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Potency matters: Thresholds govern endocrine activity

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ABSTRACT

Whether thresholds exist for endocrine active substances and for endocrine disrupting effects of exogenous chemicals has been posed as a question for regulatory policy by the European Union. This question arises from a concern that the endocrine system is too complex to allow estimations of safe levels of exposure to any chemical with potential endocrine activity, and a belief that any such chemical can augment, retard, or disrupt the normal background activity of endogenous hormones. However, vital signaling functions of the endocrine system require it to continuously discriminate the biological information conveyed by potent endogenous hormones from a more concentrated background of structurally similar, endogenous molecules with low hormonal potential. This obligatory ability to discriminate important hormonal signals from background noise can be used to define thresholds for induction of hormonal effects, without which normal physiological functions would be impossible. From such thresholds, safe levels of exposure can be estimated. This brief review highlights how the fundamental principles governing hormonal effects – affinity, efficacy, potency, and mass action – dictate the existence of thresholds and why these principles also define the potential that exogenous chemicals might have to interfere with normal endocrine functioning.

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1. Introduction

The European Commission asked DG Environment to develop a definition of and criteria for identification of endocrine disrupting chemicals (EDCs) applicable to several legislative structures, e.g., the plant protection products regulation (Reg. (EC) No 1107/2009), the biocidal products regulation (Reg. (EC) No 528/2012), and REACH (Reg. (EC) 1907/2006). Besides definitions and criteria for EDCs, the Commission intends to answer whether EDC threshold levels can be determined. Stakeholders offer different opinions on this matter and several agencies have responded to these issues. The European Food Safety Authority (EFSA) recommended clarification of issues regarding biological thresholds and the criteria for adversity versus physiological modulation and homeostatic responses (EFSA, 2013). The Swedish Chemicals Agency concluded "...that the decision on whether or not to accept a non-threshold model for EDCs has to be based on considerations of mechanism of action. Thus, the assumption of no threshold may be as valid, or questionable, for EDCs as for genotoxic carcinogens." (Keml,

2013a). The UNEP and WHO (2013) report entitled "State of Science of Endocrine Disrupting Chemicals" concluded that endocrine disruptors produce non-linear dose responses (there referring to non-monotonic dose response curves) and no threshold can be assumed. Similarly, several publications cited in these reports question the existence of thresholds and suggest that no safe dose can be defined for EDCs.

Overall, six primary considerations have been offered to refute safe threshold levels for EDCs (Keml, 2013b): (1) the complexity of the endocrine system; (2) the presence of sensitive developmental stages; (3) long intervals between the exposure event and the appearance of the adverse effect; (4) no threshold of effect for an endocrine disrupting agent added to a hormone system that is already active, where theoretically, one molecule could activate a receptor when adding to background; (5) scientific difficulties that preclude establishing safe exposure levels, especially for human and other populations, and; (6) the scientific uncertainty in predicting endocrine effects and thereby assessing risks of EDCs.

Many of these considerations can be addressed through an understanding of how the normal functioning of the endocrine system relies on fundamental principles of receptor, enzyme, and transport kinetics, upon which the fields of endocrine physiology and pharmacology are built. The fundamental principles of endocrine action dictate the existence of thresholds that determine whether and to what degree any substance – endogenous or exogenous – may affect the endocrine system. Hence, this

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present review intends to address (1) why the endocrine system could not function if thresholds did not exist; (2) how principles of endocrine pharmacology – affinity, efficacy and potency based on mass action (for reviews of receptor, enzyme and transport kinetics, see [Matthews, 1993](#); [Kenakin, 2009](#)) – dictate thresholds, and; (3) why all conceivable effects of chemicals acting through or interfering with aspects of endocrine mechanisms that rely on molecular specificity are governed by these basic rules. In short, as has been concluded for other modes of action (MOA), we assert that principles of endocrine pharmacology imply certain “...rate-limiting key events that, if not met, can lead to a threshold for the dose–response, irrespective of the MOA involved.” ([Boobis et al., 2009](#)).

