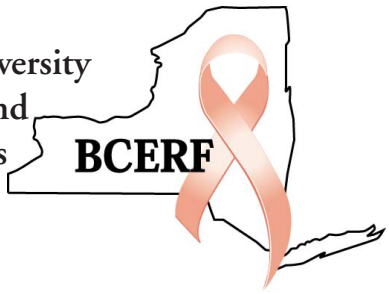
The Ribbon

A Newsletter of the Cornell University Program on Breast Cancer and Environmental Risk Factors in New York State (BCERF)



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Modeling Breast Cancer in the Mouse

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Introduction by Barbour Warren, BCERF Research Associate

Cancer formation in the breast is a complex process. Taken most simply, it involves: a) complicated interactions of different parts of the breast, b) exposure of these parts and the developing tumor to a continually changing range of hormones, and; c) the effects of a number of cancer associated genes. Because of this complexity, studies in animals, using what are called animal models, have played an important part in the current understanding of this disease and in the development of various treatments. In this issue of The Ribbon we are fortunate to have a discussion of how mice are currently being used to help understand the role of various cancer genes in breast cancer. The authors are well qualified to present this discussion. Their laboratory helped develop and is actively working with a mouse model which holds great promise for the study of genes which are lost during breast cancer formation.

Cancer formation requires a number of steps. Many of these steps involve changes in genes associated with cancer. These genes are of several types. First are genes that lead to cancer formation and whose function is increased or changed as a tumor forms. Second are the genes that keep cancer growth from happening. These are called tumor suppressor genes and they are lost or inactivated during cancer formation.

As this article succinctly details, study of cancer genes has involved either the addition or the elimination of these genes from various types of laboratory mice. The resulting animals are then studied to understand the biological function of the genes and their part in the formation of cancer. The cancer gene changes made in these mice are present throughout the developmental and adult lives of the animals. This is not optimal for two reasons. First the gene changes can result in developmental abnormalities in the animals. Second, this is different from what occurs in the formation of most human cancers where the changes are thought to happen after birth, and possibly during specific life periods. Drs. Nikitin and Shmidt describe a system their laboratory helped develop which overcomes these two problems. Using this system they are able to produce mice in which they can induce the loss of a tumor suppressor gene at any point during its life and in specific areas of its body. For example, by inducing the loss of tumor suppressor gene in animals of different ages, studies can examine effects of gene inactivation during the various phases of the development of the breast. Further, the change could be made in only some of a given animal's mammary glands, allowing comparison to breasts without the change but exposed to the same environment of hormones and other factors within the animal. This process will undoubtedly lead to advances in the understanding and treatment of breast cancer.

Breast cancer is the most common malignancy among women, affecting approximately one in nine females in Western countries in their lifetimes. In the United States, breast cancer is the leading cause of death among women between the ages 40 and 55 years. (ACS, 2000).

Modeling human cancer in laboratory animals provides unique opportunities for studying the nature of the disease, as well as for testing new diagnostic tools, drugs, therapeutic approaches, and strategies for prevention and control. In the past decades, a large variety of mouse models for breast cancer has been developed. However, until recently, most of the mouse models poorly reflected human disease and were not defined genetically.

Unlike humans, many naturally occurring breast cancers in mice are caused by tumor viruses, such as Mouse Mammary Tumor retro-Virus (MMTV). (See *The Ribbon,* Volume 5, Number 3, early Fall 2000.) These mammary neoplasias, as well as those induced by exposure to chemical carcinogens and ionizing radiation, were used until the late 1990's (Gould, 1995). Unfortunately, morphology and biological characteristics of these tumors are quite distinct from those in humans. For example, the majority of human breast tumors have a pronounced stromal reaction and frequently metastasize (spread). In contrast, mouse MMTV-induced tumors usually have very little stroma and metastasize rarely.

An alternative approach for the modeling of mammary cancer is based on preparing transplantable tumors (Heppner, 2000). In this approach, tumors arising in humans are serially transplanted into immunodeficient mice. These models allow the study of biological behaviors of human neoplasia, and are frequently used for studying new therapeutic agents. However, these models do not allow study of the organism's immune responses to the tumor. Because such responses are among well-established factors affecting tumor formation, their omission may render transplantable experiments less biologically relevant.

This difficult situation with faithful mouse models for breast cancer has changed dramatically during the past two decades due to two main reasons.

