Office of Research and Development
MultiYear Plan for Endocrine Disruptors

• **Focus Area A**
  Development/standardization of protocols to identify endocrine disrupting chemicals in the environment

• **Focus Area B**
  Long-term consequences of developmental exposure to endocrine disrupting chemicals - gonadal steroids and thyroid hormones

• **Focus Area C**
  Cumulative risk/mixtures of endocrine disrupting chemicals
  Extrapolation across species
NHEERL and STAR Thyroid Projects

Complex Mixtures of Thyroid Hormone Disruptors: Mechanisms and Predictive Modeling. Intramural Research in NTD and ETD

Low Dose Thyroid Hormone Insufficiencies and Neurological Outcomes in Rodent Models. Intramural Research in NTD

Endocrine Disrupting Chemicals and Thyroid Outcomes – Exposure-Effect Studies in Humans. STAR Grant to Dr. Henry Anderson, Wisconsin Dept Health and Family Services

Low Dose Effects of Thyroid Toxicants on Neurodevelopment – Mechanistic Studies. STAR COOP between NHEERL and Dr. Thomas Zoeller, U Massachusetts

Development of BBPK Model for the Thyroid Axis in Pregnant Rat and Fetus for Dose Response Analysis of Developmental Neurotoxicity. STAR COOP between NHEERL and Dr. Jeffrey Fisher, U Georgia
Thyroid Hormone Disruption & Neurological Dysfunction

• TH critical for brain development
  - Iodine Deficiency, Congenital Hypothyroidism

• Treatment CH still leads to subtle cognitive deficits
  (Rovet, 2000)

• Hypothyroxinemia – pregnant women with low T4, children
  ↑ incidence attention deficit disorder
  lower global IQ

  (Haddow et al., 1999; Allen et al., 2000)
Research Challenges

- Interaction in brain development is complex: Thyroid hormone serves different roles in different cells at different times.
- Many structurally diverse chemicals affect thyroid axis by interaction with a variety of targets.
- Unclear how much change is adverse. We know little at the low end of the dose-response function.
- Unclear which profiles of TH disturbance are predictive of adversity.
- Extrapolation from animal models to humans.
- Real world exposures are to complex mixtures of thyroid disrupting chemicals.
Target Sites and Early Biological Indicators
Structurally Diverse chemicals - Multiple Sites & Mechanisms

Hypothalamus
- TRH
  +
  T3 & T4

Pituitary
- T3 & T4
  +
  TSH

Thyroid Gland
- PTU, Mancozeb
- Thyroperoxidase
  T3 & T4
  I + tyrosine

Liver
- Dioxins
  Ah-Receptor
  T4
  UDPGTs
  T4-Gluc
  Biliary Excretion

Plasma/Blood
- Free-TH
- TTR/TBG
- Bound-TH

Peripherally tissues
- Deiodinase
  T4 > T3

CNS tissues
- Deiodinase
  T4 > T3

Xenobiotics?

Iodine

Perchlorate
Thiocyanate

Research Protecting the Nervous System in a Changing World
Induction of Liver Enzymes Reduces T4

Mixtures
A number of environmental contaminants induce liver glucuronidation of T4. These chemicals display distinct dose-response profiles.

Does additivity theory predict the effects of complex mixtures that induce metabolism of T4?

Species Extrapolation
Glucuronidation of T4 is differentially induced in rats and mice following exposure to chemicals.

What is the most appropriate model for extrapolation to humans?
Additivity Model predicted effects on T4 of mixtures of chemicals with common MOA at low doses, but underestimated effects at high doses.

What of mixtures with different MOA?
How do these effects extrapolate to humans?
Induction of Liver Enzymes Reduces T4

Mixtures
A number of environmental contaminants induce liver glucuronidation of T4. These chemicals display distinct dose-response profiles.

Does additivity theory predict the effects of complex mixtures that induce metabolism of T4?

Species Extrapolation
Glucuronidation of T4 is differentially induced in rats and mice following exposure chemicals.

What is the most appropriate model for extrapolation to humans? Develop reporter gene assays in hepatocytes from mouse, rat, human.
BIOLOGICALLY-BASED DOSE-RESPONSE MODEL

Exposure → Target Dose → Early Biological Effect → Altered Structure/Function → Clinical Disease

Administered Dose → Thyroid → Liver → Blood → Brain

- TH Synthesis
- Induced TH Catabolism
- Serum Transport
- TH Receptor Activation

↓ TH →

- TH-dependent Signaling, Gene Transcription
- Regulated Protein Expression
- Structural Anomalies
- Biochemical Imbalances
- Synaptic Dysfunction
- Hearing Loss
- Learning Deficits

