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 organs, 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 hold a workshop to characterize the effects from environmental exposure to endocrine disruptors on human health. The workshop provided a forum for discussion 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 workshop groups' 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 functions during development that served as the basis for the information discussed in this report.

What should be included in a baseline model to describe quantitative relationships among the processes controlling normal development?

How to 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 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 disruptors between embryos/fetus/newborn and adult; c) mechanisms of endocrine disruption; d) dose-response assessment involving examinations over a wide range of doses, from levels encountered in the environment through those 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 trait development 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 this time in life.

Information is required for both humans and other animals. Knowledge of mechanisms affected by endocrine perturbations 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 modulated by environmental (chemical) signals (6). Aqueous, parasite (such as growth factors), and endocrine (such as sex steroid) signals coordinate the direction of differentiation of tissues during critical periods in development. The differentiation of target organs that 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 factors controlling these signaling molecules and their receptors at appropriate times and appropriate rates (7-9).
androgens, and thyroid hormones, as well as their antigens. Analogous actions of the sex steroids include the male and female reproductive organs, the central nervous system, and the immune system, whereby 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 (hormonally driven) dose-response function associated with an increase in total estrogen activity in the blood. As shown here, there is already a near-maximum increase in estrogen-conjugates (estrogen) 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 Strong et al. [20]). On the basis of the assumption of a monatomic dose-response function, which may not be a valid assumption for endocrine disruptors, the conclusion would be that the total area under the fitted curve below which no effect occurs (the response is at the control level) and above which it is not testable is the area of dose in Figure 1. In this case, the NOEL observed effect level on the basis of testing three high doses is not valid if the dose-response function does not form an inverted U. Similarly, the NOEL for the estrogenic activity would not be valid if there were an inverted U (doose-response curve). The figure is based on data for pregnant adult in adult male mice following exposure to different levels of estrogenic chemicals during fetal life (7).

Examples of additional information needed on normal development include the effects on a) spatial (9) and chronic patterns of expression of relevant nuclear receptors (including androgen) and of genes known to integrate cellular processes of development, such as the homeodomain Hox, and POU, etc., and b) hormone-synthesizing and hormone-converting enzymes after treatment with hormones 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 (19). 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 androgens and estrogens (two hormones involved in differentiation of the reproductive organs) result in permanent changes in cell function. For example, the mature testis is sensitive to the presence of testosterone (1–2 ng/ml) in male mouse foetuses relative to female foetuses. Testosterone is responsible for tissue differentiation (determination of testes) and for the inability of a male to become pregnant. This sex difference in testosterone level of male and females is also mediated by this small sex difference in testosterone levels (12). In addition, a small but significant total circulating estradiol (about 50 pg/ml) permanently altered prostate size in mice (7). It is thus plausible that development of the reproductive system is irreversible during early adulthood. Estrogen can be detected in the testes of male and females at the same level throughout the developmental cycle that does not produce permanent effects.

Although development appears at a certain point of change, there are regulatory processes involved in development, such as changes in plasma-binding proteins during pregnancy, thalamus development in mice, that alter bioavailability of circulating steroids (13,14). However, the principle of hormonally sensitive tissues, which implies a level of constancy, is difficult to apply during development.

Neonatal exposure to an agent that causes the masculinization of the masculino-placental-fetal unit is not currently 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 innervation of fetal-maternal blood supplies represent important barriers to understanding the complex interactions between these systems in any 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 function of the maternal-fetal-placental unit.

What is known, however, is that regardless of the species, outcomes of endocrine manipulations in mammals are mediated by endocrine changes in fetuses (15).

