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Predictive Ecotoxicology Workshop

PREDICTING CHEMICAL IMPACTS ON VERTEBRATE ENDOCRINE SYSTEMS

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Abstract—Animals have evolved diverse protective mechanisms for responding to toxic chemicals of both natural and anthropogenic origin. From a governmental regulatory perspective, these protective responses complicate efforts to establish acceptable levels of chemical exposure. To explore this issue, we considered vertebrate endocrine systems as potential targets for environmental contaminants. Using the hypothalamic-pituitary-thyroid (HPT), hypothalamic-pituitary-gonad (HPG), and hypothalamic-pituitary-adrenal (HPA) axes as case examples, we identified features of these systems that allow them to accommodate and recover from chemical insults. In doing so, a distinction was made between effects on adults and those on developing organisms. This distinction was required because endocrine system disruption in early life stages may alter development of organs and organ systems, resulting in permanent changes in phenotypic expression later in life. Risk assessments of chemicals that impact highly regulated systems must consider the dynamics of these systems in relation to complex environmental exposures. A largely unanswered question is whether successful accommodation to a toxic insult exerts a fitness cost on individual animals, resulting in adverse consequences for populations. Mechanistically based mathematical models of endocrine systems provide a means for better understanding accommodation and recovery. In the short term, these models can be used to design experiments and interpret study findings. Over the long term, a set of validated models could be used to extrapolate limited in vitro and in vivo testing data to a broader range of untested chemicals, species, and exposure scenarios. With appropriate modification, Tier 2 assays developed in support of the U.S. Environmental Protection Agency's Endocrine Disruptor Screening Program could be used to assess the potential for accommodation and recovery and inform the development of mechanistically based models. Environ. Toxicol. Chem. 2011;30:39–51.

Keywords—Accommodation Recovery Developmental programming Endocrine disruption Endocrine axis

INTRODUCTION

Animals have evolved diverse protective mechanisms for responding to toxic chemicals of both natural and anthropogenic origin. For example, signaling pathways mediated by activation of nuclear transcription factors result in coordinated production of enzymes, binding proteins, and membrane transporters that detoxify, sequester, and eliminate foreign substances [1]. Additional pathways recognize and repair cell damage after toxicant-induced injury (e.g., the DNA-response pathway; [2]). These protective responses may allow animals to function normally despite exposure to chemicals that at higher concentrations would cause adverse toxicological effects.

For continuous or frequently occurring exposures, selection of animals that successfully respond to the insult may result in populations resistant to a specific chemical or chemical class. For example, killifish (*Fundulus heteroclitus*) and tomcod (*Microgadus tomcod*) that inhabit sites contaminated with polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), or polycyclic aromatic hydrocarbons (PAHs) become resistant to PCB-, PCDD-, or PAH-induced lethality, developmental toxicity, and induction of cytochrome P4501A (CYP1A) [3]. Similarly, aquatic insects may develop resistance to high levels of heavy metal contamination. This response depends on the magnitude, duration, and spatial extent of metal exposure, as well as movements of animals within the environment [4]. Analogous findings have been reported for target pest species (insect, fungal, and weed populations) exposed to pesticides.

From a governmental regulatory perspective, the ability of animals to accommodate and recover from chemical insults is problematic because it complicates efforts to establish acceptable levels of exposure. The challenge of dealing with these responses in the context of human health risk assessment was recognized in a report by the National Research Council (NRC) entitled *Toxicity Testing in the 21*st *Century* [5]. As presented in the NRC report, Figure 1 shows a dichotomous framework that distinguishes between normal biological

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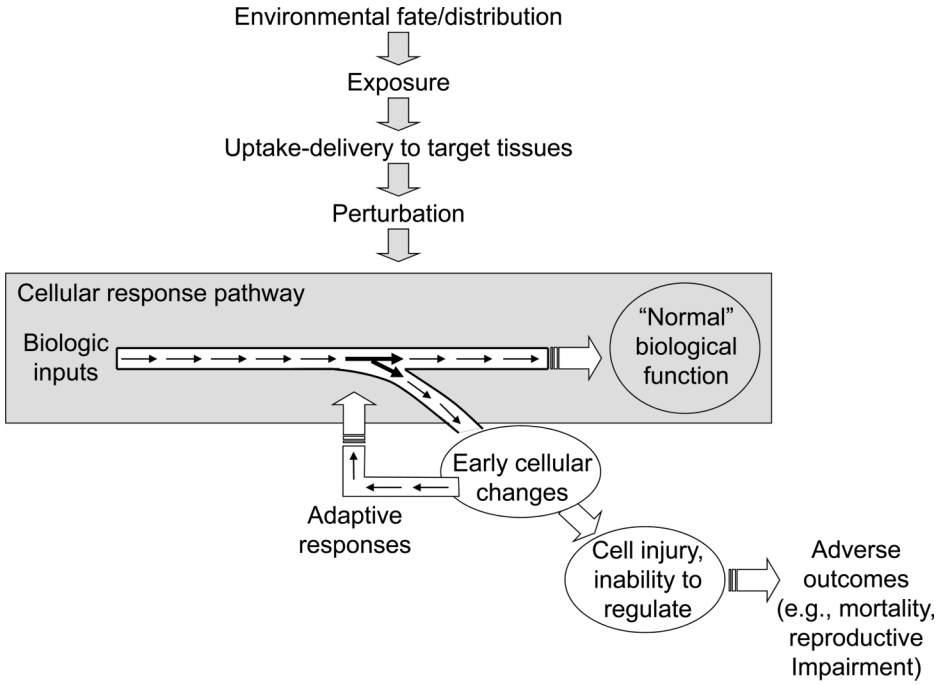

Fig. 1. Biological responses to toxic chemicals. This figure was originally presented by Andersen et al. [89] and later reproduced by the National Research Council [5]. It is reprinted here with permission. The figure highlights the role of accommodation (termed *adaptation* by the authors) in the context of chemical effects at the cellular level, and indicates that toxicity occurs only after these protective responses are overwhelmed.

function and a divergent pathway that leads to toxic effects. Within this framework, accommodation (referred to by the authors as *adaptation*) is viewed as a loop that restores the system to normal function. The report focused on toxic effects at the cellular level of biological organization, and the potential for accommodation was discussed primarily in this context. The authors noted, however, that cellular response information must be related to the response of an intact biological system. To accomplish this goal, one must understand accommodation occurring at higher levels of biological organization.

The purpose of the current paper is to summarize the findings of a workgroup that was organized as part of a SETAC Pellston Workshop entitled *A Vision and Strategy for Predictive Ecotoxicology in the 21st Century: Defining Adverse Outcome Pathways Associated with Ecological Risk* [6]. The charge to the workgroup was to better understand what differentiates successful accommodation and recovery from system failure and to discuss how this information can be incorporated into computational models of chemical effects. Chemical effects on vertebrate endocrine systems were identified as a focus for discussion, in part because of existing interest in endocrine disruption as a chemical mode-of-action and also because endocrine systems possess a demonstrated ability to accommodate and recover from chemical insults. In this report, we describe features of endocrine systems that allow them to accommodate and recover from chemical insults, review studies that illustrate these responses in ecologically relevant species, and describe efforts to model chemical effects using computational approaches. In concluding sections, we discuss accommodation and recovery in the context of ecological risk assessment and identify research needed to better predict these responses.

TERMS AND DEFINITIONS

Scientists that study the responses of biological systems to external stressors use a variety of terms to describe specific response categories. For clarity, we begin by defining our use of terms and describe their relationships to one another.

The term *accommodation* refers to any response of a biological system to an external stressor that tends to restore the system to its normal or baseline condition or establishes a new set point. Here the term *external* is relative to the level of biological organization at which accommodation occurs and could, for example, refer to a stressor operating at the cell, whole organism, or population level. In toxicology, accommodation occurs as a response to a chemical stressor and generally refers to restoration of biological function despite continued exposure to the compound. We use *accommodation* instead of *adaptation* (as used in the NRC report [5]) to describe proximate physiological responses and mechanisms so as to avoid confusion with use of the term *adaptation* in an ultimate, evolutionary context.

A diverse array of physiological systems functions to maintain relative constancy of the internal environment (e.g., in cells or the entire organism). Some of these systems tightly regulate a physical–chemical property of the organism (e.g., osmolarity, oxygen partial pressure), whereas others maintain the concentration of an endogenous substance within narrow limits (e.g., pH, glucose). This type of physiological control is termed *homeostasis*. Other systems can respond by adjusting the concentration of an endogenous substance (generally a ligand for a macromolecular receptor) or by changing the set point for one or more physiological parameters (e.g., blood pressure or heart rate). This accommodation through change is called *allostasis* [7,8]. Examples of allostatic systems include the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis. Importantly, the systems responsible for homeostasis and allostasis may themselves change during the lifetime of an animal because of developmental and aging processes, or in response to the external environment.

The term *recovery* refers to restoration of biological function after withdrawal of a stressor. Other terms describe different

outcomes after exposure to a stressor: *performance*, *fitness*, *developmental plasticity*, and *developmental programming*. *Performance* is an individual-level measure of physiological state and is what whole-animal bioassays typically measure. *Fitness* refers to the relative probability of survival and reproduction for a genotype and is inferred from an evaluation of performance measures in the context of life history and reproductive strategies. *Developmental plasticity* is the property of a given genotype to produce different phenotypes in response to environmental conditions experienced during development. Under natural conditions, developmental plasticity may generate adaptive morphological, physiological, or behavioral traits that promote survival. The term *developmental programming* encompasses a mechanism by which developmental plasticity occurs and may involve epigenetic changes that alter gene expression. The term *developmental programming* is not specific to any particular taxon. As such, it has broader application than the term *fetal programming*, which is often used to describe developmental programming in mammals.

A FOCUS ON ENDOCRINE SYSTEMS

Although chemical effects on endocrine systems have been studied for decades, current interest in this topic is attributable in large part to works published by Theo Colborn and colleagues [9]. These authors suggested that a large number of environmental contaminants may disrupt endocrine systems during critical stages of development, resulting in adverse outcomes in wildlife and humans. Scientists continue to debate whether these effects are widespread or restricted to a small number of compounds or a few geographically localized hotspots [10]. Nevertheless, governmental agencies in North America and Europe have established programs to identify and regulate compounds that have potential to impact endocrine systems (http://www.epa.gov/scipoly/oscpendo/pubs/edspoverview/finalrpt.htm; http://europa.eu/eur-lex/en/com/cnc/1999/com1999_0706en01.pdf; accessed June 26, 2009).