We attempt here to concisely describe the fundamental principles that make the case for the existence of thresholds in endocrine action, but we specifically do not represent this work as a critical treatment of all related issues or as a comprehensive review of endocrine action. Toward this end, we have cited general textbooks in several places, for two important reasons. First, some concepts would have required intricate explanations if pieced together from the primary literature that established them, thus reducing clarity and brevity. Second, we wish to emphasize that many principles discussed here are sufficiently well established in the field of endocrine pharmacology that they have been taught in standard textbooks for many years up to the present. Finally, although we make the case that thresholds are obligate for endocrine action, we do not attempt to define thresholds for adverse effects, which may be higher than the thresholds at which normal endocrine functioning can be affected due to ADME and other adaptive and protective mechanisms within animals.

2. Elementary review of endocrine pharmacology

The endocrine system provides major physiological controls in animals with critical roles in development, maturation, and maintenance of health through long-term homeostasis. These functions are accomplished through sophisticated chemical signals mediated by substances known as hormones, which are produced in and released from specific cells and transported, often via blood, to target organs or tissues where the hormonal response is produced ([Chedrese, 2009](#)). Many different types of hormonal signals are required for the complex functioning of higher mammals and more than five hundred different effector molecules have been identified in humans ([Chedrese and Celuch, 2009](#)). Hormones within related classes are usually derived from common precursors and share similar chemical structures, e.g., steroid hormones derived from cholesterol or catecholamine hormones derived from tyrosine ([Chedrese, 2009](#)). Structural similarities extend to many common endogenous molecules, including hormone precursors and metabolites and intermediates and end-products of various biochemical pathways ([Chedrese and Celuch, 2009](#)).

Typical extracellular concentrations of functionally active hormones are in the range of 10^{-11} to 10^{-9} molar whereas those of structurally similar, non-hormone molecules (e.g., sterols, amino acids, peptides) are in the range of 10^{-5} to 10^{-3} molar ([Chedrese and Celuch, 2009](#); [Grannar, 1993](#)). Given this overwhelming presence of structurally similar molecules relative to hormones, the challenge to maintaining a functional and efficient hormone-based communication system is formidable. Normal endocrine functioning requires that target cells efficiently identify and differentiate the various hormones from other molecules that are present in the extracellular fluid at molar excesses of 10^6 - to 10^9 - times ([Chedrese and Celuch, 2009](#); [Grannar, 1993](#)). Without the ability

to clearly distinguish molecules that convey critical physiological information from structurally similar molecules in the body, the endocrine system would be unable to process specific, vital signals amidst a steady roar of biological noise.

The capacity to achieve these distinctions is based on conformational matching of hormones with receptor structures present in target tissues ([Chedrese and Celuch, 2009](#)). These matches are highly selective so that only tight structural pairings produce biological effects that convey important information ([Chedrese, 2009](#); [Chedrese and Celuch, 2009](#)). Only certain hormones (called “ligands”) fit a particular class of hormone receptors with sufficient complementarity to produce receptor-mediated effects ([Chedrese and Celuch, 2009](#)).

2.1. Affinity

Affinity is a primary molecular property enabling the endocrine system to communicate vital information to different tissues of the body and to distinguish this information from biological noise. In broad terms, affinity is the strength of the molecular interaction between a receptor and its ligand ([Chedrese and Celuch, 2009](#); [Matthews, 1993](#)), conferring a tendency for the molecules to remain associated once contact has occurred. An endogenous hormone has high affinity for its conjugate receptor such that when contact occurs, a strong molecular interaction follows. Conversely, molecules with low affinity for a hormone receptor will not associate tightly and will more readily dissociate from it.

Affinity has two important consequences for hormone action. A high-affinity ligand fits the receptor well, such that any given contact event is likely to result in a conformationally correct association. This accomplishes the first step of hormone action at the target cell, called receptor binding. Second, for a given number of molecular contact events, a high affinity ligand has a much greater tendency to remain associated with its receptor than a low affinity ligand, a property typically quantified by a dissociation constant. The affinity of a ligand for its receptor determines the fraction of available receptors that will be occupied at any particular ligand concentration ([Chedrese and Celuch, 2009](#); [Matthews, 1993](#)), usually referred to as “receptor occupancy.” Thus, the greater the affinity, the lower the concentration of the ligand required to bind and occupy receptors.

The affinities of various hormone receptor-ligand combinations can vary depending on the needs of the particular hormonal pathway. Normally, affinities are finely matched with the concentration of hormones required to produce the desired response in target cells ([Chedrese and Celuch, 2009](#)). As well, the fraction of available receptors that must be activated to produce a cellular response varies with target cell and tissue type. Overall, affinity dictates whether the ligand has the opportunity to accomplish the second task of hormone action, receptor activation ([Chedrese and Celuch, 2009](#); [Matthews, 1993](#)).

