Pure Appl. Chem., Vol. 75, Nos. 11–12, pp. 2159–2166, 2003.

Topic 3.14

Endocrine active substances and dose response for individuals and populations*

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Abstract: Dose–response characteristics for endocrine disruption have been major focuses in efforts to understand potential impacts on human and ecological health. Issues include assumptions of thresholds for developmental effects, effects at low doses with nonmonotonic (e.g., "U-shaped") behaviors, population vs. individual responses, and background exposures (e.g., dietary phytoestrogens). Dose–response analysis presents a challenge because it is multidisciplinary, involving biologists and mathematicians. Statistical analyses can be valuable for evaluating issues such as the reproducibility of data as illustrated for contradictory findings on low-dose effects. Mechanistically based modeling provides insights into how perturbations of biological systems by endocrine active substances can create different dose–response behaviors. These analyses have demonstrated that higher order behaviors resulting from the interaction of component parts may appear highly nonlinear, thresholded, low-dose linear, or nonmonotonic, or exhibit hysteresis. Some effects need to be evaluated as population impacts. For example, alterations in male:female ratio may be important at the population level even though not adverse for the individual. Descriptions of the contributions of background exposures to dose–response behaviors are essential. The challenge for improving dose–response analyses is to better understand how system characteristics create different dose–response behaviors. Such generalizations could then provide useful guidance for developing risk assessment approaches.

INTRODUCTION

Estimating the potential risks for human health or environmental toxicity from exposure to chemicals that can disrupt the endocrine system requires a quantitative understanding of the relationship between the dose of the endocrine active substance and the occurrence of effects [1–4]. The controversies about endocrine disruption have been somewhat unusual in environmental toxicology because issues of dose–response relationships have played a central role, somewhat similar to the continuing debates over whether or not cancer dose response is always low-dose linear [5–7]. This represents a challenge to the toxicology, endocrinology, and regulatory communities because dose–response assessment requires collaborations between those with biological and mathematical expertise. While physics, engineering, and population biology historically have required and utilized many forms of mathematics to progress, the introduction of mathematical analyses into many areas of biology, such as physiology and toxicol-

*Report from a SCOPE/IUPAC project: Implication of Endocrine Active Substances for Human and Wildlife (J. Miyamoto and J. Burger, editors). Other reports are published in this issue, *Pure Appl. Chem.* **75**, 1617–2615 (2003).

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ogy, has been sporadic [8,9]. As reductionist biology is successfully giving scientists access to the pieces from which biological systems are composed, it is becoming increasingly apparent that understanding and fully describing behaviors of the intact biological systems will require sophisticated mathematical analyses. At the same time, the different disciplines need to understand each other's different perspectives and responsibilities. For example, pharmacologists are concerned with obtaining adequate doses to provide significant therapeutic benefits without causing unacceptable side effects, while public health professionals and risk assessors are attempting to ensure there will not be adverse impacts from relatively low doses in large populations. This review will describe some of the key issues that have been raised about the dose–response behaviors of endocrine-mediated toxicities and some of the approaches that have been used to begin to address them.

Dose–response issues for endocrine-mediated effects include a range of questions that reflect upon toxicity testing approaches and risk assessment methods.

- Is there a common dose–response shape characterized by threshold, nonlinearity, or linearity at low doses for endocrine-mediated effects? Do receptor-mediated effects exhibit a common dose–response relationship?
- Are effects during early development (i.e., in utero or postnatal through puberty) characterized by linear dose response?
- Are endocrine-mediated effects characterized by nonmonotonic (e.g. "U-" or "J-" shaped) dose–response relationships?
- Are effects commonly observed at low doses relative to the doses used in traditional toxicity studies, such that they are poorly predicted by such studies?
- When would alterations in the population distributions of characteristics (e.g., intelligence or numbers of males and females) be "adverse" even though the endpoint itself is not "adverse"?
- Are ecological species "sentinels" for potential endocrine-mediated effects in humans? How do their dose–response relationships compare?
- What are the contributions of normal dietary constituents to endocrine-mediated effects, particularly phytoestrogens?

