A biologically based computational model for the hypothalamic-pituitary-thyroid (HPT) axis in *X. laevis* larvae

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Supplemental Information SI1

In-Depth Modeling Approach and Model Calibration

In-Depth Modeling Approach

Primary regulation of the hypothalamic-pituitary-thyroid (HPT) axis in vertebrates is achieved by negative feedback of plasma thyroid hormones (THs) on secretion of thyroid stimulating hormone (TSH) by the anterior pituitary gland and feed-forward effects of TSH on TH secretion by the thyroid gland (Zoeller et al., 2007). In mammals, the effects of THs on TSH secretion are mediated in part by impacts on neurosecretory neurons located in the paraventricular nucleus of the hypothalamus (Koller et al., 1987; Segersen et al., 1987; Zoeller et al., 2007). Neurohormones produced by these neurons are transported to the pituitary via the hypothalamic-pituitary portal vessels and include both TSH releasing factors (e.g., thyrotropin releasing hormone; TRH) and release inhibiting factors (e.g., somatostatin). Thyroid hormones also have negative feedback effects directly on the pituitary gland (Franklyn et al., 1987; Mirell et al., 1987; Shupnik and Ridgway 1987; Sternberg et al., 2011; Zoeller et al., 2007). These impacts are primarily mediated by 3,5,3'-triiodothyronine (T3) derived from enzymatic deiodination of thyroxine (T4) by type 2 iodothyronine deiodinase (DIO2) within the hypothalamus-pituitary (Haselman et al., 2022; Larsen et al., 1979; Obregón et al., 1980). In amphibian larvae, corticotropin-releasing hormone (CRH) appears to be the primary hypothalamic signal that initiates TSH synthesis and release (Boorse and Denver, 2004; Okada et

al., 2007). Once the animal completes metamorphosis, control of TSH secretion is taken over by TRH.

In several previous models of HPT axis function in mammals (Degon et al., 2008; DiStefano 1985; Eisenberg et al. 2006, 2008; Kohn et al. 1996; Li et al., 1995; McLanahan et al. 2008), control of TSH secretion was keyed to circulating levels of plasma T4. Previous studies with pre- and pro-metamorphic amphibian larvae have shown, however, that inhibition of DIO2 can result in a large (>10-fold) increase in plasma T4 (Galton 1989; Haselman et al., 2022; Huang et al., 2001). If secretion of TSH is referenced to plasma T4, inhibition of DIO2 would be expected to result in a modest increase in T4 due to reduced total clearance. This increase in plasma T4 would then be predicted to result in reduced TSH secretion, which would reduce secretion of T4 by the thyroid gland, thereby tending to restore the initial condition. Instead, inhibition of DIO2, by reducing the amount of T3 in the pituitary, is more likely to result in increased TSH secretion. High levels of TSH, by feeding forward on processes responsible for T4 secretion, would then be expected to result in a large increase in circulating T4, as has been observed. In the present study, control of TSH secretion by the pituitary was referenced to the free or unbound concentration of T3 in plasma, making it possible to simulate chemical impacts on DIO2. Currently, the relationship between free T3 levels in plasma and within the pituitary gland is unknown. The decision to reference TSH secretion to the free concentration of T3 in plasma reflects an assumption that free T3 levels in plasma and within the pituitary vary in strict proportion to one another. With this assumption, the free concentration of T3 in the plasma may be viewed as a surrogate for the concentration of T3 that operates within the pituitary to control TSH secretion.

Several published models for the HPT axis in mammals have included descriptions of iodide uptake and elimination. One such model for rats was used to simulate the effects of low dietary iodine in combination with thyroid-disrupting compounds (McLanahan et al., 2008). This effort was supported by the development of physiologically based pharmacokinetic (PBPK) models for plasma iodide and perchlorate anion (Clewell et al., 2003; Merrill et al., 2003, 2005). A second model given by Degon et al. (2008) was used to describe the inhibition of TH synthesis in humans by high levels of plasma iodide (i.e., Wolff-Chaikoff block).

