

# Environmental Estrogens

*Found internally, certain compounds are important biological signals; found in the environment, they can become just so much noise*

John A. McLachlan and Steven F. Arnold

In many ways, the story of the pesticide DDT is the story of America's attitude toward synthetic chemicals in the environment. DDT was the first of many new pesticides that people hoped would improve the quality of their lives, but gradually it became clear that such progress often had a cost. DDT was one of the first environmental chemical agents to be banned in the United States. Scientists are still seeking a full understanding of how it came to have broad and unexpected environmental and health effects.

First synthesized in 1874, DDT took on its modern role in the late 1930s, when the Swiss chemist Paul Muller recognized its potential as an insecticide. It was perceived to be so beneficial for public health and military hygiene (mostly as a delousing agent), in fact, that Muller was awarded the 1948 Nobel Prize for Medicine and Physiology. In spite of occasional reports that stirred concern about potential health effects, it was used copiously in the

United States and around the world as an agricultural pesticide and a malaria-control agent (a function for which it continues to be used today in some developing countries).

As America began to closely scrutinize technology in general—and ecological agents in particular—in the late 1960s, people took a closer look at DDT. Early in that decade scientists had noticed a decrease in certain bird populations in Europe. Eventually this decrease was linked to the use of various pesticides, among them DDT. By the early 1970s the use of DDT had been banned in the U.S. and in many European countries.

Recently, the DDT story has taken on a new dimension, alerting scientists to a novel class of potential interactions between environmental agents and living creatures. Since the early 1980s, reports have been surfacing of "feminized" wildlife that have been exposed to certain chemicals in the environment. One recent report by Louis Guillette and his group at the University of Florida received a great deal of publicity when it linked DDT exposure with a growing population in a Florida lake of male alligators whose penises were smaller than those of normal males. DDT, as it turns out, can act in the body like endogenous estrogen. Or, as in the case of the alligators, its breakdown products may be estrogens or even a compound that blocks the effects of androgens, the male sex hormones.

This case and others suggest to biologists that synthetic compounds in the environment can mimic in animals the actions of natural signaling molecules, such as hormones and growth factors. But what has been particularly surprising about this discovery is that the synthetic compounds in no way resemble

the chemical structure of the natural hormones or growth factors. The classification of environmental chemicals that mimic endogenous signaling molecules opens up a whole new field of toxicology—environmental signaling. It now appears that the toxicity of some environmental pollutants may be the result of a "natural" signal being sent by an "unnatural" signaling molecule.

To be sure, environmental pollutants have been studied intensely for the past 30 or so years. But the focus of most of those studies has been on the potential of toxins to cause genetic abnormalities by damaging DNA directly. Environmental hormones, on the other hand, do not alter genes themselves, but may change the way they are expressed.

Recent reports, not only of feminized wildlife, but also of the possibility of a precipitous fall in sperm counts of people and of the rise in hormone-related cancers, such as breast cancer, have brought popular attention to environmental hormones—estrogen, in particular. But the so-called ecoestrogens may be only the most obvious of the chemical mimics in the environment. Observations of the effects of environmental estrogens are paving the way for what will undoubtedly turn out to be a larger phenomenon of environmental signaling. We believe that environmental estrogens are the paradigm for a new understanding of the health effects of external signals in the environment.

## Accidental Estrogens

The observation that DDT could behave like estrogen provided a framework for understanding that chemicals not specifically designed to possess hormonal activity may in fact have it. For example, a 1975 spill of Kepone, a

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chemical used in the manufacture of the pesticide Mirex, resulted in a lowered sperm count in men exposed to the chemical. Since natural estrogens given to men decrease their sperm count, some investigators guessed that Kepone might also be estrogenic. Subsequent studies designed to test chemicals for estrogen-like activities confirmed that indeed Kepone was a weak estrogen, but an estrogen nevertheless. The chemical structure of Kepone is even farther removed from estradiol—the main form of estrogen found in people—than is DDT.

The structural differences between natural estrogens and estrogens in the environment were sufficiently puzzling to scientists that they convened a national meeting in 1979 to discuss the subject. Investigators working on many aspects of estrogen biology and chemistry met to solve the "estrogen problem." At the time, the information avail-

able led investigators to expect the number of possible estrogenic chemicals to be fairly limited. At the meeting, the potential for adverse effects on human health was considered, but at the time, there were few examples. Nevertheless, elegant studies on Kepone in Richard Palmiter's laboratory at the University of Washington at Seattle suggested that the effects of environmental hormones might be more widespread than initially thought. His laboratory demonstrated that a chemical such as Kepone, which has no structural resemblance to an estrogen, could activate some of the same very specific estrogen-associated genes that the natural hormone activates in the oviduct of chickens. Although Kepone was much weaker as an estrogen, the functional similarity of two widely disparate chemicals was striking.

Whereas the discovery of environmental estrogens was news to scien-

tists in the middle and late 1970s, the world did have some experience with synthetic estrogens. This came in the form of diethylstilbestrol, or DES. DES was produced in 1938 in London by Sir Charles Dodds and was the first synthetic agent specifically designed to have estrogenic activity. Like many of the environmental estrogens, DES is not structurally similar to natural estrogens. This landmark study in pharmacology provided an early demonstration that compounds of diverse structures could exhibit similar biological functions.

DES also taught scientists other lessons about the possible toxic effects of estrogens. Because of its growth-promoting effects, DES was used for decades as a growth stimulant in cattle. It also had obvious clinical applications. As early as 1948, it was used to prevent miscarriages in women. In 1971 the drug became associated with a rare

