Furthermore, most studies of associations between breast cancer incidence and environmental hazards do not control for potential migration bias affecting exposure.

Research on pesticide health effects in less developed countries has been mainly focused on acute pesticide poisonings and not on long-term effects such as cancer. Epidemiologic observational studies to measure pesticide exposure are usually difficult to design and interpret because humans are rarely exposed only to one pesticide. Nevertheless, specifically in Costa Rica, pesticide use has been associated with increased cancer incidence of lung and female hormone-related cancers in the most rural areas from 1981 to 1993. Relative risk of breast cancer was 25% to 80% higher in regions with high pesticide exposure as compared to regions of low pesticide exposure (Wesseling et al., 1999).

One of the drawbacks of Wesseling and colleagues' study conducted in Costa Rica is that it only controlled for urban/rural location. It did not control for risk factors such as fertility, socio

economic status, age at first full-term pregnancy, and access to health care. Furthermore, the lack of use of spatial techniques to test for spatial autocorrelation in the study makes it difficult to discern whether they obtained appropriate estimates in their results. They also did not control for migration in this study.

My study, based on more recent breast cancer incidence data, uses spatial analysis to test for spatial autocorrelation and to rule out the heterogeneity of a possible relationship between breast cancer and pesticides. It takes advantage of the pesticide exposure index developed by Wesseling et al. (1999), and makes an important contribution by controlling for the aforementioned risk factors, as well as by mitigating the effect of migration, as it will be discussed in the following sections. Yet, while discovery of an association between pesticide exposure and breast cancer can point to needed further research, conclusions about causality can not be drawn from an ecologic approach, like the one taken in this research.

2.2. Spatial autocorrelation

The concept of spatial autocorrelation in the context of this research is relevant given that nearby areas may have similar incidence rates of breast cancer when the populations in those areas share a common exposure. Spatial effects -spatial heterogeneity and spatial dependence- are properties generally found in spatial data. These effects complicate the statistical analysis of such data. Moreover, it is not easy to discern whether the data arise from a heterogeneity or dependence data-generating process. Spatial heterogeneity results from relationships between dependent and independent variables that vary across space. Spatial dependence results from the influences of individual observations on neighboring observations.

One could think about relationships that vary across space such as pesticide use, or any other environment related characteristic, as well as one could think about processes of social and economic distance which can definitely be related to reproductive history characteristics associated to breast cancer risk. Both spatial heterogeneity and spatial dependence could be present in the spatial patterning of breast cancer risk, and that is why methods that can handle spatial autocorrelation were used in this study.

2.3. Migration bias

Breast cancer takes many years to develop, so its occurrence reflects exposure over a woman's lifetime, at diverse times and places. When trying to find associations between environmental factors and diseases with long lags between exposure and occurrence of disease -like breast cancer- migration plays a very important role. It is important to minimize migration bias, particularly in ecologic studies of health outcomes with a long latency period (Tong, 2000). Assuming that place of residence at diagnosis has always been the same place individuals have lived, and therefore the most probable place of environmental exposure, can be misleading. A way to give credence to any hypothesis regarding environmental exposure therefore should attempt to control for migration bias (Timander and McLafferty, 1998).

History of changes in place of residence is tracked neither in the National Tumor Registry –the source of case information in this research- nor in any other national register. Nevertheless, every Costa Rican citizen has a personal unique identification number (ID), which can be used to

identify place of residence using voter registration lists. For every person 18 years or older, voter registration lists provide the reported place of residence. These lists are exhaustive, and are more accurate every four years when presidential elections take place. In order to mitigate the migration bias described above, this research considers history of place of residence for up to 13 years. Voter registration lists for presidential election years were used in this analysis in order to have more accurate information. More details are presented in section 3: Methods and section 4: Results.

3. METHODS

3.1. Description of data and sources

Costa Rica's administrative divisions consist of 7 provinces, each of which is divided into counties and each county into districts. There were a total of 81 counties and 459 districts in 2000. Breast cancer incidence was analyzed at the district level for a five year period: 1996-2000. Computerized record linkage between various registries was employed using unique personal IDs in order to take advantage of all the available information. Six main sources of information were used: National Tumor Registry, Population Census, Vital Statistics, Voter Registration Lists, Access to health care data, and Pesticide exposure indicator data.

1. Cancer data. Data about cancer cases comes from the National Tumor Registry (NTR) database. The standard NTR record contains personal identification (ID), gender, age at diagnosis, tumor site and histology, year of diagnosis, and residence at diagnosis. Access to the NTR was provided by the Ministry of Health in Costa Rica. This nationwide population-based registry has been maintained by the Ministry of Health since 1977. Since 1980 all hospitals and private pathologists have agreed to report any hospitalizations or outpatient biopsies associated with a cancer diagnosis (De Bermudez, 1985). According to the last publication of "Cancer incidence in five continents" by the International Agency for Research on Cancer (IARC), this registry has high indices of data quality. A total of 90% of breast cancer cases diagnosed between 1995 and 1996 in Costa Rica were diagnosed based upon morphological verification of tissue. Also, just 3% of cases were reported based on death certificate only (Parkin et al., 1997). NTR's coverage has been estimated to be around 98% (Lee et al., 1987).

2. Population data. Population data from Costa Rica's Population Census 2000 was used. This information is publicly available at http://censos.ccp.ucr.ac.cr. I used female population by age in each geographical region, grouped in five-year age strata.

3. Vital Statistics. These include the birth and death database. These databases contain the date and place of occurrence of the event as well as the individual person's ID. This personal ID was used to validate NTR information about date of birth and death for each breast cancer case analyzed by linking the three registries. The vital statistics databases were provided by Central America Population Research Center, University of Costa Rica.

4. Voter Registration Lists. Four voter registration lists containing residence information of adult Costa Ricans with their personal ID were used: 1990, 1994, 1998 and 2002. These years were selected because they are electoral years and therefore citizens are more likely to update their place of residence in the registry. These four registries were linked to NTR in order to establish an approximate residential history for each breast cancer case. This resulted in residence history of up to 13 years. Electoral registries databases were provided by Central America Population Research Center, University of Costa Rica.

5. Access to Health Care Data. This database is a comprehensive index of geographic accessibility to health care facilities in Costa Rica for year 2000. All health care facilities are included in this index: primary health care facilities, clinics and hospitals. It was created by Rosero-Bixby (2004) using Geographic Information System (GIS) technologies and aggregating characteristics of both population and health care facilities. District level data on this index was provided by its author.

6. Pesticide Exposure Indicator Data. Pesticide exposure is a key variable in this research. I use an index which estimates the mean pesticide load per inhabitant at the county level for year 1984. It was created by Wesseling et al. (1999) as a means to investigate geographic differences of cancer incidence in Costa Rica. It uses population size in each geographic unit along with information about areas treated with pesticide and the average number of pesticide applications per year, this latter number corrected for aerial/non aerial application. County level data on this index was provided by the authors.

3.2. Statistical data analysis

3.2.1. Spatial autocorrelation test

Spatial autocorrelation was measured using the Moran's I coefficient, which is a weighted product-moment correlation coefficient in which the weights reflect geographical proximity. Moran's I is used to detect departures from spatial randomness. It is used therefore to determine whether neighboring areas are more similar than would be expected under the null hypothesis of no spatial dependency. Moran's I values and probabilities were calculated using the GeoDa 0.9.5i software (Anselin, 2005). Different weight matrices were tested for this research, with each of them yielding similar results. The statistical results in this research were obtained using a 1st order queen matrix, which is calculated taking into consideration all district neighbors having a shared boundary or otherwise touching.

