Evaluating the Effects of Endocrine Disruptors on Endocrine Function during Development

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The major concerns with endocrine disruptors in the environment are based mostly on effects that have been observed on the developing embryo and fetus. The focus of the present manuscript is on disruption of three hormonal systems: estrogens, androgens, and thyroid hormones. These three hormonal systems have been well characterized with regard to their roles in normal development, and their actions during development are known to be perturbed by endocrine-disrupting chemicals. During development, organs are especially sensitive to low concentrations of the sex steroids and thyroid hormones. Changes induced by exposure to these hormones during development are often irreversible, in contrast with the reversible changes induced by transient hormone exposure in the adult. Although it is known that there are differences in embryonic/fetal/neonatal versus adult endocrine responses, minimal experimental information is available to aid in characterizing the risk of endocrine disruptors with regard to a number of issues. Issues discussed here include the hypothesis of greater sensitivity of embryos/fetuses to endocrine disruptors, irreversible consequences of exposure before maturation of homeostatic systems and during periods of genetic imprinting, and quantitative information related to the shape of the dose–response curve for specific developmental phenomena. *Key words:* androgen, development, embryo, endocrine disruptors, estrogens, fetus, thyroid. — *Environ Health Perspect* 107(suppl 4): 613–618 (1999).
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An endocrine disruptor has recently been described as "an exogenous chemical substance or mixture that alters the structure or function(s) of the endocrine system and causes adverse effects at the level of the organism, its progeny, populations, or subpopulations of organisms, based on scientific principles, data, weight-of-evidence, and the precautionary principle" (*1*). To address concerns of potential effects of endocrine disruptors, the National Institute of Environmental Health Sciences and other co-sponsors held a workshop to characterize the effects from environmental exposures to endocrine disruptors on human health. The workshop provided a forum to discuss methods and data needed to improve risk assessments of endocrine disruptors. This article is the product of one of six subgroups from that workshop. It is based on the work group's discussion of a set of questions provided by the organizing committee of the workshop. The following is a list of questions posed to the working group on endocrine function during development that served as the basis for the information discussed in this report.

- What should be included in a baseline model to describe quantative relationships among the processes controlling normal development?
- How do perturbations at critical stages of development lead to adverse effects, e.g., impaired reproductive function, neurologic effects, cancer?
- How can these changes be quantified?
- By what mechanisms do endocrine disruptors perturb endocrine function during development and alter risks from normal levels of endogenous hormones?
- What are the principal mechanisms by which endocrine disruptors are thought to act on the developing reproductive tract?
- Are there effective repair mechanisms operating during development to reduce the effects of endocrine disruptors?
- Are there adequate/relevant animal models for evaluating potential human effects?

We focused on the regulatory processes of normal development and on how exposure to low doses (that is, doses encountered in the environment) of endocrine disruptors at critical stages of development can lead to adverse health effects. We also discussed areas where information is needed to permit better evaluation of the risks of endocrine disruptors.

The authors feel that additional research in five areas is essential: *a*) mechanisms of normal development; *b*) differences of endocrine disruptor effects between embryo/fetus/neonate and adult; *c*) mechanisms of endocrine disruption; *d*) dose–response assessment involving examination over a wide range of doses, from levels encountered in the environment through doses that produce acute toxicity; and *e*) the design of screens to accurately predict unique developmental effects.

Mechanisms of Normal Development

Basic information is needed on the normal molecular, cellular, and physiologic developmental mechanisms perturbed by altered endocrine function during organogenesis (*2–4*). Some of the resultant developmental changes may not be detectable until later in life (*5*). Also, knowledge acquired through the study of developmental perturbation is likely to lead to a better understanding of normal processes occurring during that time in life.

Information is required for both humans and other animals. Knowledge of mechanisms affected by endocrine perturbation due either to congenital defects, including experimental gene knockout systems, or to application of synthetic or naturally occurring endocrine-mimicking compounds would be useful.

We recognize that development is epigenetic, which refers to changes in gene activity during development that are mediated by environmental (chemical) signals (*6*). Autocrine, paracrine (such as growth factors), and endocrine (such as steroid) signals coordinate the direction of differentiation of tissues during critical periods in development. The differentiation of organs thus involves a complex cascade of signals whose action is dependent on being released at precise times and within a specific dose range. Coordination of these processes depends on the transcription of genes coding for these signaling molecules and their receptors at appropriate times and appropriate rates (*7–9*).