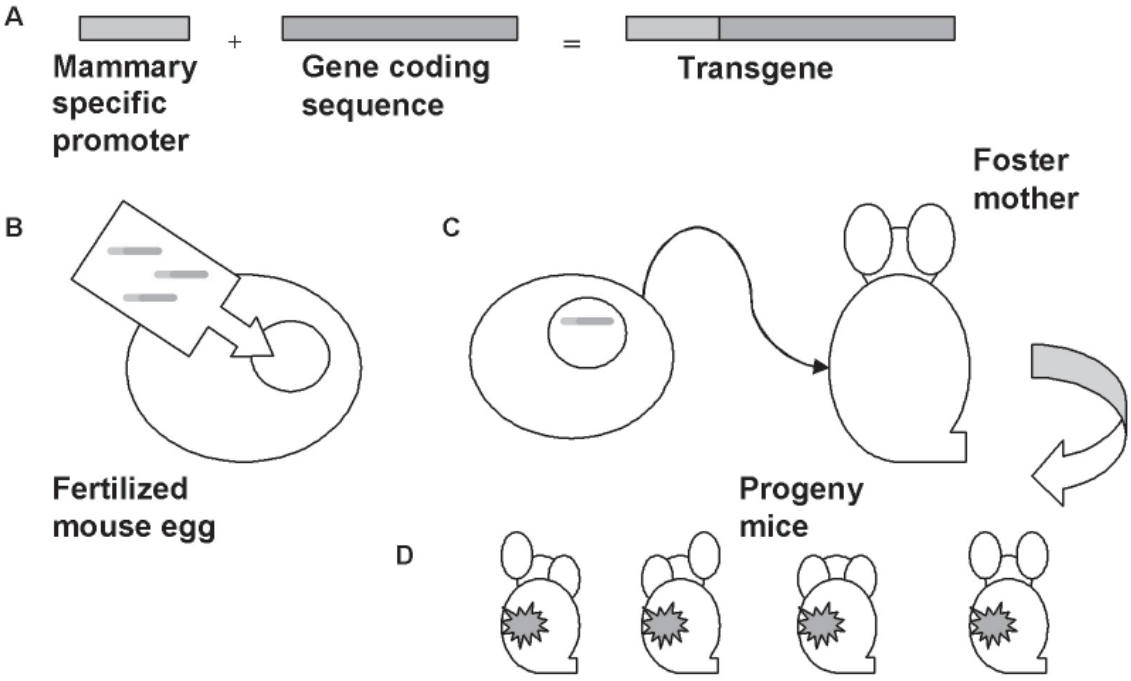
First, remarkable progress has been made in understanding genetic mechanisms of cancer. In particular, it has become clear that genes involved in carcinogenesis can be of two main types: oncogenes and tumor susceptibility genes (Vogelstein, 1993; Weinberg, 1995). Normal products of proto-oncogenes usually play roles in promoting cell proliferation and in the prevention of differentiation and programmed cell death (apoptosis). This group also includes genes that increase genetic instability and favor mutagenesis. "Gain of function" mutations, amplification (increased copy number per cell) or overexpression (increased amount of product) of known oncogenes are associated with cancer. Examples of oncogenes involved in breast cancer include *neu/erbB2/HER2*, *ras* and *myc*. In contrast to oncogenes, tumor susceptibility genes either accomplish negative control of cell proliferation and promote differentiation (tumor suppressors), or positively control genetic stability and are directly involved in repair of genetic material. Usually such genes have "loss of function" mutations in tumors. Among the best known genes of this group are retinoblastoma susceptibility gene (*Rb*), *p53*, and breast cancer susceptibility genes (*BRCA*) 1 and 2.

Second, rapid advances were made in mouse embryology and genetics. It has become possible to construct genetically accurate mouse models of human cancer by insertion, deletion, or mutation of cancer-relevant genes in the mouse genome. Thereby, the role of individual human oncogenes and tumor susceptibility genes in cancer can be tested directly.

Specifically, several approaches have been developed for in vivo study of gene functions. In **transgenic technology** (Jaenisch, 1988), an artificial DNA sequence – transgene – is engineered in a test tube and introduced into a fertilized mouse egg (Fig. 1). Foreign DNA of the transgene then randomly integrates into a chromosome and becomes a part of the mouse genome. Any transgene consists of two main genetic elements: a coding sequence for a gene of interest and a promoter that drives an expression of this gene in cells of a host animal. Some promoters are ubiquitous and direct transgene expression in every cell type of the animal. Others are more specific. For example, in targeting transgene expression to the mammary gland MMTV and WAP promoters are frequently used (Cardiff, 1993). The MMTV promoter is derived from the MMT virus known to infect mouse mammary epithelial cells, and, as discussed above, induce mammary tumors. The WAP promoter is a part of the whey acidic protein gene, which is synthesized in normal mammary epithelial cells during the last half of pregnancy and lactation period. As a result, expression of a transgene under the control of this promoter depends on the physiological status of the animal.

The transgenic technology allowed direct testing of numerous human oncogenes for their transforming

Fig. 1. Transgenic technology. (**A**) Transgene is constructed in the test tube; and (**B**) microinjected into fertilized mouse egg; (**C**) fertilized egg is transferred to foster mother; (**D**) Progeny contains transgene, which is expressed in the mammary gland.



potential in mouse mammary cells. Notably, many of those oncogenes were able to induce mammary tumors in the mouse. Such genes include mammary epithelial cell growth factors (for example, Transforming Growth Factor (TGF) α, TGF-β, heregulin, Fibroblast Growth Factors (FGF), etc.), receptors for these growth factors (TGF-β DNIIR, ErbB2, etc.), second messengers in signal transduction pathways (e.g., ras, PTEN), regulators of cell cycle (e.g. myc and cyclin D) and differentiation (e.g. WNT and NOTCH4) (Hennighausen, 2000, Balmain, 2002).

