

A Systems Approach to Characterizing and Predicting Thyroid Toxicity

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Thyroid Toxicity Research

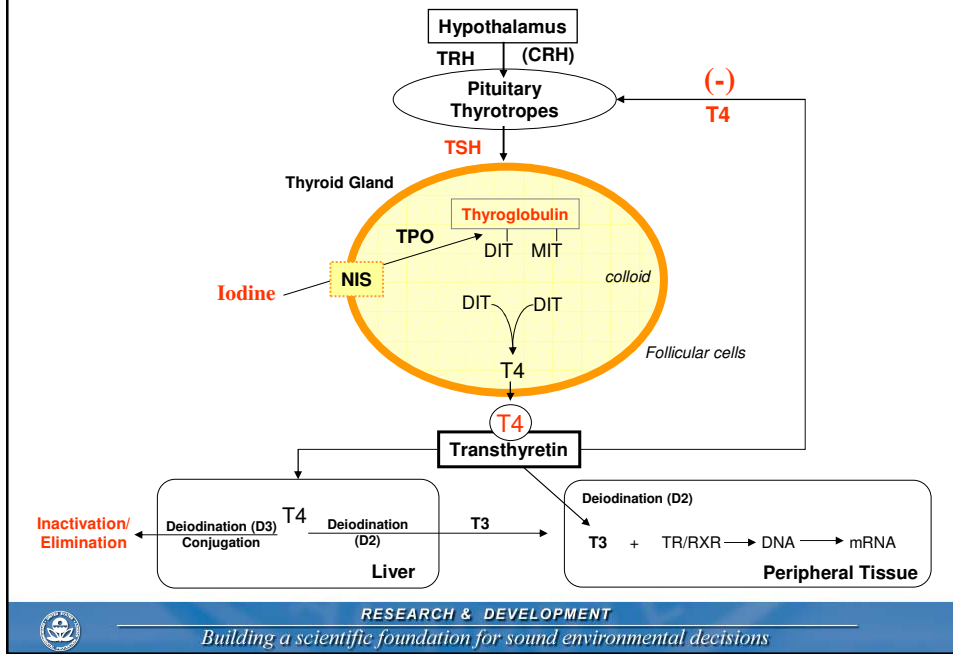
- Endocrine Disruptors
- Thyroid hormone is important for growth and development, neurodevelopment, metabolism
- To understand thyroid toxicity need to look at it in the context of the whole Hypothalamus-Pituitary-Thyroid Axis (HPT)



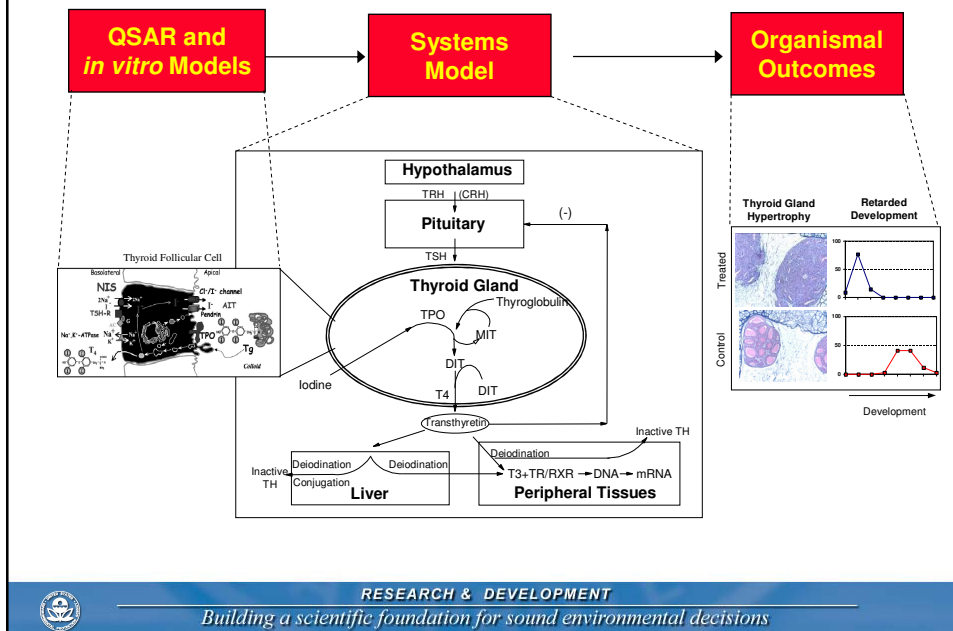
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Thyroid Hormone Regulation



Thyroid-axis Systems Model



Why an amphibian model ?