Our goal was to model the HPT axis of pro-metamorphic *Xenopus laevis* larvae used in chemical safety testing efforts. Since current toxicity testing guidelines such as the Amphibian Metamorphosis Assay (AMA; OECD 2009; U.S. EPA 2009) and the Larval Amphibian Growth and Development Assay (LAGDA; OECD 2015; U.S. EPA 2015) outline acceptable standard diets and aqueous iodine concentrations, we assumed iodide-sufficient conditions that are relatively constant. Further, we assumed that *X. laevis* larvae maintain circulating plasma iodide within a relatively narrow range by homeostatic mechanisms. As such, we chose not to model iodine uptake and elimination dynamically and instead represented circulating iodide as a fixed concentration. Experimental support for this approach is provided as Supporting Information (Section SI4).

Thyroglobulin (Tg) serves as the protein source of THs and also serves as organified iodine storage. Thyroglobulin is synthesized in the thyroid follicular cells (thyrocytes) and exocytosed to the follicular lumen where tyrosyl residues are iodinated by thyroperoxidase (TPO) enzymes located on the apical surface of the thyrocytes. In *X. laevis* larvae exposed to potent TPO inhibitors (e.g., methimazole or mercaptobenzothiazole), the iodinated species monoiodotyrosine (MIT), diiodotyrosine (DIT), T3 and T4 are substantially depleted in the

thyroid gland within 2-3 days of exposure (Haselman et al., 2020). Depletion of follicular colloid becomes apparent histologically on a similar time scale (Degitz et al., 2005; Tietge et al., 2010, 2013). In contrast, significant decreases in plasma T3 and/or T4 are not apparent until at least 6 days of exposure to potent TPO inhibitors (Haselman et al., 2020; Tietge et al., 2010). The different time frames for depletion of the gland of iodinated species and a change in the concentration of circulating THs suggests that storage of iodinated Tg in the follicular lumen buffers the system from chemical effects on TH synthesis.

The Tg molecule exists as a glycoprotein dimer with a total molecular weight of about 660 kDa. Each molecule contains a large number of tyrosyl residues, a subset of which can be iodinated by TPO. The total number of tyrosyl residues iodinated under physiological conditions appears to vary among species. Relatively low values have been reported for rat, bovine, and human Tg (12-16 for the monomer; Palumbo et al., 1990; Taurog et al., 1996; Gentile et al., 1997) while a substantially higher value was determined for mice (37; Dedieu et al., 2011). By comparison, the total number of hormonogenic sites (i.e., those tyrosyl residues associated with production of THs) appears to be relatively invariant; studies with several species suggest that each monomer contains 4 or 5 such sites (Dedieu et al., 2011; Dunn and Dunn, 2000), while Coscia et al. (2020) concluded there are 7 molecules of T4 synthesized per human Tg dimer. Moreover, these hormonogenic sites are the first to be iodinated when iodide is provided at low concentrations (Gavaret et al., 1977; Haraguchi et al., 1988; Lamas et al., 1974, 1989).

Collectively, these observations suggest that a single molecule of Tg possesses tyrosyl residues that have different chemical fates. A subset of those are iodinated by reactions exhibiting high affinity with respect to iodide. Iodo-tyrosyl residues are subsequently coupled to become T3 and T4. Additional tyrosyl residues, which are greater in number, are iodinated by

reactions exhibiting relatively lower affinity with respect to iodide and become “stand alone” MIT and DIT residues; that is, MIT and DIT represent the terminal products of this reaction sequence. Lacking detailed information for iodination of Tg in *X. laevis* larvae (e.g., affinity constants for different iodination reactions) we instead made the simplifying assumption that each category of activity results in complete iodination of all available sites corresponding to a particular pathway. Thus, iodination of one mole of Tg results in a defined ratio of iodo-tyrosyl species.

