Endocrine Disruptors and Breast Cancer

Researchers from the Silent Spring Institute search for clues to explain the higher incidence of breast cancer on Cape Cod.

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The hypothesis that endocrine-disrupting chemicals affect the risk of breast cancer is a short, logical step away from what we already know about the disease. Since the 18th century, when the Italian physician Bernardino Ramazzini observed a higher incidence of breast cancer among nuns, researchers have known that women's natural reproductive hormones affect risk. More recently, Devra Davis with the World Resources Institute and her colleagues published the troubling hypothesis that other estrogenic compounds—including synthetic estrogens found in commercial products and the environment, as well as natural phytoestrogens in food—might also affect breast cancer risk.[1]

This hypothesis poses a critical challenge concerning the possible human health effects of endocrine disruptors, and, at the same time, it opens the doors for new research such as the Silent Spring Institute's study of breast cancer on Cape Cod. This research offers the hope of identifying preventable causes of the disease. Each year, 180,000 women are diagnosed with breast cancer in the United States. Preventable environmental factors—even if they represent relatively small, incremental risks—translate into many saved lives.

Complex Hypothesis

Multiple hormone-related mechanisms appear to be involved in breast cancer risk, and there are several ways that endocrine-disrupting chemicals may affect risk as well. Women who have never given birth—like the Italian nuns—and women who were older at the birth of their first child are at higher risk because the breast is not fully developed until after a first pregnancy, and the undifferentiated cells are more vulnerable to carcinogenesis. In addition, women with fewer children and those who were younger at menarche and older at menopause are at greater risk because each menstrual cycle results in exposure to estrogen—which causes cells to proliferate—increasing the chance of mutation and promoting growth of any existing tumor.

In addition, alcohol use, lack of physical exercise, higher body mass after menopause, and weight gain—factors that appear to increase estrogen levels—are associated with higher breast cancer risk, while removing the ovaries, the body's source of estrogen, before menopause, substantially reduces risk. Estrogen-based pharmaceuticals, including birth-control pills and hormone-replacement therapy, increase breast cancer risk, while treatment for breast cancer involves drugs such as tamoxifen that block estrogen.

Given the weight of evidence that endogenous and pharmaceutical estrogens affect breast cancer risk in multiple and complex ways, other hormonally active compounds in consumer products and the environment deserve careful study.

The seemingly simple question "Do endocrine-disrupting chemicals affect breast cancer risk?" does

not lend itself to simple research methods, however. There is a large and growing list of compounds identified as endocrine disruptors. Their biological effects are diverse and as yet poorly understood, and scientists know little about how women are exposed or what aspects of exposure matter. Multiple research approaches are needed to disentangle the health effects of these compounds, and the challenge is particularly complex for diseases, like breast cancer, with long latency.

On Cape Cod, where breast cancer incidence is 20 percent higher than in the rest of Massachusetts, a multidisciplinary team, led by the Silent Spring Institute, is investigating the endocrine-disruptor hypothesis in the Cape Cod Breast Cancer and Environment Study. After three years of research, new methods and results are emerging that will inform studies of breast cancer and other hormonally mediated conditions.[2]

Cape Risk

The Cape Cod Breast Cancer and Environment Study began in 1994 when the Massachusetts Department of Public Health published age-adjusted cancer-incidence rates by town. Cape Cod stood out as a region where cancer rates in nearly all its towns were above the statewide average. Since then, detailed surveillance, using data from the Massachusetts Cancer Registry, has shown a sustained pattern of higher breast cancer incidence on the Cape. In fact, the incidence has been 20 percent higher on Cape Cod than in the rest of Massachusetts, at least since 1982, when the registry began.[3]

In another study, Kathleen Egan and Meir Stampfer at Harvard School of Public Health found that for Cape Cod women over 50, breast cancer risk remains 20-percent higher than for women elsewhere in Massachusetts even after established risk factors are taken into account.[4] This finding indicates that demographic differences do not fully explain higher breast cancer rates on the Cape.

