S-10

Risk Assessment of Thyroid Hormone Disrupter and Mixtures in Marine Biota

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ABSTRACT

Varieties of chemicals alter thyroid hormones (THs) in vertabrates. The importance of THs during neurodevelopment, suggest that these chemicals would likely be developmental neurotoxicants. A number of epidemiological studies have demonstrated associations between exposure to polychlorinated hydrocarbons, such as PCBs and dioxins, and alterations in thyroid homeostasis and neurodevelopmental delays. Ecological studies have also observed associations between environmental pollutants and thyroid hormone disruption in mammals, birds, and fish. However, the effect of thyroid disruption on population levels in ecological systems is uncertain. Several levels of uncertainty affect our understanding of the point of departure, mode-of-action, and mechanism-of-action of xenobiotics that alter THs. One uncertainty is the impact of species differences in the pharmacokinetics of THs. In rodents, the serum half-life of T4 is approximately hours whereas in humans it is 4-7 days. In humans, serum is a major storage pool for thyroid hormones. In rodents, the major storage pool is the thyroid gland. In rats, glucuronidation is a major deactivation pathway while in humans, deiodination and sulfation is more important. These differences may result in altered sensitivity to environmental chemicals. Another uncertainty is species differences due to altered sensitivity to the mechanisms of action of xenobiotics; e.g., induction of UDPGT, which increases elimination of THs, is mediated in part by CAR and PXR pathways. There are species differences in the structure activity relationship for activation of these pathways that could lead to altered species sensitivity to xenobiotics. Another uncertainty is the effects of exposure to multiple thyroid hormone disruptors. Recent studies in rodents demonstrate that exposure to mixtures of thyroid hormone disruptors has the potential for synergism at high exposures. Future studies aimed

at examining mixtures of thyroid hormone disrupters in multiple species would aid in our understanding of the potential adverse health and ecological effects of thyroid hormone disruptors.

EDUCATION

1983	Drew University Chemistry B.A.
1988	Rutgers, The State University of New Jersey Toxicology M.S.
1992	Rutgers, The State University of New Jersey Toxicology Ph.D.

EXPERIENCE

1995-2002	Toxicologist, Pharmacokinetics Branch, National Health & Environmental Effects Research Laboratory, US Environmental Protection Agency
2002-present	Branch Chief, Pharmacokinetics Branch, National Health & Environmental Effects Research Laboratory, US Environmental Protection Agency
2002-2003	North Carolina SOT Councilor
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PUBLICATION

1. Hurst HH, DeVito MJ, Setzer RW, and Birnbaum, LS. Acute administration of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) in pregnant Long Evans rats: Association of measured tissue concentrations with developmental effects. *Toxocol. Sci.* 53(2):411-20, 2000
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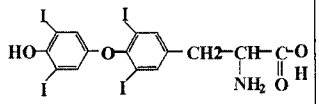
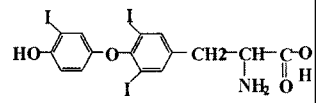
What are Thyroid Hormones

Principle Hormone released by the thyroid gland

Thyroxin (T4)



Triiodothyronine (T3)



What are the Physiological Roles of Thyroid Hormones

- Act on the Thyroid Receptor (TR)
- Regulate lipid and carbohydrate metabolism
- Necessary for normal growth and maturation
  - Not essential, but without the thyroid
    - Mental and physical retardation (developmental)
    - mental and physical slowing (adult)
    - poor resistance to cold

Main Concern is Developmental Exposures

- Developmental hypothyroidism or hypothryoxinemia are linked to irreversible neurological deficits in humans
- Developmental toxicity studies are time consuming and expensive
- The USEPA has to develop screens for endocrine active chemicals including thyroxicants

Thyroid Hormone Disruption in Wildlife

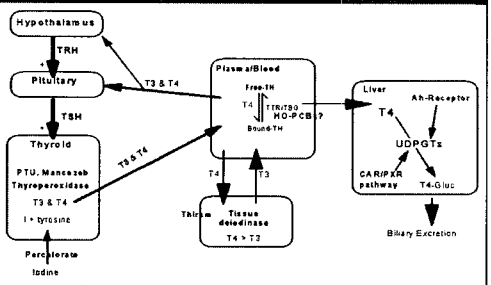
Species	Chemical	Location	Reference
Polar Bears	PCBs	Svalbard, Norway	Braathen, et al., 2004
Harbor Porpoise Beluga Whale	PCBs, OCs	St Lawrence Estuary, Canada	De Guise et al 1995
Northern Elephant Seal	PCBs	California, USA	Beckman et al 1997
Harbor Seal	PHAHs	North Sea	Schuumcher et al 1993
Cricket Frogs	Perchlorate	Texas, USA	Theodorakis et al 2006
Stonerollers (Campostoma anomalum)	Perchlorate	Texas USA	Theodorakis et al 2006
Mummichogs (Fundulus heteroclitus)	Unknown (Dioxins, PCBs)	New Jersey, USA	Zhou et al., 2000
Herring Gulls	PHAHs	Great Lakes, USA/Candada	Moccia et al 1986
Common Turn	Dioxins, PCBs	Belgium/Netherlands	Bishop et al., 1990

