

# HORMONALLY ACTIVE CHEMICALS IN THE ENVIRONMENT

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# Hormonally Active Chemicals in the Environment

BY JOEL SCHWARTZ

## Executive Summary

### A. The Debate over Hormonally Active Chemicals

The endocrine system, a system of glands and the hormones they release, regulates the development of a fetus in the womb, sexual development and reproductive function, maturation of the brain and nervous system, and energy metabolism. Some researchers have postulated that a range of natural and synthetic chemicals in the environment could damage or disrupt human and animal endocrine systems at exposure levels much lower than what previous studies and regulatory agencies have determined to be dangerous or toxic. Proponents of this hypothesis have dubbed the implicated chemicals “endocrine disruptors.”

Many chemicals can exert toxic effects at high levels of exposure. Regulatory agencies set exposure limits for chemicals intended to protect even sensitive people from adverse effects due to chemical exposure, and few people are ever exposed to chemicals at levels above these safety limits. But proponents of additional regulatory safeguards believe that hormonally active chemicals could cause harm even at very low exposure levels. They observe that:

- The endocrine system can be affected by very small amounts of certain foreign chemicals—much less than the levels tested in traditional laboratory animal toxicity studies;
- There is evidence that some hormonally active chemicals can circumvent the normal defenses of developing organisms; and
- The environmental persistence of some of these chemicals gives them more time to do damage.

Studies in the early 1990s raised concerns over whether synthetic chemicals were causing widespread harm through endocrine disruption. Researchers in Europe published a study reporting that average human sperm counts had declined by more than 40 percent between 1938 and 1990. Other researchers reported that male alligators in a pesticide-contaminated Florida lake had abnormally small penises and reduced fertility. A breast cancer study reported that a group of women with breast cancer had higher average levels of the

insecticide DDT in their bodies than a group of otherwise similar women without breast cancer. More recently, researchers have reported that some chemicals can cause changes in the size and structure of reproductive organs in laboratory animals at doses well below regulatory safety limits and near the range of typical human exposures.

However, a number of other scientists are skeptical of the extent to which endocrine disruption plays a significant role in human and wildlife health. They agree that adverse endocrine effects have been demonstrated for several synthetic chemicals in laboratory settings, and are likely to have occurred in a number of human poisoning incidents and in wildlife habitats with high contamination levels. However, these researchers question the existence and importance of health effects from the relatively low exposures to chemicals typical of the everyday environment. They raise the following objections:

- Inherent biases in human sperm count studies make them unsuitable for evaluating actual sperm-count trends. Furthermore, long-term data from farm animals show no change in sperm counts over time;
- Studies of the relationship between DDT and other organochlorine chemicals and breast cancer have been inconsistent, with most studies finding no effect;
- Although some researchers have found endocrine effects in laboratory animals with very low doses of chemicals, other laboratories have not been able to duplicate these results; and,
- Even if these low-dose effects exist, it's not clear that they should be considered harmful, because the effects are subtle, and "natural" variations in diet, stress, and other factors can cause similar effects.

Typical chemical exposures in humans are generally hundreds to thousands of times lower than exposures considered worrisome based on traditional toxicity studies. The practical importance of endocrine disruption thus depends on whether very low exposures to commonly used chemicals can cause significant harm.

## **B. Identifying and Studying Hormonally Active Chemicals**

Aside from the body's own hormones, chemicals with hormonal activity fall into three broad classes: (1) synthetic chemicals used in industry, agriculture, and consumer products; (2) synthetic chemicals used as pharmaceutical drugs; and (3) natural chemicals found in many foods, particularly soy.

The U.S. Environmental Protection Agency (EPA) sets regulatory safety limits for synthetic chemicals that are at least 100 times less than the highest dose found to be without adverse health effects in laboratory animals. EPA uses these conservative safety limits due to the uncertainty in whether humans are more sensitive than laboratory animals to certain toxic effects, and because humans vary in their sensitivity to toxic effects. Human exposures are substantially lower than regulatory safety limits. The key question then for assessing the risk of endocrine disruption is whether chemicals could cause adverse health effects at exposures below regulatory safety limits, and within the range of typical human exposures.

Scientists have developed a number of tests, or "assays" for hormonal activity. For example, relative-potency assays determine the ability of a given foreign chemical to mimic one or more effects of a particular hormone. These tests show that while some pharmaceutical hormones are as potent as natural hormones, industrial and consumer-product chemicals are generally hundreds to thousands of times weaker than natural hormones.

### C. Risks from Chemicals with Hormonal Activity

Scores of laboratory animal studies have confirmed that high doses of hormonally active environmental chemicals—that is, doses much greater than everyday environmental exposure levels—can cause a range of adverse effects. There is also limited evidence in humans of such effects due to chemical accidents and use of some pharmaceutical drugs. For example, diethylstilbestrol (DES), a synthetic estrogen roughly as potent as estradiol, was given to several million pregnant women between 1947 and 1971 in the mistaken belief that it reduced the risk of miscarriage. DES caused high rates of infertility in daughters and increased rates of undescended or abnormal testes in sons of DES-treated women.

Although these studies show hormonally active chemicals can harm a developing fetus, it is not clear if these results can be generalized to endocrine disruption by exposures to chemicals at the low levels found in the everyday environment. Both dose and potency determine toxicity, and the doses in these studies were far greater than exposures to chemicals at the low levels found in the everyday environment. Furthermore, DES is thousands of times more potent in its hormonal effects when compared to hormonally active environmental chemicals. The practical risk of endocrine disruption for human and wildlife health instead centers on the potential for adverse effects at doses well below regulatory safety limits, including exposures that could be encountered in everyday life.

Several researchers have studied low-dose effects of hormonally active chemicals, but with mixed results. For example, some researchers have found that the industrial chemical bisphenol A (BPA) increases mouse prostate gland weight at doses well below the lowest dose at which BPA had previously been found to have a physiological effect, and near the estimated “worst-case” human exposure level. However, other researchers were unable to duplicate these results. Similar discrepancies have occurred in laboratory animal tests of other chemicals.

Because of the controversy regarding low-dose effects, EPA and the National Institute of Environmental Health Sciences convened an independent expert panel to re-analyze data from 49 low-dose studies. The expert panel concluded that endocrine effects have been demonstrated for a number of chemicals at doses below their previously determined no-effect levels. Only BPA had any effects at doses near the range of human exposure. However, because the BPA effects could not be duplicated by some laboratories, the panel concluded that it is not clear whether the apparent low-dose effects of BPA represent a general property of the chemical. Because of the subtlety of the effects, the panel also concluded that it is not clear whether low-dose effects should be considered “adverse,” or merely biological changes that can’t be assigned a value label such as “bad” or “good.” For example, natural factors such as variation in diet, stress, and hormone levels during pregnancy appear to have as much influence on study results as low doses of the chemicals being tested.

Laboratory studies provide information on the types of chemical effects that are plausible at given dose levels. The next step is to go out into the world and see if there is evidence for actual endocrine disruption or other harmful chemical effects in humans or wildlife at typical chemical exposure levels. Epidemiological studies attempt to determine if particular health outcomes, such as cancer, endocrine disruption, or asthma, are associated with particular risk factors, such as diet, genetics, smoking, or exposure to environmental pollution. Although epidemiological studies are an important part of risk assessment, they are not as definitive as laboratory studies, because the subjects are not randomly assigned to “treatment” and “control” groups, introducing the potential for bias in study results.

**Neurological Effects.** Laboratory animal studies have found that exposure to a group of chemicals called polychlorinated biphenyls (PCBs) in the womb can cause later learning and behavioral disorders. Although the biochemical mechanisms for these effects are unknown, some researchers have suggested

endocrine disruption, as well as alterations in neurotransmitters (the chemical signaling mechanism in the brain and nervous system) as possible factors.

Five studies have assessed whether humans are adversely affected by exposure to PCBs and a number of other persistent chemicals at the relatively low levels encountered in the everyday environment. Some of these studies have found that children who had higher PCB exposures in the womb performed more poorly on tests of intellectual and neurological development. Where effects were observed, children in the top 5 to 20 percent of PCB exposure generally performed up to several percent worse than less-exposed children on one or more neurological tests.

The results from these studies are inconsistent regarding the type and timing of observed health effects. For example, the Lake Michigan study found declines in test performance in school-age children while the North Carolina study did not, even though the PCB exposures were similar. Effects sometimes also appeared and then disappeared, or vice versa, among the same group of children assessed at different ages. Where effects were observed, they were relatively subtle—only a few percent in most cases. One of the studies also found that the association of higher PCB exposure with lower test performance disappeared for children who were breastfed. Thus the extent to which low-level PCB exposure has permanent negative effects on children's development remains unclear, but appears at worst to be relatively small.

Another factor to consider in assessing *current* risk from PCBs is that the children in these studies were born roughly 8 to 20 years ago. PCB exposure, as well as exposure to other persistent chemicals in the environment, has declined substantially during the last 20 years, and continues to decrease. Thus, whatever the effects of these chemicals on children born in the past, current effects are now lower and will likely continue to decline.

**Male Reproductive Health.** In 1992, researchers in Denmark published an analysis concluding that human sperm counts had declined by more than 40 percent between 1938 and 1990. However, other researchers have argued that the samples of men who elect to donate semen are never representative of the general population, and that the degree of bias can vary in different directions at different times and in different places. For example, a study in Australia found that average sperm counts varied by more than a factor of two among five separate groups of sperm donors recruited by the same doctors, at the same hospital, using the same recruitment methods.

Farm animals provide a check on human sperm-count studies, both because humans and farm animals are likely exposed to most of the same chemicals present in the environment, and because studies in farm animals presumably avoid the potential for selection bias inherent in human studies with voluntary sperm donors. Researchers have found that sperm counts in farm animals have been constant during the last 70 years. Evidence for human sperm-count declines thus appears to be relatively weak.

**Female Reproductive Health.** Increased lifetime exposure to estrogen increases the risk of developing breast cancer. Because of the link between estrogen and breast cancer, some researchers have proposed that estrogenic chemicals in the environment might increase the risk of developing the disease. Although a few studies have found an association between exposure to persistent chemicals such as DDT and increased risk of breast cancer, most have not. Furthermore, the chemicals in question—DDT, dieldrin, and hexachlorobenzene—are exceedingly weak estrogens. As a result, a link between these chemicals and breast cancer appears to be biologically implausible. It thus appears that the potential for everyday exposures to estrogenic foreign chemicals to increase the risk of breast cancer has not been properly evaluated.

**Wildlife Studies.** There are a few cases in which wildlife health effects have been linked specifically to the mechanism of endocrine disruption due to environmental contamination. Examples include the following:

- In a mollusk species called the dogwhelk, the marine fungicide tributyltin (TBT) can cause females to develop masculine characteristics, such as a penis, at exposure to concentrations as low as one-billionth of a gram of per liter (one part per trillion). Some European populations of dogwhelk began to recover after regulations reduced TBT use. Nevertheless, TBT concentrations are still high enough in some regions to endanger sensitive mollusk species.
- A high rate of hermaphroditism has been found in some freshwater fish in England that live just downstream of some sewage treatment works (STW) wastewater discharges. In most cases, the causative agents appear to be natural estradiol excreted by women, and ethinyl estradiol, a synthetic estrogen in birth control pills, both of which are not completely removed by STWs. In a few other cases, estrogenic chemicals called alkylphenols, released from industrial plants, are responsible for the effects.
- Many Great Lakes birds accumulated organochlorine chemicals in their bodies by eating contaminated fish in the 1960s and 1970s. These chemicals were likely the cause of high rates of eggshell thinning, deformities, and mortality in chicks. Although some Great Lakes bird species have made dramatic recoveries due to reductions in organochlorine chemicals during the last 20 years, other species continue to decline in the most contaminated locations.

## **D. Summary**

Endocrine disruption has been unequivocally demonstrated in humans and animals at relatively high doses of chemicals—many times greater than typical human or animal exposures to environmental contaminants. Endocrine disruption has also been demonstrated in a few aquatic species due to low exposures to a few chemicals. However, the evidence for adverse hormonal effects from low-level chemical exposures in humans is much weaker. Subtle neurological effects may have occurred in some children due to PCB exposure in the womb, though the evidence is inconsistent. There does not appear to be credible evidence for hormonally active chemicals causing increases in breast cancer risk, or declines in sperm counts. Overall, the evidence suggests it is unlikely that adverse health effects due to endocrine disruption have occurred in humans from exposures to small amounts of foreign chemicals in the environment.

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## Part I

# Hormonally Active Chemicals and Why We Need A Plain-English Guide

## A. Public Policy Implications

Issues of public and environmental health increasingly involve complex scientific analysis, and neither the public nor policymakers have the time or resources to study every issue in depth. Advocacy groups publish materials promoting one side of a policy issue or the other, but generally present the evidence that supports their policy goals. Rarely do advocacy groups attempt to paint a balanced picture with suitable detail to allow for meaningful policy consideration or discussion. Scientific review bodies and blue-ribbon commissions strive for a more balanced portrayal of scientific evidence and often do so very well, but they rarely translate that information into language that the interested lay reader or policymaker can understand. The debate over environmental chemicals and their potential to disrupt human and wildlife hormone systems is one such issue.

## B. Controversy Over Hormonally Active Chemicals

Many chemicals can have toxic effects on people or animals who experience high enough levels of exposure. To protect public health, a number of federal agencies have programs in place to require testing of chemicals for toxic effects before they are used commercially, or to require testing of chemicals already in commercial circulation if an agency determines that they have not been adequately tested. The U.S. Environmental Protection Agency (EPA), the Food and Drug Administration (FDA), and the Occupational Safety and Health Administration (OSHA) set maximum safe exposure limits for chemicals based on the results of these tests.<sup>1</sup> Regulatory exposure limits include substantial safety factors intended to protect even sensitive people from adverse effects due to chemical exposure. Few people are ever exposed to chemicals at levels above these safety limits. One might therefore ask why the risk from these chemicals merits special attention.

The endocrine system, a series of glands and the hormones they release, regulates most physiological functions in humans and animals: the development of a fetus in the womb, sexual development during puberty and later reproductive function, maturation of the brain and nervous system, and energy metabolism.<sup>2</sup> A number of researchers have postulated that some natural and synthetic chemicals in the environment could interfere with or disrupt human and animal endocrine systems at exposure levels much lower than what previous studies and regulatory agencies have determined to be dangerous or toxic.<sup>3</sup> These hormonally active chemicals could then cause potentially serious damage to the reproductive success,

disease resistance, and neurological development of humans and wildlife. Proponents of this hypothesis have dubbed the phenomenon “endocrine disruption” and the implicated chemicals “endocrine disruptors.”

Proponents of this hypothesis have urged additional regulatory safeguards to protect against putative endocrine disruptors, and have advanced several reasons why endocrine disruption should be considered an imminent and serious danger to human and wildlife health:<sup>4</sup>

- The endocrine system has the potential to be affected by very small amounts of foreign chemicals—much less than the levels tested in traditional laboratory animal toxicity studies. Minute amounts of natural hormones are sufficient to produce appropriate physiologic responses in animals. Those concerned about endocrine disruption observe that if the same is true of hormonally active foreign chemicals, these chemicals might be able to adversely affect endocrine function at the low exposure levels found in the everyday environment.
- The developing embryo<sup>5</sup> and fetus could be especially vulnerable to endocrine alterations. Although embryos and fetuses have means to protect themselves from some changes in their chemical environment, there is evidence that some hormonally active chemicals can circumvent these defenses.
- Some of the suspect chemicals persist in the environment and can build up in animals’ bodies over time, particularly a group of pesticides and industrial chemicals known as persistent organochlorines.<sup>6</sup> To the extent that these chemicals cause toxic effects, their persistence gives them more time to do damage.

A number of studies in the early 1990s raised concerns over whether synthetic chemicals were indeed causing widespread harm through endocrine disruption. Researchers in Europe published a study reporting that average human sperm counts had declined by more than 40 percent between about 1938 and 1990.<sup>7</sup> Researchers in Florida found that male alligators in Lake Apopka, which had been heavily contaminated in 1980 by a spill of the insecticide DDT (dichloro-diphenyl-trichloroethane) and other chemicals, had abnormally small penises and other birth defects, and reduced fertility.<sup>8</sup> A breast cancer study reported that a group of women with breast cancer had higher average levels of DDT in their bodies than a group of otherwise similar women without breast cancer.<sup>9</sup> The book *Our Stolen Future* brought endocrine disruption to popular attention after its publication in 1996.<sup>10</sup> More recently, researchers have reported that some chemicals can cause observable endocrine changes, such as changes in the size and structure of reproductive organs, in laboratory animals at doses well below regulatory safety limits and near the range of typical human exposures.<sup>11</sup>

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As a result of the publicity and lobbying efforts regarding endocrine disruption, Congress in 1996 passed the Food Quality Protection Act and amendments to the Safe Drinking Water Act. These laws directed EPA to develop and implement a program to screen thousands of chemicals for hormonal activity and assess the potential risks posed by these chemicals to humans and wildlife.<sup>12</sup> Funding for research on endocrine disruption has increased exponentially as a result of these events, producing hundreds of studies over the last several years.

There are also, however, a number of scientists who are skeptical of the extent to which endocrine disruption plays a significant or general role in human and wildlife health.<sup>13</sup> While they agree that

adverse hormonal effects from several synthetic chemicals have been demonstrated in laboratory settings, and are likely to have occurred in a number of human-poisoning incidents and in some wildlife habitats with high contamination levels, these researchers question the existence and importance of health effects from the relatively low exposures to chemicals typical of the everyday environment. They raise the following objections:

- Inherent biases in human sperm count studies make them unsuitable for evaluating actual sperm-count trends. In addition, long-term data from farm animals show no change in sperm counts over time;
- Studies of the relationship between organochlorine chemicals and breast cancer have been inconsistent, with most studies finding no effect;
- Although some researchers have found endocrine effects in laboratory animals with very low doses of chemicals, other laboratories have not been able to duplicate these results; and
- Even if these low-dose effects exist, it's not clear that they should be considered harmful, because the effects are subtle, and "natural" variations in diet, stress, and other factors can cause similar effects.

Typical chemical exposures in humans are generally hundreds to thousands of times lower than exposures considered worrisome based on traditional toxicology studies. The importance of endocrine disruption thus depends on whether low doses of commonly used chemicals can cause significant harm. Confirmation of widespread harm from chemicals at low doses would have major consequences for public health. For the purposes of this paper, "low exposure" or "low dose," unless otherwise noted, will refer to chemical exposure levels typical of the everyday environment.

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It is also worth noting that endocrine disruption is only a proposed mechanism by which chemicals might harm humans and other animals. In some cases chemicals can be observed to cause harm, but the mechanism is unknown. Thus, a more general question for assessing the risks from chemicals is whether some chemicals can cause harm at low doses, regardless of whether the mechanism is alterations in endocrine function, or some other mode of action. Although this paper focuses on hormonally active chemicals, it will also note some related cases in which low-dose chemical effects have been reported and where endocrine disruption is a suspected mechanism, but where the actual mechanism has not been determined.

The controversy over hormonally active chemicals was reflected in the deliberations of an expert panel convened in 1995 by the National Research Council (NRC) of the National Academy of Sciences. The panel was asked to independently evaluate the state of the science on hormonally active chemicals and endocrine disruption, estimate the risk to human and wildlife health, and make recommendations on how the country should respond.<sup>14</sup> Although the panel was expected to complete its work by 1997, dissent within the panel delayed its ability to reach consensus.<sup>15</sup> The panel ultimately issued its report in 1999, finding that based on the available evidence, there was still great uncertainty regarding whether hormonally active chemicals are actually causing adverse effects in humans and animals at the low doses typical of most environmental exposures, and that more research was needed.<sup>16</sup>

## Hormones and the Endocrine System

The endocrine system regulates and coordinates growth and metabolism in many types of animals, including humans and other mammals, and includes two major components:<sup>17</sup>

1. A series of glands located throughout the body that synthesize and secrete hormones into the blood stream. These glands include a part of the brain called the hypothalamus, the pituitary, adrenals, thyroid, pancreas, ovaries and testes, among several others.<sup>18</sup> The endocrine system is ultimately controlled and coordinated by the brain.
2. A group of proteins, called receptors, that “recognize” hormones. Each hormone has its own specific receptor(s). Various types of hormone receptors are located on cell surfaces and within the fluid medium inside cells. Binding of hormone to receptor initiates a chain of events that results in the response of the target organ or tissue to a hormonal signal.

The human endocrine system includes more than a hundred different hormones. Some hormones affect many different organs and tissues, while others affect only one or a few. Major groups of hormones include the following:

1. **Steroids.** These include the sex hormones estradiol, an estrogen, or “female” sex hormone, and testosterone, an androgen, or “male” sex hormone.<sup>19</sup> The steroid hormone family also includes a group known as glucocorticoids, including cortisol, which has roles in sugar metabolism and immune function, and retinoids, which are synthesized from vitamin A.
2. **Amines.** These include the thyroid hormones thyroxine and triiodothyronine, and the adrenal hormones, adrenaline and noradrenaline.
3. **Proteins and peptides.** These include insulin and growth hormone. Peptides are short chains of amino acids, while proteins are long chains of amino acids.
4. **Eicosanoids.** These include prostaglandins and leukotrienes, and are mostly synthesized from a fatty acid called arachidonic acid. Fatty acids are components of fat molecules. Eicosanoids have a number of roles, including regulation of blood pressure and joint inflammation.

Many portions of the endocrine system are organized into a series of “feedback loops,” with each loop referred to as an axis. One such axis is the hypothalamo-pituitary-gonadal axis, which regulates levels of sex hormones as follows: the hypothalamus secretes gonadotropin releasing hormone (GnRH), which causes the pituitary gland to secrete luteinizing hormone (LH) and follicle-stimulating hormone (FSH). In females, LH and FSH cause the ovaries to produce and secrete the hormones estradiol and progesterone as part of the menstrual cycle. In males, LH causes production of testosterone, while FSH causes sperm production. Estradiol and testosterone then act back on the pituitary to reduce LH and FSH secretion, and on the hypothalamus to reduce GnRH secretion—a process known as “negative feedback.” This negative feedback system regulates circulating levels of sex hormones. Negative feedback is the most common form of control in the endocrine system.

In addition to its role in regulating and coordinating body functions in children and adults, the endocrine system plays a key role in the growth and development of animals in the womb. During pregnancy, the fetus, placenta, and mother form an interacting set of endocrine systems that coordinate the physiologic processes necessary to maintain pregnancy. Although the role of estradiol in pregnancy is not completely understood, it may stimulate production of progesterone, which is necessary for maintenance of the uterus, and may also initiate other hormonal processes necessary for the fetus to mature.<sup>20</sup>

Testosterone is a key hormone in the development of male fetuses. Starting around the ninth week of pregnancy the testes begin to secrete testosterone, which causes development of male external genitalia and the prostate gland. Female genitals would develop in the absence of testosterone, even in a fetus that is genetically male. Much of the research on hormonally active chemicals focuses on the effects of changes in estradiol and testosterone levels in developing organisms, and on the effects of foreign chemicals that either augment or detract from natural hormonal signals.

The endocrine system is integrated into virtually all aspects of an organism’s life. Endocrine function is intimately intertwined with the nervous and immune systems and changes in endocrine activity can be caused by, and in turn cause, changes in other body systems.

Even in the face of uncertainty, some argue for the preemptive banning or curtailment of the use of chemicals that might have endocrine-disruptive or other health effects at low doses. Advocates of the “precautionary principle” recommend this approach.<sup>21</sup> But the precautionary principle includes the underlying assumptions that: (1) it’s possible to know up front what approach among the many options available will provide the greatest level of safety, (2) that the suspect chemicals impose only costs on society, but provide few or no benefits, and (3) that substitutes for the banned chemicals would have fewer adverse effects. However, many of these chemicals are key components in the production of goods and services that have become integral parts of peoples’ everyday lives. Without them these goods and services might cost more, be of lower quality, or perhaps not be available at all. In addition, policy researchers have shown that when uncertainty is high, preemptive action has a significant potential to do more harm than good.<sup>22</sup> On the other hand, if there is a genuine hazard from low exposures to some widely used chemicals, the costs of their continued use could be substantial.

This study will assess the nature, magnitude, certainty, and imminence of endocrine disruption in humans and wildlife by focusing on:

1. How chemicals might be able to interfere with human and animal hormone systems;
2. The extent to which such effects have been documented in the laboratory and in the field, and their health consequences;
3. Actual risks to humans and wildlife; and
4. Uncertainties that need to be resolved with additional research.

## Part 2

# Identifying and Studying Hormonally Active Chemicals

**W**e can assess the plausibility and actual risk of endocrine disruption in humans and animals with data and analysis from the disciplines of toxicology and epidemiology. Toxicology is the study of the potential for chemicals or other environmental factors to harm living organisms. Epidemiology is the study of the degree of association between exposure to suspected toxicants and the prevalence of adverse health effects in a given group of organisms.<sup>23</sup> Risk assessment is the process of estimating the probability of adverse health effects from given levels of exposure to environmental hazards. Risk assessment combines evidence from toxicological and epidemiological studies to determine maximum safe levels of exposure to chemicals, and whether actual levels of exposure pose a threat to health. The first step is determining how different organisms respond to varying doses of a potentially toxic substance.