In addition, we found that a greater use of mammography by Cape Cod women is not a likely explanation. In recent years, the fraction of breast cancers diagnosed early—at stage one—was a few percentage points higher on the Cape than elsewhere in the state, while in earlier years the fraction diagnosed early was the same This finding indicates that greater use of mammography on the Cape may contribute somewhat to higher breast cancer rates in recent years but does not adequately explain the 20-percent gap and does not account for higher rates during earlier years.

Since established risk factors and mammography practices do not fully explain higher breast cancer risk on Cape Cod, unknown factors, perhaps in the environment, must account for the difference. Indeed, the environmental characteristics of the Cape may result in greater exposure to certain chemicals, including endocrine disruptors. As we considered how the Cape environment is different, we focused in particular on potential exposure to endocrine-disrupting chemicals from two sources: pesticides and drinking water infiltrated by wastewater.

Exposure Pathways

The Cape has a long history of pesticide use that dates back to the 1940s when trees were treated to control the gypsy moth. Pesticides were also used for cranberry cultivation and other agricultural uses, and to enhance tourism. Campgrounds, forests, gardens, golf courses, and lawns were sprayed with dozens of different chemicals, including persistent organochlorines and newer pesticides, many of which are also endocrine disruptors. Spraying was common in or near residential areas where women were likely to be exposed.

In addition to a history of widespread use of pesticides, the Cape has sandy soils over a shallow aquifer, leading to contamination of some drinking-water wells by wastewater from septic tanks.[5] Researchers in other regions have found that surface waters contaminated by wastewater contain endocrine-disrupting chemicals from commercial products and from human wastes that carry excreted pharmaceuticals and endogenous estrogen.[6] Therefore, we reasoned, if endocrine disruptors do play a role in breast cancer, the Cape's hydrogeology and land-use history, which contributed to groundwater and drinking-water contamination by wastewater, may also contribute to higher breast cancer risk.

The Cape Cod Breast Cancer and Environment Study is the first to focus on the impact of endocrine disruptors from septic systems on shallow drinking-water wells that are typical on the Cape. However, earlier studies of the impact of sewage-treatment plant effluents on surface drinking-water supplies such as lakes and rivers have found hormone-related effects in fish. For example, researchers found hermaphrodite fish downstream of sewage-discharge points, and further study of sewage identified alkylphenol ethoxylates, which are surfactants in common household detergents.

These compounds break down in the environment to form the estrogenic alkylphenols nonylphenol and octylphenol.[7] Alkylphenol ethoxylates are also found in many other products. They are used as inert ingredients in pesticides and in personal-

care products including spermicides and cosmetics.

Other endocrine-disrupting chemicals found in wastewater include ubiquitous constituents of some plastics, including phthalates and bisphenol A, and some antioxidants, like BHT, which have estrogenic effects.[8] Recent studies have also identified excreted endogenous and pharmaceutical estrogens in treated wastewater effluent at concentrations high enough to cause reproductive effects in fish.[9]

In addition to identifying estrogenic compounds in consumer products and wastewater, a few studies have measured alkylphenols in environmental samples. A survey of 30 U.S. rivers by the Chemical Manufacturers Association found a wide range of concentrations of alkyphenols in sediments and water samples.[10] Other researchers have reported detecting nonylphenol and related compounds in the effluent of sewage-treatment plants and in a New Jersey drinking-water supply.[11]

Much less information is available about groundwater contamination with alkylphenols. However, Larry Barber and colleagues at the U.S. Geological Survey have been conducting research on the fate and transport of chemicals in a wastewater plume at the Massachusetts Military Reservation on Cape Cod for many years, and they have reported nonylphenol in this plume. For a variety of reasons, including the acidic pH and low organic-carbon content of the Cape aquifer, many contaminants that typically degrade in the subsurface have persisted for more than 30 years in the Cape aquifer.[12] This information on pollutants introduced to the Cape aquifer is important for making predictions about the impacts of wastewater on drinking water.

Since consumer products, wastewater, and surface water impacted by wastewater contain substantial concentrations of estrogenic compounds, the Cape Cod study evaluated the impacts of wastewater effluents and septic systems on groundwater and drinking water. This sampling program was designed to improve our understanding of how women are exposed to compounds that may be associated with breast cancer.