A majority of research on environmental endocrine disruptors has been focused on vertebrate species. This focus is reflected within this report. The workgroup recognized, however, that endocrine systems control the development and growth of most (if not all) invertebrate species and chemicals that are used to intentionally impact hormonally regulated development in invertebrates constitute an important class of insecticides [11].

THE HPT, HPA, AND HPG AXES AS CASE EXAMPLES

To provide additional focus for discussion, the workgroup considered the hypothalamic-pituitary-thyroid (HPT), -gonad (HPG), and -adrenal (HPA) axes as case examples. Here we describe the three axes in general terms, noting shared structural attributes that allow these systems to respond to various insults.

The three endocrine axes are represented schematically in Figure 2. Each is controlled centrally by neurosecretory neurons located in the anterior preoptic region and hypothalamus of the brain. These neurons project axons to the median eminence, where neurohormones are released into the pituitary portal circulation (in bony fishes the axons terminate within the pituitary gland, directly innervating pituitary cells). The neurosecretory neurons receive inputs from higher brain centers, limbic structures, and the hindbrain and also are regulated by hormones and metabolites in the blood circulation. Neurohormones produced by these neurons regulate the secretion of pituitary hormones to the general circulation. Many pituitary

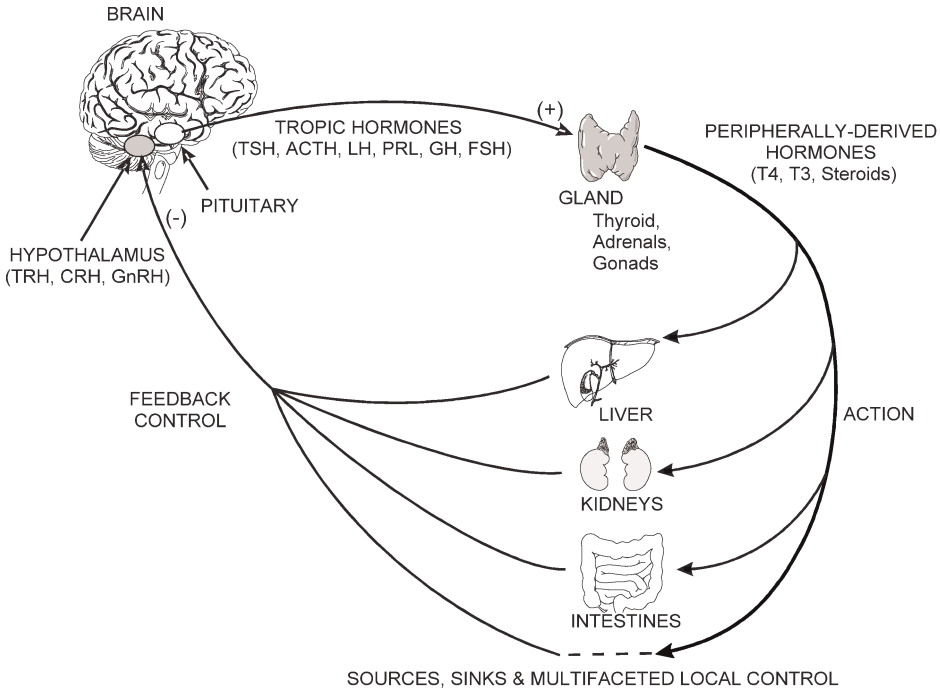

Fig. 2. Neuroendocrine regulation within the hypothalamic-pituitary-thyroid (HPT), -adrenal (HPA), and -gonad (HPG) axis feedback control systems. Hierarchical closed-loop regulation begins at the hypothalamus, which produces neurohormones that either stimulate or inhibit pituitary secretion of tropic hormones. Primary feedback regulation occurs in the hypothalamus and pituitary via intracellular signal transduction initiated by circulating thyroid, adrenal, and gonadal hormones produced and secreted by the target glands. The actions of peripherally derived hormones are also controlled via an array of regulatory mechanisms at their intracellular sites of action throughout the organism. TRH = thyrotropin-releasing hormone; CRH = corticotropin-releasing hormone; GnRH = gonadotropin-releasing hormone; TSH = thyroid-stimulating hormone; ACTH = adrenocorticotropic hormone; LH = luteinizing hormone; PRL = prolactin; GH = growth hormone; FSH = follicle-stimulating hormone; T4 = thyroxine; T3 = 3,5,3′-triiodothyronine.

hormones are under dual hypothalamic control, being stimulated by releasing factors and inhibited by release-inhibiting factors. Pituitary hormones that act on other hormone-producing glands are called *tropic* hormones. These tropic hormones control the secretion of hormones by peripheral endocrine glands such as the thyroid, gonads, and adrenal cortex. Peripherally derived hormones act in turn on various target tissues to coordinate a range of biological functions [12,13].

Peripherally derived hormones are transported in the bloodstream bound to hormone-binding proteins. The hormones enter cells in target tissues, either passively by diffusion (steroids) or by active uptake via membrane transporters (thyroid hormones), and they may be metabolized to active or inactive forms. Active hormones bind nuclear hormone receptors, which act as transcription factors to regulate gene expression. Peripherally derived hormones also may interact with receptors located on the plasma membrane, leading to activation or inhibition of intracellular kinases, or they may bind to intracellular enzymes, regulating their activity.

Primary control of each endocrine axis is achieved by negative feedback of peripherally derived hormones on the hypothalamus and pituitary, which reduces the production of tropic hormones. This closed-loop feedback structure allows the system to maintain a relatively constant plasma hormone concentration through a process of continuous readjustment. When the system receives signals from the external environment (e.g., photoperiod, temperature, hydroperiod) or from internal biological clocks, or responds to changes in circulating metabolites or hormones, plasma hormone concentrations can fluctuate above or below some mean baseline value. The baseline for any particular hormone is context dependent and is set by experience within the system extending over a period of hours to weeks (i.e., physiological set points). These systems are robust and are capable of adjusting in response to a diversity of environmental insults, including interactions of xenobiotics with one or more components of the system.

A PRESUMPTIVE SPECTRUM OF PHYSIOLOGICAL RESPONSES

The functioning parts of an endocrine axis are separated in time and space. Moreover, effects on one axis may impact the function of another axis because of shared molecular substrates, enzymatic reactions, and signaling pathways. Given this complexity, impacts on the whole organism may be difficult to predict from an impact on any single part of an endocrine axis unless the effect is of an extreme nature such as prolonged stimulation by a hormone receptor agonist or substantial inhibition of hormone synthesis.

To provide a basis for further discussion, the workgroup sought to describe a spectrum of possible responses of endocrine systems (Fig. 3). Because some of these responses are poorly described, particularly in species of ecological concern, this spectrum was labeled *presumptive*. When the magnitude of stress on an endocrine axis is low, homeostasis can be accomplished by normal feedback control, and a high level of benefit is provided to the organism at little or no cost beyond maintenance of the system itself. If stress on the system exceeds the capacity for homeostatic control, the system may generate an allostatic response, wherein the set point of the system may change, generating a new stable state [14]. Long-term, stable changes in physiological set points may involve epigenetic changes in gene regulatory regions that lead to altered gene expression [15]. These epigenetic changes include methylation

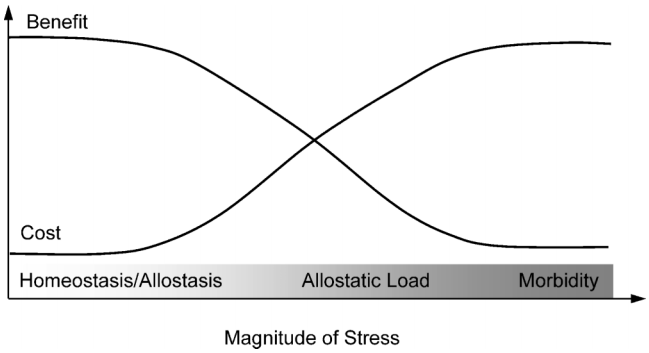

Fig. 3. Presumptive spectrum of responses to chemical impacts on endocrine systems. The costs and benefits to the organism of mounting a response are arrayed along the *Y*-axis. The magnitude of the stressor is arrayed along the *X*-axis. Terms along the *X*-axis describe a spectrum of responses and potential consequences: homeostasis = maintenance of relative constancy through normal feedback mechanisms; allostatic change = change to one or more components of the system itself as the organism strives to deal with uncompensated stress; allostatic load = permanent changes attributable to repeated cycles of allostasis.

of cytosine bases in gene promoter regions called CpG islands and post-translational modifications to histones [16]. Such changes may lead to changes in hormone receptor expression or the activities of pathways responsible for hormone biosynthesis. These changes may be beneficial (adaptive in an evolutionary sense; i.e., tending to increase fitness), provided that the functioning of the system is appropriate to the environment within which the organism lives. Mismatch between the phenotype and the environment may result in reduced fitness. Chronic stress in animals can result in permanent changes that lead to disease [17]. This stress on the system, which is often termed the *allostatic load* [8], has been documented in studies of the central nervous system, immune system, and cardiovascular system [14]. To our knowledge, the term *allostatic load* has not previously been used by toxicologists. We introduce it here and use it to describe potential impacts associated with long-term physiological responses to endocrine-disrupting compounds.

At some point, a level of stress exceeds the animal's ability to fully compensate, and benefits of the system are lost even as the cost of accommodation increases. If the magnitude of exposure is high, the animal may proceed rapidly to death (e.g., failure to complete metamorphosis). If the exposure and resulting effects occur chronically, the animal may accumulate an allostatic load, which could result in adverse fitness consequences.