## A. Key Principles and Methods of Toxicology

### 1. Dose and Potency

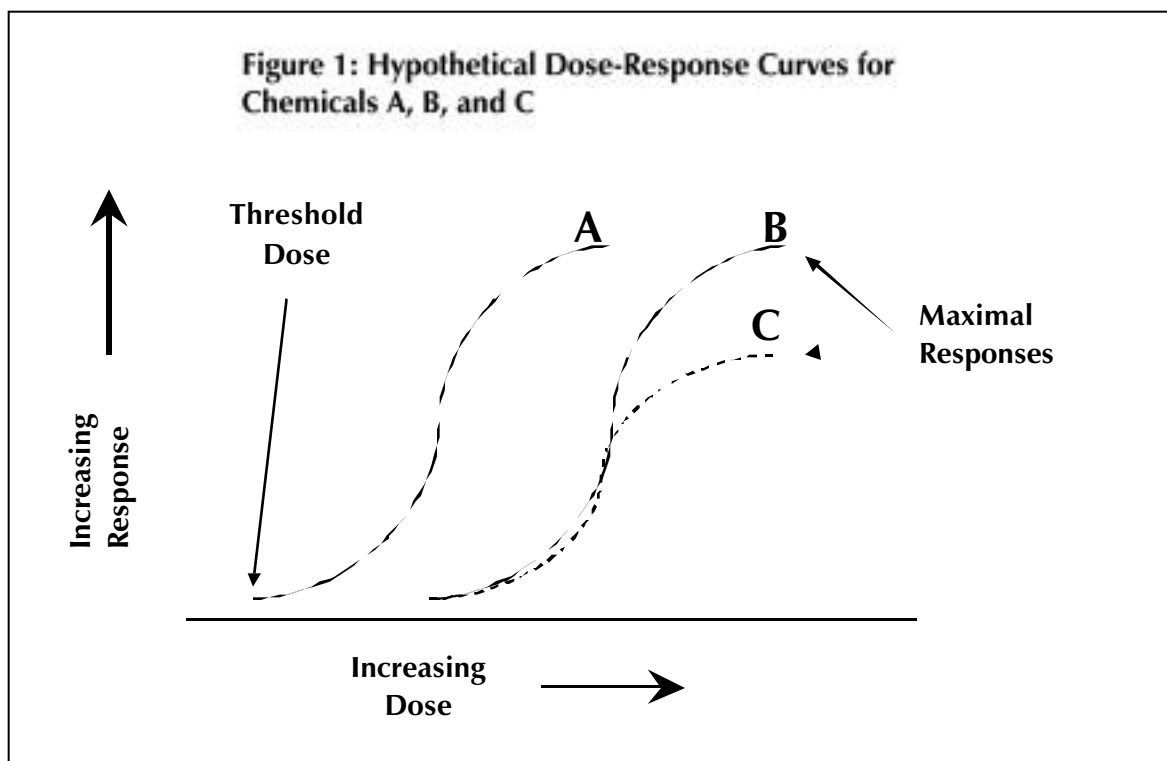
Perhaps the most important principle of toxicology is that the degree of a chemical's toxicity depends on the dose—that is, the amount of the chemical to which an organism is exposed. This is sometimes expressed in the maxim “the dose makes the poison”—or, as a recent toxicology textbook put it, “there are no toxic substances but only toxic doses of a given substance.”<sup>24</sup>

Variation of a toxic effect with changes in dose is expressed in a “dose-response curve.” Figure 1 displays examples of hypothetical dose-response curves for three different chemicals that all cause the same toxic effect. Note the following:

- The size of the effect or response increases with increasing dose;
- There is a dose below which no effect occurs, which is called the “threshold;” and
- There is a maximum level, called the “maximal response,” above which increasing the dose has no additional effect.

The ability of a chemical to elicit a toxic effect at a given dose is known as its potency. Different chemicals can have different potencies and different maximal responses. Curve A represents a chemical of greater potency than Curve B. That is, chemical A elicits a given level of toxicity at a lower dose than chemical B. Curve C represents a chemical that is just as potent as chemical B, but that elicits a smaller maximal response than either chemicals A or B.

A given chemical can cause a number of different toxic effects, each with a different dose-response curve. Although the specific shape of the dose-response curve varies for different chemicals and for different effects, effects generally increase with increasing dose up to the maximal response. In regulations designed to minimize risk, the adverse effect that has the lowest threshold dose would be used for determining safe exposure levels of the chemical.




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## 2. Testing for Toxic Effects of Chemicals

Toxicological studies attempt to determine whether and under what conditions chemical exposures can have adverse effects, and the mechanisms by which those effects occur. Because it is unethical to purposely expose humans to toxic chemicals, these studies focus on laboratory animals, such as rodents, rabbits, or monkeys. Groups of animals are randomly assigned to several treatment groups, each exposed to a different dose of a chemical, while another randomly selected group of animals, the control group, is given a fake treatment.<sup>25</sup> This type of study design is called a “randomized controlled experiment.”

The use of random assignment to treatment and control groups is intended to ensure that all aspects of the experiment are the same except for the different doses of the test chemical given to each group of animals. This ensures that any observed differences between the treated and untreated animals is actually caused by the suspect chemical, rather than by other factors. Animal studies use a range of doses in order to determine dose-response curves for the various adverse effects that might be caused by the chemical.

In the case of pharmaceutical drug development, randomized controlled trials are used in humans as the final step in determining whether a potential drug is safe and effective for widespread use. However, human trials are attempted only after results of animal studies have already provided strong evidence that the potential drug will indeed be safe and effective in humans.

### 3. Differences in Responses Among Species and Among Individuals

Sensitivity to a given toxic effect varies between species due to genetic differences in their ability to absorb, metabolize, and respond to foreign chemicals. For example, humans are about one-tenth as sensitive to the toxic effects of polychlorinated biphenyls (PCBs) as rhesus monkeys, and one one-hundredth as sensitive as rodents.<sup>26</sup> Guinea pigs are about 1,000 times less sensitive to the effects of dioxin than hamsters. Species also differ in the particular organs that are affected by dioxin.<sup>27</sup>

On the other hand, closely related families of animals such as mammals are similar in more ways than they are different. For example, rats and mice give similar results about 70 percent of the time in tests of potential carcinogens. Even here, however, there can be differences in potency—that is, in the dose of a particular chemical required to cause a tumor to form.<sup>28</sup> The key issue for toxicological research is ensuring to the extent possible that the various species in which a chemical is tested are sufficiently representative of humans. Testing a chemical in more than one species provides greater certainty when setting safe exposure limits for humans.

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Some species also come in different varieties, called “strains,” which are the result of selective breeding. Different strains can have different sensitivities to the effects of chemicals due to differences in their genetic makeup. For example, a recent study found a 16-fold variation in susceptibility between various mouse strains to changes in testes weight produced by estradiol, the most potent form of natural estrogen in animals.<sup>29</sup> Rat strains vary in their sensitivity to the uterus-enlarging effects of the chemical bisphenol A.<sup>30</sup> Thus, the presumed risk of any particular health effect can depend on the rodent strain chosen for testing.

Finally, sensitivity to toxic effects also varies among individuals of the same species. Some laboratory animal strains are inbred, which reduces individual variability, but humans and wildlife can vary a great deal in sensitivity between individuals, due to subtle genetic differences. For example, genetic differences between individuals can cause differences in their ability to biochemically neutralize or detoxify toxic substances.<sup>31</sup>

### 4. Route of Exposure Determines Toxicity

Chemicals can't cause a toxic effect unless they reach appropriate sites in the body and in levels sufficient to cause harm. The route by which a chemical enters the body affects its toxic potency. For example, for any given dose of a toxic chemical injection directly into the bloodstream generally causes the greatest toxic effect. Inhalation, ingestion, and absorption through the skin are in general progressively less effective routes of exposure for inducing toxic effects.<sup>32</sup> In order to determine the potential toxicity of a

chemical in wildlife and humans, it is important that the route of exposure in toxicity studies be similar to what could occur in real-world situations. While injection generally increases the toxic effect of a chemical, most real-world exposure occurs through ingestion or inhalation. This is a factor in endocrine disruption research, as some hormonally active chemicals have been shown to elicit a greater hormonal effect when injected, rather than ingested.<sup>33</sup>

## 5. Determining Safe Exposure Levels

One purpose of toxicity studies is to determine the dose below which a given chemical is safe for a given animal. Toxicologists have defined two dose levels to express this concept: the “no observed adverse effect level” (NOAEL) and the “lowest observed adverse effect level” (LOAEL). The NOAEL is the highest dose at which no adverse effect is observed in a given species. The LOAEL is the lowest dose at which some adverse effect is observed.<sup>34</sup> Because toxicological studies generally test only three or four doses of a chemical, and because there is always some variability in results between repeated experiments, it is generally not possible to make exact determinations of these levels.

Doses are generally reported as an amount of chemical per unit of body weight per day. So, for example, a typical dose in a toxicology study might be 100 micrograms per kilogram of body weight per day, abbreviated as 100 mcg/kg b.w./day.<sup>35</sup> To provide an everyday example, a teaspoon of sugar weighs about 4 grams, or one-seventh of an ounce. Per unit of body weight, a teaspoon of sugar per day would then equal a dose of 66,700 mcg/kg b.w./day, or 66.7 mg/kg b.w./day for a 60-kilogram (132-pound) person. Likewise, a typical dose of aspirin, 400 milligrams, would equal 6.6 mg/kg b.w./day.

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The presumed risk of any particular health effect can depend on the rodent strain chosen for testing.

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EPA uses NOAELs and LOAELs as the basis for setting safe exposure limits for humans. EPA’s safety limit for a given chemical is known as the “Reference Dose.” The Reference Dose is a daily intake of a given chemical that EPA estimates is “likely to be without an appreciable risk of deleterious effects during a lifetime.”<sup>36</sup> As noted above, sensitivity to toxic effects varies among species and among strains of the same species. EPA sets the Reference Dose by starting with the toxic effect with the lowest NOAEL or LOAEL measured in laboratory animals. EPA then applies additional safety factors for uncertainties in extrapolating from animal effects to humans, which also ensures that the Reference Dose is likely to protect even the most sensitive people. If only the LOAEL, but not the NOAEL for the chemical is known, EPA applies an additional safety factor. These safety factors usually range from 100 to 1,000.<sup>37</sup> That is, the Reference Dose is usually a factor of 100 to 1,000 times smaller than the lowest NOAEL or LOAEL found in laboratory animal toxicology studies.

In addition, the Food Quality Protection Act requires EPA to add an additional safety factor of up to 10 if it deems data on toxicity to children to be inadequate, or if children appear to be more sensitive to a given chemical’s toxic effects.<sup>38</sup> The World Health Organization (WHO) sets a similar safety limit for chemicals called the “acceptable daily intake,” defined as “the daily intake of a chemical, which during an entire lifetime appears to be without appreciable risk on the basis of all known facts at that time.”<sup>39</sup>

## 6. Types of Toxic Effects Assessed in Toxicity Studies

Various chemicals can cause a wide range of adverse effects given high enough doses. For the purposes of studying endocrine disruption, key adverse effects involve the potential for reproductive and/or developmental toxicity. Reproductive toxicity refers to damage to the sex organs that could, for example, cause reduced fertility, or a cancer such as breast or testicular cancer. Developmental toxicity refers to damage to a developing fetus or pre-pubescent that could cause, for example, spontaneous abortion or birth defects during pregnancy, or could cause problems after birth, such as neurological disorders, infertility, or cancer.

Developmental and reproductive toxicity need not involve the mechanism of endocrine disruption. For example, consumption of more than three to four ounces of alcohol per day during pregnancy can cause a condition called Fetal Alcohol Syndrome (FAS) in offspring. FAS is a complex of problems that includes severe mental retardation and facial deformity. The sedative thalidomide, marketed to treat morning sickness, was banned after it was found to cause grossly deformed arms and legs in many children born to women who took the drug.<sup>40</sup> In contrast, the health effects—or “endpoints,” in toxicology parlance—assessed in endocrine disruption studies are generally much more subtle than those caused by thalidomide and excessive alcohol consumption. Studies of hormonally active chemicals, particularly the low-dose studies needed to assess the risk from typical exposures, focus on effects such as changes in sperm count, fertility, or in the size of the prostate gland, uterus or other reproductive organs, or on changes in hormone levels, such as in the amount of thyroid hormones circulating in the blood.

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## B. How Endocrine Function Can Be Altered by Hormonally Active Chemicals

Endocrine disruption research has focused on the steroid and thyroid hormone systems, because they appear to have the greatest potential to be altered by foreign chemicals.<sup>41</sup> The steroids include the sex hormones estradiol and testosterone.<sup>42</sup> Both regulate general growth and also functioning of the reproductive system from fetus through adulthood. Thyroid hormones play a major role in regulating energy metabolism. Thyroid hormones, estradiol, and testosterone all take part in regulating development of the brain and nervous system.

Foreign chemicals could disturb the functioning of the endocrine system by interfering with any of the steps necessary to generate an endocrine response, for example:<sup>43</sup>

- Interfering with hormone synthesis or release into the blood stream, thereby changing the amount of hormone that reaches its target, which could result in an inappropriately decreased or increased response;
- Interfering with hormone transport, potentially changing the amount of natural hormone available for generating an intended effect;
- Binding to hormone receptors and either blocking the action of natural hormones or inappropriately activating a hormonal response. Hormones act by attaching, or binding, to

specific molecules on the surface of, or inside cells. This binding initiates an additional series of events that lead to the specific response of a given organ or tissue to a hormonal signal;<sup>44</sup>

- Altering the number of hormone receptors in a target tissue or organ, potentially changing the degree to which the tissue or organ responds to a hormonal signal;
- Altering cells' response to a hormone signal after hormone molecules bind to their receptors; and
- Interfering with hormone metabolism. Hormones are normally degraded into other compounds and then excreted in urine or feces after they have served their purpose. Some chemicals might be able to alter these metabolic processes, for example, by changing the rate at which hormones are degraded, or changing the types of chemical products that result from hormone metabolism. Either result could alter endocrine processes.

Although there are many ways in which the endocrine system could be disrupted, the endocrine system also has a series of feedback mechanisms that protect organisms from effects of external chemicals, particularly at the low doses encountered in the everyday environment. For example, Crisp et al. (1998) note that "hormones are highly regulated, and mechanisms for controlling modest fluctuations of hormones are in place. Therefore, minor increases of exogenous hormones following dietary absorption and [liver] detoxification of these [foreign chemicals] may be inconsequential in disrupting endocrine [balance] in adults."<sup>45</sup>

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Researchers therefore believe that the potential for harm from hormonally active chemicals centers on the degree to which the embryo and fetus are affected by low doses of chemicals to which its mother might be exposed during pregnancy.<sup>46</sup> During development in the womb, hormone signals regulate how the cells of the embryo and fetus divide, move, and differentiate<sup>47</sup> to form the overall body plan of an organism, its organs, and its physiologic systems. Interference with this process can cause permanent damage. Chemicals or other factors that can damage an embryo or fetus during development are called teratogens.

Research has shown that developing organisms are most susceptible to teratogens during critical periods of development during pregnancy. For example, human embryos are most susceptible to damage from roughly the third to the eighth week of development.<sup>48</sup> This is a period known as organogenesis, when the basic structures of most organs and physiological systems are laid out. Some organ systems are also relatively susceptible to damage even after the eighth week. For example, the critical period extends to week nine for the genitals and to week sixteen for the nervous system.<sup>49</sup>

Susceptibility to damage from teratogens is lower after critical periods have passed.<sup>50</sup> For example, a study of the fetal effects of high doses of the natural hormone retinoic acid in hamsters found that the risk of birth defects is low if treatment occurs before the beginning of organogenesis. However, the rate of birth defects increases dramatically when treatment is administered during organogenesis. Furthermore, within the organogenesis period, the timing of retinoic acid treatment determines the type of birth defects that result (for example, cleft palate, spina bifida, missing tail, etc.).<sup>51</sup> Likewise, a study in mice found that exposure of pregnant mice to alcohol on days 12 to 17 of pregnancy caused growth retardation in their offspring. However, exposure on days 5 to 10 caused no effect.<sup>52</sup>

There is also human evidence for the importance of critical periods of fetal development. The drug diethylstilbestrol (DES) was given to millions of women to reduce the risk of miscarriage until it was banned in 1971, because it was found to cause cancer and infertility in some daughters of exposed women, and genital abnormalities in some sons. Daughters' risk for DES-induced problems was greatest when their mothers were given DES during the first trimester, rather than later in pregnancy when organogenesis was completed.<sup>53</sup> For sons, the risk of genital abnormalities was greatest when mothers began DES treatment before the end of the ninth week of pregnancy.<sup>54</sup>

The endocrine disruption hypothesis is based largely on the observation that excesses of estrogen-mimicking chemicals during development in the womb can have serious health consequences for developing organisms. Indeed, in a normal pregnancy, the fetus is surrounded by a highly "estrogenic" environment from which it must protect itself. Large amounts of estradiol are necessary for the mother and the placenta to maintain the physiologic processes of pregnancy. But estradiol can also cross the placenta and enter the fetus. The fetus has specialized enzymes that convert estradiol to hormonally inactive estrone.<sup>55</sup> The fetus also produces large amounts of estriol and alpha-fetoprotein. Estriol binds to estrogen receptors, directly blocking the effects of estradiol, while alpha-fetoprotein binds to estradiol in the blood, preventing it from entering cells and activating a hormonal response.<sup>56</sup>

Although the fetus can protect itself from estradiol, one basis for concerns about hormonally active foreign chemicals is that some of them might be able to evade fetal defenses against estradiol. For example, some studies have found that steroid-binding proteins, such as alpha-fetoprotein, which bind to estradiol, do not bind as effectively to foreign chemicals that mimic the effects of estradiol.<sup>57</sup> Furthermore, although the fetus, as well as the pregnant mother and the placenta, have additional mechanisms to break down foreign chemicals,<sup>58</sup> it is not clear to what extent these mechanisms are capable of protecting the fetus from a given dose of chemical.

Although little is known regarding the ability of the fetus to protect itself specifically from hormonal effects of foreign chemicals,<sup>59</sup> there is evidence that the fetus can in some cases protect itself from low exposures to chemical toxicants. For example, the chemical 5-fluorouracil (5-FU) can block the action of an enzyme required for deoxyribonucleic acid (DNA) synthesis. DNA synthesis is necessary for new cells to form and for the fetus to grow. Toxicological studies have shown that high enough doses of 5-FU can cause low birth weight and birth defects. But 5-FU's enzyme-inhibiting effect can be observed even at doses 90 percent less than the level necessary to damage the fetus. This suggests that there is a threshold dose of 5-FU below which the fetus has some means to compensate for 5-FU's effects.<sup>60</sup> In any case, there is a biologically plausible concern that even low doses of hormonally active foreign chemicals could damage a developing embryo or fetus.

### C. Chemicals Under Study for Hormonal Effects

During the last few decades researchers have found that a number of chemicals have the potential to mimic hormones, or interfere with the actions of hormones. Foreign chemicals with hormonal activity fall into three broad classes:

1. Synthetic chemicals used in industry, agriculture, and consumer products, or generated as unwanted byproducts of production;
2. Synthetic chemicals used as pharmaceutical drugs; and
3. Natural chemicals found in fruits, grains, and vegetables.

### Molecular Mechanism of Hormone Action

The case of estradiol illustrates in general how steroid and thyroid hormones work at the molecular level. In mammals, estradiol is synthesized by the ovaries and released into the blood when stimulated by luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary gland. Estradiol in the blood is mostly attached, or bound to proteins called sex hormone binding globulins (SHBG) as well as other blood proteins that bind to steroid hormones, while a small amount of the hormone remains in its free, unbound form. These proteins protect hormones from being degraded and removed from the body before they can have an effect. They also regulate the amount of estradiol that can enter cells, as estradiol and other steroid hormones can cross cell membranes only in their unbound, free form.

Once the hormone reaches its target organ, it passes through cell membranes and into the fluid medium inside cells, called the cytoplasm. Estradiol exerts its effect by binding to specialized protein molecules in the cytoplasm and nucleus called estrogen receptors (ER). Many types of cell-signaling occur through binding of a signaling molecule to a receptor. Receptor molecules are proteins that have a shape that complements the shape of one or more specific signaling molecules, so that it will bind to those signaling molecules and not to other chemicals.

After an estradiol molecule binds to an estrogen receptor, the combined estradiol-ER complex binds to specific sequences of DNA in the cell nucleus called estrogen response elements (ERE). Binding of an estradiol-ER complex to an ERE initiates a process in which one or more genes are switched on, resulting in the synthesis of a chemical called messenger-ribonucleic acid (mRNA). The mRNA moves back into the cytoplasm where it initiates the manufacture of specific proteins that ultimately cause the observed physiologic effects of estradiol on the target organ or tissue.<sup>61</sup>

There are additional features of hormone action that complicate understanding of how hormones work at the molecular level. First, there can be more than one type of receptor for a given hormone. For example, two different estrogen receptors have been identified, dubbed ER-alpha (ER $\alpha$ ) and ER-beta (ER $\beta$ ). The amounts of each ER vary between different estrogen-sensitive tissues and organs. Estradiol has a different propensity to bind to each type of estrogen receptor.<sup>62</sup>

Second, steroid and thyroid hormone effects are also modulated—that is, increased or decreased, by other chemicals in the cell nucleus called coregulators. Coregulators come in two varieties: coactivators, which enhance a hormonal signal, and corepressors, which diminish a hormonal signal. Variation in the amounts and types of coregulators and hormone receptors in different organs and tissues can alter the effect of the same hormone in different cells or in the same cells at different times.<sup>63</sup>

Finally, until recently, it was believed that steroid hormones such as estradiol, acted only by binding to receptors inside cells and turning genes on or off. However, researchers have found during the 1990s that estradiol can have previously unrecognized effects by binding to receptors on the surface of cell membranes. Although many chemical signals are exchanged between cells through receptors on cell surfaces, estradiol had not been previously known to act through this route.<sup>64</sup>

Only organs and tissues that have estrogen receptors can respond to an estradiol hormonal signal. In general, only those organs and tissues that have the appropriate hormone receptors can respond to a given hormonal signal.

Sorting out these molecular details of steroid hormone action is crucial to understanding under what conditions foreign chemicals can alter endocrine function. For example, estradiol binds to ER $\alpha$  about 20 times more effectively than to ER $\beta$ . On the other hand, genistein, found in soy and other foods, binds about 17 times less effectively to ER $\alpha$  than ER $\beta$ . The industrial chemical bisphenol A has only a slightly greater affinity for ER $\beta$  when compared with ER $\alpha$ .<sup>65</sup> Once bound to an estrogen receptor, different chemicals also vary in their ability to recruit coregulators and to activate various estrogen response elements on genes.<sup>66</sup> Differences between chemicals in these molecular-level effects likely hold at least some of the keys to understanding how and why foreign chemicals do or do not affect hormonal activity at various doses and in various organs and tissues.

Among synthetic industrial and agricultural chemicals, the persistent organochlorine chemicals are among the most heavily studied. Large amounts of these chemicals were released to the environment between the 1940s and the 1970s, the period from their discovery as commercially useful products through the years of their peak production and use. DDT, probably the most well-known persistent organochlorine, was developed as an insecticide in 1939 and received its first large-scale use in suppression of a typhoid epidemic in Naples during the winter of 1944.<sup>67</sup> Other members of this class include the pesticides dieldrin, chlordane, heptachlor, mirex, and hexachlorobenzene (HCB), a group of closely related industrial chemicals called polychlorinated biphenyls (PCBs), and a group of unwanted byproducts of incineration and chlorinated chemical production called polychlorinated-dibenzo-dioxins (PCDDs) and polychlorinated-dibenzo-furans (PCDFs), which are both often referred to collectively as dioxins. Dioxins gained notoriety as the contaminant in “Agent Orange” that has been blamed for a number of health problems in those soldiers who were exposed to it during the Vietnam War.

Persistent organochlorines can take years to break down, and can therefore build up in the environment and in the bodies of people and animals exposed to them. Studies in the 1960s and 1970s found that high levels of contamination by these chemicals interfered with the fertility and reproductive success of a number of marine bird and mammal species, particularly those at the top of the food chain.<sup>68</sup> Since that time, use of most persistent organochlorines has been banned or restricted in the United States and many other nations, and exposure to humans and wildlife has been declining.<sup>69</sup>

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There are also many organochlorines that are not persistent, but break down relatively quickly in the environment or inside organisms that ingest them, and therefore do not accumulate over time. Some of these chemicals, such as the pesticides methoxychlor, endosulfan, and dicofol, are still in use and have also been studied for their potential to mimic hormones. A number of other industrial and commercial chemicals have hormone-altering potential as well. These include classes of chemicals known as bisphenols and alkylphenols, both of which have many commercial and consumer applications.

Table 1 lists some of the synthetic chemicals that are under study as potential endocrine disruptors, their NOAEL or LOAEL in animal studies, the EPA or WHO regulatory limit for daily exposure, and an estimate of typical daily human exposure.<sup>70</sup> As explained earlier, doses are reported in micrograms of chemical per kilogram of body weight per day, abbreviated as mcg/kg b.w./day. Note that the standard general toxicity studies used to determine these NOAELs and LOAELs assessed a wide range of potential health effects and were not limited to endocrine effects. Therefore, the particular health effect observed at the LOAEL will in many cases not be an endocrine or reproductive effect.

Note from the table that human exposures are typically substantially lower than the EPA and WHO safety limits, which are themselves 100 to 1,000 times lower than the NOAEL or LOAEL determined by general toxicity studies. The key question for assessing the importance and risk of endocrine disruption is whether these chemicals could cause heretofore unrecognized health effects at exposures substantially lower than the EPA and WHO safety limits, and within the range of typical human exposures. This issue will be explored in detail when we look at studies of the actual health effects of hormonally active chemicals at various dose levels, including the low-exposure levels found in the everyday environment.

Unlike the chemicals in Table 1, which were not intended to exhibit hormonal activity in humans and animals, a number of chemicals have been synthesized with the express purpose of altering natural hormonal activity for use as drugs. These include synthetic hormones in birth control pills, which mimic the effects of estradiol and progesterone, tamoxifen, which blocks the effects of estradiol and is used to treat breast cancer, and flutamide, which blocks the effects of androgens and is used to treat prostate cancer.<sup>71</sup> Some drugs interfere with hormonal activity indirectly. For example, so-called aromatase inhibitors inhibit the enzyme that produces estradiol from testosterone, and are also used to treat breast cancer.<sup>72</sup> Synthetic hormones, as well as estradiol itself, are also often used as growth promoters in cattle.<sup>73</sup>

**Table 1: Synthetic Chemicals Used in Industry and Consumer Products**

Chemical	Use(s)	NOAEL or LOAEL in animal studies mcg/kg b.w./day	EPA or WHO safety limit in mcg/kg b.w./day	Estimated Daily Human Exposure in mcg/kg b.w./day	% of EPA WHO Regulatory Limit	Status
<b>Persistent Organochlorines</b>						
Chlordane	Insecticide	150	0.5	0.0008 (late 1980s)	0.2	Most U.S. use ended in 1983. Banned in United States in 1988 and in Canada in 1985. Use currently being phased out in Mexico. Banned in many other countries as well.
DDT	Insecticide	50	0.5	0.011 (late 1980s)	2	Banned in United States in 1972. Canada phased out most uses in 1970s and banned it in 1985. Still used in many developing countries for malaria-carrying mosquito control, but not for agriculture.
Dieldrin	Insecticide	5	0.05	0.0025 (late 1980s)	5	Banned on food crops in United States in 1974. All other uses banned or voluntarily suspended in late 1980s. Restricted or banned in many other countries as well.
Hexachlorobenzene (HCB)	Fungicide	80	0.02	0.0006 (late 1980s)	3	Banned in the United States in 1975. Also banned in many other countries.
Lindane	Insecticide	470	1.6	0.05	3	Most uses banned in United States, but use still permitted on a small number of vegetables.
Mirex	Pesticide and flame retardant	70	0.2	NA	NA	Banned in United States in 1978.
Polychlorinated biphenyls (PCBs) (A group of more than 200 related compounds)	Electrical insulator, heat exchange fluid, plastic softener, and component of coatings and sealants	5	0.02	0.00003 (late 1980s)	0.2	Banned in United States in 1977. Banned in many other countries as well.
Polychlorinated-dibenzo-dioxins and -furans (commonly referred to as dioxins)	None. Unwanted byproduct of some types of organochlorine manufacture, and incineration.	0.0006	0.000001	0.0000001	10	NA
<b>Other Chemicals</b>						
Bisphenol A	Components of polycarbonate plastics, and some epoxy coatings and dental sealants.	50,000	50	0.1	0.2	Common industrial and commercial chemical
Nonylphenol	Component of some detergents and coatings, and "inert" carrier fluid in some pesticides.	250	NA	0.2	NA	Common industrial and commercial chemical
Butyl benzyl phthalate	Phthalates are used as plastic softeners, and additives in some makeups, soaps and fragrances.	159,000	200	25	13	Common industrial and commercial chemical
Methoxychlor	Insecticide	5,000	5	0.0003	0.006	Usage increased after ban on DDT. However, methoxychlor's "registration" (its federal permit to be sold and used) was suspended in January 2000.
Dicofol	Insecticide	120	0.4	0.08	20	Still in use
Endosulfan	Insecticide	600	6	0.02	0.3	Still in use

Sources: see Appendix D.