The transgenic approach is useful for studying oncogenes. However, for inactivation of tumor suppressor genes a different method is required. Such a method relies on **gene targeting technology** (Capecchi, 1994). In this approach, desired genetic modification is first introduced into a cloned copy of the chosen gene by recombinant DNA technology in a test tube. Then, modified DNA is transferred into cultured embryonic stem (ES) cells which are undifferentiated cells capable of multiplying and differentiating into specialized cells. In the ES cells modified DNA replaces the normal gene by homologous recombination (Fig. 2). Homologous recombination is a regular genetic event that occurs in all cell types at low frequency. Recombinant ES cells are identified and injected into mouse blastocysts (early stage of embryonic development), which are in turn surgically transferred to foster mothers. The newborn "chimeric" mice are capable of transmitting the mutant genetic locus to their offspring.

The "knock out" technology has been successfully used for inactivation of many genes. Unfortunately, many tumor susceptibility genes are vitally important and their complete inactivation results in embryonic lethality (*Rb* and *BRCA1*). Yet inactivation of some other genes, such as *p53*, leads to development of other tumors, for instance, lymphomas, prior to mammary tumor formation (Deng, 2001, Gusterson, 1999). Thus, limited conditional inactivation of such genes is required for studying their functions in the mammary epithelial cells.

A number of approaches for **conditional inactivation** of tumor susceptibility genes have been developed. In the Cre-*loxP* system (Rossant, 1999), gene deletion is accomplished by Cre-recombinase enzyme from bacterial virus (phage) P1. This enzyme is able to excise

(delete) any DNA sequence located between flanking recombinase-specific *loxP* sites. Placing Cre under the control of mammary specific promoter (see transgenic technology above) allows for expression of the recombinase followed by gene deletion only in cells of the mammary gland. For such experiments, two types of genetically engineered mouse strains are generated (Fig. 3): one contains "floxed" (flanked by *loxP* sites) gene of interest, and the other Cre-recombinase transgene. After intercrosses between these two strains, both genetic alterations appear in all cells of the progeny. As a result, Cre-recombinase that is selectively expressed in mammary epithelial cells catalyzes excision of "floxed" tumor suppressor gene in tissue-specific manner so that only mammary cells become predisposed for the cancer development. This method was successfully applied for conditional inactivation of the *BRCA1*, *BRCA2* and *p53* tumor suppressor genes in mammary epithelial cells (Xu, 1999; Jonkers, 2001). In agreement with the known role of these genes in human breast cancer, mice with targeted deletion of these genes developed mammary carcinomas.

In addition to limiting Cre expression to the mammary gland, it is often necessary to control time and anatomical area of gene deletion. For example, for understanding of mechanisms of cancer formation, it is necessary to study the effects of gene deletion at different stages of mammary gland development. Therefore, we developed an approach that combines Cre-mediated gene inactivation with tetracycline-controlled gene expression (Utomo, 1999). The tetracycline-controlled system consists of a gene controlled by the tetracycline-inducible operator. The

Fig. 2. Gene targeting ("knockout") technology. (**A**) Gene X is modified (inactivated) in the test tube. Additional genetic element (Neo), which confers antibiotic resistance, is introduced into gene during modification in order to allow for selection of modified ES cell. (**B**) Inactivated gene is introduced into ES cells. ES cells with inactivated gene are selected by adding antibiotic (G418) to cell culture. Since cells carrying inactivated gene also contain element Neo, they remain alive. (**C**) Selected ES cells are injected into mouse blastocyst. "Chimeric" blastocyst consists of normal and genetically modified embryonic cells. (**D**) The "chimeric" blastocyst is transferred to foster mother. (**E**) "Chimeric" progeny contains normal as well as genetically modified cells. (**F**) Intercrosses between "chimeric" progeny result in homogeneous genetically modified offspring.

