The activity of endocrine-active agents exhibits specificity at many levels. Differential responsiveness to these agents has been observed between different species and extends to interindividual differences within a species and between different tissues as well. In cases where they have been identified, the biologic and molecular mechanisms underlying this specificity are quite diverse. Determinants of species specificity include differences that exist in receptor binding, gene transcription, and cellular responses to endocrine-active compounds between species. Interindividual differences in responsiveness may be determined at the level of genetic polymorphisms in hormone-metabolizing enzymes, hormone receptors, and in those genes that are transactivated by these receptors, as well as during changing windows of susceptibility that occur as a function of age, such as prenatal and postmenopausal exposures. Extrinsic factors such as diet can also impact individual susceptibility to endocrine-active agents. Tissue-specific determinants of susceptibility are well documented, but little is known regarding the mechanisms underlying these different responses. Differences in the expression of accessory proteins for steroid hormone receptors and different patterns of receptor expression, estrogen receptor α and estrogen receptor β, for example, may contribute to tissue specificity, as may differences in the pattern of expression of other genes such as hormone-metabolizing enzymes. The use of animal model systems and development of appropriate mathematical models has the potential to yield additional valuable information for elucidating the role of these determinants of specificity at low-dose exposures and for improved risk assessments for the adverse health effects of endocrine-active compounds. Key words: animal models, endocrine disruptor, metabolizing enzymes, p450, polymorphisms, reproductive tract, steroid hormone receptors, susceptibility. — Environ Health Perspect 107(suppl 4):619-624 (1999).

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This article is the result of a workshop concerned with characterizing the effects of endocrine disruptors on human health at environmental exposures. This workshop provided a forum for the discussion of methods and data needed to improve risk assessments of endocrine disruptors. This working group report addresses issues related to the physiologic and biochemical basis for species, interindividual, and tissue-specific differences in response to an endocrine-disrupting chemical at environmentally relevant doses. In these discussions, group members addressed what factors have been identified that may underlie differential responsiveness at each of these levels and where questions remain to be answered regarding the basis for differences in response that should serve to direct future research initiatives. Included in this report are issues related to generic versus epigenetic phenomena, the adequacy of in vitro and in vivo models for predicting variability, and how this body of information could be used to improve risk assessments for sensitive subpopulations.

Species-Specific Factors That Can Impact Endocrine Signaling

Three levels of hormone activity at which species-specific factors may have an impact were discussed: receptor binding, gene transcription, and cellular response.

Receptor Binding

Several factors were identified that can affect receptor binding to endogenous and potentially exogenous hormonally active compounds (1,2). Such factors include serum-binding proteins (SBPs) that sequester and/or transport hormones to target cells. SBPs are differentially expressed in different species. Although in humans, steroid hormones are found primarily associated with SBPs in the blood, the rat does not express this protein. Both rats and humans express α-fetoprotein during fetal development, but this expression does not persist in the adult rat. SBPs increase/activate cyclic adenosine monophosphate (cAMP) when bound to steroid hormones in hormonally sensitive cells such as the prostate (3,4). Because endocrine disruptors exhibit differences in their ability to bind these same proteins, it would be important to assess whether they may similarly initiate this activation cascade and at what doses.

Differences also exist in the ligand-binding domain of steroid hormone receptors from different species. Whereas rodent and human estrogen receptors (ER) are essentially the same, fish and quail receptors exhibit significant variation in their ligand-binding domains compared to humans. In fact, in some species, receptors are adapted to recognize different hormones (for example, trout androgens and their cognate receptor). However, receptors from all species appear to recognize the same consensus sequence in the DNA.

Ligand-independent receptor activation also exhibits species specificity (5,6). Ligand-independent progesterone receptor (PR) activation does not occur in humans but has been observed for rodents and chickens. The androgen receptor (AR) appears to exhibit ligand-independent activation in humans but not in rats (7). However, whether this difference is real or due to interlaboratory experimental variation is unclear. Similar species differences could also exist at the level of receptor crosstalk, cAMP activation, and AP1 signaling (nontraditional promoter events), and as these differences could impact on the activity of endocrine disruptors in different species, this area warrants further exploration. In particular, ligand-independent activation is facilitated by low levels of hormones ("priming the pump"), suggesting that these nontraditional means of receptor activation may be particularly relevant for low-dose exposures.