In mammals, iodination of Tg results in substantial production of glandular T3 and T4; in rats, for example, the measured ratio of T3 and T4 in the thyroid is approximately 1:4 (Gilbert et al., 2013). In contrast to these findings, measured amounts of T3 in the thyroid gland of *X. laevis* larvae were approximately 100-fold lower than those of T4 (Haselman et al., 2020; 2022). We elected, therefore, to ignore the production of glandular T3. As such, the primary source of plasma T3 in the model derives from DIO2-mediated conversion of T4 to T3 in the plasma compartment. The static molar ratio of glandular MIT:DIT:T4, specific to *X. laevis*, used to define a fully iodinated Tg molecule here was derived based on temporal gland iodo-species data from Haselman et al. (2020). Molar ratios for glandular MIT:DIT:T4 at each of the 2, 4, 7 and 10 day timepoints were adjusted proportionally so the lowest value was at least 1. The resulting ratios were similar across the four time-points so the mean moles of each iodo-species across the four time-points were rounded to the nearest whole number and considered to be static across this period of development for the purposes of this modeling effort.

Although organification of iodide and coupling of DIT residues to form T4 occur as separate enzymatic reactions with differing kinetic parameters (Tater et al., 2021), we chose to simplify the model description of these processes by expressing iodide organification

mathematically as a single Michaelis-Menten relationship constrained by the availability of newly synthesized Tg. Simplification of this process still allows for synthesis of MIT and DIT, which serve as organic storage of iodine within the thyroid gland. In addition, it was assumed that the colloidal material within the gland consists primarily of Tg with the result that accumulation and depletion of Tg directly impact thyroid lumenal volume. Following this logic, we did not treat the follicular lumen as a model compartment with a corresponding volume but instead modeled the total amount of Tg present. This approach permits rates of Tg synthesis and proteolysis to be driven by TSH concentrations in plasma and allows iodination of Tg to be referenced to the amount of uniodinated Tg available while still retaining Michaelis-Menten kinetics based on thyrocyte iodide concentrations.

The dominant pathway for mobilization of Tg involves endocytosis of colloidal material within the follicular lumen. Thyroglobulin contained within the endocytic vesicle is then broken down enzymatically, releasing T4, T3, MIT and DIT. The hormones T4 and T3 are secreted to the plasma while iodine from MIT and DIT is recycled by iodotyrosine deiodinase (IYD) and remains within the thyrocyte (Olker et al., 2018). This iodine may then be used to synthesize new THs. The iodine recycling process represented in the model was simplified by assuming that 100% of iodine associated with MIT and DIT is liberated as a result of Tg proteolysis.

Thyroid stimulating hormone binds to G-coupled protein receptors on the basolateral membrane of thyrocytes and operates though a 2^{nd} messenger cascade to regulate the production of proteins involved in TH synthesis and secretion (Dumont et al., 1992; Vassart and Dumont, 1992). Additional regulation may be achieved by direct interactions of TSH with gene promoter sequences. Based on earlier work, Eisenberg et al. (2006) suggested that the TSH:TH secretion rate relationship in humans is a simple gain function, indicating not only that TSH feeds forward

on TH secretion but that it does so in a way that is manifested as proportional changes in all relevant processes. This suggestion is consistent with the observation (from research on rats) that a single set of transcription factors activated by TSH regulates the synthesis of Tg and TPO as well as the membrane transporter responsible for active uptake of iodide by the thyroid gland (the sodium-iodide symporter, or NIS)(Di Lauro et al., 1995; Ohno et al., 1999). Accordingly, we use similar mathematical relationships to describe the rate of Tg synthesis and the maximal activities of NIS and TPO as functions of plasma TSH concentration. Affinity constants (K_M) that describe the TSH concentration-dependence of each relationship were also set equal to one another. Previously, it was suggested that endocytosis of Tg is a constitutive process; however, other work indicates that endocytosis is regulated by TSH (Marino and McCluskey, 2000). Here we assume that endocytosis and proteolysis of Tg (as with Tg synthesis) are entirely controlled by TSH.

Thyroid glands in *X. laevis* larvae grow throughout development (Opitz et al., 2009; Tietge et al., 2010). Changes in gland size are important because these changes can be expected, by themselves, to increase the capacity for T4 synthesis and storage. Combining these changes with TSH-mediated upregulation of the same processes may therefore have a multiplicative effect. An even greater impact of TSH on TH synthesis can be expected if TSH induces growth of the thyroid gland itself. In the current model, the activities of all processes responsible for synthesis, storage, and secretion of thyroid hormones are scaled directly to the aggregated volume of thyrocytes at each time point.