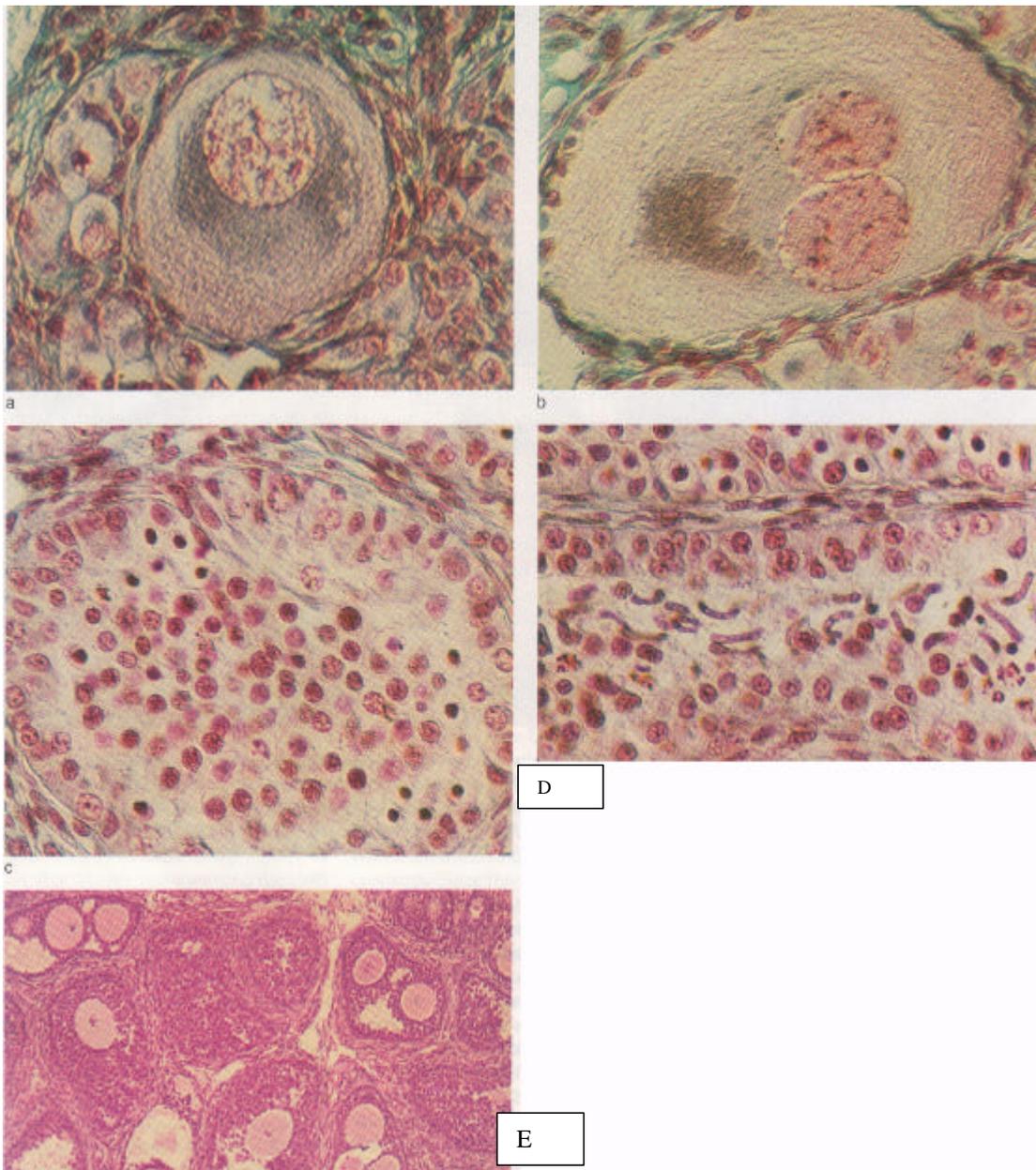


Figure 2. Environmental hormones can cause serious defects in the genitals of animals exposed to them. Ovaries from normal female alligators contain only one egg in each ovary. As shown in a histological slide made from a female alligator that lived in a relatively unpolluted lake, each normal egg cell has only one nucleus (a). In contrast, the ovaries of a juvenile female from Lake Apopka, which contains high concentrations of DDT and other pesticides, each holds more than one egg, and some of these contain more than one nucleus (b). Male alligators can also be affected by environmental hormones. The normal appearance of germ cells in the testes of a juvenile male from a clean lake are shown in (c). In juvenile males from Lake Apopka, dark bar-shaped structures of unknown origin can be seen in the tubules that carry the semen through the testes (d). Ovaries of female mice exposed to the synthetic estrogen diethylstilbestrol, or DES, contain too many eggs, just like the ovaries of female alligators (e). (Photographs a-d taken by J. Matter, e by L. Guillette. All photographs courtesy of Louis J. Guillette, Jr., University of Florida at Gainesville. Magnifications: a = 130x; b = 134x; c = 134x; d = 140x; e = 30x.)

form of vaginal cancer called clear-cell adenocarcinoma detected in some of the adolescent daughters of women who had taken DES. In addition, the drug brought about cellular changes in the vagina or Fallopian tubes of female offspring, as well as structural changes in

the uterus. Studies on this important clinical problem resulted in animal models demonstrating the effects of estrogens on the sexual development of both male and female mammals. DES was the first documented example of a human "transplacental" carcinogen—that is, a chemical, which when given to the mother, causes cancer in her daughter. The clinical and experimental studies surrounding the DES findings in the 1970s and 1980s, gave scientists a new appreciation for the effects of potent synthetic estrogens on the develop-

ment of the reproductive system and on subsequent adult health.

Estrogens also have effects on male genital development. As adults, male mice exposed *in utero* to DES had a higher-than-average frequency of undescended testicles, testicular cancer, sperm abnormalities and prostate disease. Some of these outcomes were also reported for men exposed *in utero* to DES. Even though these men had more genital-tract abnormalities as adults, the most recent studies suggest there is no loss of fertility. The doses of DES required to cause malformations of the male reproductive tract were almost the same in mice and men.

The extensive research on the effects of DES in mice and people served as a model for predicting the possible outcomes associated with estrogens of any source in many species and formed the basis for identifying chemicals in the environment that elicit changes similar to DES. The effects of such a potent estrogen actually set the standard for judging the activities, as well as outcomes, of other chemicals acting like estrogens. The fact that DES feminized the development of laboratory animals provided insight into what ecoestrogens might do to the development of many other species; almost a kind of guidebook to outcomes. It also suggested an upper limit on effects, since DES is much more potent than any single ecoestrogen.

### One Lock, Many Keys

Studies on DES, natural estrogens and early pesticides such as DDT have taught scientists that natural estrogens play an important role in the normal growth or function of many organs, including breast, bone, liver, the organs of the reproductive system and the cardiovascular system. Thanks to modern biochemistry and molecular biology, scientists can now also work out the details of how these compounds affect the cells of target organs.

Estrogens, indeed all hormones, are chemical signals, and as such are important links in the body's internal communication system, helping cells in various organs to sense and respond to changing physiological circumstances.

Endogenous estrogens are steroid hormones, produced from cholesterol in the ovaries of females and the testes of males (and possibly, the adrenal cortex in both sexes) in response to signals from the brain and other organs.

