Challenging Risk Assessment

Traditional toxicological testing cannot detect the adverse effects of very low doses of environmental chemicals.

BY FREDERICK S. VOM SAAL AND DANIEL M. SHEEHAN

Current methods of risk assessment for some manufactured chemicals may not accurately predict the risks of exposure to humans and animals. In fact, we are beginning to realize that very low levels of exposure to some chemicals present in the environment disrupt the endocrine system, particularly during fetal development, at doses to which humans and animals are routinely exposed. Recent research on environmentally relevant exposures raises great concern since endocrine signals regulate the differentiation and growth of cells in fetuses.

The endocrine system consists of cells that produce chemical signals or hormones that regulate the development and subsequent adult functioning of other cells in the body. As the fetus grows, hormones control the development of cells and thus determine the formation of all the body's organs. Adult organs may never function properly if hormonal signals are disrupted at this crucial developmental stage. Even very small changes in levels of endogenous hormones, whether natural or experimentally induced, produce significant changes in organ function throughout the remainder of life. In short, organs, especially the reproductive organs and brain, are highly sensitivity to endocrine disruption during fetal development.[1]

For this reason, the major concern regarding endocrine disruptors is with exposure during critical times in fetal development when organs are forming.[2] Exposure to endocrine disruptors at this stage may reduce or destroy reproductive capability and result in the loss of entire populations.[3]

Not only has the issue of the unique vulnerability of the fetus been ignored in some recent reviews,[4] but the term endocrine disruptor has been replaced by some authors with the term endocrine modulator. This change in terminology is intended to convey an entirely different meaning, namely, that these chemicals are causing a small adjustment in function. In fact, endocrine modulation typically occurs in adults, where alterations of the adult endocrine system, such as result from taking oral contraceptives, typically lead to transient—reversible—effects that disappear when exposure to the chemical ceases and the chemical is cleared from the body.

On the other hand, a chemical that interferes with the normal functioning of the fetal endocrine system and irreversibly alters the development of reproductive organs and the brain is appropriately described as an endocrine disruptor. We consider permanent effects of endocrine disruptors on physiological processes to be adverse.

Even more disturbing, current toxicological testing for potentially harmful substances that are being released into the environment is designed to assess very high doses of chemicals, while recent findings show that many of the most harmful effects occur at doses so low they are rarely tested.

Dilution Not the Solution

Decades ago, the Great Lakes region was home to many predatory birds that fed on fish in the lakes. In the late 1960s, wildlife biologists observed that entire populations of birds, salmon, and trout were not successfully reproducing, and their reproductive organs were abnormal. Moreover, the children of women who ate these contaminated fish from the Great Lakes displayed developmental abnormalities and a decrease in I.Q.[5] Scientists now know that these effects are related to exposure to endocrine-disrupting chemicals, chemicals that are ubiq-

uitous in the environment. These chemicals are found in plastics, pesticides, detergents, cosmetics, fabrics, and building materials such as insulation and carpets, to mention just a few examples.

In addition, endocrine-disrupting chemicals are now found in lakes, rivers, oceans, and the air, where they contaminate fish and other animals. Currently, there are about 60 known endocrine-disrupting chemicals, but only a very small number of the approximately 80,000 manufactured chemicals in use today have been tested for endocrine-disrupting effects. New findings concerning these chemicals conflict with earlier toxicology studies that concluded that concentrations of endocrine-disrupting chemicals in the environment are safe.[6]

Recent legislation, including 1996 revisions of the Safe Drinking Water Act and regulations enforced by the U.S. Food and Drug Administrations (FDA) and the Environmental Protection Agency (EPA), has resulted in a plan to reevaluate these chemicals.[7] Legislation and regulations are being implemented even though many toxicologists have yet to accept the possibility of adverse effects from low-dose exposure to environmental chemicals. In fact, the toxicological research community remains deeply divided on the assumptions that underlie risk assessment.

While some toxicologists and government regulators continue to defend the traditional model of risk assessment for all environmental contaminants, we offer a new model for endocrine-disrupting chemicals that challenges the traditional assumptions in toxicology, assumptions that have until recently governed our national regulatory processes.

