SYNOPSIS

GEOGRAPHIC PATTERNS and time trends for breast cancer suggest there are preventable causes that may include environmental factors. This article describes the development of new methods used in the Cape Cod Breast Cancer and Environment Study to investigate whether synthetic chemicals in the environment contribute to breast cancer risk.

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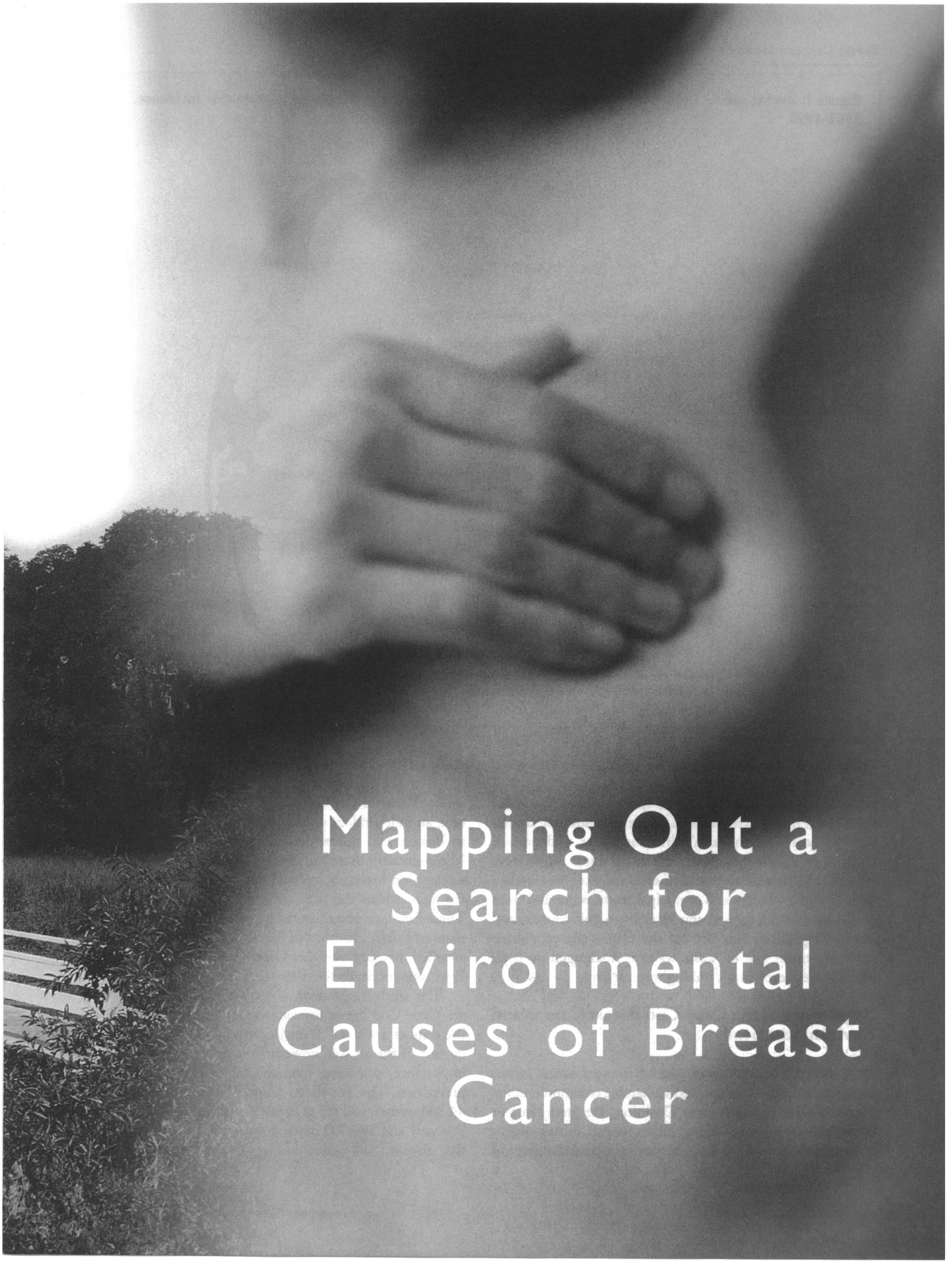
When the Massachusetts Department of Public Health published town-by-town cancer statistics for 1982–1990, the breast cancer rates for Cape Cod stood out as sharply as the peninsula itself on a state map. Among the state's 351 cities and towns, eight communities had breast cancer rates that were at least 25% higher than the state average and also met the stringent statistical significance criterion of $P \leq 0.001$ (one chance in a thousand that the town breast cancer rate differed from the statewide rate by chance alone). Of these eight towns, seven were on Cape Cod. Using the common statistical significance criterion of $P \leq 0.05$ (one chance in 20), two other Cape Cod towns also were found to have elevated breast cancer rates (Figure 1).

When elevated cancer incidence occurs within a confined geographic unit such as Cape Cod, epidemiologists begin to think about whether demographic or environmental features specific to the area may explain the pattern. Could the explanation lie in something about the people or something about the place?

Cape Cod is home to many retirees, but Massachusetts cancer figures are standardized to account for age differences, so age is not a factor. In other ways—ethnicity or income, for example—U.S. Census data show the Cape's population is not markedly different from the population of the rest of the state.

The environment of the Cape, on the other hand, is unique. The vast expanses of



Mapping Out a Search for Environmental Causes of Breast Cancer

Figure 1. Breast cancer incidence for women residing in Cape Cod towns compared to statewide incidence, 1982–1990

Breast cancer incidence not significantly higher than statewide incidence

Breast cancer incidence at least 25% higher than statewide incidence ($P \leq 0.05$)

Breast cancer incidence at least 25% higher than statewide incidence ($P \leq 0.001$)

SOURCE: Massachusetts Department of Public Health: *Cancer incidence in Massachusetts 1982–1990*; 1993 Dec.; with a later adjustment for one town by Massachusetts Department of Public Health

The incidence of breast cancer for the years 1982 to 1990 was significantly elevated in nine of Cape Cod's fifteen towns.

sandy beach that give the area its special beauty also make the Cape a fragile ecosystem. A single groundwater aquifer supplies nearly all the area's drinking water, and porous, sandy soils above the aquifer make drinking water wells especially vulnerable to environmental impact from sources such as septic systems and municipal waste water and from pesticides used on forests, cranberry bogs, golf courses, and lawns. Regulations that protect the Cape's coastal marine sanctuary mean that all waste water is discharged on land, where it may leach into drinking water sources.