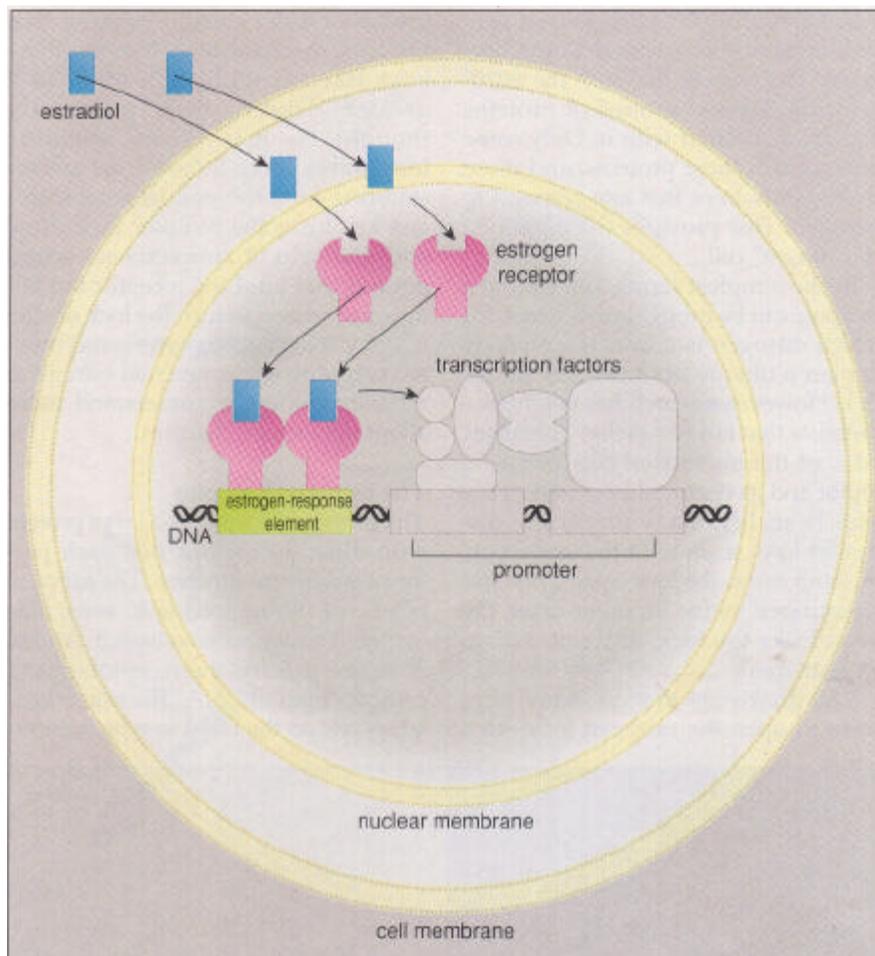


Figure 3. Natural estrogens, such as estradiol, bring about their cellular effects by altering the expression of particular genes in target cells. Estradiol is soluble in the lipids that make up the membranes surrounding cells and their nuclei, so the hormone can pass unassisted into the nucleus. Once inside, estradiol binds with an estrogen receptor, a protein molecule dissolved in the aqueous nuclear medium. Estradiol fits into a specific site on the receptor, much as a key fits a lock. Although estradiol can probably enter many cell types, only those with estrogen receptors can respond. Two occupied receptors join together to form a dimer, which then attaches to a regulatory site called the estrogen response element, or ERE, on a gene. The ERE is located within the gene's "on" switch, its promoter. The occupied and dimerized receptor molecules interact with proteins associated with transcription factors that are attached to the promoter and thus regulate gene expression. Either gene expression is turned on, or its level is modulated. In some cases, gene expression can be suppressed when estrogens bind their receptor.

Estrogens are secreted into the blood, where they are carried to the cells of target organs, such as the breasts and reproductive organs.

In the case of most chemical signal molecules, elaborate systems are required to admit the signal molecules into target cells. This is not necessary for endogenous estrogen, which is soluble in fats, such as the lipids that make up the membranes surrounding cells. Estrogen can therefore pass unaided through the cellular membrane. Once inside the cell, estrogens can also easily cross the membrane into the nucleus, the compartment that contains the cell's DNA. Inside the nucleus, estrogen binds to a protein, called an estrogen

receptor, which is dissolved in the aqueous nuclear medium. The estrogen-receptor complex can then bind to the regulatory regions of specific genes and, by this, alter the way they are expressed. The complex can either activate or repress gene expression completely, or it can alter the level at which a gene is expressed, the overall result being a change in cell programming. Among the genes regulated by the binding of the estrogen-receptor complex is the gene encoding the receptor for another hormone, progesterone, as well as genes encoding several growth factors and their receptors.

Because estrogens can easily enter many cells, and probably their nuclei,

it is at first difficult to understand why only certain cells respond to the hormone. The answer lies with the estrogen receptor and groups of proteins that are associated with it. Only some cells contain these proteins, and those are the only ones that can respond to estrogen. This provides the definition of a "target" cell.

In the simplest terms, the estrogen receptor can be thought of as a lock for which estrogen is a key. The analogy implies a unique fit of one key to one lock. However, research has taught biochemists that is a somewhat unrealistic view of the interaction between a receptor and its chemical key, called a ligand. In reality, many physically dissimilar keys seem to fit the same lock. In some cases, the lock opens; one has an estrogen mimic. In other cases, the key blocks the lock, and one has an antiestrogen.

The discovery that so many keys seem to open the estrogen lock—to a

greater or lesser extent—suggests that the lock mechanism is looser, or the keys interact with each other to a greater degree, than previously thought. For many years, scientific locksmiths have focused on understanding how the endogenous estrogen key turns the receptor lock. Now with the help of antiestrogens—compounds that bind the receptor but fail for some reason to turn the lock or elicit a physiological response—and more recently with environmental estrogens, they are coming to understand more about the lock mechanism.

