
# "The use of competition binding potency of thyroid transport proteins from *Larus argentatus* and *Larus hyperboreus* for ecotoxicology risk assessment"

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# Why thyroid hormones?

Only known iodine-containing compounds with biological activity

Developing animals and humans:

- critical determinants of normal development
- maintain metabolic homeostasis (virtually all organs)

It is formed in thyroid gland; metabolized in liver (mainly) and brain

Serum secretion precisely regulated by pituitary hormone

Predominant action: binding to nuclear thyroid hormone receptors, modulation of gene transcription; common mechanism of action as steroid hormones, vitamin D and retinoids

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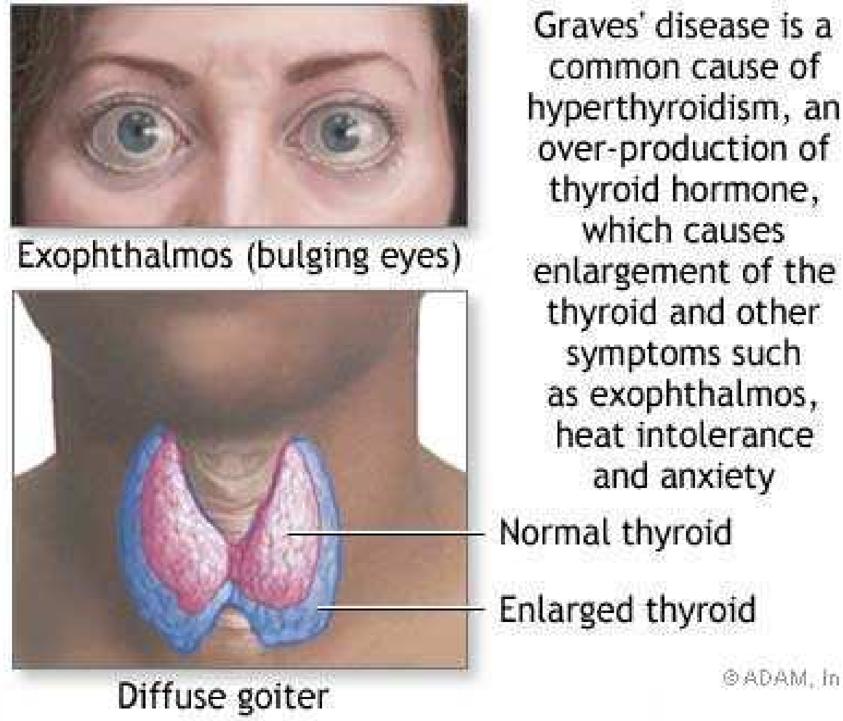
**thyroid elevation – hyperthyroidis (thyrotoxicosis, thyroid gland hyperfunction, Grave's disease)**




Goiter

Cretinism

**thyroid deficiency – hypothyroidism (e.g., congenital, goiter, mental retardation and cretinism)**

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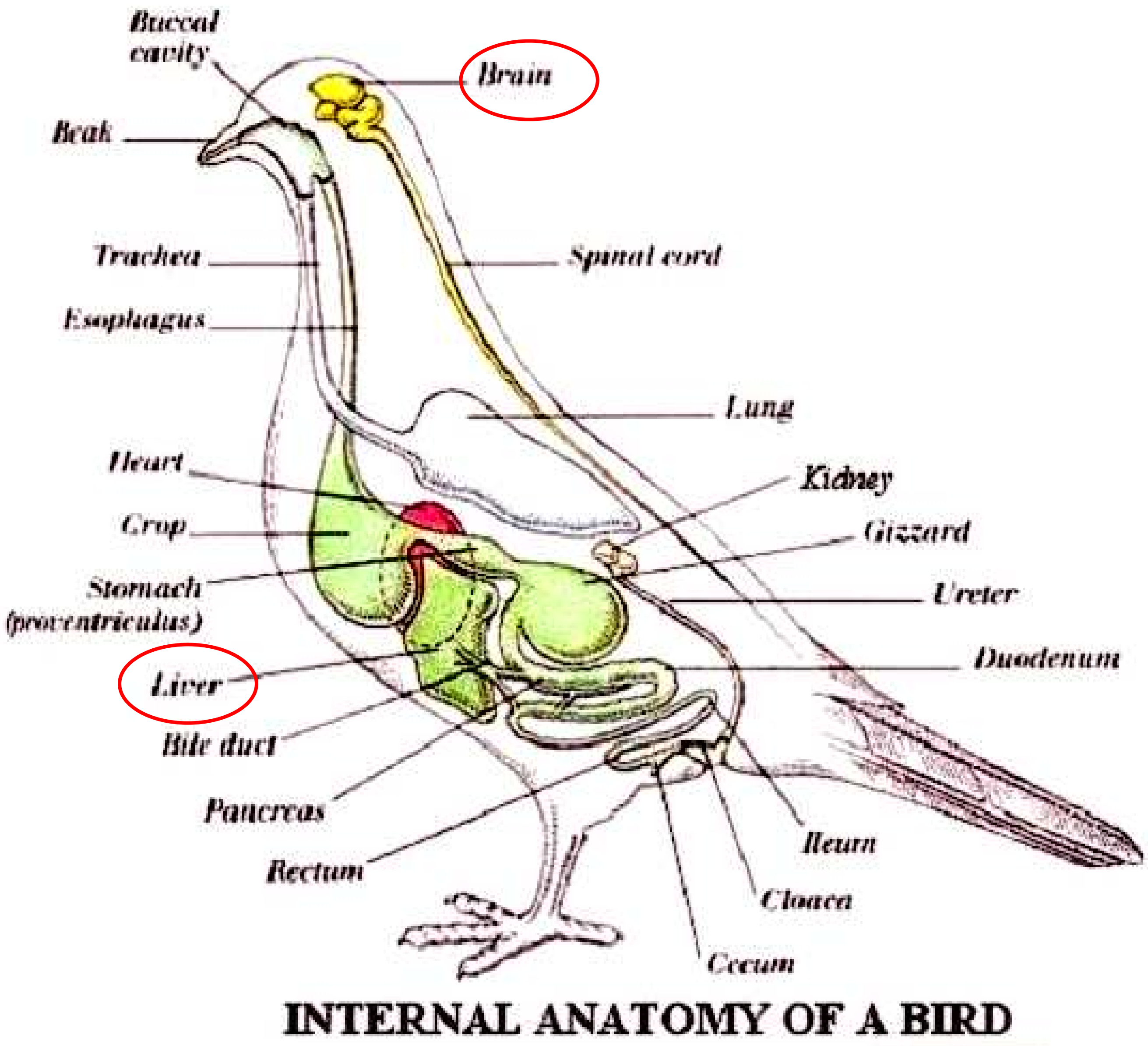