3.2.2. Regression analysis

Given that breast cancer is a "rare event", cases are assumed to be generated from a Poisson distribution. The preponderance of zeros and the small values and clearly discrete nature of the dependent variable (number of new breast cancer cases on each district), suggests that we could improve on Ordinary Least Squares (OLS) with a specification that accounts for these characteristics (Greene, 2000). This type of regression is the most commonly used when the

dependent variable is count data i.e. it takes the form of non-negative integer values. The Poisson regression method allows for the statistical modeling of data when there are a small number of events within strata. Therefore I used Poisson regression in my analysis, as similar previous studies have done (Muir et al., 2004; Hopenhayn-Rich et al., 2002; Wesseling et al., 1999). In this model, the Poisson distribution provides the probability of the number of events, and the parameters correspond to the expected number of occurrences as a function of the independent variables (Kennedy, 1998). This part of the analysis was done using Stata 7 software.

Also, since this analysis is based on geographical units, it was necessary to test for spatial autocorrelation in the model. This part of the analysis was conducted using GeoDa software (Anselin, 2005), which tests for spatial autocorrelation of error terms in regression models and also enables the running of spatial regression models. GeoDa software does not have applications to test for spatial autocorrelation in Poisson regression models. Therefore, an OLS regression model was used to test for spatial autocorrelation with GeoDa.

Further descriptive spatial analysis of the data was conducted using a geographically weighted regression approach. GWR 3.0 software was used for this purpose. Geographically weighted regression is a statistical technique to analyze spatial variations in relationships. It allows a regression to be carried out at each of the observation points in a geographic region. Since it is very likely that regression parameters in a global regression model are not constant across the whole region, exploring the data using this technique allows determining how each parameter varies across space. Geographically weighted regressions have proved to be an important tool to help to understand spatial heterogeneity in data, which justifies its use in this study.

Dependent variable

The dependent variable in the OLS regression analysis used to test for spatial autocorrelation was the age-adjusted breast cancer incidence rate in each district[1]. The national age distribution of women according to Costa Rica's 2000 Census was used as the standard population. It will be shown in the next section that there was no need to use any spatial regression model. Poisson regression models were used for further analysis. The dependent variable in the Poisson models was the count of new cases of breast cancer diagnosed in each district between 1996 and 2000. The mean age at diagnosis was 56 years (standard deviation = 14).

Using the count of cases as the dependent variable immediately suggests the need to control for female population exposed to breast cancer in each district, because each count of cases refers to areas of different underlying populations. The expected number of cases was used as an offset variable to control for population size. The standard to estimate the expected number of cases was the observed incidence for the entire country during 1996 to 2000. This reduces the potential confounding effect of different age distributions in the districts and allows for valid comparisons among geographical units. The observed number of cases b_i was the dependent

[1] Breast cancer incidence does not have a normal distribution. It rather behaves as a Poisson. Nevertheless, an OLS approach had to be used to test for spatial autocorrelation since current GeoDa software does not allow for spatial autocorrelation testing under Poisson distribution.

variable, and the expected number of cases b_i^E was the offset variable introduced in the right hand side of the Poisson regression model. Therefore:

$b_i = P\ (b_i, x)$
$b_i^E = \sum\ (M_i x * W_i^S x)$

Where: b_i is the observed number of cases at location i; P indicates a Poisson function; x is the age group; b_i^E is the expected number of cases at location i; $M_i x$ is the observed population size in location i at age x; and $W_i^S x$ is the incidence rate in the standard population at age x.

The interpretation of coefficients in Poisson regression models is different from that on OLS models because of exponentiation. Some calculus and algebra show that:

$$\frac{\partial E(y_i \mid x_i)}{\partial x_{ji}} = \exp(\beta_1 + \beta_2 x_{2i} + \ldots \beta_k x_{ki}) \times \beta_j = E(y_i \mid x_i) \times \beta_j$$

Therefore, a one unit change in the jth regressor leads to a change in the conditional mean by the amount of:

$$E(y_i \mid x_i) \times \beta_j$$

Whereas in the linear model we would simply have:

$$\beta_j$$

It has been suggested that breast cancer may have a different etiology among pre and postmenopausal women (Davis and Bradlow, 1995). Menopause is more likely to occur between 45 and 50 years of age. Therefore, age has been used as a proxy for menopausal status in population studies. Some studies have used the age of 50 as the cut point – for example Kulldorff et al. (1997)-, while others have used age 45 or older as a proxy of postmenopausal status –for example Muir et al. (2004)-. As reported by Easton et al. (1993) and by Hulka and Stark (1995), predisposing genetic factors are believed to account for a larger proportion of cases before age 45. Environmental exposure is believed to account mainly in cases diagnosed after age 45. Based on this premise, Muir et al. (2004) included only cases diagnosed at age 45 or older in their study of the association between breast cancer incidence and pesticides in England. In order to reveal whether pesticide exposure has a different effect on older women, I conducted the analysis on three sets of ages at breast cancer diagnosis: all ages, younger than 45, and women of 45 years of age or older.

Independent variables

Five independent variables were considered in the models: pesticide exposure index, access to health care, cohort total fertility rate, percentage of women with late age at first full-term pregnancy and social lag index.

1. Pesticide exposure in 1984. The pesticide exposure index (PEI) was calculated by Wesseling et al. (1999) by means of the following formula:

$$PEI = \left(\sum_{i=1}^{k} h_i n_i a_i\right) / \text{population}$$

i = crop (1, 2,...k)
h_i = hectares treated with pesticides
n_i = estimated average number of pesticide applications per year
a_i = aerial spraying correction factor

The numerator of the formula quantifies the extent of pesticide treated agricultural land, weighted by intensity of pesticide use for each crop. With the population of the county in the denominator, the PEI provides an estimate of mean pesticide load per inhabitant at the county level in 1984. Rather than providing a dose, this indicator provides a marker of the extent and intensity of use of pesticides that allows ranking counties by pesticide use. In the regression analyses I conducted, I used the decile of PEI rather than its absolute value in order to make interpretations more meaningful. Since my analysis is conducted at the district level and no data were available to calculate PEI for each district, it was assumed that all districts belonging to one county had the PEI value of their corresponding county.

The last Agricultural Census conducted in Costa Rica was in 1984. Pesticide exposure, which was calculated using information from that census, is therefore the most current information at the national level. Since breast cancer, as most cancer types, has long lag periods between exposure and occurrence of disease, this analysis benefits from the use of a time-lagged pesticide exposure. If pesticide exposure actually is associated with initiation of breast cancer, it is more likely to be related with past rather than current pesticide exposure levels (Birnbaum and Fenton, 2003). There is a temporal lag between exposure and development of breast cancer, but the length of that latent period is unknown so far. The use of PEI in 1984 allows for a time lag of up to 16 years in this analysis.

As mentioned in section 2.1.3 above, there is not conclusive evidence of an association between breast cancer and pesticide exposure. Some studies have found evidence of a positive association. Some other studies have found no evidence of such association. My hypothesis is that is that pesticide exposure is directly associated with breast cancer incidence in Costa Rica.