Approaches to evaluating these issues have included experimental studies to provide additional data, statistical analyses of the data, and development of mathematical models for the biological systems and perturbations of those systems. These approaches will be discussed, with some perspectives provided on their abilities to provide answers. The paper begins with the interplay of statistical analyses and dose–response data, particularly with regard to issues of low-dose effects and reproducibility, summarizes the state of mechanistically based modeling, and describes the challenges of developing population approaches.

STATISTICAL ANALYSES OF DOSE–RESPONSE DATA

The major methods employed for analysis of dose–response data involve statistical fitting of curves and analyses using statistics to evaluate trends or group comparisons. These approaches form the underlying basis for most regulatory risk assessment analyses for both noncancer and cancer effects and are the subject of another discussion (Topic 3.13). Such analyses can provide useful insights about the behavior of data in the range of observation and the variability of dose–response data across labs or assay protocols. But, ultimately, these approaches are not powerful tools for providing a fundamental understanding of the complex and controversial issues centered on whether various effects exhibit thresholds, highly nonlinear behaviors, or low-dose linear responses.

It had been suggested that endocrine-mediated effects contrasted with other toxicities in that effects frequently occurred at unusually low doses. An extensive analysis of this issue has recently been completed using a definition of *low dose* as approximating human exposures or below those typically used in standard toxicity testing [10]. This peer review of data concluded that there were specific cases

where low-dose effects apparently had been observed in experimental animals with estrogen receptor agonists, but that, in some cases, these findings had not been replicated, so there was no consistent or reproducible support for the existence of low-dose effects. No evidence for low-dose effects was reported for antiandrogens. The approximately 50 studies involved provide a good overview of the extensive experimental efforts to address this issue, including toxicity studies using five or six dose groups spanning several orders of magnitude in contrast to classic toxicity studies involving two or three dose groups often within a factor of 10 of each other. Clearly, there is a value to well-designed studies evaluating biological or adverse effects in the dose range to which humans are exposed, but, at the same time, it is necessary to keep in mind the significant limitations on the power of such studies due to the relatively small number of animals involved.

In addition, this peer review identified important issues for experimental design and statistical analysis of dose–response studies focusing on potential low-dose effects of endocrine active substances [11]. Many of the issues, such as handling body weight as a covariate versus calculating an organ/body weight ratio, apply much more broadly than to only endocrine-mediated effects.

Understanding the real variability of studies of endocrine-mediated responses with live animals is another difficult problem for dose–response analysis. Rarely are assays for toxicological effects repeated multiple times with the same chemical using the same exact methods, so data concerning the reproducibility of endpoints, especially their quantitative dose response, is limited. An analysis of published uterotrophic assay dose–response data following administration of estradiol found the dose–response characteristics to be highly variable [12]. The Hill equation (1)

$$\text{Response} = \text{Response}_0 + \text{Response}_{\max} \times (\text{dose}^n/(\text{dose}^n + \text{ED}_{50}{}^n) \quad (1)$$

was fitted to the data, where ED_{50} is the dose giving a response 50 % of maximal, and n is often referred to as the Hill coefficient. This equation, though loosely derived from biological underpinnings describing cooperative interactions in proteins [13], is frequently used empirically for fitting continuous response data exhibiting a maximum response. The Hill coefficient varied from 0.4 to 6.0 among the 12 uterotrophic datasets with estradiol; a coefficient of 1.0 is linear at low doses, while coefficients greater or less than 1.0 are sublinear and supralinear, respectively. The observed variability in dose response arises in part from the large number of variations in procedures for this bioassay (e.g., immature vs. ovariectomized adult females, injection vs. oral gavage). Clearly, this degree of variability and lack of assay standardization is unacceptable from the point of view of dose–response analysis, regardless of whether such data might be considered adequate for hazard characterization (i.e., qualitative characterization of the active form of a substance). To address issues of cumulative risk from compounds acting through the estrogen receptor, greater consistency is required [14].