Thyroid hormones are required for the differentiation and development of sensory function, nervous system development and control functions, muscle and skeletal development, and integumentary development including feathering in birds.

They also control the obligatory heat production that is characteristic of homeothermic vertebrates

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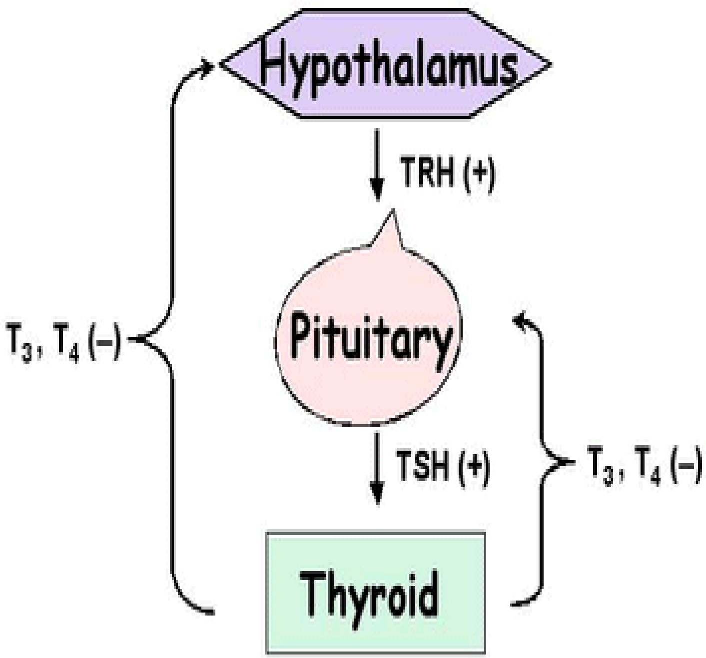
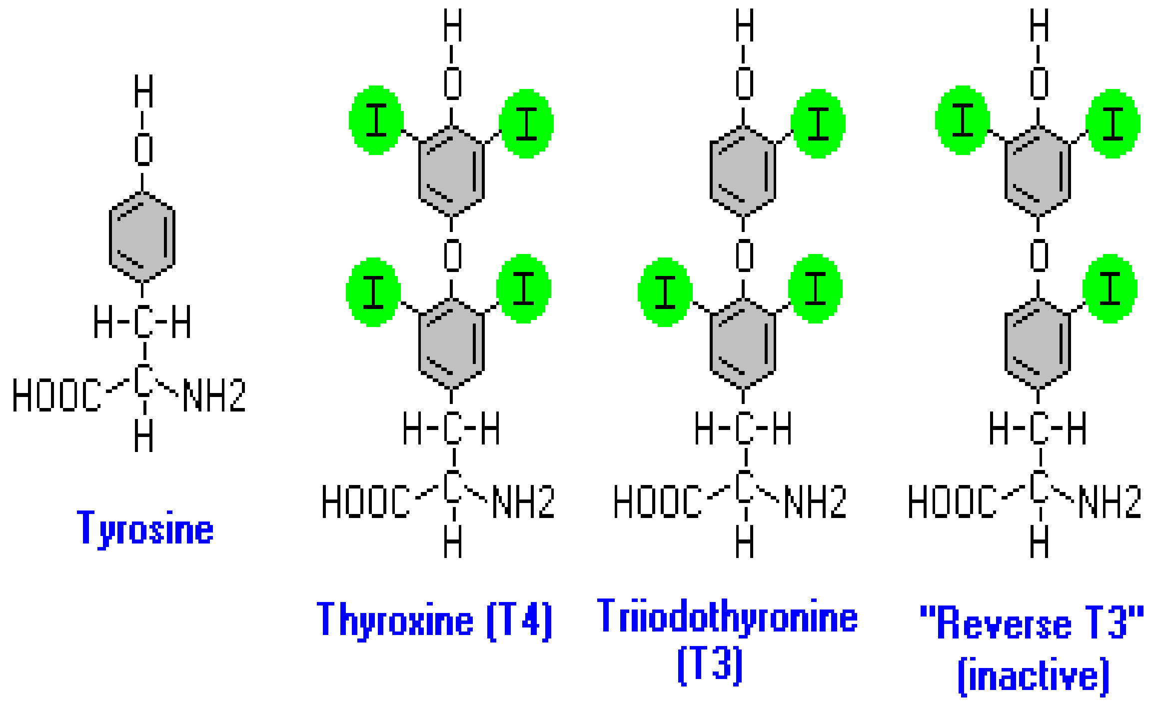
# Interconversion of Thyroid Hormones