2. Density index of access to health care services in 2000. Although access to health care is a concept with at least two dimensions: geographic and social (Donabedian, 1973), geographic access is what this index measures. Geographical access to health care facilities is measured using a comprehensive index of accessibility that results from the aggregation of all facilities weighted by their size, proximity, and characteristics of both the population and the facility.

More details about the construction of this index can be found in Rosero-Bixby (2004). The density index of access to health care services uses physician hours per capita yearly as the metric. The greater the value a district has for this index, the better access to health services has its population. In the regression analyses I conducted, I used the decile of density of access to health care.

A greater supply of family physicians is associated with an earlier detection of breast cancer (Starfield et al., 2005). Ferrante et al. (2000) found that the increase in the supply of primary care physicians is associated with a statistically significant increase in the odds of diagnosis of breast cancer in an early (rather than late) stage. Most mammograms are ordered by primary care physicians, and a physician's advice to have mammograms usually enhances their receipt (Starfield et al., 2005). Access to health care is therefore important regarding early detection of breast cancer. Regions with better access to health care may be hypothesized to have an "artificial" higher incidence because of earlier diagnoses, as compared to regions with worse health care access. My hypothesis is that after controlling for risk factors, health care access has a positive association with breast cancer incidence in Costa Rica.

3. Cohort Total Fertility Rate. Total fertility rate (TFR) was estimated using mean parity, or mean number of children ever born of the cohort of women born between 1951 and 1955 in each district. As mentioned by Preston et al. (2001), the mean parity of women who have completed childbearing is equal to the cohort total fertility rate if reporting is accurate and if there are no differentials in mortality or migration by parity. This particular cohort of women was 45 years of age or older when the census was conducted, and are considered to have completed childbearing. The cohort TFR was calculated using the question about parity in Costa Rica's Census 2000.

Evidence from previous studies also indicates that an increasing number of full-term pregnancies -i.e. higher fertility rates- decreases the risk of breast cancer. High parity acts as a protector factor for breast cancer by diminishing the cumulative number of ovulatory cycles and therefore the estrogen bioavailability. Other studies of breast cancer have usually adjusted for parity (for example Kulldorff et al., 1997). Evidence from a case-control study conducted in Costa Rica showed evidence that higher parity had a significant protective effect, which is independent of the duration of age at first full-term pregnancy (Rosero-Bixby et al., 1987). My hypothesis is therefore, that total fertility rate has a negative association with breast cancer incidence.

4. Late age at first full-term pregnancy. Proportion of women who had their first full-term pregnancy at age 30 or older was calculated only for 1951-1955 cohorts, i.e. those women who were born between 1951 and 1955 and therefore were 45 during the period of analysis: 1996 to 2000. This variable was not computed for cohorts other than 1951-1955 because later cohorts have not completed their childbearing. The numerator of this variable was obtained from the Vital Statistics databases, specifically births from 1981 to 2000. The denominator was obtained from official population projections at the district level from 1996 to 2000. Projections are only available for 5-year groups. Since it was necessary to use only the number of women who were 45 between 1996 and 2000, the "45-49" 5-year group was disaggregated into single ages using Karup-King multipliers (Shryock et al., 1976).

As it was discussed in section 2.1.1., late age at first full term pregnancy is a risk factor for breast cancer. My hypothesis is therefore that late age at pregnancy is directly associated with breast cancer incidence in Costa Rica.

5. Social lag index. A social lag index, as an approximation of socioeconomic status (SES) was calculated using education and dwellings characteristics. Two education indicators were used: (1) proportion of illiterate population and (2) proportion of population that did not attend high-school. Three dwellings indicators were used: (1) proportion of houses without electric service, (2) proportion of dwellings without drinking water, and (3) proportion of dwellings in bad condition. "Dwellings in bad condition" was defined as those dwellings with damage in two or more of three components: floor, walls and roof.

The values each district obtained in each of the five indicators were ranked in deciles. The index was calculated as the average of the five deciles for each district. This index uses two out of the three components of a more comprehensive index (González, 2004). The full González index was not used in this analysis because it included a health dimension that was better approached by the "density index of access to health care services" which was just described.

Unlike most other cancers, breast cancer is more common among women of higher socioeconomic status (SES) –as estimated by such factors as income, education, housing, etc. -(Parkin et al., 2001; Timander and McLafferty, 1998). Nevertheless most of the gradient can be explained by the differing prevalence of known risk factors between social classes and not by SES itself. For example, it is more common to have children at older ages or not have them at all for women with higher SES than for those with lower SES. Also, breastfeeding tends to be less common in high SES women. It is also possible that better access to health care and to medical screening makes some contribution to this association between high SES and higher risk of breast cancer. Although part of this relationship is likely to be confounded with a number of risk factors, several studies have found that the relationship persists after controlling for those risk factors. My hypothesis is that after controlling for other risk factors, social lag will have an inverse relationship with breast cancer incidence. That is, high SES -measured as low level of social lag- would be associated with high breast cancer incidence in Costa Rica.

4. RESULTS

The original sample size during 1996 to 2000 was 2,682 cases. 7% of those cases had no personal ID number in the National Tumor Registry database. Having IDs was necessary to link this database to the other available databases, and better locate individuals in geographic units and control for migration bias. First and last names and date of birth of these cases were used in order to find out if an ID number existed for these cases in any of Costa Rica's available registries. None of them appeared in Vital Statistics records, and 60% of them lived in counties where the percentage of foreign-born people is higher than the national level. Therefore they likely were not Costa Ricans. History of place of residence could not be tracked and, furthermore, exposure was likely to have occurred in any other country. These 192 cases were not considered in the analysis.

One woman was diagnosed twice with breast cancer during 1996 to 2000. Because the exposure to pesticides was calculated as an average per inhabitant, only the first diagnosis was

included in the analysis and the second was dropped. In addition, 17 cases had a different location in every voter registration list. These were also dropped because migration made it impossible to determine a place where they were more likely to have been exposed to pesticides. As a result, the final sample size of cases was **2,472**, 92% of the original sample. Using date of birth data from Vital Statistics records, misreported or no reported age was identified and corrected for 1% of cases in the final sample.

Cases were assigned to a district taking into consideration the information in five different points in time: 1990, 1994, 1998, 2002, and the moment of diagnosis. As has been mentioned in other studies (Wesseling, 1997, 1999) it is common practice for people to declare a place of residence in the central or near to central districts where hospitals are located, in order to receive presumably better medical attention. Therefore, individuals who had the same district in the entire 13 years covered by voter registration lists were considered to have lived in that district for the entire period, regardless of residence declared at diagnosis. Those individuals who lived in the same district for at least 13 years are the great majority of cases, 74% as shown in **Table 1.**

Individuals who did not live in the same district during the entire 13 years period include: patients who died and therefore were excluded from voter registration lists; patients who were diagnosed at very young ages and were not included in the 1990 voter list; and patients who moved to a different district during the 13 years period. Those who lived in the same district between 9 and 12 years were 11% of all cases, which is the difference between the first two categories shown in **Table 1**. Finally 15% of all cases lived in their assigned district for 5 to 8 years.

Using voter registration lists had the effect of altering the allocation data for 40% of cases (**Table 2**). For 6% of individuals who did not declare a place of residence at diagnosis, a geographical location was assigned using this additional information. For 34% of the cases, using voter lists produced a change in the place where they would have been located if only the NTR had been used. As shown in **Table 3**, most of these women lived at least in the same county they declared, but 221 cases lived in completely different areas.

