Quantitative Mechanistically Based Dose–Response Modeling with Endocrine-Active Compounds

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A wide range of toxicity test methods is used or is being developed for assessing the impact of endocrine-active compounds (EACs) on human health. Interpretation of these data and their quantitative use in human and ecologic risk assessment will be enhanced by the availability of mechanistically based dose–response (MBDR) models to assist low-dose, interspecies, and *in vitro* to *in vivo* extrapolations. A quantitative dose–response modeling work group examined the state of the art for developing MBDR models for EACs and the near-term needs to develop, validate, and apply these models for risk assessments. Major aspects of this report relate to current status of these models, the objectives/goals in MBDR model development for EACs, low-dose extrapolation issues, regulatory inertia impeding acceptance of these approaches, and resource/data needs to accelerate model development and model acceptance by the research and the regulatory community. *Key words:* endocrine-active compounds, endocrine disruptors, linkage models mechanistic dose–response modeling, pharmacodynamics, pharmacokinetics, risk assessment extrapolations. — *Environ Health Perspect* 107(suppl 4):631–638 (1999).
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The potential for various compounds to alter endocrine system function has received increasing public attention in the United States [e.g., Food Quality Protection Act, 1996 (*1*), Safe Drinking Water Act Amendments of 1996 (*2*)] and in other countries throughout the world. The recognition of this potential toxicity has led to debate about the ability of current testing methods to identify endocrine system effects throughout the full gamut of life stages. New screening assays and test protocols for reproductive and developmental toxicity have been developed and others are currently being evaluated (*3*). In addition, a large number of new mechanistic test systems have been developed to evaluate interactions of endocrine-related compounds with specific hormone receptors. Together, these efforts will provide more comprehensive characterization of the potential hazards posed by exposure to these compounds. Coordinate with development of these new tools for hazard identification and new mechanistic tests is a need to create a set of refined dose–response assessment tools that use as much of this new data as possible (*4*).

A workshop was held in May 1998 in which several subgroups focused on approaches for characterizing the effects of endocrine-active compounds (EACs) on human health at environmental exposure levels. One work group addressed issues related to development of mechanistically based dose–response (MBDR) models for EACs, emphasizing the potential role of mechanistic models in improving the scientific foundations of dose–response assessments for EACs. This report is the product of that work group.

Dose–Response Models

Currently, default dose–response assessment approaches differ for cancer and noncancer end points. Default carcinogen risk assessments assume that all doses of a carcinogenic compound carry some degree of risk (*5*). Noncancer end points, including reproductive and developmental toxicity, have traditionally been regulated by assuming that these responses have a threshold. No observed adverse effect levels obtained from toxicity tests are adjusted by the application of uncertainty factors to derive reference doses or reference concentrations. The reference concentration methodology (*6*) for inhaled compounds includes defaults to calculate doses of inhaled compounds in specific regions of the respiratory tract. The newly proposed U.S. Environmental Protection Agency guidelines for carcinogen risk assessment emphasize the role of mode of action and tissue dosimetry (i.e., mechanistic data) in supporting departure from the linear cancer defaults. Consideration of both dosimetry and mode of action is essential in producing dose–response assessments that make maximal use of available data and reduce uncertainties.

Quantitative dose–response models for toxicology relate adverse response outcome with exposure duration and intensity. These include empirical models that derive model parameters from fitting response data, models that incorporate limited mechanistic information, and finally, models that include exposure, dosimetry, tissue interactions, mode of action, and biologic responses in an integrated and more quantitative fashion. These latter models, explicitly incorporating mode of action and tissue dosimetry data, are referred to here as MBDR models. This summary of the dose–response work group provides background information regarding mechanistic models for EACs. It stresses the potential for these models to improve the precision of risk estimates below the range of sensitivity in current test methods (usually at the 5–10% incidence levels or at the 5–10% increase in a continuous measure of response) and to reduce uncertainties in risk assessments with EACs.

Empirical Dose–Response Models in Risk Assessment

Parameters derived from fitting empirical models to response data do not necessarily have specific biologic meaning or bear one-to-one relationships with particular biochemical or molecular parameters. Nonetheless, these empirical approaches are still important for assessing the range of response behaviors associated with exposure to these compounds (*7*). Empirical models are especially useful if they are capable of describing a wide range of

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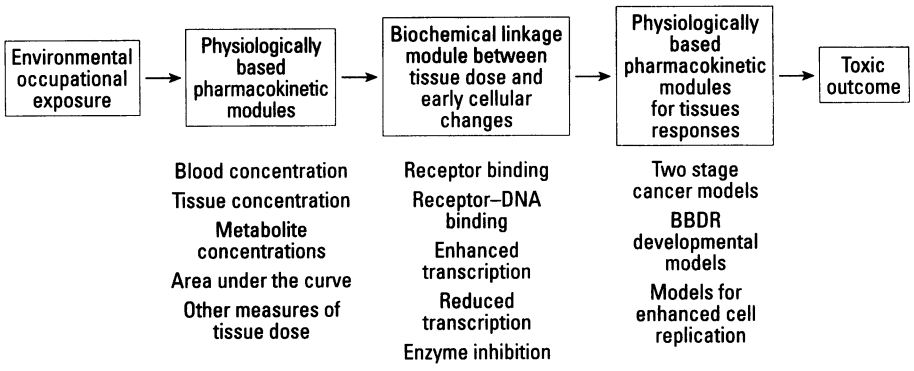