Assessing Exposure

One of the most significant barriers to learning whether endocrine-disruptors or other chemicals cause or promote breast cancer is identifying the specific chemicals to which women are exposed, understanding their biological activity, and assessing the concentrations, pathways, and timing of exposures. For example, recent research demonstrates that the *in utero* hormonal environment of the fetus affects breast cancer risk many years later in adult women, so it would be useful to know exactly which compounds women were exposed to over their lifetime, including *in utero*.[13]

Ideally, we would like to know exactly how much of each compound women were exposed to, how multiple exposures interact, and when in each woman's lifecycle exposure occurred. Data currently available for exposure assessment are far from this ideal, but researchers are beginning to piece together information from multiple sources.

Phase one of the Cape Cod study used two approaches to characterize potential exposure to endocrine-disrupting chemicals on the Cape, and we will add a third approach this year. First, we developed a sophisticated geographic information system (GIS) to integrate multiple sources of historical environmental data and develop indicators of past exposures dating from the late 1940s. Second, we developed a field-sampling program to test wastewater, groundwater, and drinking water for endocrine-disrupting chemicals and other relevant compounds.

Development of the GIS involved extensive reconstruction of pesticide-use practices and the history of drinking-water supply systems on the Cape. Development of the field-sampling program involved new methods of chemical analysis and a new method for measuring estrogenic activity using a strain of breast-cancer cells called MCF-7. Researchers use the MCF-7 breast-cancer cell assay to determine the estrogenic activity of a variety of compounds.

While we are continuing to use GIS and field-sampling methods to assess exposure, in 1998 we are adding the third approach. We plan to interview 2,500 women in a case-control study that will gather data about occupational and residential history and activities—such as use of home pest-control and gardening products—that relate to chemical exposures.

Historical Reconstruction

Conceptually, the GIS is similar to well-established epidemiologic research methods that for years used paper maps with push-pins and markers to locate cancer cases and local environmental features, but it is an even more powerful tool for analyzing larger data sets by overlaying maps from multiple sources in a computerized database. It is particularly valuable in assessing exposures to toxicants in years past—an important asset in studies of diseases, such as breast cancer, with long latency.

The Cape GIS study reaches back to the 1950s, creating a detailed view of past and present environmental events and human activities. GIS data from a dozen public agencies and academic centers, plus archival research by the Silent Spring Institute, are combined to indicate exposure to a range of factors including endocrine disruptors. The data include land-use maps derived from aerial photographs dating back to 1951, water-quality measurements for public wells dating from the 1970s and 1980s, maps of areas where pesticides were applied to forests or other lands, U.S. Geological Survey groundwater contours, and Massachusetts Department of Environmental Protection maps of contaminated groundwater and the sources of public well water.

To infer exposure to endocrine-disrupting chemicals, we combined data from multiple sources. For example, we used historical land-use maps to identify areas used for cranberry cultivation, golf courses, and other activities where pesticides are heavily applied. We used other maps collected from public records to locate areas sprayed with DDT and other chemicals for tree pests such as the gypsy moth. Then we calculated the distance from every house lot on the Cape to the nearest mapped pesticide use, taking into account whether maps showed a forest buffer to protect the home from drift. The system takes into account historical as well as spatial factors, including, for example, the year in which spraying was recorded, years of operation of cranberry bogs, and the time period in which homes were constructed.

We also used the GIS in a similar way to assess historical exposure to contaminants in drinking water. Since endocrine-disruptors have only recently surfaced as a health concern, there are no data sets recording historical levels in the environment. We inferred exposure from measurements of nitrate contamination, a widely accepted indicator of wastewater or agricultural impacts on groundwater.[14] Nitrate is limited as an indicator of endocrine disruptors, however, because nitrates are water soluble, while endocrine-disrupting chemicals vary in water solubility. Nitrates may be a better indicator for some compounds than for others.

By combining nitrate measurements for public drinking-water wells with data about pumping rates and maps of residential areas served by the water-supply districts, we were able to assess the impact of wastewater on drinking water supplies for each residence back to the 1970s. In the next phase of research, these indicators will be combined with other data in a case-control study.