Model Calibration

Model calibration was initiated with the goal of maintaining T4 and T3 concentrations in plasma at starting values, independent of the effects of organism growth, gland growth, and TSH

effects on thyrocyte biochemistry (operating through R_{NIS}, $R_{TG,\,SYN}$, R_{TPO}, $R_{TG,\,PROT}$). To accomplish this, $R_{T4,\,SEC}$, $CL_{T4,\,CONJ}$, and $V_{MAX,\,DIO2}$ were adjusted iteratively to achieve target levels of $C_{T4,\,TOT,\,PL}$ and $C_{T3,\,TOT,\,PL}$ while resulting in a desired T4 half-life of approximately 2 hours. Then, $CL_{T3,\,CONJ}$ and R_{DIO3} were initialized and $R_{T4,\,SEC}$, $V_{MAX,\,DIO2}$ and $V_{MAX,\,DIO3}$ were readjusted to maintain target hormone levels. Since the calculation of $C_{T3,\,TOT,\,PL}$ is entirely dependent on $C_{T4,\,TOT,\,PL}$ as an input, this process established an overall $R_{T4,\,SEC}$ needed to maintain both $C_{T4,\,TOT,\,PL}$ and $C_{T3,\,TOT,\,PL}$.

Thyrocyte biochemistry components were calibrated by first holding $C_{TSH,\,PL}$ constant and establishing a rate of Tg proteolysis ($R_{TG,\,PROT}$; scaled to V_{FC}, which was initially held constant) needed to achieve the $R_{T4,\,SEC}$ determined from the initial optimization step. Then, with $C_{I,\,FC}$ held constant at 20 times $C_{I,\,PL}$, the rates of Tg synthesis ($R_{TG,\,SYN}$) and iodide organification (R_{TPO}) were calibrated to exceed $R_{TG,\,PROT}$, resulting in an accumulation of MIT, DIT and T4 in the thyroid gland. Next, $V_{MAX,\,NIS}^{TSH}$, $K_{D,\,I}$ and $K_{I,\,FC}$ were initialized and adjusted to maintain $\frac{C_{I,\,FC}}{C_{I,\,PL}}$ at approximately 20 to achieve iodide organification rates sufficient for both accumulation of MIT, DIT and T4 in the gland and adequate T4 secretion to the plasma compartment.

Absent an increase in the size of the thyroid gland, an increase in body weight would result in a dilution effect that would require an increase in $R_{T4,\,SEC}$ over time to maintain increasing plasma T4 levels. However, gland growth contributes to an increasing capacity for T4 secretion, which tends to counteract the effect of organism growth. Initially, modeled simulations of thyroid gland growth were fit to measured cell number data by manipulating $R_{FCN,\,CONT}$, $R_{MAX,\,TSH}$, and the Hill equation constants, with $C_{TSH,\,PL}$ held constant. This resulted in a linear increase in cell number that was expected to change to a curvilinear increase once $C_{TSH,\,PL}$ was

simulated dynamically. Cell number predictions (FCN_{TOT}) were then linked to R_{NIS}, $R_{TG,\,SYN}$, R_{TPO}, and $R_{TG,\,PROT}$ by scaling these rates to V_{FC}. With body growth also activated, V_{MAX} values associated with these rates were readjusted to align with target trajectories.