Figure 1. Schematic of the modular components of a mechanistic model. BBDR, biologically based dose response.

curve shapes, provide a measure of a potency of response, and are useful for assessing time-dependent aspects of the test system. The benchmark dose methodology (*8–10*) applied to noncancer end points and discussed in the revised carcinogen guidelines (*5*) is an example of this class of model structures.

Other useful attributes of empirical models would include their ability to describe background information, including prior exposure, background incidence, heterogeneity, and variability, and to permit extrapolation to other groups of chemicals. Usually these empirical models are tailored to specific data sets. The accumulation of information from these independent quantitative analyses permits the integration of multiple responses, comparisons of potency, and the cataloging of the shapes of response curves that need to be evaluated in more detailed mechanism-based modeling. With limited information regarding the biologic system, it would be possible to create hybrid models that included specific biologic parameters such as protein or receptor binding affinity constants, enzyme activities, or receptor number (*11–14*). These biologic parameters would be embedded within the otherwise empirical model. Other approaches that consider the pharmacology of ligand–receptor signaling have also received attention from the pharmacology community (*15*).

Goals and Expectations for Mechanistic Models for Endocrine-Active Compounds

General Characteristics

More comprehensive mechanistic models are designed to include descriptions of both pharmacokinetics, especially the time course for distribution of compounds to target tissues, and pharmacodynamics, i.e., the interactions between compounds and target tissues. Biochemical linking models connect the pharmacokinetic and pharmacodynamic models. The linking models specify the manner in which the chemical alters critical biochemical processes and initiates the series of steps leading to toxicity. Thus, a more complete mechanistic model for EACs should consist of a number of modular elements, e.g., a pharmacokinetic module, a linkage module for receptor-based interactions, and a module for tissue response (Figure 1). Obviously, the parameters in these models are expected to correspond with specific biochemical, physiologic, and anatomic characteristics of the test system and test compound.

The advantage of a modular structure is to show clearly the manner in which data from multiple studies and disciplines become tightly integrated into the overall MBDR model. The goal in pursuing development of these integrated models is to gain insight from the quantitative mechanistic analysis of responses in animals to predict exposure outcomes for the health of human and wildlife populations. In this regard, inclusion of population characteristics such as genetic variability may be essential for ensuring that these mechanistic models become more useful for risk assessment. MBDR models with these general characteristics are intended to play specific roles in chemical risk assessment. Five of the more important roles are *a*) understanding the expected shape of the response curve based on biologic principles; *b*) understanding differences in response for structurally related compounds, different genders, strains, organ systems, life stages, and animal species; *c*) applying better extrapolations for specific exposure situations in exposed human populations; *d*) accounting for nonlinearities in pharmacokinetics and pharmacodynamics; and *e*) accounting for genetic variability and other factors that contribute to differential sensitivities among subpopulations.

Although MBDR models attempt to capture the salient features of a given process and/or effect, they are always simplifications of the real world. Nonetheless, even simplistic mechanistic models may be useful in summarizing information, generating hypotheses, challenging scientific knowledge, and clarifying the key issues to consider when extrapolating across routes, across species, to lower exposures, and across different ages (*16–18*). In their initial stages, MBDR models are designed to explain limited sets of data and are limited in the conclusions they can support. They have to be modified as new information becomes available, as interest in a given effect is increased by either scientific curiosity or regulatory scrutiny, and most especially, as our understanding of basic endocrinology and biology advances. The molecular biology revolution of the past 20 years has provided a broad new set of tools for analyzing molecular signaling and cellular control processes. The insights and data arising from use of these biologic techniques will provide continuing challenges for refining and extending MBDR models at all levels of structural organization. In MBDR models the normal biology should be described quantitatively and then the impact of the xenobiotic examined as a perturbation of the normal state.