A complicating feature of endocrine systems is that effects on a specific axis may be buffered by attributes of the axis itself or the systems that it influences. For example, the thyroid gland stores precursors to 3,5,3′-triiodothyronine (T3) and thyroxine (T4) as iodinated species within a protein matrix called colloid. This storage form of thyroid hormones may be sufficient to satisfy the needs of an organism for days, weeks, or months (depending on the organism, its age, and other factors), protecting the animal from short-term inhibitory effects of chemicals on thyroid hormone synthesis. In addition, effects elicited by a hormone may persist over time even when its production declines because of the persistence of cellular and molecular changes in target tissues. For example, although thyroid hormone plays a critical role in amphibian metamorphosis, the effect of a thyroid hormone synthesis inhibitor on metamorphosis tends to be stage dependent, and an exposure that occurs late in development may have little or

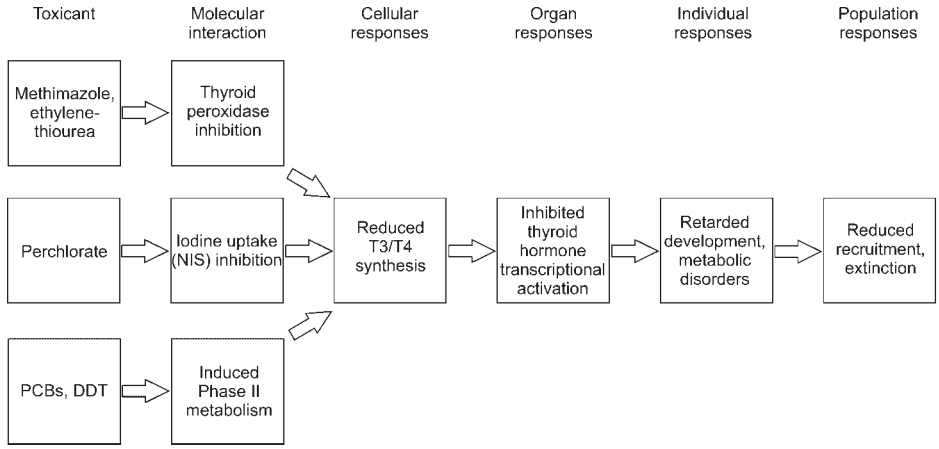

Fig. 4. Adverse outcome pathways for several chemicals that may impact the thyroid axis. Toxicants acting via different modes action may reduce thyroid hormone production, resulting in similar consequences for the exposed organism. PCBs = polychlorinated biphenyls; DDT = dichlorodiphenyltrichloroethane; NIS = sodium-iodide symporter; T3 = 3,5,3′-triiodothyronine; T4 = thyroxine.

no impact because the genetic program for metamorphosis has already been initiated [18,19].

ACCOMMODATION AND RECOVERY OF ENDOCRINE SYSTEMS

A large number of compounds have been shown to impact one or more endocrine axes. Figure 4 uses the adverse outcome pathway [20] concept as an organizing principle to illustrate this point. In this example, adverse outcome pathways are shown for five compounds that can impact the HPT axis via three different mechanisms. Methimazole and ethylenethiourea inhibit thyroid peroxidase, an enzyme that catalyzes the incorporation of iodide into thyroglobulin, the protein precursor for T3 and T4. Perchlorate competitively inhibits the sodium-iodide symporter, a protein that transports plasma iodide into the thyroid follicle cell, whereas PCBs and DDT induce phase II metabolic pathways in the liver that clear T3 and T4 from plasma. All three mechanisms can reduce circulating levels of T3 and T4, potentially resulting in adverse outcomes such as impaired development [21] and metabolic dysfunction [22].

Although these adverse outcome pathways provide plausible linkages between a specific initiating event and an adverse outcome, they do not directly address the possibility of accommodation to these chemical insults. Instead, the arrows linking molecular interactions to higher-order responses (cell → organ/tissue → individual) presume that the dose is sufficient to cause effects at all levels of biological organization. Accommodation may be viewed, therefore, as a response that operates against one or more of the arrows shown in Figure 4.

By its nature, accommodation to a chemical exposure is difficult to demonstrate. How does one demonstrate that an animal has responded to an insult when it appears to be unaffected? Dose–response studies often result in identification of treatment levels below which no effects were observed. Traditionally, toxicologists have interpreted this finding in terms of a threshold for toxicity. In most instances, however, a range of dose levels may be present to which the animal successfully responds, resulting in no measured effects (as defined by the test endpoints). The apparent toxicity threshold is therefore defined by the test endpoints as well as by the animal's ability to respond to real, albeit uncharacterized, effects.

One way to demonstrate that accommodation has occurred is to characterize the physiological response itself, including its time course in relation to that of well-defined apical endpoints. The structure/function of the HPT axis in amphibians is well known, as is the mode of action by which many thyroid-disrupting compounds act (Fig. 4). By integrating this information, one may hypothesize likely elements of a compensatory response and develop research tools needed to measure them. Using this approach, several research groups have characterized the effects of thyroid hormone synthesis inhibitors on metamorphosis in *Xenopus laevis* tadpoles [23–25]. In each of these studies, the authors observed histological changes (follicle cell hypertrophy or hyperplasia) consistent with an increase in circulating levels of thyroid stimulating hormone, as would be expected if circulating T3/T4 levels declined (because of reduced negative feedback). Importantly, these changes were observed at treatment levels that resulted in impaired metamorphosis as well as one or more treatment levels that had no discernible effect on development.

In recent work, the time course for this response and its underlying mechanistic basis have been studied in detail. Optiz et al. [26] found that genes that code for the sodium-iodide symporter, thyroid peroxidase, and the thyroid-stimulating hormone receptor were significantly up-regulated in tadpoles exposed to perchlorate or ethylenethiourea. These responses were observed at early time points (3–5 d), well in advance of any effects on metamorphosis. Tietge et al. [27] exposed tadpoles to concentrations of methimazole, perchlorate, or 6-propyluracil that had been shown to inhibit metamorphosis after 14 d of exposure. Circulating levels of T4 declined after 6 d of exposure. This effect was accompanied by an increase in thyroid cell number, suggesting compensation via cell proliferation. Both observations were preceded in time, however, by a decrease in T4 stored within the gland (exposure day 2). This observation suggests that, despite a progressive decrease in stored T4, animals were able to maintain circulating levels of T4 for several days.

Several studies have demonstrated that endocrine systems can recover after brief exposures to various chemical classes. For example, rainbow trout exposed for 30 d to an environmentally relevant mixture of PCBs exhibited a decreased level of deiodinase activity and an increased number of thyroid epithelial cells [28]. Both measures of thyroid system status returned to control levels after a 20-d depuration period. Li et al. [29] found that partial-to-full recovery of the rare minnow

(*Gobiocypris rarus*) thyroid system (as measured by liver histopathology, plasma thyroid hormone, transthyretin deiodinase, and thyroid hormone receptor messenger RNA in the liver and brain) occurred during a 28-d depuration period in clean water after 28 d of exposure to 3-amino-1,2,4 triazole (amitrole). The extent of recovery was dependent, however, on sex and the endpoint measured. Villeneuve et al. [30] reported that endocrine-related effects resulting from a short-term (8-d) exposure of fathead minnows to fadrozole were fully reversible after an 8-d recovery period in clean water.

The effect of 17α-ethynylestradiol (EE2) on the HPG axis in male zebrafish provides a particularly good example of recovery from exposure to an endocrine-disrupting compound. EE2 is a synthetic estrogen receptor agonist that is widely used as a reference reproductive toxicant in vertebrate animal models. Two studies have examined recovery after short-term exposure of juvenile or adult males to EE2. Van den Belt et al. [31] reported that 6-to-8-month-old adults exposed to 10 or 25 ng/L EE2 for 24 d exhibited a significant decrease in the testis somatic index and presence of mature sperm; however, all fish fully recovered after a 24-d recovery period in clean water. Maack and Segner [32] exposed 43 d postfertilization (dpf) zebrafish to 1.7, 3, or 10 ng/L EE2 for 28 d, resulting in 85, 100, and 100% of fish, respectively, with ovarian tissue. Despite a complete reversal in gonadal phenotype at the two highest treatment levels, these authors reported that, after a 119-d recovery period, male fish were present, and testicular tissue morphology was not significantly different from that of controls.

Five additional studies have evaluated recovery after long-term exposures of young (0–2 dpf) zebrafish to the juvenile hermaphroditic stage (75–90 dpf) or premature/mature gonad stage (120 dpf). Van den Belt et al. [31] found that exposure of newly hatched zebrafish to 0.1, 1, 10, or 25 ng/L EE2 for 90 days resulted in approximately 40, 40, 70, and 100%, respectively, of total fish with undifferentiated gonads. After a recovery period of 150 d, all male gonads were fully differentiated, and there were no significant differences in testis somatic index compared with controls. In contrast to these findings, Nash et al. [33] exposed 1 dpf zebrafish to 0.5 or 5 ng/L EE2 for 75 d and then allowed them to recover for 150 d. At the end of the recovery period, male fish exhibited histological alterations in the testis, including extensive sperm duct malformations, presence of ovarian cavities, variations in the frequency of testicular cell types, and ciliation of sperm ducts. Xu et al. [34] exposed 2 dpf zebrafish to 0.4, 2, and 10 ng/L EE2 for 88 d followed by a recovery period of 90 d. Exposure to 2 and 10 ng/L EE2 resulted in a significant increase in the number of fish containing ovarian or undifferentiated tissue at the end of the exposure period. Although no significant differences in sex ratios were seen after the recovery period, a significant percentage of males exhibited malformations of the sperm duct, altered proportions of germ cell types, and reduced number of spermatozoa. Larsen et al. [35] observed partial recovery of 0 dpf zebrafish exposed for 120 d to 5 ng/L EE2 and allowed to recover for 8 months.

Longer exposure or varying recovery periods do not appear to affect the ability of male zebrafish to recover from EE2 exposure. Schäfers et al. [36] exposed 0 dpf zebrafish to 10 ng/L EE2 for 177 d followed by a recovery period of 108 d. Although 100% of fish contained ovarian tissue after EE2 exposure, 25% of these fish recovered and developed mature testicular tissue. Fenske et al. [37] exposed 0 dpf zebrafish to 3 ng/L EE2 for 42 d followed by a recovery period of 33, 76, or 134 d; these authors also exposed 0 dpf zebrafish to 3 ng/L EE2 for 118 d followed by a 58-d recovery period. Although gonadal effects were not measured at the end of the exposure period, these authors reported that, based on gonadal histology, partial-to-full recovery occurred for all exposure and recovery periods.