- Metamorphosis is controlled by thyroid hormone
- Simple apical endpoint to monitor disruption *in vivo*
- Molecular events are well characterized
- Easy to raise and test in the laboratory
 - *Xenopus laevis*

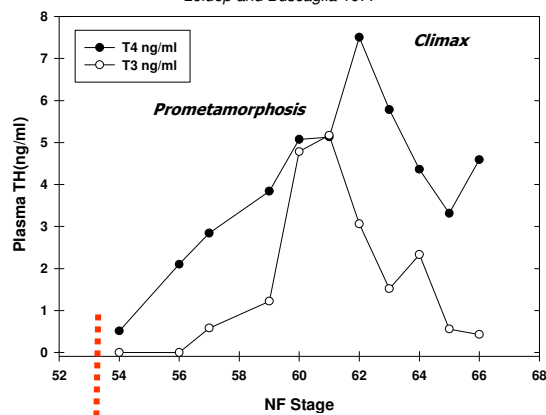


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Xenopus Metamorphosis

X. laevis Plasma Thyroid Hormones
Leluop and Buscaglia 1977



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MED Thyroid Project Objectives

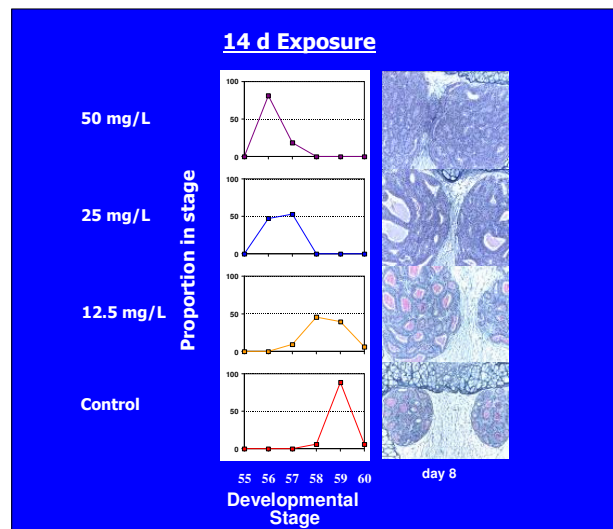
- Conduct studies with known HPT disruptors
 - Inhibitors of thyroid hormone synthesis
 - Thyroid Peroxidase: Methimazole, Propylthiouracil
 - Sodium Iodide Symporter: Perchlorate
- Develop diagnostic measures
 - What are the appropriate tissue level endpoints?
 - Histology, T4, TSH
 - Can gene and protein expression be used as indicators of thyroid axis disruption?
- Develop assays to enable ranking and prioritization of chemicals



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Effect of Methimazole on Development and Thyroid Histology



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Summary of Metamorphosis Assay

- *X. laevis* is sensitive to model thyroid pathway modulators
 - Methimazole, 6-PTU, Perchlorate
- Early stage tadpoles (stg 51-54) can be arrested in development by T4 synthesis inhibitors, stage 60 is not
- Thyroid histology is an essential component of assay
 - More sensitive than developmental rate (d8)
 - Diagnostic



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Diagnostic Research Approach

- Link Chemical-Biomolecular Interaction to Organism Response
 - Examine gene expression during normal metamorphosis and following chemical exposure
 - Examine protein changes
 - Circulating T4 and TSH
- Responses of tissues isolated from compensatory mechanisms
 - Pituitary explant culture: TSH – T4 feedback
 - Thyroid explant culture: TSH stimulation, chemical inhibition of T4 release
- Develop computational – predictive approaches