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 ancitators that can be used in specific screens and tests. End points for the three hormone systems that are the focus here are also the focus of new regula- tions currently being developed by the U.S. Environmental Protection Agency (U.S. EPA) under congressional mandate and through the Endocrine Disruptor Screening and Testing Program (1). Examples of end points include the following:

- Steroids (Estrogen/Androgens)
- Receptor binding and function; this is an indicator of activation and inhibition and is an important mechanistic of endocrine disruption (16,17). Steroid synthesis inhibition. This is a well-known mechanism of endocrine disruption (18). The use of methods that are specific for the hormones or estrogenic or androgenic systems have been disrupted (17).
- Plasma levels and rates of metabolism and clearance. An example is the free concentration (not bound to plasma-binding proteins) in blood, which changes dramatically between development and adulthood in rodents (19). Differences between endogenous androgens and endocrine disruptors in binding to plasma-binding proteins can dramatically alter the potency of endocrine disruptors compared to the hormone, such as estradiol, which is 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 thyroid binding to the thyroid hormone receptor.
Synthesis inhibitors. Several classes of endocrine disruptors fall into this category, including compounds that block thyro- 
oids (4.37, 130), iodide uptake, and the desensitization (29). 
 Plasma transport and rate of metabo-
ilmation and diffusion. These factors affect the ability of the hormone to be carried through the blood to serum pro-
 teins. Some endocrine-disrupting chemicals (polychlorinated biphenyls and dioxins) inhibit thyroid hormone binding to plasma proteins, resulting in more rapid clearance and reduced thyroid hormone levels (27).
 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 (21). Similarly, some estrogenic endocrine disruptors, such as the bis-phosphoryl metabolite of the isocoumarin methoxyestrone, also bind to the androgen receptor (23, 24). Endocrine disruptors that bind to steroid receptors such as α, β, or δ T3 (the persistents in xenobiotic of the inos-
 uciduciduct DTD) also show the highest affinity for one steroid receptor (in this case, androgen receptors) but also show a lower binding affinity for other receptors (testosterone recep-
tors) (25). For example, dioxin may influence the affinity of estrogen receptors in the liver, and estrogen receptors 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 di-
 fferent from and may not be a reliable predic-
tion of the response to a lower dose. Endocrine disruptors that act to disrupt the estrogen, androgen, and thyroid systems have been linked to the following: the design of worker tests and tests for detecting potential endocrine-
disrupting chemicals (25, 27, 30). However, we know that these mechanisms do not repre-
sent the full range of potential endocrine dis-
 ruption. 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 mecha-
nisms 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 pro-
cedures adequately sensitive to the effects of endocrine disruptors by examining only a few doses that may be billions of times higher than the toxicological exposure to human or wildlife? Second, there has been considerable interest in the shape of dose-
response curves for endocrine disruptors to determine whether the exposures for endogenous telepathy is being remixed or antagonized. In a multigenerational study in which the effects of endocrine disruptors in the chemical before and during the production of off-
spring, and then the offspring continue to be used after weaning (the procedure is then repeated for two generations), three doses are usually examined (39). 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, it is important to note that the relative potency of the endocrine disruptor and the endogenous hormone it mimics. This issue is the type of health risk posed by endocrine disruptors has generated much discussion. There is evidence that endocrine-disrupting compounds may alter functional end points, such as neurotransmission. It has been suggested that endocrine disruptors may have adverse effects on organ function (1). Traditional approaches to determine the development 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 toxicology tests. Such tests are referred to as in several studies in which the endocrine disruptor interacts with a given enzyme or pathway. 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 disrupter in question provide a basis for predicting the types of adverse effects that may occur. These studies, the types of effects that have not been available for most multigenera-
tional studies that have been conducted, or if known, were not applied in the determina-
tion of doses to be examined for contrasting approaches in examining a chemical used in plant biophotol (44, 45). As point, lim-
itigating 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 ease of use of these 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 accompa-
nied by the use of smaller numbers of animals per group. A resolution of these complex issues will require more information that is now inadequate.