I have discussed sample size at some length because the individual cases are important in constructing my dependent variable. This research is not based on individual level characteristics. Rather, I take an ecological approach using districts as my units of analysis. This results in a sample size of 459 districts. Each district has observations for total age-adjusted population and for the five independent variables described in the previous section: pesticide exposure, access to health care, fertility rate, late age at first pregnancy, and social lag index. A descriptive analysis of the variables is presented in **Table 4**. In this descriptive analysis, cases and population are not presented as different variables, but rather as age-adjusted rates.

Use of maps helps to visualize that the dependent as well as the independent variables are not randomly distributed along the country. Although the analysis was done at the district level, maps are presented at the county level in order to better visualize the general distributional pattern of the variables. As shown in **Map 1**, breast cancer incidence is higher in the central zone of the country, where the capital city is located, which is mainly urban. Nevertheless, higher incidence is also evident in the some areas in the east, southeast and southwest parts of the country, which are mainly rural areas.

Average pesticide exposure per inhabitant is higher in the most rural areas of Costa Rica, close to borders (**Map 2**). The locations of the main crops in Costa Rica overlap largely; many

pesticides have been applied on multiple crops, and multiple pesticides on all crops. But in general, coffee has been grown mainly in the inland, and pesticide intensive rice and banana production predominated alongside the entire Pacific Coast and alongside the Atlantic Coast respectively (**Map 3**). The amount of active ingredients used per hectare varies widely by crop. But it has been estimated that on average, in 1984 it was 6.5 kg for coffee, 10 kg for rice and 45 kg for banana (Wesseling, 1997)**.** It can be noticed from both **Map 2** and **Map 3**, that areas of greater pesticide exposure per inhabitant are in the banana and rice plantations regions of the country.

Map 4 shows how poorer access to health care is more concentrated in rural areas, with very good access to health care in the central zone of Costa Rica. Cohort total fertility rate is presented in **Map 5**, where a clear spatial pattern can be observed with the lowest cohort TFRs in the metropolitan area, and higher TFRs as we move away from the capital. Similarly, **Map 6** shows the highest proportions of women having children at age 30 or older mainly in the central metropolitan zone of the country. As presented in **Map 7**, highest indicators of social lag -measured using education and dwelling characteristics- are in the most rural and agricultural regions of Costa Rica.

While maps are useful for visualizing spatial patterns, a more precise means of identifying departure from random spatial patterning is desirable. Moran's I is a commonly used statistic for measuring departures from spatial randomness, or spatial autocorrelation. A test for spatial autocorrelation among my regression residuals will be the primary diagnosis to determine whether a spatial analysis approach is necessary for analyzing these spatially referenced data. Moran's I value is usually in the range of -1 to +1; although it might be slightly higher than +1 or slightly lower than -1. When Moran's I value approaches zero, it indicates no spatial autocorrelation. When it approaches +1, it is an indication of strong positive spatial autocorrelation -i.e. spatial clustering either of high or of low values-. Similarly, when Moran's I value approaches -1, it is interpreted as strong negative spatial autocorrelation -i.e. a pattern of mix of high and low values-.

The dependent and each of the independent variables had a positive and significant Moran's I value (**Table 5**), which means that there is a pattern of spatial clustering in individual variables. Therefore, it is worth to run a regression model and pay attention to the diagnosis for spatial dependence once the explanatory variables are included in the model. This part of the analysis was conducted using GeoDa software. As I discussed in section 3.2.2., my data is assumed to have a Poisson rather than normal distribution. Nevertheless, GeoDa software does not have applications to test for spatial autocorrelation in Poisson regression models. Therefore, an Ordinary Least Square (OLS) regression model was used to test for spatial autocorrelation.

Results in **Table 6** show that there is no spatial dependence in the errors after controlling for the independent variables in the model (Moran's I probability = 0.72). An interpretation to this result is that the independent variables have taken care of the original spatial autocorrelation in the dependent variable, so that very little spatial autocorrelation remains after the regression model is run. Neither a spatial error regression model nor a spatial lag regression model could be considered as proper alternatives (Anselin, 2005). I conducted Poisson regression analyses in my final models. More details on why I chose this regression model were discussed in section 3.2.2.

Results of Poisson regression models are shown in **Table 7**. As it may be observed from these results, breast cancer in women younger than 45 years seems to be explained differently

than in older women. This is consistent with the literature in this field which states that breast cancer at younger ages is better explained by genetic factors, of which I do not have information in this study (see section 2.1.2.).

For young women, parity –measured by TFR- and access to health care services have the unexpected direction. Pesticide exposure has a marginally significant direct association. The reported coefficient can be interpreted by the incidence relative ratio (IRR), which is 1.03 for PEI coefficient, meaning that after controlling for other risk factors, moving a district to the next decile of pesticide exposure was associated with 3% increase in breast cancer incidence for women younger than 45.

Nevertheless, given that etiology of breast cancer is different in younger women, it does not seem accurate to analyze the effect of pesticides for all women together, or for young women only. Rather, these results justify the stratification by age and show that the relationships between breast cancer incidence and the analyzed risk factors are completely different in young women as compared to old women. The most appropriate strategy is analyzing the subgroup of 45 years or older, where a possible effect of pesticide has been reported to be more likely. I will focus therefore in this subgroup.

For women of 45 years of age or older, the effect of the two reproductive risk factors tested: parity and late age at first full-term pregnancy were significant and in the hypothesized direction. Parity is a protective factor, which was measured through cohort TFR. It had an inverse relationship with breast cancer incidence, which means that having a higher number of children is associated with lower incidence of breast cancer. Late age at first full-term pregnancy is a risk factor. It showed a direct relation with the dependent variable. Therefore, districts with higher percentage of women who had their full-term pregnancy after the age of 30 had higher incidence of breast cancer, after controlling for other risk factors.

Both access to health care services and socioeconomic status had a significant effect in the unexpected direction. That is, better access to health care services was associated with lower breast cancer incidence rates and low socioeconomic status was associated with high incidence rates. These are interesting but hard to interpret results. Access should theoretically be associated with higher incidence because of early detection, and there is nothing intrinsic to low socioeconomic status that could be hypothesized to be related to breast cancer. Measurement errors are likely to be playing a role.

Pesticide exposure index (PEI) had a statistically significant direct association with breast cancer. The corresponding incidence rate ratio (IRR) for pesticide exposure index is 1.29. This means that after controlling for other risk factors, moving a district to the next decile of pesticide exposure was associated with 29% increase in breast cancer incidence for women of 45 years of age or older[2]. As mentioned in section 3.2.2, this analysis benefits from using this time-lagged pesticide exposure index. Breast cancer is more likely to be related with past rather than current pesticide exposure levels. PEI calculated for 1984 allows for a time lag of up to 16 years in this analysis. Nevertheless, this index was calculated at a higher geographical unit (county level) and

[2] Wesseling et al. (1999) analyzed the effect of pesticide exposure for all women regardless their age, for the tertile of most rural counties. As a means of comparison, I ran a regression for women 45+ controlling only for PEI and urban/rural classification for the entire set of districts. This resulted in an IRR of 4.13 for PEI. This overestimation of the effect of pesticide exposure illustrates the importance of controlling for other risk factors.

assumed to be the same for all the districts that are part of each county. The effect this could introduce will be discussed in the next section.