False Assumptions

For many years toxicologists have studied adverse effects of chemicals that are hormonally active either through hormone receptors or other mechanisms.[8] Yet in spite of our extensive knowledge of how certain hormones act naturally, toxicological studies of chemicals that mimic or block these hormones have not always taken this basic information into account. As a result, toxicology will now have to reevaluate the assumptions underlying current testing strategies for chemical toxicity.

Almost all prior studies of hormonally active chemicals used study designs that were assumed to be appropriate for chemicals that do not directly damage genes—which endocrine disruptors are generally thought to be.

These methods are based on the assumption that high-dose testing predicts low-dose outcomes; in other words, information from testing a chemical at doses that may be millions of times higher than those encountered in the environment can be used to predict effects at environmental exposure levels. This, in turn, is based on the assumption that a threshold exists below which no adverse effects are seen and that there is a monotonic dose-response cure; that is, as dose increases the response increases or stays the same, but the response never first increases then decreases. If either of these assumptions is incorrect with regard to an endocrine-disrupting chemical, then the acceptable safe dose derived from high-dose studies will be incorrect. This would be the case, for example, if the response to a chemical were to first increase at very small doses and then actually decrease at much higher doses—a non-monotonic dose-response curve. Recent research has shown that this, in fact, does occur.

It is inappropriate for regulatory agencies to base acceptable exposure levels on an assumption applied uniformly for all classes of chemicals when existing data contradicts that assumption in specific cases. Nonetheless, many toxicologists vigorously defend the concept of a measurable threshold for adverse effects and a simple, monotonic dose-response function for all chemicals. They do so because the assumptions appear reasonable on the surface and because, over many years, a variety of toxic and carcinogenic substances have been found to actually behave this way. However, in the case of endocrine-disrupting chemicals, recent research sheds doubt on the conventional wisdom that the "dose makes the poison."[9]

No Safe Dose

Traditional toxicological studies aim largely at establishing a no-observed-adverse-effect level. To do so, researchers study very high doses that produced acute toxic effects—such as a decrease in body weight—in laboratory animals and then lower the dose to a point where no adverse effects are observed. There is seldom greater than a 50-fold difference between the lowest and highest doses in these studies. This type of testing, though apparently simple, is not without its problems.

For example, the validity of this type of test is statistically limited by the number of animals required by regulatory agencies to be tested in each dose group. The smaller the group size, the more difficult it is to reliably detect an adverse effect. Moreover, because the purpose of testing is to establish a safe level, doses below the apparent level at which there is no acute toxic effect are rarely tested; therefore, the assumption that very low doses are

safe has rarely been investigated.

Toxicologists Edward Calabrese and Linda Baldwin estimate that fewer than 2 percent of toxicological studies have been designed to assess the possibility that effects might occur at doses below those that do not produce acute toxicity. As a result, there has been a dramatic miscalculation of the doses of endocrine disruptors that can result in abnormal development in animals.

The Numbers Game

Some endocrine disruptors represent a unique class of toxicants that can act via the same mechanisms—for example by binding to a hormone receptor—as endogenous hormones.[10] These chemicals provide a model to examine the validity of the two assumptions of a threshold dose and a monotonic dose-response function. We will review evidence from two experimental systems showing that current toxicological testing methods used to examine environmental chemicals are inappropriate for determining endocrine-disrupting effects at environmentally relevant doses. Additionally, these examples, along with other information we will present, provide an explanation for the dramatic miscalculation of the doses of endocrine disruptors that can result in abnormal development in animals.

In our research, we have shown how exposure to environmental chemicals that mimic the hormone estradiol affects the development of the prostate gland in male mice. The prostate develops from the embryonic tissue that forms a portion of the vagina in females, and like the vagina, it has estrogen receptors and responds to estrogen.

To determine whether an environmental chemical has estrogenic activity, we first measured the effects of elevating the natural estrogen estradiol in male mouse fetuses via maternal treatment to levels at the high end of the normal physiological range. Next, we looked for similar effects from administering a dose of a chemical thought to mimic this very small increase in estradiol.

Specifically, we first increased the levels of estradiol in male mouse fetuses by an infinitesimal amount as measured in picograms, or trillionths of a gram. We increased the amount of free, biologically active estradiol in the blood of male mouse fetuses during the time that the prostate is forming—from the natural levels of 0.2 picograms per milliliter of serum in untreated males to 0.3 picograms per milliliter, a 50-percent increase of only one-tenth of a trillionth of a gram of estradiol per milliliter of serum.