### The Estrogen Receptor

The estrogen receptor is a large protein with different regions that each performs a different function. The function of one of the regions is to recognize and bind endogenous estrogen. Another segment helps the receptor-ligand complex bind to DNA. The exact regulatory site on the DNA to which the es-

trogen receptor-ligand complex binds is called an estrogen-response element, or ERE. Once the complex binds to DNA, particular sites on the estrogen receptor allow the complex to interact with proteins attached to an adjacent regulatory site on the gene, called the promoter. The estrogen receptor is a transcription factor and interacts with the promoter-bound proteins to somehow bring about a change in the gene's expression—either to activate or suppress gene expression, or to change the level at which the gene is expressed. It is believed that the longer the receptor-ligand complex remains attached to the ERE, the longer the complex modulates gene activity. Once the complex is removed from the ERE, gene regulation also ceases. Although the steps leading to the initiation of an estrogen response have been extensively studied, little is currently known about how the response is turned off.

It is likely that some environmental estrogens bring about their estrogen response by replacing endogenous estrogens in the signaling pathway. For some, this means direct interaction with the estrogen receptor. Most of the ecoestrogens tested, however, bind the receptor with only a fraction of the strength—anywhere from one-fiftieth to one-ten-thousandth—of the natural hormones. But, as it turns out, receptor binding is only one factor of many that predict how well an ecoestrogen mimics the physiological response of the natural hormone.

Although ecoestrogens may be more weakly binding than natural estrogens, they may be more effective at gaining access to the receptor, or they may block the natural hormone's access. For example, some estrogens may be able to bind to proteins other than the estrogen receptor. Some of these proteins, such as serum albumin, sex-hormone-binding globulin and alpha fetoprotein, are dissolved in the blood serum, the extracellular fluid bathing the cell. As a result, the actual concentration of an estrogen-like chemical found inside the cell is a function, in part, of the affinity the chemical has for proteins outside the cell. If the chemical binds very strongly to extracellular proteins, fewer molecules move inside the cell. If, on the other hand, the chemical in question has a greater binding affinity for the estrogen receptor, more of it is found inside the cell than out. Because different estrogenic chemicals differ sig-

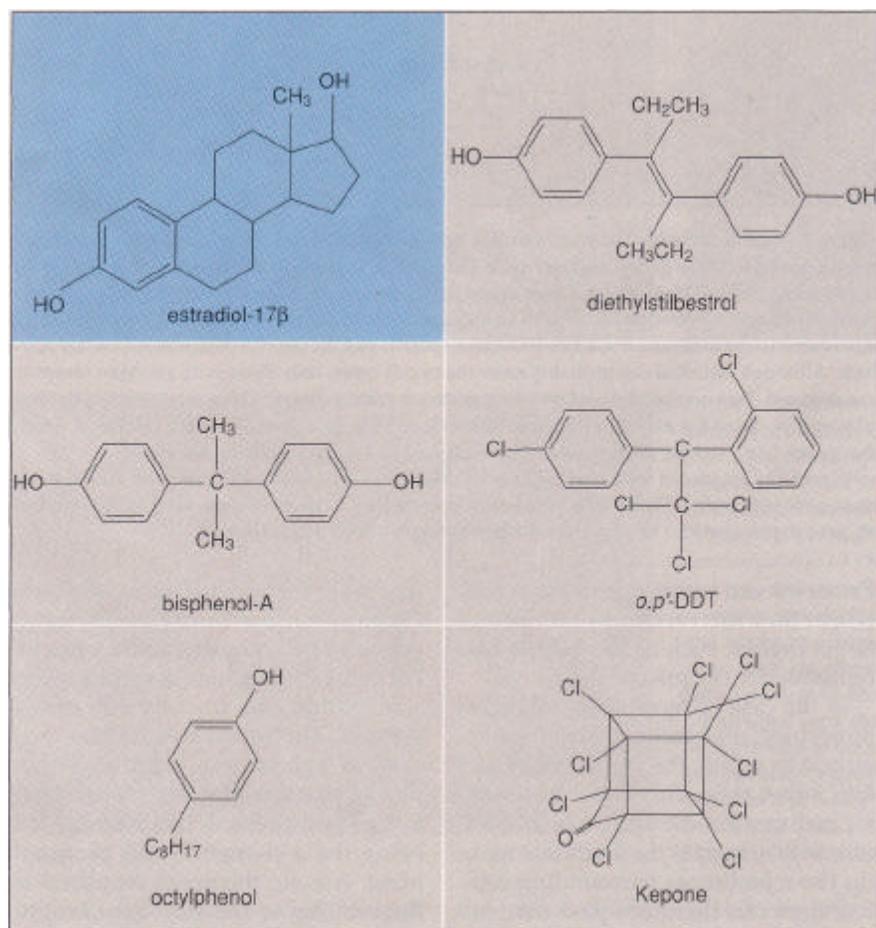


Figure 4. Many ecoestrogens also bind the estrogen receptor. Yet a comparison of the structures of estradiol and those of the ecoestrogens shows how physically dissimilar they are. For this reason, it is difficult to understand how so many differently shaped keys can fit the same lock. Standard receptor-binding assays therefore may not always give accurate information about the potential strength of an environmental estrogen.