Beginnings of the Cape Cod Breast Cancer and Environment Study

Concerned about this picture of elevated breast cancer rates coupled with a unique and vulnerable environment, the Massachusetts Breast Cancer Coalition, the state's largest breast cancer advocacy organization, began to ask urgent questions about possible links between the environment and women's health. The Coalition founded Silent Spring Institute, an independent scientific research organization linked to Boston area universities, in 1994. That same year, the Masasachusetts state legislature earmarked $1.5 million for research on breast cancer and the environment in areas of the state where the incidence of breast cancer was high. These funds are administered by the Massachusetts Department of Public Health, and Silent Spring Institute has been awarded funding for the Cape Cod Breast Cancer and Environment Study for three years.

The study began with a specific local question, "Why are Cape Cod breast cancer rates different from those for the state as a whole?" If environmental factors are found to be part of the answer, however, our results will be of broad importance, providing fundamental clues to etiology and prevention. The American Cancer Society estimates that 44,300 women will die of breast cancer in the United States this year and 184,300 more women will be diagnosed with the disease,[1] so understanding environmental causes—

which may be preventable—could be a powerful tool for improving women's health.

Our decision in the Cape Cod Study to look for answers in the environment reflects a national trend among breast cancer activists to focus resources on prevention. As a nation, we have invested heavily in early detection and treatment. Yet it is only in the last few years, according to Eric Feuer at the National Cancer Institute, that a decline has been reported in U.S. breast cancer mortality. And even with better treatment options, a diagnosis of breast cancer brings suffering to women and their families. Regional studies, including ours on Cape Cod and others beginning on Long Island and in the San Francisco Bay Area, offer new hope that we can learn from the areas with high breast cancer incidence how to prevent this disease.

The Cape Cod Breast Cancer and Environment Study is in mid-course now, with results expected next year. This article traces how we developed our approach to the study and shows how we are using new computer mapping methods to analyze the relationships between breast cancer data and environmental data, including both historical records dating back to the 1950s and results from new tests for hormone-related chemicals. Our experience highlights the knotty methodological problems involved in studying breast cancer and the environment. We hope our progress will suggest fruitful strategies for others to use in expanding the scope of research into preventable causes of the disease.

Clues to Environmental Causes: Time Trends and Geographic Patterns of Breast Cancer

The impetus to look for environmental causes of breast cancer comes from data showing that breast cancer incidence varies across time periods and geographic areas. If breast cancer rates can change over time and place, it may be possible to find out why by studying the patterns of change.

Questions about why breast cancer rates are elevated on Cape Cod are part of a larger international puzzle about patterns of breast cancer incidence and mortality. A comprehensive review of breast cancer rates for 1955 to 1990 in 11 countries found four- to five-fold differences in breast cancer incidence by country, with the lowest rates in Asia and the highest in North America and Western Europe.[2] Incidence also showed an increasing trend in the United States during most of this period but has been relatively flat since about 1988.[3]

Part of the historical increase in reported breast cancer rates and the generally higher rates in developed than in developing nations is undoubtedly due to greater access to screening through both mammography and physical exams.[2] But screening is not the whole story. Better diagnosis cannot explain increased breast cancer mortality for older women (ages 65 to 74) in the United States or substantial mortality increases in some countries, including the 50% to 60% increases in mortality from 1955 to 1990 in Japan, Singapore, and Hungary.[2] Greater access to screening also does not explain the increased incidence for younger U.S. women (ages 35 to 54), an age group less likely to use mammography, or the dramatic increases in breast cancer rates in developing countries with low screening rates.[2] These statistics about breast cancer incidence and mortality across time and geography suggest that preventable factors—perhaps including environmental factors—may be at work.

Studies of women who move from one geographic area to another are particularly thought-provoking. For example, when women move from Asia, a low risk continent, to the United States, a high risk country, their breast cancer rates increase over successive generations until they approximate the rates for U.S. whites.[4] Similarly, a study of migrants to Australia and Canada found that breast cancer mortality rates for women from lower and higher risk countries shifted toward rates in their new homes.[5]

While these migration studies, along with other research on historical and geographic patterns, also add to the case for preventable causes of breast cancer, they provide very little guidance about what those causes may be. Recognized risk factors, such as reproductive history, that are affected in part by cultural differences play some role. But, like screening, they cannot "explain away" differences in breast cancer rates. In a study of regional differences in breast cancer in the United States, 50% to 90% of regional differences in mortality were unexplained after rates were adjusted for a long list of recognized risk factors and predictive variables, including mammography screening and reproductive and family history.[6] Researchers estimate that 50% to 70% of breast cancer cases cannot be explained by recognized risk factors.[7]

The frustration of thousands of women diagnosed with breast cancer who have no known risk factor has fueled a new urgency to find better explanations for the patterns of breast cancer rates. New environmental research offers several interesting hypotheses.

Hypotheses about Environmental Causes of Breast Cancer

Researchers have focused on ionizing radiation, electromagnetic fields (EMFs), and synthetic chemicals, particularly organochlorine pesticides, as possible environmental causes of breast cancer. In the early stages of the Cape Cod Study, we explored the strength of each of these research areas and its likely relevance to women on Cape Cod.

Radiation. Exposure to ionizing radiation is now well established as a risk factor for breast cancer, based on studies of medical uses of radiation and the after-effects of the atomic bombs.[8] In addition, questions have been raised about exposure to radiation from nuclear power plants, including the Pilgrim Nuclear Power Plant in Plymouth, Massachusetts. However, the seven towns most likely to be exposed to emissions from the Pilgrim plant[9] are not on Cape Cod and have not historically shown elevated incidence of breast cancer, so we gave low priority to radiation as a likely explanation for the high rates on Cape Cod.

EMFs. Research interest in a relationship between EMFs and breast cancer stems from a study that showed increased breast cancer risk for men who worked as electricians, telephone linemen, electric power workers, and radio workers.[10] Based on laboratory studies, researchers theorized that elevated breast cancer risk might result from the effects of EMFs on the hormone melatonin.[11] Elevated breast cancer risk has not been consistently reported in studies of EMF exposure in women, but a large study now underway in Washington state may be informative, and additional laboratory studies are in progress at the National Toxicology Program,[12] an interagency Federal effort. On Cape Cod most of the primary power lines are located away from population centers. EMFs fall off rapidly with distance from the source, so the EMF hypothesis, like the radiation hypothesis, was given low priority in our study. (See *PHR* March/April 1996 "EMFs: Cutting Through the Controversy" for an overview of research on the health effects of EMFs.)