This very small increase in an already very low endogenous estradiol concentration resulted in significant changes in the prostate of treated males. There were 40 percent more glandular ducts and double the number of androgen receptors per cell. Androgen receptors determine the capacity of cells to respond to male sex hormones, and also mediate the effects of male sex hormones on the development and functioning of the prostate. Of great importance, the abnormal enlargement of the prostate in treated males was permanent and persisted into adulthood.[11]

This experiment illustrates an important principle in developmental toxicology. Brief exposures to endocrine-disrupting chemicals that interfere with fetal hormones—even to low doses—during a critical period in development can result in irreversible lifetime effects, even though no trace of the chemical that caused this effect can be detected in the adult. Only if chemicals persist for a very long time after entering the body might some trace remain from exposure during fetal development.

We next examined the effects on prostate development in mice of an estrogen-mimicking chemical called bisphenol A, which is the monomer used to manufacture polycarbonate plastic bottles, including baby bottles, the lining of food and beverage cans, dental sealants, and many other products. Free, biologically active bisphenol A is released into food and drinks stored in plastic containers or in cans and is also released from dental sealant placed on teeth.

In 1996, the plastics industry reported to the U.S. government that the dose of bisphenol A that produced no effect in their standard toxicological studies was 50 milligrams—or thousandths of a gram—per kilogram of body weight per day.[12] We fed pregnant mice amounts of bisphenol A within the range being consumed per day by people through dietary and environmental exposure. These doses were thousands of times lower than those used in previous standard toxicological tests reported to have no effect. Pregnant females were fed bisphenol A at a dose of either 2 or 20 micrograms, or millionths of a gram, per kilogram of body weight per day for only seven days during the time that the prostate and other reproductive organs in male fetuses are forming.

We observed numerous effects in adult male offspring, including permanent enlargement of the prostate and a decrease in daily sperm production.[13] In female offspring, we found an earlier onset of puberty. Other effects of bisphenol A on the breast and pituitary gland at doses below the previously reported no-dose-effect level of 50 micrograms have also recently been reported in studies with rats.[14] The 2-micro-

gram dose of bisphenol A that significantly altered fetal development is 25,000 times below the 50-milligram dose that the plastics industry found to produce no adverse effects in animals tested by the traditional high-dose toxicological testing methods.

Margin of Error

How can there be such discrepancies between the effects of seemingly safe, high levels of endocrine-disrupting chemicals and drastically smaller doses of the same compounds? An experiment we conducted with the manufactured estrogenic chemical diethylstilbestrol (DES) provides an explanation for the huge error that can occur in estimating safe doses of endocrine-disrupting chemicals in traditional toxicological tests.

DES is often used in toxicological studies as a control estrogen, since it is as potent as estradiol, but unlike estradiol, DES can be administered orally, which is the route of exposure for chemicals such as bisphenol A. When pregnant mice were fed very low doses of DES—0.02, 0.2 or 2.0 micrograms per kilogram of body weight per day for seven days—using the same procedures as those for bisphenol A, we found a significant increase in prostate size in the adult male offspring relative to untreated males. Like estradiol and bisphenol A, then, DES administered during fetal development can, even at very low doses, produce significant adverse effects, in this case permanent enlargement of the prostate. However, with a higher dose of DES, 20 micrograms per kilogram, the prostate was actually smaller than in the low-dose animals and was no different from controls. Finally, at a dose of 200 micrograms per kilogram, a dose 10,000-times higher than that which increased prostate size, the adult mouse prostates were significantly smaller than controls, after correcting for body weight. In addition, at this very high dose, the mice exhibited a 10-percent decrease in body weight relative to controls.

By plotting these results on a graph, it's easy to see that administration of very low to very high doses of DES produce an inverted-U dose-response curve. That is, low doses stimulated inappropriate enlargement of the prostate while high doses inhibited normal prostate development, and males exposed to some intermediate doses did not differ significantly from controls. An inverted-U dose-response function was also found for adult prostate weight following prenatal treatment with different doses of estradiol.[15] If low doses had not been investigated, the very small, 20-microgram per kilogram dose of DES would have been considered a no-adverse-effect-level dose.