Early Biological Effect → Altered Structure/Function → Clinical Disease
Low Level Thyroid Disruption and Neurodevelopment

- Subclinical hormone reductions in pregnant women leads to IQ deficits in offspring
- Developing fetus and newborn are populations of concern
- Cognitive function is endpoint of concern
- Evaluation at low levels of hormone disruption is needed
Critical Factors Impacting Outcome: Magnitude, Timing and Duration of TH Insufficiency

Neuronal Histogenesis
Neuronal Migration

Neuronal Maturation
Dendritic Arborization, Synapse Formation

Oligodendrocyte Development
Proliferation, Migration, Maturation, Myelination

Cerebellar & Hippocampal Granule Cell
Proliferation, Migration, Maturation

Rat
0 5 10 15 20 25 30
Gestational Age in Days
5 10 15 20 25 30
Postnatal Age in Days

Human
0 3 6
Gestational Age in Months
0 3 6
Postnatal Age in Months

Adapted from Anderson et al. (2003) Thyroid, 13:1039-1056
Timing and Duration are Important Elements for Consideration

- **Prenatal** hypothyroidism – errors in migration? Functional Implications?

- **Postnatal** PV-Immunohistochemistry – altered neuronal phenotype? Functional Implications?

- **Postnatal** Cochlear Development – hair cell loss Functional Implications?
Prenatal: Cortical Malformations

Cortical Malformation

Dose-Dependent

Neuronal

Persistent TH-Dependent
Postnatal: Parvalbumin Expression

Density PV-IR Neurons in Hippocampus

- DG
- CA1
- CA3

0 ppm (n=5)
3 ppm (n=5)
10 ppm (n=6)

*Significant differences

Hippo

Cortex

A
B
C
D
E
F

Interpulse Interval (msec)

Pulse 2/Pulse 1 x 100

Mean +/- SE Cell Density X 10^-6

Density PV-IR Neurons in Hippocampus

Early Inhibition (100 mV)
Facilitation (70 mV)
Late Inhibition (150 mV)

20% Max

Interpulse Interval (msec)
Postnatal: Cochlear Damage

Postnatal PCB exposure reduces T4, induces hair cell loss in the basal turn of the cochlea

Low frequency hearing loss is evidenced by behavioral measures of acoustic startle reflex
Dose-Response Relationships: Thyroid Hormones and Hearing Loss

Developmentally-induced reductions in T4 on PN15 are predictive of hearing loss.
Sensitivity of Cognitive Endpoints?

Cellular Models

Behavioral Models
Hippocampal Synaptic Transmission is Impaired

Normalized Spike vs EPSP

Normalized Population Spike

Normalized Spike vs EPSP
- 0 ppm
- 3 ppm
- 10 ppm

Research Protecting the Nervous System in a Changing World
Synaptic Plasticity is Impaired

- Long term potentiation, a cellular model of learning and memory, is reduced in a dose-dependent manner.

[Graph showing EPSP slope and population spike with data points for different concentrations and time post training.]
Hippocampal Learning is Impaired

Morris water maze acquisition and reversal learning are impaired in adult offspring.

Trace fear conditioning is impaired in adult offspring.
STAR Thyroid Projects

Endocrine Disrupting Chemicals and Thyroid Outcomes – Exposure-Effect Studies in Humans. STAR Grant to Dr. Henry Anderson, Wisconsin Dept Health and Family Services

Low Dose Effects of Thyroid Toxicants on Neurodevelopment – Mechanistic Studies. STAR COOP between NHEERL and Dr. Thomas Zoeller, U Massachusetts

Development of BBPK Model for the Thyroid Axis in Pregnant Rat and Fetus for Dose Response Analysis of Developmental Neurotoxicity. STAR COOP between NHEERL and Dr. Jeffrey Fisher, U Georgia
Exposure-Dose-Response Model

**Pharmacokinetic Effort**

- PBPK
- Administered Dose
- Target Dose

**Pharmacodynamic Effort**

- BBDR Thyroid axis
- Describes the perturbations in the thyroid axis via MOA.
  - U Georgia COOP
  - U Mass COOP
  - NTD/ETD

- BBDR Developmental Neurotoxicity
- Describe alterations in CNS development due to altered hormones
  - U Mass COOP
  - NTD

- Simulate exposure-induced changes in thyroid dosimetrics associated with CNS toxicity.
  - U Georgia COOP
Low Dose Effects of Thyroid Toxicants on Neurodevelopment – Mechanistic Studies.

EPA STAR COOP
Dr. Thomas Zoeller
U Massachusetts
Development of BBPK Model for the Thyroid Axis in Pregnant Rat and Fetus for Dose Response Analysis of Developmental Neurotoxicity

EPA STAR COOP
Dr. Jeffrey Fisher
University of Georgia