MECHANISTICALLY BASED DOSE–RESPONSE ANALYSES

Mathematical descriptions of the biological processes and their perturbation by endocrine active substances can provide insights into how different dose–response behaviors are created. These analyses have demonstrated that quite varied dose–response behaviors can be obtained, depending upon the description of the system and the quantitative values for the parameters (e.g., affinities of ligand binding to receptors, Hill coefficients) [13,15].

A driving force behind the development of mechanistically based mathematical analyses is that statistical curve fitting approaches and qualitative evaluations do not appear able to provide sufficient insights and often reach contrasting conclusions that reflect the perspectives of the analysts [16–18]. Mechanistically based mathematical analyses provide a method to make an explicit description of biological processes (e.g., receptor–ligand binding, clearance processes, feedback regulation) that are hypothesized to be important and, thus, obtain understanding of the underlying basis for the observed dose–response behavior. For endocrine active substances, this requires describing the normal biological processes and their perturbation by the exogenous compounds. These models ultimately link models for

the pharmacokinetics of the endogenous hormones, the pharmacokinetics of the exogenous chemical, and the pharmacodynamics of the endogenous system and its perturbation (e.g., Fig. 1).

The limitation of the mechanistically based analyses is that adult hormonal regulation is a complex process, and the changes occurring during fetal development and puberty are even more complex. Thus, strikingly different conclusions have been reached about the dose–response behaviors for dioxin-induced effects, by far the most extensively studied and mathematically modeled receptor-mediated processes in toxicology and risk assessment (though not, strictly speaking, hormonal responses). Some analyses have concluded that the effects, particularly induction of cytochromes P450, were ultimately hyperbolic (often described as Michelis–Menten, although this actually refers to enzyme kinetics, not receptor–ligand interactions) and, thus, low-dose linear [19–21]. In contrast, other analyses have focused upon the apparently "all or none" nature of induction in adjoining or nearby hepatocytes, which would have essentially the same free concentration of chemical, although the total concentration is affected by induction of P450s [22–26]. These analyses find the dose response for enzyme induction to be highly nonlinear, though hyperbolic when averaged over the entire liver. While this may appear to be the same position that is obtained from statistical curve fitting, the advantage is that the discussion is focused upon biological processes, which may be experimentally measurable so that, with time and resources, the questions of dose–response behavior could ultimately be resolved for that system. Thus, mechanistically based modeling may be able to provide insights into when and how threshold, highly nonlinear, or linear dose–response behaviors are created, so that the impact of chemicals could be evaluated, as appropriate, based upon these insights.

Mechanistically based models have been developed for a number of developmental and hormonal systems, as summarized in a workshop report on the topic [18]. This report also provides recommendations for risk assessment and research. While it has sometimes been assumed that linear systems of differential equations necessarily resulted in a low-dose linear behavior, this is not necessarily true for feedback systems. This is readily illustrated for positive feedback (sometimes referred to as feed-forward or autoinduction) systems in which the receptor concentration or the production of a high-affinity ligand (e.g., conversion of testosterone to dihydrotestosterone) is regulated by the ligand [15]. Not surprisingly, systems described with Hill equations (which are nonlinear if the exponent is significantly greater than 1.0) can give a range of dose–response behaviors that depend, in part, on the value of the