In traditional studies of the effects of estrogens on the prostate, only high doses were used, and only the decrease in prostate weight was observed. However, other effects have been observed in adult mice that received DES in low doses during fetal life. A similar inverted-U dose-response curve was observed for a behavioral effect of the same prenatal doses of DES. Specifically, at low doses of DES, adult mice displayed increased territorial behavior, but at the highest dose, territorial behavior declined significantly.[16]

The current risk-assessment model—the basic design for toxicological tests—that assumes a simple monotonic relationship between dose and response cannot accommodate a data set in which response first increases and then decreases as the dose increases.

Circular Reasoning

In the traditional model, information from the high-dose study is used to predict effects at lower doses found in the environment, which are virtually never directly tested.[17] This constitutes circular reasoning. That is, the assumptions drive the study design, and researchers simply don't examine low doses. Failure to directly study low-dose effects reinforces the misconception that there is, in fact, a safe dose for certain endocrine-disrupting chemicals. This has lead to false conclusions concerning safe doses of these substances.

We have found permanent, and thus adverse, effects for every one of the endocrine-disrupting chemicals we tested at doses below the supposedly safe levels derived from results of very high-dose studies, including the plastic monomer bisphenol A[18] and two insecticides, DDT and methoxychlor.[19] Methoxychlor is still in use in agricultural and other applications. It is likely that other endocrine-disrupting chemicals will also be found to produce adverse effects at doses below the established safe-intake level.

Depending on the dose used in a toxicological study, very different conclusions about the effects of an endocrine-disrupting chemical can be reached. This leads to the question of how doses are selected in traditional toxicological studies and how the data from these studies are used in risk assessment.

In the traditional model, the highest dose is established at a level that produces acute toxicity, such as a 10-percent reduction in body weight. The middle dose is typically about five times lower than the highest dose, and the lowest dose used is typically 10 to 50 times lower than the highest dose. The lowest of these three doses is ex-

pected to be the no-observable-adverse-effect level. To account for several uncertainties inherent in extrapolating the results from animals to humans, and because individuals vary in their response to chemicals, this level is reduced by a factor of 100. This adjusted dose is supposedly safe and predicted not to increase risk from exposure to the chemical.

From these data, EPA establishes a level of safe exposure—a reference dose—and the FDA establishes an acceptable daily-intake dose. Is this method of calculating risk adequate to predict the possibility that much lower doses found in the environment might have effects mediated by hormonal response systems that operate at millions of times lower doses?

In our study with DES, we found that the prostate of the developing fetus was permanently enlarged by a maternal dose 10,000 times lower than the 200-microgram-per-kilogram dose that caused an acute toxic effect, including decreased body weight and an inhibition of normal prostate development. We also found that a dose 10 times lower than the acutely toxic 200-microgram-per-kilogram dose did not significantly stimulate or inhibit prostate development relative to untreated males and did not significantly decrease body weight.[20] This dose would traditionally be considered the no-observed-adverse-effect level, and would be used as the basis for calculating safe exposure levels. Correcting 100-fold for uncertainties, the predicted safe dose of DES would still be 100-times higher than the maternal dose that permanently enlarged the prostate in male offspring.

Is it reasonable to assume that other estrogenic chemicals will also show an inverted-U dose-response relationship similar to that for DES and estradiol? Unless all environmental estrogenic endocrine disruptors behave differently from our natural estrogen estradiol and the manufactured estrogenic chemical DES, we have to assume that in a traditional toxicology study design, the endocrine-disrupting effects at low doses—in many cases similar to amounts being consumed by people—could be missed. We propose that until endocrine-disrupting chemicals are tested using doses within the environmentally relevant range that people and wildlife are being exposed to, we cannot assume that these exposure levels are safe. Current testing procedures need to be revised, and chemicals in use today that are being released into the environment need to be tested for endocrine-disrupting effects at environmentally relevant doses.