Models of Endocrine System Function

Endocrine organs secrete hormones that travel throughout the body, affecting distant target cells (endocrine), neighboring cells (paracrine), and cells of origin (autocrine). These hormones control processes involved in maintaining normal development and function of the organism. The molecular machinery of the target organs transduces the hormonal signals to create biologic responses. EACs have the potential to alter these signaling, recognition, and transduction processes, leading to mild perturbations, altered biologic function, or overt toxicity. MBDR models of endocrine function eventually include all these molecular characteristics, as well as information on the distribution of the key hormones to active tissues, hormone synthesis and metabolism, hormone binding to plasma proteins, interactions of multiple receptor isoforms (e.g., estrogen receptors α and β) and physiologic interactions that serve to maintain homeostasis. Key aspects of these processes include receptor synthesis, recycling, and degradation; tissue-specific regulation and activation of receptors during different life stages; and control of enzymes that synthesize or metabolize ligands. Homeostasis in adult organisms is regulated by important feedback controls for many endocrine processes. In addition, an important aspect of modeling in the endocrine system is consideration of multiple time-dependent phenomena. These behaviors include dynamics of organism development, circadian rhythms, puberty, estrous or menstrual cycles, and reproductive senescence

and aging. In these life stages, the coordination and timing of sequential events by a variety of receptors and natural ligands organizes a set of events that evolves over time.

Once the normal endocrinology has been considered in a model for effects of EACs, inclusion of the major biologic processes affected by individual EACs and their metabolites becomes the key focus of the next stage of modeling. This concept of the characterization of the major steps deserves special emphasis in regard to an evolving understanding of signal transduction pathways, including gene transcription, translation, and posttranslational modification and regulation. It would be difficult or impossible to account for the behavior of all these factors in any contemporary model. However, identifying and modeling the essential characteristics that account for the potency of exogenous compounds in the mechanistic description may be sufficient to significantly improve the scientific credibility of current risk assessments. Many biologic factors may contribute to the major potency-determining steps in a process, and the specific step involved may vary for different exposure situations. Among the factors that may have to be considered are timing of exposure, amplification of multistep cascades, multiple receptor isoform interactions, and the presence of tissue-specific accessory proteins, such as co-activators and co-repressors.

When empirical models are applied to multiple data sets, there is no reason to expect consistency in the estimates of parameters across different experiments. With MBDR models, there should be consistency in values of specific parameters. Although receptor number varies across age, gender, and species and is affected by cycling and circadian rhythms, basic parameters such as receptor number, affinities, and metabolic characteristics should be similar as long as the same age, gender, tissue, and species of animal are used for studies. In this fashion, MBDR models have the ability to synthesize a broad range of scientific observation into a coherent description of normal and altered biologic states. As such, these models reflect the present state of knowledge about EAC toxicity and provide a quantitative organization of prevailing, dominant hypotheses regarding the modes of action of hormones and EACs on endocrine-regulated processes. The ability to create quantitative hypotheses of the mode of action and the use of these hypotheses to design critical experiments for their verification are simply the applications of the scientific method to problems in toxicology and risk assessment. One of the strongest arguments for expanding the use of MBDR models in EAC risk assessments is that they allow development of testable hypotheses related to mode of action and to the shape of the dose–response curve at low doses.

Endocrine systems and the impact of EACs on these systems appear to be readily amenable to MBDR modeling techniques. Much is known about the regulation of organ system function by natural hormones and about the interactions among various endocrine systems. This body of information can be combined with data on the perturbations of the endocrine system by specific classes of EACs to develop comprehensive and testable hypotheses. It is important to realize that these organized mechanistic models are a first-step hypothesis compilation. Optimally, these models should be used in experimental design in confirming or refuting the original hypothesis and for risk assessments based on the most plausible prevailing perception of modes of action. To accumulate appropriate data for improving the biologic basis of risk assessment with these EACs, special effort is necessary to develop protocols that simultaneously fulfill specific data needs for modeling and specific data needs for regulatory testing requirements.

Model Evaluation

The ability to organize and explain a broad array of diverse scientific findings is a primary goal of mechanistic modeling and is a key component for achieving broad scientific acceptance. A difficulty in gaining acceptance for these models occurs because of the very requirement that the models organize and integrate such a wide variety of experimental data. These ambitious structures may adequately describe the majority of studies but fail to match all observed results. It will be necessary to use a broad array of scientific evidence in characterizing and assessing the success of mechanistic models. The degree of emphasis to be placed on particular studies and the congruence of the model with the observed results will depend on both statistical issues and scientific judgment.

In many situations, it has been difficult to characterize how scientific judgment affects a regulatory decision because of the inability to express quantitatively the different risk estimates derived from differing assumptions regarding modes of action for xenobiotics. MBDR modeling uses diverse expertise to determine key biologic aspects of a model such as causal linkages between exposure changes and biologic effect. The explicit articulation of the mechanistic assumptions in these models shows their impact on the risk predictions. Thus, the process of risk assessment becomes more transparent (objective) and the ability to test these assumptions (challenging the model and/or validating its use) is greatly enhanced. The integration and analysis of hypotheses can provide a more explicit incorporation of scientific judgment into the process of evaluating the impact of specific mechanistic assumptions in risk assessments. The coupling of modeling results with elicitation of expert judgment has been considered a potential tool for achieving consensus in the use of mechanistic data and mechanistic models in risk assessment.