In the field of endocrine disruption, particular regulatory emphasis has been placed on processes or tissues affected by estrogens,

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androgens, and thyroid hormones, as well as their antagonistic analogs. Organ systems responsive to the sex steroids include the male and female reproductive organs, the central nervous system, and the immune system, whereas thyroid hormone affects most tissues. The work group focused only on these three hormone groups. This decision was based on the extensive literature that is available regarding these developmentally important signaling molecules. The current identification of particular endocrine-disrupting chemicals as mimics or antagonists of the sex steroid (estrogen and androgen) and thyroid hormones, and their respective functions, facilitates the work group's goal toward an understanding of the mechanism of action of these known endocrine disruptors.

Quantitative aspects of these three components of the endocrine system must be carefully considered to determine if certain developmental events and tissues are particularly sensitive to the test compounds. With specific regard to the dose issue, a critical question that remains to be resolved is whether higher doses may actually inhibit some responses that are stimulated by much lower doses, causing what has been described as an inverted U-shaped dose–response curve (*7*). To understand this phenomenon, the normal concentration range for hormones being disrupted must be characterized with regard to a variety of responses (Figure 1).

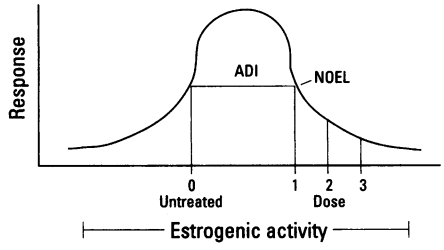

Figure 1. An inverted-U (nonmonotonic) dose–response function associated with an increase in total estrogenic activity in the blood. As shown here, there is already a response occurring at zero dose of exogenous estrogenic endocrine disruptor, due to the presence of endogenous estrogen that is circulating in blood at a concentration above the threshold for the response [based on data in Sheehan et al. (*38*)]. On the basis of the assumption of a monotonic dose–response function, which may not be a valid assumption for endocrine disruptors, the conclusion would be that dose 1 represents a threshold dose below which no effect occurs (the response is at the control level), and lower doses are then not tested. The labeling of dose 1 in this figure as the NOEL (no observed effect level) on the basis of testing three high doses is only valid if the dose–response function does not form an inverted U. Similarily, the use of the NOEL to estimate an acceptable daily intake (ADI) dose would be invalid if there were an inverted -U dose–response curve. The figure is based on data for prostate weight in adult male mice following exposure to different doses of estrogenic chemicals during fetal life (*7*).

Examples of additional information needed on normal development include the effects on *a*) spatial (*9*) and chronologic patterns of expression of relevant nuclear receptors (including isoforms) and of genes known to integrate cellular processes of development, such as the homeobox genes Hox, Wnt, Pit-1, Pou, etc., and *b*) hormone-synthesizing and hormone-catabolizing enzymes after treatment with hormone analogs or endocrine-disrupting chemicals (*4,8*). Quantitative analyses of such responses should be stressed in an attempt to allow formulation of predictive hypotheses.

Differences between the Embryo/Fetus/Neonate and Adult

During the differentiation of reproductive organs, hormones, growth factors, and other endogenous chemical mediators regulate gene expression and direct differentiation (*10*). One marked difference between exposure to endocrine disruptors during critical periods in development versus during adulthood is the irreversibility of an effect during development (*9,11,12*).

Evidence indicates that changes in concentrations of androgen and estrogen (two hormones involved in differentiation of the reproductive organs) result in permanent changes in cell function. For example, the higher circulating levels of testosterone (by 2–3 ng/ml) in male mouse fetuses relative to female fetuses result in the differentiation of tissue in the cranial region of the urogenital sinus into prostatic tissue as opposed to vaginal tissue. Many other differences between males and females are also mediated by this small sex difference in testosterone (*12*). In addition, a small increase in total circulating estradiol (about 50 pg/ml) permanently altered prostate size in mice (*7*). It is thus plausible that disruption of the action of estrogen or androgen during critical periods can lead to permanent alterations in the development of reproductive organs and other tissues with receptors for these hormones. Some of these effects can be unique to the time during development in which the hormonal alteration occurred (*11*). This contrasts with cyclic changes in hormones that occur normally in adult females during the menstrual cycle that do not produce permanent effects.