Synthetic chemicals. For the Cape Cod region, with its history of agricultural and other pesticide uses and waste water disposal into the aquifer, the possibility of risks from exposure to synthetic chemicals forms a more plausible hypothesis. To evaluate this hypothesis, we looked to earlier studies of synthetic chemicals and breast cancer risk in laboratory studies of cells and animals as well as epidemiologic studies.

Out of approximately 1000 chemicals tested in laboratories internationally, scientists have identified nearly 150 as causing mammary gland cancer in animals. In the United States, the National Toxicology Program identified 36 of 425 agents studied as causing mammary gland cancer in rodents.[13] Mary Wolff and her colleagues recently summarized research on these chemicals,[12] including polycyclic aromatic hydrocarbons (PAHs), which are found in tobacco smoke and air pollution, and certain solvents, including chloroethylenes, noting that these animal mammary carcinogens are an important starting point in looking for the causes of breast cancer in women.

So far, however, relatively few studies have made the link between animal mammary cancers and human epidemiology. One recent finding suggests how the two areas of research can be intriguingly complementary. Ann Aschengrau and David Ozonoff, who are researchers at the Boston University School of Public Health and also part of the Silent Spring Institute study team, found a modest association between breast cancer and living near the gun and mortar positions at the Massachusetts Military Reservation on Cape Cod.[14] The odds ratio was statistically unstable but was higher for subjects living closer to the gun and mortar sites; the finding is interesting because dinitrotoluene, a propellant used at the Reservation, is on the National Toxicology Program list of animal mammary carcinogens.

Of the chemicals suspected as human breast carcinogens, organochlorines—including DDT, DDE, and PCBs—are probably the best-studied in animals. DDT causes tumors of the liver and other organs in rats and mice, but not mammary tumors.[15] Three case-control studies have linked organochlorines and breast cancer in human populations.[12] A fourth shows similar results for African Americans and Caucasians but not for Asians.[16,17] Two-to ten-fold increased breast cancer risks associated with high serum concentrations of organochlorines have been

Examples of hormonally active chemicals

Chemical	*Sources of exposure*
Chemicals that mimic estrogen	
Alkylphenols	Surfactants in detergents; inert ingredients in pesticides
Bisphenol A and certain phthalates	Plastics
Amsonic acid	Optical brighteners
Phenylphenol	Cleaners, disinfectants
DDT Dieldrin Chlordecone (Kepone) Endosulfan Methoxychlor Toxaphene	Pesticides
Some PCBs	Electrical transformers
Pharmaceutical estrogens (DES,estrogen replacement therapy, birth control pills)	Pharmaceuticals
Phytoestrogens such as genistein	Active compounds in plants: soy, others
Chemicals that affect hormone systems in other ways	
Atrazine Chlordecone (Kepone) Endosulfan DDT	Pesticides
Some PCBs	Electrical transformers
Some PAHs	Air pollution, other sources
Indole-3-carbinol and others	Active compounds in plants: cabbage, broccoli, others

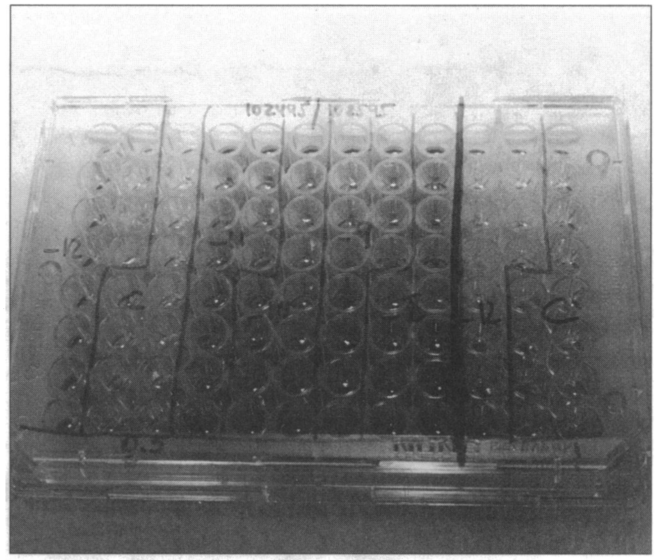
reported in this and other studies.[12] These are among the higher risks observed for breast cancer in epidemiologic research. Earlier studies, with small numbers of cases, failed to find any association between breast cancer and organochlorines.[12]

The two most recent of these epidemiologic studies of organochlorines and breast cancer capitalize on the fact that many of these chemicals are highly persistent and bioaccumulate and that humans worldwide harbor residues in their bodies. The New York University Women's Health Study touched off a flurry of additional research when the authors reported higher levels of organochlorines in blood drawn from 58 women later diagnosed with breast cancer than from controls.[18] The study was criticized because blood was drawn within six months of diagnosis, when cancers were probably present although not yet diagnosed. Nancy Krieger, now at the Harvard School of Public Health, improved on the NYU study, using blood drawn in the 1960s for the Kaiser Foundation multiphasic health examination. She and her colleagues initially reported no overall difference between 150 women who eventually developed breast cancer and matched controls in DDE or PCB levels.[16] But when their data were analyzed separately by ethnicity, an association between organochlorines and breast cancer risk emerged for black and white women, though not for Asians.[17] A number of studies are now underway to further explore the relationship between body burdens of organochlorines and breast cancer. A newly initiated set of studies of Long Island, New York, is of particular interest. Long Island, like Cape Cod, has sandy hydrogeology, a history of substantial pesticide use, and elevated breast cancer rates. The new studies will examine in detail the body burdens of selected compounds, including pesticides, in a sample of Long Island women.

Taken together, studies of possible environmental causes of breast cancer add up to only a small fraction of breast cancer research, and results do not yet present a coherent picture. In contrast, research about individual risk factors—for example, hereditary and reproductive risks—is more advanced and may, indeed, lead the way to the most promising environmental hypotheses.