As a further descriptive spatial analysis, I conducted a geographically weighted Poisson regression to examine spatial variations in the relationship between breast cancer and pesticide exposure in Costa Rica. Just as the Poisson regression ran with Stata software, an offset variable of the expected number of cases was used in this regression model ran with GWR software. Significance values for PEI coefficient are presented in **Map 8**, which shows that the significance of this variable is not homogenously distributed in the country. Rather, it has significant coefficients only in certain regions of the country, after controlling for other risk factors.

5. DISCUSSION AND CONCLUSION

Controlling for migration in this study increases the validity of the results obtained. The restriction of cases to those that could be assigned in the same geographical location for at least five years -and most of them for up to 13 years- increases the validity of results by diminishing the effect of migration. Although cases were restricted, it still did not mean losing a big part of the original sample and allowed for a reasonably robust statistical analysis.

There are some characteristics that may be related to breast cancer but were not included in this research, such as: age at menarche, age at menopause, nulliparity, birth spacing, breastfeeding and use of hormone replace therapy. Variables on other more controversial characteristics like oral contraceptives use, family history, alcohol intake and diet were not included either. Data on these characteristics were not available. Even though the Sexual and Reproductive Health Survey conducted on Costa Rica in 1999, gathered most of this information, it was representative at the national, but not at the district level. So, it could not be used in this research.

Other reproductive characteristics like parity (measured by cohort TFR) and late age at first full-term pregnancy were analyzed, and showed to be significant as expected. That is, higher total fertility rates are associated with lower breast cancer incidence rates; and the greater the proportion of women who had their first full-term pregnancy at age 30 or older, the higher the probability of high incidence of breast cancer in Costa Rica's districts.

A social lag index was used as an indicator of the general socioeconomic status (SES) of people in each district. According to the literature, breast cancer incidence is higher in women at high SES levels. However, as it was mentioned before, this could actually be a confounding factor since most of risk factors are more prevalent in high SES women. These results showed that after controlling for risk factors, lower SES is actually associated with high breast cancer incidence rates. Access to health care services was according to this study associated with lower incidence.

Pesticide exposure in the population as measured by PEI was statistically significant and directly associated with breast cancer incidence. PEI variable was available only at the county level and assumed to be the same in all districts of a county. This introduces less variability to the analysis, which is not desirable. As a mean exposure of the county, PEI reflects the combined exposure to many pesticides with varying carcinogenic properties. Data are not available to assess the impact of this potential bias. There is a temporal lag between exposure and

development of breast cancer, but the length of that latent period is unknown so far. Although individual dose of exposure to pesticides and duration of that exposure still remain an issue, using this pesticide exposure indicator in the population had the strength of allowing for a time lag of up to sixteen years in the analysis.

Pesticide exposure also had a significant and positive relationship with breast cancer in specific rural and agricultural regions of the country. This relationship was clearly heterogeneous along the country. This was shown by the results of a spatially weighted Poisson regression. These results suggest that breast cancer may be explained differentially in the country. This is consistent with other research. Wesseling and colleagues (1999) used cancer data from Costa Rica from 1981 to 1993. They found that in the tertile of most rural counties (low incidence) breast cancer was associated with excess risks due to exposures to pesticides, which was not observed in urban counties. In recently published research conducted in two counties of England, no spatial association was found between breast cancer incidence rates and application of pesticides in urban areas. Those findings however, revealed a spatial association between breast cancer and pesticides in the rural areas (Muir et al., 2004).

There is a plausible biological explanation of the role of hormones like estrogen in the causal pathway to breast cancer. This is true not only to endogenous hormones, but also to exogenous chemical compounds in pesticides that mimic hormones and may induce breast cancer. Nevertheless, public health policy has had a unifocal emphasis on early detection of breast cancer through screening programs and self examination, rather than primary prevention (Potts, 2004). Breast cancer is projected to remain the most common cancer in women in the next half century. Rapidly increasing rates of breast cancer incidence in many developing countries suggest that the burden of the disease will be much greater than projections based on demographic change alone imply (Parkin et al., 2001). Therefore, of particular interest is the identification of potentially modifiable risk factors.

An ecological approach like this is able to rule out strong associations. A finer assessment of risks due to pesticide exposure would require a large study in individuals, perhaps a case-control or cohort approach, which would definitely be expensive, but worth it. Results of this research give a hint that there may be an actual relation between breast cancer and pesticides. Prevention of breast cancer is difficult because many associated factors are endogenous and thus difficult to manipulate. Nevertheless, pesticide exposure is preventable and thus an important public health issue to be debated.

As pointed out by Rosser (2000), many critics of breast cancer research is that it has basically been conducted within the biomedical model, and therefore it has focused its attention on the cellular, hormonal, and genetic causes of the disease at the expense of attention to behavioral, social and environmental causes. Biomedicine traditionally researches disease and how to cure it, rather than studying health and how to prevent illness, which places “responsibility” at the individual level rather than pointing to society responsibility as a whole for addressing environmental causes. Demographic and epidemiologic approaches, like the present research make a contribution to a broader understanding of the etiology of breast cancer.

In order to induce a fundamental social change, it will be necessary to make prevention the goal of research and clinical practice. To achieve this, I think it is preponderant to study and communicate in an effective way the possible environmental connections to breast cancer. Reasons for not giving credence to a relationship between environmental factors and any disease

are usually grounded on a number of aspects, ranging from the need of more study, to conclusions that risk has been exaggerated, or that damage is actually trivial, and many times because of considerations of the economic impact of regulating environmental exposures. Paying more attention to health consequences that derivate from environmental exposure would imply a shift toward the application of the precautionary principle.

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7. Tables, Graphs and Maps

Table 1. Migration patterns of breast cancer cases from 1990 to 2002

Period of time cases lived in their assigned district	Relative distribution (n=2472)
13 years or more	74%
9 years or more	85%
5 years or more	100%

Table 2. Effect of using voter registration lists as source of information to locate cases in geographic units

Effect	Relative distribution (n=2472)
Did not change declared place of residence	60%
Changed place of residence	34%
Gave information on place of residence that was not declared	6%
Total cases	**100%**

Table 3. Effect of using voter registration lists on cases where place of residence was changed

Effect	Relative distribution (n=850)
Same province and county, but different district	45%
Same province, but different county and district	29%
Different province, county and district	26%
Total cases	**100%**

Table 4. Descriptive analysis of variables used in the models for the 459 districts. Costa Rica: 1996-2000

Variable	Mean	Standard deviation	Median	Minimum	Maximum
Age-adjusted incidence rate of breast cancer (per 100.000 women)	14.9	19.8	11.11	0	217.40
Cohort Total Fertility Rate	5.6	1.2	5.53	2.73	8.98
Women late age at 1st pregnancy (%)	5.6	5.5	4.74	0	55.30
Median of access to health services (MD hrs/capita/year)	0.4	0.2	0.3	0	1.5
Social lag index	5.5	2.4	5.2	1	10
Pesticide exposure index *	31.8	55.6	12.8	0.00	280.53