Mechanistic models, by their nature, attempt to describe in mathematical detail the processes involved in generating an adverse health effect from an environmental exposure. This has several clear advantages. First, when data are available on interindividual variation in response to any element of the process, this information can be directly incorporated into the use of the model for prediction using various population-oriented methods (*19,20*). Second, mechanistic modeling provides the potential for generalizing results from one chemical to other agents. For example, key components of a model, such as the mechanism for stimulation of follicle growth and ova release by follicle-stimulating hormone and luteinizing hormone, once characterized, can be used in models for other environmental agents that affect the same mechanisms. Additionally, once models have been developed for prototypical agents in a class of chemical agents, structure–activity relationships can be used to determine pharmacokinetic model parameters for other congeneric compounds (*21,22*) or binding parameters for other chemicals with similar biologic activity (*23,24*). More simple applications of mechanistic data may provide reduction in experimental costs, i.e., using existing models to estimate toxic potency on the basis of alterations in key parameters. Predictive models may find use in prioritizing compounds for testing. High-exposure compounds that are predicted to have high potency would be candidates for immediate testing. Streamlined approaches become possible when the components of the model describe mechanisms that can be clearly identified as parts of the cascade of events leading to toxicity for other agents.

Risk Assessment Applications

MBDR modeling can provide scientific support for the shape of the dose–response curve in the low-dose (or low-incidence) region. A representation of the potential role of biomarkers and mechanistic models in extending the region of dose where confident extrapolations are possible is shown in Figure 2.

Mechanistic studies can be used to determine elements of the system that give rise to the shape of the dose–response curve below the range of observation of overt toxic effects, e.g., the studies that evaluated the shape of the protein induction curve for CYP1A1 by dioxin at doses much below those that caused

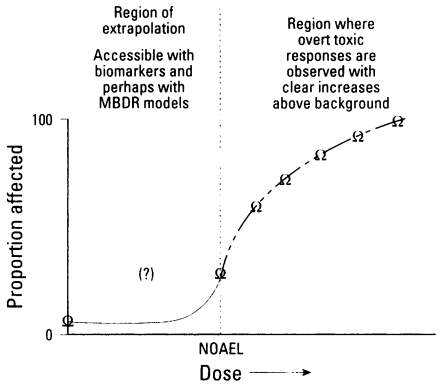

Figure 2. Range of incidences where validated MBDR models coupled with biomarker measurements could improve predictions of shapes of dose–response curves. MBDR, mechanistically based dose response; NOAEL, no observed adverse effect level.

overt toxicity (*25*). Carefully designed experiments and quantitative organization of the experimental data into a model should provide a more precise determination of the expected risks at different exposure levels. More generally, the development of mechanistic models can actually provide support for the relationship between responses and biomarkers at most levels of exposure.

Mechanistic modeling has other uses in addition to dose–response assessment. These models have been used to identify data gaps in our understanding of the toxicity of EACs and to identify key experiments to fill these gaps. In addition, by locating the critical elements governing the potential potency of an agent for a given effect, mechanistic modeling aids in identifying useful short-term, cost-effective testing strategies that directly contribute to the prediction of risks and strengthen the level of evidence needed to determine if a hazard exists. Optimally, several mechanistic models with differing underlying biologic mechanisms can be compared against various data sets to discriminate between competing mechanisms (*25*). Experimental simulations with these competing models prior to acquisition of new data can identify the parameters with the greatest impact on model predictions. These parameters should be investigated initially to provide the greatest ability to discriminate between competing hypotheses. With EACs, this could involve comparing models, assuming receptor agonist activity, antagonist activity, or mixed activities before deciding on a specific experimental design to test the predictions.

Additionally, mechanistic models offer a logical framework in which to link exposure assessment and dose–response modeling. Extrapolation issues, when properly considered prior to developing a mechanistic model, can be more readily addressed. These include cross-species, cross-organ, cross-route, and cross-compound extrapolations. Specific data will be required for these model extrapolations; however, the amount of experimentation should be considerably less than required in the initial model development. Furthermore, issues associated with exposure to multiple agents working additively or synergistically can be addressed through model simulations that are followed by targeted experimentation to verify and/or refine these assumptions.

Other uses are in improving design of toxicity tests and creating modules useful for multiple chemicals. Regulatory agencies require that the chemical industry generate specific types of data on many compounds. Mechanistic models could greatly improve the value of these mandated toxicity testing results for risk assessment. This interaction, however, would only be possible if there were more flexibility in establishing protocols for toxicity testing. In addition, experimentation with prototype endogenous hormones (e.g., estradiol, testosterone) could provide information helpful in predicting the response to many other compounds with similar activities. Such generic, natural hormone models would include modules containing pharmacokinetics, receptor interaction, and tissue-response portions. This information would be important for future studies with agonist/antagonists of these native hormones.