(–) = inhibition; (+) = stimulation

**The structure of the thyroid hormones.**

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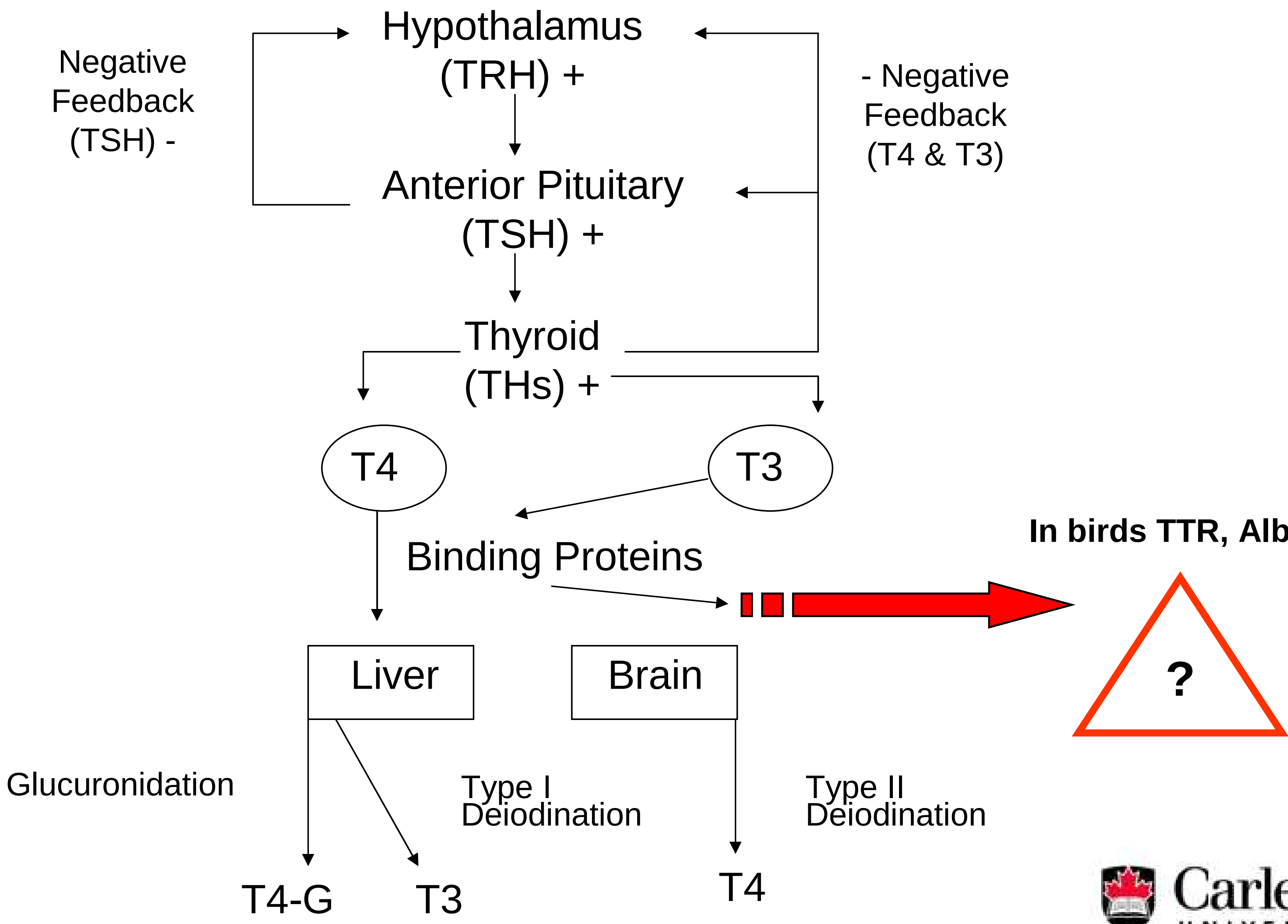
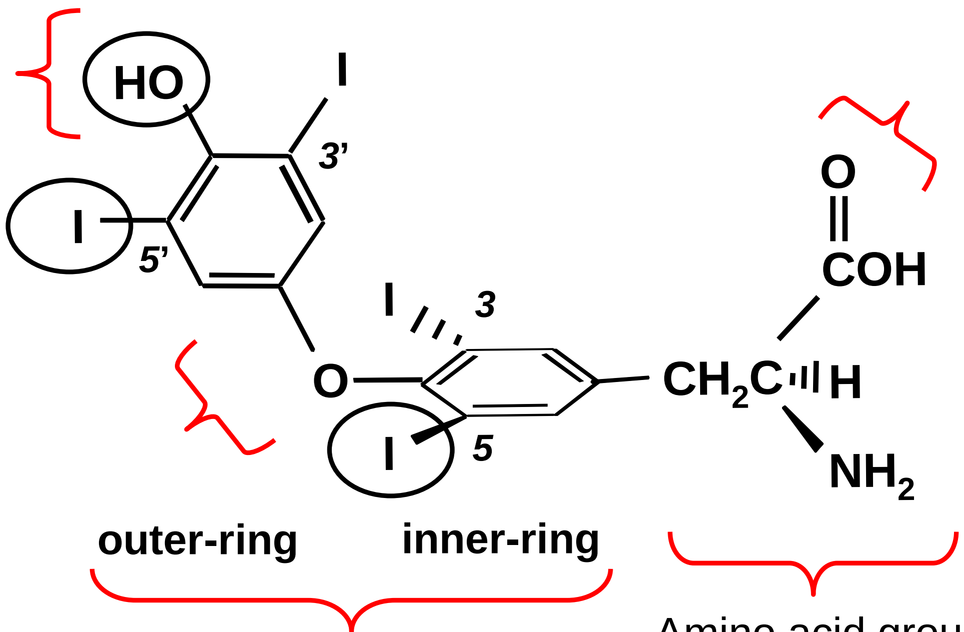


Negative Feedback (TSH) -
Hypothalamus (TRH) +
- Negative Feedback (T4 & T3)
Anterior Pituitary (TSH) +
Thyroid (THs) +
T4
T3
Binding Proteins
In birds TTR, Albumin
?
Liver
Brain
Glucuronidation
Type I Deiodination
Type II Deiodination
T4-G
T3
T4

# Metabolic Pathways for Thyroid Hormones

Phenolic group:
**<u>Conjugation</u>**
(glucuronidation, sulfation)

Carboxylic group:
**<u>Oxidation decarboxylation</u>**
(tetaiodothyroacetic acid, triiodothyroacetic acid)