*county level

Table 5. Spatial descriptive analysis of variables used in the models

Variable	Moran's I	P-value
Age-adjusted incidence rate of breast cancer (per 100.000 women)	0.099	0.003
Cohort Total Fertility Rate	0.745	0.001
Women late age at 1st pregnancy (%)	0.167	0.001
Median of access to health services (MD hrs/capita/year)	0.259	0.001
Social lag index	0.577	0.002
Pesticide exposure index	0.472	0.002

Table 6. Ordinary Least Squares Regression Results and test of spatial autocorrelation

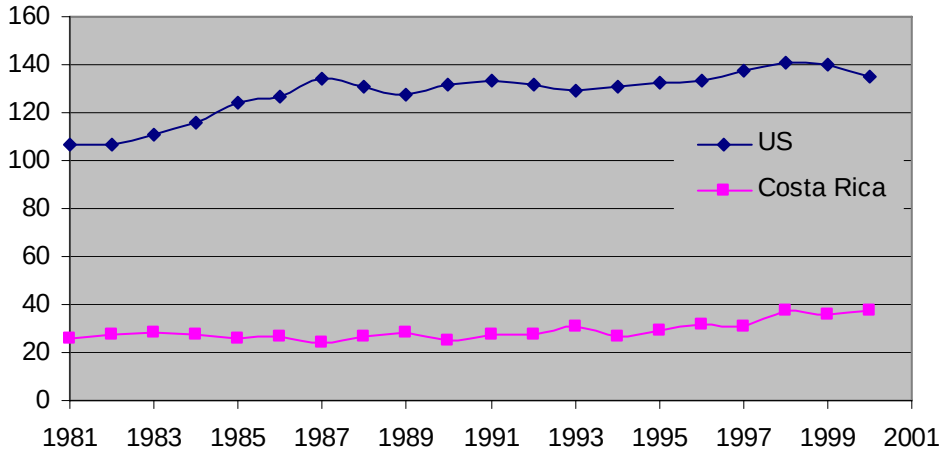
Variable	Coefficient	Std error	p-value
Constant	37.85	5.21	0.000
Cohort Total Fertility Rate	-2.71	1.30	0.038
Late age at 1st pregnancy	0.65	0.18	0.000
Access to health services	5.67	2.49	0.023
Social lag index	-0.82	0.69	0.234
Pesticide exposure index	0.02	0.02	0.254
R^2	0.15		
Adjusted R^2	0.14		
n	459		
Moran's I probability	0.72		

Table 7. Poisson Regression Results by group of age

Variable	All ages		<45 years of age		>=45 years of age	
	Coef	Std error	Coef	Std error	Coef	Std error
Cohort Total Fertility Rate	** -4.732	0.112	** 1.396	0.105	** -5.930	0.108
Late age at 1st pregnancy	** 0.892	0.003	** 0.195	0.006	** 0.626	0.003
Access to health services	** -3.677	0.022	** -0.328	0.029	** -2.696	0.023
Social lag index	** 4.079	0.035	** -0.362	0.057	** 4.209	0.039
Pesticide exposure index	** -0.029	0.010	* 0.031	0.017	** 0.254	0.010
Constant	*** -10.40*	*0.519*	*** -8.973*	*0.504*	*** -2.635*	*0.497*
N =459 Pseudo R2 =	0.302		0.259			0.333
Offset variable = expected number of cases						
** p=0.000 * p=0.071						

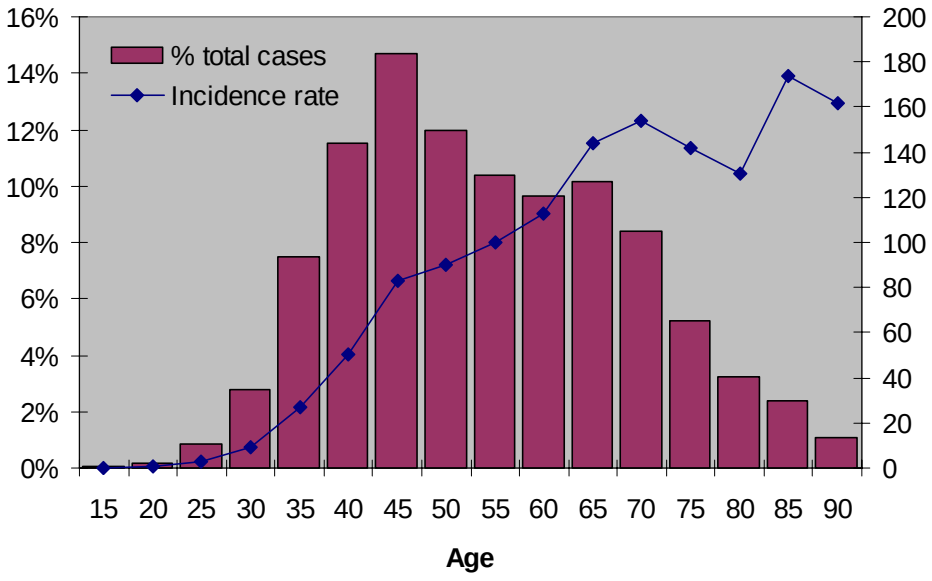
Graph 1. Age adjusted incidence rates of breast cancer in women. Costa Rica and US: 1981-2000

(rates per 100.000, adjusted to IARC world standard population)



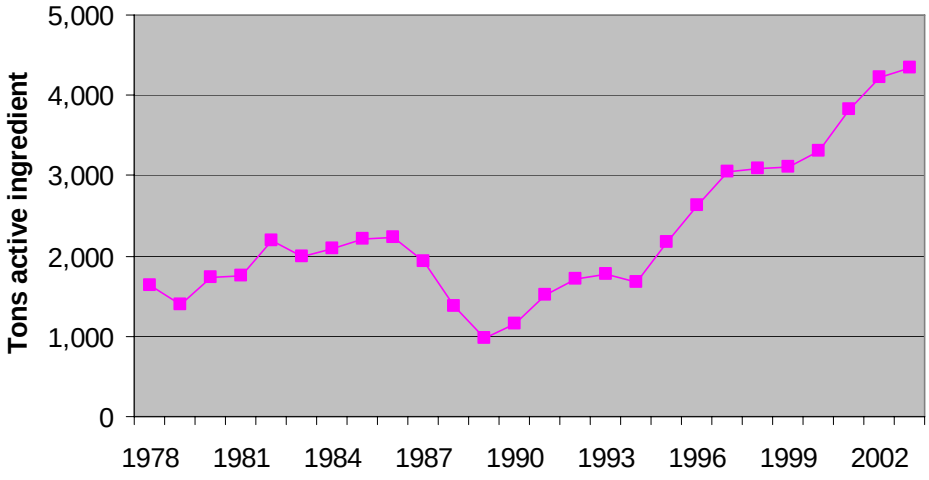
Source: Central American Population Center http://censos.ccp.ucr.ac.cr (Costa Rica), SEER, National Cancer Institute http://seer.cancer.gov (US)

Graph 2. Breast cancer incidence rates and distribution of cases by age. Costa Rica 1996-2000