There are also a number of natural chemicals in fruits, grains, and vegetables that have been found to have hormonal activity. Humans and animals are exposed to many of these chemicals through the foods they eat. A number of these plant chemicals mimic the action of estradiol and are known as phytoestrogens. A few of the many phytoestrogens are listed in Table 2 along with estimates of their prevalence in various foods. There is also evidence that genistein and daidzein can under some circumstances inhibit the production of thyroid hormones.<sup>74</sup>

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There are also a number of natural chemicals in fruits, grains, and vegetables that have been found to have hormonal activity.

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<b>Table 2: Plant Chemicals With Hormonal Activity</b>		
<b>Chemical</b>	<b>Sources</b>	<b>Food content*</b>
Genistein	Soy and other legumes; small amounts in cabbage and other cruciferous vegetables	Tofu: 27–52 mg Soy Milk: 5 mg Kidney Beans: 0.03–0.9 mg
Daidzein	Soy and other legumes	Tofu: 10-20 mg Soy Milk: 1 mg Kidney Beans: 0.01–0.07 mg
Naringin	Grapefruit	Grapefruit Juice: 17–136 mg
Quercetin	Fruits, vegetables, black and green tea	Onion: 48–83 mg Apple: 3.6–12 mg Red wine: 0.7–2.7 mg
Secoisolariciresinol	Grains, berries, seeds, nuts, black and green tea	Flaxseed: 140 mg Strawberries: 2 mg

\* All values are in milligrams of phytoestrogen per 6-ounce serving. Kidney bean and strawberry values are based on dry weight. Sources: see Appendix D.

## D. Identifying Hormonal Activity of Chemicals

Evidence that some chemicals have the potential to alter endocrine function prompted researchers to begin broader screening of chemicals for hormonal activity. Scientists use a set of laboratory screening tests called assays as the first step in determining the hormonal activity of a particular chemical. These assays come in two major types, called by their Latin names *in vivo* and *in vitro*. *In vivo* assays are performed on intact living organisms such as rats or mice. *In vitro* assays use cells or tissues from living organisms placed in a nutritive medium called a cell culture.

Chemicals that are found to mimic a natural hormonal effect are called “agonists,” while chemicals that decrease or prevent a natural hormonal effect are called “antagonists.” For example, a chemical that has effects similar to that of estradiol would be called an “estrogen agonist” or simply “estrogenic.” To mark the fact that it is not a natural hormone in human or animal physiology, it would be called an “environmental estrogen” or a “xenoestrogen.” A chemical that blocks the effects of estradiol would be called an “estrogen antagonist” or “antiestrogenic” and would be classified as an “environmental antiestrogen.”

There are several cell-culture assays to determine whether a chemical can act like estradiol. These include (1) the relative ability to bind to the estrogen receptor when compared with estradiol—a so-called relative binding affinity assay, (2) the ability to induce cells to divide and grow, an effect known as cell proliferation, (3) the ability to turn on genes known to respond to estradiol, and (4) the ability to induce the synthesis of proteins whose synthesis is normally induced by estradiol.<sup>75</sup>

Table 3 displays results of cell culture tests of several chemicals currently under study for hormonal activity. The table displays the relative potency (RP) of these chemicals compared to estradiol, the most potent natural estrogen.<sup>76</sup> For example, if estradiol is assigned an RP of 100, then for a chemical with an RP of 0.01, 10,000 times as much of the chemical is necessary to cause the same effect (for example, cell proliferation, or activating a particular gene) as a given amount of estradiol.<sup>77</sup> The table gives a range of values for some of the chemicals. This range reflects the variability of results using different test methods and between different laboratories using similar methods.

As the table shows, there are many chemicals that demonstrate estrogenic activity in cell culture. However, most of these chemicals are very weak hormones when compared with estradiol. Estradiol is hundreds to tens of thousands of times more “estrogenic” than the pesticides and industrial chemicals that have estrogenic activity. The natural plant chemicals are more estrogenic than the synthetic chemicals, but still less so than estradiol.

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## The natural plant chemicals are more estrogenic than the synthetic chemicals.

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The data in Table 3 represent tests of individual chemicals. However, humans and animals are never exposed to only a single chemical in the real world, but are instead exposed to low levels of several different chemicals present in the everyday environment. This has prompted researchers to test the hormonal activity of more than one chemical at a time. There are two concerns regarding the effects of chemical mixtures. First, are the effects of chemical mixtures similar to what would be expected by simply adding together the effects expected from each chemical individually? This would be called an additive effect. However, it is also possible that there could be synergistic effects, in which the hormonal activity of a mixture is much greater than the sum of the individual effects. There could also be antagonistic effects between chemicals, resulting in a smaller overall effect than would be expected from additivity. Second, could mixtures of chemicals generate a hormonal response even when each chemical is present at a level below the threshold dose at which that chemical normally begins to have an effect?

Cell-culture experiments have shown that mixtures of estrogenic chemicals can indeed generate an estrogenic response, in this case inducing cells to grow and divide, even when each of the chemicals is present at a level below the minimum necessary to cause an effect on its own.<sup>78</sup> On the other hand, studies with a wide range of chemicals so far suggest that the overall hormonal effect of mixtures of estrogenic chemicals is additive, rather than synergistic or antagonistic, including at least one study in live animals, rather than in cell culture.<sup>79</sup> This is a relatively new area of investigation that will require additional studies, particularly with live animals, before firm conclusions can be drawn.

Although chemicals with estrogenic or anti-estrogenic properties are among the most heavily studied, some foreign chemicals can affect other hormone systems as well. For example, p,p'-DDE, although not estrogenic, is a potent anti-androgen. That is, it blocks the action of male sex hormones like testosterone. Likewise, the herbicide linuron and metabolic breakdown products of the fungicide vinclozolin are anti-androgens.<sup>80</sup> Some chemicals can also affect other steroid hormones, as well as thyroid hormones.<sup>81</sup>

<b>Table 3: Potency of Selected Chemicals Relative to Estradiol</b>		
<b>Chemical</b>	<b>Description</b>	<b>Relative Potency Compared to Estradiol</b>
Estradiol	Principal and most potent natural estrogen	100
Estrone	Another form of natural estrogen	4–10
Estriol	Another form of natural estrogen	0.6–25
DES	Banned drug once used as a synthetic estrogen	16–74
Ethinyl estradiol	Drug used as a synthetic estrogen in birth control pills	89–112
Bisphenol A	Industrial chemical	0.002–0.005
Butyl benzyl phthalate	Industrial chemical	0.0003–0.0004
4-nonylphenol	Industrial chemical	0.002–0.003
o,p'-DDT <sup>82</sup>	Insecticide	0.0001–0.002
p,p'-DDT	Insecticide	0–0.0004
p,p'-DDE	Breakdown product of DDT	0–0.0001
Dieldrin	Insecticide	0.0001
Methoxychlor	Insecticide	0.0005–0.003
2,3,4,5,6-Pentachlorobiphenyl (a PCB)	Industrial chemical	0
2',3',4',5'-tetrachloro-4-hydroxybiphenyl (a PCB)	Industrial chemical derivative	0.001
Coumestrol	Naturally present in alfalfa, legumes, and soybeans	0.7–1
Zearalenone	Produced by molds commonly found in grains	0.3–6
Genistein	Naturally present in soybeans and grains	0.01–0.05

Sources: see Appendix D.

Results of cell-culture studies should be interpreted with caution because they provide information outside the context of a living organism. For example, the relative binding affinity assay determines whether a chemical binds to the estrogen receptor, but not whether it is an agonist or an antagonist.<sup>83</sup> Tests of a chemical's ability to induce cells to divide and grow can give different results depending on the type of cells used and the specific conditions of the cell culture. Test results can vary based on the density of cells in the culture, or due to differences in the nutritive medium in which the cells grow.<sup>84</sup>

Hormonal activity in cell culture shows that a chemical has the potential to exhibit hormonal effects in a living organism, but several additional factors determine whether a chemical will actually have a physiological effect in a live animal. When a chemical is ingested, it has to make it into the circulatory system from the digestive tract in order to have an effect, which is not possible for some chemicals.

Mammals also have a so-called “first-pass” metabolism system in the liver, that can break down some chemicals before they can make it into the blood stream.<sup>85</sup> On the other hand, some chemicals are not hormonally active themselves, but are converted into hormonally active substances through metabolic processes.<sup>86</sup>

In the blood, chemicals will have different abilities to bind to hormone transport proteins, which will affect the availability of the chemical to exert a hormonal effect, and could also affect the availability of natural hormones.<sup>87</sup> Some chemicals, such as the persistent organochlorines, can remain in the body for years, while others are removed rapidly in urine or feces, giving them less chance to have an effect. Cell-culture studies also do not provide information on what dose of a chemical might be necessary to cause an adverse effect. Because the detailed mechanisms of hormone action are only partially understood, there probably are also other factors that affect the ability of a chemical to alter endocrine function. Hormonal activity observed in cell-culture studies must therefore be confirmed by studies of living organisms.<sup>88</sup>

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Researchers have developed a number of tests to study potential hormone-altering effects of foreign chemicals in living organisms. For example, the uterotrophic assay determines whether female rodents fed a given chemical develop a larger uterus when compared with animals that are not fed the chemical. Other tests assess changes in the growth of other hormone-responsive organs, such as the prostate, or changes in the production of certain proteins that are normally influenced by the action of hormones.<sup>89</sup>

As noted earlier, toxic effects can vary depending on the species of animal tested, and can also vary depending on where in an organism one looks. For example, the drug tamoxifen is estrogenic in the uterus, but anti-estrogenic in breast tissue.<sup>90</sup> Cell-culture tests can be similarly ambiguous. For example, in the presence of estradiol, low concentrations of a weakly estrogenic chemical could add to the activity of estradiol, increasing the estrogenic effect. However, high concentrations of the same weakly estrogenic chemical could block the activity of estradiol by competing with estradiol to bind to a limited number of estrogen receptors.<sup>91</sup> A battery of tests, both in cell culture and in living organisms, may be necessary to characterize the potential hormonal effects of chemicals because of the limitations of each individual test.

## Part 3

# Exposure to Hormonally Active Chemicals

This section summarizes human and wildlife exposure, as well as environmental levels of four groups of chemicals:

1. Persistent organochlorines;
2. Other pesticides and industrial chemicals;
3. Synthetic pharmaceutical hormone-mimics and natural hormones; and
4. Plant chemicals with hormonal activity.

Long-term trend data are available for virtually all chemicals in the first group and a few in the second group, but we will be limited to discussion of current exposure measurements for other chemicals.

## A. Exposure to Persistent Organochlorines

In general, worldwide levels of persistent organochlorines in the environment and in the bodies of animals and humans have decreased substantially since their peaks in the 1960s and 1970s, though there remain some animal populations and locations where levels remain high enough to threaten health. The declines are due mainly to restrictions or bans, since the 1970s, on the use of these chemicals, and restrictions on the manner in which the chemicals are disposed.<sup>92</sup>

***Trends in Wildlife Exposure and Levels in the Environment.*** Researchers have been measuring levels of several organochlorines in wildlife and waterways since the 1960s. Chemicals measured include PCBs, dioxins, DDE, DDT, hexachlorocyclohexane, hexachlorobenzene, toxaphene, and chlordane. PCBs are a group of industrial chemicals that had many uses, but were used mainly as an insulating fluid in transformers and as a heat-exchange fluid in cooling systems. DDE is a persistent breakdown product of DDT. Dioxins are an unwanted byproduct of some manufacturing processes and of incineration. The rest of these chemicals are pesticides. They have been measured in a wide range of environments, mainly in Europe and North America, and parts of Asia.

Among environments and species exposed to persistent organochlorines, levels declined by 30 to more than 99 percent between the 1970s and the 1990s in most environments and species that have been measured.<sup>93</sup> Here are a few specific examples:

- In North America, DDT levels in waterfowl across the United States declined by 85 to 99 percent between 1966 and 1985.<sup>94</sup> PCBs and several organochlorine pesticides declined by 30 to 99 percent in a number of Great Lakes fish and bird species between the 1970s and 1990s. Pesticide concentrations in Great Lakes waters and sediment also declined significantly.<sup>95</sup> Some, though not all, Great Lakes bird species have recovered, concomitant with declines in organochlorine levels.<sup>96</sup>
- In Europe, studies of Baltic Sea porpoises showed declines of 75, 65, and 65 percent, respectively, in DDT, PCBs and dioxins between 1978 and 1990.<sup>97</sup> A study of organochlorines in fat tissue of pigs and cows in Sweden measured declines of 4 to 17 percent per year between 1991 and 1997.<sup>98</sup> DDE and dieldrin levels in otters in southwest England declined by more than 90 percent between 1988 and 1996, while PCB levels declined by about 80 percent. Dieldrin had been implicated in the decline of the otter population and was heavily restricted in 1981 and banned in 1989. English otters have now recovered concomitant with the declines in dieldrin levels.<sup>99</sup>
- In Asia, DDT and PCBs declined by 95 and 50 percent, respectively, in female northern fur seals near Japan between the 1970s and 1980s.<sup>100</sup>

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Although there has been a general decline in persistent organochlorines, this is not true of all species or environments. Animals at the top of the food chain, such as sea mammals and fish-eating birds, are at particular risk, because they consume fish that might themselves have accumulated pollutants over time through ingestion of contaminated animals and absorption from water.<sup>101</sup> For example:

- PCBs and DDT levels in Greenland walrus were constant between 1978 and 1988, though previous studies had found declines during the 1970s. Levels of dieldrin, hexachlorocyclohexane and toxaphene increased by 50 to 200 percent in females, but not males during the study period.<sup>102</sup>
- Although DDT levels in dolphins near southern Spain declined by 50 percent between 1984 and 1996, PCB levels stayed the same.<sup>103</sup>
- Levels of DDT and PCBs in whales near Japan were steady between 1979 and 1986.<sup>104</sup>

***Trends in Human Exposure.*** A number of countries collect trend data on human chemical exposure by measuring chemical levels in food, human tissue samples, and in breast milk. The chemicals under study for hormonal effects tend to dissolve in fats and oils, rather than in water. As a result, they are usually found in the body in fatty tissue and in breast milk, which has a high fat content.

Human chemical exposure data generally show large declines in persistent organochlorine levels between the 1970s and 1990s. For example, the federal Food and Drug Administration (FDA) has performed its Total Diet Study (TDS) over the last few decades to assess average daily intake of a number of synthetic chemicals, mainly pesticides. TDS data show that organochlorine pesticide intake through food, the main route of exposure, declined by 50 to more than 90 percent for dieldrin, chlordane, DDT, hexachlorobenzene, methoxychlor, and pentachlorophenol between 1982 and 1991.<sup>105</sup> PCB and dieldrin intake declined by 95 percent between 1971 and 1987.<sup>106</sup> Typical exposure to persistent organochlorines was well below EPA and WHO safety limits (see Table 1).<sup>107</sup> A Total Diet Study in England also found substantial reductions

in human organochlorine intake. For example, PCB and dioxin intakes both declined by about 60 percent between 1982 and 1992.<sup>108</sup>

Along with an overall reduction in ingestion of organochlorines through food, these chemicals are also detected much less frequently in food samples. For example, in 1970 DDT was detected in 33 percent of food samples, but in only 4 percent by 1981. Likewise, PCBs were detected in about 50 percent of samples in 1970, but only 10 percent by 1981.<sup>109</sup> A study of hexachlorocyclohexane levels in food in China found reductions of 80 to more than 99 percent in various foodstuffs between 1978 and 1992.<sup>110</sup>

Another way to measure trends in human exposure to chemicals is to measure concentrations in blood, breast milk or body tissues such as adipose (fat) tissue, where many of these chemicals accumulate. The National Human Adipose Tissue Survey (NHATS) gathered data on the prevalence of various chemicals in a representative sample of people in the United States.

NHATS found that dieldrin, hexachlorocyclohexane, and DDT levels in human fat tissue declined by 60, 70 and 80 percent, respectively, between 1970 and 1983. Hexachlorobenzene and chlordane were unchanged, although levels of these two chemicals were both very low to begin with. Toxaphene was undetectable throughout the study period. A similar study in Canada found that DDT levels declined by more than 95 percent.<sup>111</sup>

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Human chemical exposure data generally show large declines in persistent organochlorine levels between the 1970s and 1990s.

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NHATS also measured PCB levels in human adipose tissue. The fraction of people with a PCB concentration greater than one part per million decreased from 70 percent in 1972 to 5 percent in 1983, indicating a substantial decline in human PCB exposure.<sup>112</sup> Other studies have also found large decreases in PCBs. For example, a recent study of human exposure to PCBs and dioxins in Western Europe found that exposure had dropped by 50 percent between the early and late 1990s.<sup>113</sup>

A number of studies have looked at trends in persistent chemicals in breast milk:

- A review of worldwide trends in DDT levels in breast milk found large reductions in most parts of the world, most likely due to bans or restrictions on DDT use. On average, DDT levels in breast milk declined by about 80 percent within eight years after restrictions went into effect. DDT levels declined even in tropical areas of South America and Asia where DDT is still used to control the mosquitoes that spread malaria, though the declines were more shallow than in North America and Europe.<sup>114</sup>
- A study of levels of eight organochlorine chemicals in Canada found declines of 45 percent to 95 percent between 1967 and 1992. PCB levels rose between 1970 and 1982 and then dropped back to just above the 1970 value by 1992.<sup>115</sup>
- A study in northern Germany found that PCB levels declined by 60 percent, while organochlorine pesticides declined by 80 to 90 percent between 1986 and 1997.<sup>116</sup> Another German study found that dioxin levels declined by more than 30 percent between 1987 and 1994.<sup>117</sup>
- A review of several studies in Sweden found that PCB levels declined by 70 percent between 1972 and 1997 while dioxins declined by more than 75 percent. Levels of several organochlorine pesticides, including DDT, DDE, dieldrin and hexachlorobenzene, declined by 85 to 95 percent between the late 1960s and 1997.<sup>118</sup>

Although persistent organochlorines have declined in most human populations that have been assessed, certain people are still at risk for high exposures. For example, people who consume large amounts of contaminated seal or whale blubber, such as some Eskimos, and people who eat significant amounts of fish from contaminated rivers or lakes have higher exposures than the general population.<sup>119</sup> Nevertheless, there appears overall to have been a substantial decline in human exposure to persistent chemicals in large areas of the world during the last 25 years.

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Although persistent organochlorines have declined in most human populations that have been assessed, certain people are still at risk for high exposures.

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## B. Exposure to Pesticides and Industrial Chemicals

Dozens of chemicals are suspected of having hormonal activity based on laboratory tests and an exposure assessment for all of them is beyond the scope of this paper. This section instead summarizes exposure data for a few of the most common among these chemicals. Trend data exist for only a few chemicals, mainly pesticides, while only current exposures can be estimated for other chemicals.

**Dicofol.** Dicofol is an insecticide used mainly to protect cotton and citrus crops and, less often, various fruits and vegetables from insect pests. Although chemically related to DDT, dicofol is less persistent in the environment, having a half-life<sup>120</sup> of weeks to months.<sup>121</sup> Based on TDS data, dicofol exposure increased by about 50 percent between the early and late 1980s. Daily exposure during the late 1980s was estimated to be 0.008 mcg/kg b.w./day, which is 98 percent lower than the EPA Reference Dose.<sup>122</sup> In a more recent assessment, EPA estimated typical human exposure to dicofol to be 0.08 mcg/kg b.w./day—10 times greater than the FDA Total Diet Study estimate, but 80 percent less than the EPA Reference Dose.

**Methoxychlor.** Use of this insecticide increased after DDT was banned in 1972. Although chemically similar to DDT, it has relatively low toxicity and a half-life of one week to three months in the environment.<sup>123</sup> The FDA TDS found that methoxychlor exposure through food declined by 75 percent between the early and late 1980s. Typical exposure through food in the late 1980s was estimated to be 0.0003 mcg/kg b.w./day—more than 10,000 times less than the EPA Reference Dose. Presumably as a result of low exposure levels and its lack of persistence, methoxychlor was undetectable in fat tissue of people tested by NHATS throughout the 1970s.<sup>124</sup>

**2,4-D.** 2,4-D is an herbicide used in forest management and in a number of common home and garden herbicide products. It has relatively low toxicity, a half-life in the environment of about one week, and in humans and animals of 10 to 20 hours.<sup>125</sup> Based on FDA's TDS, typical human exposure to 2,4-D through food was 0.0001 mcg/kg b.w./day in the late 1980s. Another study estimated 2,4-D exposure through food for young children at 1.3 micrograms per day, or about 0.14 mcg/kg b.w./day for a 20-pound child.<sup>126</sup> However, there is greater potential for 2,4-D exposure from residential use. A two-year study of seven homes in Ohio found that regular users of herbicides containing 2,4-D can track the chemical into their homes.<sup>127</sup> The study found a maximum 2,4-D exposure in young children of about 7.6 micrograms per day, or 0.84 mcg/kg b.w./day for a 20-pound child. Median exposure was only about 1.1 micrograms per day, much less than the maximum exposure. Combining dietary exposure and the maximum exposure after herbicide application results in a total maximum exposure of about 9 micrograms per day, or

1 mcg/kg b.w./day. This is 90 percent lower than the EPA Reference Dose of 10 mcg/kg b.w./day. This exposure level occurred only during the few days immediately after application of the herbicide. Total 2,4-D exposure at other times averaged 1.4 micrograms per day, or about 0.16 mcg/kg b.w./day for a 20-pound child—about 98 percent lower than the Reference Dose.

**Chlorpyrifos.** Chlorpyrifos is one of a class of insecticides called organophosphates that are widely used in agriculture and in residential pest control. Chlorpyrifos is not persistent, having a half-life of a few days in humans and one to three months in the environment.<sup>128</sup> A recent risk assessment by EPA found that children aged one to six are on average exposed to about 0.024 mcg/kg b.w./day, while the general population is exposed to 0.01 mcg/kg b.w./day. Children's exposure is about 20 percent lower than the EPA Reference Dose, and 1,200 times lower than the NOAEL from animal studies.<sup>129</sup>

**Bisphenol A.** Bisphenol A (BPA) is used to make polycarbonate plastics for a wide range of products, including drink containers, household appliances, and automotive and electrical parts, and to make lacquers used to coat the insides of metal food cans. BPA is also used in some dental sealants. A number of recent studies have found that BPA can in some cases leach from the walls of food and drink containers into food, and can leach from dental sealants into saliva.

A study of BPA concentrations in canned vegetables found that BPA ranged from 0 to 33 micrograms per can. Canned peas and artichokes had the highest levels (an average of 23 and 19 micrograms per can, respectively), while BPA was undetectable in canned tomatoes and asparagus.<sup>130</sup> Another study measured BPA in canned fish, soda, and concentrated liquid infant formula. BPA was not detected in infant formula or cola cans. Canned tuna in oil was found to contain roughly 11 micrograms of BPA, while anchovies ranged from 750 to 3,500 micrograms (0.75 to 3.5 milligrams) per can.<sup>131</sup>

The worst-case ongoing exposure based on these results would be 33 micrograms per day for someone eating one can of food with the liquid each day.<sup>132</sup> This would be a dose of about 0.5 mcg/kg b.w./day for a 60-kg (132-pound) adult. Typical exposures would probably be considerably less than this. The worst-case exposure level is 99 percent less than the EPA Reference Dose. Nevertheless, as we will see later, some recent studies have reported endocrine effects of BPA in rodents at doses as low as 2 mcg/kg b.w./day, which is relatively close to the worst-case exposure level. A recent study of BPA exposure reported a typical human exposure of 0.1 mcg/kg b.w./day, with a worst-case level of 1 mcg/kg b.w./day.<sup>133</sup> These numbers are consistent with the estimates of adult BPA exposure from food sources discussed above.

BPA can also be released from dental sealants. The estimated exposure level is 90 to 931 micrograms in the first hour after the sealant is applied, or a one-time dose of 1.5 to 16 mcg/kg b.w.<sup>134</sup> This is greater than lowest dose found in some studies to cause endocrine effects in the offspring of pregnant rodents, though in the rodent studies, animals were dosed for several days during critical periods of fetal development, while dental sealants would presumably supply a one-time exposure.

Many baby bottles are made from polycarbonate plastics, creating the possibility that BPA could leach from these bottles into infant beverages. A number of studies have assessed this possibility with widely varying results. A study by researchers at the British Ministry of Agriculture, Fisheries and Food of 24 different brands of baby bottle found that BPA did not migrate into liquids placed in the bottles, even after the bottles were sterilized by washing at high temperature.<sup>135</sup> A study of five baby bottles by researchers at the Japanese National Institute of Health Sciences found that BPA was undetectable in four samples, and reached a level of 0.8 micrograms per kilogram of liquid in a fifth sample.<sup>136</sup> A study by researchers from the FDA found that, under normal-use conditions, BPA levels in formula ranged from undetectable in one experiment to 2 micrograms per kilogram of liquid in a second experiment.<sup>137</sup> The latter level would expose a bottle-fed infant to roughly 0.1 mcg/kg b.w./day of BPA—500 times less than the EPA Reference

Dose and 95 percent less than the lowest dose found to have endocrine effects in animal studies. *Consumer Reports* also studied leaching of BPA from baby bottles when formula was sterilized by heating it in the bottles and found a similar BPA leach rate.<sup>138</sup>

Two other studies have found that, while new baby bottles leach little or no BPA, some heavily used and scratched baby bottles can leach greater amounts. These studies suggest worst-case BPA exposure to infants could be as high as 1 mcg/kg b.w./day of BPA.<sup>139</sup> An FDA analysis found similar worst-case results.<sup>140</sup> It thus appears that typical and worst-case BPA exposures may be similar for adults and formula-fed infants.