Collectively, research on zebrafish has shown that animals exposed to EE2 can recover from effects on the HPG axis. Interestingly, although most of these studies demonstrated that fish can partially or fully recover based on gonadal histology and fecundity tests (as assessed by female egg production), most efforts showed that short- or long-term EE2 exposure can result in long-term impacts on male fertility (as assessed by fertilization success [32–34,36,37]). Thus, these studies highlight the importance of endpoint selection for evaluation of recovery and illustrate the importance of assessing potential fitness costs associated with chemically mediated disruption of the HPG axis. This work also shows the value of using standardized testing protocols to facilitate comparisons among efforts.

Endocrine disrupting effects on developing systems

Epigenetic mechanisms of gene regulation acting during early development play an important role in programming phenotypic expression later in life [38]. In humans, developmental programming may occur as a response to systemic stress (e.g., malnutrition) and can cause adverse long-term health effects [15,39]. Similar mechanisms may, however, contribute to species survival by providing a means by which animals match their phenotype to prevailing environmental conditions, an outcome commonly referred to as developmental plasticity. Developmental plasticity has been demonstrated in virtually all groups of plants and animals, although particularly good examples come from research on amphibians. Studies by amphibian ecologists have shown that environmental conditions experienced during the larval stage can affect metamorphic timing, body size, and the morphology of tadpoles and adults [19,40]. This developmental plasticity may confer a selective advantage to individuals that respond in an appropriate manner to changing conditions (e.g., accelerated metamorphosis in response to declining water levels), although changes such as reduced body size also may result in reduced fitness.

Research with mammals has shown that the HPA axis plays an important role in developmental programming that occurs as a response to stress. The molecular and physiological mechanisms involved in this response appear to be conserved in lower vertebrates. For example, *X. laevis* tadpoles placed on a restricted diet exhibited a reduced body size at metamorphosis and elevated levels of whole body corticosterone when compared with controls [41]. Exposure of *X. laevis* tadpoles to exogenous corticosterone resulted in similar impacts on metamorphosis. Previous studies with other Anuran species have resulted in similar findings [42,43]. Hu et al. [41] went further, however, and evaluated the long-term recovery of frogs after metamorphosis. Tadpoles placed on a restricted diet exhibited catch-up growth when transferred to a normal diet but exhibited long-term elevations in whole-body corticosterone content. Subsequent immunohistochemical studies showed that early exposure to elevated levels of corticosterone as a tadpole resulted in decreased glucocorticoid receptor densities in discrete regions of the central nervous system of juvenile frogs. It is not clear whether these changes would have adverse health consequences for adult amphibians. Research with mammals suggests, however, that early life nutritional deficits can result in profound effects on adult physiology and behavior [44].

The potential significance of developmental programming to the current discussion of accommodation and recovery is threefold. First, because developmental programming can be influenced by hormones, chemicals that impact endocrine axes could impair the ability of animals to respond to changing environmental conditions. Second, because impacts on developmental programming are thought to be limited to early life stages, compounds that impair endocrine function may produce different effects in early versus late developmental stages. Third, phenotypic changes associated with developmental programming may result in the establishment of a new steady-state condition, distinct from the steady-state of unstressed animals.

The toxicological relevance of developmental programming has been demonstrated in several studies with mammals [16]. These efforts showed that exposure to endocrine-disrupting chemicals during early development can result in permanent functional changes that are not realized until later in life, including reproductive dysfunction and tumor promotion. Similar data from ecologically relevant species are more limited. King Heiden [45] showed that transient exposure of zebrafish to 2,3,7,8- tetrachlorodibenzo-*p*-dioxin during early development resulted in latent toxicity in adults, and it was suggested that developmental programming was responsible for this outcome. Surprisingly, the offspring of these fish also exhibited increased mortality, suggesting that chemical effects were transgenerational. Although this result may have been caused by direct effects on the genome (i.e., mutations), epigenetic changes are also heritable [46,47].

PREDICTING CHEMICAL IMPACTS ON ENDOCRINE SYSTEMS

An expanded view of accommodation and recovery

Using as a starting point the paradigm for toxicity described in the NRC report [5] and shown here as Figure 1, we sought to develop a broader description of accommodation and recovery relevant to ecological risk assessment of chemicals that impact vertebrate endocrine systems. Figure 5 illustrates this broader vision. In Figure 5, the spectrum of physiological responses described previously (homeostasis/allostasis → allostatic load → morbidity) is arrayed along the line that represents a departure from the original stable state. This figure also includes the possible emergence of new stable states (phenotypes B, C. . ., and so forth) that arise as a consequence of developmental programming or epigenetic changes associated with chronic accommodation (i.e., constituting an allostatic load). Homeostasis and allostasis restore the system to a stable state; however, allostatic responses function in part to maintain the integrity of homeostatic systems and are viewed as possessing a broader scope for accommodation. Both homeostasis and allostasis may operate to restore either the original stable state or any new stable states that emerge from stable epigenetic changes.

Figure 5 is generic with respect to levels of biological organization. Although homeostasis and allostasis ultimately result in changes at the cellular level, coordinated control of these changes is likely to involve multiple tissues and organs operating as part of a physiological system. The altered phenotypes in Figure 5 could refer to whole organisms. These new phenotypes would be expected, however, to contain altered cells and tissues.

Implied by an explicit recognition of altered phenotypes in Figure 5 is a departure from the NRC figure, which relates to differences between the goals of human health and ecological risk assessment. In general, human health risk assessment tends to focus on protection of individuals, whereas ecological risk assessment tends to focus on protection of populations and ecosystems (with the exception of threatened and endangered species). This difference follows from historic legislation authorizing these activities and reflects a fundamental difference in the valuation of humans and wildlife.

As a practical matter, directly evaluating chemical impacts on populations or ecosystems is difficult. The potential for adverse ecological consequences is therefore largely inferred from information obtained by testing individual organisms, as

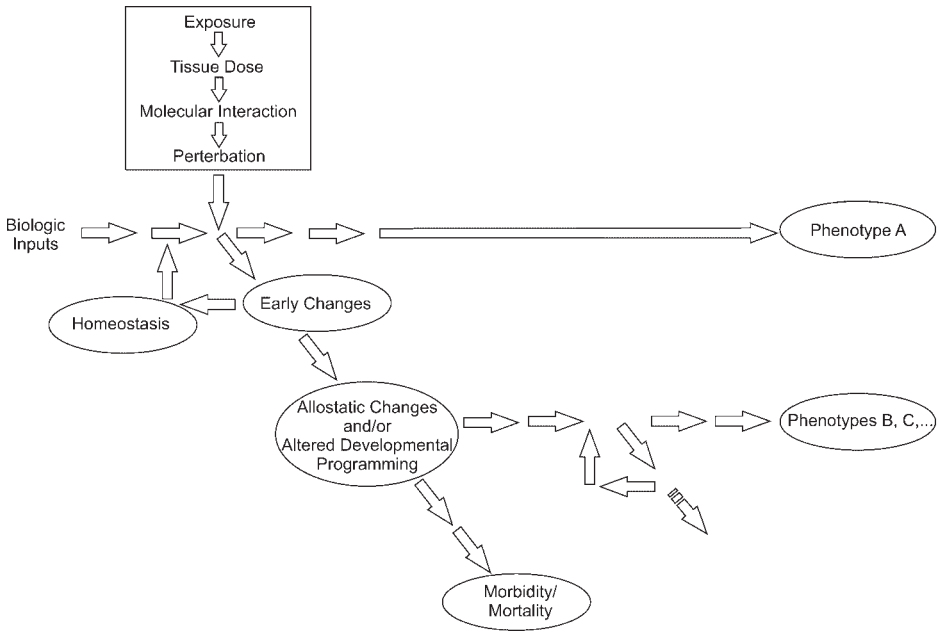

Fig. 5. Expanded vision of accommodation and recovery for chemicals that impact endocrine systems. Homeostasis = maintenance of relative constancy through normal feedback mechanisms; allostatic change = change to one or more components of the system itself as the organism strives to deal with uncompensated stress; developmental programming = epigenetic changes that may occur in response to chemical exposure during early development resulting in the production of new phenotypes. The relative fitness of phenotypes A, B, and C depends on the environment within which the phenotype is expressed.

is the case for research conducted in support of human health risk assessment. Therefore, the primary difference between these activities relates to the identification and use of specific toxicological endpoints. For human health risk assessment, demonstrated toxic effects of any kind are generally viewed as unacceptable. As such, endpoints used to support this activity are often more sensitive than traditional apical endpoints. For ecological risk assessment, acceptable endpoints must be relevant to the maintenance of populations and ecosystems. This has led to a reliance on endpoints such as mortality, reproduction, and development [48]. Given these differences, human health risk assessors would be unlikely to view the emergence of an altered phenotype as an acceptable outcome. Therefore, the simpler structure represented by Figure 1 may suffice for the purpose of assessing chemical risks to humans. How altered phenotypes might be viewed within the context of an ecological risk assessment is unclear. We are not aware of any risk assessments that have directly addressed this issue. A critical question in this case would be whether these changes have clear impacts on the standard set of assessment endpoints (mortality, reproduction, and development).

Relating exposure to effect

Exposure patterns for anthropogenic chemicals in terrestrial and aquatic ecosystems often possess a significant degree of temporal and spatial variability. This variability is a function of many factors, including chemical production and use patterns, local climate, watershed hydrology, and soil characteristics, as well as intrinsic physicochemical properties (e.g., photolysis, volatilization, solubility, and potential for microbial degradation) that, in combination with local conditions, influence the degree of chemical transport and persistence.

The normal functioning and dynamics of endocrine systems in ecologically relevant taxa is likely to exhibit significant temporal, intraspecies, and interspecies variability, depending on local environmental and ecological conditions. Chemical exposure frequency, magnitude, duration, and timing may determine whether these systems can accommodate or recover from chemical exposure, and whether these responses result in a selection pressure that results in sustained change at the population level. A better understanding of temporal and spatial variability of chemical exposure relative to the dynamics of vertebrate endocrine systems is therefore needed to accurately predict the potential for short- or long-term adverse effects.

Points of regulation and potential disruption

An endocrine axis may possess numerous points of regulation, including tissues within the central nervous system that control the release of neurohormones, the pituitary gland, other hormone-producing glands, and tissues responsible for clearance of both tropic and peripherally derived hormones. Biochemical pathways operating within these tissues provide, in turn, a large number of potential molecular targets for disruption of the axis. For a comprehensive listing of known targets for chemicals that act on endocrine axes we refer the reader to Diamanti-Kandarakis et al. [49].