Although development is a period of change, there are regulatory processes involved in developmental processes, such as changes in plasma-binding proteins during pregnancy, that alter bioavailability of circulating steroids (*13,14*). However, the principle of homeostasis, which implies a level of constancy, is difficult to apply during development.

Diczfalusy (*15*) initiated the concept of the maternal–placental–fetal unit. It is now accepted that pregnancy in mammals represents the interaction of three endocrine systems, all of which are changing throughout pregnancy. The vast differences in gestation length, hormone production, and the degree of intimacy of fetal–maternal blood supplies represent important barriers to understanding the complex interactions between these systems in one species on the basis of information obtained in another. Little is known concerning the regulation of protein and steroid hormones by the placenta in most species, and this lack of information limits predictions concerning the effects of endocrine-disrupting chemicals on the functioning of the maternal–fetal–placental unit. What is known, however, is that regardless of the species, outcomes of endocrine manipulations in adults are not predictive of endocrine changes in fetuses (*11*).

Mechanisms of Endocrine Disruption

Numerous mechanisms of endocrine function have been disrupted by endocrine disruptors. Consideration of these end points allows the identification of end point measures that can be used in specific screens and tests. End points for the three hormonal systems that are the focus here are also the focus of new regulations currently being developed by the U.S. Environmental Protection Agency (U.S. EPA) under congressional mandate and named the Endocrine Disruptor Screening and Testing Program (*1*). Examples of end points include the following.

Steroids (Estrogen/Androgen)

Receptor binding and function. This includes both activation and inhibition and is an important mechanism of endocrine disruption (*14,16*).

Steroid synthesis inhibition. This is a well-known mechanism by which steroid (estrogen/androgen) hormone systems are disrupted (*17*).

Plasma transport and rate of metabolism and clearance. An example is the free concentration of steroid (not bound to plasma-binding proteins) in blood, which changes dramatically between development and adulthood in rodents (*18*). Differences between endogenous steroids and endocrine disruptors in binding to plasma-binding proteins can dramatically alter the potency of endocrine disruptors compared to the hormone, such as estradiol, being mimicked by the endocrine disruptor (*19*). Endocrine disruptors may require metabolic activation in order to interact with one of these mechanisms (*16*).

Thyroid

Receptor binding and function. Currently there are no reports of xenobiotics binding to the thyroid hormone receptor.

Synthesis inhibitors. Several classes of endocrine disruptors fall into this category, including compounds that block thyroperoxidase (TPO), iodide uptake, and the deiodinases (*20*).

Plasma transport and rate of metabolism and clearance. Thyroid hormone must be carried through the blood on serum proteins. Some endocrine-disrupting chemicals (polychlorinated biphenyls and dioxin) inhibit thyroid hormone binding to plasma transport proteins, resulting in more rapid clearance and reduced thyroid hormone levels (*21*).

Several types of endogenous hormones and endocrine disruptors have been found that interact with more than one component of the endocrine system. An example involves compounds, such as genistein in soy, that are weak estrogens but that also block TPO (*20*). Another example is that at a higher than physiologic concentration, estradiol binds to androgen receptors (*22*). Similarly, some estrogenic endocrine disruptors, such as the bis-hydroxy metabolite of the insecticide methoxychlor, also bind to the androgen receptor (*23,24*). Endocrine disruptors that bind to steroid receptors such as *p,p'*-DDE (the persistent *in vivo* metabolite of the insecticide DDT) thus show the highest affinity for one steroid receptor (in this case, androgen receptors) but also show a lower binding affinity for other receptors (estrogen receptors) (*25,26*). As the dose of *p,p'*-DDE or methoxychlor increases, they will thus bind to multiple receptors. As a result, the change in some end point to increasing doses of an endocrine disruptor may reflect its action on different components of the endocrine system, and each component may contribute to a composite dose response. For this reason, the response to a dose on the high end of the dose–response curve may be qualitatively different from and may not be a reliable predictor of the response at much lower doses.

Endocrine disruptors that act to disrupt the estrogen, androgen, and thyroid systems have been the focus of the design of screens and tests for detecting potential endocrine-disrupting chemicals (*25,27,28*). However, we know that these mechanisms do not represent the full range of potential endocrine disruption. Therefore, it is essential to recognize that endocrine disruptors may interfere with hormone actions in ways that would not be identified in the assays currently contained in the new U.S. EPA testing program (*1*). Moreover, there are many potential mechanisms by which endocrine disruptors could produce nonlinear dose–response curves (*29*).