No Threshold Dose

Not only do we find that very low doses of estrogenic chemicals can have significant adverse effects, we also maintain that there is no threshold, or level of exposure below which there is no effect. The no-threshold-dose hypothesis has been tested in sex-determination studies in turtles and in breast cancer studies in women. These studies have shown that there is no threshold below which there is no response to estrogenic chemicals. There may also be no threshold for a response to other endocrine disruptors that operate through different endogenous response mechanisms.

The concept of a threshold for any potential toxicant is a theoretical concept because no matter how low the dose that gives a statistically significant response, it can always be argued that the threshold lies somewhere below that dose and was missed by the experiment. Also, even if a large number of animals are examined in a study, research results may show no significant difference between the control and the animals that received the dose. This may suggest that there is a threshold. However, by further increasing the number of animals in these studies, a significant response may be revealed no matter how small the dose. In many cases, therefore, the question of whether a threshold exists cannot be resolved.

However, in some circumstances, it is possible to show that a threshold dose will not be observed in an experiment involving administration of a chemical that mimics the activity of a natural hormone—such as estradiol—that is already present at levels above the threshold for responses. In this situation, whether or not there is a threshold for a particular response to the endogenous hormone is irrelevant, because responses to the hormone are being observed. For example, consider the situation in which the maternal ovaries and placenta are secreting estrogen that passes into the fetal circulation—which occurs in a human pregnancy—and estrogen causes irreversible developmental effects. The threshold for an estrogenic effect is already exceeded by the animal's own estrogens before any dose of an estrogenic endocrine-disrupting chemical is administered to the animal.

In rodents, for example, endogenous estradiol influences development of numerous tissues and already exceeds the threshold dose. Very small changes in circulation of estradiol produce lifetime differences in the functioning of organs.[21] This natural estrogen thus causes irreversible effects. Any added dose, no matter how low, of an estrogenic chemical that increases the number of estrogen receptors activated by estrogen will permanently alter the course of development, and thus experi-

mentally no threshold dose will be observed.[22]

Of Turtles and Women

In some species such as turtles and lizards and in all crocodilians, temperature determines whether the animal develops testes or ovaries.[23]

Unlike mammals and birds, these animals possess no X,Y chromosomes that determine sex. Instead, the incubation temperature of the egg in which the embryo develops determines gonadal sex. For the red-eared slider turtle, for example, during embryonic life, low temperatures stimulate production of testes and produce males, while higher temperatures spur production of ovaries and produce females. At intermediate temperatures, different proportions of males and females are found, with the proportion of females increasing with temperature. The evidence is that the level of endogenous estrogen production in the egg is controlled by temperature and that estrogen mediates sex determination.

If estradiol or other estrogenic chemicals are applied to the eggshell at temperatures that would normally lead to the development of testes, the undifferentiated embryonic gonads develop into ovaries. As temperature increases, lower doses of exogenous estrogen are required to produce ovaries in the embryos that would otherwise become males, because background levels of endogenous estradiol increase as temperature increases. Conversely, when eggs are incubated at the higher temperatures that normally produce all females, administration of an inhibitor of the enzyme aromatase—the critical enzyme involved in the synthesis of estradiol—results in a decrease in the proportion of embryos with ovaries. Instead, embryos develop testes.

Incubation of eggs at temperatures that produce a minority of females meets the criteria for testing the threshold-dose hypothesis. At these temperatures there is a measurable background incidence of sex reversal—formation of ovaries rather than testes. This is due to the elevated circulating level of endogenous estrogen in the eggs that occurs because of a slight increase in temperature over levels that result in 100-percent males.

Analysis of three published estradiol dose-response data sets reveals no threshold dose.[24] This means that any dose, no matter how low, will result in some response, and that the incidence of the response at any dose below the lowest one used in the experiment can be directly calculated.

Thus, a dose that produces no effect does not exist. There can be no safe dose.

Numerous human studies have established that some women may develop breast cancer even if they are never exposed to any exogenous, endocrine-disrupting chemicals.[25] Likewise, in animals, ovarian estrogens increase the incidence of breast cancer in a variety of experimental models, and the incidence of breast cancer is markedly lower in ovariectomized animals.[26] Treatment of ovary-intact animals with any one of a wide variety of estrogens increases the incidence of breast cancer above that found in controls because there is an increase in estrogenic stimulation of the breast. Therefore, women, as a population, are already above the threshold dose for estrogen-induced disease, and exposure of breast tissue to estrogenic endocrine disruptors at any dose should increase the risk for breast cancer.