2.2. Efficacy

The degree of receptor binding and occupation achieved by low concentrations of high affinity endogenous hormone ligands could theoretically be augmented by a proportionately greater concentration of low affinity ligands, and thus, might lead to cellular responses. However, affinity is not the only determinant of how effectively a ligand activates a receptor. The ability of a bound ligand to efficiently activate a receptor and trigger a cellular response is called “efficacy.” There are several theories on the molecular nature of efficacy, including receptor occupancy theory and conformational models, with contributions from post-receptor events ([Clarke and Bond, 1998](#); [Kenakin, 2004](#)). Efficacy can range

from negative to positive values where a ligand with high (positive) efficacy is capable of eliciting the maximal cellular response.¹

2.3. Potency

Together, affinity and efficacy determine the potency of a ligand to activate specific hormone receptors and to elicit specific cellular responses in target tissues. Because the manifestation of these properties involves a variety of molecular interactions, potency and efficacy may not be tightly coupled across dose–response ranges and among different tissues and hormone receptor types (Kenakin, 2009; Simons, 2008). However, both properties are essential for hormonal activity (Kenakin, 2009), and endogenous hormones tend to be very potent because they typically possess both high affinity and high efficacy. Pharmacologically, these are referred to as potent hormone receptor agonists. Molecules with high affinity but no efficacy are receptor antagonists, i.e., they block the action of endogenous hormones because they interact with and occupy the receptor, preventing its occupation by ligands with efficacy, but themselves produce no response.

Endogenous hormones have high potency and so produce a greater cellular response for a given concentration than lower potency ligands. For example, in a yeast reporter assay, the endogenous estrogen 17 β -estradiol achieves one-third maximal activation of the native human estrogen receptor at a concentration of 10⁻¹⁰ molar and maximal activation at 10⁻⁸ molar. In contrast, testosterone produces no measurable activation of that receptor at concentrations less than 10⁻⁶ molar, and its highest achievable activation requires 10⁻⁵ molar but is only one-third maximal. Progesterone is inactive at all concentrations in this system (Chen et al., 2004). On the basis of this assay, testosterone exhibits a relative potency of about 1 \times 10⁻⁵ (one one-hundred thousandth) that of 17 β -estradiol.

A chemical with low affinity can produce a cellular response if it has efficacy and if a sufficient concentration can be achieved at the receptor site. However, at relatively low concentrations, such chemicals would lack detectable endocrine activity against the background of endogenous hormones already occupying receptors. For instance, even in the treatment of hormone-deficiency disorders, where the background concentrations of natural hormone are low, only potent molecules have been found to be effective therapeutically. Similarly, during a woman's lifetime, potency differences dictate which estrogenic hormone is dominant – 17 β -estradiol > estrone > estradiol – yet even these differences span less than two orders of magnitude (Chen et al., 2004; Kuiper et al., 1997). In contrast, putative environmental estrogens exhibit potencies three or more orders of magnitude below that of 17 β -estradiol (Borgert et al., 2003; Wu et al., 2008), indicating a low potential for estrogenic activity. Since circulating endogenous estrogen concentrations are several hundred times greater in women compared to rodent test species, an inference of human risk from low-potency chemicals based on endocrine disruptive effects observed in those species would be speculative (Witorsch, 2002).

2.3.1. Thresholds

The differences in affinity and efficacy between hormones and structurally similar endogenous molecules that do not act as hormones imply potency thresholds in the activation of cellular responses (Borgert et al., 2012). Although such biological thresholds would vary for different types of hormone receptors and with the degree of receptor activation required to induce cel-

lular responses, any detectable hormonal activity will require an appropriate concentration of ligand with sufficient potency. These sufficiency requirements amount to thresholds for activation. Target cells may have receptors for various hormones, each present in a finite number at any given time (Chedrese and Celuch, 2009). However, target cells do not respond to receptor activation on an individual basis, but read the status of receptor activation collectively. An example of this is the regulation of gene expression, where on average, at least 5 transactivators need to be activated simultaneously to induce gene expression for any gene. Many, if not most, of these transactivator activations are the result of activity in the endocrine system (Nelson and Cox, 2008). Thus, hormonal responses in target organs, tissues or groups of cells require coordinated changes in the status of receptor activation. This requirement for a coordinated change in receptor activation creates a second threshold mechanism by which the endocrine system distinguishes important signals from biological noise (Matthews, 1993).