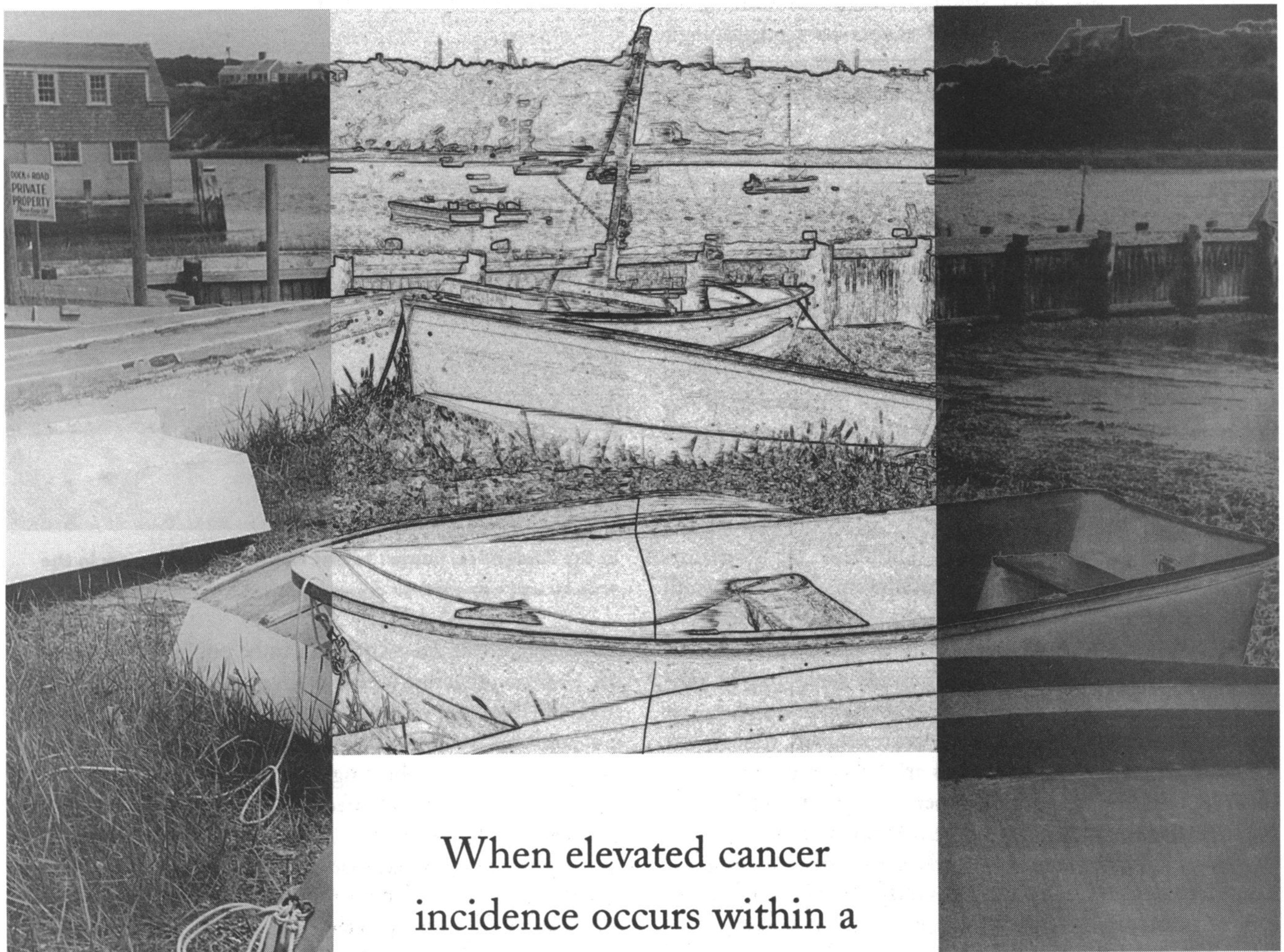
Research about Estrogen-Related Risk Suggests a New Hypothesis about Synthetic Endocrine Disrupters

Scientists have reached a consensus that many of the established individual risk factors for breast cancer are related to lifetime exposure to estrogen through the menstrual cycle; exposure to other hormones has also been considered.[19] These established risk factors include several that relate to menstrual and reproductive history—age at menarche and menopause, age at first full-term pregnancy, number of births, history of lactation.[20,21] Both oral contraceptives and estrogen replacement have been associated with an increased risk of breast cancer.[2,21,22] A reanalysis of epidemiologic evidence worldwide, to be published in the journal *Contraception* just as *Public Health Reports* goes to press, suggests that oral contraceptive use is not associated with increased breast cancer risk 10 or more years after stopping use. Newer studies showing increased risk associated with alcohol and a protective effect for physical exercise may also be explained by estrogen. Alcohol appears to increase estrogen exposure, while exercise may reduce estrogen, perhaps by lowering body weight.[23,24] Studies of traditional risk factors associated with estrogen may, in fact, be the strongest impetus to explore a powerful hypothesis about synthetic chemicals in the environment that act like estrogens or interfere with hormone activity.



In the laboratory, human breast cancer cells will grow in the presence of estrogen or compounds that mimic estrogen.

Scientists have proposed three mechanisms for the effect of hormones on breast cancer. The best studied hypothesis is that hormones affect breast cancer by promoting cell proliferation. Estrogen is the primary hormone that signals breast cells to proliferate rapidly during puberty and pregnancy, signals changes during the menstrual cycle, and maintains the adult structure of the breast. Progesterone and growth factors may also play a role. When hormones signal breast cells to divide more rapidly, scientists hypothesize, they increase breast cancer risk by causing precancerous or cancerous cells to multiply. They may also increase the chance of a spontaneous mutation or a mutation prompted by exposure to a carcinogen and decrease the chance that the cell will repair itself before it divides again and replicates the error. In addition, scientists hypothesize that hormones may initiate breast cancer directly by causing DNA damage or that they may predispose cells to become cancerous at a later time, as was found for DES.[25] With this understanding of how natural estrogens may affect breast cancer, it makes sense to take a close look at chemicals, synthetic or naturally occurring, that mimic estrogens or otherwise affect hormone systems.



When elevated cancer incidence occurs within a confined geographic unit such as Cape Cod, epidemiologists begin to think about whether demographic or environmental features specific to the area may explain the pattern.

Since World War II, dozens of newly manufactured compounds that act like hormones or interfere with hormone metabolism have entered the environment through the synthetic chemicals used in some pesticides, detergents, and plastics. This class of chemicals, called endrocrine disrupters, is just beginning to be studied as a possible cause of breast cancer and of differences in rates between developed and developing nations.

Researchers have known for years that some of the postwar wonder chemicals are endocrine disrupters that mimic natural estrogens. DDT and its analogs were first identified as estrogenic (acting like estrogen) in 1969,[26] as was kepone in 1979.[27] Alkylphenols, used in detergents and plastics, were observed to interact with the estrogen receptor in 1977, thus disrupting normal estrogen activity. DDT and kepone were found to be estrogenic in animals, not just in tissue culture, during the 1970s, and octylphenol was shown to be estrogenic in animals last year.[28]

In 1995, DDT was found to be a potent anti-androgen, another hormonal effect. When anti-androgens bind to the androgen receptor, natural androgens cannot access the receptor. Since men and women have a greater than 100-fold difference in breast cancer risk, scientists speculate that higher androgen levels in men may be protective. Chemicals that block androgens could increase risk. The 26-year lag between the discovery that DDT is estrogenic and the finding that it is also an anti-androgen illustrates the startling gaps in this field of research. These gaps represent a failure to systematically study the hormonal effects—and particularly possible links to women's health—of compounds entering the environment in massive quantities.