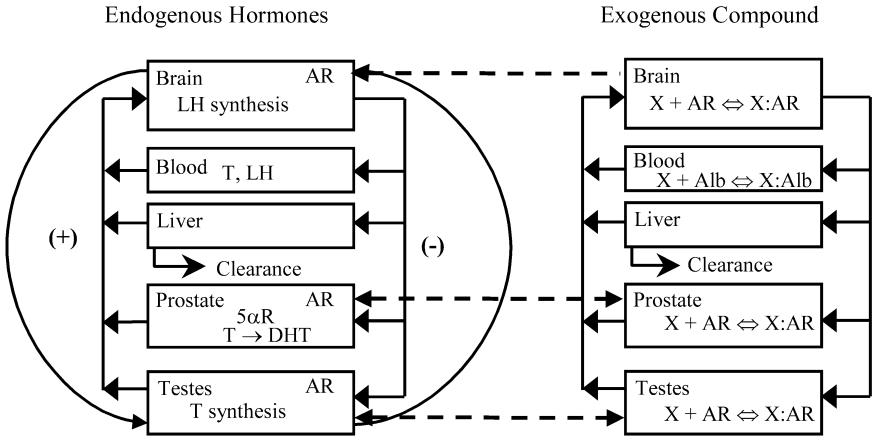

Fig. 1 *Pharmacokinetic and pharmacodynamic modeling for endocrine active substances*. Pharmacokinetic models for the endogenous hormones and exogenous compounds are needed to determine the appropriate tissue dosimetry. The endogenous hormone model incorporates feedback pharmacodynamic processes regulating its production (curved arrows). The exogenous compound, illustrated as an androgen receptor antagonist, interacts with the receptor and affects the pharmacodynamics processes. This affect will be in either the local tissue binding to the androgen receptor or, in the case of the brain, the feedback regulation of the endogenous hormone.

Hill coefficient or exponent [13]. This analysis found low-dose linear, nonmonotonic ("U-shaped"), and highly nonlinear dose response arising from a receptor-mediated system involving positive cooperativity, whether described with a Hill equation or two DNA binding sites for the receptor-ligand complex. Nonmonotonic behaviors can arise from other processes. For example, it has been hypothesized that the in vitro observation of a U-shaped dose response for androgen receptor-mediated response in the presence of dihydrotestosterone and hydroxyflutamide [27] might occur because the steroid receptor dimer bound to either the endogenous hormone, or an exogenous agonist was active for gene activation, but the dimer and one of each ligand were inactive (Rory Conolly, personal communication). Broader impacts on the animal resulting from altered endocrine function, such as caloric restriction due to reduced feeding, have the potential to create nonmonotonic behaviors (sometimes described as hormesis) and should also be considered [28]. Negative feedback is widely recognized to be involved in the creation of homeostatic conditions that are often associated with thresholds for toxic effects [29]. Finally, hysteresis (i.e., different dose–response behaviors depending upon progression from high-to-low dose or low-to-high dose) also was observed to result from positive feedback systems regulating receptor concentration and production of a high-affinity ligand [15].

Mathematical descriptions of the biology underlying the creation of varied dose–response behaviors are increasingly being explored for a wide range of biological systems, at levels of organization ranging from cells to whole organisms to populations of each of these [9]. Studies of embryonic development and cell cycling will clearly be highly relevant to gaining greater understanding of those situations where endocrine-mediated effects give different dose–response behaviors [30,31]. In particular, they will likely identify common types of processes used for creating those behaviors, even when factors such as the specific effector molecules or cell types are all different [15]. It is the interactions of these components in a higher-order system that generally creates the system dose–response behaviors.

POPULATION DOSE RESPONSE VS. INDIVIDUAL DOSE RESPONSE AND BACKGROUND EXPOSURES

Chemical risk assessments, particularly for noncarcinogenic effects, essentially evaluate the dose–response behavior for individuals representative of sensitive populations rather than estimate population risks. It has been suggested that endocrine-mediated effects may, in part, be evidenced as shifting distributions of a characteristic in the population rather than as directly causing an adverse effect. One example of this would be altered ratios of male and female offspring among turtles born from eggs exposed to estrogenic compounds [32–34]. It is interesting to note that temperature- and steroid-dependent turtle egg sex determination appears to be a highly nonlinear process at the individual level, involving positive feedback on both the steroid receptor and the high-affinity hormone synthesis enzymes in order to drive the turtle's development to be either male or female. However, the observation at the population level is that exogenous compounds can influence the chances that the positive feedback will proceed toward one sex or the other [34].