From a risk assessment perspective, an important question is whether the number of toxicologically important sites for disruption of an endocrine axis (each of which would result in a unique adverse outcome pathway) is large or small. All three endocrine axes described in this report operate through homologously structured nuclear receptors that recognize and bind the appropriate hormone. Receptors for both estrogen and thyroid hormone have been shown to bind a substantial number of compounds, and research is underway to predict this binding from a quantitative analysis of chemical structure [50,51]. Chemicals may interact with other molecular components of both axes, however. Examples from the environmental toxicology literature include perchlorate inhibition of iodide uptake by the thyroid gland (attributable to competitive inhibition of the sodium–iodide symporter [25]); and inhibition of aromatase (CYP19; an enzyme responsible for synthesis of 17β-estradiol from its androgen precursor testosterone) by the model chemical fadrozole and the pesticide prochloraz [30,52–54]. Whether these nonreceptor mechanisms of action are relevant for a large number of compounds or constitute a small but important response category remains to be determined. The answer to this question has obvious relevance, however, for development of high-throughput testing programs (e.g., what mechanisms does one evaluate?) as well as efforts to build mechanistic models of the major endocrine axes (see later sections).

Compared with the HPG and HPT axes, the HPA axis has received little attention with respect to potential effects of endocrine-disrupting compounds [55]. The HPA axis depends on the activity of CYP enzymes for corticosteroid biosynthesis. Therefore, like the HPG axis, the HPA axis is a potential target for compounds that disrupt steroid biosynthesis. In addition to its central role in coordinating organism responses to stress, the HPA axis strongly impacts animal development, and altered HPA function can lead to preterm birth and intrauterine growth retardation, as well as changes in developmental programming that lead to long-term health consequences (see previous discussion). Indeed, of the three endocrine axes described here, the HPA axis provides some of the best-known examples of physiological accommodation to nonchemical stressors. Presently, the potential for chemical effects on the HPA axis represents an understudied field of investigation.

Fitness costs associated with accommodation and recovery

The evolution of resistance to toxic chemicals has been investigated in a variety of microorganisms, invertebrates, plants, and vertebrate pest species. This work suggests that initial emergence of a resistance trait is generally associated with negative fitness costs for the resistant organisms (e.g., decreased growth or reproduction [56,57]). Termination of the exposure at this time can be expected, therefore, to result in disappearance of the trait from the population. If the exposure is continued for an extended period, however, resistant populations may exhibit compensatory evolution, and initial fitness costs may be reduced or eliminated altogether [58,59]. Under these circumstances, chemical resistance may become fixed within a population.

As indicated previously, killifish that inhabit highly contaminated sites may develop resistance to chemicals present at those locations [3]. Recent work suggests that this resistance is not associated with a negative fitness cost [60]. The mechanistic basis for chemical resistance in killifish remains unclear. However, Meyer et al. [61] found that laboratory-reared, first-generation offspring of feral killifish from a highly contaminated site were resistant to PAH-mediated CYP1A induction, whereas CYP1A induction in second-generation offspring was similar to that of second-generation offspring of killifish collected from clean reference sites. Thus, although chemical resistance in killifish

may develop over a long period, the underlying basis may be independent of changes in the genome.

A number of endocrine-active compounds have been shown to cause nonlethal effects that could be expected to reduce the fitness of individual organisms. Examples include reduced production of fertile eggs in female fish exposed to estrogen receptor agonists such as EE2 [62] and decreased oocyte production in fish exposed to fadrozole [52] or 17β-trenbolone (an androgen receptor agonist [63]). In each of these studies, the dose exceeded the animal's ability to compensate for effects on the target axis. Less well known is whether successful accommodation to or recovery from a chemical exposure results in fitness costs for the exposed organisms. Studies of male zebrafish exposed to EE2 suggested that restoration of normal gonadal histology after withdrawal of the compound was accompanied by impacts on male fertility (see earlier discussion). This is not surprising, given that the impacted organ is the site of sperm formation. A more challenging question is whether recovery from chemical effects on nongonadal tissues can result in negative fitness costs, or alternatively whether fitness costs are associated with long-term accommodation that is not accompanied by any discernible impact on tissues and organs. These questions are largely unexplored, and efforts to address them would require the use of long-term, low-level exposure protocols as well as sensitive measures of reproductive success.

Computational modeling of endocrine systems

Mathematical modeling is a key component of strategies proposed by the NRC for toxicity testing in the 21st century [5]. The authors of the report discussed how computational models could be used to improve risk assessments for chemicals and identified three complimentary modeling approaches: physiologically based toxicokinetic (PBTK) modeling; toxicity pathway modeling; and biologically based dose–response (BBDR) modeling. Physiologically based toxicokinetic models translate measured or predicted exposure information into dose metrics that can be related to in vitro and in vivo testing data. Because they are based on a mechanistic description of processes responsible for chemical uptake, distribution, and elimination, PBTK models provide a rational basis for extrapolating dosimetry information from tested to untested species and among different dosing scenarios. A review of PBTK modeling in fish was provided by Kleinow et al. [64].

As defined by the NRC, toxicity pathway models describe molecular events at the subcellular level that result in the expression of cellular toxicity. These models provide a mechanistic basis for interpreting the results of in vitro cellular assays, and their development for major mechanisms of action was viewed by the NRC as a relatively short-term goal. In contrast, BBDR models relate toxicity pathway perturbations to apical responses in intact animals. As such, BBDR models encompass the potential complexity of a complete biological system, including important susceptibility factors (e.g., presence/absence of a detoxifying enzyme) and compensatory responses operating at the tissue, organ, and organ systems levels. A toxicity pathway model can be viewed, therefore, as a module that exists within the framework of a BBDR model. The ultimate goal of this approach is to use linked PBTK/BBDR models to predict toxic impacts on humans from knowledge of in vitro chemical effects (preferably obtained from human cell cultures) and a measured or hypothesized exposure. Not surprisingly, the development of BBDR models was viewed by the NRC as a longer-term goal.

Because the needs of human health and ecological risk assessors differ, we may expect that the role of mathematical models in support of each activity also will differ. The workgroup therefore discussed how mathematical models could be used to support ecological risk assessments for compounds that impact endocrine systems. Some of the resulting conclusions overlap with statements made in the NRC report, whereas others are more pertinent to ecological risk assessment and the science that supports it.

In the near term, computational models of endocrine systems can be used to better understand the results of whole-animal chemical exposures. The major challenge of this type of modeling is to relate a specific molecular interaction to a chain of events that leads to an adverse outcome. Progress on the development of these models is likely to be iterative and may require supporting data from a variety of in vitro and in vivo studies. Because the modeled system is highly dynamic, incorporating control elements that describe this behavior as well as behaviors that occur as a response to chemical insults will be necessary.

Models developed in this manner may have relatively little value for quantitative extrapolation of toxicity test results beyond the species and lifestage for which data were collected. Nevertheless, these models could provide a basis for qualitative comparisons among species. For example, demonstrating the conservation of molecular targets and general system behavior in multiple species may be important, because this implies that chemicals impacting one species can be expected to impact others in a similar manner. Alternatively, differences among species with respect to a critical molecular target (presence/absence of the target or important structural differences) may explain observed differences in sensitivity.

As noted previously, chemical effects on endocrine systems may be highly time dependent. This time dependence includes the length of time before an exposure elicits a measurable effect as well as the time before measured effects become manifested as apical endpoints of regulatory concern, and is attributable to attributes of the systems themselves (e.g., hormone storage and the capacity for compensation) and the persistence of genetic programs initiated by hormones. Models that incorporate these time dependencies could be used to improve the design of test protocols, ensuring that tests capture the effects of interest without incurring unnecessary costs.

Finally, the temporal nature of chemical effects on endocrine systems must be viewed within the context of complex natural systems. Mechanistic models of these processes could be combined with relevant life-history information (e.g., timing of metamorphosis or reproduction) and information on the likely timing of a chemical exposure (e.g., application of a pesticide within an agroecosystem) to optimize the design of field sampling efforts.

Over time we anticipate the development of a suite of validated BBDR models for key species, including those selected as test animals to screen for chemicals with endocrine disrupting activity (see following discussion). The use of these models within a regulatory context would require in vitro test systems, quantitative structure–activity approaches, or short-term assays to identify chemicals with potential to impact specific endocrine axes (initial prioritization). This information would be used to determine which models were needed and whether additional data were required to make predictions (e.g., relating to exposure dynamics). The selected models could then be used to predict species and life-stage sensitivities and address questions related to possible accommodation and recovery (see also Villeneuve and Garcia-Reyero [6]).

As indicated previously, BBDR models for most chemical modes of action represent a longer-term goal. This may not be true, however, for endocrine systems. The need is for models that adequately represent essential features of whole-organism physiology and pathophysiology in the presence of toxicants and that are sufficiently predictive. Fortuitously, computational models possessing these attributes are fairly well developed—particularly for mammals—in the systems physiology and pharmacology literature, with perturbations represented primarily by disease processes and drugs. Several of these models have been used also to predict the toxicity of environmental toxicants. Here we summarize published models for the HPG, HPT, and HPA axes, emphasizing those that are based on a mechanistic description of the underlying biology as well as those developed to predict the effects of endocrine-active chemicals in ecologically relevant species.

Models of the HPG axis. Models of the HPG axis have been developed to predict plasma steroid hormones and vitellogenin concentrations in male fathead minnows exposed to 17α-ethinylestradiol and 17β-estradiol [65], as well as vitellogenin concentrations in male sciaenid fish exposed to PCBs and Cd [66]. A third model for fish was developed to describe normal functioning of the HPG axis in salmon [67]. A model of the HPG axis in rats was developed to simulate hormone levels in testes and blood [68]. Models of the HPG axis in humans have been developed to describe changes in luteinizing hormone and testosterone concentrations after treatment with the gonadotropin-releasing hormone agonist triptorelin, and the gonadotropin-releasing hormone receptor blocker degarelix [69], as well as the luteinizing hormone–releasing hormone antagonist cetrorelix [70].