Perhaps the spotty attention to endocrine disrupters results, in part, from disciplinary boundaries that fragment the study of this large class of natural and synthetic compounds. The broad class of hormonally active chemicals includes natural substances produced by the body; pharmaceuticals, including DES, estrogen replacements, and birth control pills; phytoestrogens in plants; and manufactured chemicals in pesticides, detergents, and plastics. Some signal cells in the same way as natural hormones, effectively increasing hormone exposure; others, particularly in plants, may block signals from natural hormones, effectively decreasing exposure. The box lists some chemicals shown to be endocrine disrupters. Few researchers studying natural hormones have made the connection between human hormone function and the synthetic chemicals in the environment that mimic or interfere with natural hormones.

Two scientists who have made the link are Ana Soto and Carlos Sonnenschein at Tufts University Medical School, who are members of the Silent Spring Institute study team. In 1991, in the course of routine studies of reproductive biology, they found that tissue cultures of human breast cancer cells were proliferating wildly in their laboratory. After painstaking efforts to decontaminate their lab, they found the source of the problem was plastic test tubes leaching a chemical, later identified as nonylphenol, that mimicked natural estrogen. Other researchers, too, have accidentally found estrogen mimics, also called xenoestrogens, in their labs.[29,30] Laboratory tests show that a variety of alkylphenols, phthalates, pesticides, and PCBs show estrogenic activity, as do pharmaceutical estrogens and rat chow, which contains soy, a phytoestrogen.[31,32] The growing list of findings lends support to the idea that exposure to estrogen mimics, as well as to natural estrogens, may contribute to breast cancer risk or affect hormone systems in other ways.

Other research suggests another way this process might work. Bradlow and Davis, drawing an analogy to "good" cholesterol and "bad" cholesterol, describe "good" and "bad" metabolic pathways for estradiol (natural estrogen).[33] In the "good" pathway, estrogen is metabolized to 2-hydroxyestrone (2-OHE1), which does not damage DNA and has minimal estrogenic activity. In the "bad" pathway, estrogen is metabolized to 16-alpha-hydroxyestrone (16α-OHE1), which can damage DNA and is strongly estrogenic. Bradlow and Davis describe a class of compounds that includes substances in plants such as broccoli and cauliflower that increase the production of "good" estrogens in the body and may be protective for breast cancer. Another class of compounds, some of which also mimic estrogen, includes many pesticides and other synthetic chemicals (Table 1). These increase production of "bad" estrogens, so they may increase breast cancer risk.

Further support for hormonal effects of synthetic chemicals comes from observations in wildlife populations. Hypotheses about endocrine disrupters made headlines this year with the publication of *Our Stolen Future*,[34] a book that describes their reproductive and developmental effects in wildlife. The discovery of hermaphrodite fish downstream from a sewage treatment plant[35,36] is an example of wildlife research that is particularly disturbing because waste water is a likely route for exposure to endocrine disruptors in humans as well. In tests of 20 compounds that are common constituents of waste water, about half were found to interact with the estrogen receptor in some way.[37]

Although some endocrine disrupters, including DDT and PCBs, are currently banned in the United States, they are highly persistent. They are already found in human fat even in the most remote areas of the Arctic, and new exposures are occurring from contaminated food, soil, and water and continued international use. Other xenoestrogens, such as bisphenol A and alkylphenols, may be less persistent, but exposure is ubiquitous from their use in modern life. Given the widespread potential exposure to xenoestrogens and new discoveries about how they disrupt natural hormones, careful study of the link between these endocrine disrupters and breast cancer is a top priority.

The Silent Spring Institute Approach

While the endocrine disrupter hypothesis is thought-provoking, it is hard to test using traditional methods given the current state of scientific knowledge. Because this area of science is new, we face problems common to epidemiologic research on emerging hypotheses.

A traditional case-control epidemiologic study would test the endocrine disrupter hypothesis by comparing the exposure to synthetic endocrine disrupters for women with and without breast cancer. This design involves a series of decisions about how to define "exposure to synthetic endocrine disrupters." For example, researchers must decide which chemicals are endocrine disrupters, which women are exposed to meaningful amounts of these chemicals, and what time frame is significant. Misjudgments would lead to research failures in identifying true effects.

Given this array of pitfalls, we needed to develop the best method to advance scientific knowledge in the field. In this section, we explore how we approached these methodological questions and how our understanding of the state of the science led to our ecologic epidemiology design, which focuses on populations, rather than to a case-control study of individuals. We discuss what we know about how to define exposure to hormone disrupters and why we believe that exposure at particular points in the life cycle may be important, and we raise the possibility that some women may be differentially affected. Next, we describe how our research approach begins to address these challenges by using two new methodologies: a geographic information system that superimposes detailed maps of exposure-related environmental data on patterns of breast cancer incidence and a program of environmental sampling for endocrine disrupters. Using this information, we address our key research questions: Is breast cancer incidence on Cape Cod associated with exposure to hormone disruptors in drinking water infiltrated by waste water? Is it associated with exposures to pesticides used in cranberry cultivation, insect control, golf course maintenance, or commercial lawn care? Finally, we discuss how our approach incorporates some consideration of individual risk factors, though the core analyses are ecologic and focus on environmental risk factors.

Defining exposure to endocrine disrupters. Defining what constitutes exposure to synthetic endocrine disrupters is central to studying their effects on breast cancer, but it is not easy to tease out such a definition. Of the tens of thousands of chemicals currently in commerce, only about 100 have been tested for hormonal effects. Even for these 100 compounds, it is problematic to find out whether women have been exposed. Clearly, we cannot ask women themselves about their experience with nonylphenol or amsonic acid, as we could in a standard epidemiologic survey of lifestyle risk factors such as alcohol.