Plasma TSH concentrations were expected to increase approximately 3- to 4-fold over the 10-day simulation while also being negatively regulated by increasing $C_{T3,\,FF,\,PL}$ (recall the paradoxical rise of both hormones in the plasma during metamorphosis). With $C_{T3,\,FF,\,PL}$ set to the concentration determined in previous calibration exercises, $K_{M,\,TSH}$ was increased linearly approximately 20-fold over the course of the 10-day simulation by applying a fixed rate constant ($R_{K_{M,\,TSH}}$). This promoted a modest increase in $C_{TSH,\,PL}$ over time. When $C_{T3,\,FF,\,PL}$ is allowed to increase over time, negative feedback on TSH increases and a substantial increase in $C_{TSH,\,PL}$ is only possible if $V_{MAX,\,TSH}$ increases. This was accomplished by applying a second fixed rate constant ($R_{V_{MAX,\,TSH}}$). Simultaneous increases in $K_{M,\,TSH}$, $V_{MAX,\,TSH}$ and $C_{T3,\,FF,\,PL}$ resulted in a generally logarithmic increase in $C_{TSH,\,PL}$ of the desired magnitude. When linking the model components together, however, the feedback between $C_{T3,\,FF,\,PL}$ and $C_{TSH,\,PL}$ caused nearly linear increases in $C_{T4,\,TOT,\,PL}$ and $C_{T3,\,TOT,\,PL}$, whereas the calibration data exhibited curvilinear increases in plasma TH levels. Applying a third rate constant ($R_{R_{K_{M,\,TSH}}}$) to adjust $R_{K_{M,\,TSH}}$ imparted a 2nd order polynomial relationship that resulted in a later onset acceleration to the increase in $K_{M,\,TSH}$ (overall 25-fold increase) that optimized the fit of the simulation profiles for $C_{T4,\,TOT,\,PL}$ and $C_{T3,\,TOT,\,PL}$.

REFERENCES

Boorse, G.C. and Denver, R.J., 2004. Expression and hypophysiotropic actions of corticotropin-releasing factor in *Xenopus laevis*. General and Comparative Endocrinology, 137(3), pp.272-282.

Clewell, R.A., Merrill, E.A., Yu, K.O., Mahle, D.A., Sterner, T.R., Mattie, D.R., Robinson, P.J., Fisher, J.W. and Gearhart, J.M., 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. Toxicological Sciences, 73(2), pp.235-255.

Coscia, F., Taler-Verčič, A., Chang, V.T., Sinn, L., O'Reilly, F.J., Izoré, T., Renko, M., Berger, I., Rappsilber, J., Turk, D. and Löwe, J., 2020. The structure of human thyroglobulin. Nature, 578(7796), pp.627-630.

Dedieu, A., Gaillard, J.C., Pourcher, T., Darrouzet, E. and Armengaud, J., 2011. Revisiting iodination sites in thyroglobulin with an organ-oriented shotgun strategy. Journal of Biological Chemistry, 286(1), pp.259-269.

Degitz, S.J., Holcombe, G.W., Flynn, K.M., Kosian, P.A., Korte, J.J. and Tietge, J.E., 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. Toxicological sciences, 87(2), pp.353-364.

Degon, M., Chipkin, S.R., Hollot, C.V., Zoeller, R.T. and Chait, Y., 2008. A computational model of the human thyroid. Mathematical Biosciences, 212(1), pp.22-53.

Di Lauro, R., Damante, G., De Felice, M., Arnone, M.I., Sato, K., Lonigro, R. and Zannini, M., 1995. Molecular events in the differentiation of the thyroid gland. Journal of Endocrinological Investigation, 18(2), pp.117-119.

DiStefano III, J.J., 1985. Modeling approaches and models of the distribution and disposal of thyroid hormones. In ''Thyroid Hormone Metabolism''(G. Hennemann, Ed.).

Dumont, J.E., Lamy, F., Roger, P. and Maenhaut, C., 1992. Physiological and pathological regulation of thyroid cell proliferation and differentiation by thyrotropin and other factors. Physiological Reviews, 72(3), pp.667-697.

Dunn, J.T. and Dunn, A.D., 2000. Thyroglobulin: chemistry, biosynthesis, and proteolysis. LE Braverman, and RD Utiger, eds. Werner and Ingbar's The Thyroid: A Fundamental and Clinical Text, pp. 91-104.

Eisenberg, M., Samuels, M. and DiStefano III, J.J., 2006. L-T4 bioequivalence and hormone replacement studies via feedback control simulations. Thyroid, 16(12), pp.1279-1292.