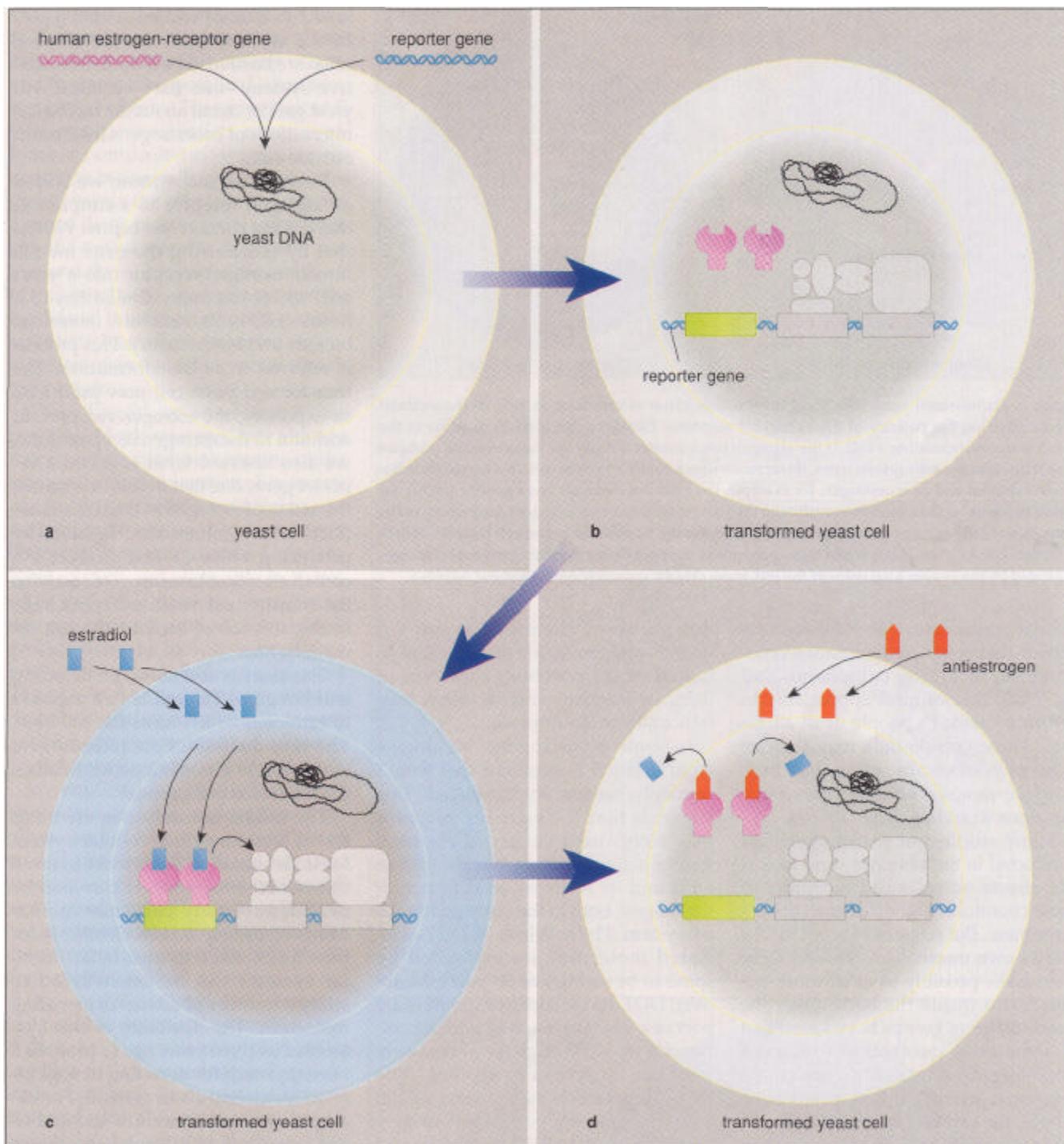


Figure 5. Yeast-estrogen response system used by the authors gives scientists the opportunity to study the whole-cell response to natural and synthetic estrogens in a real-world context. Yeast cells do not normally manufacture estrogen receptors, so the system is constructed by transferring the gene for the human estrogen receptor into a yeast cell (a), which can then manufacture the receptor proteins (b). In addition, a reporter gene is transferred into the yeast cell. The reporter gene contains an estrogen-response element, so it senses the presence of an estrogen bound receptor and reports it in a color-coded fashion. The yeast cell containing the transferred genes, a transformed yeast cell, turns blue to indicate an estrogenic response in the presence of natural (c) or synthetic estrogens. Some environmental compounds act as antiestrogens, substances that bind to the estrogen receptor but fail to activate an estrogenic response. An antiestrogen can in some cases displace estrogens bound to the receptor and curtail the estrogenic response (d), turning the cell from blue to white.

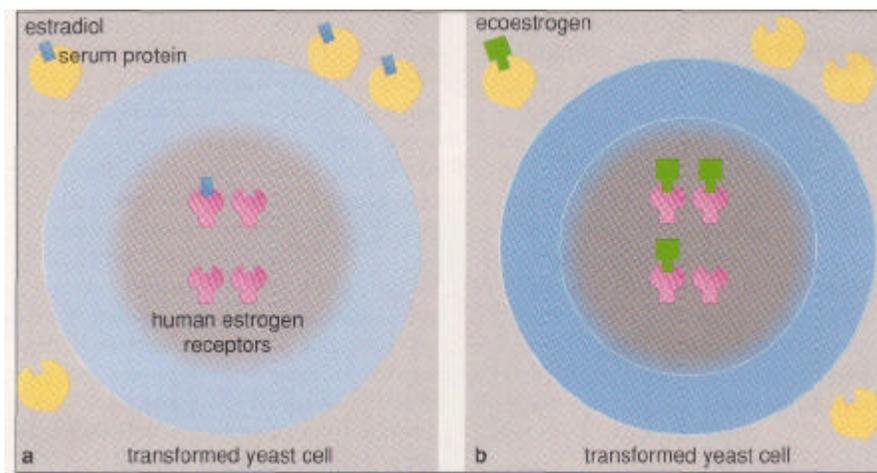
nificantly in their binding affinities for extracellular proteins, their intracellular concentrations vary accordingly.

The natural hormone, estradiol, exhibits extensive binding to extracellular

proteins, whereas the synthetic hormone, DES, has little affinity for them. Thus at an equivalent concentration in the blood, more DES enters the cell than does estradiol. In effect, DES is a func-

tionally more efficient estrogen than is the natural hormone.

In simple terms, one may ask whether ecoestrogens are more DES-like or more estradiol-like in their binding to extra-



**Figure 6.** Transformed yeast cells yield information about extracellular as well as intracellular events affecting the potency of the estrogenic response. Estrogens can bind to proteins in the blood serum surrounding a cell. If the estrogen has a greater affinity for these external proteins than it does for the estrogen receptor, fewer molecules actually enter the cell. In a comparison between estradiol and an ecoestrogen, for example, the estradiol molecule has a greater affinity for serum proteins (a) than does an ecoestrogen (b). Even though the two estrogens are present in the same concentration, more ecoestrogen molecules enter the transformed yeast cell than do estradiol molecules. As a result, the ecoestrogen produces a stronger than expected estrogenic response, indicated by the deeper blue color of the cell, more closely approaching the natural estrogen.

cellular constituents. We have recently shown that some representative ecoestrogens, including octophenol and o,p'-DDT, do not bind appreciably to serum proteins in people or in alligators. These compounds may thus be more physiologically active than their estrogen-receptor binding characteristics alone would predict.

Many studies on ecoestrogens are conducted in the laboratory and look at the effects on cells and receptors of these chemicals as they exist in the environment. But scientists know that the body's own metabolism can alter these chemicals, possibly to even more potent forms inside the body. Over the years, different groups have found that in some cases metabolism converts a nonestrogenic substance to one that has hormonal activity. This is true, for example, for certain polycyclic aromatic hydrocarbons, which become estrogenic after a hydroxyl group is added to them metabolically. Hydroxyl groups also enhance the estrogenic activity of polychlorinated biphenyls, popularly known as PCBs, which are common environmental contaminants.