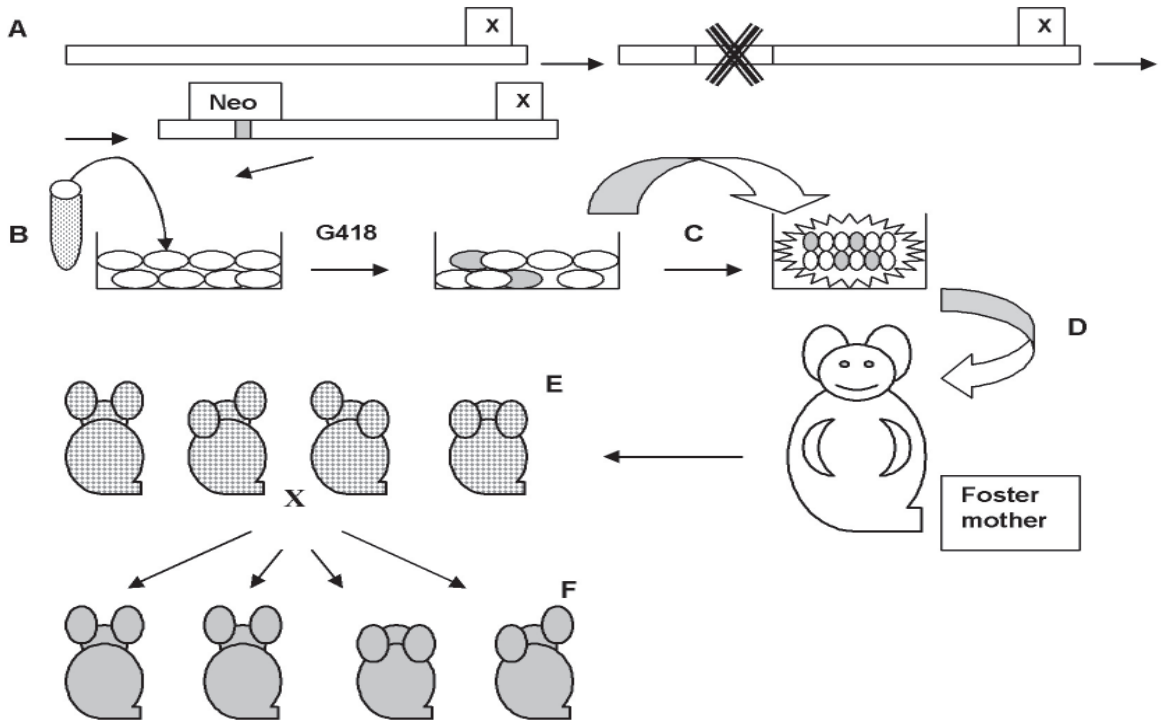
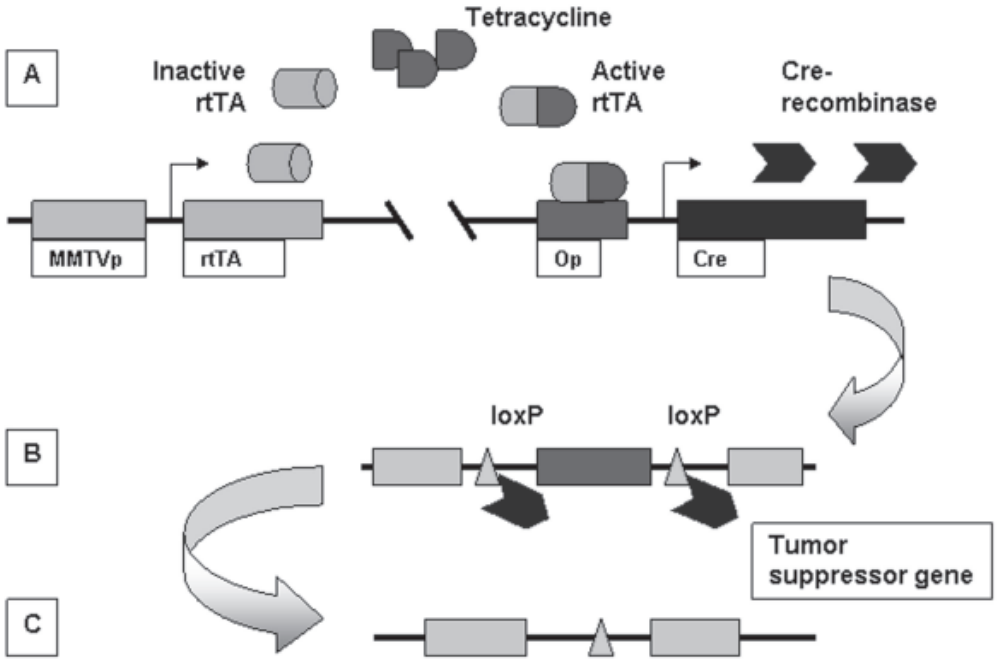


Fig. 3. "Conditional" technology. (**A**). Inactive form of tetracycline-responsive activator of transcription (rtTA) is constantly produced in the mammary epithelial cells of genetically modified mice under the guidance of selective mammary epithelium-specific MMTV promoter (MMTVp). After tetracycline administration rtTA binds antibiotic and becomes active. Activated form of rtTA binds to the operator sequence (Op) and induces Cre-recombinase (Cre) expression in mammary epithelium. (**B**). Cre-recombinase catalyzes excision of "floxed" (flanked by loxP sites) fragment of a tumor suppressor gene. (**C**). Inactivated tumor suppressor gene with only one loxP site left in place of the fragment. Although all genes that participate in this series of events are presented in all cells of genetically engineered mice, deletion takes place specifically in breast epithelium as a result of mammary-specific expression of rtTA.



operator is a special DNA sequence which is activated by the tetracycline-sensitive regulator protein (tetracycline-responsive activator), which is expressed under the control of a cell-specific promoter (Baron, 2000). Administration of antibiotic tetracycline activates tetracycline activator, and subsequent expression of the target gene, for example Cre recombinase, occurs. Because, tetracycline can be applied at any time, our approach allows initiation of Cre-recombinase-mediated gene deletion by administration of tetracycline whenever it is required. Furthermore, direct injection of tetracycline allows for gene inactivation in a limited area of the mouse body. Thus direct comparison of the mammary gland with and without gene deletion is possible in the same mouse.