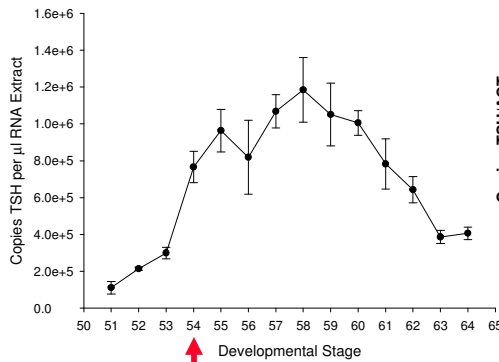


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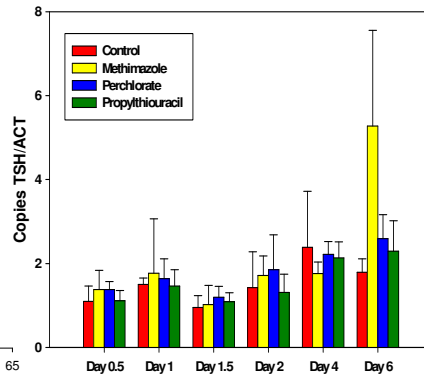
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In vivo Pituitary Gene Expression: Thyroid Stimulating Hormone

Developmental Expression



Chemical Exposure

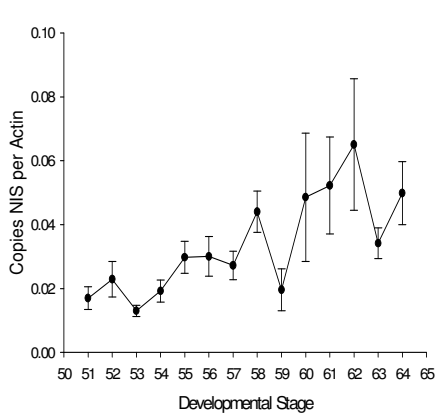


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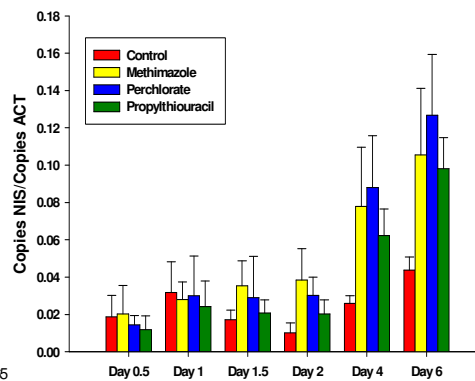
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In Vivo Thyroid Gland Gene Expression Sodium/Iodide Symporter

Developmental Expression



Chemical Exposure



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Pituitary Explant Culture

Objective:

Characterize function of the pituitary during development and the relationship between T4 and TSH

Method:

Culture pituitaries from tadpoles at multiple stages of development

Measure TSH expression in the pituitaries
Gene expression or T4 release in thyroid glands treated with media conditioned by pituitary culture

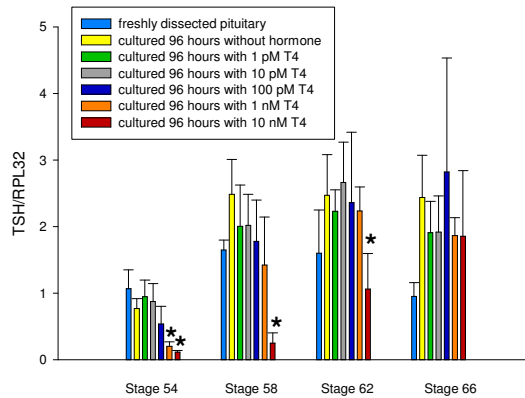


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Pituitary Explant Culture

TSH mRNA is repressed by T4



- Negative feedback mechanism is functional throughout development although the setpoint changes
 - sensitivity to T4 decreases



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Thyroid Gland Explant Culture

Objective:

Define thyroid-specific outputs in response to TSH and xenobiotics in the absence of whole organism compensatory response

Method:

Culture thyroid glands from prometamorphic tadpoles and treat with TSH and T4 synthesis inhibitors