BPA has also been detected in some rivers and in drinking water supplies. A study in southern Germany found that BPA levels in drinking water ranged from 0.0003 to 0.002 micrograms per liter.<sup>141</sup> The high end of this range would translate into a dose of roughly 0.0002 mcg/kg b.w./day<sup>142</sup>—hundreds of times lower than exposures through food. Studies of BPA in German rivers found levels ranging from 0 to 0.7 micrograms per liter, with one study finding the median level to be 0.02.<sup>143</sup>

Recently, two groups of researchers were able to measure for the first time actual BPA levels in human umbilical cord blood from babies shortly after birth. BPA levels in umbilical cord blood represent the degree of BPA exposure to the fetus at the time of measurement. Levels ranged from 0 to 3.1 micrograms of BPA per kilogram of blood.<sup>144</sup> The question for assessing risk is what BPA dose to the pregnant mother results in the measured BPA levels in the fetus? It's not clear whether doses in the range of a few tenths of a mcg/kg b.w./day—the estimated range of human BPA exposure—could result in the measured BPA levels in umbilical cord blood. If not, then there would have to be other sources of previously unrecognized BPA exposure. On the other hand, it is also possible that the BPA exposures estimated above are sufficient to result in the measured levels in umbilical cords. This is an area for future research.

**Phthalates.** Phthalates include a number of related chemicals used as plastic softeners in a wide variety of consumer products, including vinyl flooring, detergents, cosmetics, food packaging and plastic toys, as well as in some types of medical equipment.<sup>145</sup> Phthalates are not persistent and are rapidly eliminated from the body.<sup>146</sup> A recent study by the Centers for Disease Control measured levels of phthalate metabolic breakdown products in the urine of 289 randomly selected people. Based on the measurements of urinary levels, the average person is exposed to phthalates at a level 97 percent less than the EPA Reference Dose.<sup>147</sup>

A small percentage of people were found to be exposed to phthalates at levels slightly greater than the Reference Dose. For example, the person with the highest exposure to dibutylphthalate (DBP) exceeded the Reference Dose by 17 percent, with an estimated exposure of 117 mcg/kg b.w./day. However, this exposure level is more than 3,000 times less than the NOAEL for endocrine effects in rodent toxicity studies with DBP.<sup>148</sup> The person with the highest exposure to diethylhexylphthalate (DEHP) exceeded the Reference Dose by 80 percent, with an estimated exposure of 38 mcg/kg b.w./day. However, this exposure level is about 40,000 times less than LOAEL for endocrine effects in rodent toxicity studies.<sup>149</sup> The results suggest that even the few people exposed to phthalates at levels slightly greater than the Reference Dose are nevertheless nowhere near exposure levels that could have an endocrine effect. In addition, because the measurements were taken at a single point in time, it is unclear whether the highest measured exposures represent one-time events, or ongoing elevated phthalate exposure.

**Alkylphenols.** Alkylphenols are used in a wide range of consumer products, including detergents and spermicides, and are also used as an inert carrier in some pesticide sprays. Nonylphenol is the most common among this class of compounds. Exposure data are sparse, but a recent study reported estimates of nonylphenol exposure ranging from 10 micrograms per day on average, to a worst-case exposure of 160 micrograms per day.<sup>150</sup> These exposures translate into daily doses of 0.2 and 2.7 mcg/kg b.w./day,

respectively, for a typical adult woman. The worst-case exposure is 99 percent lower than the NOAEL determined from animal toxicity studies.<sup>151</sup> There is no EPA Reference Dose for nonylphenol.

Alkylphenols enter river water after being discharged in waste from sewage treatment plants. A study of rivers in southern Germany found nonylphenol levels ranging from 0.006 to 0.14 micrograms per liter.<sup>152</sup> Nonylphenol in drinking water ranged from 0.002 to 0.015 micrograms per liter. The high end of this range translates into a dose of roughly 0.002 mcg/kg b.w./day—a negligible exposure level.

Nonylphenol has also been detected in rivers and lakes in the United States. Measurements in several areas of Lake Mead in Nevada and the Detroit River in Michigan found nonylphenol levels ranging from zero up to 1.2 micrograms per liter.<sup>153</sup>

A study of airborne nonylphenol levels in the New York-New Jersey harbor region, found average nonylphenol levels ranging from 8 to 25 nanograms per cubic meter.<sup>154</sup> The high end of this range would result in inhalation of about 0.12 micrograms per day, or 0.002 mcg/kg b.w./day—once again, a negligible exposure level.<sup>155</sup>

### C. Exposure to Natural Hormones and Synthetic Pharmaceutical Hormone-Mimics

A number of estrogens, androgens, and other steroid hormones are naturally present in meat and dairy products. Whole milk is by far the largest source, and can provide up to 0.25 micrograms of estrogens in an 8-ounce glass.<sup>156</sup> This is a significant percentage of the estimated amount of estrogens adults produce internally each day. For children, estrogens from milk might even exceed the amount produced internally, though childrens' estrogen production is uncertain.<sup>157</sup>

Orally ingested natural hormones are generally destroyed before entering the bloodstream, and therefore likely have little or no chance to influence endocrine activity.<sup>158</sup> However, some dairy estrogen is in the form of estrone sulfate, which can enter the body and be converted to hormonally active estradiol. Some researchers have hypothesized that humans' exposure to estrone sulfate from milk has increased markedly during the last century due to new methods of cattle farming that allow cows to produce milk even during pregnancy, when internal hormone levels are high.<sup>159</sup>

Cattle in the United States and several other countries are also generally treated with growth-promoting drugs, including natural estradiol, the testosterone-mimic trenbolone acetate, and the progesterone-mimic melengestrol acetate. A number of studies have assessed whether such treatment increases hormone levels in meat products. Estradiol levels can be up to several times greater in estradiol-treated cattle.<sup>160</sup> Even so, estradiol exposure from meat is still many times lower than exposures through milk and eggs, suggesting that this isn't a potential health risk.<sup>161</sup>

In the case of trenbolone, assessments have found that levels in meat are many times less than FDA regulatory limits when used as directed, and do not exceed these limits even when administered at three-times the approved dose.<sup>162</sup> Melengestrol levels are also below FDA regulatory limits when used as directed. However, levels can slightly exceed FDA limits at three-times the approved dose.<sup>163</sup> Even here, melengestrol exposure from consuming treated meat would be more than 200 times lower than the NOAEL found in animal experiments.

Cattle and other livestock also excrete hormones in their manure and urine. These hormones can end up in streams and rivers due to water runoff from livestock feedlots and manure-treated fields. Few studies have assessed this phenomenon, but it appears that streams receiving livestock runoff can contain up to twice the

amount of hormones found in areas upstream of feedlots.<sup>164</sup> No hormones were detected in drinking-water sources near the runoff-receiving streams, however.<sup>165</sup> A study of synthetic growth-promoting hormones found that they can persist for up to several months in soils treated with hormone-containing manure.<sup>166</sup>

Women excrete natural estradiol as well as the synthetic hormone ethinyl estradiol, used in birth control pills. Both chemicals can make their way into rivers from sewage treatment plants. Though they degrade relatively rapidly in the environment, because they are very potent hormones (see Table 3), even very low concentrations can have an effect on exposed animals.<sup>167</sup>

Studies in German rivers have found levels in river water ranging from undetectable in central and northern Germany, to between 0.0002 and 0.005 micrograms per liter in southern Germany.<sup>168</sup> Concentrations in drinking water ranged from 0.0001 to 0.002 micrograms per liter in southern Germany.<sup>169</sup> A study of various locations in Lake Mead in Nevada and the Detroit River in Michigan found estradiol levels ranging from zero up to 0.003 micrograms per liter, while ethinyl estradiol was undetectable in most areas, but reached 0.0005 micrograms per liter in a few locations. These levels are similar to those in Germany.<sup>170</sup> A study in England found river-water estradiol concentrations to be tens of times greater than in southern Germany or the United States.<sup>171</sup> Neither the United States nor British studies measured drinking water concentrations.

#### **D. Exposure to Hormonally Active Plant Chemicals**

Human exposure to phytoestrogens, that is, estrogenic plant chemicals, varies based on diet. Diets high in soy, such as a typical Asian diet, include roughly 25 to 200 milligrams of phytoestrogens per day (about 0.4 to 3.3 mg/kg b.w./day for an adult woman), while a typical western diet includes only a few milligrams of phytoestrogens per day (on the order of 0.02 to 0.2 mg/kg b.w./day).<sup>172</sup> People who take phytoestrogen-containing supplements can be exposed to phytoestrogens at levels several times greater than would be possible from dietary sources alone.<sup>173</sup> Babies fed soy-based infant formulas are also exposed to high levels of phytoestrogens. Two recent studies estimated that four-month-olds fed a diet of soy-based formula receive a phytoestrogen dose of 3 to 8 mg/kg b.w./day. Genistein and daidzein are the principal phytoestrogens in soy.<sup>174</sup>

## Part 4

# Risks from Hormonally Active Chemicals

The following factors and questions determine the risks to humans and wildlife from hormonally active chemicals:

- **General Plausibility.** Is it possible for some chemicals to adversely affect endocrine function?
- **Low-Dose Plausibility.** Can some chemicals cause adverse endocrine (or other) health effects at the relatively low exposure levels typically encountered in the everyday world, in addition to the higher doses known to be dangerous based on traditional toxicology studies?
- **Observation of Actual Adverse Effects.** To what extent have adverse effects actually been observed in human and wildlife populations? Is there evidence of the agents that caused the observed effects?

## A. General Plausibility of Endocrine Disruption

### 1. Human Evidence

That some foreign chemicals can disrupt endocrine function is without doubt. DES, a synthetic estrogen that is roughly as potent as estradiol, was given to several million pregnant women between 1947 and 1971 in the United States in the mistaken belief that it reduced the risk of miscarriage. The children of these women, who were exposed to DES in the womb, have suffered a number of adverse health effects as a result.

Roughly 50 percent of daughters exposed to DES in the womb exhibit structural abnormalities of the vagina, cervix, and uterus. DES-exposed women are also more likely than unexposed women to have reproductive difficulties, including spontaneous abortion (1.8 times as likely), ectopic pregnancy<sup>175</sup> (8.6 times as likely), and premature delivery (4.7 times as likely). Roughly one in 1,000 exposed women also suffered a rare form of vaginal cancer as young adults—an unusually high rate for this age-group.<sup>176</sup>

Studies of sons exposed to DES in the womb have also found increased rates of abnormalities including undescended or abnormal testes, and epididymal cysts.<sup>177</sup> Some studies have also found lower sperm counts and a high percentage of abnormally shaped sperm, but these did not appear to affect fertility.<sup>178</sup>

The DES experience demonstrates a number of key factors for assessing hormonal effects of chemicals. First, both the total dose and the timing of exposure have a substantial effect on health outcomes. For example, the negative effects of DES were more likely to occur with higher doses, and with exposure earlier

in pregnancy.<sup>179</sup> Second, the DES doses were enormous considering that DES has similar estrogenic potency to estradiol itself. Although DES doses varied a great deal between hospitals, a typical DES dosing regimen started out at 5 milligrams per day around the time the embryo was seven weeks old, and increased by 5 mg every two weeks until the thirty-fourth week.<sup>180</sup> This amounts to a dose ranging from 80 up to 1,000 mcg/kg b.w./day for a 130-pound woman. Even the lower dose is thousands of times greater than doses of DES, estradiol, and ethinyl estradiol found to have physiological effects in animal studies.<sup>181</sup>

The prevalence of abnormal reproductive tracts also increased with earlier exposure in the womb. Eighty-percent of daughters exposed earlier than the ninth week of pregnancy, but none who were exposed after the twenty-second week of pregnancy were affected.<sup>182</sup> The period from three to eight weeks of pregnancy, called organogenesis, is when the basic structures of the body and its organs are forming.<sup>183</sup> Although additional development continues throughout pregnancy, the human embryo and fetus is generally more susceptible to toxic effects during earlier periods of development.<sup>184</sup> Thus, the DES data showing greater effects for exposure earlier in pregnancy support the hypothesis that adverse endocrine effects are more likely during critical periods in the womb. In addition, offspring whose mothers received relatively low doses of DES were much less likely to suffer many of the effects of DES exposure, even when exposed early in pregnancy.<sup>185</sup> This suggests that there could be a threshold below which DES does not induce at least some of the adverse effects seen at higher doses, even during critical periods in the womb.

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The total dose and the timing of exposure have a substantial effect on health outcomes.

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The effects of DES clearly show that foreign chemicals can disrupt human hormone systems, with adverse consequences for the developing fetus. However, it is not clear to what extent the DES results can be generalized to endocrine disruption by environmental pollutants. As noted earlier, both dose and potency determine toxicity. DES is thousands of times more potent in its estrogenic effects when compared with most other chemicals studied for potential endocrine disruption (see Table 3). Furthermore, the doses of DES administered to pregnant women were much larger than typical exposures to environmental pollutants.

## 2. Laboratory Evidence

While there are few confirmed examples of endocrine disruption in humans, scores of laboratory studies have documented the potential for environmental chemicals to interfere with endocrine function in animals and to cause adverse developmental effects.

These toxicological studies are of the randomized controlled variety discussed earlier. That means that some animals are exposed to various doses of a chemical, while another control group receives a fake treatment. Some of these studies also include a positive control group that is exposed to a known potent hormone, such as DES. The positive control group is expected to show the effects of estrogen exposure and thus ensure that the experiment is working properly.

Table 4 summarizes some of the endocrine effects observed in animal studies, as well as the mode of action (where known), and doses necessary to cause each effect. The studies listed in Table 4 represent only a fraction of the chemicals and biological effects that researchers have studied and reported on in the scientific literature. These are all “high-dose” studies in the sense that they used doses much larger than those to which humans or wildlife are typically exposed. As a result, these studies demonstrate the potential for endocrine disruption, but not actual risk at real-world exposure levels.

## B. Extent of Endocrine Disruption from Low Doses of Chemicals

The importance of endocrine disruption for human and wildlife health centers on the potential for hormonally active chemicals to cause adverse health effects at doses well below EPA and WHO safety limits, including exposures that could be encountered in everyday life. The logic behind the low-dose hypothesis is that animal hormone systems require exceedingly small amounts of hormones in order to generate normal endocrine responses. Furthermore, because hormones are often present in the body at high enough levels to generate an endocrine response, the endocrine system is frequently operating at a level above its activation threshold. If this is the case, *any* addition of foreign chemicals that have hormonal effects could inappropriately add to or detract from natural hormonal processes.<sup>186</sup> In addition, as noted earlier, there is evidence that some foreign chemicals can evade maternal and fetal defenses, possibly allowing even low doses to alter endocrine activity during development in the womb.

This section assesses the reality of and potential health risk from low doses of foreign chemicals. “Low dose” is a relative term, but for our purposes, low dose will be taken to mean exposure levels typical of the everyday environment of many animals and/or people. Low-dose studies have focused on a number of chemicals that are widely used in consumer products, agriculture, and industry, in particular bisphenol A, alkylphenols, and phthalates, as well as some pesticides, synthetic hormones developed for use as drugs, and phytoestrogens.

Part 3 and Table 1 provide estimates of human exposures to several chemicals that have demonstrated hormonal activity, along with doses that elicit toxic effects in traditional toxicology studies. The table shows that typical human exposures are low when compared to EPA and WHO safety limits, and very low when compared with levels that caused toxic effects in animal studies. Nevertheless, a number of recent animal studies have reported evidence for biological effects of some chemicals at doses much lower than the presumed NOAEL or LOAEL.

In 1995, Sharpe et al. published the first test for low-dose endocrine effects, in this case with the chemicals butylbenzylphthalate (BBP) and octylphenol (OP).<sup>187</sup> The study found that exposing male rats to BBP and OP in the womb at doses on the order of 100 to 350 mcg/kg b.w./day caused reductions in testes weights and sperm production. While these doses are substantially greater than typical human exposures, they are much lower than what was believed to be the lowest level that might cause observable changes in physiology.

Two independent research groups repeated the experiments of Sharpe et al. (1995) on BBP but failed to find any effect. Sharpe et al. were also unable to reproduce their own results. In addition, several months after their original study was published, the testes weights of the untreated control group animals in that study began to decrease. The decrease was comparable in size to the decrease that was apparently caused by treatment with BBP and OP. The researchers were unable to explain the decline, but noted that it occurred after a change in the water supply to their animal facility. Furthermore, in the repeat study, not only did they fail to find a decrease in testes weights in the animals exposed to BBP and OP, they actually found an increase. Sharpe et al. concluded, “these findings add to growing awareness that endocrine disruption data may be difficult to reproduce in different laboratories or between different studies, although the reason for these inconsistencies remains obscure.”<sup>188</sup>

**Table 4: Selected Studies Showing Laboratory Evidence for Endocrine Disruption**

Chemical (description)	Mechanism	Exposure Period and Type of Animal	Dose Range	Effects (relative to untreated control-group animals)
Estradiol (the principal natural estrogen)	Estrogenic	One generation Rat	0.003 to 4.12 mg/kg b.w./day	Infertility at doses greater than 0.8 mg/kg b.w./day. At 0.015 mg/kg b.w./day delay in male foreskin separation and delay in vaginal opening (both measures of sexual maturation); decreased testes size; uterine enlargement.
DES (drug used as a synthetic estrogen)	Estrogenic	Prenatal days 9 to 16 Mouse	0.00001 to 0.1 mg/kg b.w./day	Infertility in males and females at 0.1 mg/kg b.w./day. Reduced fertility in females at all other doses.
Methoxychlor (insecticide)	Estrogenic Anti-androgenic	One generation Rat	18 to 300 mg/kg b.w./day	Accelerated puberty and reduced fertility in females and reduced sperm count in males at 50 mg/kg b.w./day. Delayed puberty in males at 100 mg/kg b.w./day and reduced growth at 25 mg/kg b.w./day.
Vinclozolin (fungicide)	Anti-androgenic	Prenatal day 14 to postnatal day 3 Rat	100 or 200 mg/kg b.w./day	In males at both doses: Reduced distance between anus and genitals (a "feminizing" effect), and caused hypospadias <sup>*</sup> and formation of vaginal pouches. In females, reduced distance between genitals and anus only in period just after birth.
p,p'-DDE (metabolic breakdown product of DDT)	Anti-androgenic	Prenatal days 14 to 18 Rat	10 or 100 mg/kg b.w./day	Highest dose reduced distance between anus and genitals and increased retention of nipples (which are normally not retained) in males.
Dioxin (contaminant created in some industrial processes)	Aryl hydrogen receptor agonist <sup>§</sup>	Prenatal day 15 Rat	0.00005 to 0.001 mg/kg b.w./day	Decreased distance between anus and genitals in males and females at highest dose. Delayed puberty in females at 0.0008 mg/kg b.w./day and in males at 0.0002 mg/kg b.w./day. Reduced sperm count at 0.00005 mg/kg b.w./day.
Bisphenol A	Estrogenic	One-time dose to immature female rats	37.5 to 150 mg/kg b.w.	Induced cell growth and division in the uterus and vagina (an effect of estradiol) at all doses. Effects increased with increasing dose.
Polychlorinated biphenyls (PCBs) (electrical insulator, heat exchange fluid and other commercial and industrial uses)	Unknown. Hypotheses include (1) competition with thyroid hormone for binding with blood transport proteins, (2) direct damage to thyroid gland, and/or (3) inducing faster metabolic degradation of thyroid hormone	Prenatal day 6 through postnatal day 21 Rat	1, 4, or 8 mg/kg b.w./day	Reduced circulating thyroid hormones. Effect increased with dose and disappeared at age 45 days. 20% and 50% pup mortality, respectively, in the two highest dose groups. Permanent hearing loss in the two highest dose groups. Thyroid hormone replacement therapy eliminated the negative effects.
Lindane (pesticide)	Unknown. Might be indirect effect on endocrine system.	Conception to 67 weeks of age Ewe lambs	1 mg/kg b.w./day	Slightly lower body weight up to 42 weeks of age. 20% reduction in thyroxine levels until 10 weeks of age when they returned to normal.
Pentachlorophenol (pesticide)	Unknown. Might be indirect effect on endocrine system.	Conception to 67 weeks of age Ewe lambs	1 mg/kg b.w./day	Significantly lower body weight throughout experiment. Ongoing 30% drop in thyroxine levels.
Genistein (phytoestrogen in soy and other legumes; small amounts in some vegetables)	Estrogenic	Prenatally through puberty Rat	Approx. 2.5 to 50 mg/kg b.w./day	At highest dose, decreased body weight gain, increased uterus weight relative to body weight, and accelerated puberty; 12% increase in distance between anus and genitals in females at lowest dose (the lowest dose was more typical of genistein levels in a regular diet).
Genistein	Estrogenic	Postnatal days 1 to 5 Mouse	50 mg/kg b.w./day	35% of treated animals developed uterine cancer, compared with no control group animals.

\* Hypospadias is a condition in which the urinary tract opening is displaced away from the tip of the penis to somewhere along the penile shaft.

§ The aryl hydrogen receptor (AhR) is known as an "orphan receptor" because no one knows which natural hormone(s), if any, bind to it. However, dioxin does bind to AhR and the adverse health effects of dioxin are believed to result from this.

Sources: see Appendix D.

Shortly after Sharpe et al. reported their original low-dose results, Nagel et al. (1997) reported that Bisphenol A in doses of 2 or 20 mcg/kg b.w./day, well below the EPA regulatory limit and near the range of typical human exposures, caused increases of 30 and 35 percent, respectively, in the weight of mouse prostate glands for male mice exposed in the womb, when compared with unexposed mice. OP at the same doses had no effect.<sup>189</sup>

In a second study, the same group of researchers also found an atypical dose-response relationship for prostate weight in male mice exposed in the womb to the pharmaceutical estrogen DES at several doses between 0.02 and 200 mcg/kg b.w./day.<sup>190</sup> High doses of estrogen in the womb have been found in the past to decrease prostate size by inhibiting the action of male sex hormones. However, in this study *the lower doses increased prostate size while the highest dose decreased prostate size*. Toxic effects generally don't occur until the dose rises above some threshold, and effects then increase with increasing dose. In this case, DES had opposite effects at low and high doses. This result suggested that even very small increases in hormone levels could cause potentially irreversible changes to the fetus during development. Based on these results, vom Saal and Sheehan (1998) challenged the validity of the traditional toxicological assessment process for chemical hazards.<sup>191</sup> Because these results were unexpected in the toxicology community, and because they could have serious implications for both public health and for the financial health of companies that manufacture products with these chemicals, a number of researchers attempted to confirm the results in repeat studies.

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Ashby et al. (1999) and Cagen et al. (1999) were unable to reproduce the results of Nagel et al. and vom Saal et al. using bisphenol A and DES at low doses.<sup>192</sup> In contrast to Nagel et al. and vom Saal et al., they found no difference in reproductive organ weights between mice exposed in the womb to bisphenol A or DES, and unexposed control mice. These studies also used larger numbers of animals than the original studies in order to increase the statistical reliability of the results. Researchers in Japan and at the Research Triangle Institute in North Carolina have also failed to find effects from low-dose bisphenol A exposure.<sup>193</sup>

On the other hand, two recent studies have found low-dose effects, one with bisphenol A and the other with OP. In the first study, a dose of 50 mcg/kg b.w./day bisphenol A (2.5 times higher than the highest dose in the original study by vom Saal et al., but still much lower than the presumed NOAEL) increased prostate weights in male mice.<sup>194</sup> In the second, a dose of 10 mcg/kg b.w./day of OP reduced the time to puberty by 14 percent in female offspring of pigs. However, this dose had no effect on any other measured parameters in the offspring, including birth weight, genital structure, or semen production.<sup>195</sup>

Because of the controversy and potential importance of low-dose effects, EPA and the National Institute of Environmental Health Sciences convened an independent expert panel called the Endocrine Disruptors Low Dose Peer Review (hereafter, the "Low Dose Panel") to evaluate the available data from low-dose studies.<sup>196</sup> Rather than relying on only published results in the research literature, the Low Dose Panel took the extraordinary step of reanalyzing the raw data from 49 different low-dose studies, and asking the researchers who submitted the data to provide detailed answers to questions about how the studies were

performed. The studies included treatments with chemicals such as bisphenol A and nonylphenol, the pesticide methoxychlor, and phytoestrogens such as genistein, as well as studies of the effects of diet. The Low Dose Panel recently released its report and concluded overall that low-dose endocrine effects have been demonstrated for a number of chemicals, but that it is not clear whether the low-dose effects should be considered “adverse,” or merely to be biological *changes* that can’t be assigned a value label like “bad” or “good.”<sup>197</sup>

Looking at specific chemicals, the Low Dose Panel concluded that there is evidence for effects induced by genistein, methoxychlor, and nonylphenol, but not octylphenol, at doses below the presumed NOAELs for these substances.<sup>198</sup> Methoxychlor showed effects down to a dose of 1 mg/kg b.w./day. This is 80 percent lower than the NOAEL determined in previous toxicology studies.<sup>199</sup> However, it is still hundreds of thousands of times greater than typical human exposures measured in the late 1980s.<sup>200</sup>

For nonylphenol (NP), no effects were observed at a dose of roughly 0.25 mg/kg b.w./day, the lowest dose tested. This is about 90 times greater than the estimated worst-case daily human exposure to NP.<sup>201</sup> The panel concluded that additional research is needed at typical human exposures in order to determine if there is a risk to people from NP. Thus, although the Low Dose Panel concluded that health effects have been demonstrated at doses below the previously determined NOAELs for a few chemicals, the required doses were still well above typical human exposures.

For bisphenol A (BPA), the panel concluded, “there is credible evidence that low-doses of BPA can cause effects on specific endpoints.<sup>202</sup> However, due to the inability of other credible studies in several different laboratories to observe low dose effects of BPA, and the consistency of these negative studies, the Subpanel is not persuaded that a low dose effect of BPA has been conclusively established as a general or reproducible finding. In addition, for those studies in which low dose effects have been observed, the mechanism(s) is uncertain (that is, hormone related or otherwise) and the biological relevance is unclear.”