The majority of mouse genetic models have been prepared relatively recently, and their complete characterization is still in progress. However, even the limited available observations indicate that genetically modified mice represent an extremely promising tool for modeling human breast cancer. For example, it was demonstrated that the activation of oncogenes and inactivation of tumor susceptibility genes identified in humans initiates breast cancer in mice. Importantly, alterations of these genes in the mouse gives rise to distinct tumor types, which are morphologically alike to known human breast cancer variants with similar genetic alterations (Cardiff, 2001, our unpublished observations). Furthermore, unlike spontaneous neoplasms, genetically defined tumors are frequently metastatic, similarly to their human counterparts. Since similar genetic alterations produce comparable phenotype both in the men and in the mouse, it is reasonable to expect parallel biological responses to molecular and pharmacological agents for cancer treatment and prevention.

Perhaps the main future challenge in the mouse modeling is to recapitulate complex natural settings of tumor formation. Breast cancer represents a heterogeneous group consisting of different sets of genetic alterations, histopathological types, and metastatic potential. Therefore, future experiments will be aimed at deciphering the crosstalk between individual cancer genes, and, hopefully, will help in pinpointing the most significant genetic alterations. At the same time, since breast cancer formation is also influenced by a number of non-genetic factors, such as diet and hormonal status (Alberg, 1999), mouse models shall be very useful for evaluation of the complex interplay between genetic and non-genetic alterations.

Taken together, rapid advances in molecular biology and embryology have allowed generation mouse models for breast cancer which in many respects are similar to human disease. Availability of such models will further facilitate our understanding of breast cancer, and will provide an invaluable tool for developing new rationally designed approaches for diagnosis, treatment, and prevention of this debilitating disorder.

Acknowledgements

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Research Commentary

Two Studies Compare Levels of Contaminants in Farmed versus Wild Salmon

Sandra Steingraber, Visiting Assisant Professor, BCERF

Easton, M.D.L., Luszniak, D., Von der Geest, E. Preliminary examination of contaminant loadings in farmed salmon, wild salmon and commercial salmon feed. Chemosphere 46 (2002):1053-1074.

Iacobs, M., Ferrario, J., Bryne, C. Investigation of polychlorinated dibenzo-p-dioxins, dibenzo-p-furans and selected coplnar biphenyls in Scottish farmed Atlantic salmon (Salmo salar). J Chemosphere 47 (2002):183-191.

Reports about chemical contaminants in popular seafoods have made many women wonder if farmed fish might well be a safer dietary choice than wild fish. Two new studies from Canada and Scotland indicate that, at least in salmon, they are not. Indeed, the results of these two investigations show that salmon raised in fish farms have significantly higher levels of dioxins, chlorinated pesticides, and PCBs than their free-swimming counterparts. Both studies, conducted independently and published in recent issues of the environmental science journal Chemosphere*, trace the source of the contamination back to commercial salmon feed.*

Fish: Health food or conduit of contaminants?

Fish is the last form of wildlife many Americans still eat. Those who have never called a deer or a wild boar dinner may well enjoy the occasional trout. Those who have never tasted jugged hare, squirrel stew, or broiled grouse may be on very familiar terms with baked haddock, poached halibut, or tuna salad.

And fish is good food. It is low in saturated fat and high in protein, vitamin E, and selenium. Fish oils prevent blood platelets from sticking together, which lowers risk of stroke. Fish is also a leading source of omega-3 fatty acids, which reduce blood pressure and cholesterol, thereby promoting cardiovascular health. These same nutrients also help build healthy brains in our children. During the last trimester of pregnancy, when the fetal brain undergoes a big growth spurt, omega-3 fatty acids are required for the proliferation of neurons and blood vessels.

But fish has also become some of the most chemically contaminated of all human foods. Methylmercury, PCBs, pesticides, and dioxins are just a few of the toxic trespassers found in fish and seafood. Fish advisories now warn women and children against eating sport-caught fish in all of New England's waterways, for example, so contaminated are every single one of them with mercury.

The International Joint Commission of the United States and Canada, which manages our border's water systems, has issued similar warnings for the entire Great Lakes basin. It also recommends that the Canadian and US governments issue advisories directly to women that would plainly state that eating Great Lakes sport fish may lead to birth defects and other serious health problems for children and women of reproductive age. (So far, this directive has gone unheeded.) Human studies in the Great Lakes area clearly show elevated levels of PCBs in the umbilical cord blood of babies whose mothers consumed even moderate amounts of Great Lakes fish during their pregnancies. And PCB exposures during prenatal life are known to place children at risk for cognitive deficits, like lowered I.Q. and shortened attention spans.