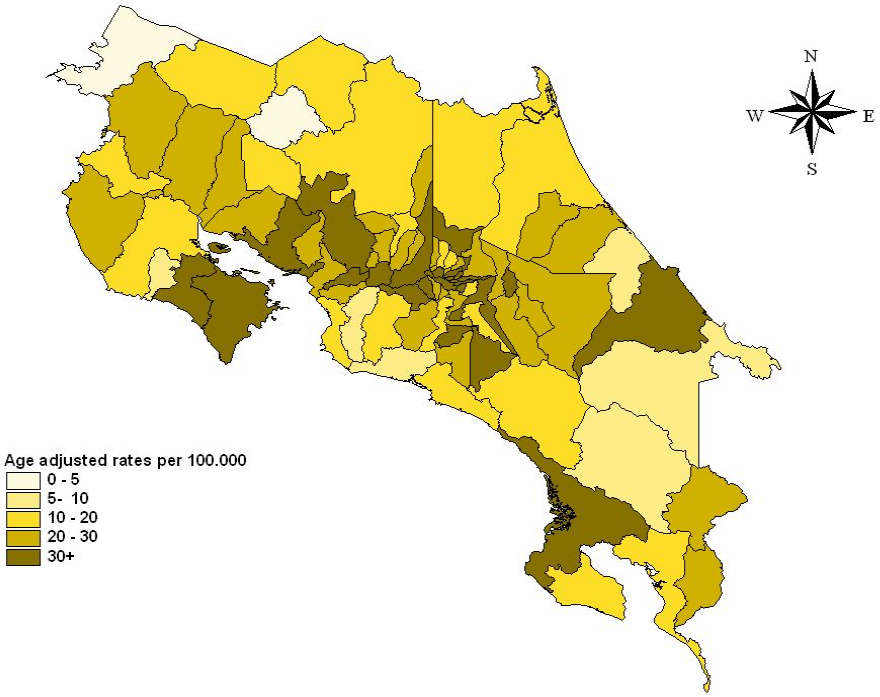
Graph 3. Imported carcinogenic pesticides. Costa Rica: 1978-2003

(EPA categories A, B1and B2)

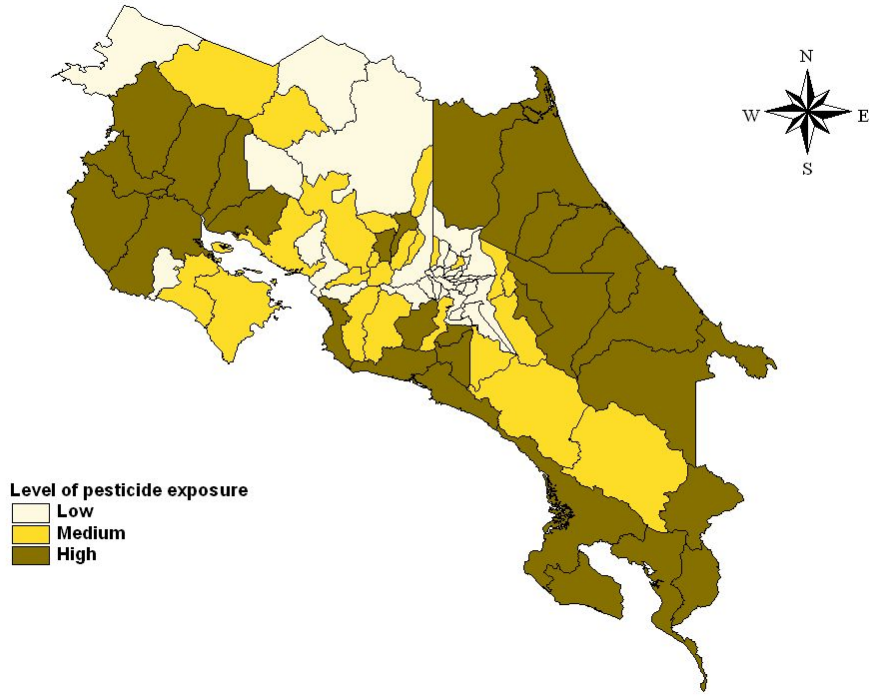


Source: IRET-Universidad Nacional, Costa Rica

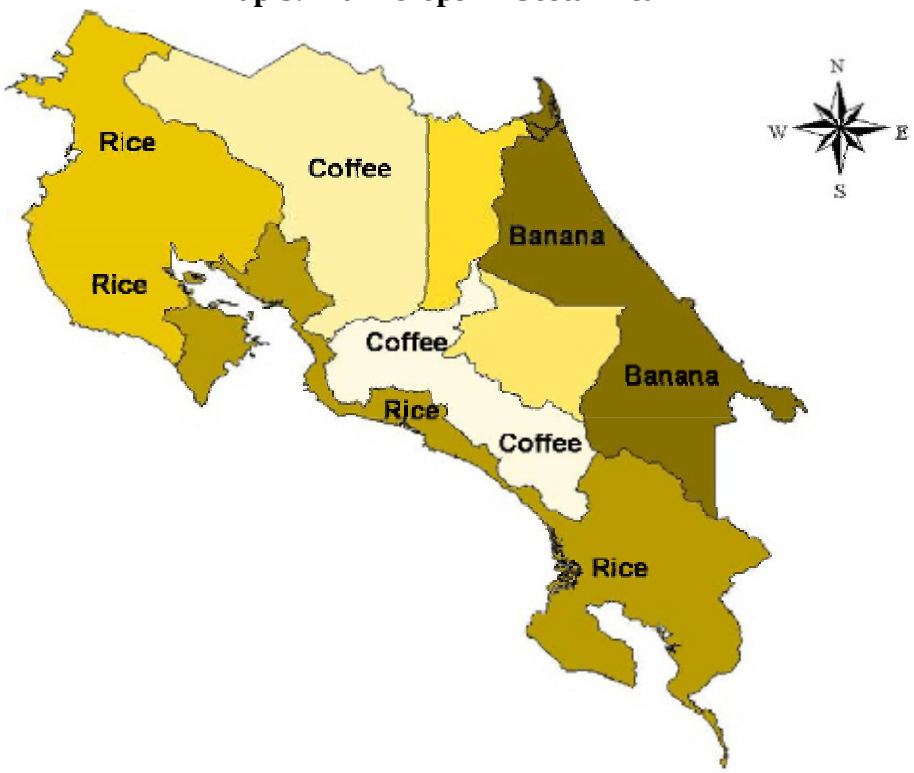
Map 1. Breast cancer age-adjusted incidence rates. Costa Rica: 1996-2000



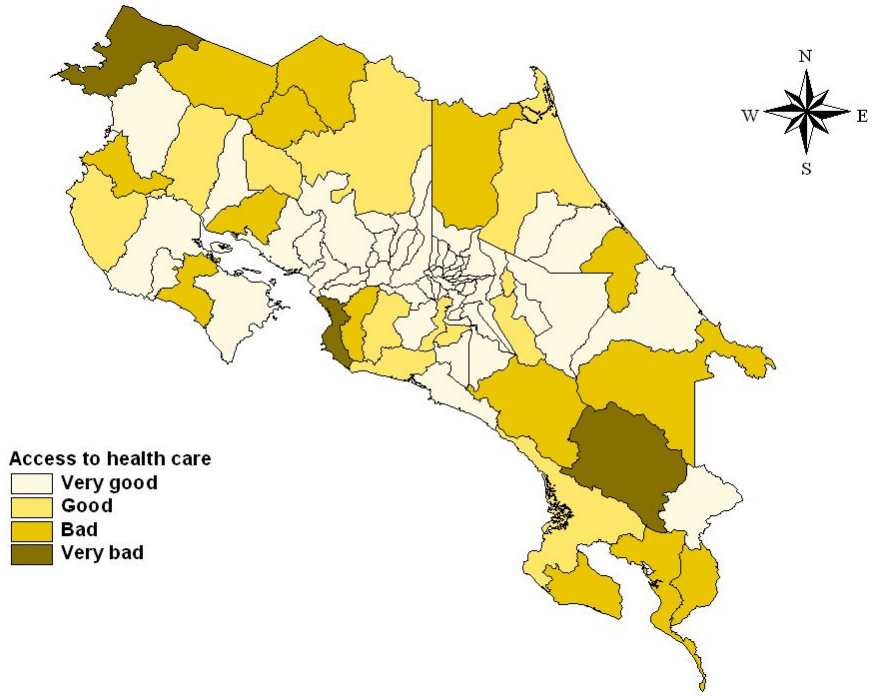
Map 2. Population pesticide exposure. Costa Rica: 1984