A study published *after* the Low Dose Panel released its report has added a new wrinkle to the assessment of endocrine effects of BPA. Sakaue et al. (2001)<sup>203</sup> found that doses of BPA of 20 mcg/kg b.w./day up to 200 mg/kg b.w./day decreased sperm production in adult male rats by about 30 percent. There was also suggestive evidence of smaller decreases in sperm production from even lower doses of BPA, but there were not enough animals in the experiment to be statistically certain that this wasn’t a chance effect. The researchers also repeated their experiment and got the same results, lending strength to their findings. Nevertheless, other studies using the same rat strain and larger numbers of rats—which gives them greater statistical reliability—have not found that BPA affects sperm production, either at low doses or at doses tens to hundreds of times greater than 20 mcg/kg b.w./day.<sup>204</sup>

Because some laboratories have been unable to replicate some of the low-dose findings, the Low Dose Panel recommended that studies be undertaken to determine whether the findings can be consistently replicated. In addition, the panel recommended that researchers evaluate whether the observed low-dose effects have any long-term health consequences.

No one is certain what is causing the variability among studies and among different laboratories. Randomized controlled experiments are intended to remove all potential sources of variability from a study in order to ensure that any observed effect can be ascribed solely to the chemical(s) in question. Nevertheless, there might be subtle differences between laboratories, or between experiments in the same laboratory, that could obscure the real effects of the chemicals, or identify effects that occur only under specific conditions. Research has shown that several factors can complicate the interpretation of low-dose studies. For example:

**Diet.** The content of laboratory animals' diets affects the outcome of experiments. Researchers use standardized rodent diets with arcane names like Purina 5001, AIN-76A, or RM-1 when conducting laboratory studies. Many of these diets include soy and alfalfa, which contain phytoestrogens—that is, plant chemicals with estrogenic properties. Both Purina 5001 and RM-1 contain phytoestrogens, while AIN-76A does not. Nevertheless, all three diets cause growth of the uterus—an estrogen-like effect—in a test called the immature rat uterotrophic assay, and each diet has a different effect on the time to puberty in immature rats. Thus, even though one of the diets contains no phytoestrogens, they all induce what appears to be an estrogenic effect in a test explicitly designed to test for estrogenic effects.<sup>205</sup>

The diet fed to pregnant rats can also affect fetuses exposed in the womb. One study found that various organ weights, overall body weights, and time to puberty differed significantly between groups of rodents fed diets containing differing levels of phytoestrogens.<sup>206</sup> Another study found that different diets could also change estradiol levels in fetuses, and growth rates and sex organ sizes after birth. A change in diet had the same effect as treating animals with bisphenol A.<sup>207</sup> Ashby et al.'s attempt to reproduce the low-dose results of Nagel et al. (see above), might also have been affected by differing diets between the two experiments. It turned out that the diet used by Nagel et al. contains more phytoestrogens than the one used by Ashby et al.<sup>208</sup> These results suggest that (1) the outcome of endocrine toxicity studies can be affected by the type of diet the rodents are fed, and (2) changes in diet can cause effects similar in magnitude to the effects of low doses of certain hormonally active chemicals.

Brown and Setchell (2001) also point out that the companies that supply animals for laboratory experiments have fed multiple generations of animals with diets high in estrogenic plant chemicals.<sup>209</sup> These diets result in blood concentrations of phytoestrogens greater than levels previously shown to have a range of physiological effects. Specifically, such diets can reduce or enhance the sensitivity of the animals to a given estrogenic chemical in laboratory experiments. The authors conclude, “studies of hormone-dependent conditions, including animal models of cancer, and investigations of potential endocrine-disrupting compounds may be seriously compromised by high dietary phytoestrogen exposure.”

**Uncontrolled Variability of Some Experimental Parameters.** As noted earlier, Sharpe et al. (1998) found anomalous and unexplained variations in the testes weights of their control group (that is, untreated) animals.<sup>210</sup> Similar anomalies have been seen in other studies. The sources of this anomalous variability in the reproductive parameters of untreated animals needs to be understood in order for researchers to be certain of when they are seeing a real effect in treated animals.<sup>211</sup>

**Influence of Animal Strain.** Like rodent diets, laboratory rodents come in different varieties called strains, which are the result of selective breeding. Different strains have different sensitivities to the effects of toxic chemicals, presumably due to differences in their genetic makeup. As noted earlier, a recent study found a 16-fold variation in susceptibility of various mouse strains to changes in testes weights produced by estradiol. In addition, the study on bisphenol A by the Research Triangle Institute used a rat strain called “Sprague-Dawley” (SD) and found no effects due to bisphenol A exposure. However, other studies have found that SD rats are less sensitive to some estrogenic effects than other rat strains.<sup>212</sup> Thus, the presumed risk of any particular health effect is dependent on the strain of rodent chosen.<sup>213</sup> Differences between strains also make it difficult to compare results from laboratories that test the same chemicals on different strains. There is even some circumstantial evidence that genetic makeup might vary among members of the same strain.<sup>214</sup>

**Position in the Uterus During Pregnancy.** Vom Saal and coworkers have shown that position in the uterus affects the hormonal environment of the mouse fetus.<sup>215</sup> For example, male mice that develop next to two female fetuses (“2F males”) are exposed to about 35 percent more estradiol than male mice next to two male fetuses (“2M males”). 2F males end up with prostate glands that are about 35 percent larger than 2M males. Recall that vom Saal et al. (1998) also showed that very low doses of the drug DES

increased prostate weight, and higher doses decreased prostate weight.<sup>216</sup> These results lend support for the existence of low-dose effects, but also suggest that “natural” variation in hormonal environment in the womb has effects similar to those of low doses of weakly estrogenic chemicals.

***Animal Stress Level.*** In another study of position in the uterus, vom Saal et al. (1990) showed that female mouse fetuses that are located next to other females in the womb have shorter estrous cycles and a shorter distance between their anus and genitals than females located next to males in the womb.<sup>217</sup> However, this effect disappears—that is, the females next to females in the womb look the same as those who are next to male—if the mother is stressed during pregnancy by being placed under floodlights for two 45 minute periods each day for several days. This suggests that changes in stress can cause effects similar in magnitude to those produced by low doses of hormonally active chemicals.

The Low Dose Panel assessed study design issues and concluded that factors such as diet, rodent strain, how the animals are caged (such as alone or in groups, which affects stress levels), and seasonal variation (which can affect the ratio of males to females in offspring) can have a significant influence on the results of low-dose endocrine studies.<sup>218</sup>

The conclusions of the Low Dose Panel leave the issue of the significance of low-dose effects as an open question in need of further study. Most of the “low-dose” effects validated by the panel involved doses that are still well above typical human exposures. For doses near the level of typical human exposures, “natural” factors such as diet, stress, and, for mice, position in the womb appear to have as much influence on study results as the chemicals being tested. This calls into question whether low-dose effects should be considered “adverse.” It is also not clear whether the low-dose effects can be reliably reproduced. Additional carefully controlled research will be necessary to determine whether low-dose effects are generally reproducible, and whether the observed effects should be considered harmful.

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### **C. Effects in Humans and Wildlife at Typical Environmental Exposures**

The preceding discussion shows that many chemicals can cause adverse endocrine effects at high enough doses. In addition, some chemicals can cause observable biological changes at low doses, but the low-dose peer review panel was not sure if these changes are biologically significant—that is, whether they are harmful. The next step is to go out into the world and see if there is evidence for endocrine disruption or other harmful chemical effects at typical environmental exposures. Studies in wildlife and people cannot be done under the controlled laboratory conditions of toxicology studies. Instead, researchers use an additional set of methods that together form the field of epidemiology.

Epidemiological studies attempt to determine if particular health outcomes, such as cancer, endocrine disruption, or asthma, are associated with particular risk factors, such as diet, genetics, smoking, or exposure to environmental pollution. Epidemiologists don’t have the luxury of randomly assigning people to treatment and control groups, and must instead look for associations between health effects and potential causes in natural populations.

One example of an epidemiological study is as follows.<sup>219</sup> Researchers want to find out whether there is an association between exposure to polychlorinated biphenyls (PCBs) in the womb, and lowered performance on neurological and behavioral tests in childhood. Some types of Great Lakes fish are known to be contaminated with PCBs, so the researchers recruit pregnant volunteers from families that catch and eat a significant amount of Lake Michigan sport fish each year. They also recruit another group of pregnant women who live near Lake Michigan but who don't eat Lake Michigan fish, in order to have a group of people less likely to be exposed to PCBs, and to therefore act as a natural comparison group. By this recruitment method, the researchers hope to end up with a group of people who represent a wide range of exposures to PCBs.

To estimate children's exposure to PCBs in the womb, the researchers measure PCB levels in umbilical cord blood of newborns, and in the mother's blood and breast milk. To estimate exposure after birth, they measure PCB levels in the breast milk of women who breastfeed their children. The researchers also perform several neurological tests on the children at birth and over the next several years. These tests include measurements of birth weight and reflexes in the newborns, and tests of behavior, physical coordination, and intellectual performance as the children grow up.

The researchers then look for associations between PCB exposure and results on the neurological tests. But they can't just compare the relationship between PCBs and neurological outcomes directly, because the people in the study were not randomly assigned to treatment and control groups. There could be other differences between people who go sport fishing in the Great Lakes and people who don't. For example, if women from families who go sport fishing are also more likely than other women to drink or smoke, or use medications during pregnancy, then it's possible that a portion or even all of any observed effects in their children are due to alcohol or smoking, rather than to PCBs. Alcohol consumption and smoking are therefore called "confounding factors," or simply "confounders," because they have the potential to create an apparent relationship between PCBs and neurological test results when there might not really be one.

In an attempt to account for these potential confounding factors, the researchers gather information on smoking, alcohol, and medication consumption by the mothers during pregnancy, as well as information on educational backgrounds, incomes, and professions of the parents. Previous research has shown that all of these factors are correlated with child health and development and are therefore potential confounders. Other potential confounders include additional pollutants present in Lake Michigan fish, such as methylmercury and DDT, which might also cause neurological harm. The researchers try to assess exposure to these other chemicals and account for them in their analysis. The researchers try to identify and account for as many potential confounding factors as they can in order to increase the likelihood that any residual association that remains between the neurological test scores and PCB exposure is likely to be due to a genuine cause and effect relationship.

Additional factors can influence the interpretation of the study results as well. For example, at each follow-up period after birth, not all families in the original study can be located for testing, and some no longer wish to participate. This can create bias in the study results because the families that continue to participate might differ in important ways from those that drop out of the study.

After following this group of families for 11 years, the researchers find that the children with the highest PCB exposure are about 3.5 times as likely to be in the "low-normal" IQ range as other less exposed children.

As the above example shows, epidemiological studies are not as definitive as randomized controlled experiments in demonstrating a causal relationship. They are subject to various kinds of confounding and bias because the study subjects are not randomly assigned. Because of the inherent weaknesses of epidemiological studies, experts in the field generally require that several conditions be satisfied before they

accept an epidemiological association between a chemical exposure and a health outcome as representative of a genuine cause and effect relationship.<sup>220</sup>

First, there should be a strong association between the health outcome and the proposed risk factor. For conditions that are either “on” or “off” like cancer, the strength of association is reported as the relative risk. For example, a study might find a relative risk of 1.6 for rectal cancer among people who eat a high-fat diet. This means that the study found a 60 percent increase in risk.<sup>221</sup> In the study of PCBs and intellectual performance discussed above, the relative risk of having an IQ in the low-normal range was found to be 3.5 for children in the high PCB exposure group when compared to other children.

Because epidemiological studies almost always suffer from some degree of bias and/or confounding, epidemiologists don’t consider an association between a risk factor and a health outcome to be strong until the relative risk is significantly greater than one, usually at least 3 or 4.<sup>222</sup> A relative risk of, say, 2 represents a 100 percent increase in one’s chances of suffering a particular condition. Surely, this must be considered a serious increase in health risk. If it’s real, it is indeed a large increase, especially for a common condition like breast cancer or heart disease. But epidemiologists’ criterion for strength of association is not based on *how large* the increase in health risk is, but on *how likely the results of the study are to represent a genuine cause-and-effect relationship*, rather than the spurious result of undetected bias or confounding. High relative risk is key because the likelihood of confounding goes down as the strength of association goes up.

Second, the study methodology should be free from obvious weaknesses. Weaknesses might include bias in the selection of study subjects, significant attrition rates of the study population over time, unreliability of the measures of exposure or of health outcomes, or sample sizes too small to provide a statistically valid result. If there are serious problems with the study methodology it will not be a valid study even if it fulfills the other conditions.

Third, there should be a plausible biological mechanism for how the proposed risk factor causes the disease condition. For example, the fact that PCBs have been found to cause learning deficits in monkeys under controlled laboratory conditions suggests that PCBs could also do the same in closely related species such as humans.

Finally, there should be consistency of results between studies. Because of the pitfalls of epidemiological studies, a single study is almost never sufficient to convincingly demonstrate a causal relationship between a disease and a potential causal factor. Until an association between a risk factor and a disease is shown consistently in a number of different groups of people, preferably using a range of different methods, epidemiologists generally don’t consider a causal relationship to have been definitively demonstrated. These limitations of epidemiological studies should be kept in mind as we review the potential for health effects of low-level chemical exposure below.

## 1. Neurological Effects

Few environmental contaminants have been studied for potential effects on the brain and nervous system at exposure levels encountered in typical human and wildlife environments. PCBs have received the most attention, followed by dioxins and DDT, along with breakdown products of DDT, such as DDE. Laboratory studies in rodents and monkeys indicate that exposure to PCBs in the womb at relatively high doses (greater than 1 mg/kg b.w./day) can cause learning and behavioral deficits. Feeding rodents a diet of fish contaminated with several pollutants, including PCBs, DDT, and DDE, likewise causes impaired learning in offspring. Although the biochemical mechanisms for these effects are unknown, researchers have suggested endocrine disruption, as well as alterations in neurotransmitters, the chemical signaling

mechanism in the brain and nervous system, as possible factors.<sup>223</sup> As noted at the beginning of this paper, endocrine disruption is one among many mechanisms by which adverse health effects can occur. If low-level exposures to some chemicals can cause heretofore unrecognized adverse effects, this would be of concern regardless of the mechanism.

Accidental poisoning incidents in Japan in 1968, and Taiwan in 1979 highlighted the dangers of high doses of PCBs and dioxins in humans. In both cases, rice bran oil with undetected PCB and dioxin contamination was sold to several thousand people who used it for cooking. Exposed people suffered overt toxic symptoms, including headaches, nausea, and a skin condition known as chloracne, a sign of poisoning by organochlorine chemicals.<sup>224</sup> Children born to exposed women suffered lowered IQs, growth retardation, higher levels of bronchitis, and abnormal skin pigmentation.<sup>225</sup> The estimated total PCB dose in the Taiwan incident was about one gram, or roughly 17 mg/kg b.w. for a 130 pound woman.<sup>226</sup> The total dose of dioxins was estimated to be about 150 millionths of a gram or 0.0025 mg/kg b.w.<sup>227</sup>

Studies of the health outcomes of the poisoned women suggest that dioxins, rather than PCBs, might be the main cause of the adverse effects. Studies of women occupationally exposed to PCBs show that these women had much less severe symptoms than the rice oil-poisoned women, even when blood PCB levels were comparable between the two groups.<sup>228</sup> In addition, there was little relationship between blood PCB levels and adverse health effects.<sup>229</sup> Although the dioxin exposure was much lower than the PCB exposure, animal studies suggest that dioxins are 100 to 500 times more toxic than PCBs on a gram-per-gram basis (that is, dioxins are more potent toxicants).<sup>230</sup>

The doses of PCBs and dioxins in these poisoning incidents were very large compared to typical exposures. For example, the PCB dose was about one hundred times greater than the estimated annual PCB exposure for a typical person in the United States in the 1980s.<sup>231</sup> The dioxin levels in the bodies of the exposed women were about 200 times the levels measured in women in North America in the late 1980s.<sup>232</sup> Thus, although these tragic incidents provide information on toxic effects at very high exposures, they don't appear to be directly relevant for assessing the potential health effects of typical human exposures, which are much lower.

More recently, a number of epidemiological studies have assessed whether humans are adversely affected by exposure to PCBs and other persistent chemicals at the relatively low levels encountered in the everyday environment. There are five major studies of the effects on child development of PCB exposure in the womb. Although there are differences in detail between the studies, in general they are all epidemiological studies that attempted to determine whether there is an association between prenatal PCB exposure, and later intellectual and behavioral development. All five studies are of a type known as "prospective cohort" studies, because the same group, or cohort, of children was assessed several times over a period of years from birth onward.<sup>233</sup> Two of the studies drew their study populations from areas around the Great Lakes, including Lake Michigan and Lake Ontario, one from North Carolina, one from Rotterdam and Groningen, The Netherlands, and one from Düsseldorf, Germany. Table 5 summarizes the results of these studies.

As the table shows, the studies found that children who had higher PCB exposures in the womb sometimes performed more poorly on tests of intellectual and neurological development. Where effects were observed, children in the top 5 to 20 percent of PCB exposure generally performed up to several percent worse than less exposed children in the various tests. Although all studies found some deficits associated with PCB exposure, the studies are inconsistent regarding the type and timing of the deficits. In addition, effects sometimes appeared and then disappeared, or vice versa, among the same group of children at different points in time.

<b>Table 5: Summary Results of Human Epidemiological Studies of Neurological Effects of Prenatal PCB Exposure</b>			
<b>Outcome Measure</b>	<b>Age at Measurement</b>	<b>Location of Population Studied</b>	<b>Effect of Greater PCB Exposure</b>
Reflexes	Between one day and two weeks after birth	Lake Michigan	None
		North Carolina	Slower reflexes
		Netherlands	None
		Lake Ontario	Abnormal reflexes
Muscle Tone	Between one day and two weeks after birth	Lake Michigan	None*
		North Carolina	Less muscle tone
		Netherlands	Less muscle tone
		Lake Ontario	None*
Psychomotor Development	6 and 12 months	North Carolina	Delayed
	3 months	Netherlands	Delayed
	42 months	Netherlands	None
	7 months	Germany	None
	30 and 42 months	Germany	Delayed
Mental Development	6 and 12 months	North Carolina	None
	3 months	Netherlands	None
	42 months	Netherlands	Delayed
	7 months	Germany	Delayed
	30 and 42 months	Germany	None
Visual Recognition Memory	7 months	Lake Michigan	Decreased
	7 months	Germany	None
	6 and 12 months	Lake Ontario	Decreased
Short-Term Memory	4 years	Lake Michigan	Decreased
	3, 4 and 5 years	North Carolina	None
IQ	11 years	Lake Michigan	Decreased

\* Mothers' fish consumption, rather than direct measurements of PCB levels, was used as a surrogate for children's prenatal PCB exposure in assessing these outcomes.

Sources: see Appendix D.

Only two studies have so far followed children up to school age. The Lake Michigan study measured children up to age 11 and found that children at the highest prenatal exposure range had IQs 6 points (roughly 6 percent) lower than other children. The North Carolina study followed children through age five and found that associations of test performance with PCB exposure disappeared after the age of two. PCB exposures appear to be roughly similar between the two studies.<sup>234</sup> The other studies have so far followed children up to about the age of four or less, though school-age results should be available in the future.

There are several reasons why the results from these studies should be treated cautiously. First, although all of the studies find PCBs associated with some developmental deficits, there are inconsistencies between them, with some finding effects and others not on any given measure. The size of the effects in all of the studies is also relatively small compared to what would normally be considered a strong effect by epidemiological standards.

Second, there are the conflicting school-age results between the North Carolina and Michigan studies—the only ones so far to assess children at school age. The Michigan IQ test results are also based on only 30 children in the high exposure category, which makes them of questionable applicability to the general population.

Third, with the exception of the test of visual recognition memory,<sup>235</sup> the early childhood test results do not correlate well with performance on later school-age tests, such as IQ tests, so it is not clear what the measured deficits before school age mean for later development.<sup>236</sup> An answer will have to wait until additional groups of children are assessed at school age.

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The extent to which low-level PCB exposure has permanent negative effects on children's development remains unclear, but appears at worst to be relatively small.

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Fourth, the Dutch study found that the association of higher PCB exposure with lower test performance disappeared for children who were breastfed. This might have been due to the breast feeding itself, and/or to the more intellectually stimulating home environment that was found to be associated with women who breastfed. Thus, any effect of higher PCB exposure might be counteracted by breastfeeding or intellectually stimulating parental behavior. As a result of these caveats, the extent to which low-level PCB exposure has permanent negative effects on children's development remains unclear, but appears at worst to be relatively small.

Some of the studies also looked for associations between neurological development and exposure to other chemicals in the womb. The North Carolina study assessed exposure to DDT and DDE. The Lake Ontario study assessed exposure to DDT and DDE, mirex, methylmercury, hexachlorobenzene, and lead. Neither study found an association between exposure to any of these chemicals and neurological test performance. Most of the studies also assessed postnatal PCB exposure through breastfeeding and generally did not find a relationship between postnatal PCB exposure and developmental deficits.

Another factor to consider in assessing current risk from PCBs is the timing of these studies. The children in these studies were born roughly 8 to 20 years ago. As discussed in Part 3 Section A, PCB exposure, as well as exposure to other persistent organochlorines in the environment, has declined substantially during the last 20 years. This suggests that whatever the effects of PCBs or other persistent chemicals on children born in the past, current effects from these chemicals are now lower and will likely continue to decline.<sup>237</sup>

Nevertheless, although the risk to the general population from PCB exposure at current levels appears to be small, there are still some places where people are at risk of PCB exposure at levels that might affect health. For example, Inuit Eskimos, who consume large amounts of seal and whale blubber, as well as people who consume significant amounts of fish from relatively contaminated areas of rivers and lakes, are at risk of greater-than-average PCB exposure.<sup>238</sup>

## 2. Male Reproductive Health

Recent concern regarding endocrine disruption has focused in part on the possibility that environmental contaminants have caused a worldwide decline in human sperm counts. In 1992, Carlsen et al. published an analysis of 61 studies of sperm counts spanning the years 1938 to 1990 and several countries that concluded that sperm counts had declined by more than 40 percent during the period.<sup>239</sup> This analysis became the focus of intense controversy regarding technical aspects of the statistical methodology, potential variation in the way men were selected for inclusion in each of the studies, and potential for other factors (that is, confounding factors) such as geographic variation, changes over time in the age of the men in the study, the percent with proven fertility, and/or their abstinence time before providing semen, to account for the apparent decline in sperm counts.

Swan et al. (2000) attempted to address these concerns with a reanalysis of the Carlsen et al. data and also included additional studies of sperm counts not included by Carlsen et al., bringing the total number of studies to 101.<sup>240</sup> This study concluded that, even after accounting for all of the above concerns, there was still a downward trend in sperm counts between 1938 and 1990 in the United States and between 1970 and the 1990s in northern Europe, but that a paucity of data precluded drawing conclusions regarding other areas of the world.

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If human sperm counts have been falling due to ubiquitous environmental chemical exposures, one would expect similar effects in farm animals.

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Other researchers have argued that analyzing trends in sperm counts is inherently futile because the samples of men who volunteer to donate semen are never representative of the general population, and the degree of bias can vary in different directions at different times and in different places. As a result, any apparent trend in the data is the result of sample bias, rather than of actual trends in sperm counts.<sup>241</sup> For example, a study in Sydney, Australia found that average sperm counts varied by more than a factor of two among five separate groups of sperm donors recruited by the same doctors, at the same hospital, using the same recruitment methods.<sup>242</sup> This study illustrates the danger of extrapolating findings from self-selected volunteers to the general population.

Sperm-count trends in farm animals can serve as an additional check on the validity of reports of sperm-count declines in humans. If human sperm counts have been falling due to ubiquitous environmental chemical exposures, one would expect similar effects in farm animals. In addition, because there are no sociological barriers to semen collection from farm animals, sampling is likely to be more representative of the general farm-animal population than is the case with humans. Studies of trends in farm animals have found that sperm counts have not declined during the last 70 years. The studies assessed bulls, boars, and rams between 1932 and 1995, dairy bulls between 1962 and 1995, and stallions between 1981 and 1996.<sup>243</sup> Sperm counts in rams actually rose slightly during the period studied. Thus, if human sperm counts have declined, it is due to some factor that is not affecting farm animals.

Four additional male reproductive health problems have also received attention in relation to endocrine disruption: undescended testes (known medically as cryptorchidism), hypospadias (displacement of the urinary-tract opening away from the tip of the penis), prostate cancer, and testicular cancer.<sup>244</sup>

Paulozzi (1999) analyzed worldwide trends in rates of undescended testes and hypospadias between the 1960s and 1990s in more than 20 countries based on data collected by the International Clearinghouse for

Birth Defects Monitoring Systems of the WHO.<sup>245</sup> This study found an overall increase in hypospadias in North America and Western Europe up until the mid-1980s when rates leveled off or declined. However, even among these nations, the report found wide variations. For example, rates decreased slightly in Sweden but doubled in Norway and Denmark between the late 1960s and mid 1980s. Some countries and regions, such as Australia, Alberta, Canada, and Atlanta, Georgia, experienced large upward and downward swings within the study period. Hypospadias rates in less affluent regions, such as China and South America, were generally more stable than in the more affluent countries.

For undescended testes, Paulozzi concluded “there is no indication of a generalized increase in cryptorchidism rates over time since 1970, although data on this defect is much more limited...Since 1985, rates in most systems have actually declined.”<sup>246</sup> However, the study did find that rates in the United States appear to have increased.

Prostate cancer is now the most common cancer in men. The incidence of prostate cancer rose by 178 percent between 1973 and 1991 in the United States. Other countries have also seen large increases. However, the apparent rise in prostate cancer appears to be largely the result of improvements in detection, rather than increases in the actual incidence of the disease.<sup>247</sup>

To the extent that real prostate cancer rates have risen, many factors have been implicated as potential contributors. For example, diets high in fat or low in fiber and vegetables are associated with increased risk.<sup>248</sup> Japan has the lowest incidence of prostate cancer in the world. But “westernization” of the Japanese diet since World War II to include more animal fat and fewer grains could be a contributing factor to rising prostate cancer incidence there.

Some researchers have even theorized that milk consumption could be contributing to increases in prostate cancer and other male reproductive disorders through a hormonal mechanism.<sup>249</sup> As noted in Part 3 Section C, unlike milk produced a century ago, modern milk contains high levels of estrone sulfate, which is converted to hormonally active estradiol in the body. However, whether hormones in milk are actually a plausible cause of endocrine-related diseases can be determined only through further research.