Regulatory Acceptance

There is a degree of skepticism about the willingness of regulatory bodies to make decisions based on mechanistic models. Sometimes these concerns are caused by the unfamiliarity of regulators and many research scientists with these modeling techniques. It deserves emphasis that regulatory acceptance of MBDR models should be secondary, following broader acceptance of these techniques by the scientific community.

Within the regulatory structures, there is appreciation that current risk assessments have many uncertainties. These uncertainties often lead to legislation requesting new mechanistic data. However, the new data are only infrequently incorporated in the risk assessment because the entire package of results is still regarded as incomplete or appears to have been collected in the absence of a clear context for its use. MBDR models are an important avenue for providing the context for successful incorporation of various new data.

Another concern has been that the use of models may provide a level of confidence that is not justified because of the uncertainties in biologic knowledge. In practice, the reverse appears true. These models define the individual parameters that comprise the overall function of the endocrine system within an organism. Each parameter in the model then has associated variability and uncertainty, together with uncertainty associated with the choice of mode of action. Frequently, the mere identification of the parameters in the models and the appreciation of associated uncertainties has provided an impression in the regulatory community of an increased level of uncertainty when these models are proposed. This perception was voiced in the initial attempts to incorporate physiologically based pharmacokinetic models in health risk assessments. However, the perception is based on an inappropriate comparison of MBDR approaches and default risk assessment models. Default methods are simply sets of experiential rules. Although the risk assessments derived from application of the default procedures may lack precision in defining the true risk, there is no way to assess inherent uncertainties in the default process. The MBDR modeling approach does uncover areas of uncertainty but explicitly defines those areas that need the most attention in assessing uncertainties.

In addition to risk assessment applications, mechanistic dose–response models might also play a role in clinical diagnosis and management of specific disease states. For example, mechanistic models might be especially useful in assessing the degree of risk associated with hormone imbalance in certain human endocrinopathies. An important clinical problem that might approached in this way is hyperandrogenism in women.

Comparisons with Contemporary Approaches

In their applications to risk assessment, MBDR models should be compared and contrasted with the current default assumptions. The hurdle for application of mechanistic models and mechanistic data should not be set so high as to disqualify all but the most sophisticated and detailed of these models from application in risk assessments. Clearly, the utility of any quantitative model will be derived from a clear articulation of the reasons for constructing the model and the range of detail that is successfully captured in the model structure. The level of biologic detail in mechanistic models varies depending on the present state of knowledge of the normal biology and for the biology of specific EACs. Although it is a laudable goal to include all of the details of the expected biologic interactions, this level of detail cannot be provided at this time or in the foreseeable future. Hence, procedures must be in place to accept MBDR models as a means of improving risk assessments as part of a reiterative hypothesis-generating process, where continuing changes in these models may occur as our understanding of complex biologic systems improves. No risk assessment

should be regarded as the final word on a compound, as long as toxicologic research and toxicity testing continues to accumulate new findings and data. Risk assessments and MBDR models are simply recapitulations of the state of the science for a compound at a particular point in time.

Information and Data Needs

Many types of information could be organized and included within structured mechanistic models. Because of the breadth of studies that could be completed, it is important to prioritize efforts and to remain focused on the most pressing uncertainties and data needs. The role of background hormone concentrations in regulating and eliciting specific responses needs to be included in these MBDR models in order that estimates of risk above background can be evaluated. A challenge in assessing normal function of the endocrine system is in defining the range of normal function and the perturbations of this range of normal function that would be regarded as an adverse response. This consideration is important for individual responses and for responses of populations. Thus, the inclusion of the behavior of endogenous hormones serves as a critical element of the overall model structure, providing important perspective to the perturbations caused by incremental increases in agonists or antagonists. The discovery that compounds serve as effectors for multiple receptors, e.g., methoxychlor and its metabolites display both estrogen-agonist and androgen-antagonist activity, counsels some caution in assigning single mechanisms of action to compounds that have not been adequately screened for interactions with these various systems. In assessing data needs for MBDR models, it is possible to focus on specifics in minute detail, such as the presence of hormone receptors in specific tissues with estimates of binding constants, receptor number, etc. Another approach is to broadly list the characteristics that appear to be important in constructing these mechanistic models in order to identify critical response-limiting parameters. In real-world applications, model building, data accumulation, and model testing and refinement will usually help guide discussion about the level of detail required for use of the models in risk assessment.

Current emphasis is on potential effects of estrogenic, antiandrogenic, and thyroid-mimetic compounds on human health. Assessments for estrogenic compounds require information on normal effects of estrogen during different life stages (*26,27*). Modeling studies with other endocrine systems include androgen control of spermatogenesis (*4*) and thyroid hormone-mediated control processes (*28*). The estrogenic system has multiple interactions with other hormonal regulatory systems, and it might be that simpler systems could be examined to develop strategies and modeling techniques in more sparsely connected hormonal systems. Blumenthal et al. (*29*) reported on the development of a pharmacokinetic model for the pineal hormone melatonin. Androgen function in males is also likely to be more tractable than the time-dependent control of cycling and pregnancy in females. A tabulation of some of the characteristics of available MBDR models for endocrine related toxicities appears in Table 1.