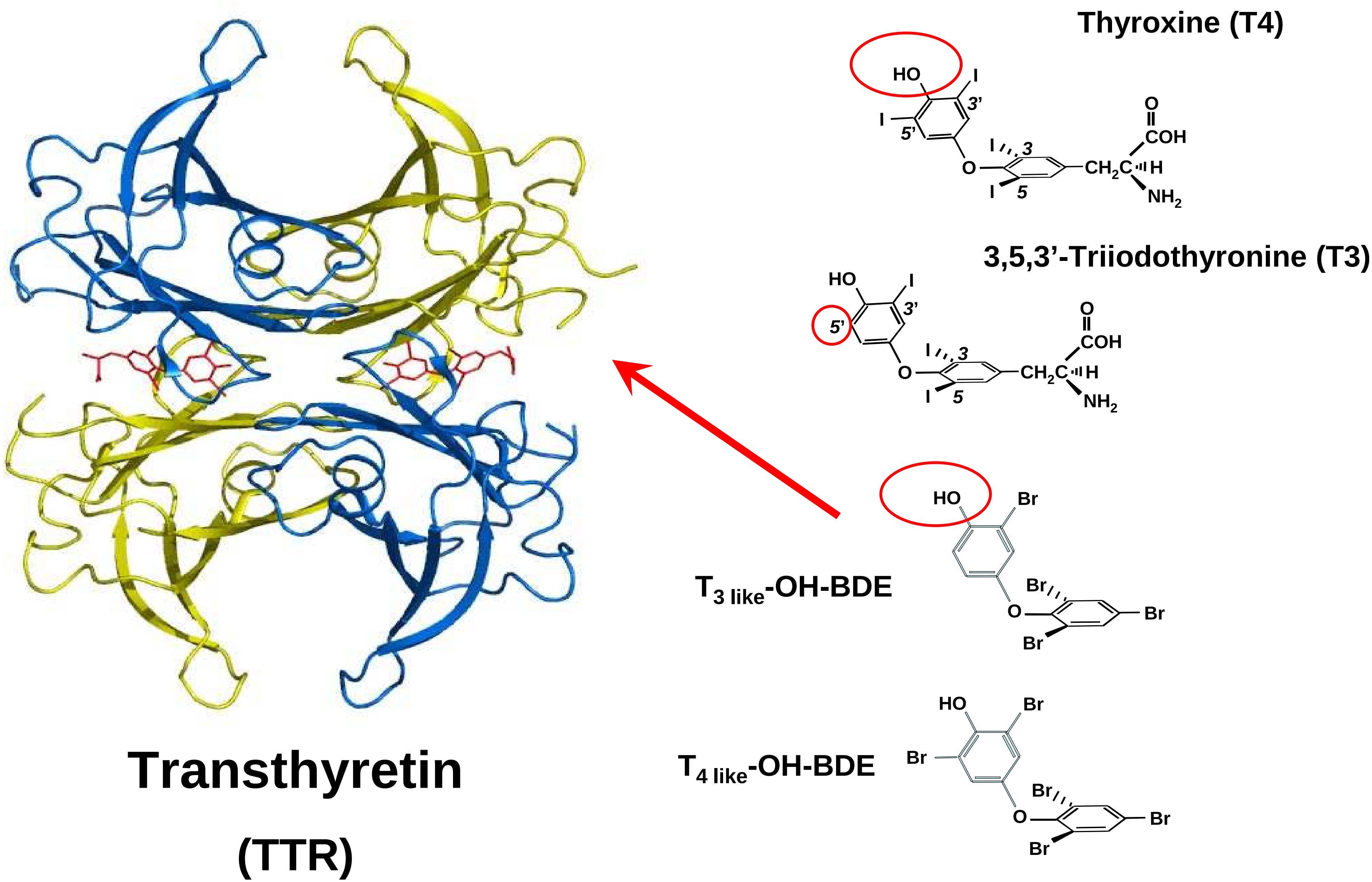



Ether linkage:
**<u>Ether cleavage</u>**
(monoiodotyrosone (MIT), diiodotyrosine (DIT))

**<u>Deiodination</u>**

Amino acid group

**e.g., Thyroxine (T4)**


Thyroxine (T4)
HO
I
3'
I
5'
I
3
O
COH
CH2C
H
I
5
NH2
3,5,3'-Triiodothyronine (T3)
HO
I
3'
5'
I
3
O
COH
CH2C
H
I
5
NH2
T3 like-OH-BDE
HO
Br
Br
O
Br
Br
T4 like-OH-BDE
HO
Br
Br
Br
O
Br
Br
Transthyretin
(TTR)

## Localization of Glaucous & Herring gull habitat samples


**Why?**

- Contrasting exposure and well represented ecosystem, top predators,
- Transport Thyroid Hormones = homeostasis,
- PCBs, PBDEs have potential to modulated the TH homeostasis *in vitro* studies with vertebrata, and have been reported in these sites
- lack of documentation in the transportation of thyroid hormones

# Rationale

- Traditionally human TTR has been used to assess risk studies in birds and other animals.
- Humans have 3 transport proteins, birds have 2.
- This study will address some basic questions about transport proteins and their possible disruption by a selected set of currently detected organohalogen compounds.
- A methodology to address ecotoxicological problems.
- The normal binding of transthyretin with thyroxine and triiodothironine in gulls of two different species and ecosystems.

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# Hypothesis

a) **Differences? –** Conservation of the TTR nucleotide sequence as inter-avian divergence among 2 predator avian models & between brain – liver.

b) **Relation? -** between OH's, Me O and TTR competition in binding T3 / T4 essential for avian homeostasis.

c) **Differences? -** in the T3 / T4 and human / bird TTR activity for proper assessment of toxicological risks.