Nonetheless, it would be fair to ask, can the thresholds of biological potency and for a coordinated change in receptor activation status in target tissues and organs could be overcome by a molar excess of molecules that have low affinity but high efficacy, particularly if the endogenous hormone concentration is augmented by continuous, long-term exposure to environmental chemicals with similar properties? Some assert that because the endocrine system is already stimulated by endogenous hormones, the threshold for activation is already exceeded and therefore, any potential hormonal activity that is introduced, no matter how slight, will increase (or decrease) this baseline activity. This is the foundational hypothesis of the endocrine disruptor theory – usually termed “additivity to background” – and asserts that because concentrations of endogenous hormones are low and fluctuate widely, small additions or subtractions of even a single molecule will result in altered hormonal responses (Hass et al., 2013). A few simple calculations, however, illustrate why one or a few molecules added to an existing level of molecules will not change receptor occupancy in any detectable way, and why the molar excess of low potency ligands would need to be substantial to alter hormonal responses.

If the endogenous hormone is present at 10 parts per quadrillion, and we assume it has a molecular weight of 100 mass units, its concentration is 1 \times 10⁻¹³ molar, or 6 \times 10¹⁰ molecules per liter. This is at the low end of the effective physiologic concentration range for even the most potent endogenous hormones. The diameter of typical eukaryotic cells ranges from 10 to 100 μ m. Choosing a value near the center of this range gives a radius of 20 μ m, and assuming the cell is roughly spherical gives a volume for a single cell of about 3 \times 10⁻¹¹ liters, which translates to about 2 molecules per cell. At 1 \times 10⁻¹³ molar, a hormone with an affinity constant of 1 \times 10⁻¹¹ molar would produce only about 1% receptor occupancy. If the endogenous hormone is present at its K_D concentration (1 \times 10⁻¹¹ molar) receptor occupancy would be at 50%. Adding an equipotent ligand at a concentration of 1 \times 10⁻¹³ molar would increase receptor occupancy to 50.25%. If we add a ligand with high efficacy but with an affinity 10³ lower than the endogenous ligand, it would require 250 times more of that molecule (2.5 \times 10⁻¹¹ molar) to increase receptor occupancy by the same amount. If instead we add a ligand with the same affinity as the endogenous hormone but with low efficacy at a concentration of 1 \times 10⁻¹³ molar, this molecule would behave as a competitive antagonist and it would decrease receptor occupancy by the endogenous hormone by 0.25%. Similarly, lower concentrations of added ligands would have proportionally smaller effects. Therefore, any added receptor ligand, endogenous or exogenous, highly potent or not, would need to approach at least the 1 \times 10⁻¹³ molar level to have any measurable or detectable influence on receptor occupancy.

¹ Efficacy is technically considered a product of a ligand's ability to stimulate a receptor, termed “intrinsic activity,” and the ability of the stimulated receptor to elicit the cellular response. The distinction is not essential for understanding hormone action and thresholds.

This molar concentration translates to approximately 2 trillion molecules in the body water compartment (about 33 liters) of an average sized woman (66 kg), and more in men, in whom body water is typically a higher percentage of the body weight. This disputes the no threshold effect level hypothesis, given that a receptor is necessary for the effect, and negates the notion that a single molecule could produce an effect, even theoretically. If this were not so, normal, small changes in the intra- or extracellular milieu – i.e., in the concentrations of hormone precursors, metabolites, metabolic intermediates, etc., many of which possess low affinity and low efficacy for hormone receptors – would be detected as hormonal signals and produce measurable tissue and organ disturbances.