The end points of interest with agonists and antagonists of sex-steroid action are reproduction, development, immunologic function, neurologic, and neoplasia. Models for reproduction and development are still in their formative stages compared to risk models for cancer. Despite some activities in mechanistic models for developmental responses (*39*), none of these models have been organized to correlate the concentrations of

Table 1. Attributes of MBDR models including some used to describe endocrine-related processes.

	Examples	ADME	Receptor/(ligand) interactions	Altered cell functions	Altered biologic responses	Reference
Dosimetry modeling	Chloroform	PBPK	Metabolizing enzymes	Cell killing by metabolites	Toxicity/carcinogenicity	(30)
	Formaldehyde	CFD/PBPK	DNA–protein cross-links, tissue CH_2O	Cytotoxicity, mutation due to CH_2O itself	Clonal growth models for nasal tumors	(*31*)
	Methylene chloride	PBPK	GST-mediated metabolism, GSH metabolite	Presumed initiation potential	LMS cancer models	(*32*)
Endocrine-related physiologic process models	Melatonin	Natural hormone, PBPK				(*29*)
	Testosterone regulation, antiestrogen	Feedback (T), PBPK model	Putative brain T-receptor, testicular LH receptor	T-LH-FSH feedback	Altered hormone levels	(*4*)
	Estrus cycle atrazine	Empirical model, (E_2) PK	Putative hypothalamic E_2-receptor	LH surge	Persistent anovulatory estrus, mammary tumors	(*33*)
Toxicants affecting endocrine status	5-Fluorouracil	Compartmental PK model	Enzyme inhibition	Altered nucleotide pools	Fetal growth Malformations	(*11*)
	TCDD-NIEHS	PBPK	Ah-receptor binding estradiol, metabolism EGF-receptor, enzyme induction	Cell cycle alterations Thyroid status TSH levels	Thyroid tumors, liver tumors, and clonal growth	(*34*)
	TCDD	PBPK	Ah-receptor, CYP1A1 protein induction	Hepatic cell phenotype changes	Clonal growth of initiated cells	(*35,36*)
Endocrine-related physiologic effect models	Thyroid homeostasis (e.g., thionamide)	NA	TRH, TSH, T_4, T_3 (interruption of normal inhibitory feedback mechanism)	TSH-stimulated DNA synthesis and cell division	Follicular cell tumors	(*37*)
	Leydig cell regulation (e.g., cimetidine, linuron, procymidone)	A	LH, T (interruption of normal regulatory feedback loops)	Elevated LH or Leydig cell responsiveness to increased LH	Leydig cell tumors	(*38*)
SAR models	QSAR	—	Thyroid hormone AhR, AR, ER receptors	—	Comparative evaluations of potency	
Developmental models	CH_3HgCl and fetal growth	PBPK model for CH_3HgCl	Not receptor based	Cell growth	Fetal weight	(*13*) (*39*)

Abbreviations: ADME, absorption, distribution, metabolism, and excretion; AhR, arylhydrocarbon receptor; AR, androgen receptor; CFD, computational fluid dynamics; EGF, epidermal growth factor; ER, estrogen receptor; E_2, 17β-estradiol; FSH, follicular-stimulating hormone; GSH, glutathione; GST, glutathione *S*-transferase; LH, luteinizing hormone; LMS, linearized multistage; NIEHS, National Institute of Environmental Health Sciences; PBPK, physiologically based pharmacokinetics; PK, pharmacokinetics; SAR, structure–activity relationship; TCDD, tetrachlorodibenzo-*p*-dioxin; QSAR, quantitative structure–activity relationship; T, testosterone; TRH, thyroid-releasing hormone; TSH, thyroid-stimulating hormone; T_3, triiodothyronine; T_4, tetraiodothyronine.

morphogens, including ligands and receptor molecules, with the occurrence of specific structural abnormalities. Models for development need to consider feedback loops and potentially include differential transcriptional activities of individual agonists for hormone receptors in different cell types, in different regions of the embryo, and at different times during gestation. Models for developmental stages also have the challenge of accounting for a dynamic system where sensitivity of the tissue to the hormone changes markedly over time (*40*).

Low-Dose Extrapolations with Endocrine-Active Compounds

The default position in noncancer risk assessments assumes a threshold, i.e., a dose below which there are negligible risks. A combination of empirical modeling and mechanistic data has been marshaled to suggest that there are certain developmental end points for which thresholds are unlikely to occur and others for which thresholds would not be at all unexpected (*41*). There do appear to be situations in which it would be realistic to consider that there is a continuum of responses (i.e., no threshold dose for the observed effects) for added hormone agonists. For instance, when the natural outcome in adult reproductive system structure and function is determined by the *in utero* exposures to natural hormones, the addition of exogenous agonists should cause alterations in the response incidence. This expectation is consistent with additivity to background and with results of studies of the effect of uterine position on adult reproductive system parameters in rodent species (*42*).