In general, hydroxylation seems to enhance the affinity a chemical has for the estrogen receptor. Recently it was shown by investigators in Sweden that the hydroxylated form of PCBs is retained more than the unhydroxylated form within the serum of seals and people. The relative estrogenicity of

this group of chemicals is not yet known, and Steve Safe at Texas A & M has raised the possibility that some of them are inactive, whereas others may be actually antiestrogenic.

In some ecoestrogens, a chlorine atom is found in positions that would normally become hydroxylated. This suggests that the estrogen receptor may recognize a variety of chemical species. Chlorinated chemicals, such as DDT and its relatives, tend to persist the longest, both in the body and in the ecosystem. The half-lives of DDT-associated molecules are estimated by some to be as high as 50 years. Moreover, DDT and related compounds are part of a global system in which compounds move through the atmosphere from one ecosystem to another. Thus these compounds may persist within an individual or a population, or they may persist globally. The widespread and persistent nature of some hormonally active compounds demands improved methods for their detection, removal (where possible) or prevention (where necessary).

#### **Cellular Litmus Test**

The test routinely used to determine the presence and relative strength of ecoestrogens has been to assay a compound's binding affinity for the estrogen receptor. As we have pointed out, this approach excludes many mitigating factors that ultimately determine

how a substance will act within a cell. In our laboratory we are developing what we believe to be a more informative system—one that we hope will yield greater detail about the molecular interactions of ecoestrogens inside and outside cells.

To construct our system, we added an estrogen receptor to a simple cell that did not contain one before. We did that by transferring the gene for the human estrogen receptor into a yeast cell, which has many similarities to a human cell in its structure, molecular biology and biochemistry. This process is referred to as transformation. The transformed yeast cell now produces, or expresses, the estrogen receptor. In addition to the estrogen receptor gene, we also inserted what is called a reporter gene, one that in this case senses the action of estrogen in the cell and reports it in a color-coded fashion, by producing a blue-colored product. If a certain chemical has estrogenic activity, the transformed yeast cell turns blue; in the absence of activity, the cell remains white.

This assay is also useful for detecting antiestrogens. The cell is first exposed to estradiol, which turns the cell blue. The introduction of an antiestrogen, which blocks estrogenic activity, causes the blue color to disappear.

The model not only demonstrates the utility of a simple cellular system for understanding the activity of environmental estrogens, it can also be modified to study particular mechanisms or to help screen chemicals for their functional activities. In fact, similar systems can be constructed to study a number of classes of signaling molecules. The flexibility of this system led us three years ago to propose a new approach for detecting biologically active chemicals in general. For example, one can introduce the androgen receptor into mammalian or even yeast cells and then use them to assess the androgenicity of a substance. Very recently this exact approach was taken by Bill Kelce at the U.S. Environmental Protection Agency and Betty Wilson at the University of North Carolina, with the result that an antiandrogenic chemical was identified, providing the first example of a steroid-hormone-like activity outside of the estrogen-antiestrogen family.

In the future, the yeast-estrogen system can be used to identify chemicals in the environment having activities

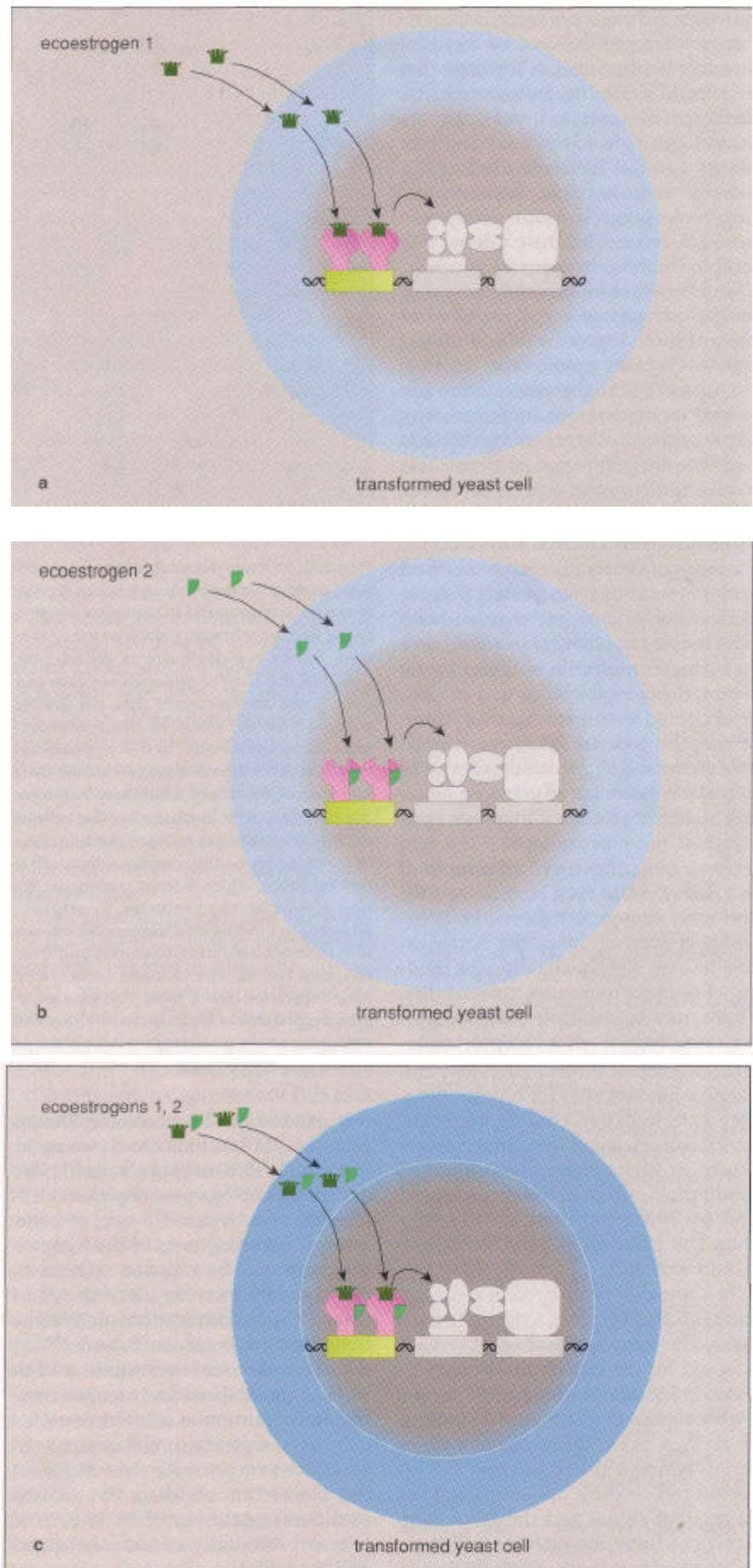
that mimic other hormones and biomolecules, such as progestins, glucocorticoids or retinoids, among others, and to assess their potential adverse or beneficial effects. This experimental system allows scientists to approximate a comprehensive cellular response to biologically active molecules and to assess the effects of the expression of drug-metabolizing enzymes, specific serum proteins or growth factors and their receptors. One can think of this as a "Lego" system approach to model-cell building because it permits scientists to make a stepwise reconstruction of an overall hormone-signaling system.