Gene Transcription

In terms of specific gene transcription, the work group identified a need to assess the available literature on gene transcription in different species in response to steroid hormones. This discussion led further to a consensus that a hormone-responsive gene chip would be very useful for making this assessment. Such a chip, containing a battery of hormone-responsive genes, could be used to quantify changes in the expression of these genes in different species in response to...
endogenous and exogenous hormones in human, rat, mouse, and fish (fathead minnow). Patterns of gene expression could then be compared across multiple species to identify similarities and differences in hormonally regulated gene expression that could be later correlated with species-specific responses.

**Cellular Response**

Differences in hormone responses have been observed between different species in several hormone-responsive tissues that may be relevant to low-dose effects. In the rat, the development of mammary gland tumors is enhanced by pituitary prolactin production. For example, estradiol-induced tumors in AxC and Noble rats can be inhibited by hypo. In contrast, secretion of pituitary prolactin is not required for tumorigenesis in humans but may in fact be compensated for by the endogenous production of prolactin by the tumors themselves. Thus, as a target for endocrine-disrupting chemicals, altered pituitary function may have quite a different impact in rats than in humans.

Other species differences exist in the timing of windows of susceptibility to the effects of endocrine-disrupting chemicals. For example, in the mouse a critical window for estrogen exposure in terms of changes in prostate weight occurs postnatally, whereas in the rat, postnatal exposures have the most dramatic effects on the prostate. Treatment of rats on postnatal day 3 with either diethylstilbestrol (DES) or estradiol produces a decrease in prostate size and increased dysplasia and carcinoma development in the mature prostate gland.

The existence of the species-specific differences described above underscores the fact that mechanistic information will be necessary to make informed choices regarding the appropriateness of a given animal model for modeling and testing of adverse human health effects as a result of exposure to endocrine disruptors.

**Intrinsic/Genetic Factors Responsible for Interindividual Differences**

Individuals may exhibit differences in susceptibility to endocrine disruptors during different stages of their life cycle relative to adult exposures, and this information should be factored into human risk assessments. Different susceptibilities may exist for prenatal, postnatal, peripubertal, adult, and aged subpopulations (8,9). Prenatally, uterine position effects that have been documented for rodents suggest that very low levels of androgens, and by inference endocrine disruptors, may have effects on the organization of neural and other tissues and may have permanent masculinizing consequences. Variability in anatomical, physiologic and behavioral characteristics of mouse, rat, and gerbil as a consequence of fetal androgen exposure has also been observed. A window of susceptibility has been documented for postnatal exposures to polychlorinated biphenyls (PCBs) and mercury in terms of behavioral/neurologic effects in humans. Experience with DES exposures in both humans and rodents indicates that similar windows of susceptibility exist for the induction of reproductive tract abnormalities and cancer during pre- and postnatal periods of development. The progressive decrease in age of menarche in women that has occurred over previous decades may result in an increased time until first pregnancy if maternal age at conception remains the same. This population shift toward early menarche-late pregnancy could result in an increase in breast cancer risk within the population. This same change, however, could also prove protective for other endocrine-related processes such as osteoporosis. In aged populations, decreased repair enzyme function due to oxidative inactivation decreased detoxification capability, and changes in endogenous hormone levels or metabolites may place this subpopulation at increased risk for the adverse health effects of endocrine disruptors.

Along these same lines, issues were raised in the work group related to cyclical versus persistent exposures. All steroids are released in a rhythmic fashion and some receptor systems show rhythmic changes. It is thus possible that low-dose effects could occur if they are persistent in a system in which endogenous hormone levels exhibit peaks and valleys. Although the amount of endocrine disruptor present might be low relative to peak hormone levels, it could have a biologic impact if exposure occurs during a time in which endogenous hormones themselves are at very low levels or if the exposure to an endocrine disruptor occurs at a susceptible period during cyclical changes in hormone levels. In a similar vein, an interesting question was raised regarding circadian rhythms and whether there were any data to suggest that hormones could have different effects as a function of these rhythms. It was noted that there is an evolutionary link between transcription factors associated with dioxin activity (an endocrine-disrupting compound) and factors involved in regulating circadian rhythms.