Another aspect involving populations is the widespread, but varied, exposures of populations to backgrounds of endocrine active substances, including persistent bioaccumulative compounds (e.g., dioxins/furans) and phytoestrogens. It has been suggested that the dose response could be low-dose linear if the endogenous hormones or the background exposures already resulted in some incidence of the effect in the population, and additional exposure worked through that common mechanism [35]. One complication with hormones and endocrine-mediated effects is that the same hormones (or exogenous compounds such as phytoestrogens) often appear to be responsible for both beneficial and adverse effects (e.g., associations of elevated estrogen status in women with increased risk of some cancers, decreased risks of others, decreased risks of osteoporosis). Thus, assessing the population impact of exposures to endocrine active substances is an area deserving effort, but requiring development of innovative tools and approaches. It should be particularly focused on bringing together knowledge about public health and toxicology.

RESEARCH PRIORITIES AND OTHER RECOMMENDATIONS

Research priorities in the area of dose–response assessment for endocrine active substances have been described previously [10,18,36]. Largely, the priorities previously outlined remain needs, as these were generally longer-term efforts. It has been recommended that prototype case studies implementing mechanistically based dose–response modeling be developed for endocrine-mediated effects [18]. Case studies have been suggested for impacts on adult prostatic function from in utero estradiol exposure or developmental antiandrogen exposures and the association of estrogen exposure and mammary/breast cancer, among others. This clearly continues to be one of the highest priority needs as this approach will build the understanding necessary to begin to discern how to appropriately generalize from these case examples to broader ranges of substances or broader ranges of effects.

A strong recommendation of one previous effort was that development of mechanistically based dose–response models should be a routine part of the risk assessment process [18]. Models for the pharmacokinetics of compounds are generally more readily developed than for their pharmacodynamic processes. Pharmacokinetic models can assist in addressing extrapolation issues that frequently arise in risk assessments, such as those across routes of exposure, among exposure regimens (e.g., continuous vs. episodic), and among species [3,37]. These models are also valuable for evaluating how internal doses would change with different exposures at different ages (e.g., children or the elderly). Selection of the appropriate internal dose-metric for such extrapolations is facilitated by knowledge of the pharmacodynamic processes, but doesn't require the same level of quantitative information as is often required to develop a mechanistically based pharmacodynamic model. Thus, implementation of mechanistically based modeling as a way of explicitly incorporating scientific data into risk assessment can and should proceed as an incremental process replacing default assumptions.

SUMMARY AND CONCLUSIONS

Issues of dose–response behavior are critically important to risk assessment for chemical-mediated toxicities [3]. The standard paradigm for chemical risk assessment in the United States involves an explicit dose–response assessment step. Continued progress toward better understanding dose–response behaviors is essential for moving risk assessments from default assumptions to more scientifically based approaches.

Theoretical mechanistically based modeling has clearly demonstrated that a range of dose–response behaviors can be obtained from receptor-based feedback-regulated systems, depending upon the specific characteristics of the system and the values of the parameters that describe it (e.g., affinity constants) [13,15,29]. These behaviors include: (1) highly nonlinear dose response, for example, created by positive feedback, (2) thresholds created by negative feedback regulation, (3) low-dose linear behavior when a system is essentially determined by the interaction of a ligand with a receptor, (4) nonmonotonic ("U-shaped) dose response arising from positive cooperativity in specific biological steps such as protein synthesis or from activity of dimers with the same ligand, but inactivity of mixed dimers with different ligands, and (5) hysteresis (dose–response behaviors that vary as dose increases or decreases) due to positive feedback on the receptor and synthesis of a high-affinity ligand. Thus, the challenge now is to better define when these different dose–response behaviors arise so that risk assessment approaches for endocrine active substances can be tailored appropriately.

ACKNOWLEDGMENTS

The critical review and input of Woody Setzer are acknowledged. The information in this article has been subjected to review by the National Health and Environmental Research Laboratory and approved for publication. Approval does not signify that the contents reflect the views of the Agency.

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