Evidence of Thyroid Hormone Disruption in Humans

Population	Chemicals	Location	Reference
Adult Male fish-eaters	PCBs	Great Lakes, USA/Canada	Turyk, et al., 2006 Persky et al., 2001
General Population	HCB	Spain	Sala et al 2001
Children	PCBs	Germany	Osius et al 1999
Pregnant Women and infants	Dioxins/PCBs	Netherlands	Koopman-Esseboom et al, 1994
Yusho	PCBs/Dioxins	Yusho, Japan	Murai et al., 1987
Pregnant Women General Population	PCBs	Taiwan	Wang et al 2005

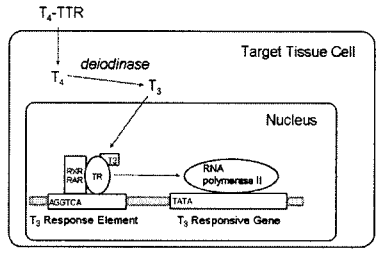
Hypothalamic-Pituitary-Liver Axis

Cumulative Risk / Species Differences



Structurally diverse chemicals decrease TH through their actions at diverse sites and through *multiple mechanisms*.

Thyroid Hormone Receptors and Thyroid Response Elements



Possible Targets for Environmental Chemicals

- **Thyroid Gland**
  - **→uptake process – perchlorate, bromate, chlorate**
  - **→organification – amitrole, PTU**
  - **→release - lithium**
- **Plasma Transport Proteins – OH-PCBs**
- **Tissue Deiodinases – OH-PCBs, mancozeb**
- **Hepatic Catabolism – Dioxins, PBDEs, PCBs, DDE, pesticides**

Species Differences in Mode of Action

Mode of Action	Prototype Chemical	Species Differences
Iodide Uptake	Perchlorate	No known significant species differences
Synthesis inhibition	PTU	Primates may be less sensitive than rodents
Serum Transporters	Hydroxy PCBs	TTR vs TBG
Hepatic Catabolism	AhR, PXR, CAR PPAR, BXR agonists	• SAR for receptor binding • Role of UGTs
Deiodinase inhibitors	FD&C red dye	Uncertain
Hepatic transporters	PCBs? Pesticides?	uncertain

Species Differences in Serum Transport Protein

Species	Thyroid binding Globulin	Transthyretin	Albumin
Mammals			+
humans	++	+	+
monkeys	++	+	+
cattle	++	++	+
dogs	+	+	+
cats	+	+	+
rats		++	+
mice		++	+
Aves		++	+
Reptile		++	+
Amphbia		++	+
Pisces		++	+

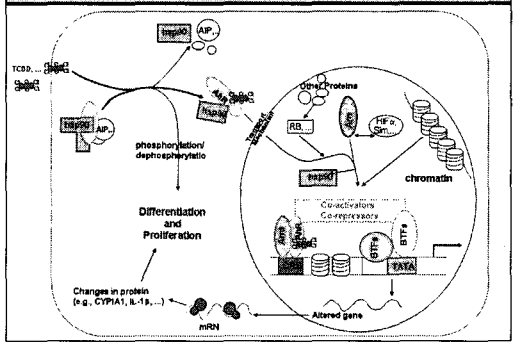
Species Differences in Hepatic Thyroid Hormone Catabolism

- Glucuronidation vs Sulfation
  - Humans use sulfation
  - Glurcuronidation more important for most other species
- Nuclear Receptors
  - SAR differences for ligand activation
- Transporters
  - Recent evidence in rodents suggest that some chemicals increase T4 secretion into the bile, thus lowering serum hormones

Nuclear Receptors

- Ligand activated transcription factors in the orphan receptor family
- Promiscuous receptors
- Control xenobiotic metabolizing enzymes and other genes
- Regulate UGTs and transporters
- Species differences in ligand activation
- Includes
  - Pregnane X Receptor (PXR)
  - Constitutive Androstane Receptor (CAR)
  - Benzoate X Receptor (BXR)
  - PPAR – peroxisome-proliferators activator receptor

Mechanism of Action of Dioxin



What about species extrapolations?