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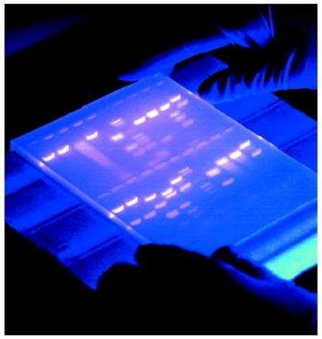
# AIM1-Methodology: Cloning and Sequencing TTR

**Herring gull and Glaucous gull**

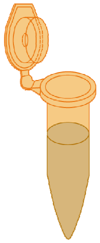
**Frozen samples from Brain and Liver**

**Obtaining RNA From Frozen Tissues, using TRIZOL Reagent**

**cDNA from RNA Using iScript cDNA Synthesis Kit**

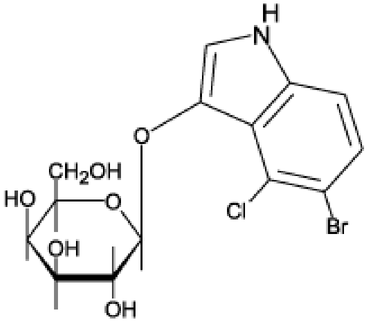


**DNA extraction from GEL using QIAEX IIc**

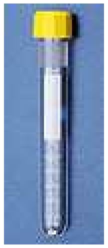


**T4 ligase + buffer, Mg2, Salt ATP, Water overnight**

**Prepare Petri Dish with X-gal**




**Select white colonies and transfer to Tube with LB media to let them grow overnight at 37℃ shaking**



**Plasmid Isolation by Genelute Plasmid Miniprep KIT (SIGMA)**

**DNA Sequencing + Expression**

## RESULTS: Cloning

**Herring gull Liver TTR amino acid sequence**

SHGSVDSKCPLMVKVLDAVRGSPAANVAVKVFKKAADGSWQDFATGKTTEYGEIHELTTE
EQFVEGIYRVEFDTSSYWKGLGLSPFHEYADVVFTANDSGHRHYTIAALLSPFSYSTTAV
VSDPQE

**Glaucous gull Liver TTR amino acid sequence**

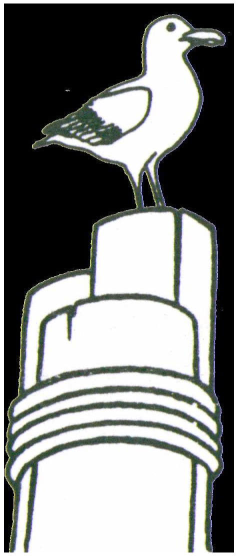
SHGSVDSKCPLMVKVLDAVRGSPAANVAVKVFKKAADGSWQDFATGKTTEYGEIHELTTE
EQFVEGIYRVEFDTSSYWKGLGLSPFHEYADVVFTANDSGHRHYTIAALLSPFSYSTTAV
VSDPQE

**Herring gull Brain TTR amino acid sequence**

SHGSVDSKCPLMVKVPDAVRGSPAANVAVKVFKKAADGSWQDFATGKTTEYGEIHELTTE
EQFVEGIYRVEFDTSSYWKGLGLSPFHEYADVVFTANDSGHRHYTIAALLSPFSYSTTAV
VSDPQE

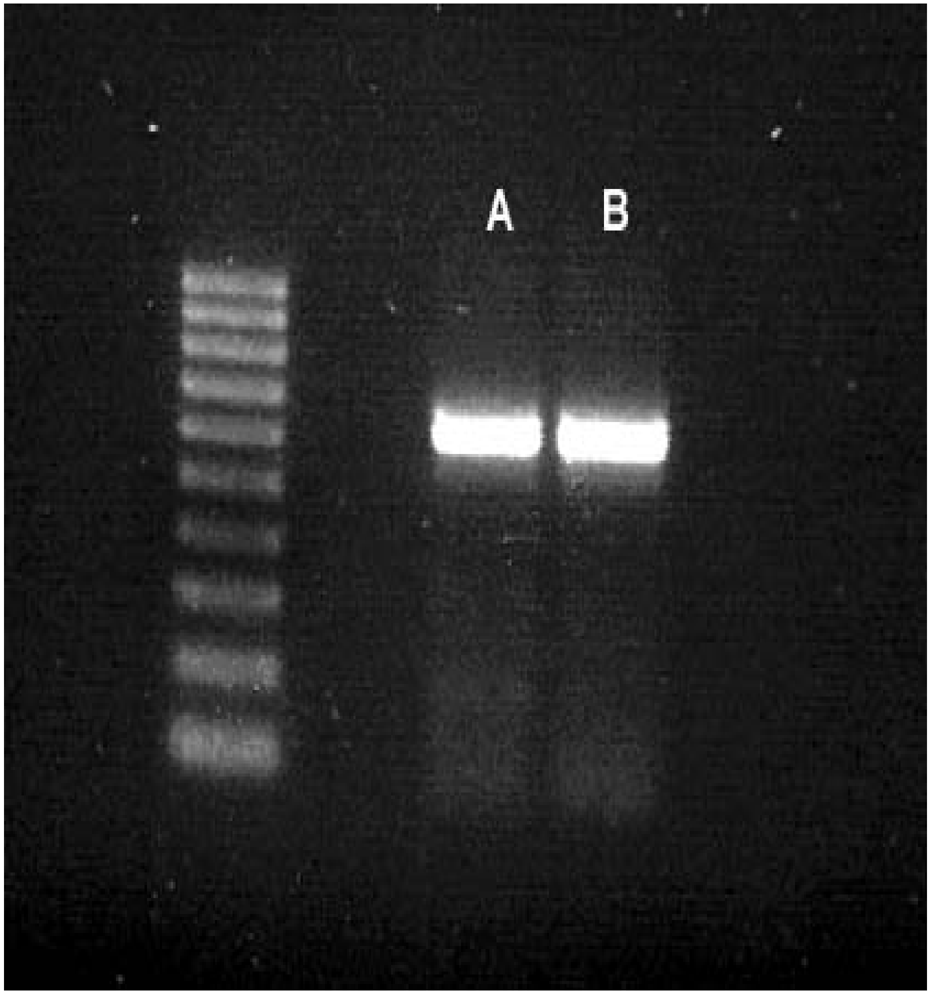
**Glaucous gull Brain TTR amino acid sequence**

SHGSVDSKCPLMVKVLDAVRGSPAANVAVKVFKKAADGSWQDFATGKTTEYGEIHELTTE
EQFVEGIYRVEFDTSSYWKGLGLSPFHEYADVVFTANDSGHRHYTIAALLSPFSYSTTAV
VSDPQE



- Comparing the length of hormone transport protein transthyretin in Brain (A) and Liver (B) tissues from Herring gull (*Larus argentatus*)

- BLAST DNA (cDNA) cloning comparison showed a large degree of nucleotide and amino acid sequence similarity (> 95%) between the brain and liver, and between the tissues from the two gull species but different with other species.