As the thousands of advisories for US lakes, rivers, and streams attest, fish that live in fresh water are those most profoundly affected by toxic contamination. But neither are saltwater fish exempt from problems. Since 1991, the Institute of Medicine, a non-profit group affiliated with the National Academy of Sciences, has warned women even *considering* pregnancy to avoid swordfish because of high levels of mercury contamination. Last year, this recommendation was underscored by the Food and Drug Administration, which extended the warning to shark, swordfish, king mackerel, and tilefish. The

FDA is now considering whether restrictions on tuna fish consumption during pregnancy is also prudent. (Several states have already enacted tuna advisories for children, pregnant women, and nursing mothers.) Even Alaskan wild salmon, considered among the cleanest fish in the world, are known to contain persistent organic pollutants at levels sufficient to raise contaminant levels in the lakes into which they migrate and spawn.

How fish concentrate environmental poisons

Fish—farmed or wild—are vulnerable to toxic contamination because watery environments enhance the ability of persistent organic pollutants to biomagnify.

The ecological principle of biomagnification refers to the fact that persistent organic pollutants, such as PCBs or dioxins, concentrate as they move up the food chain. Whether on land or at sea, organisms at the top invariably end up with the highest levels of contamination. Biomagnification follows from two simple laws of physics: the idea that matter can neither be created nor destroyed, and the contrasting proposition that some amount of useable energy is always lost whenever it is transformed from one type to another.

Taken together, these principles mean that fewer and fewer individuals can occupy each ascending link of the food chain because fewer and fewer calories of energy are available to feed them. The total amount of a persistent pollutant doesn't change, however. Thus, as the rarer members of the higher links dine upon the commoners below them, poisons dispersed among the many are drawn up into the bodies of the few. As a general rule, persistent toxic chemicals concentrate by a factor of 10 to 100 with every link ascended.

In water, toxic substances can concentrate to higher levels because food chains are longer than they are on land. The buoyancy of water allows aquatic organisms to survive on comparatively fewer calories than their gravity-bound counterparts. Because they spend less energy holding themselves up, the transfer of energy between one link of the chain to the next is more efficient. With less energy lost between links, more links can be added. Terrestrial food chains rarely have more than three links. Aquatic ecosystems can easily support food chains with six links, and some are known to have as many as twelve.

As a general rule, whenever persistent pollutants are released into the environment at large, people who eat a lot of fish or other aquatic animals will receive the highest exposures. For example, all other things being equal, nursing mothers who are frequent eaters of fish and seafood have higher levels of organic pollutants in their breast milk than women who are meat eaters. (Mothers who have adhered to vegan diets throughout their adult lives, eating no fish or animal products of any kind, have the lowest levels.)

The Canadian study: weekly eaters of farmed salmon at risk

The first study, "Preliminary Examination of Contaminant Loadings in Farmed Salmon, Wild Salmon and Commercial Salmon Feed," examined toxic contaminants in salmon collected from the Pacific Coast of Canada. Its lead author is Dr. Michael Easton, an eco-toxicologist funded by the David Suzuki Foundation of Vancouver. Easton's objective was to survey individual farmed fish, commercial salmon feed, and some wild salmon to learn whether differences in contaminant levels occur among these groups. He and his colleagues were inspired to undertake this investigation by an unexpected discovery in 1987: bottom-dwelling organisms living near salmon pens in British Columbia were found to have significantly elevated levels of PCBs. Subsequent investigation revealed that the source of the contamination was the salmon feed itself.

Because this study was undertaken as a pilot project, the sample sizes are admittedly small. The researchers analyzed five types of commercial salmon feed, four farmed salmon obtained from retail fish outlets in Vancouver, and four wild salmon purchased from a Vancouver fish company. The researchers tested each for many different contaminants: 112 PCB congeners, 41 polybrominated diphenylethers (PBDEs) (flame retardants), 25 different organochlorine pesticides, 20 types of polycyclic aromatic hydrocarbons (PAHs), as well as methyl and inorganic mercury.

The results were striking. While methyl mercury was not notably different between the wild and farmed fish, the farmed salmon showed consistently higher levels of PBDEs and pesticides (except toxaphene). Most dramatically, levels of PCBs were ten times higher in farmed fish. Elevated levels of contaminants were also found in all types of five salmon feed.

The authors' discussion focuses on the human health implications of eating farmed salmon. None of the salmon collected for analysis in this study had levels of contaminants that exceeded government-approved

safety levels. However, individuals who consume farmed salmon on a regular weekly basis would, according to this analysis, easily exceed the World Health Organization's tolerable daily intakes for persistent organic pollutants.

The Scottish study: the culprit is fish oils in feed

Like the Canadian study, the Scottish study compared contaminant loads in wild and farmed salmon obtained from commercial fish markets. The sample size was nine. In this investigation, however, the fish were analyzed only for dioxins, furans, and seven different PCB congeners. Miriam Jacobs, the leading investigator in this study, "Investigation of Polychlorinated Dibenzo-*p*-dioxins, Dibenzo-*p*-furans and Selected Coplanar Biphenyls in Scottish Farmed Salmon (*Salmon salar*)", a toxicologist at the University of Surrey, worked in conjunction with the US Environmental Protection Agency.