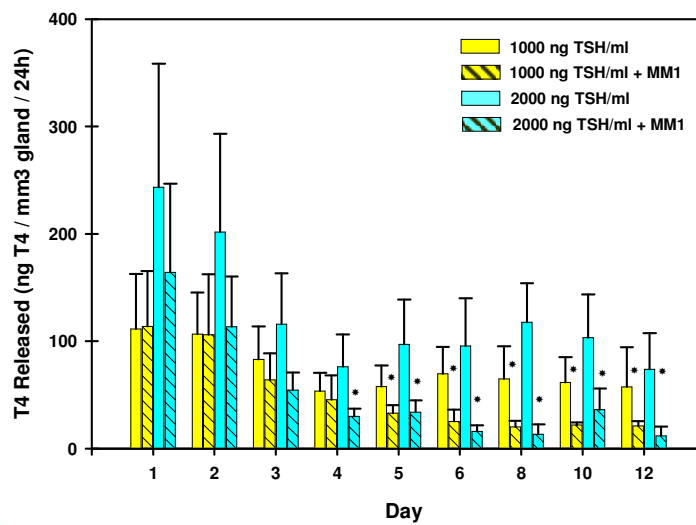
Measure T4 release and gene expression



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Thyroid Gland Explant Culture: Time relationship of T4 release inhibition



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Pituitary Explant Culture

- Feedback mechanisms in the pituitary
 - Negative feedback by T4 on the pituitary is present in metamorphosis
 - Sensitivity of the pituitary to this inhibition decreases over time
 - in early metamorphosis prevent excess T4
 - allow more T4 later to complete metamorphosis



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Thyroid Explant Culture

Interpretation of compensatory and direct effects

In vitro...

- Release T4 in response to TSH is dose related
- T4 reserves must be depleted before synthesis inhibition significantly affects T4 release

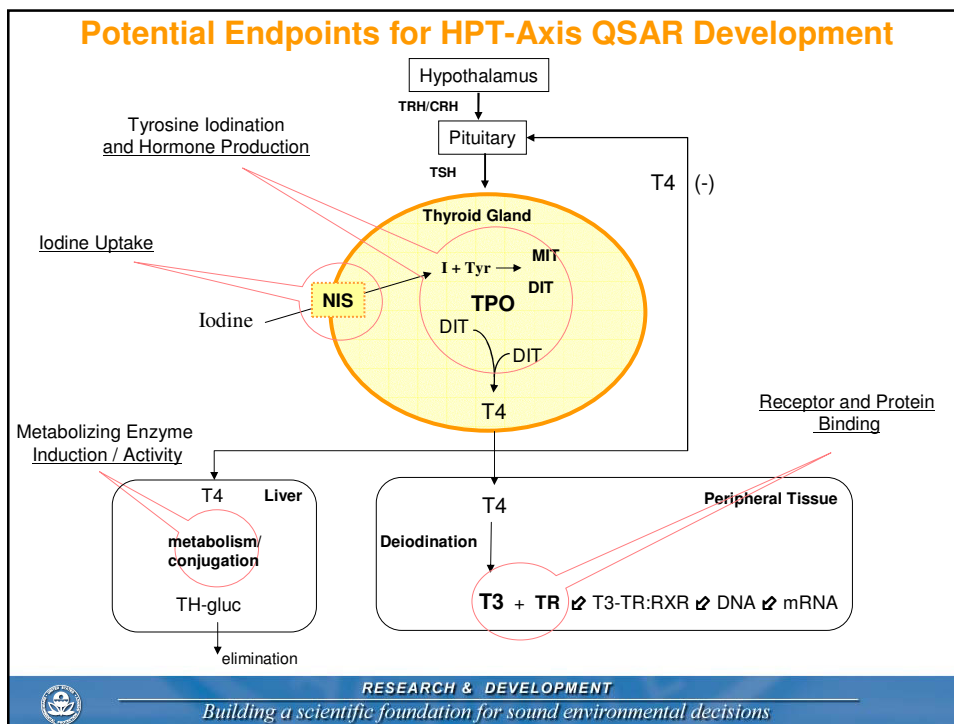
In vivo...