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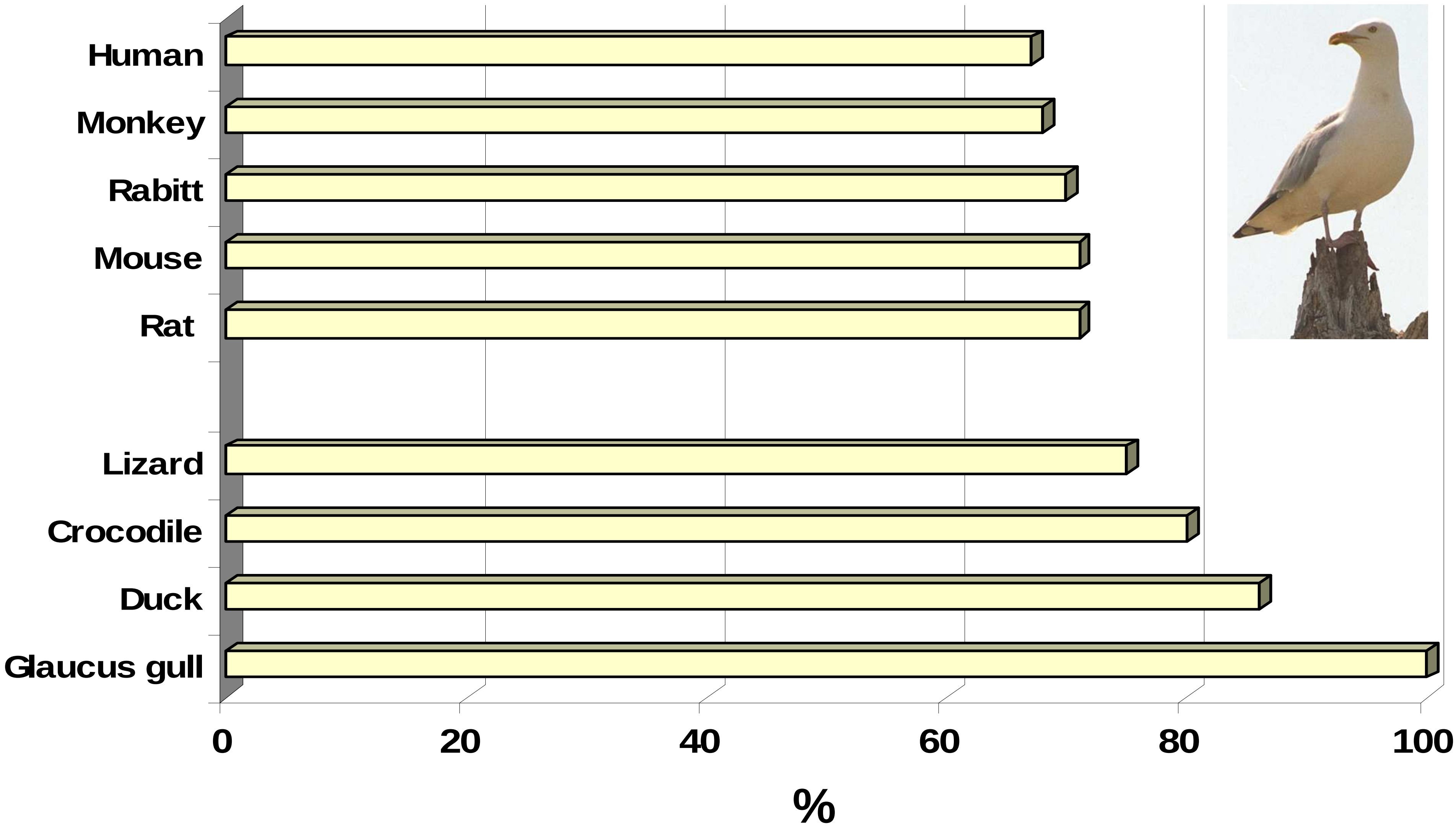
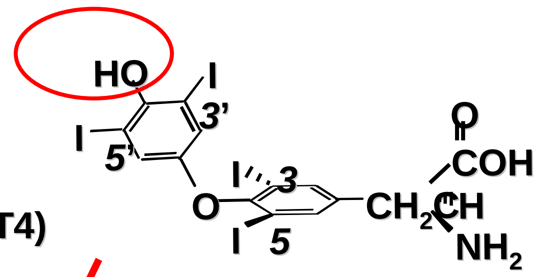
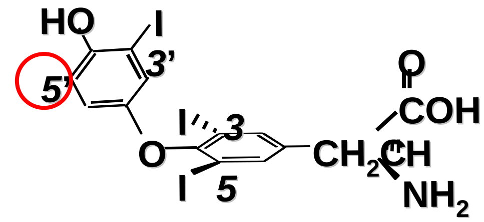
# TTR Comparison with other Species