Studies of occupational exposures can often be a useful source of information about the relationships between chemicals and health. As a partial solution to identifying women exposed to endocrine disrupters, we used existing information about the occupations of women with breast cancer and a comparison group from an earlier study of Upper Cape Cod by Aschengrau and Ozonoff. Working with Margaret Quinn, an industrial hygienist at the University of Massachusetts-Lowell, they have now classified women's job titles by their likely exposure to chemicals identified as xenoestrogens. Exposure to alkylphenols and bisphenol A appears to be common in women's work settings, and we will know more later this year about which workplaces are the most likely sources of xenoestrogen exposure. But the painstaking process of assessing xenoestrogen exposure through job histories or surveys about the use of familiar consumer products is hampered by our limited knowledge of the full range of substances used in commercial processes and products. For example, the estrogenic compounds in some pesticides may not be the active ingredient named on the label but rather an unnamed surfactant, and manufacturers' formulas for pesticides and consumer products are often protected as trade secrets. In addition, we have occupational data only for the relatively small number of Upper Cape Cod women who were included in the earlier study.

Multiple exposures to compounds with different kinds of hormonal activity further complicate the picture. For example, the family of PCBs includes some compounds that are estrogenic and others that inhibit extrogenic activity, perhaps explaining why epidemiologic studies of PCBs and breast cancer risk have been inconclusive.[12] Exposure to synthetic estrogens in the environment comes along with exposure to endogenous estrogens (those produced by the body) and phytoestrogens in the plants we eat, and to pharmaceutical hormones such as oral contraceptives and estrogen replacements, so disentangling environmental effects is difficult. Researchers speculate that lower breast cancer rates for Asians exposed to DDT than for people of other ethnic groups with comparable exposures may be due to a soy-rich diet with protective phytoestrogens.[12] Studies of drinking water are complicated by the possibility that exposure to endocrine disrupters may come from contaminated tap water or from water bottled in plastic.

The consequences of multiple exposures are highlighted in laboratory research in which known chemicals are combined and their effects on cell growth observed. Researchers at Tulane recently reported the dazzling finding that chemicals which showed very low estrogenic activity when applied to cells one at a time in a yeast bioassay were highly estrogenic when applied in combination.[38] Soto and Sonnenschein have also reported similar effects. Their E-SCREEN (estrogenicity screen) bioassay, which captures the combined estrogenic effect of mixtures of chemicals, is an important part of our approach to defining exposure, as will be seen below.

Timing of exposure. To the complications of defining what constitutes exposure to synthetic endocrine disrupters, we must add another question: Does the timing of exposure affect breast cancer risk? Many cancer studies share uncertainties about the relevance of timing because of the latency period for disease—the length of time between the initiation of a tumor and its clinical detection. Latencies of 20 to 30 years have been hypothesized for breast cancer.[12] On the other hand, studies of the relationships between hormone replacement therapy and breast cancer suggest that estrogens may be cancer promoters that affect the course of disease with exposures as recent as five years prior to diagnosis.[8]

The specific stage in her life cycle when a woman is exposed may also be important, since hormone systems are especially vulnerable during the prenatal period, adolescence, and pregnancy.[39] Effects of prenatal exposure to the pharmaceutical estrogen DES vividly illustrate the possibility of a sensitive period. The women who were exposed prenatally to DES may not yet be old enough to show the drug's potential effects on breast cancer risk, but other cancers and reproductive effects are well documented in these women. In addition, research suggesting a protective effect of physical exercise during adolescence, which may reduce estrogen exposure, supports the idea of one or more critical periods. Similarly, women exposed to radiation at younger ages are at greater risk for radiation-induced breast cancers than those exposed later in life; the greatest risk is observed for girls exposed before age four.[40] The use of historical environmental data in our geographic information system, described below, is a beginning step toward addressing the timing of exposures.

Exposure of susceptible groups. Recent research on genes and breast cancer suggests that some groups of women may be particularly susceptible to any environmental risk factors for the disease, though a family history of breast cancer still explains only 5% to 10% of all breast cancer cases. The politics of breast cancer sometimes pit genetic theories against environmental ones, but it is more likely that multiple causes are at work. It may be that environmental factors are particularly important for women of specific genotypes. An example of how this might work comes from research on smoking: smokers of one genotype have a five to ten times higher risk of lung cancer than smokers of a different genotype.[41] Another possibility is that environmental factors are risks for only certain forms of the disease. Small sample sizes have hindered exploration of the plausible hypothesis that xenoestrogens promote cancers in women with estrogen-receptor-positive tumors.[42]

The interactions between environmental and genetic factors is an important area for future research.

The decision to look for answers in the environment reflects a national trend among breast cancer activists to focus resources on prevention.