The question that arises as a natural consideration of these data is how much more susceptible might individuals be during these different life stages? Additional analysis of available data may give some indication of the magnitude of this increased susceptibility, much as earlier analyses for dioxin (in which good quantitation for species and age effects was available) helped quantify dose-response effects for this compound. However, this is clearly one area in which additional research will be necessary to understand which adverse health effects resulting from exposure to endocrine disruptors occur as quantitative alterations in different susceptible subpopulations and which effects are qualitative in nature and specific for a given window of exposure.

Polymorphisms in steroid hormone-metabolizing genes also represent genetic factors that can predispose to adverse health effects of endocrine disruptors (10-12). 5α-Reductase levels can be altered by the presence of TA dinucleotide repeats in the 3' region of the gene and changes in these levels can impact the conversion of testosterone to dihydrotestosterone (DHT), the active form of this hormone. These polymorphisms have also been linked to increased risk of prostatic carcinoma, although this is somewhat controversial (13-15). The V89L substitution can also affect 5α-reductase activity. Polymorphisms in several cytochrome P450 genes, including Cyp1B1 and Cyp17α (aromatase) as well as catechol-OH-transferase (COMT), have been linked to increased risk of hormone-dependent cancers including breast cancer. Similarly, deletions in glutathione transferase, a detoxifying enzyme for xenobiotics that is present in some individuals, put them at increased risk for breast and prostate cancer, possibly as a result of increased endogenous/exogenous hormone levels (16).

Receptor polymorphisms may also increase susceptibility to endocrine disruptors via changes in the regulation or function of steroid hormone receptors (10). AR hypersensitivity is a function of the length of CAG nucleotide repeats, with individuals carrying shorter length repeats expressing AR that are more sensitive to androgens (17). Polymorphisms in the PR have been associated with increased risk for ovarian cancer, possibly due to increased activity or stability of the receptor. Similar polymorphisms may exist for ER-α and ER-β.

Target gene polymorphisms can also predispose to hormonally related diseases such as breast cancer (18). Individuals carrying BRCA1 mutations, for example, are refractory to the protective effects of pregnancy on breast cancer risk. Whether such target gene mutations would predispose individuals to the adverse health effects of endocrine disruptors is not known at this time. Mutations in other potential target genes may also soon be identified through the Environmental Genome Project, and these will need to be investigated to determine if they can potentially affect susceptibility to low-dose exposures.

Several of the genetic alterations and polymorphisms noted above may contribute
to the observed ethnic differences in risk for hormonally related diseases such as breast cancer and prostate cancer. The frequency of higher activity alleles of Cyp1B1 that result in increased 4-OH-estradiol (4-OH-E2) levels, which in turn are associated with increased potential for free radical damage to the DNA and more potent activation of the ER than 17β-estradiol (17BE2), are more prevalent in African Americans than Caucasians and may contribute to increased breast cancer risk (19). Similarly, alleles of COMT with decreased activity could also increase 4-OH-E2 levels and increase risk for developing postmenopausal breast cancer (11). There is evidence that endocrine disruptors can modulate the activity of estradiol-metabolizing enzymes, with indole-3-carbinol increasing the extent of 2-hydroxylation of estradiol and decreasing mammary tumor incidence and multiplicity in mice (20), whereas PCBs can increase the production of 16α-OH metabolites of estrogen that can bind to the ER and form a protein-reactive Schiff base. The ratio of 2-OH to 16α-OH metabolites is thought to be one determinant of breast cancer risk, with compounds that shift the balance toward 2-OH being protective and those that produce increases in 16α-OH increasing cancer risk.

Several research needs were identified as a result of these discussions related to possible genetic determinants of susceptibility. First, many of the studies relating genetic polymorphisms in the population to increased risk for a particular disease are quite controversial and conflicting data sets have been reported. Therefore, a primary research need is to confirm these studies and resolve conflicting data presented in the literature. Second, the impact of receptor polymorphisms on receptor activation by endocrine disruptors should be investigated to determine if any of these polymorphisms may predispose individuals to the adverse health effects of these chemicals. Finally, the functionality of genetic polymorphisms in metabolizing genes or other relevant genes, especially in terms of how they affect the dose–response curves for endogenous or exogenous compounds resulting in shifts in sensitivity or response levels, needs to be determined.