The "Lego," or interconnecting building block strategy, has already helped us discover new aspects of the estrogen-signaling system. For example, it was generally assumed that one molecule of endogenous estrogen binds one receptor molecule. Using the yeast system, we were able to determine that, in some cases, introducing two ecoestrogens produced a response greater than the simple sum of that produced by each molecule. In other words, certain combinations of these chemicals work synergistically to produce a result greater than would be expected from the sum of their inputs.

We studied four weakly estrogenic pesticides—dieldrin, endosulfan, toxaphene and chlordane—in our yeast model, and showed that, indeed, they produce a very low-level response when were tested singly. However, when we tested combinations of these chemicals, the estrogenicity jumped by 160 to 1,600 times their individual potencies.

On a molecular level, there are several possible explanations for this. One is that the chemicals may physically combine to form an estrogen-like molecule. Another is that various ecoestrogens and natural estrogens bind to one or both of the receptor subunits that join together to form a functional receptor pair or dimer. A third possibi-

**Figure 7. Surprising synergy of some ecoestrogens was recently demonstrated in the authors' laboratory using the yeast-estrogen system. Each of two ecoestrogens acting alone caused a very weak estrogenic response, as indicated by the pale blue color of the transformed yeast cells (a and b). Acting together, the two ecoestrogens produce an estrogenic response greater than the sum of the individual responses (c). In fact, the combined response can range from 160 to 1,600 times the individual responses.**



ty, which is the one we favor, is that the estrogen receptor has two or more interactive binding sites, a situation that may build flexibility and control into the response system. It may also account for the great structural diversity of estrogen-like molecules. In fact, Elwood Jensen, one of the "fathers" of estrogen-receptor research, recently proposed a second binding site on the receptor that recognizes antiestrogens. The question of multiple binding sites on the estrogen receptor may be important in endogenous as well as exogenous signaling pathways.

Our work with the yeast system has helped us expand our understanding of the subtle relations between ligands and the estrogen receptor. It may also inadvertently reveal aspects of the endogenous estrogen system that we had previously not known. For example, the levels of synergy we have observed may come about when two protein molecules bind together to act as a unit. In one hypothetical scenario, after an estrogen molecule binds to its receptor, the complex binds to a second, unoccupied estrogen receptor. Once bound, the unoccupied receptor molecule changes its shape in such a way as to make it easier for an estrogen molecule to bind to it than it than to an unattached receptor molecule. The two receptor molecules together, referred to as a dimer, could then bind to the ERE and modulate gene expression. Ecoestrogens may influence the dimerization process itself, facilitating the binding of receptor molecules to each other. There may be multiple binding sites for ecoestrogens on a receptor molecule, or receptor dimerization may create new binding sites for ecoestrogens. Hence, ecoestrogen binding might enhance dimerization, and dimerization might in turn enhance ecoestrogen binding. As a result, the ecoestrogens end up being more potent together than the binding of any one alone would indicate.

In a sense, the entire receptor-ligand complex itself becomes a signaling molecule. The complex effectively becomes a ligand for the estrogen response element. In this way a hierarchy of signals within signals is set up. At the simplest level, there is the intramolecular interaction between the ligand and the receptor, followed by the interaction between that signal and the DNA. The nature of these interactions and their subsequent outcomes depends on the

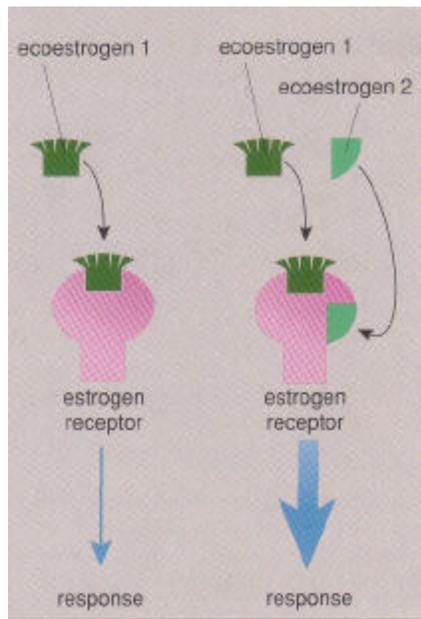


Figure 8. At least two models may be developed to describe the binding relationship between ecoestrogens and the estrogen receptor. In the first, an estrogen, either natural or synthetic, binds to a single site on the receptor, possibly the site for endogenous estrogen (left). Based on their recent data, the authors propose a second model in which ecoestrogens act synergistically. In this hypothetical model, each binds to distinct sites on the same molecule (right), one of which may be the endogenous estrogen binding site. The authors are exploring whether estrogen binding regulates other sites on the receptor, called activation functions, which interact with transcription factors on the promoter. Synergistic ecoestrogen binding may increase the interaction between activation functions and transcription factors. The outcome is increased gene expression and a more vigorous estrogenic response than is produced by either two estrogens acting at a single site or a single ecoestrogen acting alone.

original estrogen-like molecule. That is, each ecoestrogen binds to the receptor in a slightly different fashion, giving the overall ligand-receptor complex a different shape. Under different circumstances, different areas of the receptor molecule may be exposed or hidden, or its charge may be altered. All of these subtle modifications determine how the complex interacts with DNA. One complex may stimulate a high level of gene expression; another complex may stimulate a lower level or may repress gene expression altogether. It is even possible that different complexes can modulate the activity of different genes entirely, which, of course, profoundly affects subsequent cellular activities.