Models of the HPT axis. A generalized feedback control system model of the HPT axis in humans was published by Eisenberg et al. [71]. This model builds on work by the same author [72] as well as earlier models by DiStefano for several species (e.g., [73]). At the core of each model is a nonlinear, six-compartment submodel that describes the distribution and elimination of T3 and T4. A similar approach was used to model thyroid hormone kinetics in fasted trout [74] and tilapia [75]. Kohn et al. [76] linked a PBTK model for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to a description of the thyroid gland to simulate the effects of 2,3,7,8- tetrachlorodibenzo-*p*-dioxin on thyroid hormones in the rat. A highly detailed model of the human thyroid gland was published by Degon et al. [77]. The model was developed to describe the effect of dietary iodide on thyroid hormone secretion, including the decrease in hormone production that occurs when dietary iodide levels are elevated. A BBDR model of the HPT axis in adult rats was presented by McLanahan et al. [78] and was used to simulate the effects of dietary iodide deficiency. This model builds on earlier PBTK models for perchlorate-induced inhibition of iodide uptake in rats and humans [79–81].

Models of the HPA axis. A feedback model of the HPA axis in humans was developed to describe the pulsatile behavior of this system as well as its delayed responsiveness to different pathological conditions [82]. A similar model was used by Bairagi et al. [83] to simulate the effects of different surgical (e.g., adrenalectomy, hypophysectomy) and pharmacological interventions (e.g., infusion of different hormones). Two additional models were developed to describe the oscillatory behavior of HPA axis hormones [84,85]. A stability analysis showed that these oscillations occur in response to an external pulse activator [85,86]. A model that incorporates pituitary glucocorticoid receptor synthesis was used to describe the observed bistability of the HPA axis [87]. This model predicted the low levels of cortisol seen in some stress-related disorders (e.g., chronic fatigue syndrome). To our knowledge, no models have been developed to describe the HPA axis in ecologically relevant species or the response of this system to environmental contaminants

RESEARCH NEEDS

Short-term research needs (5–10 years)

Research is needed to improve current understanding of endocrine axes in selected ecological animal models (e.g., sensitive life stages or species used for conventional chemical hazard testing), as well as effects of endocrine-active chemicals on these axes. Initially, the baseline dynamics of endocrine systems under unstressed conditions must be well understood to develop and calibrate computational models of the HPG, HPT, and HPA axes. To maximize their utility for ecotoxicology, these models should be structured to allow for prediction of chemical effects from knowledge of specific molecular interactions (e.g., chemical binding to a receptor). Once baseline model outputs for selected species and endocrine axes have been validated, dose–response relationships for apical and nonapical endpoints after exposure to endocrine-active compounds must be defined across multiple exposure and recovery durations to document the spectrum of possible effects (homeostasis/allostasis → allostatic change → morbidity; Fig. 3). These studies would have as complimentary goals the need to determine whether these effects occur across a relatively narrow or wide range of dose levels (thereby defining the risk assessment challenge); evaluate how dose–response relationships change with exposure or recovery duration; and incorporate chemical-specific dose–response relationships into computational models of endocrine axes for selected species. Once these species-, axes-, and chemical-specific relationships have been integrated within a modeling framework, the models may be used to predict individual-level impacts of endocrine-disrupting compounds for different exposure concentrations and durations.

Long-term research needs (10+ years)

Prediction of population-level impacts will require information from multigenerational studies that addresses the potential fitness costs of accommodation and recovery. Studies with mammals have shown that prolonged allostatic change within the HPA axis (as well as other physiological systems) can result in production of new phenotypes, including several disease states. Whether these or other manifestations of allostatic load can be elicited in ecologically relevant species and whether such changes have associated fitness costs are unknown. Other studies with mammals suggest that chemical impacts on the endocrine system of very young animals can cause developmental programming that results in adverse health effects later in life. Nonchemical stressors have been shown to impact developmental programming in several ecologically important taxa, and epigenetic mechanisms that underlie these changes may play a key role in the response of individuals and populations to changing environmental conditions [88]. The potential for adverse effects on these species because of chemical impacts on developmental programming is largely unexplored. These impacts could include both inappropriate developmental responses and prevention of appropriate changes in response to changing environmental conditions.

A significant challenge will be to use output from computational models of endocrine axes to predict possible impacts on fitness and, by extension, populations. This will require a linkage of models described previously under *Short-term research needs* with additional models that relate apical endpoints to organism fitness. Unlike mechanistic models for individual endocrine axes, models that predict organism fitness from apical endpoints are likely to be based, at least initially, on empirical correlations.

An additional challenge will be to characterize and model interactions among the major endocrine axes. For example, corticosteroids produced by tadpoles during periods of environmental stress are thought to synergize with thyroid hormones to accelerate metamorphosis [19]. The ecotoxicological significance of this type of interaction is essentially unknown.

Need for both standardized and nonconventional testing

The use of identical test species, reference chemicals, experimental designs, and endpoints—such as studies conducted according to hazard testing guidelines for chemical registrations—will be critical for characterizing short- and long-term responses after time-varying chemical exposures. Data from well-defined experimental systems also are required to facilitate the calibration and validation of computational models. Obtaining much of this information may be possible by appropriate modification of assays under development as part of the U.S. Environmental Protection Agency's (U.S. EPA) Endocrine Disruptor Screening Program (http://www.epa.gov/endo/). In response to 1996 congressional mandates (Bill number PL 104-170 Food Quality Protection Act of 1996; 1996 Amendments to the Safe Water Drinking Act, PL 104-182), the U.S. EPA established the Endocrine Disruptor Screening Program to identify chemicals with potential endocrine effects. Under guidance from the Endocrine Disruptor Screening and Testing Advisory Committee, the U.S. EPA designed and implemented a two-tiered testing strategy. Short-term tier 1 in vitro and in vivo assays were selected to identify chemicals that can disrupt the estrogen, androgen, or thyroid hormone systems through classical endocrine-related modes of action (e.g., estrogen receptor activation), whereas long-term tier 2 assays were selected to assess the potential for effects on reproduction and development over one or two generations.

Although validation and peer review are nearly complete for the tier 1 screening battery, tier 2 assays are still in development. Proposed tier 2 assays include the amphibian development/reproduction assay; avian two-generation assay; fish life-cycle assay; and aquatic invertebrate life-cycle assay. As currently envisioned, these assays include assessments of morphological-, biochemical-, and molecular-based endpoints that are linked to apical endpoints such as development, growth, and reproductive fitness. These endpoints would be evaluated under constant concentrations and exposure durations throughout multiple life stages (including sensitive developmental windows). In principle, modifying these assays to address uncertainties pertaining to accommodation and recovery might be possible. Alternatively, assays developed by the U.S. EPA could be used as a basis for follow-up work that employs more sophisticated exposure protocols. An important advantage of this approach is that it would build on other data collected using the same test system, increasing the environmental relevance of these observations.

Finally, laboratory bioassays are generally conducted under ideal conditions that control for experimental variables (e.g., temperature, light) and chemical exposure duration. To better define the limits of accommodation and recovery, and to provide a stronger basis for relating test results to environmentally relevant conditions, using nonconventional approaches to evaluate effects of endocrine-disrupting compounds will be necessary. For example, testing the effects of chemicals on the HPT axis of fish under conditions under which dietary iodide is restricted may be important. Similarly, limitations on the amount of food provided to test animals may help determine whether endocrine-active chemicals have subtle effects on growth and fitness.

SUMMARY AND CONCLUSIONS

Toxicologists have long recognized the ability of animals to accommodate and recover from chemical exposures. From a governmental regulatory perspective, these responses complicate efforts to establish acceptable levels of chemical exposure and generate reliable risk assessment decisions. This report presents the findings of a workgroup that was organized as part of a SETAC Pellston Workshop [6]. The workgroup discussed accommodation and recovery within the context of chemical effects on vertebrate endocrine systems. This effort resulted in an expansion of a vision for accommodation and recovery that was provided in a recent NRC report [5]. The expanded vision recognizes the possible emergence of new phenotypes that arise from developmental programming or allostatic changes that occur as a consequence of repeated cycles of accommodation.

Research to define the limits and biological costs of accommodation and recovery using standardized test designs is needed. Ecological risk assessors generally focus on the potential for chemicals to adversely affect animal populations. In most cases, the fitness costs of successful accommodation and recovery in individual organisms are unknown. An evaluation of these fitness costs will require information from long-term exposures, including multigenerational studies. Studies of ecologically relevant species have shown that nonchemical stressors acting on the HPA axis during early development may cause changes in developmental programming. Additional work with mammals has shown that exposure to endocrine-active compounds during early development may result in adverse health impacts that are not realized until adulthood. Research is needed to determine whether chemical effects on developmental programming represent an important mode of action in ecotoxicology.

The workgroup strongly endorsed the development and validation of mechanistically based computational models of HPT, HPG, or HPA axes in ecologically relevant species to better predict accommodation and recovery of endocrine systems, and as a basis for understanding the dynamics of these systems in relation to complex environmental exposures. In the short-term (5–10 years), these models can be used to design experiments, interpret study findings, and predict the spectrum of possible individual-level impacts of endocrine-disrupting compounds for different exposure concentrations and durations. Over the long-term (10+ years), a set of validated models could be used to extrapolate limited in vitro and in vivo testing data to a broader range of untested chemicals, species, and exposure scenarios. With appropriate modification, tier 2 assays developed in support of the U.S. EPA's Endocrine Disruptor Screening Program could be used to assess the potential for accommodation and recovery, and to inform the development of mechanistically based models.