Field Work

To complement historical information provided by the GIS studies, we designed field-sampling methods to assess the types and concentrations of estrogenic chemicals currently found in wastewater, groundwater, and drinking water on the Cape. We wanted to learn which estrogenic compounds are found in the highest concentrations in Cape Cod septic systems and wastewater and whether these compounds have leached into groundwater and drinking water.

To identify specific endocrine disruptors and overall estrogenic activity, we used two approaches. First, we used chemical analysis to identify a range of pesticides, plastics, and surfactants previously identified as estrogenic, including the alkylphenol ethoxylates and their degradation products.

Next, in collaboration with Ana Soto and Carlos Sonnenschein at Tufts Medical School, we tested environmental samples such as wastewater and groundwater through a bioassay that measures total estrogenicity.[15] The ability to measure estrogenicity without knowing the identity of every estrogenic chemical that may be found in the samples is important, since many chemicals have not yet been tested for estrogenicity and therefore could not be selected as target chemicals for analysis.

The bioassay method using the MCF-7 breast cancer cell was originally used by pharmaceutical companies to identify synthetic estrogens for therapeutic uses and involves laboratory observation of the effects of different compounds on breast cell growth. In the Cape study, we applied the bioassay to water samples that contain a mixture of contaminants, rather than to pure chemicals, and this adapted method may also be used more widely to test a variety of environmental samples.[16] For example, we will be using it in 1999 to test for estrogenicity in dust and air samples from Cape Cod homes.

Pesticide Use

We reconstructed the history of pesticide use on the Cape through contacts with dozens of organizations and individuals who helped us find records of wide-area spraying with known endocrine disruptors, including atrazine, chlordane, DDT, dieldrin, heptachlor, methoxychlor, and toxaphene. Many other pesticides used on the Cape have not been tested for endocrine activity. But even this list of known endocrine disruptors indicates that previous studies of breast cancer and DDT do not tell the full story of possible effects of multiple pesti-

cides on this disease. Perhaps, for example, cumulative exposure to a combination of pesticides or other chemicals that act additively can increase breast cancer risk. In addition, inert ingredients in pesticides, including alkylphenol ethoxylates, may also be a source of exposure to endocrine disruptors, but information about inert ingredients in pesticide formulations is difficult to obtain because of trade-secret protections.

Another difficulty in studying possible effects of DDT and other chemicals on breast cancer is that there may not be enough variation in exposures to allow researchers to observe differences in effects. DDT was first used on the Cape in 1948, when federal and state officials attempted to totally eradicate the gypsy moth by spraying all 265,000 acres of Cape Cod. Cape-wide spraying was conducted in 1949 and 1950, and more limited spraying was repeated in later years. Thus, although some areas of the Cape were sprayed more frequently than others, we cannot compare women exposed to DDT with unexposed women within Cape Cod. In fact, all of us have the DDT metabolite DDE in our bodies, so a completely unexposed group cannot be identified in any study population.

Other limitations come from incomplete records of pesticide use. The primary large-scale uses included wide-area spraying of forests for gypsy moth and other tree pests; mosquito control in wetlands; and applications on cranberry bogs, lawns, and golf courses. Records appear to be reasonably complete for large-scale federal and state spraying for gypsy moth, and land-use maps allow for assessment of exposures from specific uses, such as cranberry cultivation. However, records of spraying for other purposes are inadequate. For example, mosquito-control spraying is not mapped to specific areas within towns. Private chemical use on lawns and gardens is, of course, unrecorded, although we plan to gather partial information by interviewing Cape residents in the case-control study we are just beginning.

Despite these limitations, record-based exposure assessment has some strengths in comparison with other methods, including interviews and blood measurements. Records provide information about a larger number of different chemicals than most blood measurements, and records also provide information that women themselves do not have and so cannot be ascertained in interviews. In addition, records identify exposures to specific chemicals in specific years, an advantage if the timing of exposure in a woman's life makes a difference in risk, as some researchers hypothesize. Still, the limitations of historical records will make it difficult to find associations between pesticide exposures and breast cancer, even if the association exists.

Groundwater

In addition to pesticides, wastewater is also a possible source of exposure to endocrine disruptors. Our field-sampling program confirmed that wastewater and septic systems contain high concentrations of estrogenic compounds and high levels of estrogenic activity. Chemical analyses detected high concentrations of alkylphenol ethoxylates in samples from septic systems and other estrogenic compounds such as phthalates, bisphenol A, and organochlorine pesticides at lower concentrations. Groundwater from areas known to be polluted by wastewater showed lower levels of many of the same chemicals.