Although rare, testicular cancer is the most common cancer in young men. The rate of testicular cancer varies from country to country, but rates increased during the twentieth century in all countries that collected data. Data go back as far as the 1930s for Denmark, the 1950s for Finland, Norway and East Germany, and the early 1960s for many other nations and regions.<sup>250</sup> The reasons for increasing incidence of testicular cancer are unknown.

Trends in sperm counts, undescended testes, hypospadias, and cancer can tell us what is happening, but by themselves cannot provide information on the underlying causes. A number of scientists have proposed endocrine disruption by environmental contaminants as a potential cause for increasing rates of male reproductive health problems.<sup>251</sup> The hypothesis is plausible, as evidence from men exposed to DES in the womb and evidence from laboratory animals tested with several different chemicals shows that chemicals with estrogenic or anti-androgenic activity can interfere with development of the male reproductive tract. However, these effects required doses much higher than would be normally encountered in the everyday world.<sup>252</sup>

A recent study did find that rates of undescended testes, but not hypospadias, were higher in male children of women working in gardening or farming, though the effect was small.<sup>253</sup> However, these women were presumably occupationally exposed to significantly higher levels of pesticides than most people would typically encounter.

To demonstrate a causal link between male reproductive health and low-level chemical exposure, there would need to be studies of the degree of association between exposures to suspect chemicals and male reproductive

health trends in various regions. Such studies have not yet been performed. In addition, two factors suggest caution in presuming that male reproductive health trends are affected by low-level exposure to chemicals.

First, the apparent trends might not be real for at least some of these effects. As noted earlier, the evidence for a sperm-count decline in humans appears to be relatively weak, based on the great potential for biased sampling of semen donors, and the lack of sperm-count decline in several species of farm animal. For other measures of male reproductive health, apparent trends could be caused by changes in diagnosis or reporting over time rather than real changes in incidence. For example, with regard to hypospadias and undescended testes, Paulozzi (1999) notes that several artificial factors could have resulted in the apparent increases.<sup>254</sup> These include changes in definitions to include less severe forms of the conditions, and gradual improvement in the degree to which physicians document cases. It is also possible that criticism in the 1980s of early reports on rates of these conditions prompted stricter and more standardized diagnosis and reporting systems in some regions. This possibility is consistent with the flattening of the trend during the mid-1980s.

A recent study of hypospadias trends in Finland also lends credence to these concerns.<sup>255</sup> Birth registry data suggested a 50 percent increase in hypospadias between the mid-1970s and the mid-1980s in Finland. To assess the accuracy of the registry, the researchers identified everyone who was surgically treated for hypospadias in Finland between 1970 and 1986. They found not only that the rate of hypospadias was constant during this period, but also that the actual rate was about three times higher than the rate suggested by the birth registry.

Second, testicular cancer rates, though clearly increasing, might have begun increasing before the widespread use of synthetic chemicals in industry, farming, and consumer products. Synthetic chemical production was less than a billion pounds in 1920, increased by more than a factor of 20 by 1945, and continued to increase through the second half of the twentieth century.<sup>256</sup> Testicular cancer, though rare, is most common among men in their twenties and thirties. If synthetic chemicals were causing the increase, we would expect to see increasing rates of testicular cancer 25 to 30 years after chemical exposure began to increase. However, data from Denmark already show an increasing trend in the 1930s, Finland, Norway and East Germany in the 1950s, and other industrialized countries in the 1960s.<sup>257</sup> Increases in rates of testicular cancer might thus have in some cases preceded increases in exposure to synthetic chemicals. Testicular cancer could even have been increasing earlier in some or all of these countries, but data are not available to make a determination.

### 3. Female Reproductive Health

Increased lifetime exposure to estrogen is associated with increased risk for developing breast cancer,<sup>258</sup> though it is not known whether increased prenatal exposure to estrogen also increases breast cancer risk.<sup>259</sup> Because of the link between estrogen and breast cancer, some researchers have proposed that estrogenic chemicals might increase the risk of developing the disease.<sup>260</sup>

A number of studies have looked at the link between blood levels of PCBs, DDT, and DDE and breast cancer in the general population, but with mixed results—some studies finding a weak association of these chemicals with breast cancer and most finding no association.<sup>261</sup> However, two studies stand out for their strong epidemiological designs.<sup>262</sup> First, these studies were prospective. That is, the blood samples were drawn long before any of the subjects developed breast cancer. Second, the blood samples were drawn as part of a general physical and not due to a concern regarding cancer risk. Both of these factors reduce the potential for bias in the way the study subjects were selected. Third, there were many years between the time the blood samples were drawn and the follow-up period in which the researchers checked to see who had developed cancer. This allowed the researchers to select large numbers of both cases (that is, those with breast cancer) and controls (that is, those without breast cancer). Including a larger number of subjects improves the ability of a study to detect an effect, should one exist. Both studies found no

relationship between levels of PCBs or DDT and breast cancer. These results are also consistent with studies of women exposed occupationally to high levels of PCBs and DDT.<sup>263</sup>

However, in a follow-up study of the same women, Hoyer et al. (2000) used two measurements of PCBs and DDT in blood samples drawn about five years apart.<sup>264</sup> This study found that women in the top 25 percent for p,p'-DDT exposure were 3.6 times as likely to develop breast cancer as the low-exposure group. There was still no association of breast cancer with PCB exposure. Using two measurements of exposure taken at different times likely provides a better estimate of total exposure than a single measurement, so the results of this study should be considered stronger than the results based on a single measurement.

A third study of the potential link between breast cancer and either PCBs or DDT, although not prospective, stands out for including an extraordinarily large number of subjects, improving the chances of detecting any real effects.<sup>265</sup> In this case, PCB and DDE levels were not associated with an increased breast cancer risk.

Overall, these results suggest that PCBs and DDT do not increase the risk of breast cancer. Vom Saal et al. (1998) have also pointed out the biologic implausibility of the hypothesis that PCBs and DDT could be a cause of breast cancer.<sup>266</sup> DDT is not estrogenic (see Table 3) and PCBs are a mixture of many closely related chemicals, some of which are estrogenic, some of which are anti-estrogenic, and some of which are neither. They conclude that studies of PCBs, DDT and breast cancer do not even test the hypothesis that estrogenic foreign chemicals could increase breast cancer rates.

Breast cancer studies have also looked at the association of other persistent organochlorines with breast cancer. Hoyer et al. (1998) measured blood levels of the pesticides beta-hexachlorocyclohexane (HCH) and dieldrin. There was no association of HCH with breast cancer, but women in the top 25 percent for blood dieldrin levels were about twice as likely to develop breast cancer as women in the bottom 25 percent. A later study by the same research group also found that the likelihood of death from breast cancer increased with higher blood dieldrin levels.<sup>267</sup>

Two studies have assessed whether the pesticide hexachlorobenzene (HCB) is associated with an increased risk of breast cancer. One study found that women with high HCB levels have 1.8 times the risk of developing breast cancer, while the other study found no increase in risk.<sup>268</sup>

Although both dieldrin and HCB levels had a positive relationship with breast cancer in one study each, the strength of the associations is weak by epidemiologic standards. In addition, there is once again a lack of biologic plausibility for dieldrin or HCB exposure increasing breast cancer risk. Dieldrin is an exceedingly weak estrogen (see Table 3), and HCB is not estrogenic.<sup>269</sup> It thus appears that the potential for everyday exposures to estrogenic foreign chemicals to increase the risk of breast cancer has not been properly evaluated.

#### **4. Immunological Effects**

There are few human studies of the effects on the immune system of chemical exposure in the womb or through breastfeeding. The Japanese and Chinese rice bran oil PCB/dioxin poisoning incidents showed that very high doses of these chemicals can result in greater susceptibility to respiratory and ear infections in exposed offspring.<sup>270</sup> Exposed children also had lower blood levels of immune cells.<sup>271</sup> Two recent studies have looked at whether low-level PCB or DDT exposure is associated with immunological effects. The Inuit people in the Canadian Arctic are at greater risk for exposure to organochlorine chemicals due to their high consumption of seal and whale blubber. Persistent organochlorines tend to accumulate in these animals due to their ecological position at the top of the food chain. A study of one group of Inuits found

that among breastfed infants, those in the top third of exposure to persistent organochlorines were about 1.5 times as likely to have ear infections as those in the bottom third. However, the study population might not be representative of other populations because ear infections are 1.5 to 2.5 times more common among Inuit children than among children in industrialized nations. The study also included biochemical measurements of immune function, such as numbers of various types of immune cells, but found no association of these immune measures with organochlorine exposure.<sup>272</sup>

A study in Dutch children found that higher PCB body levels were associated with an increased risk of contracting ear infections and chicken pox. On the other hand, higher PCB levels were also associated with a decrease in the risk of developing allergies. In addition, higher prenatal PCB and dioxin exposures were associated with slight deficits in immune parameters, which could indicate an increased susceptibility to infections. All of these effects appeared to be counteracted by breastfeeding.<sup>273</sup>

## 5. Wildlife Studies

There are a number of cases in which wildlife health effects have been linked specifically to the mechanism of endocrine disruption due to environmental contamination. These studies have typically focused on aquatic environments, including both environments with “background” levels of pollution and “hot spots” such as sites of industrial or sewage discharge or relatively contaminated bodies of water.<sup>274</sup>

One case concerns the fungicide tributyltin (TBT), a constituent of some marine paints, which causes female mollusks, such as snails, to develop a penis and other masculine characteristics—a condition known as imposex. In a species called the dogwhelk, concentrations as low as one-billionth of a gram of TBT per liter (one part per trillion) are sufficient to cause at least some imposex. Local extinction due to female sterility has occurred in areas where concentrations reached six to eight billionths of a gram per liter.<sup>275</sup> At least 150 mollusk species in the wild show some degree of imposex. The mechanism is not known precisely, but the weight of evidence suggests that TBT inactivates the enzyme that converts testosterone to estradiol (which is the normal route of estrogen synthesis), resulting in a buildup of testosterone in females.<sup>276</sup> Some European populations of dogwhelk began to recover after TBT was banned for ships smaller than 80 feet long. Nevertheless, concentrations are still high enough to endanger sensitive species.<sup>277</sup>

Two lines of evidence suggested that some freshwater fish in England were experiencing the effects of endocrine disruption.<sup>278</sup> First, a field study in England documented an unusually high incidence of hermaphroditism<sup>279</sup>—up to 5 percent—in a type of fish called roach living just downstream of a sewage treatment works (STW) wastewater discharge. Second, male fish were found to be producing vitellogenin, an egg yolk protein whose production is stimulated by estradiol. Experiments in which fish were placed in effluents from 28 different STWs confirmed that these effects were widespread. Similar results have been found in rivers in Germany, France, and the United States.

For the majority of the rivers in England, the causative agents appear to be natural estradiol excreted by women, and ethinyl estradiol, a synthetic estrogen in birth control pills, both of which are incompletely removed by STWs. In a few other cases, estrogenic chemicals called alkylphenols, released from industrial plants, are responsible for the effects.<sup>280</sup> Experiments have shown that alkylphenol concentrations need to be more than 1,000 times greater than that of estradiol to induce the same effects, because of their lower potency when compared with estradiol.<sup>281</sup> A recent study of estrogenic chemicals in several lakes and rivers around the United States also found that estradiol and ethinyl estradiol accounted for almost all of the estrogenic potential of the water samples, while alkylphenols accounted for less than 0.5 percent.<sup>282</sup>

Hormone-containing runoff from livestock feedlots could also be causing endocrine alterations in some aquatic animals. For example, a recent study found that female turtles in ponds within cattle farms show

signs of endocrine alteration.<sup>283</sup> Also, as noted in Part 3 Section C, there is evidence that runoff from livestock farms can elevate hormone levels in local streams and rivers by as much as a factor of two.<sup>284</sup> More research is necessary to determine the extent and severity of endocrine effects due to livestock farm runoff.

Additional evidence comes from rivers in the United States that receive effluent from paper and pulp mills. Bleaching of wood pulp releases natural chemicals in the wood called sitosterol and stigmastanol that have estrogenic properties. When compared with fish from uncontaminated sites, fish swimming in so-called bleached “kraft mill” effluent (BKME) are more likely to have altered hormone levels, smaller sex organs, lower fertility and enlarged livers. However, these effects did not appear to be related to the degree to which the BKME was diluted, nor to whether the plant used chlorine bleaching or whether it treated its effluent before releasing it to the river.<sup>285</sup>

Fish-eating birds around the Great Lakes accumulated organochlorine chemicals in their bodies by eating contaminated fish in the 1960s and 1970s. These chemicals, particularly DDT and its breakdown products, as well as dioxins, were likely the cause of high rates of deformities and mortality in chicks.<sup>286</sup> The chemical p,p'-DDE, but not p,p'-DDT or o,p'-DDE, is the likely cause of eggshell thinning in these birds.<sup>287</sup> Fish-eating birds have suffered similar health effects in other contaminated areas of the world.<sup>288</sup> Although some Great Lakes bird species have made dramatic recoveries due to reductions in organochlorine chemicals during the last 20 years, other species continue to decline in the most contaminated locations.<sup>289</sup>

Alligators in Lake Apopka, Florida were found to be experiencing high rates of infertility and genital abnormalities, including male alligators with small penises, and high death rates of hatchlings. These effects were likely caused by a large spill of the insecticide DDT and other pesticides from a nearby chemical plant that contaminated the lake.<sup>290</sup> The chemical p,p'-DDE, a breakdown product of DDT that is persistent in the environment and in animals, is likely the cause of the observed health effects. The chemical p,p'-DDE is anti-androgenic—it blocks the effects of male sex hormones. “Feminized” male alligator eggs at Lake Apopka were found to contain about 5.8 milligrams of p,p'-DDE per kilogram of egg—about 90 times more than necessary to elicit an anti-androgenic response in a laboratory cell culture.<sup>291</sup>

Toxicological and epidemiological evidence also supports this explanation. For example, injecting alligator eggs with a dose of p,p'-DDE of between 3 and 10 billionths of the weight of the eggs caused female characteristics in what would otherwise have become male alligators.<sup>292</sup> In addition, recent studies comparing alligators from lakes with varying levels of chemical contamination also support the hypothesis of high levels of p,p'-DDE as the causal agent.<sup>293</sup>

Epidemiological studies of Baltic Sea seals have found a strong association between reproductive failure and high PCB exposure. Although these seals are now increasing in number, they still show physiological signs of PCB exposure.<sup>294</sup> In addition, “semi-field” studies<sup>295</sup> of harbor seals found that seals fed contaminated fish from the Baltic Sea were more likely to develop reproductive problems and to show symptoms of a suppressed immune system than seals fed uncontaminated Atlantic fish. Once again, PCBs were implicated as the causal agent.<sup>296</sup>

The alligator, seal, and bird studies assessed animals exposed to high levels of organochlorine chemicals that are now banned or restricted, and whose concentrations in most environments are dropping. As a result, many animal populations are recovering, albeit slowly in some cases. The fish and mollusk studies provide evidence of endocrine disruption from chemicals that are still in use, and effects that can occur at low doses, particularly in sensitive species. The effects in fish appear to be mainly due to natural estradiol and synthetic hormones in birth control pills released as sewage treatment waste, with alkylphenols also accounting for estrogenic effects in a few instances.

## 6. Health Effects of Hormonally Active Plant Chemicals

Many food plants, particularly soy, contain chemicals known as phytoestrogens that mimic the effects of estradiol (see Table 2). All people are exposed to some phytoestrogens in their diets. However, diets rich in soy-based foods such as tofu, miso, and soy milk, result in the largest phytoestrogen exposures. A soy-rich Asian or vegetarian diet can include 25 to 200 milligrams per day of phytoestrogens, while a typical western diet includes only a few milligrams per day. Phytoestrogens were shown decades ago to have physiological and hormonal effects when it was observed that sheep that graze on a species of clover called subterranean clover can become permanently infertile—a condition known as “clover disease.” The cause appears to be high levels of phytoestrogens in the clover interfering with sheep reproductive systems.<sup>297</sup>

Epidemiologic studies in humans suggest that phytoestrogens can have a number of beneficial effects when consumed at levels typical in an Asian or vegetarian diet. For example, Asians have a lower risk of breast and prostate cancer than westerners. This lower risk is correlated with high levels of phytoestrogens in blood and urine. Toxicological studies have shown that the phytoestrogens in soy can reduce cancer rates in laboratory animals and can inhibit the growth of cancer cells in cell culture.<sup>298</sup> A study of women who ate 45 grams per day of soy phytoestrogens as part of their diet for a month (a dose of roughly 0.75 mg/kg b.w./day) found increases in the length of the menstrual cycle. Increased exposure to estradiol increases the risk of developing breast cancer and a longer menstrual cycle reduces lifetime exposure to estradiol. The researchers thus concluded that this could be one mechanism by which phytoestrogens might reduce breast cancer risk.<sup>299</sup> Epidemiologic studies also suggest lower risk of other cancers, heart disease, and postmenopausal osteoporosis associated with greater phytoestrogen consumption.<sup>300</sup>

Phytoestrogens have also been found to damage health under some circumstances. A high dose of genistein (50 mg/kg b.w./day), a major phytoestrogen in soy, increased uterine cancer in rats, for example.<sup>301</sup> However this study used a dose many times greater than dietary levels, and also administered the dose by injection, rather than through the diet, which would tend to increase the observed effects.<sup>302</sup> Researchers have also expressed concern regarding high doses of phytoestrogens in dietary supplements. Supplements typically contain several hundred to thousands of milligrams of phytoestrogens, which is several times more than could be consumed through dietary sources. Although the effects of such high doses are uncertain, there is evidence that they could have negative effects, including increasing the risk of cancer.<sup>303</sup>

Not all of the effects of phytoestrogens are hormonal. Phytoestrogens have many physiological effects, complicating understanding of how they work and under what circumstances they are beneficial or harmful. For example, some phytoestrogens have been found to inhibit the action of enzymes necessary for cell growth and division, which could be one of the mechanisms by which phytoestrogens reduce the risk of cancer—a disease characterized by uncontrolled cell growth.<sup>304</sup>

Phytoestrogens also appear to differ from synthetic hormonally active chemicals in their estrogenic effects. For example, recall that estradiol acts by binding to molecules in cells called estrogen receptors (ER).<sup>305</sup> There are two types of receptors, called ER-alpha (ER $\alpha$ ) and ER-beta (ER $\beta$ ). Estradiol binds more strongly to ER $\alpha$ , as do most synthetic chemicals with estrogenic activity. However, a number of phytoestrogens, including genistein, bind more strongly to ER $\beta$ . Some researchers have suggested that this could in part explain differences between the hormonal effects of phytoestrogens and other hormonally active chemicals.<sup>306</sup>

## Part 5

# Summary

**E**ndocrine disruption has been unequivocally demonstrated in humans and animals at relatively high doses of chemicals—much higher than typical human or animal exposures to environmental contaminants. These high exposures were associated with the drug DES and chemical accidents in humans, and with contaminated bodies of water, such as the Great Lakes, in wildlife. Many wildlife populations are recovering as contamination levels decrease. However, some species at the top of the food chain, such as some populations of whales, seals, and fish-eating birds, still show high levels of chemical contamination in some regions.

There are also examples of endocrine disruption in wildlife at very low exposures to some chemicals. Natural estradiol, synthetic pharmaceutical hormones, and, in a few cases, the industrial chemical nonylphenol in sewage treatment waste, have “feminized” male fish in some areas. Tributyltin still poses a threat to many mollusk species.

In humans, the evidence for endocrine disruption, or other significant health effects from everyday low-level chemical exposure is relatively weak. Studies of neurological effects of low-level exposure to PCBs, DDT, DDE, and a few other contaminants have inconsistently found small neurological deficits in early childhood associated with higher PCB exposure. These deficits, of up to a several percent reduction in performance on neurological tests, were found in children in the top 5 to 20 percent among typical PCB exposures during the 1980s and early 1990s. These studies should be interpreted cautiously because of the inconsistency among their results, and the subtlety of the measured effects.

Studies of male reproductive health indicate that testicular cancer increased in the industrialized world during the twentieth century. However, the increasing trend may have begun before widespread use of synthetic chemicals. The cause of increased testicular cancer remains unknown. Although some researchers have argued that human sperm counts have declined, the evidence for this appears to be relatively weak due to unavoidable bias in samples of semen donors, and also due to the lack of a corresponding declining trend in the sperm counts of various species of farm animal.

Trends in hypospadias and undescended testes vary by region and have both risen and fallen during the last 20 to 30 years in some regions. Some apparent trends might not be real due to potential biases in data collection. No one has yet done studies to determine whether there is a causal link between regional male reproductive health trends and environmental chemical exposures.

Most studies have failed to find a link between breast cancer and organochlorine chemical exposure. Where associations have been found, the results are inconsistent between studies, and the associations are not strong by epidemiological standards. The chemicals studied so far are barely or not at all estrogenic. Given that increased breast cancer risk is associated with greater lifetime exposure to estradiol, a link

between low-level exposures to non-estrogenic chemicals and breast cancer appears to be biologically implausible.

The effects of low-level chemical exposure in humans have been studied mainly for organochlorines—the persistent pesticides and industrial chemicals that have been banned or restricted during the last 30 years. Where adverse effects have been confirmed, they are due to exposures substantially greater than typical environmental exposures. To the extent that low doses of these chemicals are causing health damage, the effects are subtle. Organochlorine exposures have dropped substantially, suggesting that to the extent low exposures do harm human health, their effects have also declined.

A number of recent laboratory studies have found low-dose effects of some chemicals that are still in wide use in many commercial and industrial applications. While these chemicals are not persistent like many of the organochlorines, many people are exposed to them at low levels on an ongoing basis.

The Endocrine Disruptors Low Dose Peer Review found that some of these chemicals cause subtle physiological changes in rodents at doses lower than the previously determined no-effect level. However, none besides bisphenol A appeared to cause any effects at doses near the worst-case human exposure level. Because the bisphenol A effects could not be duplicated by some laboratories, the panel concluded that it is not clear whether the apparent low-dose effects of bisphenol A represent a general property of the chemical. It is also not clear whether these subtle effects should be considered harmful, because “natural” factors such as changes in diet or stress level, or natural variation in exposure to estradiol in the womb, can elicit similar effects. Research is under way to gather additional data on the extent of low-dose effects from common chemicals and the degree to which they pose a risk to humans.

Overall, the evidence suggests it is unlikely that adverse health effects due to endocrine disruption have occurred in humans from exposures to small amounts of foreign chemicals in the environment.

## About the Author

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## Appendix A

# Glossary

**Agonist:** in general, a chemical that activates a physiological process; in the case of hormonal activity, a chemical that mimics the effect of a natural hormone. For example, a chemical that has effects similar to that of estradiol would be called an “estrogen agonist” or simply “estrogenic.”

**Antagonist:** in general, a chemical that diminishes a physiological process; in the case of hormonal activity, a chemical that diminishes a natural hormonal effect. For example, a chemical that blocks the effects of estradiol would be called an “estrogen antagonist” or an “antiestrogen.”

**Androgen:** a “male” sex hormone, such as testosterone.

**Aromatase inhibitors:** drugs that interfere with the enzyme aromatase, which facilitates the conversion of testosterone to estradiol. Such drugs are used to treat breast cancer.

**Assay:** a laboratory screening test. In the case of hormonal effects, various assays are used to assess the ability of chemicals to mimic or antagonize the effects of natural hormones.

**Cell proliferation:** the process of cell growth and division.

**Confounding factor:** an uncontrolled or random factor in a study that has the potential to create an apparent relationship between an agent, such as a toxic chemical, and a health outcome, such as performance on a neurological test, when such a relationship might not actually exist. Also called a “confounder.”

**Control group:** in toxicology testing or drug development, a randomly selected group of subjects that are given a fake treatment to compare with other randomly selected groups given actual treatments.

**Coregulator:** a chemical in a cell nucleus that modulates, that is, enhances or diminishes, the action of a steroid or thyroid hormone. Coregulators come in two varieties: coactivators, which enhance a hormonal signal, and corepressors, which diminish a hormonal signal.

**Differentiation:** the process by which cells in the embryo and fetus, which start out as generic cells, become specialized for particular functions, such as skin cells or heart muscle cells.

**Dose-response curve:** the relationship between the dose of an agent administered and the response triggered by that dose. Dose-response curves are used to chart the effects of toxic chemicals and therapeutic drugs with changes in dose.

**Endocrine disruptor:** a hormonally active chemical that could alter natural hormonal processes, possibly damaging reproductive or neurological health, or disease resistance.

**Endpoint:** a physiological or toxic effect assessed as part of a toxicology study.

**Epidemiology:** the study of the degree of association between exposure to suspected toxicants and the prevalence of adverse health effects in a given group of organisms.

**Estrogen:** the generic term for “female” sex hormones. Estradiol is the principal and most potent form.

**Estrogenic:** having effects similar to estradiol.

**Half-life:** the estimated time for something to diminish by 50 percent. For example, the time it takes for an organism to eliminate 50 percent of a chemical from its body. For a chemical with a half-life of one month, 50 percent of the chemical would be gone after one month, 75 percent after two months, 87.5 percent after three months, and so on.

**Hermaphroditism:** a condition in which an animal develops both male and female sex organs.

**Hormone:** a chemical produced by a given gland that causes changes in the activity of cells, tissues, or organs in other parts of an animal’s body. Major hormone systems studied for effects of hormonally active chemicals include the steroid and thyroid hormones.

**Imposex:** a condition wherein female mollusks develop a penis and other masculine characteristics.

**“Lowest observed adverse effect level” (LOAEL):** the lowest dose of a chemical at which some adverse effect is observed in a given species.

**Maximal response:** the dose of a chemical above which increasing the dose has no additional effect.

**Negative feedback:** the process by which a hormone “acts back” on a gland to reduce secretion of that hormone, thus regulating levels of hormones in circulation. This is the most common system by which organisms control circulating levels of hormones.

**“No observed adverse effect level” (NOAEL):** the highest dose of a chemical at which no adverse effect is observed in a given species.

**Organogenesis:** the period of development in the womb during which the basic structures of most organs and physiological systems are laid out. Organogenesis lasts from roughly the third to the eighth week of pregnancy in humans. Organisms are most susceptible to damage during this so-called critical period.

**Persistent organochlorine:** a group of pesticides and industrial chemicals that persist in the environment and in animals’ bodies for long periods of time. These are also sometimes referred to as “polychlorinated aromatic hydrocarbons” or “persistent organic pollutants.” Most of these chemicals have been banned or heavily restricted in most countries.