A second area of research needs relates to epigenetic effects on responsiveness to endocrine disruptors. Hormonal exposure early in development has organizational and lasting effects on later sensitivity to hormones that activate hormone-dependent physiologic and behavioral functions. For example, it would be important to learn whether exposure of a fetus to one or more natural or xenobiotic endocrine-active substances has long-term effects on susceptibility, i.e., endocrine imprinting (10,12,21).

Extrinsic Factors Affecting Susceptibility

Several extrinsic factors such as diet, socioeconomic status, and obesity affect the risk for hormonally related diseases such as breast cancer. Dietary history and previous chemical exposures can produce a biologic imprint that can persist even in the absence of the continued presence of the causative agent. These types of historical exposures may be very difficult to assess in human populations but must be considered as important contributing risk factors. Obesity can have dramatic effects on the hormonal milieu, especially in postmenopausal women in which aromatization of fat stores can significantly alter estrogen levels, particularly levels of estril and estrone. Similarly, increased weight gain in adolescent girls appears to be one of the contributing factors to early onset of puberty. Many of these extrinsic factors are not evenly distributed across ethnic and socioeconomic populations and may contribute to the observed decreased breast cancer risk in Asian women (caloric restriction) and increased risk for breast and uterine cancer in African American women (obesity and early menarche). By definition, these extrinsic factors would be considered epigenetic contributors to increased risk.

Factors Affecting Tissue Specificity

These factors can be broadly grouped into those that modulate the transcriptional activation function of steroid hormone receptors and those that occur as a result of altered patterns of gene expression in specific tissues. A new and important area of investigation in the former category is steroid hormone receptor accessory proteins that can function as either coactivators or corepressors for gene transcription. Very little information is presently available on how these proteins might confer tissue-specific responsiveness, and more research is needed on a) whether polymorphisms in these accessory proteins exist that might have functional consequences for their activity, b) whether expression levels are different in different tissues and how the ratio of coactivators to corepressors affects receptor activation, and c) whether tissue-specific modifications such as splicing variants or phosphorylation might affect their activity. These accessory proteins may participate in nontraditional receptor activation pathways, which as mentioned above, may be particularly sensitive to low-dose exposures. SRC-1 can facilitate ligand-independent activation of steroid hormone receptors and in combination with NCoR can act as a determinant of agonist or antagonist activity for ligands such as tamoxifen (ER) and RU486 (PR) and therefore, possibly for endocrine disruptors as well (22–24).

Tissue-specific receptor distribution or number may also influence the response of different tissues to endocrine disruptors (25). ER-α and ER-β receptors exhibit tissue-specific patterns of expression, with ovary, prostate, testis, brain and bone being primarily driven by ER-β (25). As ER-α and ER-β have different agonist and antagonist activities for the same ligand, this expression pattern could ultimately influence whether a specific ligand acts as an agonist or antagonist in a given tissue (26). The mammary gland also exhibits changes in ER and PR expression as a function of age and differentiation status (for example, during pregnancy or neonatal estrogen exposure) that can alter its susceptibility to induction of breast cancer; similar changes may occur in the uterus as well (27). It should also be recognized that there are numerous members of the nuclear receptor family that are termed orphan receptors because their ligands or functions are unknown. However, it is evident from those ligands that have been identified that ligands are typically small hydrophobic molecules. The identification of orphan receptors that bind progestosterone or androgen metabolites suggests that some of these receptors (e.g., the steroid and xenobiotic-sensing nuclear receptor) may also be targets of endocrine disruptors (28). Further research to determine if these orphan receptors contribute to the adverse health effects of endocrine disruptors will be needed.