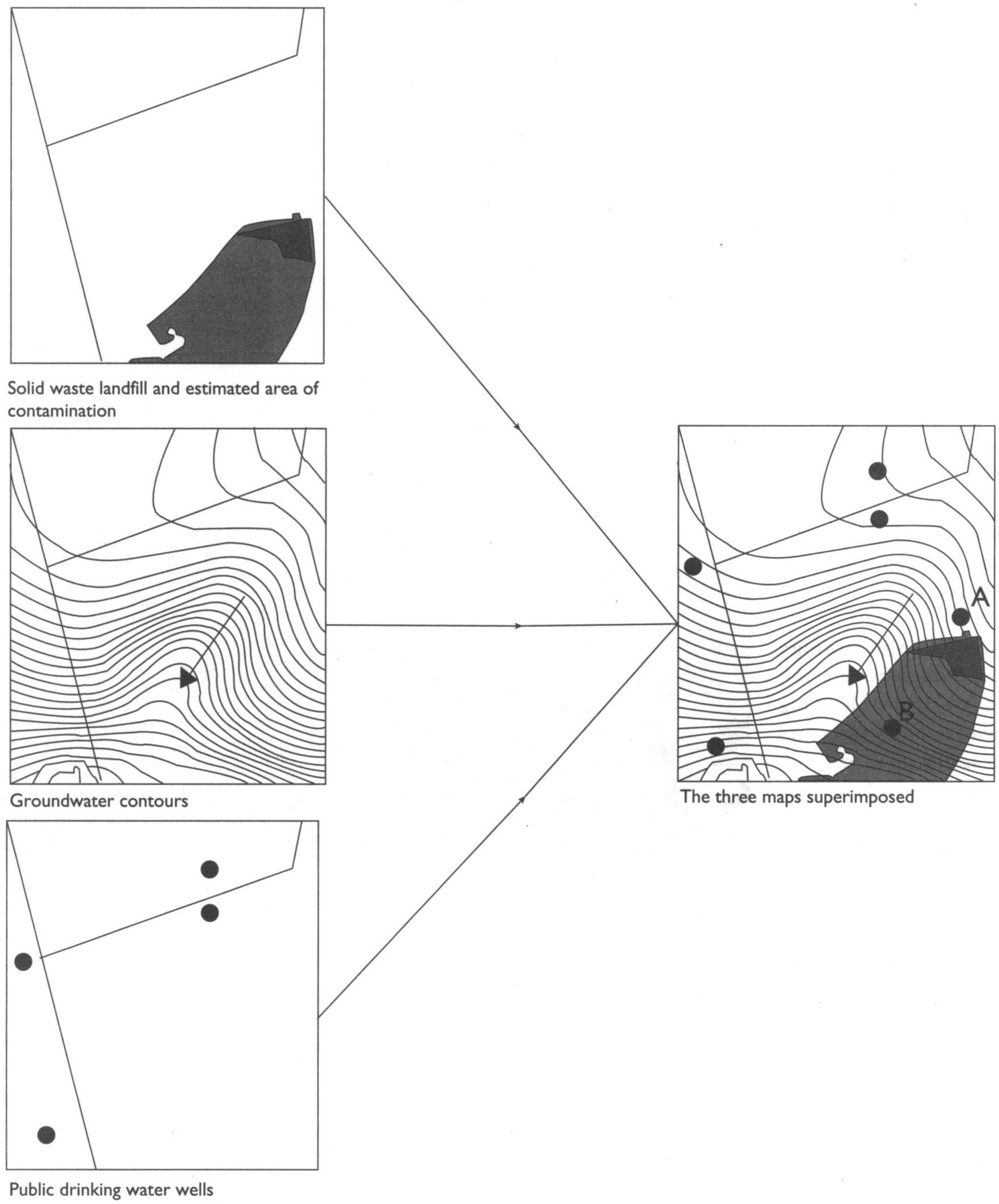
The ecologic epidemiology approach. Because we are still in the early stages of learning *what* environmental exposures may increase breast cancer risk, *when*, and *for whom*, researchers face a difficult challenge in defining the most fruitful research questions. Ecologic epidemiology—a study design that determines exposure and disease in populations rather than in individuals—is particularly useful in this phase. Indeed, this type of epidemiology has often generated the first findings to suggest a relationship between exposure and disease. As our colleague David Ozonoff tells his students at Boston University School of Public Health, an ecologic finding can serve to focus future research, like a sign in a vast terrain that says, "Dig here." In the Cape Cod study, the ecologic design allows us to study more than 2000 women with breast cancer and to explore numerous possible exposures at different periods over a long time span—a much broader view than could be taken, as a practical matter, if we interviewed individual women, as in a case-control study.

New strategies incorporating computer mapping techniques. While capitalizing on the strengths of ecologic epidemiology, the Cape Cod Study also aims to develop new strategies to bring greater power and subtlety to this method by incorporating a geographic and historical perspective. Our key data management and analytical tool is a sophisticated Geographic Information System (GIS) used to map breast cancer cases and extensive environmental data for the Cape. Using the GIS enables us to explore the relationship between where women lived when they were diagnosed and where we estimate the greatest potential for environmental exposures. Conceptually, the research design is analogous to mapping zones of likely environmental contamination and then superimposing the addresses of women with breast cancer to examine whether we find increased breast cancer risk in the same places we expect greater environmental impact.

Using the GIS as an innovative tool to define exposure, we will map zones of environmental impact to address our primary research questions. One set of exposure zones will assess likely exposure to endocrine disrupters from waste water in drinking water supplies. Another will assess areas of likely exposure to pesticides that have been widely used on the Cape.

The ability of the GIS to handle multiple data sources is critical to this task. Our environmental characterization of the Cape draws on data from the state Department of Environmental Protection, including locations of solid and hazardous waste sites, known plumes of groundwater contamination, and zones within an aquifer that feed into public drinking water wells. We are also using groundwater contours from the U.S. Geological Survey and University of Massachusetts land use data for five specific years dating back to 1951. This information will be combined with data we are collecting about pesticide applications and the historical development of water systems, including water test results, hook-up dates for supply wells, and the composition of water pipes—information gleaned from Cape Cod's regional planning agency, individual town governments, and our own fieldwork.

Figure 2. Using the Geographic Information System to map environmental data



These maps illustrate the use of the Geographic Information System to improve our understanding of which locations are likely to be exposed to environmental impact. The map at the top left shows the location of a solid waste landfill (dark shading) and the estimated area of contamination (lighter shading) leaching from the landfill. Below that is a map of the groundwater contours for the same location; the arrow shows the direction of groundwater movement. The map at the bottom left shows the locations of drinking water wells. The right-hand map shows these three types of data superimposed. A home located at point "A" is quite close to the landfill but is unlikely to be affected. A home at point "B," farther away, is more likely to be affected. If a drinking water well fell within an area of likely contamination, we could use our maps of the water systems to learn which homes are served by that well.

Figure 2 illustrates how layering this information together improves substantially on traditional environmental exposure assessment techniques. The map shows a known area of contamination from a landfill together with groundwater contours and the zones that feed into public drinking water wells. Traditional epidemiology typically uses distance from a source—in this case, a solid waste disposal site—as a proxy for exposure. Figure 2 shows that a residence would not be exposed if drinking water comes from a well close to but up gradient from the source of contamination. Conversely, exposure could be significant if drinking water came from farther away but downgradient from the source. Thus, the GIS allows definition of exposure zones that are environmentally relevant rather than being driven by arbitrary boundaries such as town lines or relatively crude proxies such as distance.

As noted above, the GIS further allows the possibility of defining exposure zones that are not contiguous. For example, one exposure zone might include all areas across the Cape where certain pesticides were applied for insect control during certain years. By aggregating these noncontiguous zones and calculating a single outcome measure, we improve statistical power, increasing our ability to find a true effect.

In addition, by incorporating diverse environmental data, some of it dating back to 1951, we gain remarkable flexibility in exploring exposures to possible risk factors for specific population groups. Thus, we can empirically test different ways to specify research questions, a useful exercise given the limited prior research about the relationship between breast cancer and environmental risk factors.

So far, we have discussed the strengths of GIS in defining exposure. It is also useful in analyzing patterns of disease. Independent of the exposure zone mapping, we added data on cancer incidence to the GIS so that areas of environmental impact and locations of breast cancer cases can be compared. Using data from the Massachusetts Cancer Registry together with town records and other sources, we located street addresses for 96% of the 2200 Cape Cod women diagnosed with breast cancer (regardless of where diagnosed) from 1982 through 1992, then mapped these cases.