Hypothesis

- Decreases in serum T_4 concentrations are related to increased T_4-glucuronidation in both C57BL/6J mice and Long Evans rats following administration of PCB 126 and PCB 153

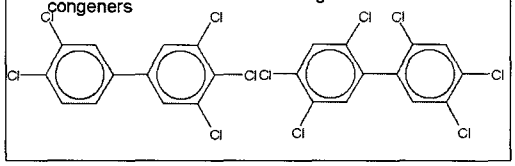
Two Specific PCB Congeners

PCB 126

- TCDD-like
- Activates aryl hydrocarbon (Ah) receptor
- One of most toxic congeners

PCB 153

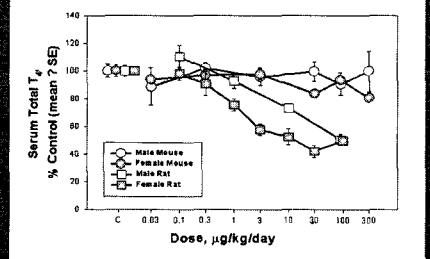
- Phenobarbital-like
- Acts through a phenobarbital response unit (PBRU)
- One of most abundant congeners



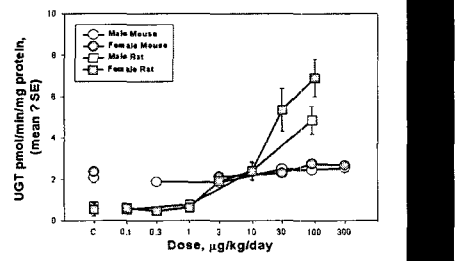
Dosing Protocol

- Young adult male and female Long-Evans rats and C57BL/6J mice
- 4-day dosing regimen
- Animals sacrificed 24 hours after last dose
  - → serum and livers collected

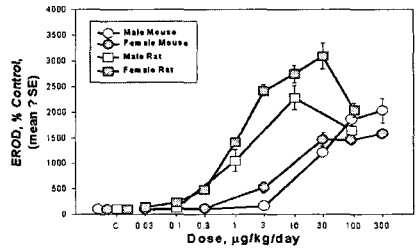
PCB 126: T_4 Data



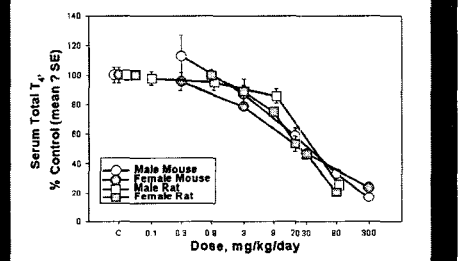
PCB 126: UGT Activity



PCB 126: EROD Activity



PCB 153: T_4 Data



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Results Summary

T_4	*T4UGT*
▪ PCB 126 → No effect in mice decreases in rats	▪ PCB 126 → No effect in mice increased in rats
▪ PCB 153 → Decreased in rats and mice	▪PCB 153 → Increased in rats and mice

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Are the effects of multiple thyrotoxicants additive

- A number of environmental toxicants decrease thyroid hormones in experimental animals
- What are the effects of exposure to multiple thyrotoxicants

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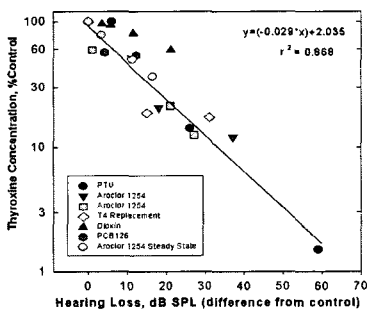
The Relationship between serum thyroxin concentrations in neonates and hearing loss (Crofton, 2005)



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Experimental Design for Mixtures Study

- Short-term assay
  - Long Evans Female Rats (28 days old)
  - Dose for 4 days and kill animals on day 5
  - Determine serum T4 and T3 and hepatic enzyme activities
- Focus on dioxins and non-dioxin-like PHAHs (Dioxins, Dibenzofurans and PCBs)
  - collect individual dose response information
  - prepare and test a mixture of these chemicals

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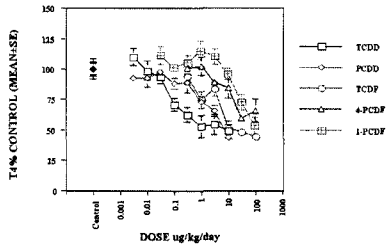
The effects of PCDDs and PCDFs on Serum T4