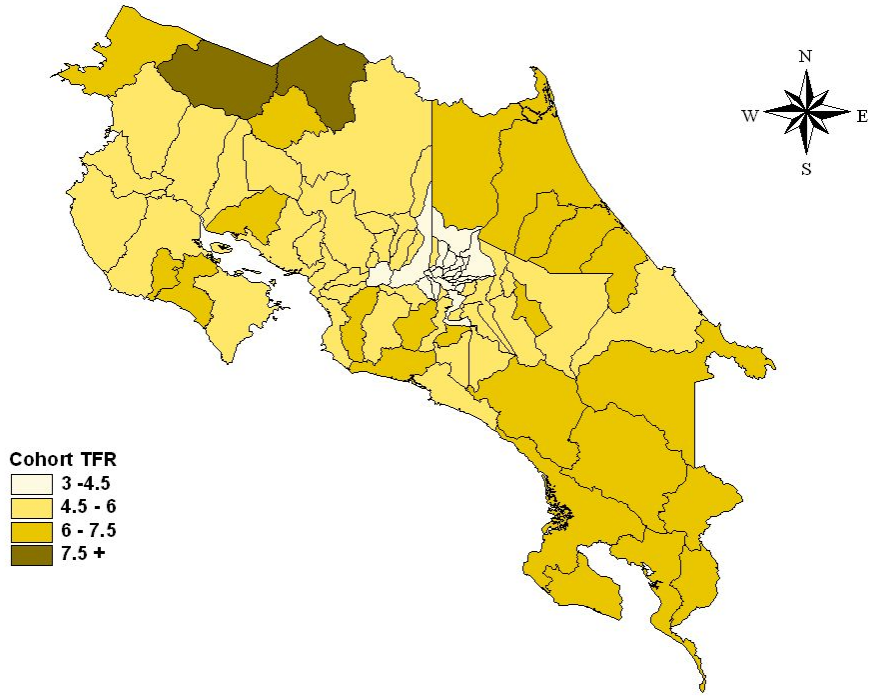
Map 3. Main crops in Costa Rica



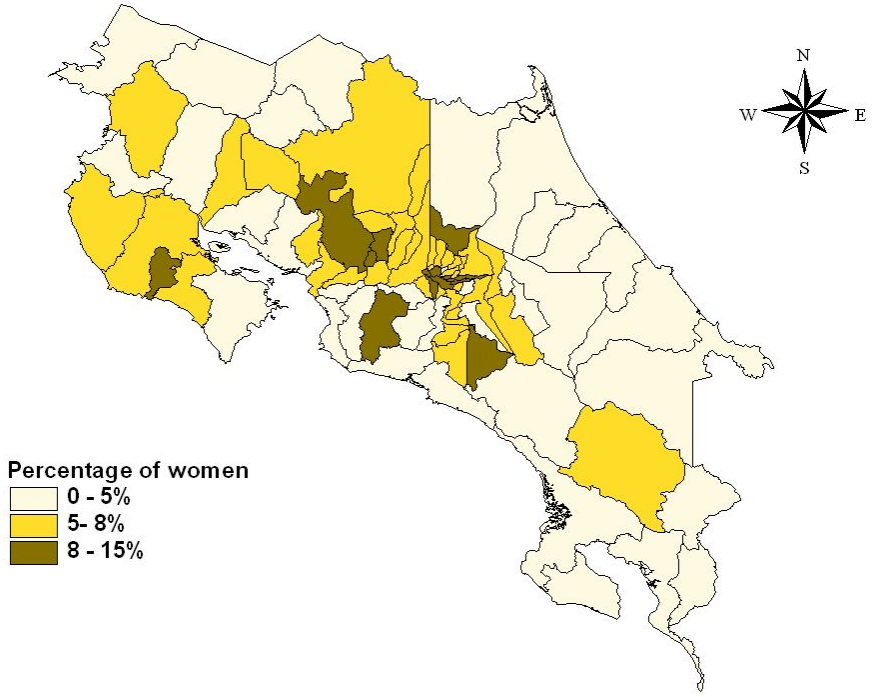
Map 4. Access to health care services. Costa Rica: 2000



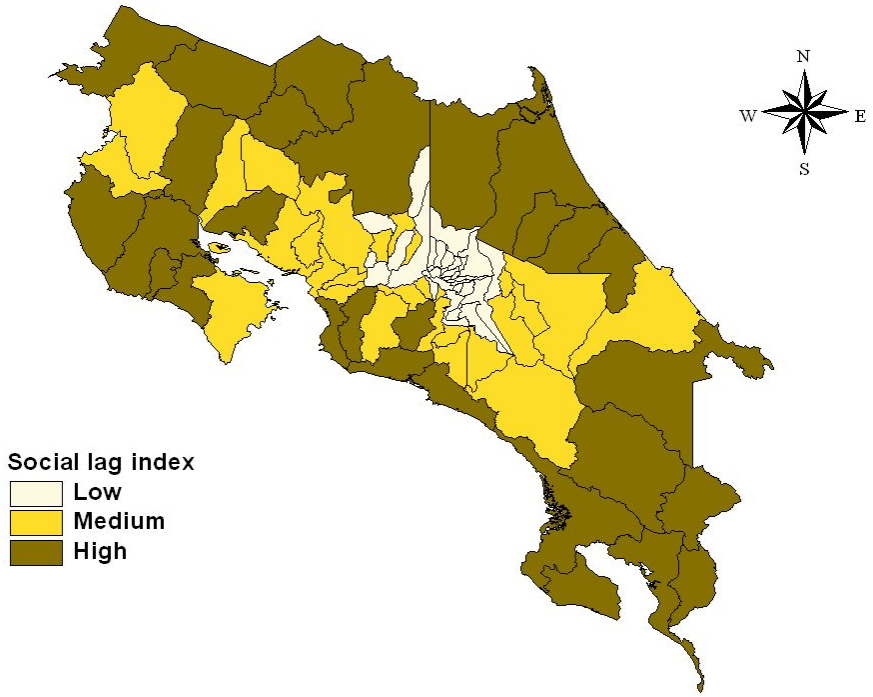
Map 5. Total Fertility Rate for the cohort 1951-1955. Costa Rica



Map 6. First full-term pregnancy at age 30+ for the cohort 1951-1955. Costa Rica



Map 7. Social lag index. Costa Rica: 2000



Map 8. Significance of Pesticide Exposure Index (PEI) estimated with geographically weighted Poisson regression