Along similar lines, it has been shown that the activity of a given receptor can have quite different effects in different tissues and in different species. An example of this would be endometrium and breast, where estrogen priming (or possibly low-dose estrogen disruption exposure) followed by progesterone results in a mitogenic stimulus, whereas in the ovary, progesterone induces an apoptotic response. Species-specific behavioral responses mediated by the PR have been observed at the level of the brain. In rodents for example, estrogen priming followed by progesterone (Pg) is required for behavioral sexual estrus, whereas in primates, expression of sexual behavior is inhibited by Pg.

Differential gene expression in various target tissues should also be considered as a determinant of tissue-specific response (12). Extrahepatic estrogen-metabolizing enzymes display tissue-specific patterns of expression resulting in different profiles or activity of endogenous hormones in different tissues. Exposure to endocrine disruptors that impact the activity of these metabolizing enzymes could therefore exhibit a tissue-specific target cell pattern. Another example of tissue-specific protein expression affecting response
Asa WALKER decreased viability of by decreased blood that found although it have been the important body endocrine estrogens Gender thymus, different mechanisms to that exist. Examples shown that concentrations of genistein, which has been synthesized to an increased responsiveness of AR polymorphisms, which affect receptor activity. The use of mechanistic information to identify the critical rate-limiting step for a model of endocrine disruption, and use of a quantitative structure–activity relationship (QSAR) approach for modeling the activity of endocrine disruptors.

In this regard, in vivo models of cancer susceptibility with relevance to adverse health effects of endocrine disruptors are also available and may be used to address some of these questions. These include the Noble rat for prostate and breast cancer, Sprague-Dawley rat for breast cancer, F344 rat for pituitary tumors, and the Eker rat model for uterine fibroids (32–35). These models have been well characterized for their sensitivity to steroid hormones, and research opportunities exist for investigating the effects of endocrine disruptors on the specific target tissues that are susceptible to endocrine modulation in these animal models. Some mouse models are also available that may be useful for this purpose, such as the T-ramp murine prostatic carcinoma model and the mouse mammary tumor virus (MMTV)-aromatase transgenic mouse model for breast and testicular cancer. The sensitivity of these models to hormone-induced tumor development may make them particularly useful for studying low-dose effects of endocrine disruptors. More research in this area is recommended. In particular, these in vivo models may provide additional dosimetry data that could be useful for modeling low-dose exposures to these compounds.

An additional area for consideration of model development is one or more behavioral tests for endocrine disruptors. Alterations in behavior are the outcome of a cascade of effects at the molecular, cellular, and organ levels. This is both a blessing in that it sums across many effects and a curse in that it is hard to attribute changes to specific internal effects. Another issue to be addressed in the development of behavioral models would be when one considers the behavioral alteration to be adverse. This topic may be more suitable to a small workshop of its own rather than a workshop on a specific model.

**Incorporation of This Information into Improved Risk Assessments**

Three paradigms for translating relevant information from the discussion above into risk assessments for sensitive populations were discussed: (a) the use of quantitative information related to a receptor polymorphism that affects receptor activity, (b) use of mechanistic information to identify the critical rate-limiting step for a model of endocrine disruption, and (c) use of a quantitative structure–activity relationship (QSAR) approach for modeling the activity of endocrine disruptors.

The example of AR polymorphisms associated with increased risk for prostatic cancer was discussed as an example of how this type of information would be incorporated into a risk assessment model. Decreases in the polyglutamine repeat length in this receptor are associated with increased cancer risk, with loss of each repeat contributing an additional 3% increase in relative risk. Mechanistic data suggest that the $K_d$ of these receptor variants is unchanged but that transactivation function of the receptor is functionally different. Information needed for modeling this polymorphism could be acquired by establishing a quantitative dose–response relationship for these receptor variants (Figure 1) to determine if transactivation activity by these variants differed in sensitivity or responsiveness to an endocrine disruptor and the magnitude of these changes. This information could then be translated to a population in which these receptor variants were distributed with a given frequency to model the effect of these polymorphisms on the response of an exposed population.

is metallothionine expression in the testis, where expression of this protein is very low. As a result of this low expression level, the testis displays an increased sensitivity to Cd that results from a cascade of events initiated by decreased blood supply to this tissue, decreased viability of Leydig cells, and ultimately decreased production of testosterone. Membrane-bound receptors for steroid hormones are also differentially expressed and are found primarily on sperm and neurons. These receptors could potentially mediate endocrine disruptor activity in these cells, although research addressing this point is lacking. Finally, the testis is another site of SBP expression in addition to the liver, where it is known as androgen-binding protein (ABP). Here it primarily binds testosterone and, in contrast to the production of SBP in the liver, is synthesized in both rat and human testis. Different effects of xenobiotes have been observed on the binding of 5α-DHT to rat ABP or human sex hormone-binding globulin (29).