Eisenberg, M., Samuels, M. and DiStefano III, J.J., 2008. Extensions, validation, and clinical applications of a feedback control system simulator of the hypothalamo-pituitary-thyroid axis. Thyroid, 18(10), pp.1071-1085.

Franklyn, J.A., Wood, D.F., Balfour, N.J., Ramsden, D.B., Docherty, K., Chin, W.W. and Sheppard, M.C., 1987. Effect of hypothyroidism and thyroid hormone replacement in vivo on pituitary cytoplasmic concentrations of thyrotropin-β and α-subunit messenger ribonucleic acids. Endocrinology, 120(6), pp.2279-2288.

Galton, V.A., 1989. The role of 3, 5, 3'-triiodothyronine in the physiological action of thyroxine in the premetamorphic tadpole. Endocrinology, 124(5), pp.2427-2433.

Gavaret, J.M., Dème, D., Nunez, J. and Salvatore, G., 1977. Sequential reactivity of tyrosyl residues of thyroglobulin upon iodination catalyzed by thyroid peroxidase. Journal of Biological Chemistry, 252(10), pp.3281-3285.

Gentile, F., Ferranti, P., Mamone, G., Malorni, A. and Salvatore, G., 1997. Identification of Hormonogenic Tyrosines in Fragment 1218-1591 of Bovine Thyroglobulin by Mass Spectrometry: Hormonogenic Acceptor TYR-1291 and D TYR-1375. Journal of Biological Chemistry, 272(1), pp.639-646.

Gilbert, M.E., Hedge, J.M., Valentin-Blasini, B.C., Kannan, K., Tietge J., Zoeller, R.T., Crofton, K.M., Jarrett, J.M., and Fisher, J.W., 2013. An animal model of marginal iodine deficiency during development: The thyroid axis and neurodevelopmental outcome. Toxicological Sciences, 132(1), pp.177-195.

Haraguchi, K., Endo, T., Onaya, T., Sho, K., Ohmiya, Y. and Kondo, Y., 1988. Evidence for a preferential iodination site within the thyroglobulin molecule. Molecular and Cellular Endocrinology, 59(1-2), pp.111-115.

Haselman, J.T., Olker, J.H., Kosian, P.A., Korte, J.J., Swintek, J.A., Denny, J.S., Nichols, J.W., Tietge, J.E., Hornung, M.W. and Degitz, S.J., 2020. Targeted pathway-based in vivo testing using thyroperoxidase inhibition to evaluate plasma thyroxine as a surrogate metric of metamorphic success in model amphibian *Xenopus laevis*. Toxicological Sciences, 175(2), pp.236-250.

Haselman, J.T., Olker, J.H., Kosian, P.A., Korte, J.J., Denny, J.S., Tietge, J.E., Hornung, M.W. and Degitz, S.J., 2022. Characterization of the mechanistic linkages between iodothyronine deiodinase inhibition and impaired thyroid-mediated growth and development in *Xenopus laevis* using iopanoic acid. Toxicological Sciences, 187(1), pp.139-149.

Huang, H., Cai, L., Remo, B.F. and Brown, D.D., 2001. Timing of metamorphosis and the onset of the negative feedback loop between the thyroid gland and the pituitary is controlled by type II iodothyronine deiodinase in *Xenopus laevis*. Proceedings of the National Academy of Sciences, 98(13), pp.7348-7353.

Kohn, M.C., Sewall, C.H., Lucier, G.W. and Portier, C.J., 1996. A mechanistic model of effects of dioxin on thyroid hormones in the rat. Toxicology and Applied pharmacology, 136(1), pp.29-48.

Koller, K.J., Wolff, R.S., Warden, M.K. and Zoeller, R.T., 1987. Thyroid hormones regulate levels of thyrotropin-releasing-hormone mRNA in the paraventricular nucleus. Proceedings of the National Academy of Sciences, 84(20), pp.7329-7333.

Lamas, L., Taurog, A., Salvatore, G. and Edelhoch, H., 1974. Preferential synthesis of thyroxine from early iodinated tyrosyl residues in thyroglobulin. Journal of Biological Chemistry, 249(9), pp.2732-2737.