Breast cancer incidence maps will be used to further explore geographic patterns within Cape Cod. With the U.S. Census population data also in the system, we can study patterns of breast cancer by calculating standardized incidence ratios (SIRs) at multiple levels from the town level to census tracts and census block groups. Thus, we can empirically explore trade-offs between larger sample size and greater statistical power on the one hand and the potentially greater explanatory power of smaller geographic units on the other. Historically, mapping of small geographic units to find disease clusters has been a useful way for epidemiologists to generate hypotheses about causes of disease. In addition, we are extending the traditional cancer surveillance approach by applying it to environmentally meaningful units such as groundwater zones and public water supply districts and even to noncontiguous units. For example, we will aggregate areas served by private wells and compare them with areas served by public water supplies.

Environmental sampling, chemical analysis, and the E-SCREEN bioassay. A further innovation in the Cape Cod Study involves sampling and testing water sources on the Cape. Data from these tests will contribute to defining environmental exposure using the GIS. Testing Cape Cod water samples is useful because the hypothesis relating breast cancer with exposure to endocrine disrupters, potentially important worldwide, is particularly relevant to the Cape, where the sandy soil and sole-source aquifer make drinking water vulnerable to contamination from pesticides and from waste water containing endocrine disrupters.

The E-SCREEN bioassay developed at Tufts University Medical School[32] is a new tool to study estrogenic activity in water, opening research opportunities that were impossible just a year ago. The test involves growing human breast cancer cells in culture plates, then exposing them to suspected estrogenic compounds. The breast cancer cells proliferate only in the presence of natural estrogen or estrogen mimics.

Using E-SCREEN together with chemical analyses, we have begun to test samples of waste water and drinking water on the Cape, with our first 50 samples collected during the summer of 1996. This is the first time E-SCREEN has been used to test samples from the environment for estrogenic activity. The beauty of the bioassay is that it can test compounds and mixtures of unknown estrogenicity, avoiding some of the problems of defining exposure.

Complementary chemical analysis using gas chromatography/mass spectrometry will enable us to study compounds such as atrazine and dinitrotoluene that are not estrogenic but are identified as disrupters for other hormones or as possible causes of breast cancer. Chemical analysis will also help us identify the likely sources of estrogenicity in samples that are positive on the E-SCREEN.

These test results will give us important information about whether there is estrogenic activity in drinking water supplies and what specific compounds are present. However, we will not be able to go directly from lab results to exposure assessment. Costs of testing are too high to allow us to evaluate a representative sample of water supplies. Instead, we will be looking for correlations between our limited water test results and more extensive GIS data on water quality and land use. Because we hypothesize that waste water is a source of endocrine disrupters, we expect to find associations between estrogenicity and nitrates, a well accepted marker for waste water contamination. Such an association will strengthen the argument for using historical nitrate concentrations as a surrogate for historical exposure to xenoestrogens, allowing us to make use of our GIS data, which includes historical data on nitrates in drinking water, and take account of breast cancer latency.

Revisiting earlier case-control data to explore individual risk factors and small clusters. The strength of the Cape Cod Study comes from detailed information about the environment. For nearly every Cape Cod woman diagnosed with breast cancer from 1982 through 1992, we will know a lot about which water wells supplied her tap, the water quality in those wells, and what types of pesticides were applied during which years in her area. Our limitation comes from knowing very little about each woman as an individual. We will not know whether she drank bottled water as well as tap water from the wells we are assessing. We will not know whether she drank alcohol, a risk factor for breast cancer. We will not know if she was exposed to chemicals at work or how long she lived at her address before diagnosis. Just as studies of individual risk factors do not assess environmental effects, we cannot account for breast cancer risk from individual lifestyles and genetic factors or study how these factors interact with the environment.

We can address this limitation indirectly, however, by using existing case-control datasets to explore how much of the variation in breast cancer risk within the Cape, and how much of the difference between the Cape and the rest of Massachusetts, can be explained by individual risk factors. In addition, to explore how well regional differences in mammography screening rates can explain differences in breast cancer incidence, we will analyze variations in stage at diagnosis, that is, the physician's classification in the Cancer Registry files of whether a tumor was identified "earlier" or "later." If more extensive screening—detecting tumors earlier—contributes to higher reported incidence on Cape Cod, we would expect to see more tumors identified at an earlier stage for women there than elsewhere in Massachusetts.

Finally, new information will come from earlier case-control studies that included women on the Cape. David Ozonoff, Chris Paulu, and Tom Webster at Boston University School of Public Health are developing promising statistical methods using data from the Upper Cape Cancer Incidence Study. A persistent problem in studying environmental health effects is the possibility of highly localized impacts from point sources of pollution. With small-scale impacts, statistical power may be inadequate to identify meaningful patterns. New mathematical techniques to identify clusters of cases and to evaluate different cut-points for defining exposure across place and time are promising tools to link exposure and disease.[43,44]

Partnership with Activists and the Community

While our discussion so far has focused on the scientific challenges of our work, we are always aware that the science of breast cancer has personal and political dimensions. A productive partnership among scientists, activists, public health officials, and the Cape Cod community is central to our work. Activists and the community are crucial advocates for financial support and access to data, and they are ultimately the ones who make our results meaningful.

Activists played a critical role from the start in getting the Cape Cod Study funded and underway. While commercial interests invest in mammography, genetics, and chemotherapy, no one makes big bucks from environmental research, so funding for environmental studies is often driven by activists' concerns. At the national level as well as in Massachusetts, women with breast cancer and their families, friends, and colleagues have been advocates for shifting research priorities toward the environment in a search for preventable causes.

Collecting data about the places where people live further engages the community and requires public support. Dozens of women and men on Cape Cod have helped us by searching town records, reconstructing historical information, and giving permission to test their wells. Our work began with focus groups and interviews to ensure that our study addressed the questions people wanted us to ask and was informed by long-time residents' knowledge of local history.

The critically important enthusiasm and support of Cape residents and breast cancer activists statewide bring with them a "social contract" between researchers and the community that includes a responsibility to keep people informed about our work. Explaining xenoestrogens and standardized incidence ratios and nitrate loadings isn't easy. But developing new ways to fulfill our social contract is as much a part of our mission and commitment to innovation as developing the E-SCREEN assay.

As results come in, we face the dual tasks of interpreting our complex findings and communicating accurately what we have found, including the limitations and uncertainties in our results. We know that our ecologic study, although it is ambitious in scope, won't have the definitive answer we all want to the question of whether there are environmental causes of breast cancer. We are committed, though, to narrowing uncertainty and laying a groundwork on which answers can be built during coming years. We are sure, at least, that our question is an important one.

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