The immune system is another potentially important but understudied target tissue for endocrine disruptors. There is now a large body of literature supporting the concept that estrogens are potent immunomodulators. Gender differences exist in both normal physiology of the immune system as well as the elaboration of diverse autoimmune diseases. Furthermore, it is now clear that there are bidirectional interactions between the immune system, central nervous system, and endocrine system. For example, castration of males results in marked hyperplasia of the thymus, whereas administration of estrogens or androgens induces thymic atrophy. Conversely, neonatal thymectomy has been shown to result in ovarian dysgenesis, autoimmunity, oophenitis, and autoimmune thyroiditis. Given the fact that estrogen and progesterone modulate inflammatory activity in the mouse uterus (30), it is plausible that xenoestrogens and other endocrine disruptors that affect the endocrine system will likely impact the immune system as well.

Tissue-specific differences in response to steroid hormones underscore the fact that endocrine disruptors may use multiple cellular mechanisms to produce an adverse cellular response, and these mechanisms may be different at low- versus high-dose exposures. Examples of this would be high- versus low-dose effects of genistein, which has been shown to have agonist effects mediated via the ER but can also have growth inhibitory effects that are not receptor mediated, such as inhibition of protein tyrosine kinase activity at high concentrations of this compound (31). Thus it will be important to achieve an understanding of these and other tissue-specific determinants of responsiveness to various endocrine disruptors and to identify which pathways are used at different dose levels.

**Utility of Available Model Systems**

Short-term in vitro assays that utilize reporter genes may be useful tools for determining a) the functional consequences of receptor polymorphisms, interactions, and number; b) the functional impact of polymorphisms in metabolic enzymes; and c) the dose–response relationships between promoter structure and gene expression. The development of in vitro assays that can address these questions would be particularly useful for studying how these parameters affect response to low doses of endocrine disruptors, and should be a research priority. Such assays may also prove useful for assigning functionality to gene polymorphisms identified through the Environmental Genome Project, which might impact responsiveness to endocrine disruptors.

Several in vitro and in vivo model systems that focus primarily on cellular responses such as cell proliferation are currently available and these may also be useful for studying the effects of endocrine disruptors on susceptible populations. Breast cell lines from individuals with inherited cancer susceptibilities such as Li-Fraumeni syndrome (p53) and BRCA1 mutations are available that display differential responsiveness to chemical carcinogens. These cell lines may be useful for studying the impact of low-dose exposures on susceptible populations at increased risk for adverse effects of endocrine disruptors due to inherited mutations in relevant target genes. However, the use of both short-term assays and cell lines will have limited utility for extrapolating how these susceptibility factors contribute to the variability observed in heterogeneous human populations and for understanding the biologic basis of adverse health effects observed in individuals exposed to endocrine disruptors.