An example of this phenomenon involves the demonstrated ability of essential fatty acids to stimulate proliferation (Rose and Connolly, 1989) and selectively modulate estrogenic responses (Menendez et al., 2004) in estrogen-sensitive human breast cancer cells in culture, and to bind estrogen receptors and induce certain estrogen responsive genes in other *in vitro* assays (Liu et al., 2004). Nonetheless in women administered flaxseed supplementation, a rich source of these essential fatty acids, no significant changes are seen in serum hormones or biochemical markers of bone metabolism, both of which would be expected from estrogenic action (Brooks et al., 2004). Flaxseed supplementation does not alter follicle stimulating hormone or estradiol levels or produce clinically important estrogenic effects on the vaginal epithelium or endometrium in women (Colli et al., 2012), or alter uterine responses to estradiol in rats (Sacco et al., 2012). Insufficient potency appears to underlie the inability of even high levels of essential fatty acids to exhibit hormonal effects, despite activity *in vitro*. Another recently published example of this principle shows that metabolites of dehydroepiandrosterone (DHEA) lack sufficient potency via androgen or estrogen receptors to account for their biological activities, even though their potencies are within roughly three orders of magnitude of the principal endogenous hormones (Shaak et al., 2013). Although neither example precludes all possible modes of endocrine action, they clearly illustrate the difficulty of reconciling the additivity to background hypothesis with the ability of hormones to convey meaningful biological information amidst the high background of endogenous biological noise due to structurally related endogenous molecules.

Based on the above considerations, in order for an exogenous chemical substance to be able to alter the normal physiological functioning of the endogenous endocrine system, it is necessary for that chemical to achieve a sufficient activity level. This activity level will depend upon the ability of the chemical substance to interact with and modify the activity of one or more components of the endogenous endocrine system, its affinity for such interactions, and its concentration. To be sure, the existence of thresholds for endocrine activity is demonstrable from theory based on established principles of hormone action, as we have argued. The quantitative magnitude of a particular threshold is both calculable from theory, as our earlier example indicates, and empirically estimable. The minimum level of endocrine activity capable of altering physiological functioning can be used to quantify a biological potency threshold, the range of which is estimable from empirical measurements relevant to any specific endocrine activity. Recognizing that no biological measurement is without technical limitations and some uncertainty, conservative thresholds could be estimated based on the life stage or condition at which the activity level of the primary endogenous ligand is lowest. Defining endocrine thresholds in this manner identifies the types of endpoints useful for interpreting biologically meaningful effects, i.e., those that allow measurement of relative potency for a specific hormonal effect. We have not attempted to define thresholds for adverse effects, which may be higher than the thresholds at which normal

endocrine functioning may be affected, i.e., biological potency thresholds, due to ADME and other adaptive and protective mechanisms within animals.

2.4. Signal amplification, regulation of receptor number and sensitivity, cross-talk, and feedback

Admittedly, an adequate description of endocrine mechanisms and responses is more complex than the recognition of thresholds and laws of mass action (Falkenstein et al., 2000; Björnström and Sjöberg, 2005). Not only are hormonal signals filtered from background biological noise by potency differences, but hormonal signals are amplified, receptor numbers are up- and down-regulated, there is cross-talk between different hormonal receptors, and hormones themselves are controlled by negative feedback loops (Chedrese, 2009). All of these processes are governed by the kinetic principles explained above, and may be important in further differentiating hormonal signals from endogenous and exogenous biological noise. Hormonal signals are enhanced by modifying factors within cells, a feature that allows the endocrine system to efficiently convey nuanced biological information through a single receptor-ligand system (Simons, 2008; Grone-meyer et al., 2004). The variety and complexity of hormone signal enhancement is beyond the scope of this simple review, but its significance cannot be underestimated; signal modification further differentiates biological information from background noise, making the endocrine system even more resilient to spurious interruption, not more sensitive to it. To provide one brief example, the influence of modulatory factors, including coactivators, co-repressors, and other transcriptional modifiers may influence the shape of the dose–response curve for gene expression and may underlie apparent differences in agonist EC₅₀ values for inducing different genes via a single hormone receptor. Interestingly, the same factors that decrease the EC₅₀ values for agonists usually increase the amount of partial agonist activity for antagonists, and this inverse relationship suggests that the two behaviors are tightly coupled (Simons, 2006). Thus, the dynamic sensitivity of hormone receptors to ligand activation appears to be coordinated in such a way that potency differentials are maintained, and also therefore, protection against spurious perturbation. The ability of endocrine signaling to convey these distinctions is critical to survival.