Reception Problems

The breast cancer example shows that exposure to endogenous hormones at concentrations within the normal range can, at some stages of life, induce adverse effects and contribute to the background prevalence of adverse effects within a population. Another example is that the incidence of testicular cancer in males has been related to fetal exposure to levels of natural estrogen.[27]

These findings lead to two conclusions. First, since the background incidence of adverse effects such as breast and testicular cancer is already high, there is no threshold dose for exogenous estrogenic chemicals to increase the likelihood of these adverse effects. This lack of a threshold dose is of profound importance. It means that every dose carries risk. There is no safe dose; there are only levels of risk that society deems acceptable.

Second, inverted-U dose-response curves are a consequence of normal physiological regulatory mechanisms under the control of endogenous hormones. Such nonmonotonic, inverted-U curves are commonly encountered in endocrinology.

One reason for a lower response at high doses is that high doses can decrease the numbers of hormone receptors in hormone-responsive cells while much lower doses of the hormone can produce an increase in receptors.[28] Also, hormones usually bind to their corresponding receptors: estrogen to estrogen receptors, androgens to androgen receptors.

At high, toxicological doses, hormones may bind to inappropriate receptors. For example, at high doses, estradiol binds not just to estrogen receptors, but also to androgen receptors that normally bind to the masculinizing hormone testosterone. This interferes with the effects of androgen in cells. During fetal life, this permanently interferes with normal masculinization in

males. Thus, at high doses, estrogenic chemicals interfere with a cell's ability to respond normally to other hormonal signals.

Within the normal physiological range of hormonal activity, there is a delicate equilibrium that hormone-mimicking environmental chemicals disrupt. High or toxic doses of natural hormones or hormone-mimicking chemicals can produce effects that are dramatically different from low doses that disrupt this balancing act.

An important issue with regard to the concern over permanent effects of endocrine disruptors during fetal life is that effects in fetuses can be quite different from effects of the same chemicals in adults. This finding relates to whether the principle of homeostasis, the regulatory mechanisms that govern the maintenance of a constant internal environment, can be applied to embryos and fetuses based on studies of these same mechanisms in adults. While some regulatory mechanisms exist in the developing organism, homeostasis as described in adults does not.

For example, administration of a high dose of estradiol to pregnant mice results in an increase in circulating testosterone in male fetuses, while high doses of the same hormone in adults result in a decrease in testosterone.[29] In addition, female fetuses have high levels of pituitary gonadotropic hormones, which regulate estrogen levels in adults, together with high levels of circulating estrogen. This is clearly inconsistent with the homeostatic mechanisms of the adult female endocrine system in which estrogen exerts a negative feedback effect on the pituitary and suppresses the gonadotropic hormones.[30] Therefore, studies of adults cannot predict how hormones and hormonally active chemicals will behave in the fetus.

No Safe Dose

Recent research has revealed new information about the way estrogenic endocrine-disrupting chemicals behave. This information seriously jeopardizes the validity of traditional toxicological studies. In fact, we can now say that there is a biologically plausible basis for the hypothesis that there is no safe or threshold dose of these chemicals. These issues, which have now been addressed for two classes of endocrine disruptors—estrogenic chemicals and dioxin-like chemicals—now need to be explored in many other experimental systems and for other classes of endocrine disruptors.[31]

As we obtain more information and a better understanding of how these previously unanticipated phenomena occur, the procedures for establishing socially acceptable exposure levels will likely change. If we accept that endocrine-disrupting chemicals do not show a response threshold in some circumstances, we will have to abandon the traditional concept that there are safe exposure levels that pose no increase in risk. We should acknowledge that there are risks involved in the use of products containing endocrine-disrupting chemicals and establish levels of risk that society deems acceptable.■

Frederick S. vom Saal is a reproductive endocrinologist in the Division of Biological Sciences at the University of Missouri, Columbia, and Daniel M. Sheehan is a developmental toxicologist at the National Center for Toxicological Research, U.S. Food and Drug Administration in Jefferson, Arkansas.[29]

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32. The opinions expressed here do not necessarily represent those of the Food and Drug Administration.