Using methods of analysis similar to those employed by the Canadian group, the researchers obtained similar results. Farmed salmon had significantly higher levels of dioxins, furans, and PCBs. As in the Canadian study, PCB levels were roughly ten-fold higher in the farmed fish. And once again, the authors deduce, regular consumption of farmed salmon could lead to intakes above the tolerable weekly intake for these chemicals, especially for PCBs and especially for children under five.

What makes the Scottish study particularly interesting reading is its discussion of the fish meal manufacturing industry and the salmon aquaculture industry. Farmed salmon are fed a diet far richer in fish oils than their wild cousins enjoy. This lipid-rich diet allows them to grow more quickly and reach market size sooner. The typical oil fed to salmon is herring oil, which can come from many different parts of the world, depending on price and availability. Because herring is a naturally oily fish, it is known to be comparatively high—both on a whole weight and lipid-adjusted basis—in dioxins and PCBs, which are oil-soluble substances. The authors favor an aquaculture diet based more heavily on vegetable oils, which, they contend, have fatty acid compositions more closely resembling the invertebrates that comprise the natural diet of salmon in the first place.

Final thoughts

It is easy to convince ourselves that we can somehow opt out of food chain contamination by selecting dietary items, like farmed fish, that are produced under controlled conditions. But all fish, including those confined to watery pens, have to eat. And whenever animals are raised for human consumption, the economic incentive to speed growth by offering a high-fat, high-energy diet means that they are vulnerable to contamination by fat-soluble pollutants. At the same time, economic globalization means that animal feeds can derive from ingredients gathered from all over the world. Thus, a farmed salmon bought in a Canadian fish market may well contain PCBs from herring caught in the Baltic Sea. As noted by the authors of the Scottish study, the farmed fish on your dinner plate may actually be part of many more marine food chains than a wild fish.

The accumulation of environmental contaminants by fish has been a concern for many years and these examinations strongly suggest that food formulation in aquaculture needs to be monitored to utilize uncontaminated oil sources. Hopefully these reports will spur such change.

Although the problem of toxin accumulation in fish has long existed, no association between the amount of fish in the diet and breast cancer risk has been reported by almost all the epidemiological studies that have examined this issue. Thirteen studies have examined fish consumption and breast cancer risk and ten of them reported no association. Of the studies that did see an association between eating fish and breast cancer risk, two reported an increase in risk and one reported a decrease in risk.

There is also evidence that fish oils slow the development of mammary cancer in animals but the research in humans is less clear. This is an active area of research.

Barbour Warren, BCERF Research Associate

"We Need to Know"

Ad Hoc Discussion Group

"Learning Together"

The Ad Hoc Discussion Group meeting took place on June 28, 2002 with over 50 people attending. We were happy to be hosted by Hillary Rutter and the Adelphi New York Statewide Breast Cancer Hotline and Support Program, and welcomed by Senator Carl Marcellino. We met in the Alumni House at Adelphi University, in Garden City, Long Island. The agenda for this program was based on a needs assessment conducted by Cornell student Bonnie Berger, who touched base with many Long Island stakeholders to find out what they were interested in having BCERF address. Based on this valuable feedback from approximately 30 individuals representing diverse groups, we organized the following program:

- Local Updates, with six short presentations
- Breast Imaging and the Mammography Debate, Dr. Corrine Tobin, Nassau Radiologic Group
- Long Island Breast Cancer Study Update, Dr. Steve Stellman, Columbia University
- Why Are There Concerns About Chemicals and Breast Cancer Risk? Dr. Suzanne Snedeker, BCERF
- Panel Discussion, with Drs. Stellman and Snedeker, plus Dr. Barbour Warren and Dr. Sandra Steingraber, both of BCERF.

Some local updates and special guest. Although there is never enough time to hear about all the valuable projects and the concerns of participants, we do try to organize at least six updates by participants at the beginning of the program. At this meeting we heard from Barbara Balaban of the National Breast Cancer Coalition (NBCC), Neal Lewis of the Neighborhood Network, Laura Weinberg of the Great Neck Breast Cancer Coalition, Christine Mancuso of the Nassau County Breast Health Partnership, and Gail Frankel, the Long Island field coordinator for NBCC. In addition, were very fortunate to be joined by Danny Meneses of the Phillipine Breast Cancer Network who is carrying on the critical work of his late wife Rosa in the Phillipines.