Our results demonstrate that endocrine disruptors were mobile and persistent in groundwater. This finding is of particular concern because groundwater is the sole source of drinking water for the Cape, and septic systems serve 95 percent of the local population. A few of the 25 private drinking-water wells that we tested had extremely low levels of bisphenol A and breakdown products of alkylphenol ethoxylates, suggesting that impacts to drinking-water wells were limited for the target chemicals we analyzed.

While none of the drinking-water wells we tested showed measurable estrogenic activity in the MCF-7 bioassay, contaminated groundwater and wastewater samples contained estrogenic activity at levels similar to those found to induce feminization in male fish.[17] We are continuing to analyze the data to assess whether the samples also contain estrogenic activity from chemicals that were not target compounds of our analyses, for example, pharmaceutical and endogenous estrogens.

Although the levels of endocrine disrupting chemicals we detected in drinking water are very low, endocrine disruptors may act cumulatively, so total exposure could be important. In addition, numerous human and wildlife biology studies show that very low concentrations of hormones—parts per trillion and lower—can induce important biological effects. For example, the concentration of circulating free estradiol during sexual development in male mouse fetuses is less than 0.2 parts per trillion, and very small increases in estradiol during fetal development disrupt normal sexual development.[18]

Future Research

While the Cape Cod study breaks new ground in describing how

women may be exposed to estrogenic compounds in the environment, it leaves many questions unanswered. Given the small number of water samples tested, we still do not know how widespread contamination of drinking-water wells by endocrine disruptors from wastewater may be on Cape Cod. In addition, we targeted some synthetic chemicals known to be estrogenic and likely to be found in commercial products but did not target pharmaceutical or endogenous estrogens or other synthetic chemicals that may play a role.

Since we found high concentrations of endocrine-disrupting chemicals in septic-system samples but not in well water, there may be other important routes of exposure. Since wastewater on the Cape comes primarily from residential sources, the endocrine disruptors we detected in wastewater originated from household use. Perhaps, then, direct routes of exposure to consumer products are more significant than drinking water exposures.

Could these other exposures contribute to higher breast cancer incidence on Cape Cod? Focus-group discussions at the outset of the study indicated that products to control mold and insects that thrive in the moist Cape Cod climate may be more commonly used than elsewhere in Massachusetts, and these products may be a source of exposure to endocrine-disrupting chemicals. We plan to explore this possibility further over the next three years in a continued sampling program that will test for animal mammary carcinogens, endocrine disruptors, and estrogenic activity in dust and air in Cape Cod homes.

The search for causes of elevated breast cancer rates on the Cape could be greatly enhanced by other types of research in addition to our study, since many basic research questions about endocrine-disrupting chemicals remain unanswered. It would be enormously useful, for example, to be able to narrow our list of target compounds to chemicals shown in other studies to cause or promote breast cancers. More than 100 chemicals, some of them endocrine disruptors, have been identified internationally as causing mammary tumors in animals; however, only a small fraction of the 80,000 chemicals in commercial use have been tested, and recent research raises doubts about whether standard cancer bioassays in animals are designed to be sensitive to agents that cause mammary tumors.[19]

Equally pressing are questions about how synthetic endocrine disruptors, naturally occurring phytohormones, and endogenous hormones differ from each other, how they act in combination, and how the timing of exposure influences health effects. Because breast cancer is such an urgent public health problem, concurrent investigation of these questions, using multiple approaches to supplement and guide epidemiologic studies, is essential.[20]

Money Talks

While we as researchers are grappling in minute detail with methodological questions about whether nitrate measurements predict the presence of endocrine-disrupting chemicals in drinking water, or which pesticide exposures should be combined into a single exposure measure, or whether use of the spermicide nonoxynol-9 might represent an important source of exposure to endocrine disruptors, the compelling question for breast cancer activists—do chemicals in the environment cause breast cancer?—is still unanswered almost 30 years after Richard Nixon declared war on cancer. Why is the Cape Cod study one of only a few exploring whether endocrine disruptors, which have become ubiquitous in the environment, contribute to a higher incidence of breast cancer?

