Predicting Health Effects of Potential Thyroid Hormone Disruptors

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1 Introduction

Clinical and subclinical hypothyroidism diseases in women are prevalent in 4.3% of pregnancies (Silva et al. 2018). There is a wealth of examples for xenobiotic induced hypothyroidism reported for pregnant laboratory animal studies (Miller et al. 2009). Epidemiological studies have reported associations between serum biomarkers of thyroid function and exposure to chemicals (Sauer et al. 2020). Maternal thyroid hormones are critical during the first trimester when the fetus relies on placental transfer of maternal thyroxine (T4). Thyroid hormones are crucial for proper development of the fetus. Decreases in maternal serum T4 levels have been associated with reduced IQ scores in children (Miller et al. 2009).

ScitoVation has recently developed an approach for addressing whether observed thyroid hormone disruption in animal studies is relevant to humans. The approach offers a cost-efficient in both time and resources to address the concerns of a chemical on reproductive development due to thyroid hormone disruption.

An outstanding question related to the biology of the hypothalamic-pituitary-thyroid (HPT) axis and its potential disruption by chemical exposures is the extent to which experiments based on animal pregnancy models reflect hazard in pregnant humans (Figure 1). Broadly, differences in molecular mechanisms and physiology between humans and rodents have been elucidated for a variety of chemical modes of action. The degree of enzyme expression in the liver, particularly related to phase II conjugation, varies between species. Furthermore, phase II enzymes can be induced by drugs and other compounds, modulating the metabolism of hormones—including the circulating thyroid hormone T4—to different degrees. Whether this alteration of serum T4 levels results in potential health effects depends on the degree of serum thyroid hormone deficiency experienced by the fetus during development. A biologically based dose response (BBDR) model for the HPT axis in the pregnant woman will provide estimates of maternal thyroid hormone deficiency resulting from exposure to chemicals. By coupling measurements of enzyme induction performed in human and rodent hepatocyte systems with BBDR models, we can better understand the relationships between HPT effects

observed in rodents and potential health effects in humans. To this end, in vitro experiments can be used to fill data gaps and provide insight into mode of action and species relevance.

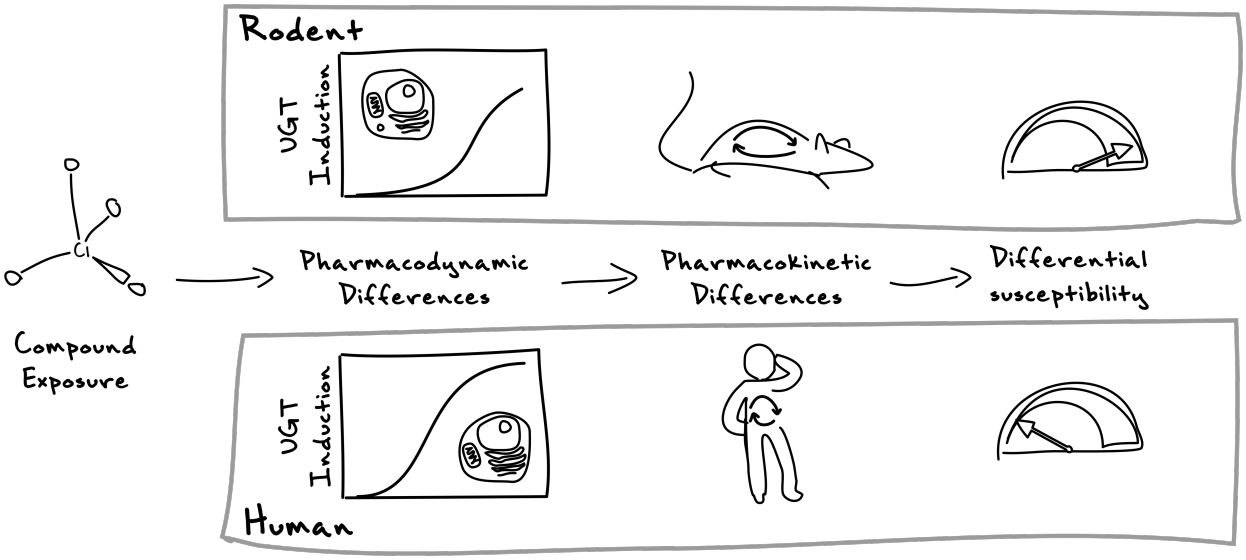

Figure 1. Differences between rodents and humans can drive differential susceptibility to HPT disrupting compounds. Outcomes from in-life testing in some cases will not translate to humans.

The three-part framework presented includes a rigorous characterization of possible differences in pharmacodynamics and pharmacokinetics for rodents and humans:

1. A pharmacokinetic model capturing human and rodent physiology for the compound of interest to determine the concentration in the liver for relevant exposure scenarios.
2. In vitro exposures of human and rat hepatocytes in concentration response to the compound of interest. we measure the expression (via PCR) and activity (via ELISA) of UGT isoforms known to play a role in metabolism of circulating thyroid hormones. These experiments assess the ability of the compound of interest under physiologically relevant concentrations to impact thyroid levels in the two species.
3. Changes in UGT expression serve as inputs for a physiologically based pharmacokinetic model that captures the dynamics of circulating thyroid hormone levels, the clinical indicator of hypothyroxinemia.