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REFERENCES

1. Aranda A, Pascual A. 2001. Nuclear hormone receptors and gene expression. *Physiol Rev* 81:1269–1304.
2. Nordstrand LM, Ringvoll J, Larsen E, Klungland A. 2007. Genome instability and DNA damage accumulation in gene-targeted mice. *Neuroscience* 145:1309–1317.
3. Wirgin I, Waldman JR. 2004. Resistance to contaminants in North American fish populations. *Mutat Res* 552:73–100.
4. Groenendijk D, Lucker SM, Plans M, Kraak MH, Admiraal W. 2002. Dynamics of metal adaptation in riverine chironomids. *Environ Pollut* 117:101–109.
5. National Research Council Committee on Toxicity Testing and Assessment of Environmental Agents. 2007. *Toxicity Testing in the 21st Century: A Vision and a Strategy*. National Academies Press, Washington, DC.
6. Villeneuve DL, Garcia-Reyero N. 2011. A vision and strategy for predictive ecotoxicology testing in the 21st century. *Environ Toxicol Chem* 30:1–8 (this issue).
7. Sterling P, Ayer J. 1988. Allostasis: A new paradigm to explain arousal pathology. In Fisher S, Reason J, eds *Handbook of Life Stress, Cognition and Health*. John Wiley & Sons, New York, NY, USA, pp 629–649.
8. McEwen BS, Seeman T. 1999. Protective and damaging effects of mediators of stress. Elaborating and testing the concepts of allostasis and allostatic load. *Ann NY Acad Sci* 896:30–47.
9. Colborn T, vom Saal FS, Soto AM. 1993. Developmental effects of endocrine: Disrupting chemicals in wildlife and humans. *Environ Health Perspect* 101:378–384.
10. Tyler CR, Jobling S, Sumpter JP. 1998. Endocrine disruption in wildlife: A critical review of the evidence. *Crit Rev Toxicol* 28:319–361.
11. Tarlochan SD, Richard KJ. 1999. Non-steroidal ecdysone agonists: New tools for IPM and insect resistance management. *Pestic Sci* 55:357–359.
12. Norris D. 2006. *Vertebrate Endocrinology*, 4th ed. Academic, New York, NY, USA.
13. Kronenberg HM, Melmed S, Polonsky KS, Larsen PR. 2007. *Williams Textbook of Endocrinology*, 11th ed. Saunders, Philadelphia, PA, USA.
14. Schulkin J. 2004. *Allostasis, Homeostasis, and the Costs of Physiological Adaptation*. Cambridge University Press, Cambridge, UK.
15. Meaney MJ, Szyf M, Seckl JR. 2007. Epigenetic mechanisms of perinatal programming of hypothalamic-pituitary-adrenal function and health. *Trends Mol Med* 13:269–277.
16. Dolinoy DC, Jirtle RL. 2008. Environmental epigenomics in human health and disease. *Environ Mol Mutagen* 49:4–8.
17. Romero LM. 2004. Physiological stress in ecology: Lessons from biomedical research. *Trends Ecol Evol* 19:249–255.
18. Denver RJ, Pavgi S, Shi YB. 1997. Thyroid hormone-dependent gene expression program for *Xenopus* neural development. *J Biol Chem* 272:8179–8188.
19. Denver RJ. 2009. Stress hormones mediate environment-genotype interactions during amphibian development. *Gen Comp Endocrinol* 164:20–31.
20. Ankley GT, Bennett R, Erickson RJ, Hoff DJ, Hornung MW, Johnson RD, Mount DR, Nichols JW, Russom CL, Schmieder PK, Serrano JA, Tietge JE, Villeneuve DL. 2010. Adverse outcome pathways: A conceptual framework to support ecotoxicology research and risk assessment. *Environ Toxicol Chem* 29:730–741.
21. Crofton K. 2009. Developmental neurotoxicity and thyroid hormone disruptors: Past, present and future. *Neurotoxicol Teratol* 31:237–238.
22. Brucker-Davis F. 1998. Effects of environmental synthetic chemicals on thyroid function. *Thyroid* 8:827–856.
23. Degitz SJ, Holcombe GW, Flynn KM, Kosian PA, Korte JJ, Tietge JE. 2005. Progress towards development of an amphibian-based thyroid screening assay using *Xenopus laevis*. Organismal and thyroidal responses to the model compounds 6-propylthiouracil, methimazole, and thyroxine. *Toxicol Sci* 87:353–364.
24. Opitz R, Hartmann S, Blank T, Braunbeck T, Lutz I, Kloas W. 2006. Evaluation of histological and molecular endpoints for enhanced detection of thyroid system disruption in *Xenopus laevis* tadpoles. *Toxicol Sci* 90:337–348.
25. Tietge JE, Holcombe GW, Flynn KM, Kosian PA, Korte JJ, Anderson LE, Wolf DC, Degitz SJ. 2005. Metamorphic inhibition of *Xenopus laevis* by sodium perchlorate: Effects on development and thyroid histology. *Environ Toxicol Chem* 24:926–933.
26. Opitz R, Schmidt F, Braunbeck T, Wuertz S, Kloas W. 2009. Perchlorate and ethylenethiourea induce different histological and molecular alterations in a non-mammalian vertebrate model of thyroid goitrogenesis. *Mol Cell Endocrinol* 298:101–114.
27. Tietge JE, Butterworth BC, Haselman JT, Holcombe GW, Hornung MW, Korte JJ, Kosian PA, Wolfe M, Degitz SJ., 2010. Early temporal effects of three thyroid hormone synthesis inhibitors in *Xenopus laevis*. *Aquat Toxicol* 98:44–50.
28. Buckman AH, Fisk AT, Parrott JL, Solomon KR, Brown SB. 2007. PCBs can diminish the influence of temperature on thyroid indices in rainbow trout (*Oncorhynchus mykiss*). *Aquat Toxicol* 84:366–378.
29. Li W, Zha J, Spear PA, Li Z, Yang L, Wang Z. 2009. Changes of thyroid hormone levels and related gene expression in Chinese rare minnow (*Gobiocypris rarus*) during 3-amino-1,2,4-triazole exposure and recovery. *Aquat Toxicol* 92:50–57.
30. Villeneuve DL, Mueller ND, Martinovic D, Makynen EA, Kahl MD, Jensen KM, Durhan EJ, Cavallin JE, Bencic D, Ankley GT. 2009. Direct effects, compensation, and recovery in female fathead minnows exposed to a model aromatase inhibitor. *Environ Health Perspect* 117:624–631.
31. Van den Belt K, Verheyen R, Witters H. 2003. Effects of 17alpha-ethynylestradiol in a partial life-cycle test with zebrafish (*Danio rerio*): Effects on growth, gonads and female reproductive success. *Sci Total Environ* 309:127–137.
32. Maack G, Segner H. 2004. Life-stage-dependent sensitivity of zebrafish (*Danio rerio*) to estrogen exposure. *Comp Biochem Physiol C* 139:47–55.
33. Nash JP, Kime DE, Van der Ven LT, Wester PW, Brion F, Maack G, Stahlschmidt-Allner P, Tyler CR. 2004. Long-term exposure to environmental concentrations of the pharmaceutical ethynylestradiol causes reproductive failure in fish. *Environ Health Perspect* 112:1725–1733.
34. Xu H, Yang J, Wang Y, Jiang Q, Chen H, Song H. 2008. Exposure to 17alpha-ethynylestradiol impairs reproductive functions of both male and female zebrafish (*Danio rerio*). *Aquat Toxicol* 88:1–8.
35. Larsen MG, Bilberg K, Baatrup E. 2009. Reversibility of estrogenic sex changes in zebrafish (*Danio rerio*). *Environ Toxicol Chem* 28:1783–1785.
36. Schafers C, Teigeler M, Wenzel A, Maack G, Fenske M, Segner H. 2007. Concentration- and time-dependent effects of the synthetic estrogen, 17alpha-ethinylestradiol, on reproductive capabilities of the zebrafish, *Danio rerio*. *J Toxicol Environ Health A* 70:768–779.
37. Fenske M, Maack G, Schafers C, Segner H. 2005. An environmentally relevant concentration of estrogen induces arrest of male gonad development in zebrafish, *Danio rerio*. *Environ Toxicol Chem* 24:1088–1098.
38. Jaenisch R, Bird A. 2003. Epigenetic regulation of gene expression: How the genome integrates intrinsic and environmental signals. *Nat Genet* 33 (Suppl):245–254.
39. Waterland RA, Michels KB. 2007. Epigenetic epidemiology of the developmental origins hypothesis. *Annu Rev Nutr* 27:363–388.
40. Denver RJ. 1996. Neuroendocrine control of amphibian metamorphosis. In Gilbert LI, Tata JR, Atkinson BG, eds *Metamorphosis: Postembryonic Reprogramming of Gene Expression in Amphibian and Insect Cell Lines*. Academic, San Diego, CA, USA, pp 433–464.
41. Hu F, Crespi EJ, Denver RJ. 2008. Programming neuroendocrine stress axis activity by exposure to glucocorticoids during postembryonic development of the frog, *Xenopus laevis*. *Endocrinology* 149:5470–5481.
42. Darras VM, Van der Geyten S, Cox C, Segers IB, De Groef B, Kuhn ER. 2002. Effects of dexamethasone treatment on iodothyronine deiodinase activities and on metamorphosis-related morphological changes in the axolotl (*Ambystoma mexicanum*). *Gen Comp Endocrinol* 127:157–164.
43. Hayes T, Chan R, Licht P. 1993. Interactions of temperature and steroids on larval growth, development, and metamorphosis in a toad (*Bufo boreas*). *J Exp Zool* 266:206–215.
44. Metcalfe NB, Monaghan P. 2001. Compensation for a bad start: Grow now, pay later? *Trends Ecol Evol* 16:254–260.
45. King Heiden TC, Spitsbergen J, Heideman W, Peterson RE. 2009. Persistent adverse effects on health and reproduction caused by exposure of zebrafish to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin during early development and gonad differentiation. *Toxicol Sci* 109:75–87.
46. Johannes F, Porcher E, Teixeira FK, Saliba-Colombani V, Simon M, Agier N, Bulski A, Albuisson J, Heredia F, Audigier P, Bouchez D, Dillmann C, Guerche P, Hospital F, Colot V. 2009. Assessing the impact of transgenerational epigenetic variation on complex traits. *PLoS Genetics* 5:e1000530.