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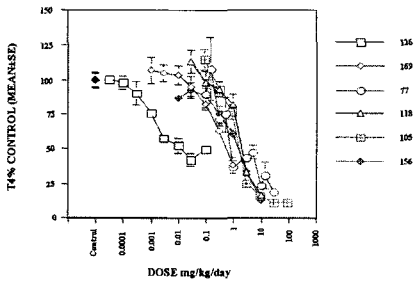
The Effects of Dioxin-like PCBs on Total Serum T4



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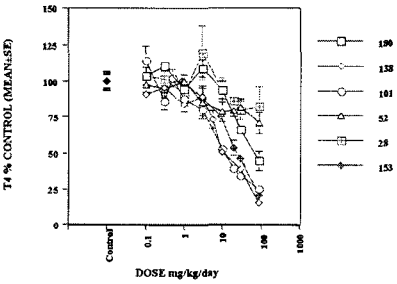
The effects of Non Dioxin-Like PCBs on Serum T4



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Mixture Composition

Chemical	Daily Dose (ug/kg)	Relative to TCDD
TCDD	0.013	1.0
PCDD	0.013	1.0
TCDF	0.019	1.5
1-PCDF	0.006	0.5
4-Pcdf	0.026	2.0
OCDF	0.06	4.7
77	1.9	147
105	77	6000
118	387	30000
126	0.6	47
138	387	30000
153	387	30000
156	13	1008
169	0.4	31
180	387	30000

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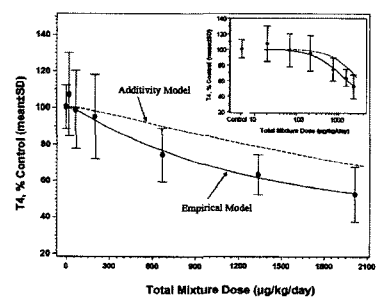

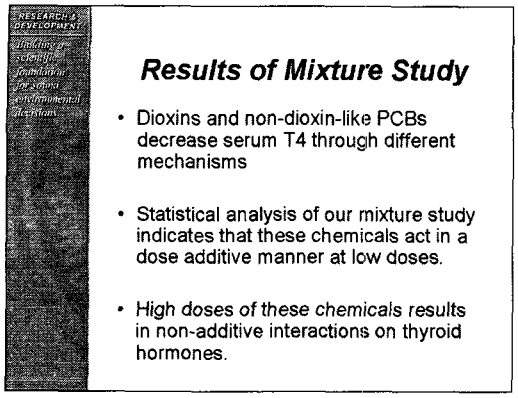
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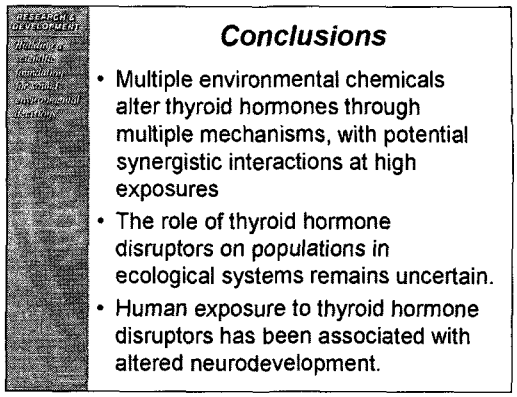
Results of Mixture Study
• Dioxins and non-dioxin-like PCBs decrease serum T4 through different mechanisms
• Statistical analysis of our mixture study indicates that these chemicals act in a dose additive manner at low doses.
• High doses of these chemicals results in non-additive interactions on thyroid hormones.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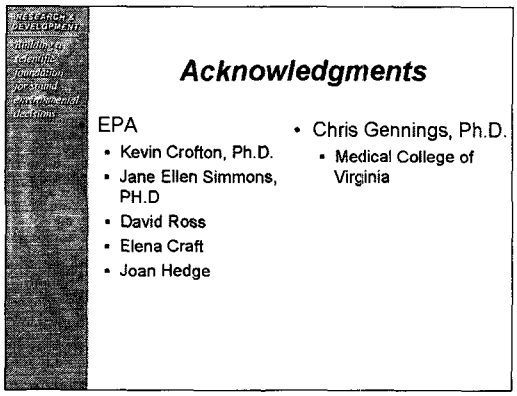
Conclusions
• Multiple environmental chemicals alter thyroid hormones through multiple mechanisms, with potential synergistic interactions at high exposures
• The role of thyroid hormone disruptors on populations in ecological systems remains uncertain.
• Human exposure to thyroid hormone disruptors has been associated with altered neurodevelopment.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