The influence of processes without clear thresholds for risk assessment must still consider the severity (adversity) of the responses. Some effects, such as prostate size, represent changes in specific phenotypic characteristics that are themselves variable in the adult population. Risk assessment for these end points will revolve around definitions of adversity. The issues surrounding altered distribution of normal characteristics in the population are complex, requiring both technical input about adversity and public policy input on the level of tolerance for changes in these distributions by the public.

Several end points for which thresholds are uncertain were discussed in our deliberations. These responses included changes in androgen receptor number and prostate weight in adult male mice following *in utero* estrogen exposures (*42*) and turtle sex ratios following egg painting with estrogens (*43,44*). Other examples were also discussed, including fertility in a continuous breeding study in females exposed to DES *in utero* (*45*) and vaginal threads in female mice exposed to dioxin *in utero* (*46*). Although these effects appear consistent with absence of threshold, they have not been examined statistically to determine the minimal threshold that would be consistent with the data. Statistical analyses of this kind would be informative and should be performed routinely.

Some of the molecular characteristics of gene transcriptional control by hormones and their receptors are expected to give rise to highly nonlinear dose–response characteristics due to positive feedback loops, receptor autoregulation, phosphorylation cascades, and control of enzymes involved in synthesis of high-affinity ligands (*47*). These molecular behaviors can give rise to biologic switches, i.e., to the ability to abruptly change from one biologic condition to another over a very small change in ligand concentration. Examples appear to include estrogen receptor autoregulation during vitellogenesis in some fish and frog species (*48*) and thyroid hormone receptor upregulation in frog tadpoles during metamorphosis (*49*). Highly nonlinear effects were also reported in progesterone-mediated maturation of *Xenopus* oocytes, a response mediated via mitogenic-activated protein kinase (*50*). Many of these nonlinear switching mechanisms are expected to produce nonlinear dose–response curves for the action of native ligands. However, the dose response for effects of exogenous compounds, even when biologic switches are present, still depends on the combination of effects of the native ligand and perturbations of the EAC on the specific biologic effect.

Another aspect of the debate surrounding endocrine-active compounds is the concept of pharmacokinetic thresholds. Even in cases in which there is likely to be a linear dependence of response on added hormone-mimetic xenobiotics, such as turtle sex determination, stochastic principles determine the distribution among competing binding sites, i.e., native receptor, shell surface structures, nonreceptor binding sites, etc., at low doses. Thus, not all added hormone or xenobiotic would be available for receptor binding. In addition, because many pharmacokinetic processes are nonlinear, it would be simplistic to assume that all EACs act additively.

Recommendations

- Make MBDR model development a routine part of the risk assessment process. The strongest recommendation is that appropriate MBDR models be developed in concert with accumulation of data on mechanistic end points and with data from new screening and toxicity testing protocols. Improved hazard identification methods alone will, in isolation, do little to assess low-dose risk situations. The use of MBDR models will provide perspective and context to hazard identification studies and improve the quantitative significance of these studies in contemporary risk assessment. Other reports from the workshop appearing in different chapters in this monograph aid in identifying specific topical areas for pursuing compound-specific MBDR models for toxic responses.
- Organize and present contemporary examples. There are examples of mechanistic models that have been incorporated into risk assessment applications (Table 1). Fairly complete mechanistic models for dioxin include pharmacokinetics, gene induction, cell proliferative responses, and tumor formation (*34–36,51*). One version of the dioxin mechanistic model has been described in the dose–response chapter of the dioxin reassessment (*34*). A second model structure with similar pharmacokinetic and gene induction modules was based on different assumptions of the characteristics of cells at risk for transformation in treated rats (*52*). On a more limited basis, pharmacokinetic modules have been used in risk calculations with several halogenated hydrocarbons. Linkage models have been developed and proposed for risk assessment use for cytotoxicity with chloroform (*30*) and acrylic acid (*53*). A model has been described that explains the effects of serum binding on estrogen potency during development (*26*). A more comprehensive biologically based dose–response model for developmental effects has recently been completed using methylmercury as the prototypical compound (*39*).

  Mechanistic information has been used in semiquantitative fashion for responses such as thyroid carcinogenicity (*37*). Although less effort has been focused on mechanistic models of endocrine system function, a number of first-generation models have been published in recent years (*4,27–29*). Examples should be compiled and made available in document form to indicate the manner in which these models are constructed and their potential applications. The availability of such a document would aid in explanation of the process of mechanistic modeling in risk assessments and provide an opportunity to learn from past efforts (Table 2).
- Select prototype compounds/mixtures for model development. For a limited number of case studies, mechanistic model development should be pursued to collect appropriate data for developing each of the modular components in the exposure, dose, linkage, response

Table 2. Characteristics for selecting prototype compounds for model development.