## Other Pathways

Research in our lab and many others has started to suggest that estrogens may exert some additional influence through signaling pathways other than the one directly involving the estrogen receptor. There is, for example, evidence to suggest that estrogens act, in part, through signaling pathways usually activated by growth factors such as epidermal growth factor (EGF), transforming growth factor alpha (TGF $\alpha$ ) or insulin-like growth factor (IGF).

Growth factors, unlike steroid hormones, are not fat-soluble, and therefore do not pass unaided through lipid membranes. Instead, a protein receptor must be present in the membrane for the factor to have an effect in a particular cell. These receptor molecules span the length of the membrane, with the growth-factor binding site located on the external membrane face. The growth factor does not actually have to enter the cell in order to activate the signaling pathway. Rather, when the factor binds the external portion of the receptor molecule, changes take place on the portion of the receptor that lies inside the cell. These changes initiate a biochemical chain reaction, where each molecule in the pathway is stimulated to activate the next molecular signal until the final signal results in some sort of cellular activity. One of the endpoints of various growth-factor signaling cascades is, apparently, the estrogen receptor.

This raises the possibility that some ecoestrogens may bring about their effect by interacting with a growth factor or with the appropriate growth-factor receptor to change the activity of the estrogen receptor. One possible outcome of such a signal may be to alter the binding activity of other ecoestrogens to the estrogen receptor. If this is the case, nature, by regulating estrogen action through hierarchies of signals, has provided additional possibilities for environmental mimicry. It is getting hard to tell the dancers from the dance.

## Ecoestrogens in Sickness and in Health

Since it now appears that ecoestrogens can bring about many of the same effects as the endogenous hormone, scientists must consider the consequences of exposure to these compounds on the health of people and animals. Since endogenous and pharmaceutical estrogens are associated with various diseases and dysfunctions, including

breast and endometrial cancer, lactation suppression, endometriosis and uterine fibroids, the possibility that ecoestrogens may also be associated with these disorders must be considered.

So far, studies linking ecoestrogenic chemicals in the blood to breast cancer have been equivocal—some have shown an association, but others could not find any of significance. Two studies, one in North Carolina, the other in Mexico by the epidemiologist Walter Rogan, demonstrated that estrogenic pesticides decreased the length of time women breast fed their infants, suggesting an estrogen-related suppression of lactation.

Ecoestrogens are not only potentially harmful to adults; they may also affect developing embryos, sometimes with lifelong consequences. The known effects of prenatal exposure to DES on sperm production later in life have led to the hypothesis that exposure to environmental hormones early in life may be partly to blame for a reported decrease in semen quality worldwide. The decline in semen quality first described in 1992 by Niels Skakkebaek in Copenhagen as well as any role for environmental factors, however, remains highly controversial.

Although the effects of ecoestrogens on human health remain, for the most part, uncertain, stronger reasons for concern have been found in other species. Scientists have seen the harmful effects of ecoestrogens both in natural settings and in laboratory studies. In one case, male fish living in polluted water produced abnormally high amounts of vitellogenin, the egg-yolk protein normally found only in female fish that are laying eggs. This therefore strongly suggests that the males had been exposed to some kind of estrogen-like molecule. In another study conducted by Stephen A. Bortone of the University of West Florida in Pensacola, female fish were masculinized following exposure to environmental wastes.

Alligators living in Florida's Lake Apopka, which had been extensively contaminated with DDT-related compounds and other agricultural chemicals, experienced a sharp population decline following the contamination. Subsequent studies by Louis Guillette have shown less than half the normal levels of the male sex hormone, testosterone, were present in the blood of the males. These data, along with the observed reduction in the size of these animals' genitals, lead to the conclusion that the alligators were "feminized."

Important work done by D. Michael Frye and his colleagues at the University of California at Davis showed that sea-gull eggs exposed to DDT developed as females, no matter what their genetic sex. This was one of the early works demonstrating the feminizing effects of environmental chemicals.

More recently, in collaboration with David Crews and Judy Bergeron at the University of Texas, we showed the developmental consequences of exposing turtles to estrogenic chemicals. Normally, sexual differentiation in turtles is dependent on the temperature at which the eggs develop. Eggs incubated at 31 degrees Celsius become females; eggs incubated at 26 degrees Celsius become male. Eggs incubated at the male-producing temperature, however, develop as females when they are exposed to natural estrogen. The same effect was produced when the eggs were exposed to estrogenic PCBs. Strikingly, as with our molecular biological studies in yeast cells, we could demonstrate a synergistic effect of ecoestrogens on sex reversal in turtles. Taken altogether, these field and laboratory studies strongly suggest that ecoestrogens are capable of altering sexual development in a manner consistent with their hormonal activity and in some cases the hormonal activity of mixtures of chemicals is greater than additive.

This knowledge provides the basis for the new science of environmental signaling. It seems that biological mimicry is a defense strategy adopted by some plants and fungi that may inadvertently be exhibited by classes of pesticides and other synthetic chemicals. Unlike the rational synthesis of DES as a synthetic hormone, there is little evidence that pesticides and other industrial chemicals that have hormonal activity were synthesized for this purpose; nor does it appear that estrogenicity was related to the way in which pesticides worked on pests. Nevertheless, as we have shown, environmental chemicals may function as signals, implying that they must interact with a particular cellular receptor and thus demonstrate some degree of inherent specificity. The outcomes of these interactions then become reasonably predictable.

We have seen that many natural and synthetic compounds in the environment can function as estrogens or antiestrogens. The recent interest in environmental chemicals as estrogens has

stimulated thinking about how synthetic chemicals may interact with biological systems. The demonstration that an environmental chemical can function as an antiandrogen portends that there may be more hormonally active chemicals in the ecosystem. It also suggests approaches to look for other unintentional environmental signals.

It is possible that other environmental signaling molecules are mimicking hormones, neurotransmitters, growth factors, or other important biological functions. The work with ecoestrogens certainly raises these possibilities. But the work on estrogenic agents also gives us experimental methods for approaching this possibility. The work also provides new insights into the mechanism of estrogen action itself and points the way to a new understanding of the relationship between people and their chemical environment at a cellular level. The more we comprehend the mechanism, the better able we are to predict and, where possible, prevent adverse effects.

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