Economic interests have a major role in shaping what we know and don't know about endocrine disruptors. Enormous resources are invested in research to understand the mode of action and biological effects of pharmaceutical hormones used for birth control, hormone-replacement therapy, and breast-cancer treatment and prevention using drugs such as tamoxifen, which may prevent or slow the development of breast cancer.

Conversely, consumer products as common as detergents and baby bottles are scarcely ever thoroughly evaluated for biological effects, since the manufacturers funnel their research dollars toward marketing. For example, research related to fluorescent whitening agents in laundry detergents has focused on studies of consumer preferences for different shades of white, rather than on the possibility that the synthetic stilbenes used to synthesize whitening agents might break down into endocrine disruptors in laundry or in wastewater. The Environmental Defense Fund recently reported that basic toxicological data are available for only 29 percent of the 3,000 highest-production-volume chemicals in the United States, and even fewer have been screened for effects on endocrine response or breast cancer.

Public Pressures

Activist initiatives that lead to public funding for breast cancer research often stem from fears about excess incidence in a particular region, together with a sense that

breast cancer deaths have been ignored for far too long. Indeed, the Cape Cod Breast Cancer and Environment Study and the Long Island Breast Cancer Study Project, two nationally significant studies of possible environmental causes of breast cancer, sprang from activist pressures for public funds for environmental research. Citizens feel an urgent need for answers *now*.

Sadly, studies of endocrine disruptors probably will not bring a quick answer in communities with a high incidence of breast cancer, because no single study can firmly document a link between endocrine disruptors and disease. Rather, multiple approaches—including epidemiology, toxicology, and exposure-assessment research—will be needed to establish a causal relationship, if indeed endocrine disruptors do cause or promote breast cancer.

Responsible Science

In stark contrast to the extensive research on hormonally active pharmaceuticals, gaping holes exist in our knowledge about endocrine disruptors in the environment. For example, the pharmaceutical hormones tamoxifen and raloxifene have become familiar across the country as women search the news headlines for a cure for breast cancer. Yet other estrogenic compounds—such as bisphenol A, nonylphenol, and methoxychlor—remain largely unknown to the public, although they are already common in consumer products in our homes. As scientists, we have an obligation to educate activists, public officials, and the public at large. We also need to demand that the synthetic estrogens we buy unknowingly at the grocery store are as intensively researched as are the designer estrogens in drugs.

For years, research has documented that endogenous estrogens increase breast-cancer risk, and associations with endogenous estrogen underlie nearly all of the known risk factors for the disease. At the same time, research about pharmaceutical estrogens demonstrates different biological effects from different types of estrogens: tamoxifen acts as an antiestrogen in the breast and an estrogen in the uterus, and raloxifene is an antiestrogen in the breast and an estrogen in bone. For diethystilbestrol (DES), we learned too late that exposure *in utero* would ultimately cause many reproductive-system problems, including cancers. Discoveries of the complex effects of these pharmaceutical hormones demonstrate the need to fully understand the equally complex effects of the many other synthetic estrogens to which we are commonly exposed as well.

As we pursue a better understanding of environmental factors on Cape Cod, we call on others to initiate complementary research. Decades of breast cancer research have uncovered many established risk factors, most of them tied to natural estrogens. With a similar investment in studies of environmental estrogens, perhaps we will find additional, *preventable* causes of breast cancer.■

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NOTES

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4. A more detailed discussion of these findings may be found in the Silent Spring Institute's "Cape Cod Breast Cancer and Environment Study Final Report" (Newton, MA: Silent Spring Institute, 1997). The Collaborative Breast Cancer Study takes into account risk factors such as age, education, Jewish ethnicity, family history of breast cancer, recent body-mass index, height, age at menarche, parity, age at first full-term pregnancy, history of benign breast disease, menopausal status, age at menopause, recent alcohol consumption, smoking, physical activity, and carotenoid consumption as described in M.P. Longnecker et al., "Risk of breast cancer in relation to lifetime alcohol consumption," *Journal of the National Cancer Institute* 82 (1995), pp. 923-929.

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