In this regard, in vivo models of cancer susceptibility with relevance to adverse health effects of endocrine disruptors are also available and may be used to address some of these questions. These include the Noble rat for prostate and breast cancer, Sprague-Dawley rat for breast cancer, F344 rat for pituitary tumors, and the Eker rat model for uterine fibroids (32–35). These models have been well characterized for their sensitivity to steroid hormones, and research opportunities exist for investigating the effects of endocrine disruptors on the specific target tissues that are susceptible to endocrine modulation in these animal models. Some mouse models are also available that may be useful for this purpose, such as the T-ramp murine prostatic carcinoma model and the mouse mammary tumor virus (MMTV)-aromatase.
As part of these discussions it was also noted that some important biologic responses occurring as a result of endocrine disruptor exposure, particularly those that are qualitative in nature, cannot be predicted by quantitative models. In many of these cases, empirical observations related to the impact of critical species or tissue-specific factors as the underlying causes of divergent biologic responses to endocrine modulation will be required to recognize these phenomena. Two examples illustrate this point. For breast cancer development, the relevant target-cell population are the terminal end buds (TEB) (rat) or type I lobules (human), and quantitating the number of such end buds or lobules exposed to carcinogens or endocrine disruptors can model cancer risk. However, as discussed above, the response of these cells to steroid hormones or endocrine disruptors is different at different stages of development. Thus, data on the ability of estrogenic compounds to promote tumor development would not have predicted the observation that perinatal exposure to genistein is protective for mammary carcinogenesis. This protective effect is due to the induction of differentiation of the TEB in the mammary epithelium to a more mature differentiated state that is refractive to tumor induction \((36)\). This emphasizes the importance of considering windows of susceptibility and the mechanistic basis responsible for differential susceptibility (in this case, stage of life cycle) in modeling the activity of endocrine disruptors. Similar examples can be described for species-specific determinants of variability. For example, in rats, perinatal exposures to low-dose estrogens results in decreased prostate weight in the adult and a decrease in testis size \((37)\). In contrast, in the mouse, a similar perinatal exposure produces an increase in the size of the adult prostate \((38)\). This illustrates how qualitative species-specific determinants of susceptibility must be recognized and incorporated into risk assessment models.

However, once these qualitative differences have been elucidated, this mechanistic understanding of the biologic basis for the observed effect may then be incorporated into a risk assessment model. This involves identification of the rate-limiting step in this process and constructing a dose–response relationship that can describe this process. For instance, in the above example, the decrease in prostate size is a result of disrupted tissue architecture caused by estrogen exposure in the prostate. Normally in the rat, during the early postnatal period a testosterone surge occurs that determines prostate architecture. The presence of estrogens during this critical period of development interferes with this surge, impairing the development of the prostate. The identification of this as the rate-limiting step for the activity of exogenous estrogens focuses the quantitation of the impact of dose of estrogen (or endocrine disruptor) on testosterone levels. This quantitative information can then be incorporated into a biologically based model to predict the response of this tissue to exogenous estrogen exposure during the perinatal period (Figure 2).

Finally, a QSAR approach to modeling the behavior of endocrine disruptors was recognized as being valuable in terms of incorporating molecular or biologic factors that act as determinants of susceptibility or variation in the population \((39)\). For instance, several receptor properties related to receptor–ligand interactions that were discussed above could produce shifts in the dose–response curve. An example of such a factor would be differences in the affinity of steroid hormone receptors for a given ligand. For example ER-\(\alpha\) and ER-\(\beta\) bind xenoestrogens with different affinities. If these receptors were present in different ratios in different cell types, the response of these cells to the same ligand could be quantitatively different. Similarly, differences between the off-rates of ER ligands such as zeralanol or estrogen metabolites relative to estradiol could also shift the dose–response curve and have an impact at low-dose exposures.

**Conclusion**

Understanding the biologic and molecular basis of species, interindividual, and tissue-specific effects of endocrine-active agents will be critical for predicting responses at these different levels. The potential adverse health impact of low-dose exposure to endocrine-active agents will vary between individuals and between different target tissues, and our ability to extrapolate data relevant to human health from studies in animal model systems will require an improved understanding of species-specific determinants of response. Additional research will be needed to elucidate how patterns of gene expression in response to endocrine-active compounds differ across species and how polymorphisms at the receptor level and in hormone-metabolizing genes influence individual response to endocrine-active compounds. More information is also needed on how expression of receptor accessory proteins such as coactivators and corepressors differs between tissues and the functional consequences these expression patterns may have on receptor activity. Orphan receptors may also interact with endocrine-active agents, and additional research is needed to determine if these receptors contribute to the adverse health impact of endocrine disruptors. The development of new in vitro and animal models will be crucial for advancing our understanding of the effects of these and other determinants of specificity yet to be identified. Equally important will be the development of mathematical models that can be used to incorporate information on species, interindividual, and tissue-specific determinants in order to accurately predict adverse health outcomes at low-dose exposures.

**Figure 2.** Quantitation of the impact of xenoestrogens on effective testosterone levels and subsequent impact on prostate development.
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