Dose–Response Assessment

The dose issue refers to the application of the previous concepts to characterize the full spectrum of the dose–response curve for endocrine disruptors. The issues are as follows: first, are current risk assessment procedures adequately evaluating the adverse effects of endocrine disruptors by examining only a few doses that may be millions of times higher than those typical of exposure by human or wildlife? Second, there has been considerable interest in the shape of dose-response curves for endocrine disruptors that bind to intracellular receptors for endogenous steroid hormones. However, until now, the establishment of the dose range in toxicologic studies on these chemicals has not been based on an estimation of whether the doses administered would result in doses within target tissues that would be below or above levels that would saturate available receptors for the endogenous hormone(s) being mimicked or antagonized. In a multigenerational study in which adults are administered a chemical before and during the production of offspring, and then the offspring continue to be dosed after weaning (the procedure is then repeated for two generations), three doses are usually examined (*30*). The lowest dose in these experiments is typically a maximum of 50-fold below the highest dose. The highest dose used in toxicologic experiments is based on some index of acute toxicity, such as a decrease in body weight without other signs of overt toxicity.

With regard to the shape of the dose-response curve at low levels for endocrine disruptors that interact reversibly with hormone receptors (and other regulatory macromolecules such as enzymes), consideration should be given to characterizing the dose–response curve within the predicted dose range for regulating receptor activity on the basis of the relative potency of the endocrine disruptor and the endogenous hormone it mimics.

Third, the issue of the type of health risk posed by endocrine disruptors has generated much discussion. There is evidence that endocrine disruptors pose risks to functional end points, such as neuromuscular and behavioral changes (*21,31,32*), and organ function (*5,7,33*). On the basis of these findings, the U.S. EPA will now require tests for endocrine disruptors that focus on adverse effects on organ function (*1*).

Traditional approaches to determine deleterious effects on the developing fetus focused on high doses of compounds that may cause fetal death, malformations, or complete loss of function (such as infertility) (*34,35*). Tests commonly employed include classical teratology tests. Such tests are referred to in the industry as Segment 2 studies in which gross malformations or death are the end points.These studies involve administration of a chemical for a short period in pregnancy. Multigenerational studies have been conducted for relatively few of the chemicals that will be screened by the U.S. EPA for endocrine-disrupting activity (*36*). Whether multigenerational studies conducted with a few high doses will detect effects similar to those seen with much lower doses is currently being investigated for a few endocrine-disrupting chemicals in studies being conducted by the National Toxicology Program within the National Institute of Environmental Health Sciences.

Data for the mechanism of action of the endocrine disruptor in question provide a basis for predicting the types of adverse effects that may occur. However, these types of data have not been available for most multigenerational studies that have been conducted, or if known, were not applied in the determination of doses to be examined [for contrasting approaches in examining a chemical used in plastic, bisphenol A (*14,34*)]. At present, limiting factors in using multigenerational studies to determine adverse developmental effects include the time required to complete these studies, interpretation of the extensive amount of data generated, and cost effectiveness of such studies with respect to the knowledge gained about the effects. An increase in the number of doses used in these studies would increase costs unless accompanied by the use of smaller numbers of animals per group. A resolution of these complex issues will require more information than is now available.

The limitations of traditional teratologic and multigenerational studies led the working group to suggest the following research needs: first, relevant and sensitive quantitative end points must be identified and tested over a much wider range of doses than have previously been examined. Second, the design of these experiments should require knowledge of the variability of the end points in the control population to adequately assess the numbers of animals that should be examined (i.e., conduct statistical power analysis). Third, the shape of the dose–response curve for specific responses should be determined with respect to endocrine disruptors within a particular class (for example, endocrine disruptors that bind to estrogen receptors and show full agonistic activity). Fourth, the mechanisms of receptor binding and activation (and other mechanisms) should be determined over the full range of dose responses. And finally, new strategies and models for dose–response assessment should be developed as data become available.

In toxicologic studies, the current model for endocrine disruptors is based on the hypothesis that *a*) as dose increases, response will increase or stay the same (a monotonic dose–response curve is assumed), and *b*) a threshold exists below which there is no

increase in risk (relative to controls) due to exposure (*37*). These assumptions, which are based on studies conducted with high doses of chemicals, have been challenged by the results of experiments involving low doses of endogenous hormones and endocrine disruptors (*7,14,29,38*).