- Compound represents a human health concern
- Metabolism and the potency of metabolites are understood
- Target tissue dose can be measured for compound/metabolites
- Intermediate markers of response are available for study
- Results are expected to be generalizable to other compounds
- Latency from exposure to response is relatively short
- A high penetrance rate for the effect can be achieved
- There is a relevant animal model to study
- Can make predictions of dose response for risk and precursor states.
- Predictions verifiable from intermediate responses and biomarkers.

cascade. Criteria for selecting these prototype chemicals and responses must be carefully considered. Among these criteria are the broader applicability of individual components, e.g., modules that provide for the synthesis and elimination of estrogen, progesterone, or testosterone. The influence of *in utero* estradiol exposure of males on adult prostatic function should be an important case study for examining the potential nonmonotonic dose–response curves for EACs. Another potential case study for assessing threshold behavior and the role of nonlinear positive feedback loops in dose–response relationships is temperature-dependent sex determination in some reptiles (*43*).

Several other candidate responses/ compounds were noted: estrogen exposure in relation to mammary tissue neoplasia in rats (*54,55*) and dopaminergic prolactinemia and amenorrhea. Other candidate examples should also be identified based on the deliberations of the other work groups. These prototype examples should be sketched out to indicate the connections of the modular portions to the more complete model and data needs identified based on the available data for each and the ease with which the data can be used in a quantitative fashion. The prototypes should include a compound(s) with more than one mode of action and examples with mixtures of compounds with different modes of action affecting a common end point. A suggestion was provided of the effects of a mixture of dioxin, dibutylphthalate, and an androgen antagonist on male reproduction. These compounds appear to have very different modes of action expressed as the same kind of functional deficit. Alternatively, prototype mixtures present in the environment could be used (*56*).

- Foster intimate interdisciplinary communication. The ability to develop and utilize these quantitative models requires new team building between individuals with training in such fields as toxicology, endocrinology, pharmacokinetics, statistics, and biomathematics. Appreciation for the risk assessment process needs to be broadly conveyed to groups collecting critical mechanistic data. In return, the MBDR modelers need to be immersed in the biological tools that provide the information for successfully coordinating the modular components.
- Promote education on multiple fronts. Three education-based activities need to move ahead in tandem. They are development of case studies to demonstrate the process, education of scientific community and regulatory bodies with regard to the application of these models, and introduction of quantitative mechanistic modeling programs as a more routine part of curricula in toxicology and risk assessment. This latter recommendation is a long-term activity but important for ensuring an environment that encourages the introduction of biologic data and mechanistic modeling in quantitative dose–response assessments.
- Encourage development and use of modular components of mechanistic models. To fully benefit from the use of MBDR modeling in science and risk assessment, there must be a forum for the review and acceptance of mechanistic models and their component parts. Critical, open peer review will not only guarantee better models for risk assessment but will encourage the use of components of these models in the development of models for numerous agents. The use of common modules for multiple compounds, in turn, will simplify the review task, improve the quality of risk assessments, and encourage the further development of mechanistic models. Development of these models and modules was a priority area for research identified in the Endocrine Disruptor Work Group of the Committee on Environmental and Natural Resources (*57*).
- Develop funding resources to support MBDR model development. If efforts to create MBDR models as dose–response assessment tools for EACs are to succeed, modeling activities will have to be more explicitly encouraged as an essential part of the present initiative to expand the toxicity databases for these compounds. This support should come in the form of funds specifically earmarked for model development, training, and education. With any attempt to provide these resources, it must be recognized that the skills to collect important data and develop model structures for hypothesis testing and risk assessment may well reside in different locales. Attention must be given to encouraging multidisciplinary activities within single institutions and multi-institutional activities for successful completion of these types of modeling initiatives.

Summary and Conclusions

MBDR models are promising tools for improving risk assessments with EACs. The technology and biology required to develop these models is advancing rapidly, providing many opportunities for model-building efforts with diverse EACs. In the absence of these MBDR models, the abundance of hazard identification data being collected will serve to indict specific EACs without providing insight regarding the exposure conditions likely to pose any significant level of risk in exposed populations. The successful development of these models requires close cooperation between the modelers and the laboratories and individuals collecting biologic data. In the early stages of model building, emphasis should be placed on prototypical compounds and on endogenous hormones themselves. Accurate dose–response models of endogenous hormones and the processes controlled and organized by these hormones are a necessary prerequisite for understanding the perturbations associated with EAC exposures. The potential of MBDR models will only be fulfilled if resources are made available for model building, along with the resources for toxicity testing and mechanistic research. A series of recommendations for expediting the development and application of MBDR models focuses on education, fiscal and data resources, and creation of a library of examples of the model-development process.

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