3. Other arguments against thresholds

The sensitivity of developmental life stages to endocrine-mediated perturbations is one of the arguments used most often as proof against endocrine thresholds. While true that hormonal activity is critical and even vital during development, one must ask whether an increased sensitivity to the severity of a perturbation equates to a lower threshold for that perturbation. These would seem to be distinctly different phenomena that should be distinguished when considering thresholds for endocrine disrupting effects. Similarly, while there is little disagreement that thresholds for endocrine-mediated adverse effects will vary depending on many factors, there is little evidence suggesting that the fundamental rules governing endocrine function cease to apply or that endocrine thresholds disappear altogether during certain periods of life. Indeed, thresholds for reproductive toxicity are the norm (Piersma et al., 2011). Moreover, it has long been known that, although oral contraceptives are embryo lethal at one hundred times the human contraceptive dose, fetuses that survive the exposure are not adversely affected (Prahallada and Hendrickx, 1983).

Indeed, endocrine pharmacotherapy could not be as effective as it is, regardless of whether natural or synthetic remedies are used, if effective and safe doses could not be predicted. The rare

occurrence of adverse effects that become evident only after extensive post-marketing surveillance would contravene this maxim only if those adverse events were produced by the primary hormonal mechanism targeted by the medication. In contrast, whereas toxic effects of drugs are dose-related, mechanistically predictable exaggerations of the desired therapeutic effect, untoward side effects and rare adverse drug reactions occur by some other mechanism and may or may not be dose-related (Edwards and Aronson, 2000).

Finally, citing the example of hormone-dependent cancers of the breast and prostate, the argument is often advanced that since adverse effects already occur at endogenous hormone levels, any change, no matter how small, portends additional disease. This argument depends on the logic that because growth and spread of these cancers depends on hormonal stimulation, endogenous hormones are the determinative factor in causing the cancer. However, that logic runs counter to most common rules of causal argumentation, which require a counterfactual demonstration, and contravenes current theories of cancer progression.

Current theories regarding the role of hormones in carcinogenesis posit that cellular abnormalities in hormone-responsive tissues, caused irrespective of hormonal involvement, produce cells whose response to hormones becomes increasingly aberrant, and eventually, neoplastic (Li et al., 1993). For example, the cancer stem cell theory posits that malignant breast stem cells, present in early development before estrogen receptors are expressed, play a key role in breast cancer development (Eden, 2010). These theories explain several observations, including why many individuals with similar or greater hormonal exposure fail to develop cancer. The observed correlation between lifetime estrogen exposure and breast cancer is logical since aberrant cells dependent on estrogen would be expected to grow more rapidly in the presence of more estrogen, or to out-compete normal cells whose replication number is limited by estrogen exposure.

4. Conclusion

The manifestation of a detectable hormonal response at the tissue and physiologic level in humans or animals depends on whether: (a) a sufficient number of specific cellular receptors are occupied by ligand molecules of sufficient specificity and potency to induce individual cells to respond to a given hormonal signal and (b) a sufficient number of cells respond to a given hormonal solicitation, enough to manifest a detectable physiologic effect at the tissue or organism level. These fundamental principles are derived directly from established knowledge about hormonal mechanisms. Normal functioning of the endocrine system thus requires precise discernment of ligand potency and amount to enable transmission of vital signals amidst an endogenous background of spurious molecular interactions. This ability to discern defines the threshold. Potency differences, laws of mass action, and the basic design and physiological functions of the endocrine system require and ensure the presence of thresholds.

Without thresholds, there would be chaos in cellular and tissue responses under normal physiological conditions, even absent exogenous EDCs, and there could be no regulated progression of signals and functions compatible with reproduction, development, behavior, repair, immunity, and life itself. It thus seems intuitive that if chemicals are to have a chance to disrupt natural endocrine signals, their doses/concentrations and potencies ought to be similar to or stronger than the natural hormones (Dietrich, 2010; Golden et al., 1998; Marty et al., 2011). This strength of potency and amount defines a minimum requirement for influencing endocrine activity, which implies that defining either an endocrine hazard or a potential therapeutic effect requires an evaluation of potency and

physiologically achievable concentrations. These principles have successfully guided endocrine pharmacology (Cleve et al., 2012), wherein it is recognized that natural hormones and their specific modifiers are already present at concentrations that occupy the available cellular receptors and are well controlled to support normal physiological functioning. A reasoned assessment of the mechanisms of hormone signaling and processing shows that safe levels of exposure can be set for endocrine active substances based on biological and pharmaceutical principles, the empirical data on the doses at which adverse effects can be observed, and an appropriate degree of conservatism (Borgert et al., 2012; Caldwell et al., 2012).

Conflict of interest

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