47. Slatkin M. 2009. Epigenetic inheritance and the missing heritability problem. *Genetics* 182:845–850.
48. Bradbury SP, Feijtel TC, Van Leeuwen CJ. 2004. Meeting the scientific needs of ecological risk assessment in a regulatory context. *Environ Sci Technol* 38:463A–470A.
49. Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, Zoeller RT, Gore AC. 2009. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. *Endocr Rev* 30:293–342.
50. Shi L, Tong W, Fang H, Xie Q, Hong H, Perkins R, Wu J, Tu M, Blair RM, Branham WS, Waller C, Walker J, Sheehan DM. 2002. An integrated ''4-phase'' approach for setting endocrine disruption screening priorities--phase I and II predictions of estrogen receptor binding affinity. *SAR QSAR Environ Res* 13:69–88.
51. Schmieder PK, Ankley G, Mekenyan O, Walker JD, Bradbury S. 2003. Quantitative structure-activity relationship models for prediction of estrogen receptor binding affinity of structurally diverse chemicals. *Environ Toxicol Chem* 22:1844–1854.
52. Ankley GT, Kahl MD, Jensen KM, Hornung MW, Korte JJ, Makynen EA, Leino RL. 2002. Evaluation of the aromatase inhibitor fadrozole in a short-term reproduction assay with the fathead minnow (*Pimephales promelas*). *Toxicol Sci* 67:121–130.
53. Ankley GT, Bencic D, Cavallin JE, Jensen KM, Kahl MD, Makynen EA, Martinovic D, Mueller ND, Wehmas LC, Villeneuve DL. 2009. Dynamic nature of alterations in the endocrine system of fathead minnows exposed to the fungicide prochloraz. *Toxicol Sci* 112:344–353.
54. Ankley GT, Jensen KM, Durhan EJ, Makynen EA, Butterworth BC, Kahl MD, Villeneuve DL, Linnum A, Gray LE, Cardon M, Wilson VS. 2005. Effects of two fungicides with multiple modes of action on reproductive endocrine function in the fathead minnow (*Pimephales promelas*). *Toxicol Sci* 86:300–308.
55. Harvey PW, Everett DJ, Springall CJ. 2007. Adrenal toxicology: A strategy for assessment of functional toxicity to the adrenal cortex and steroidogenesis. *J Appl Toxicol* 27:103–115.
56. Hebert PD, Luiker MM. 1996. Genetic effects of contaminant exposure: Towards an assessment of impacts on animal populations. *Sci Total Environ* 191:23–58.
57. Coustau C, Chevillon C, ffrench-Constant R. 2000. Resistance to xenobiotics and parasites: Can we count the cost? *Trends Ecol Evol* 15:378–383.
58. Maisnier-Patin S, Andersson DI. 2004. Adaptation to the deleterious effects of antimicrobial drug resistance mutations by compensatory evolution. *Res Microbiol* 155:360–369.
59. Wijngaarden PJ, van den Bosch F, Jeger MJ, Hoekstra RF. 2005. Adaptation to the cost of resistance: A model of compensation, recombination, and selection in a haploid organism. *Proc Biol Sci* 272:85–89.
60. Nacci D, Huber M, Champlin D, Jayaraman S, Cohen S, Gauger E, Fong A, Gomez-Chiarri M. 2009. Evolution of tolerance to PCBs and susceptibility to a bacterial pathogen (*Vibrio harveyi*) in Atlantic killifish (*Fundulus heteroclitus*) from New Bedford (MA, USA) harbor. *Environ Pollut* 157:857–864.
61. Meyer JN, Nacci DE, Di Giulio RT. 2002. Cytochrome P4501A (CYP1A) in killifish (*Fundulus heteroclitus*): Heritability of altered expression and relationship to survival in contaminated sediments. *Toxicol Sci* 68:69–81.
62. Caldwell DJ, Mastrocco F, Hutchinson TH, Lange R, Heijerick D, Janssen C, Anderson PD, Sumpter JP. 2008. Derivation of an aquatic predicted no-effect concentration for the synthetic hormone, 17 alpha-ethinyl estradiol. *Environ Sci Technol* 42:7046–7054.
63. Ankley GT, Jensen KM, Makynen EA, Kahl MD, Korte JJ, Hornung MW, Henry TR, Denny JS, Leino RL, Wilson VS, Cardon MC, Hartig PC, Gray LE. 2003. Effects of the androgenic growth promoter 17-beta-trenbolone on fecundity and reproductive endocrinology of the fathead minnow. *Environ Toxicol Chem* 22:1350–1360.
64. Kleinow KM, Nichols JW, Hayton WL, McKim JM, Barron MG. 2008. Toxicokinetics in fishes. In Di Giulio R, Hinton D, eds *The Toxicology of Fishes*. Taylor and Francis, New York, NY, USA, pp 55–152.
65. Watanabe KH, Li Z, Kroll KJ, Villeneuve DL, Garcia-Reyero N, Orlando EF, Sepulveda MS, Collette TW, Ekman DR, Ankley GT, Denslow ND. 2009. A computational model of the hypothalamic-pituitary-gonadal axis in male fathead minnows exposed to 17{alpha}-ethinylestradiol and 17{beta}-estradiol. *Toxicol Sci* 109:180–192.
66. Murphy CA, Rose KA, Thomas P. 2005. Modeling vitellogenesis in female fish exposed to environmental stressors: Predicting the effects of endocrine disturbance due to exposure to a PCB mixture and cadmium. *Reprod Toxicol* 19:395–409.
67. Kim J, Hayton WL, Schultz IR. 2006. Modeling the brain-pituitary-gonad axis in salmon. *Mar Environ Res* 62 (Suppl):S426–432.
68. Barton HA, Andersen ME. 1998. A model for pharmacokinetics and physiological feedback among hormones of the testicular-pituitary axis in adult male rats: A framework for evaluating effects of endocrine active compounds. *Toxicol Sci* 45:174–187.
69. Tornoe CW, Agerso H, Senderovitz T, Nielsen HA, Madsen H, Karlsson MO, Jonsson EN. 2007. Population pharmacokinetic/pharmacodynamic (PK/PD) modelling of the hypothalamic-pituitary-gonadal axis following treatment with GnRH analogues. *Br J Clin Pharmacol* 63:648–664.
70. Pechstein B, Nagaraja NV, Hermann R, Romeis P, Locher M, Derendorf H. 2000. Pharmacokinetic-pharmacodynamic modeling of testosterone and luteinizing hormone suppression by cetrorelix in healthy volunteers. *J Clin Pharmacol* 40:266–274.
71. Eisenberg M, Samuels M, DiStefano JJ 3rd. 2008. Extensions, validation, and clinical applications of a feedback control system simulator of the hypothalamo-pituitary-thyroid axis. *Thyroid* 18:1071–1085.
72. Eisenberg M, Samuels M, DiStefano JJ III. 2006. L-T4 bioequivalence and hormone replacement studies via feedback control simulations. *Thyroid* 16:1279–1292.
73. DiStefano JJ III. 1985. Modeling approaches and models of the distribution and disposal of thyroid hormones. In Hennemann G, ed, *Hormone Metabolism*. Marcel Dekker, New York, NY, USA, pp 39–76.
74. Sefkow AJ, DiStefano JJ III, Himick BA, Brown SB, Eales JG. 1996. Kinetic analysis of thyroid hormone secretion and interconversion in the 5-day-fasted rainbow trout, *Oncorhynchus mykiss*. *Gen Comp Endocrinol* 101:123–138.
75. DiStefano JJ 3rd, Ron B, Nguyen TT, Weber GM, Grau EG. 1998. 3,5,3′-Triiodothyronine (T3) clearance and T3-glucuronide (T3G) appearance kinetics in plasma of freshwater-reared male tilapia, *Oreochromis mossambicus*. *Gen Comp Endocrinol* 111:123–140.
76. Kohn MC, Sewall CH, Lucier GW, Portier CJ. 1996. A mechanistic model of effects of dioxin on thyroid hormones in the rat. *Toxicol Appl Pharmacol* 136:29–48.
77. Degon M, Chipkin SR, Hollot CV, Zoeller RT, Chait Y. 2008. A computational model of the human thyroid. *Math Biosci* 212:22–53.
78. McLanahan ED, Andersen ME, Fisher JW. 2008. A biologically based dose-response model for dietary iodide and the hypothalamic-pituitary-thyroid axis in the adult rat: Evaluation of iodide deficiency. *Toxicol Sci* 102:241–253.
79. Clewell RA, Merrill EA, Yu KO, Mahle DA, Sterner TR, Mattie DR, Robinson PJ, Fisher JW, Gearhart JM. 2003. Predicting fetal perchlorate dose and inhibition of iodide kinetics during gestation: A physiologically-based pharmacokinetic analysis of perchlorate and iodide kinetics in the rat. *Toxicol Sci* 73:235–255.
80. Merrill EA, Clewell RA, Gearhart JM, Robinson PJ, Sterner TR, Yu KO, Mattie DR, Fisher JW. 2003. PBPK predictions of perchlorate distribution and its effect on thyroid uptake of radioiodide in the male rat. *Toxicol Sci* 73:256–269.
81. Merrill EA, Clewell RA, Robinson PJ, Jarabek AM, Gearhart JM, Sterner TR, Fisher JW. 2005. PBPK model for radioactive iodide and perchlorate kinetics and perchlorate-induced inhibition of iodide uptake in humans. *Toxicol Sci* 83:25–43.
82. Lenbury Y, Pornsawad P. 2005. A delay-differential equation model of the feedback-controlled hypothalamus-pituitary-adrenal axis in humans. *Math Med Biol* 22:15–33.
83. Bairagi N, Chatterjee S, Chattopadhyay J. 2008. Variability in the secretion of corticotropin-releasing hormone, adrenocorticotropic hormone and cortisol and understandability of the hypothalamic-pituitary-adrenal axis dynamics--a mathematical study based on clinical evidence. *Math Med Biol* 25:37–63.
84. Kyrylov V, Severyanova LA, Vieira A. 2005. Modeling robust oscillatory behavior of the hypothalamic-pituitary-adrenal axis. *IEEE Trans Biomed Eng* 52:1977–1983.
85. Savic D, Jelic S. 2005. A theoretical study of hypothalamo-pituitary-adrenocortical axis dynamics. *Ann NY Acad Sci* 1048:430–432.
86. Savic D, Jelic S. 2005. A mathematical model of the hypothalamo-pituitary-adrenocortical system and its stability analysis. *Chaos Solitons Fractals* 26:427–436.
87. Gupta S, Aslakson E, Gurbaxani BM, Vernon SD. 2007. Inclusion of the glucocorticoid receptor in a hypothalamic pituitary adrenal axis model reveals bistability. *Theor Biol Med Model* 4:8.
88. West-Eberhard MJ. 2003. *Developmental Plasticity and Evolution*. Oxford University Press, New York, NY, USA.
89. Andersen ME, Dennison JE, Thomas RS, Conolly RB. 2005. New directions in incidence-dose modeling. *Trends Biotechnol* 23:122–127.