This approach allows quantitative assessment of (a) whether a compound has the potential to cause hypothyroxinemia by inducing UGT enzymes, (b) whether there are species differences in the potential impact of the compound on the HPT axis (and whether those differences result from pharmacodynamic or pharmacokinetic effects), and (c) the external dose of a compound of interest that would cause a clinically relevant decrease in thyroid hormone levels.

2 Background

To evaluate potential impact of individual chemicals or drugs on the human HPT axis, 'whole-body' mathematical representations have been used in clinical (Dietrich et al. 2016) and environmental (Lumen et al. 2013; Fisher et al. 2016) chemical exposure settings. These mathematical models vary in complexity but are usually composed of pharmacokinetic descriptions for drugs or chemicals, such as physiologically based pharmacokinetic (PBPK) models that describe absorption, distribution, metabolism, and elimination (ADME) of compounds. The PBPK models are then linked with a mathematical description of the HPT axis. The mathematical description of the HPT axis is like a pharmacokinetic description of a chemical in that rate equations may describe the endogenous production and elimination of thyroid hormones (thyroid gland),

including the signaling molecule thyroid stimulating hormone (TSH, pituitary gland) and thyroid releasing hormone (TRH) in the hypothalamus gland. The combined mathematical descriptions of the HPT axis and a chemical is referred to as a biologically based dose response (BBDR) model for the HPT axis. The interaction of a chemical with the HPT axis is described quantitatively, if known. This is an approach that has been recently used by the United States Environmental Protection Agency for the HPT axis in the pregnant woman to characterize the fetal health risks associated with maternal hypothyroxinemia of the environmental contaminant, perchlorate (https://www.epa.gov/sdwa/perchlorate-drinking-water).

3 ScitoVation Solution

This approach to understanding species differences in the effects of a target compound on the HPT axis via perturbation of Phase II metabolism combines in vitro bioactivity experiments with BBDR modeling. The complexity of the feedback in the HPT system necessitates consideration of both pharmacodynamic (e.g., the potential ability of compounds to alter glucuronosyltransferase (UGT) expression) and pharmacokinetic (e.g., the impact of any altered UGT expression on circulating hormone levels) facets (Figure 2). Pharmacodynamic assessment uses hepatocytes to measure changes in UGT expression and activity in response to compound exposure. Pharmacokinetic assessment integrates these changes in activity into a BBDR model of the HPT axis to determine the impact on circulating T4 levels and thyroid hormone receptor activity.

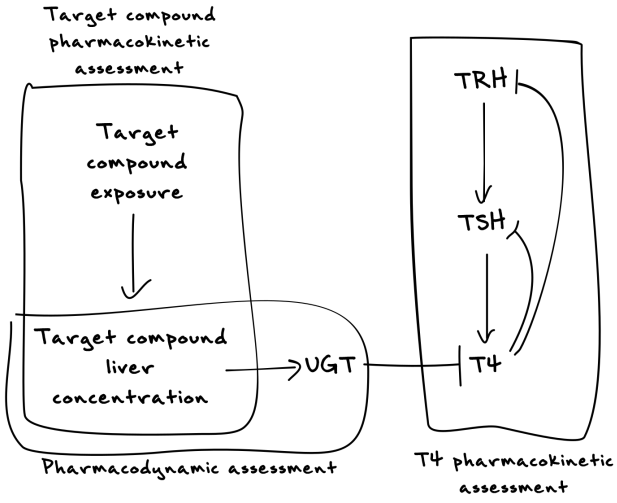

Figure 2. Understanding the impact of potential thyroid hormone disruptors requires assessment pharmacodynamic changes in hepatic metabolism, and its resulting effects on whole-body dosimetry of circulating thyroid hormones.

The framework integrates information from three components. This approach can be adapted for a variety of applications based on client needs and data available on the compound of interest.

Target compound pharmacokinetic assessment. Understanding the concentration of the test article in the liver is essential for estimating compound exposures that lead to changes in metabolism in rats and humans. There are a number of options for determining target compound pharmacokinetics. The preferred approach is to use a physiologically based pharmacokinetic model to perform in vitro-to-in vivo extrapolation. A suitable model may already exist for the compound, or it could be developed. Alternatively, a generalized PBPK model such as ScitoVation's PLETHEM can approximate liver concentrations. Lastly, in some applications, existing in-life pharmacokinetic experiments may provide sufficient information to estimate in vitro-to-in vivo relationships.

Pharmacodynamic assessment. The upregulation of UGT enzymes by the test compound will be determined using in vitro exposures of human and rat hepatocytes. PCR of relevant UGT isoforms and ELISA-based

measurement of UGT activity will quantitatively assess the impact of the target compound on phase II metabolism at relevant internal concentrations.

T4 pharmacokinetic assessment. The phase II metabolism information from the pharmacodynamic assessment provides the necessary input for the T4 BBDR models. The outputs of these models are predictions of circulating maternal thyroid hormone concentrations. Serum T4 concentrations are the clinical biomarkers of hypothyroidism.

This project can be customized to add additional rigor to the modeling. For example, while UGT expression and activity with a general substrate will be sufficient for many applications to characterize the pharmacodynamics, it is possible to measure the specific rate of T4 conjugation as modulated by the test article. Furthermore, upregulation of Phase II enzymes by the target compound could potentially influence its own metabolism. The assumption is this impact is negligible, but further characterization and modeling could be optionally pursued. Finally, initial pursuit of this project requires repurposing exiting BBDR models of T4 dynamics. After adapting and validating these, the cost of modeling additional test articles decreases substantially.

4 References

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