**Phytoestrogens:** plant chemicals found to have hormonal activity similar to that of estrogen. Humans and animals are exposed to many of these chemicals through the foods they eat.

**Potency:** the relative ability of a chemical to elicit a toxic effect at a given dose.

**Receptor:** a protein in cells that binds to a specific hormone. Each hormone binds preferentially to one or more specific receptors. Binding of hormone to receptor initiates a series of processes that eventually cause the physiological effect(s) of the hormone.

**Reference dose:** the maximum daily intake of a chemical that the U.S. EPA estimates is “likely to be without an appreciable risk of deleterious effects during a lifetime.”

**Relative binding affinity:** the ability of a chemical to attach or bind to a hormone receptor, such as the estrogen receptor, relative to the binding affinity of natural hormones, such as estradiol.

**Relative potency:** the ability of a chemical to induce a hormonal response, such as causing cells to divide and grow, relative to a natural hormone, such as estradiol.

**Strain:** in the case of laboratory test animals, a variety of a species, such as a mouse or rat, produced by selective breeding.

**Synergistic effect:** in the case of hormonal effects, a circumstance in which the effect of a mixture of hormonally active chemicals is greater than what would be predicted by adding the individual effects of the components of the mixture.

**Teratogen:** a chemical or other factor that can damage an embryo or fetus during development.

**Threshold dose:** the minimum dose of a chemical necessary to elicit a physiological effect.

**Toxicology:** the study of the potential for chemicals or other environmental factors to harm living organisms.

## Appendix B

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## Appendix D

# Sources for Information in Tables

### Table 1. Synthetic Chemicals Used in Industry and Consumer Products

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# Endnotes

- <sup>1</sup> For example, the FDA regulates pharmaceuticals, food additives, and food-contact materials, such as packaging and cosmetics, while EPA regulates pesticides. These chemicals receive the greatest scrutiny, because they have the greatest potential for human exposure and harm. EPA also has authority under the Toxic Substances Control Act to regulate existing and new industrial chemicals and to require testing of chemicals in cases where it believes toxicity data are lacking. Likewise, the European Union (EU) also regulates existing and new chemicals, and requires basic toxicity and carcinogenicity testing of all new industrial chemicals produced in amounts greater than one ton per year. Although most of the tens of thousands of chemicals in commercial use have not received the level of scrutiny applied to drugs, food additives, and pesticides, almost all chemicals have been subjected to some degree of toxicity testing. See C. D. Klaassen ed., *Casarett and Doull's Toxicology: The Basic Science of Poisons* (New York: McGraw-Hill, 1996) and U.S. EPA, *Status Report on the High Production Volume (HPV) Challenge Program* (Washington, D.C., October 2001), [www.epa.gov/opptintr/chemrtk/hpvstat.pdf](http://www.epa.gov/opptintr/chemrtk/hpvstat.pdf) (cited November 13, 2001).
- <sup>2</sup> See text box “Hormones and the Endocrine System” on page 4 for a brief overview.
- <sup>3</sup> See, for example, T. Colborn et al., “Developmental Effects of Endocrine-Disrupting Chemicals in Wildlife and Humans,” *Environmental Health Perspectives*, vol. 101, no. 5 (1993), pp. 378–384; F. S. vom Saal and D. M. Sheehan, “Challenging Risk Assessment: Traditional Toxicological Testing Cannot Detect the Adverse Effects of Very Low Doses of Environmental Chemicals,” *Forum for Applied Research and Public Policy*, vol. 13, no. 3 (1998), p. 11.
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- <sup>5</sup> Medical researchers have defined a number of stages during development in the womb. The entire period of pregnancy from egg fertilization to birth is known as gestation. For humans, gestation normally lasts about 38 weeks. The first eight weeks after fertilization are known as the embryonic period, and the developing organism is called an embryo. At the beginning of the embryonic period, the fertilized egg divides into progressively larger groups of cells and implants itself in the wall of the uterus. Most major body structures and organs begin to form during weeks three through eight of the embryonic period, a process known as “organogenesis.” Beginning around week nine, the embryo begins to look recognizably human and is now called a fetus. The fetal period is one of overall body growth and continued development of organs and other body structures. See R. O’Rahilly and F. Muller, *Human Embryology and Teratology* (New York: John Wiley and Sons, 2001).
- <sup>6</sup> This class of chemicals is also sometimes referred to as “polychlorinated aromatic hydrocarbons” or “persistent organic pollutants.”
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- <sup>10</sup> Colborn et al., *Our Stolen Future*.
- <sup>11</sup> See, for example, S. C. Nagel et al., “Relative Binding Affinity-Serum Modified Access (RBA-SMA) Assay Predicts the Relative *in Vivo* Bioactivity of the Xenoestrogens Bisphenol A and Octylphenol,” *Environmental*

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- <sup>12</sup> Endocrine Disruptor Screening and Testing Advisory Committee, *Final Report: Volume I* (Washington, D.C.: 1998).
- <sup>13</sup> S. H. Safe, “Endocrine Disruptors and Human Health—Is There a Problem? An Update,” *Environmental Health Perspectives*, vol. 108, no. 6 (2000), pp. 487–493; D. R. Juberg, “An Evaluation of Endocrine Modulators: Implications for Human Health,” *Ecotoxicology and Environmental Safety*, vol. 45, no. 2 (2000), pp. 93–105; T. M. Crisp et al., “Environmental Endocrine Disruption: An Effects Assessment and Analysis,” *Environmental Health Perspectives*, vol. 106, suppl. 1 (1998), pp. 11–56; G. H. Degen and H. M. Bolt, “Endocrine Disruptors: Update on Xenooestrogens,” *International Archives of Occupational and Environmental Health*, vol. 73, no. 7 (2000), pp. 433–441; J. Ashby, “Getting the Problem of Endocrine Disruption into Focus: The Need for a Pause for Thought,” *APMIS: Acta Pathologica, Microbiologica, et Immunologica Scandinavica*, vol. 108, no. 12 (2000), pp. 805–813; D. J. Handelsman, “Myth and Methodology in the Evaluation of Human Sperm Output,” *International Journal of Andrology*, vol. 23, suppl. 2 (2000), pp. 50–53.
- <sup>14</sup> National Research Council, Committee on Hormonally Active Agents in the Environment, *Hormonally Active Agents in the Environment* (Washington, D.C.: National Academy Press, 1999).
- <sup>15</sup> C. MacIwain, “U.S. Panel Split on Endocrine Disruptors,” *Nature*, vol. 395 (1998), p. 828.
- <sup>16</sup> National Research Council, *Hormonally Active Agents in the Environment*.
- <sup>17</sup> Unless otherwise noted, the information in this section is based on C. Brook and N. Marshall, *Essential Endocrinology* (Oxford, England: Blackwell Science, 1996), and Endocrine Disruptor Screening and Testing Advisory Committee, *Final Report: Volume I*.
- <sup>18</sup> In some cases, hormone-producing cells are not collected into glands, but are instead found as single cells within another organ. For example, specialized endocrine cells in the stomach secrete the hormone gastrin, which stimulates acid secretion for digestion.
- <sup>19</sup> “Male” and “female” are in quotes in this sentence because both estradiol and testosterone actually play key roles in both sexes.
- <sup>20</sup> E. D. Albrecht et al., “Central Integrative Role of Oestrogen in Modulating the Communication between the Placenta and Fetus that Results in Primate Fetal-Placental Development,” *Placenta*, vol. 20 (1999), pp. 129–39.
- <sup>21</sup> A generic statement of the Precautionary Principle is as follows: “When an activity raises threats of harm to human health or the environment, precautionary measures should be taken even if some cause and effect relationships are not established scientifically.” See C. Raffensperger et al., eds., *Protecting Public Health and the Environment: Implementing the Precautionary Principle* (Washington, D.C.: Island Press, 1999).
- <sup>22</sup> A. Wildavsky, *Searching for Safety* (New Brunswick, NY: Transaction Publishers, 1988).
- <sup>23</sup> Although both disciplines deal broadly with any factor that can affect the health of humans and other organisms—such as foreign chemicals, diet, and exposure to radiation, and lifestyle factors such as stress or risky behaviors—the discussion here will be limited to the applications of toxicology and epidemiology to chemical exposure.
- <sup>24</sup> H. Marquardt et al., eds., *Toxicology* (New York: Academic Press, 1999).
- <sup>25</sup> For example, the treatment group in an animal experiment might be fed the test chemical dissolved in vegetable oil. The control group would then be fed an equal amount of vegetable oil without the test chemical.
- <sup>26</sup> W. R. Swain, “Effects of Organochlorine Chemicals on the Reproductive Outcome of Humans Who Consumed Contaminated Great Lakes Fish: An Epidemiologic Consideration,” *Journal of Toxicology and Environmental Health*, vol. 33, no. 4 (1991), pp. 587–639.
- <sup>27</sup> Klaassen ed., *Casarett and Doull’s Toxicology*.
- <sup>28</sup> *Ibid.*
- <sup>29</sup> J. L. Spearow et al., “Genetic Variation in Susceptibility to Endocrine Disruption by Estrogen in Mice,” *Science*, vol. 285, no. 5431 (1999), pp. 1259–1261.
- <sup>30</sup> C. M. Markey et al., “The Mouse Uterotrophic Assay: A Reevaluation of its Validity in Assessing the Estrogenicity of Bisphenol A,” *Environmental Health Perspectives*, vol. 109, no. 1 (2001), pp. 55–60.
- <sup>31</sup> Klaassen ed., *Casarett and Doull’s Toxicology*.
- <sup>32</sup> *Ibid.*
- <sup>33</sup> J. Ashby, “Testing for Endocrine Disruption Post-EDSTAC: Extrapolation of Low Dose Rodent Effects to Humans,” *Toxicology Letters*, vol. 120, nos. 1–3 (2001), pp. 233–242.

- <sup>34</sup> Because chemicals can sometimes elicit biological effects that are not obviously “adverse,” toxicology studies sometimes report results as the “lowest observed effect level,” or LOEL, or the corresponding “no observed effect level,” or NOEL.
- <sup>35</sup> A milligram is one-thousandth of a gram, a microgram is one-millionth of a gram, a nanogram is one-billionth of a gram, a kilogram is one thousand grams. To put these units in more familiar terms, there are 28 grams per ounce, and 2.2 pounds per kilogram.
- <sup>36</sup> U.S. EPA, “EPA Integrated Risk Information System (IRIS),” [www.EPA.Gov/Ngispgm3/Iris/Index.html](http://www.EPA.Gov/Ngispgm3/Iris/Index.html) (cited September 10, 2001).
- <sup>37</sup> Ibid.
- <sup>38</sup> C. A. Kimmel and S. L. Makris, “Recent Developments in Regulatory Requirements for Developmental Toxicology,” *Toxicology Letters*, vol. 120, nos. 1–3 (2001), pp. 73–82.
- <sup>39</sup> Marquardt et al., *Toxicology*.
- <sup>40</sup> K. L. Moore and T. V. N. Persaud, *The Developing Human: Clinically Oriented Embryology* (Philadelphia: W. B. Saunders Company, 1993).
- <sup>41</sup> National Research Council, *Hormonally Active Agents in the Environment*.
- <sup>42</sup> Testosterone is one of a group of “male” sex hormones collectively referred to as androgens. Estradiol is the main “female” sex hormone and is the principal and most potent natural form of estrogen. Estrone and estriol are other estrogens found in animals and are much less potent than estradiol. Although the term “estrogen” is often used generically to refer to female sex hormones, the different forms of estrogen have different potencies and differing physiological roles and effects. As a result, we will refer to these chemicals by their individual names to avoid confusion.
- <sup>43</sup> G. Solomon and T. Schettler, “Environment and Health: 6. Endocrine Disruption and Potential Human Health Implications,” *Canadian Medical Association Journal*, vol. 163, no. 11 (2000), pp. 1471–1476; R. Bigsby et al., “Evaluating the Effects of Endocrine Disruptors on Endocrine Function During Development,” *Environmental Health Perspectives*, vol. 107, suppl. 4 (1999), pp. 613–618; Endocrine Disruptor Screening and Testing Advisory Committee, *Final Report: Volume I*.
- <sup>44</sup> See text box “Molecular Mechanism of Hormone Action” on page 13 for a brief introduction to how hormones elicit their effects and why such an understanding is important for assessing the risk from hormonally active compounds.
- <sup>45</sup> Crisp et al., “Environmental Endocrine Disruption.”
- <sup>46</sup> Ibid.; L. J. Guillette et al., “Organization Versus Activation: The Role of Endocrine-Disrupting Contaminants (EDCs) During Embryonic Development in Wildlife,” *Environmental Health Perspectives*, vol. 103, suppl. 7, nos. 3–4 (1995), pp. 157–164; S. C. Nagel et al., “Developmental Effects of Estrogenic Chemicals Are Predicted by an *in Vitro* Assay Incorporating Modification of Cell Uptake by Serum,” *Journal of Steroid Biochemistry and Molecular Biology*, vol. 69, nos. 1–6 (1999), pp. 343–357.
- <sup>47</sup> “Differentiation” refers to the process by which cells in the embryo and fetus, which start out as generic cells, become specialized for particular functions, such as skin cells or heart muscle cells.
- <sup>48</sup> Moore and Persaud, *The Developing Human*.
- <sup>49</sup> Ibid.; A. J. Wilcox et al., “Fertility in Men Exposed Prenatally to Diethylstilbestrol,” *New England Journal of Medicine*, vol. 332, no. 21 (1995), pp. 1411–1416.
- <sup>50</sup> Moore and Persaud, *The Developing Human*.
- <sup>51</sup> Klaassen ed., *Casarett and Doull’s Toxicology*.
- <sup>52</sup> J. L. Jacobson and S. W. Jacobson, “Sources and Implications of Interstudy and Interindividual Variability in the Developmental Neurotoxicity of PCBs,” *Neurotoxicology and Teratology*, vol. 18, no. 3 (1996), pp. 257–264, discussion on pp. 271–276.
- <sup>53</sup> S. H. Swan, “Intrauterine Exposure to Diethylstilbestrol: Long-Term Effects in Humans,” *APMIS: Acta Pathologica, Microbiologica, et Immunologica Scandinavica*, vol. 108, no. 12 (2000), pp. 793–804.
- <sup>54</sup> Wilcox et al., “Fertility in Men Exposed Prenatally to Diethylstilbestrol.”
- <sup>55</sup> J. Takeyama et al., “17beta-Hydroxysteroid Dehydrogenase Types 1 and 2 in Human Placenta: An Immunohistochemical Study with Correlation to Placental Development,” *Journal of Clinical Endocrinology and Metabolism*, vol. 83, no. 10 (1998), pp. 3710–3715; J. Takeyama et al., “17beta-Hydroxysteroid Dehydrogenase Type 1 and 2 Expression in the Human Fetus,” *Journal of Clinical Endocrinology and Metabolism*, vol. 85, no. 1 (2000), pp. 410–416.
- <sup>56</sup> M. Neves-e-Castro, “The Possible Role of High Estriol Levels in Pregnancy,” *Medical Hypotheses*, vol. 1 (1975), pp. 132–134; M. Melamed et al., “Molecular and Kinetic Basis for the Mixed Agonist/Antagonist

- Activity of Estriol,” *Molecular Endocrinology*, vol. 11, no. 12 (1997), pp. 1868–1878; D. M. Sheehan, “Activity of Environmentally Relevant Low Doses of Endocrine Disruptors.”
- <sup>57</sup> S. C. Nagel et al., “The Effective Free Fraction of Estradiol and Xenoestrogens in Human Serum Measured by Whole Cell Uptake Assays: Physiology of Delivery Modifies Estrogenic Activity,” *Proceedings of the Society for Experimental Biology in Medicine*, vol. 217, no. 3 (1998), pp. 300–309; Nagel et al., “Relative Binding Affinity-Serum Modified Access (RBA-SMA) Assay.”
- <sup>58</sup> Z. Gregus and C. D. Klaassen, “Hepatic Disposition of Xenobiotics During Prenatal and Early Postnatal Development,” in *Fetal and Neonatal Physiology*, eds. R. A. Polin and G. A. Fox (Philadelphia: W.B Saunders Company, 1998), pp. 1472–1493; Klaassen eds., *Casarett and Doull's Toxicology*.
- <sup>59</sup> Crisp, et al. “Environmental Endocrine Disruption.”
- <sup>60</sup> Klaassen ed., *Casarett and Doull's Toxicology* .
- <sup>61</sup> Endocrine Disruptor Screening and Testing Advisory Committee, *Final Report: Volume I*; T. Zacharewski, “*In Vitro* Bioassays for Assessing Estrogenic Substances,” *Environmental Science and Technology*, vol. 31, no. 3 (1997), pp. 613–623.
- <sup>62</sup> B. Gutendorf and J. Westendorf, “Comparison of an Array of *in Vitro* Assays for the Assessment of the Estrogenic Potential of Natural and Synthetic Estrogens, Phytoestrogens and Xenoestrogens,” *Toxicology*, vol. 166, nos. 1–2 (2001), pp. 79–89.
- <sup>63</sup> X. Zhang et al., “A Nuclear Receptor Corepressor Modulates Transcriptional Activity of Antagonist-Occupied Steroid Hormone Receptor,” *Molecular Endocrinology*, vol. 12, no. 4 (1998), pp. 513–524; B. S. Katzenellenbogen et al., “Molecular Mechanisms of Estrogen Action: Selective Ligands and Receptor Pharmacology,” *Journal of Steroid Biochemistry and Molecular Biology*, vol. 74, no. 5 (2000), pp. 279–285.
- <sup>64</sup> M. Luconi et al., “Effects of Estrogenic Compounds on Human Spermatozoa: Evidence for Interaction with a Nongenomic Receptor for Estrogen on Human Sperm Membrane,” *Molecular and Cellular Endocrinology*, vol. 178, nos. 1–2 (2001), pp. 39–45.
- <sup>65</sup> Gutendorf and Westendorf, “Comparison of an Array of *in Vitro* Assays.”
- <sup>66</sup> Katzenellenbogen et al., “Molecular Mechanisms of Estrogen Action: Selective Ligands and Receptor Pharmacology.”
- <sup>67</sup> Klaassen ed., *Casarett and Doull's Toxicology* .
- <sup>68</sup> Ibid.
- <sup>69</sup> See Part 3 for data on trends in environmental pollution levels.
- <sup>70</sup> The exposure estimates here are for ingestion in food or water, but do not include exposure through inhalation.
- <sup>71</sup> M. Namer, “Clinical Applications of Antiandrogens,” *Journal of Steroid Biochemistry*, vol. 31, no. 4B (1988), pp. 719–729; H. M. Kuerer et al., “Biologic Basis and Evolving Role of Aromatase Inhibitors in the Management of Invasive Carcinoma of the Breast,” *Journal of Surgical Oncology*, vol. 77, no. 2 (2001), pp. 139–147; Brook and Marshall, *Essential Endocrinology*.
- <sup>72</sup> Kuerer et al., “Biologic Basis and Evolving Role of Aromatase Inhibitors.”
- <sup>73</sup> R. W. Stephany, “Hormones in Meat: Different Approaches in the EU and in the USA,” *APMIS: Acta Pathologica, Microbiologica, et Immunologica Scandinavica*, vol. 109, suppl. 103 (2001), pp. S357–S364.
- <sup>74</sup> R. L. Divi et al., “Anti-thyroid Isoflavones from Soybean: Isolation, Characterization, and Mechanisms of Action,” *Biochemical Pharmacology*, vol. 54, no. 10 (1997), pp. 1087–1096.
- <sup>75</sup> Zacharewski, “*In Vitro* Bioassays for Assessing Estrogenic Substances.”
- <sup>76</sup> Relative potency is a measure of how much more of a test chemical is necessary to cause the same effect as a reference chemical. In this case, the reference chemical is the hormone estradiol.
- <sup>77</sup> Calculate this result as follows: estradiol’s potency is set to a benchmark level of 100. For a chemical that has a relative potency of 0.01, estradiol is  $100/0.01 = 10,000$  times more effective at inducing an estrogenic response.
- <sup>78</sup> A. M. Soto et al., “The E-Screen Assay as a Tool to Identify Estrogens: An Update on Estrogenic Environmental Pollutants,” *Environmental Health Perspectives*, vol. 103, suppl. 7, no. 12, Part 2 (1995), pp. 113–122; J. Payne et al., “Prediction and Assessment of the Effects of Mixtures of Four Xenoestrogens,” *Environmental Health Perspectives*, vol. 108, no. 10 (2000), pp. 983–987.
- <sup>79</sup> Payne, et al., “Prediction and Assessment of the Effects of Mixtures of Four Xenoestrogens;” J. Payne et al., “Mixtures of Four Organochlorines Enhance Human Breast Cancer Cell Proliferation,” *Environmental Health Perspectives*, vol. 109, no. 4 (2001), pp. 391–397; N. Rajapakse et al., “Defining the Impact of Weakly Estrogenic Chemicals on the Action of Steroidal Estrogens,” *Toxicological Sciences*, vol. 60, no. 2 (2001), pp. 296–304; C. A. Harris et al., “The Estrogenic Activity of Phthalate Esters *in Vitro*,” *Environmental Health Perspectives*, vol. 105, no. 8 (1997), pp. 802–811; K. Graumann et al., “Monitoring of Estrogen Mimics by a

- Recombinant Yeast Assay: Synergy between Natural and Synthetic Compounds?" *Science of the Total Environment*, vol. 225, nos. 1–2 (1999), pp. 69–79; S. R. Milligan et al., "Relative Potency of Xenobiotic Estrogens in an Acute *in Vivo* Mammalian Assay," *Environmental Health Perspectives*, vol. 106, no. 1 (1998), pp. 23–26. Arnold et al. did report synergistic effects of a mixture of estrogenic chemicals. However, neither the original laboratory nor other researchers were able to reproduce these results and Arnold et al. withdrew their original paper (S. F. Arnold et al., "Synergistic Activation of Estrogen Receptor with Combinations of Environmental Chemicals," *Science*, vol. 272 (1996), pp. 1489–1492; J. McLachlan, "Synergistic Effect of Environmental Estrogens: Report Withdrawn," *Science*, vol. 277 (1997), pp. 462–463).
- <sup>80</sup> A. O. Cheek and J. A. McLachlan, "Environmental Hormones and the Male Reproductive System," *Journal of Andrology*, vol. 19, no. 1 (1998), pp. 5–10.
- <sup>81</sup> Ibid.; A. O. Cheek et al., "Potential Mechanisms of Thyroid Disruption in Humans: Interaction of Organochlorine Compounds with Thyroid Receptor, Transthyretin, and Thyroid-Binding Globulin," *Environmental Health Perspectives*, vol. 107, no. 4 (1999), pp. 273–278.
- <sup>82</sup> The letters "o" and "p" and the apostrophe designate closely related versions, or "isomers," of DDT and DDE. The slight structural differences between each of these molecules results in different chemical properties, including type and potency of hormonal activity. "o" stands for "ortho," "p" for "para," and the apostrophe for "prime." Thus, o,p'-DDT is pronounced "ortho-para-prime-DDT." So-called technical grade DDT is actually a mixture of the o,p'-DDT and p,p'-DDT, with the latter making up the majority. In the environment and in the bodies of animals, isomers of DDT are broken down into isomers of DDE, which is the chemical that actually persists for long periods in the environment.
- <sup>83</sup> As discussed earlier, a chemical that binds to the estrogen receptor can block the action of estradiol—that is, act as an estrogen antagonist—if it binds to the receptor, but then does not initiate some or all of the subsequent events that normally occur after estradiol binds to its receptor.
- <sup>84</sup> T. Zacharewski, "In Vitro Bioassays for Assessing Estrogenic Substances."
- <sup>85</sup> Marquardt et al., *Toxicology*.
- <sup>86</sup> W. R. Kelce et al., "Environmental Hormone Disruptors: Evidence that Vinclozolin Developmental Toxicity Is Mediated by Antiandrogenic Metabolites," *Toxicology and Applied Pharmacology*, vol. 126, no. 2 (1994), pp. 276–285; D. Kupfer and W. H. Bulger, "Metabolic Activation of Pesticides with Proestrogenic Activity," *Federation Proceedings*, vol. 46, no. 5 (1987), pp. 1864–1869.
- <sup>87</sup> Nagel et al., "Relative Binding Affinity-Serum Modified Access (RBA-SMA) Assay."
- <sup>88</sup> Zacharewski, "In Vitro Bioassays for Assessing Estrogenic Substances."
- <sup>89</sup> Markey et al., "The Mouse Uterotrophic Assay: A Reevaluation of Its Validity in Assessing the Estrogenicity of Bisphenol A"; vom Saal et al., "Prostate Enlargement in Mice Due to Fetal Exposure to Low Doses of Estradiol or Diethylstilbestrol and Opposite Effects at High Doses."
- <sup>90</sup> L. Kangas, "Agonistic and Antagonistic Effects of Antiestrogens in Different Target Organs," *Acta Oncologica (Stockholm, Sweden)*, vol. 31, no. 2 (1992), pp. 143–146.
- <sup>91</sup> T. Zacharewski, "Identification and Assessment of Endocrine Disruptors: Limitations of *in Vivo* and *in Vitro* Assays," *Environmental Health Perspectives*, vol. 106, suppl. 2, nos. 1–2 (1998), pp. 577–582.
- <sup>92</sup> This section summarizes more than 40 research papers. In the interests of space and flow, references to papers not explicitly cited here are listed in Appendix B, on page 54.
- <sup>93</sup> Percentage changes vary by location and species.
- <sup>94</sup> Council on Environmental Quality, *Environmental Quality Statistics* (Washington, D.C., 1993).
- <sup>95</sup> Batelle, *Great Lakes Binational Toxics Strategy: The Level I Pesticides in the Binational Strategy* (prepared for U.S. EPA, Great Lakes National Program Office, Washington, D.C., March 2000), [www.epa.gov/glnpo/bns/pesticides/finalpestreport.html](http://www.epa.gov/glnpo/bns/pesticides/finalpestreport.html) (cited May 25, 2001).
- <sup>96</sup> J. Giesy et al., "Deformities in Birds of the Great Lakes Region: Assigning Causality," *Environmental Science and Technology*, vol. 28, no. 3 (1994), pp. 128A–135A.
- <sup>97</sup> P. Berggren et al., "Patterns and Levels of Organochlorines (DDTs, PCBs, non-Ortho PCBs and PCDD/Fs) in Male Harbour Porpoises (*Phocoena Phocoena*) from the Baltic Sea, the Kattegat-Skagerrak Seas and the West Coast of Norway," *Marine Pollution Bulletin*, vol. 38, no. 12 (1999), pp. 1070–1084.
- <sup>98</sup> A. W. Glynn et al., "PCB and Chlorinated Pesticide Concentrations in Swine and Bovine Adipose Tissue in Sweden 1991–1997: Spatial and Temporal Trends," *Science of the Total Environment*, vol. 246, nos. 2–3 (2000), pp. 195–206.
- <sup>99</sup> V. R. Simpson et al., "A Long-Term Study of Vitamin A and Polychlorinated Hydrocarbon Levels in Otters (*Lutra Lutra*) in South West England," *Environmental Pollution*, vol. 110 (2000), pp. 267–275.