- Early stages are more sensitive to arrested metamorphosis by T4 inhibitors than late stages
- At late prometamorphosis, thyroid glands are larger and reserve T4 is sufficient to complete metamorphosis
- Exposure time 0 does not equal effect time 0 for circulating T4
- Need to measure circulating hormone levels to interpret gene expression and protein responses in vivo



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HPT-Axis QSAR Development

Comparison of Endpoints of T4 Synthesis Inhibition

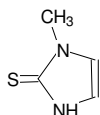
NIS activity	TPO activity
<p>Membrane protein transports iodine into the follicular cell</p> <ul style="list-style-type: none"> Limited data on chemical inhibitors of NIS - mostly monovalent anions of similar size as iodide Lack of data makes it difficult to make informed chemical selection Difficult assay to transform to high throughput format 	<p>TPO iodinates tyrosine and couples iodotyrosines to produce thyroid hormone</p> <ul style="list-style-type: none"> TPO inhibition data available for more chemicals & classes of chemicals <ul style="list-style-type: none"> Methimazole – PTU Flavonoids Resorcinols More data aids chemical selection process and QSAR model development Spectrophotometric determination of iodination of tyrosine to MIT Potential for conversion to high throughput assay

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HPT-Axis QSAR Development

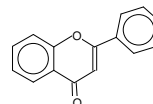
TPO Inhibitors

Methimazole

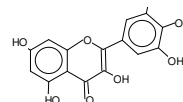


Plant Flavonoids

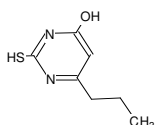
flavone



myricetin

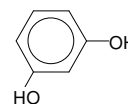


Propylthiouracil



Resorcinol & Derivatives

resorcinol

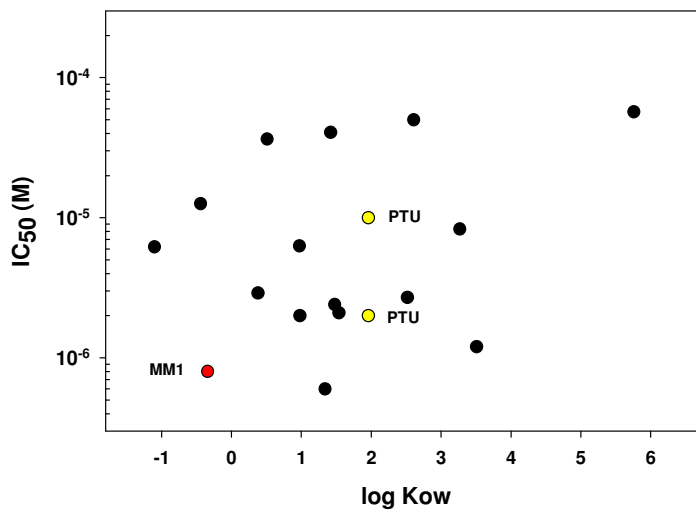


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Thyroid Peroxidase Inhibition

Literature Data



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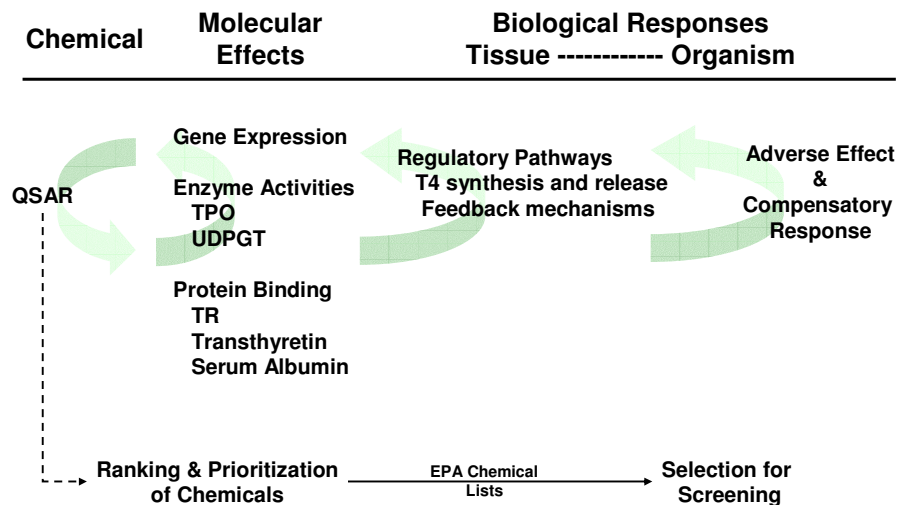
- Develop *Xenopus*-based *in vitro* assay to begin to test known inhibitors of TPO activity
- Expand the range of chemicals and classes
- Select from EPA Chemical Lists
- Predictive Linkages
in vitro → *ex vivo* (explant culture) → *in vivo*



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Systems Approach to Predicting Thyroid Toxicity



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