# Methodology: Selecting Competitors for Binding Assay



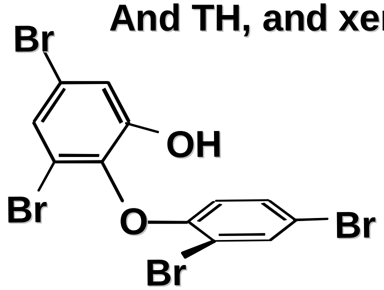
**Thyroxine (T4)**



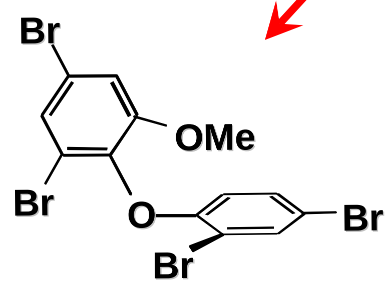
**3,5,3'-Triiodothyronine (T3)**

**Importance of the structure**
**And TH, and xenobiotics**

**Differences, THs-transport %**
**Birds / mammals / fish**



**6-OH-BDE47**
(3,5-dibromo-2-(2',4'-dibromophenoxy)phenol)



**6-MeO-BDE47**
(2-(2',4'-dibromophenoxy)-4,6-dibromoanisole)

- **Brominated flame retardants** (BFRs) replaced **Polychlorinated biphenyls** (PCBs) as the major chemical **flame retardant** in 1978 and are an effective **flame-retardant**

Bromine based flame retardants are applied to 2.5 million tons annually, with the annual consumption of PBDE’s alone being in excess of 40,000 metric tons.

PCBs:

CB101 (2,2',4,5,5')

**CB187 (2,2',3,4',5,5',6)**

OH-PCBs:

**4-OH-CB187 (2,2',3,4',5,5',6)**

4-OH-CB146 (2,2',3,4',5,5')

MeO-PCBs:

**4-MeO-CB187 (2,2',3,4',5,5',6)**

4-MeO-CB146 (2,2',3,4',5,5')

PBDEs:

**BDE47 (2,2',4,4')**

BDE49 (2,2',4,5')

OH-PBDEs:

**6-OH-BDE47 (2,2',4,4')**

3-OH-BDE47 (2,2',4,4')

**4'-OH-BDE49 (2,2',4,5')**

2'-OH-BDE68 (2,3',4,5')

MeO-PBDEs:

**6-MeO-BDE47 (2,2',4,4')**

3-MeO-BDE47 (2,2',4,4')

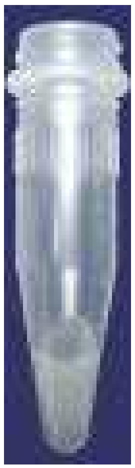
4'-MeO-BDE49 (2,2',4,5')

2'-MeO-BDE68 (2,3',4,5')

Polybrominated diphenyl ethers (**PBDE),**
Polychlorinated biphenyls (**PCBs**)

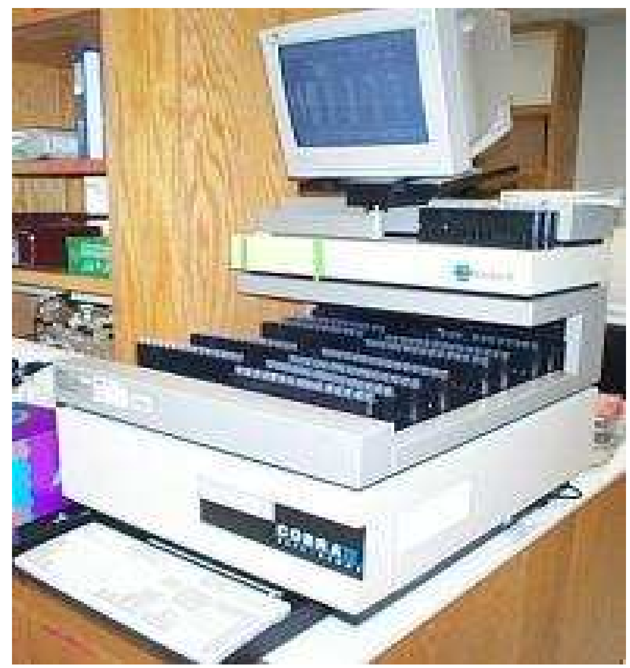
# AIM 2 and 3 : Competitive binding studies with TTR