There are currently only a few ongoing studies, including multigenerational studies, that have been designed to address some of these modeling needs and questions. By addressing these issues, information will be provided concerning the need to expand the dose range for some chemicals. It will be important to determine which properties of chemicals might predict whether their dose–response relationships will behave in a complex fashion. Finally, regulatory agencies will have to assess the impact that this information will have on regulatory policies that drive the design of toxicologic studies (*1*).

Ability of Screens to Predict Embryonic Effects

Current hazard identification (for example, identification of whether a chemical is an endocrine disruptor) and, more generally, risk assessment paradigms need to be reevaluated to determine their effectiveness at assessing effects of low doses of potential endocrine disruptors on the developing organism. Although screening systems can be designed to identify endocrine-disrupting chemicals that elicit effects at low doses, an additional concern is whether there are unique effects of exposure to these endocrine disruptors during critical periods of development (i.e., organogenesis). The concern is that effects caused by exposure to endocrine disruptors during critical periods in development may not be predicted by studies conducted at later times in life (after weaning) and also may not be detected by *in vitro* screens. There are data that support this possibility (*5,17,24,39–41*). Additionally, the identification of which end points in which tissues should be evaluated for unique effects due to exposure during development needs to be more carefully examined.

Proposed Chemicals to Address the Issue of Dose in Tests for Endocrine Disruptors

Considerably more empirical data are needed that directly compare the high end of the dose–response curve with the low end. To address this issue, the work group suggested the following compounds for initial evaluation: diethylstilbestrol (DES), methoxychlor, bisphenol A, octylphenol, phthalates, ketoconazole, flutamide, propylthiouracil (PTU), and genistein. These compounds are proposed because much is already known about their effects and mechanisms of action and because they present different spectra of effects and mechanisms. Specifically, DES is a potent ligand for the estrogen receptor (ER). Methoxychlor has both estrogenic and antiandrogenic effects and must be metabolized to be active (*24*). Bisphenol A is an estrogenic chemical that binds to the ER with modest affinity (*14*) and has been reported to result in prostate enlargement and other changes in the reproductive system in mice (*42*) and changes in pituitary function in rats (*43*). Octylphenol also binds to the ER and is estrogenic in *in vitro* and *in vivo* assay systems (*44*) but shows significantly different binding to plasma steroid-binding proteins than bisphenol A (*14*). Some phthalates, such as dibutyl phthalate, show evidence of nonreceptor-related effects on the androgen system (*17*). Ketoconazole blocks androgen synthesis and thus is antiandrogenic by a receptor-independent mechanism (*17*). Flutamide is a relatively pure androgen receptor blocker and provides a positive control antiandrogen (*25*). PTU produces thyroid effects by inhibiting thyroid hormone synthesis (*45*). Genistein has many actions, among which are binding to and activation of the ER as well as tyrosine kinase inhibition (*19, 46*). Thus, while a common thread of hormone-related activities runs through this group of chemicals, they present a sufficient spectrum of effects to allow a more broad assessment of the possibility of low-dose effects and qualitative differences in response across the dose–response curve associated with nonmonotonic functions.

The doses used for these *in vivo* studies should cover the dose ranges from just below overtly toxic (using the current method of high dose selection) to approximately 6 orders of magnitude lower. This dose range should be sufficient to provide some information on the likelihood of nonmonotonic dose–response functions.

Although the end points measured in these studies should be relevant to the compound being tested, whenever possible, an attempt to link end points to currently accepted indices of toxicity should be made. At least some of the end points measured should take advantage of what is known about the molecular effects and mechanisms of each compound (i.e., levels of hormones being mimicked, receptor number and action in specific target tissues), whereas others should be more organ-level and whole-animal-level end points (i.e., development of the reproductive or thyroid systems, gamete numbers, or rate of growth). The purpose of examining more sensitive end points for each compound against the more traditional end points in toxicologic studies is to establish whether an effect is adverse by traditional criteria. However, it is also recognized that part of the new paradigm that has been developed by the U.S. EPA in its endocrine disruptor screening and testing program is a focus on a different set of outcomes from those previously used in most toxicologic studies (*1*).

The development of this database will provide important information regarding the prevalence of nonmonotonic dose–response curves and unique low-dose effects. As this information becomes available, it can be decided if current dose–response assessment, hazard identification, and risk assessment paradigms need to be further modified. This will be possible, as the endocrine disruptor screening and testing program is designed to be a process that can be modified as new information becomes available [*1*). Future decisions must be based on data, not on presumption and extrapolation.