Lamas, L., Anderson, P.C., Fox, J.W. and Dunn, J.T., 1989. Consensus sequences for early iodination and hormonogenesis in human thyroglobulin. Journal of Biological Chemistry, 264(23), pp.13541-13545.

Larsen, P.R., Dick, T.E., Markovitz, B.P., Kaplan, M.M. and Gard, T.G., 1979. Inhibition of intrapituitary thyroxine to 3.5. 3'-triiodothyronine conversion prevents the acute suppression of thyrotropin release by thyroxine in hypothyroid rats. Journal of Clinical Investigation, 64(1), pp.117-128.

Li, G., Liu, B. and Liu, Y., 1995. A dynamical model of the pulsatile secretion of the hypothalamo-pituitary-thyroid axis. Biosystems, 35(1), pp.83-92.

Marinò, M. and McCluskey, R.T., 2000. Role of thyroglobulin endocytic pathways in the control of thyroid hormone release. American Journal of Physiology-Cell Physiology, 279(5), pp.C1295-C1306.

McLanahan, E.D., Andersen, M.E. and Fisher, J.W., 2008. A biologically based dose-response model for dietary iodide and the hypothalamic-pituitary-thyroid axis in the adult rat: evaluation of iodide deficiency. Toxicological Sciences, 102(2), pp.241-253.

Merrill, E.A., Clewell, R.A., Gearhart, J.M., Robinson, P.J., Sterner, T.R., Yu, K.O., Mattie, D.R. and Fisher, J.W., 2003. PBPK predictions of perchlorate distribution and its effect on thyroid uptake of radioiodide in the male rat. Toxicological Sciences, 73(2), pp.256-269.

Merrill, E.A., Clewell, R.A., Robinson, P.J., Jarabek, A.M., Gearhart, J.M., Sterner, T.R. and Fisher, J.W., 2005. PBPK model for radioactive iodide and perchlorate kinetics and perchlorate-induced inhibition of iodide uptake in humans. Toxicological Sciences, 83(1), pp.25-43.

Mirell, C.J., Yanagisawa, M., Lau, R., Pekary, A.E., Chin, W.W. and Hershman, J.M., 1987. Influence of thyroidal status on pituitary content of thyrotropin β-and α-subunit, growth hormone, and prolactin messenger ribonucleic acids. Molecular endocrinology, 1(6), pp.408-412.

Obregón, M.J., Pascual, A., Mallol, J., Escobar, M.D. and Rey, F.E.D., 1980. Evidence against a major role of L-thyroxine at the pituitary level: studies in rats treated with iopanoic acid (telepaque). Endocrinology, 106(6), pp.1827-1836.

OECD. 2009. Test No. 231: Amphibian Metamorphosis Assay, OECD Guidelines for the Testing of Chemicals, Section 2. OECD Publishing, Paris.

OECD. 2015. Test No. 241: The Larval Amphibian Growth and Development Assay (LAGDA), OECD Guidelines for the Testing of Chemicals, Section 2. OECD Publishing, Paris.

Ohno, M., Zannini, M., Levy, O., Carrasco, N. and Di Lauro, R., 1999. The paired-domain transcription factor Pax8 binds to the upstream enhancer of the rat sodium/iodide symporter gene and participates in both thyroid-specific and cyclic-AMP-dependent transcription. Molecular and Cellular Biology, 19(3), pp.2051-2060.

Okada, R., Miller, M.F., Yamamoto, K., De Groef, B., Denver, R.J. and Kikuyama, S., 2007. Involvement of the corticotropin-releasing factor (CRF) type 2 receptor in CRF-induced thyrotropin release by the amphibian pituitary gland. General and Comparative Endocrinology, 150(3), pp.437-444.

Olker, J.H., Haselman, J.T., Kosian, P.A., Donnay, K.G., Korte, J.J., Blanksma, C., Hornung, M.W. and Degitz, S.J., 2018. Evaluating iodide recycling inhibition as a novel molecular initiating event for thyroid axis disruption in amphibians. Toxicological Sciences, 166(2), pp.318-331.