Breast imaging and the mammography debate. BCERF frequently provides a forum for information exchange and discussion on topics which may not be those we ourselves directly work on. Breast imaging and the mammography debate is an example. This topic was widely requested in the needs assessment conducted prior to the meeting. We called upon a Long Island practitioner, Dr. Corrine Tobin, Director of Mammography at the Nassau Radiologic Group in Garden City to make a presentation to the group. Dr. Tobin reviewed all the technologies currently in use including diagnostic mammography, breast ultrasound, digital mammography, computer-aided diagnosis and breast MRI. She then reviewed the "mammography debate," i.e. the discussion generated after recent scientific papers questioned whether mammography does indeed reduce breast cancer mortality. Dr. Tobin's assessment is that the randomized trials may underestimate screening benefits due to shortcomings in the study, as well as improvements that have been made to the protocol since the time of the studies.

Long Island Breast Cancer Study Update. As most readers are aware, in the past weeks, many more results of the Long Island Breast Cancer Study have been made available. We were very fortunate in our June meeting to be joined by Dr. Steven Stellman, Professor of Epidemiology at Columbia University, to share background, results, and current dilemmas in environmental exposure/breast cancer risk research. For example, Dr. Stellman discussed some of the possible explanations for the negative findings in some of the previous organochlorine and breast cancer risk studies. He mentioned the possibility of too few cases and controls, the narrow range of exposures examined, not enough attention paid to multiple exposures, and inadequate or incomplete exposure assessment. Clearly there is also the possibility that these exposures are not related to breast cancer risk; since all women have measurable levels of organochlorines in their fat and blood, this is a very challenging area of research. Gammon et al. write in their very recently published results ("Environmental Toxins and Breast Cancer on Long Island. II. Organochlorine Compound Levels in Blood," *Cancer Epidemiology Biomarkers and Prevention.* Vol. 11, 686-697, August 2002 and also available on line at http://cebp.aacrjournals.org/cgi/content/full/11/8/686), "in conclusion, in this large population-based case-control study among women on Long Island, breast cancer risk was not increased in relation to serum organochlorine levels." They go on to point out, "these data do not rule out the possibility, however, that breast cancer risk is elevated by high organochlorine exposures several decades earlier that, through variations in individual metabolism, now measure as low body-burden levels," as well as some other directions for needed future research.

Why Are There Concerns About Chemicals and Breast Cancer Risk? BCERF staff are very pleased to now have fact sheet #45, "Environmental Chemicals and Breast Cancer Risk: Why is there Concern?" now available. In it, BCERF Associate Director for Translational Research Dr. Suzanne Snedeker outlines existing, emerging, and needed data on this question. Suzanne was able to present this information at this Ad Hoc meeting, in a session very complementary to Steven Stellman's. Because the BCERF translational research project brings into consideration all types of research — epidemiologic (human), animal and other lab studies — Suzanne is able to provide an overview of all the scientific knowledge available on risk factors that her project has examined. But, as she points out in the fact sheet, "while human studies are given the greatest weight when deciding whether or not a chemical causes cancer, there is little or no information on the cancer-causing potential of most chemicals in people." She mentions that of the 509 chemicals tested by the National Toxicology Program for their cancer-causing potential, 42 were found to cause breast tumors in laboratory animals. In addition, human studies in these areas are needed, as are studies of critical windows of exposure, and studies to further understanding of endocrine-disrupting chemicals and gene-environment interactions.

Panel Discussion with Drs. Stellman and Snedeker, plus Dr. Barbour Warren and Dr. Sandra Steingraber, both of BCERF. Many interesting questions were posed to the group, including questions for Sandra on breastfeeding with regard to the chemical contamination of breast milk. A question was posed to Dr. Stellman — who also works on issues related to the chemical Agent Orange — about the wide-scale testing of Agent Orange in the Phillipines. The meeting ran behind-schedule, but many of the participants were eager to stay extra to provide this panel more time. Clearly this kind of interactive time with scientists is valuable to Ad Hoc meeting participants.

Evaluation and next meeting. We were able to prepare brief mail-back evaluation cards for this meeting. These evaluations inquired as to whether participants thought adequate time was allowed for discussion, whether they would have preferred more or less time for local updates, and which of the various presentations were of value. At the time of this writing we received nineteen responses, all of which were overwhelmingly positive on most questions. We hope to receive more, and then draw conclusions. We are currently working on plans for an October meeting in Rochester.

The Breast Cancer Coalition of Rochester (BCCR) is seeking proposals for breast cancer research. One grant will be awarded in December 2002 in the amount of $25,000. The competition is open to researchers based in the Greater Finger Lakes Region of Upstate New York. BCCR priorities include exposures, causation, prevention and risk assessment. Applications due October 18, 2002. See http://www.bccr.org/applications/BCCR%20RFP%208-8-02-FINAL.pdf or call BCCR at (585) 473-8177

Looking for news on hormone replacement therapy?

We have just revised two of our fact sheets:

- Fact Sheet #10 on 'Estrogen and Breast Cancer Risk: Exposure to Estrogen'
- Fact Sheet #40 on 'Hormones and Breast Cancer Risk'

Please find these updated fact sheets on our web site at: http://www.cfe.cornell.edu/bcerf/ or call the BCERF office if you have no web access and need a printout. (BCERF will no longer be printing fact sheets. Limited supplies of some previous fact sheets will continue to be available.)

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