The limitations of traditional teratogenic and multigenerational studies led the working group to suggest that the first, relevant and sensitive quantitative end points must be identified and tested over a wide range of dose levels. In multigenera-
tional studies, the design of these experiments should require knowledge of the variability of the end points in the control population to adequately assess the num-
ber and type of necessary experiments. These values should be reduced to a level where the dose-response curves can be found in the dose-response curve for specific end points. The goal should be to reduce the number of end points and end points that are related to endocrine disruptors within a particular class (for example, endocrine disruptors that bind to estrogen receptors) and have high appre-
ciatory activity. Fourth, the mechanisms of receptor binding and activation (and other mechanisms) should be determined over the full range of dose responses. And finally, much wider ranges of doses should be used in the studies. At the current time, the dose-response curve is based on the hypothesis that the response will increase at the same (a monotonically dose-response curve is assumed), and a threshold exists below which there is no
increase in risk (relative to controls) due to exposure (17). 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 (15,19).

There are currently only a few ongoing studies, including multigenerational studies, that have been able 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 be in a constant 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 (16).

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 there are screens to detect 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 in development (24,25,28,29). The concern is that effects caused by exposure to endocrine disruptors during critical periods in development may be missed by studies conducted at later times in life (after weaning) and also may not be detected by in vitro screens. These are data that support this possibility (5,17,24,39,41). Additionally, the identification of which endpoints in which tissues should be evaluated for unique effects due to exposure during development needs to be determined.

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

Considering 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, biphenyl A, atrazine, phthalates, ketone, coumazole, frutamide, 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). Biphenyl A is an estrogenic chemical that binds to the ER with high affinity (14,16). The mechanism is that it is estrogenic in its own right and in zebrafish males it is antiandrogenic (14). Oxyphenbutazone also binds to the ER and is estrogenic in some in vivo and in vitro systems (16) but shows significantly different binding to plasma versus binding proteins than biphenyl A (16). Some phthalates, such as dibutyl phthalate, show evidence of receptor-related effects in the androgen system (17). Keratinocytes block androgen metabolism in tissues and thus is anti-androgenic by a receptor-independent mechanism (17). Fluoride is a relatively pure estrogen 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 screening of the effects and qualitative differences in response across the dose-response curve associated with nonmonotonic functions.

The doses used for these in vitro studies modestly overlap the dose range from non-toxic to overly toxic (using the current method of high dose selection) to approximately 6 orders of magnitude for each compound. This 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, whatever possible, as an attempt to link end points to currently accepted indices of reproductive function. At least some of the end points measured should take advantage of what is known about the molecular actions and mechanisms of each compound (i.e., levels of hormones being mimicked, receptor number and action in specific target tissues, whether: others should be more organ level and whole-animal level end points (i.e., development of the reproductive or thyroid systems, growth rate, etc.). The purpose of measuring more sensitive end points for each compound against the more traditional end points in toxicologic studies is to establish a common and effective way to evaluate traditional toxicity. However, it is also recognized that part of the new paradigms that has been developed by the U.S. EPA in its endocrine disrupter screening and testing program is to focus on a different set of outcomes from those previously used in toxicologic studies (15). The development of this database will provide important information regarding the dose, response, and testing program is designed to be a process that can be modified as new information becomes available (13). Future decisions must be based on data, nor on presumption and assumption.

The question of whether mixtures of compounds have a profile of toxicity different from that of its components has been a concern with regard to endocrine disruptors. This question of specificity is given new regulatory implications (i.e., Food Quality Protection Act) to carry out risk assessments based on the accumulated exposure to agents that exert toxicity by a common mechanism. In practice, the default approach as cumulative risk assessments is to consider the effects of different components to be additive if they are similar and to be synergistic if they are not. This approach is not always appropriate, particularly if it is determined that endocrine disrupters have complex, nonmonotonic dose-response relationships.

Prostate Development as an Endocrine Disruptor: Mediated Process Subject to Endocrine Disruption

Prostate development in the male mouse serves as a good example of the potential action sites 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 testicular androgen dihydrotestosterone (DHT) by 5x-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 prostatic ossification (10).
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. Mitigatory approaches have been the only efforts at 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 so that a variety of compound-specific endocrine disruptor activity, particularly during critical periods in organism.

REFERENCES AND NOTES


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