The question of whether mixtures of compounds have a profile of toxicity that differs qualitatively from that of its components has also been a concern with regard to endocrine disruptors. This question is of special importance given new regulatory mandates (i.e., Food Quality Protection Act) to carry out risk assessments based on the accumulated exposures to agents that exert their toxicity by a common mechanism. In practice, the default approach to cumulative risk assessments is to consider the effects of individual components to be additive if they induce similar effects and there is no contradictory evidence to suggest a nonadditive interaction. However, if the dose–response relationships are complex and nonlinear for the components, then this practice would not be appropriate. This issue must be addressed, particularly if it is determined that endocrine disruptors have complex, nonmonotonic dose–response relationships.

Prostate Development as an Example of an Endocrine-Mediated Process Subject to Endocrine Disruption

Prostate development in the male mouse serves as a good example of the potential seriousness of endocrine disruptors for the developing fetus. The prostate gland develops from the urogenital sinus (UGS) under the influence of androgens. In the day-14 male mouse embryo, testicular testosterone secretion increases, but testosterone must be converted to 5α-dihydroxytestosterone (DHT) by 5α-reductase for normal prostate development to occur. DHT stimulates androgen receptor-positive mesenchyme cells to induce glandular epithelial budding. Thus, the critical parameters for modeling are fetal circulating testosterone levels, UGS mesenchymal 5α-reductase activity, androgen receptor content of UGS mesenchyme, and mass of UGS mesenchyme at the time of initial prostate organogenesis (*10*).

With regard to the measurement of estradiol and testosterone in plasma, the total concentration in plasma can be misleading. In rodents, the concentration of estrogen binding plasma proteins (alphafetoprotein) that modify uptake into tissues is dramatically higher in fetuses than adults. As a result, the free (unbound to plasma proteins) concentration of estradiol is approximately 10-fold lower in fetuses than in adults (*18*). This is important because endocrine-disrupting chemcials show significant differences in binding to these plasma proteins relative to estradiol, and thus show substantially different uptake into fetal tissues than predicted by *in vitro* assays that do not take this into account in assessing the potency of these chemicals (*19*). In sharp contrast, the levels of free testosterone are quite high in fetuses, as rodents do not have a high-affinity plasma-binding protein for testosterone (*47*).

A small increase in circulating estradiol in male fetuses permanently increases adult prostate weight via an increase in gland genesis (*7*). Specifically, a 50% increase in circulating estradiol led to a 40% increase in the number of prostatic epithelial buds at the end of the first day of prostate differentiation. Fetal testosterone levels were not increased by estradiol treatment, but prostatic androgen receptors were permanently increased (*7*). However, whether UGS 5α-reductase activity was influenced by estradiol treatment and whether there was any change in the mass of underlying UGS mesenchyme has not been examined. Quantitative analyses of these additional parameters would assist in formulating predictive hypotheses.

The relevance of the prostate model to the assessment of endocrine-disrupting compounds is that it provides an example of an organ that has been extensively studied with regard to the impact of alteration in enzyme activity (5α-reductase) and circulating steroid levels (namely, testosterone and estradiol). Levels of these steroids can be added to or interfered with by endocrine disruptors that bind to their receptors and act as mimics or antagonists, respectively. Alternatively, circulating levels of these steroids could be altered by disruption of synthesis of or by competition for binding to plasma steroid-binding proteins. The importance of the interaction of mesenchyme and epithelium in the UGS has been studied in great detail, and there are data on the ontogeny of steroid receptors (*4,48,49*). A detailed understanding of the mechanisms of development is required to fully understand the mechanisms of endocrine disruption, particularly with regard to understanding in molecular detail the possibility that low doses of a particular chemical might interfere with normal developmental processes (*30*).

Conclusions

Much of the controversy surrounding the problem of endocrine disruptors in the environment is related to potential effects on the embryo and fetus. This working group determined that we have limited information on both the normal role of the hormones in development and on potential endocrine disruptors. Multigenerational assays have been the only means of assessing the potential for disrupting normal development by endocrine-disrupting chemicals. The principal conclusion is that there is a need for more basic information about hormonal involvement in development and for new methods to assess a variety of compounds for endocrine disruptor activity, particularly during critical periods in organogenesis.

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