Opitz, R., Schmidt, F., Braunbeck, T., Wuertz, S. and Kloas, W., 2009. Perchlorate and ethylenethiourea induce different histological and molecular alterations in a non-mammalian vertebrate model of thyroid goitrogenesis. Molecular and Cellular Endocrinology, 298(1-2), pp.101-114.

Palumbo, G., Gentile, F., Condorelli, G.L. and Salvatore, G., 1990. The earliest site of iodination in thyroglobulin is residue number 5. Journal of Biological Chemistry, 265(15), pp.8887-8892.
Taurog, A., Dorris, M.L. and Doerge, D.R., 1996. Mechanism of simultaneous iodination and coupling catalyzed by thyroid peroxidase. Archives of Biochemistry and Biophysics, 330(1), pp.24-32.

Segerson, T.P., Kauer, J., Wolfe, H.C., Mobtaker, H., Wu, P., Jackson, I.M. and Lechan, R.M., 1987. Thyroid hormone regulates TRH biosynthesis in the paraventricular nucleus of the rat hypothalamus. Science, 238(4823), pp.78-80.

Shupnik, M.A. and Ridgway, E.C., 1987. Thyroid hormone control of thyrotropin gene expression in rat anterior pituitary cells. Endocrinology, 121(2), pp.619-624.

Sternberg, R.M., Thoemke, K.R., Korte, J.J., Moen, S.M., Olson, J.M., Korte, L., Tietge, J.E. and Degitz Jr, S.J., 2011. Control of pituitary thyroid-stimulating hormone synthesis and secretion by thyroid hormones during *Xenopus* metamorphosis. General and Comparative Endocrinology, 173(3), pp.428-437.

Tater, A., Gupta, A., Upadhyay, G., Deshpande, A., Date, R. and Tamboli, I.Y., 2021. In vitro assays for characterization of distinct multiple catalytic activities of thyroid peroxidase using LC-MS/MS. Current Research in Toxicology, 2, pp.19-29.

Taurog, A., Dorris, M.L. and Doerge, D.R., 1996. Mechanism of simultaneous iodination and coupling catalyzed by thyroid peroxidase. Archives of Biochemistry and Biophysics, 330(1), pp.24-32.

Tietge, J.E., Butterworth, B.C., Haselman, J.T., Holcombe, G.W., Hornung, M.W., Korte, J.J., Kosian, P.A., Wolfe, M. and Degitz, S.J., 2010. Early temporal effects of three thyroid hormone synthesis inhibitors in *Xenopus laevis*. Aquatic Toxicology, 98(1), pp.44-50.

Tietge, J.E., Degitz, S.J., Haselman, J.T., Butterworth, B.C., Korte, J.J., Kosian, P.A., Lindberg-Livingston, A.J., Burgess, E.M., Blackshear, P.E. and Hornung, M.W., 2013. Inhibition of the thyroid hormone pathway in *Xenopus laevis* by 2-mercaptobenzothiazole. Aquatic Toxicology, 126, pp.128-136.

U.S. EPA. 2009. OCSPP 890.1100: Amphibian Metamorphosis Assay (AMA), Endocrine Disruptor Screening Program Test Guidelines, 890 Series. Available at: www.regulations.gov, ID: EPA-HQ-OPPT-2009-0576-0002. Accessed March 20, 2020.

U.S. EPA. 2015. OCSPP 890.2300: Larval Amphibian Growth and Development Assay (LAGDA), Endocrine Disruptor Screening Program Test Guidelines, 890 Series. Available at: www.regulations.gov, ID: EPA-HQ-OPPT-2014-0766-0020. Accessed March 20, 2020.

Vassart, G. and Dumont, J.E., 1992. The thyrotropin receptor and the regulation of thyrocyte function and growth. Endocrine Reviews, 13(3), pp.596-611.

Zoeller, R.T., Tan, S.W. and Tyl, R.W., 2007. General background on the hypothalamic-pituitary-thyroid (HPT) axis. Critical Reviews in Toxicology, 37(1-2), pp.11-53.