- <sup>100</sup> Tanabe et al., "Global Contamination by Persistent Organochlorines and their Ecotoxicological Impact on Marine Mammals," *Science of the Total Environment*, vol. 154, nos. 2-3 (1994), pp. 163-177.
- <sup>101</sup> The process by which animals at progressively higher levels on the food chain accumulate persistent pollutants is called biomagnification.
- <sup>102</sup> D. C. Muir et al., "Temporal and Spatial Trends of Persistent Organochlorines in Greenland Walrus (*Odobenus Rosmarus Rosmarus*)," *Science of the Total Environment*, vol. 245, nos. 1-3 (2000), pp. 73-86.
- <sup>103</sup> A. Borrell et al., "Organochlorine Compounds in Common Dolphins (*Delphinus Delphis*) from the Atlantic and Mediterrean Waters of Spain," *Environmental Pollution*, vol. 114 (2001), pp. 265-274.
- <sup>104</sup> Tanabe et al., "Global Contamination by Persistent Organochlorines and Their Ecotoxicological Impact on Marine Mammals."
- <sup>105</sup> E. L. Gunderson, "FDA Total Diet Study, April 1982-April 1984, Dietary Intakes of Pesticides, Selected Elements, and Other Chemicals," *Journal of the Association of Official Analytical Chemists*, vol. 71, no. 6 (1988), pp. 1200-1209; E. L. Gunderson, "FDA Total Diet Study, July 1986-April 1991, Dietary Intakes of Pesticides, Selected Elements, and Other Chemicals," *Journal of the Association of Official Analytical Chemists*, vol. 78, no. 6 (1995), pp. 1353-1363; E. L. Gunderson, "Dietary Intakes of Pesticides, Selected Elements, and Other Chemicals: FDA Total Diet Study, June 1984-April 1986," *Journal of the Association of Official Analytical Chemists*, vol. 78, no. 4 (1995), pp. 910-921.
- <sup>106</sup> U. G. Ahlborg et al., "Organochlorine Compounds in Relation to Breast Cancer, Endometrial Cancer, and Endometriosis: An Assessment of the Biological and Epidemiological Evidence," *Critical Reviews in Toxicology*, vol. 25, no. 6 (1995), pp. 463-531; Gunderson, "FDA Total Diet Study, July 1986-April 1991."
- <sup>107</sup> Gunderson, "FDA Total Diet Study, July 1986-April 1991."
- <sup>108</sup> N. Harrison et al., "Time Trends in Human Dietary Exposure to PCDDs, PCDFs and PCBs in the UK," *Chemosphere*, vol. 37, nos. 9-12 (1998), pp. 1657-1670.
- <sup>109</sup> Ahlborg et al., "Organochlorine Compounds in Relation to Breast Cancer, Endometrial Cancer, and Endometriosis."
- <sup>110</sup> Y. F. Li, "Technical Hexachlorocyclohexane Use Trends in China and Their Impact on the Environment," *Archives of Environmental Contamination and Toxicology*, vol. 35, no. 4 (1998), pp. 688-697.
- <sup>111</sup> United Nations Environment Program/Global Monitoring System, *Environmental Data Report 1991-92* (Cambridge, U.K.: Blackwell, 1991).
- <sup>112</sup> F. W. Kutz et al., "Organochlorine Pesticides and Polychlorinated Biphenyls in Human Adipose Tissue," *Reviews of Environmental Contamination and Toxicology*, vol. 120 (1991), pp. 1-82.
- <sup>113</sup> A. K. Liem et al., "Exposure of Populations to Dioxins and Related Compounds," *Food Additives and Contaminants*, vol. 17, no. 4 (2000), pp. 241-259.
- <sup>114</sup> D. Smith, "Worldwide Trends in DDT Levels in Human Breast Milk," *International Journal of Epidemiology*, vol. 28, no. 2 (1999), pp. 179-188.
- <sup>115</sup> A. G. Craan and D. A. Haines, "Twenty-Five Years of Surveillance for Contaminants in Human Breast Milk," *Archives of Environmental Contamination and Toxicology*, vol. 35, no. 4 (1998), pp. 702-710.
- <sup>116</sup> G. Schade and B. Heinzow, "Organochlorine Pesticides and Polychlorinated Biphenyls in Human Milk of Mothers Living in Northern Germany: Current Extent of Contamination, Time Trend from 1986 to 1997 and Factors That Influence the Levels of Contamination," *Science of the Total Environment*, vol. 215, nos. 1-2 (1998), pp. 31-39.
- <sup>117</sup> O. Papke et al., "PCDD/PCDFs in Humans, Follow-up of Background Data for Germany, 1994," *Chemosphere*, vol. 32, no. 3 (1996), pp. 575-582.
- <sup>118</sup> K. Noren and D. Meironyte, "Certain Organochlorine and Organobromine Contaminants in Swedish Human Milk in Perspective of Past 20-30 Years," *Chemosphere*, vol. 40, nos. 9-11 (2000), pp. 1111-1123.
- <sup>119</sup> E. Dewailly et al., "Susceptibility to Infections and Immune Status in Inuit Infants Exposed to Organochlorines," *Environmental Health Perspectives*, vol. 108, no. 3 (2000), pp. 205-211; D. M. Steenport et al., "Fish Consumption Habits and Advisory Awareness among Fox River Anglers," *Wisconsin Medical Journal*, vol. 99, no. 8 (2000), pp. 43-46.
- <sup>120</sup> The half-life is the estimated time for 50 percent of a chemical to be degraded. For example, for a chemical with a half-life of one month, 50 percent of the chemical would be gone after one month, 75 percent after two months, 87.5 percent after three months, and so on.
- <sup>121</sup> U.S. EPA, *Reregistration Eligibility Decision (RED): Dicofol* (Washington, D.C., 1998).
- <sup>122</sup> Recall that the EPA Reference Dose is a maximum daily dose of a chemical that is "likely to be without an appreciable risk of deleterious effects during a lifetime," and that the Reference Dose is set at least a factor of 100 below the highest dose found to have no adverse health effects in animal toxicology studies.

- <sup>123</sup> M. A. Kamrin, *Pesticide Profiles: Toxicity, Environmental Impact and Fate* (New York: Lewis Publishers, 1997).
- <sup>124</sup> Ahlborg et al., “Organochlorine Compounds in Relation to Breast Cancer, Endometrial Cancer, and Endometriosis.”
- <sup>125</sup> EXTOXNET, “Pesticide Information Profiles: 2,4-D,” [ace.ace.orst.edu/info/extoxnet/pips/24-D.htm](http://ace.ace.orst.edu/info/extoxnet/pips/24-D.htm) (cited September 3, 2001).
- <sup>126</sup> M. G. Nishioka et al., “Distribution of 2,4-D in Air and on Surfaces inside Residences after Lawn Applications: Comparing Exposure Estimates from Various Media for Young Children,” *Environmental Health Perspectives*, vol. 109, no. 1 (2001), pp. 1185–1191.
- <sup>127</sup> Ibid.
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- <sup>146</sup> B. C. Blount et al., “Levels of Seven Urinary Phthalate Metabolites in a Human Reference Population,” *Environmental Health Perspectives*, vol. 108, no. 10 (2000), pp. 979–982.
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- <sup>151</sup> National Institute of Environmental Health Sciences, National Toxicology Program, *Endocrine Disruptors Low Dose Peer Review* (Washington, D.C., August 2001), [ntp-server.niehs.nih.gov/htdocs/liason/LowDosePeerFinalRpt.pdf](http://ntp-server.niehs.nih.gov/htdocs/liason/LowDosePeerFinalRpt.pdf) (cited September 10, 2001).
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- <sup>153</sup> S. A. Snyder et al., “Analytical Methods for Detection of Selected Estrogenic Compounds in Aqueous Mixtures,” *Environmental Science and Technology*, vol. 33 (1999), pp. 2814–2820.
- <sup>154</sup> J. Dachs et al., “Occurrence of Estrogenic Nonylphenols in the Urban and Coastal Atmosphere of the Lower Hudson River Estuary,” *Environmental Science and Technology*, vol. 33, no. 15 (1999), pp. 2676–2679. As noted earlier, a nanogram is one-billionth of a gram. A teaspoon of sugar weighs about 4 grams, or 4 billion nanograms.
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- <sup>158</sup> Ibid.
- <sup>159</sup> D. Ganmaa et al., “Is Milk Responsible for Male Reproductive Disorders?” *Medical Hypotheses*, vol. 57, no. 4 (2001), pp. 510–514.
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- <sup>161</sup> Stephany, “Hormones in Meat;” Henricks et al., “Residues from Anabolic Preparations after Good Veterinary Practice.”
- <sup>162</sup> Henricks et al., “Residues from Anabolic Preparations after Good Veterinary Practice;” I. G. Lange et al., “Hormone Contents in Peripheral Tissues after Correct and Off-Label Use of Growth Promoting Hormones in Cattle: Effect of Implant Preparations Finaplix-H<sup>®</sup>, Ralgro<sup>®</sup>, Synovex-H<sup>®</sup> and Synovex Plus<sup>®</sup>,” *APMIS: Acta Pathologica, Microbiologica, et Immunologica Scandinavica*, vol. 109 (2001), pp. 53–65.
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- <sup>164</sup> B. Jégou et al., “Existing Guidelines for the Use of Meat Hormones and Other Food Additives in Europe and USA,” *APMIS: Acta Pathologica, Microbiologica, et Immunologica Scandinavica*, vol. 109, suppl. 103 (2001), pp. S531–S536.
- <sup>165</sup> Ibid.
- <sup>166</sup> B. Schiffer et al., “The Fate of Trenbolone Acetate and Melengestrol Acetate after Application as Growth Promoters in Cattle: Environmental Studies,” *Environmental Health Perspectives*, vol. 109, no. 11 (2001), pp. 1145–1151.
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- <sup>169</sup> Kuch and Ballschmitter, “Determination of Endocrine-Disrupting Phenolic Compounds in Surface and Drinking Water.”
- <sup>170</sup> Snyder et al., “Analytical Methods for Detection of Selected Estrogenic Compounds in Aqueous Mixtures.”
- <sup>171</sup> E. J. Routledge et al., “Identification of Estrogenic Chemicals in STW Effluent, 2: *In Vivo* Responses in Trout and Roach,” *Environmental Science and Technology*, vol. 32 (1998), pp. 1559–1565.
- <sup>172</sup> Fritsche and Steinhart, “Occurrence of Hormonally Active Compounds in Food.”
- <sup>173</sup> Skibola and Smith, “Potential Health Impacts of Excessive Flavonoid Intake.”
- <sup>174</sup> K. D. Setchell et al., “Exposure of Infants to Phyto-Oestrogens from Soy-Based Infant Formula,” *Lancet*, vol. 350, no. 9070 (1997), pp. 23–27; C. H. Irvine et al., “Daily Intake and Urinary Excretion of Genistein and Daidzein by Infants Fed Soy- or Dairy-Based Infant Formulas,” *American Journal of Clinical Nutrition*, vol. 68, no. 6 (suppl.) (1998), pp. 1462S–1465S.
- <sup>175</sup> This is a condition in which the embryo implants outside the uterus.
- <sup>176</sup> Swan, “Intrauterine Exposure to Diethylstilbestrol: Long-Term Effects in Humans;” S. Barlow et al., “Teratology Society Public Affairs Committee Position Paper: Developmental Toxicity of Endocrine Disruptors to Humans,” *Teratology*, vol. 60, no. 6 (1999), pp. 365–375. The adverse reproductive effects of prenatal DES exposure remained undiscovered until seven young women turned up at a single hospital in the late 1960s with the same rare vaginal cancer, prompting a search for potential causes and the identification of DES as the culprit. This prompted large-scale screening of DES-exposed women for other reproductive health problems.
- <sup>177</sup> The epididymis is a gland in the scrotum where sperm mature after they are produced by the testes. W. B. Gill et al., “Association of Diethylstilbestrol Exposure *in Utero* with Cryptorchidism, Testicular Hypoplasia and Semen Abnormalities,” *Journal of Urology*, vol. 122, no. 1 (1979), pp. 36–39.
- <sup>178</sup> Wilcox et al., “Fertility in Men Exposed Prenatally to Diethylstilbestrol.”
- <sup>179</sup> *Ibid.*; Swan, “Intrauterine Exposure to Diethylstilbestrol: Long-term Effects in Humans.”
- <sup>180</sup> Gill et al., “Association of Diethylstilbestrol Exposure *in Utero* with Cryptorchidism, Testicular Hypoplasia and Semen Abnormalities.”
- <sup>181</sup> See, for example, Thayer et al., “Altered Prostate Growth and Daily Sperm Production in Male Mice Exposed Prenatally to Subclinical Doses of 17 $\alpha$ -Ethinyl Oestradiol,” *Human Reproduction*, vol. 16, no. 5 (2001), pp. 988–996, and vom Saal et al., “Prostate Enlargement in Mice Due to Fetal Exposure to Low Doses of Estradiol or Diethylstilbestrol and Opposite Effects at High Doses.” Low-dose effects will be discussed in detail below.
- <sup>182</sup> Swan, “Intrauterine Exposure to Diethylstilbestrol: Long-Term Effects in Humans.”
- <sup>183</sup> M. Brookes and A. Zietman, *Clinical Embryology* (New York: CRC Press, 1998).
- <sup>184</sup> *Ibid.*
- <sup>185</sup> Swan, “Intrauterine Exposure to Diethylstilbestrol: Long-Term Effects in Humans.”
- <sup>186</sup> F. Brucker-Davis et al., “Significant Effects of Mild Endogenous Hormonal Changes in Humans: Considerations for Low-Dose Testing,” *Environmental Health Perspectives*, vol. 109, suppl. 1 (2001), pp. 21–26.
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- <sup>188</sup> R. M. Sharpe et al., “Endocrine Disruptors and Testis Development,” *Environmental Health Perspectives*, vol. 106, no. 5 (1998), pp. A220–A221.
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- <sup>190</sup> vom Saal et al., “Prostate Enlargement in Mice Due to Fetal Exposure to Low Doses of Estradiol or Diethylstilbestrol and Opposite Effects at High Doses.”
- <sup>191</sup> vom Saal and Sheehan, “Challenging Risk Assessment.”
- <sup>192</sup> Ashby et al., “Lack of Effects for Low Dose Levels of Bisphenol A and Diethylstilbestrol on the Prostate Gland of CF1 Mice Exposed *in Utero*,” S. Z. Cagen et al., “Normal Reproductive Organ Development in CF-1 Mice Following Prenatal Exposure to Bisphenol A,” *Toxicological Sciences*, vol. 50, no. 1 (1999), pp. 36–44; S. Z. Cagen et al., “Normal Reproductive Organ Development in Wistar Rats Exposed to Bisphenol A in the Drinking Water,” *Regulatory Toxicology and Pharmacology*, vol. 30, no. 2, part 1 (1999), pp. 130–139.
- <sup>193</sup> M. Ema et al., “Rat Two-Generation Reproductive Toxicity Study of Bisphenol A,” *Reproductive Toxicology*, vol. 15, no. 5 (2001), pp. 505–523; R. Tyl et al., “Three-generation Reproductive Toxicity Study of Bisphenol

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- <sup>194</sup> C. Gupta, “Reproductive Malformation of the Male Offspring Following Maternal Exposure to Estrogenic Chemicals,” *Proceedings of the Society for Experimental Biology in Medicine*, vol. 224, no. 2 (2000), pp. 61–68.
- <sup>195</sup> I. B. Bogh et al., “Endocrine Disrupting Compounds: Effect of Octylphenol on Reproduction over Three Generations,” *Theriogenology*, vol. 55, no. 1 (2001), pp. 131–150.
- <sup>196</sup> National Institute of Environmental Health Sciences, National Toxicology Program, *Endocrine Disruptors Low Dose Peer Review*.
- <sup>197</sup> Ibid.
- <sup>198</sup> As noted earlier in this section, octylphenol was found to induce effects at low doses in pigs in a study published in winter 2001. This study was not considered in the Low Dose Peer Review, presumably because it was either not completed or was unknown to the panel when it began its analysis during the fall of 2000.
- <sup>199</sup> U.S. EPA, EPA Integrated Risk Information System (IRIS), [www.epa.gov/Ngisp3/Iris/Index.html](http://www.epa.gov/Ngisp3/Iris/Index.html).
- <sup>200</sup> Gunderson, “FDA Total Diet Study, July 1986–April 1991.” P. Palanza et al. (1999) found that a methychlor dose as low as 0.02 mg/kg b.w./day can cause behavioral changes in rodents exposed in the womb. Nevertheless, this is still tens of thousands of times greater than estimated daily human exposure. This study appears not to have been considered by the Low Dose Panel. (P. Palanza et al., “Prenatal Exposure to Endocrine Disrupting Chemicals: Effects on Behavioral Development,” *Neuroscience and Biobehavioral Reviews*, vol. 23, no. 7 (1999), pp. 1011–1027).
- <sup>201</sup> Bolt et al., “Comparative Assessment of Endocrine Modulators with Oestrogenic Activity.”
- <sup>202</sup> “Endpoints” is toxicology jargon for “types of physiological or toxic effects.” Thus, changes in prostate weight or sperm production are two types of endpoints assessed in toxicology studies.
- <sup>203</sup> M. Sakaue et al., “Bisphenol-A Affects Spermatogenesis in the Adult Rat Even at a Low Dose,” *Journal of Occupational Health*, vol. 43 (2001), pp. 185–190.
- <sup>204</sup> Tyl et al., “Three-generation Reproductive Toxicity Study of Bisphenol A (BPA) Administered in the Diet to CD (Sprague-Dawley) Rats;” Ema et al., “Rat Two-Generation Reproductive Toxicity Study of Bisphenol A.”
- <sup>205</sup> Ashby, “Getting the Problem of Endocrine Disruption into Focus.”
- <sup>206</sup> J. Odum et al., “Effect of Rodent Diets on the Sexual Development of the Rat,” *Toxicological Sciences*, vol. 61, no. 1 (2001), pp. 115–127.
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- <sup>208</sup> Ashby, “Testing for Endocrine Disruption Post-EDSTAC.”
- <sup>209</sup> N. M. Brown and K. D. Setchell, “Animal Models Impacted by Phytoestrogens in Commercial Chow: Implications for Pathways Influenced by Hormones,” *Laboratory Investigation*, vol. 81, no. 5 (2001), pp. 735–747.
- <sup>210</sup> Sharpe et al., “Endocrine Disruptors and Testis Development.”
- <sup>211</sup> Ashby, “Getting the Problem of Endocrine Disruption into Focus.”
- <sup>212</sup> Tyl et al., “Three-generation Reproductive Toxicity Study of Bisphenol A (BPA) Administered in the Diet to CD (Sprague-Dawley) Rats;” R. Steinmetz et al., “The Environmental Estrogen Bisphenol A Stimulates Prolactin Release *in Vitro* and *in Vivo*,” *Endocrinology*, vol. 138, no. 5 (1997), pp. 1780–1786; R. Steinmetz et al., “The Xenoestrogen Bisphenol A Induces Growth, Differentiation, and c-Fos Gene Expression in the Female Reproductive Tract,” *Endocrinology*, vol. 139, no. 6 (1998), pp. 2741–2747.
- <sup>213</sup> Spearow et al., “Genetic Variation in Susceptibility to Endocrine Disruption by Estrogen in Mice.”
- <sup>214</sup> Ashby, “Getting the Problem of Endocrine Disruption into Focus.”
- <sup>215</sup> F. S. vom Saal et al., “Paradoxical Effects of Maternal Stress on Fetal Steroids and Postnatal Reproductive Traits in Female Mice from Different Intrauterine Positions,” *Biology of Reproduction*, vol. 43, no. 5 (1990), pp. 751–761; Nagel et al., “Developmental Effects of Estrogenic Chemicals.”
- <sup>216</sup> vom Saal et al., “Prostate Enlargement in Mice Due to Fetal Exposure.”
- <sup>217</sup> vom Saal et al., “Paradoxical Effects of Maternal Stress.”
- <sup>218</sup> National Institute of Environmental Health Sciences, National Toxicology Program, *Endocrine Disruptors Low Dose Peer Review*.

- <sup>219</sup> This example is a summary of a series of studies that will be discussed in detail below.
- <sup>220</sup> G. Taubes, "Epidemiology Faces its Limits," *Science*, vol. 269, no. 5221 (1995), pp. 164–169.
- <sup>221</sup> Likewise, a relative risk of 1.0 would mean that there is no difference in cancer risk between people who do or do not eat a high-fat diet.
- <sup>222</sup> Taubes, "Epidemiology Faces its Limits."
- <sup>223</sup> National Research Council, *Hormonally Active Agents in the Environment*. Understanding PCB effects in particular is complicated by the fact that the class of PCBs includes more than 200 different, but closely related chemicals. Commercial PCB formulations each included several PCBs, so people and wildlife are generally exposed to a number of different PCBs at a time. PCBs vary in their persistence in the environment and in their biological effects. For example, so-called "non co-planar" PCBs (co-planar designates those PCBs with a chemical structure such that all atoms in the PCB molecule lie in a plane) are believed to be responsible for PCB-induced neurological damage, while co-planar PCBs do not have neurological effects, but cause other kinds of health damage (H. A. Tilson and P. R. Kodavanti, "The Neurotoxicity of Polychlorinated Biphenyls," *Neurotoxicology*, vol. 19, nos. 4–5 (1998), pp. 517–525).
- <sup>224</sup> J. J. Ryan et al., "Human Body Burden of Polychlorinated Dibenzofurans Associated with Toxicity Based on the Yusho and Yucheng Incidents," *Fundamental and Applied Toxicology*, vol. 15, no. 4 (1990), pp. 722–731.
- <sup>225</sup> S. L. Schantz, "Developmental Neurotoxicity of PCBs in Humans: What Do We Know and Where Do We Go from Here?" *Neurotoxicology and Teratology*, vol. 18, no. 3 (1996) pp. 217–227 (discussion pp. 229–276).
- <sup>226</sup> National Research Council, *Hormonally Active Agents in the Environment*.
- <sup>227</sup> Ryan et al., "Human Body Burden of Polychlorinated Dibenzofurans."
- <sup>228</sup> M. Ikeda, "Comparison of Clinical Picture between Yusho/Yucheng Cases and Occupational PCB Poisoning Cases," *Chemosphere*, vol. 32, no. 3 (1996), pp. 559–566.
- <sup>229</sup> Y. L. Guo et al., "Different Congeners of PCBs/PCDFs May Have Contributed to Different Health Outcomes in the Yucheng Cohort," *Neurotoxicology and Teratology*, vol. 18, no. 3 (1996), pp. 255–256 (discussion pp. 271–276).
- <sup>230</sup> Schantz, "Developmental Neurotoxicity of PCBs in Humans."
- <sup>231</sup> E. J. Delzell et al., "Interpretive Review of the Potential Adverse Effects of Chlorinated Organic Chemicals on Human Health and the Environment: Report of an Expert Panel," *Regulatory Toxicology and Pharmacology*, vol. 20, no. 1, part 2 (1994), pp. S1–S1056.
- <sup>232</sup> Ryan et al., "Human Body Burden of Polychlorinated Dibenzofurans."
- <sup>233</sup> Because of the large number of papers published for each of these studies, the references are listed in Appendix C.
- <sup>234</sup> Schantz, "Developmental Neurotoxicity of PCBs in Humans."
- <sup>235</sup> The full name of this test is the "Fagan Test of Visual Recognition Memory."
- <sup>236</sup> T. Darvill et al., "Prenatal Exposure to PCBs and Infant Performance on the Fagan Test of Infant Intelligence," *Neurotoxicology*, vol. 21, no. 6 (2000), pp. 1029–1038; S. L. Schantz, "Developmental Neurotoxicity of PCBs in Humans;" J. L. Jacobson and S. W. Jacobson, "Sources and Implications of Interstudy and Interindividual Variability in the Developmental Neurotoxicity of PCBs," *Neurotoxicology and Teratology*, vol. 18, no. 3 (1996), pp. 257–264, discussion pp. 271–276.
- <sup>237</sup> J. L. Jacobson and S. W. Jacobson, "Dose-Response in Perinatal Exposure to Polychlorinated Biphenyls (PCBs): The Michigan and North Carolina Cohort Studies," *Toxicology and Industrial Health*, vol. 12, nos. 3–4 (1996), pp. 435–445.
- <sup>238</sup> Dewailly et al., "Susceptibility to Infections and Immune Status in Inuit Infants Exposed to Organochlorines;" D. M. Steenport et al., "Fish Consumption Habits and Advisory Awareness among Fox River Anglers," *Wisconsin Medical Journal*, vol. 99, no. 8 (2000), pp. 43–46.
- <sup>239</sup> E. Carlsen et al., "Evidence for Decreasing Quality of Semen During Past 50 Years," *British Medical Journal*, vol. 305, no. 6854 (1992), pp. 609–613.
- <sup>240</sup> S. H. Swan et al., "The Question of Declining Sperm Density Revisited: An Analysis of 101 Studies Published 1934–1996," *Environmental Health Perspectives*, vol. 108, no. 10 (2000), pp. 961–966.
- <sup>241</sup> D. J. Handelsman, "Myth and Methodology in the Evaluation of Human Sperm Output," *International Journal of Andrology*, vol. 23, suppl. 2 (2000), pp. 50–53.
- <sup>242</sup> D. J. Handelsman, "Sperm Output of Healthy Men in Australia: Magnitude of Bias Due to Self-selected Volunteers," *Human Reproduction*, vol. 12, no. 12 (1997), pp. 2701–2705.
- <sup>243</sup> J. L. Van Os et al., "Long-term Trends in Sperm Counts of Dairy Bulls," *Journal of Andrology*, vol. 18, no. 6 (1997), pp. 725–731; L. Multigner et al., "Secular Sperm Trends in Stallions between 1981 and 1996," *Journal*

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- <sup>245</sup> L. J. Paulozzi, “International Trends in Rates of Hypospadias and Cryptorchidism,” *Environmental Health Perspectives*, vol. 107, no. 4 (1999), pp. 297–302.
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