**TTR 30 nM → Dissolve in 0.1M Tris-HCl, 0.1 nM NaCl**
**1 mM EDTA Buffer, pH 8.0**



**Incubate 125I-T4/T3**
**(70,000 counts/min) 55nM**
**In Tris-HCl Buffer**

**Competitors**
**Dissolve in 5uL at increasing concentrations**
***controls are 5uL of methanol**

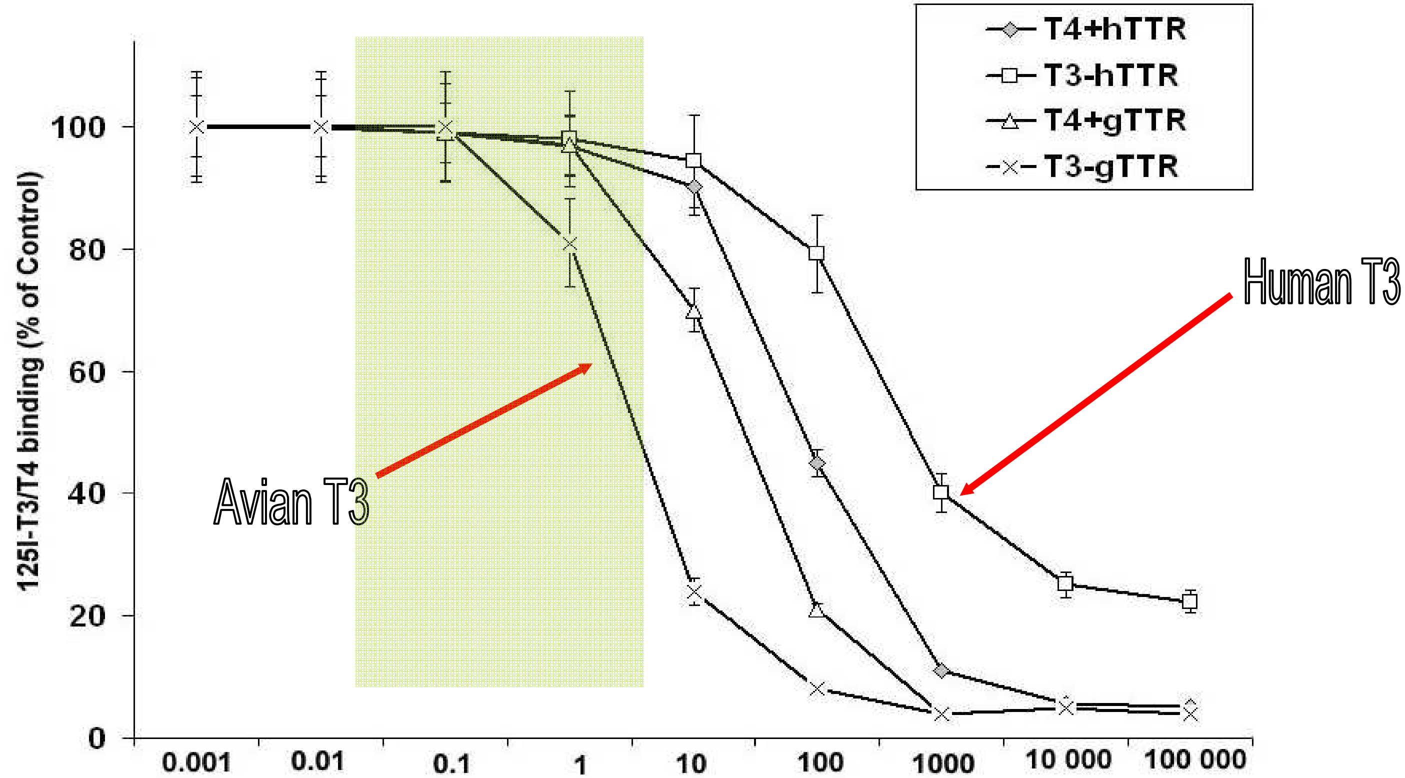


*Incubations are left for equilibrium overnight at 4 C*

**Total 125-I radioactivity (CPM)**
**X each incubation are checked by**
**gamma counting (T4-bind-TTR)**

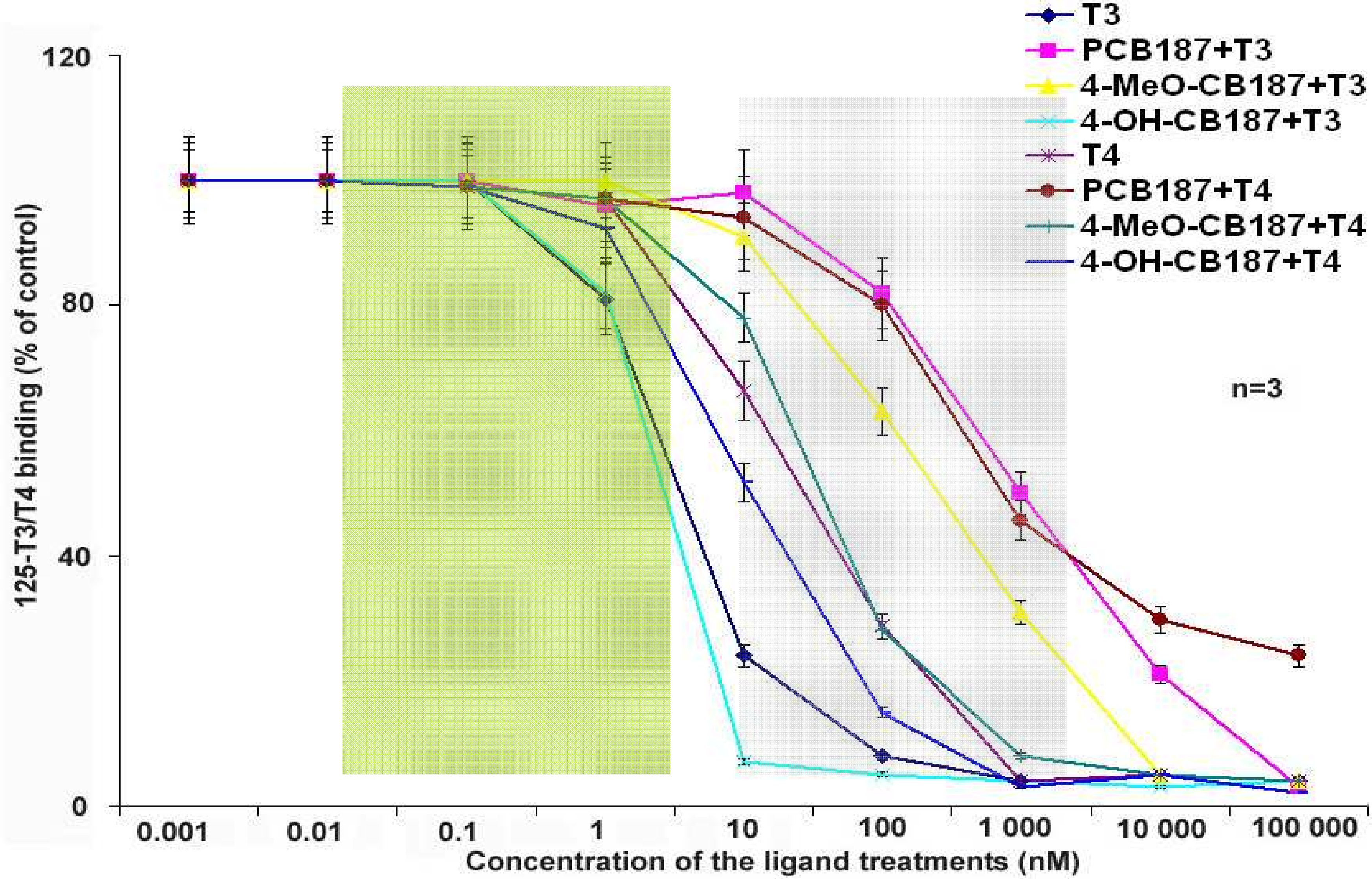
Methodology from:
Meerts *et al*,  2000. *Toxicol Sciences* 56: 95-104
Lans *et al*, 1993. *Chem-Biol Interact* 88:7-21

Concentration-dependent binding of humans transthyretin versus Herring gull transthyretin T3 andT4.

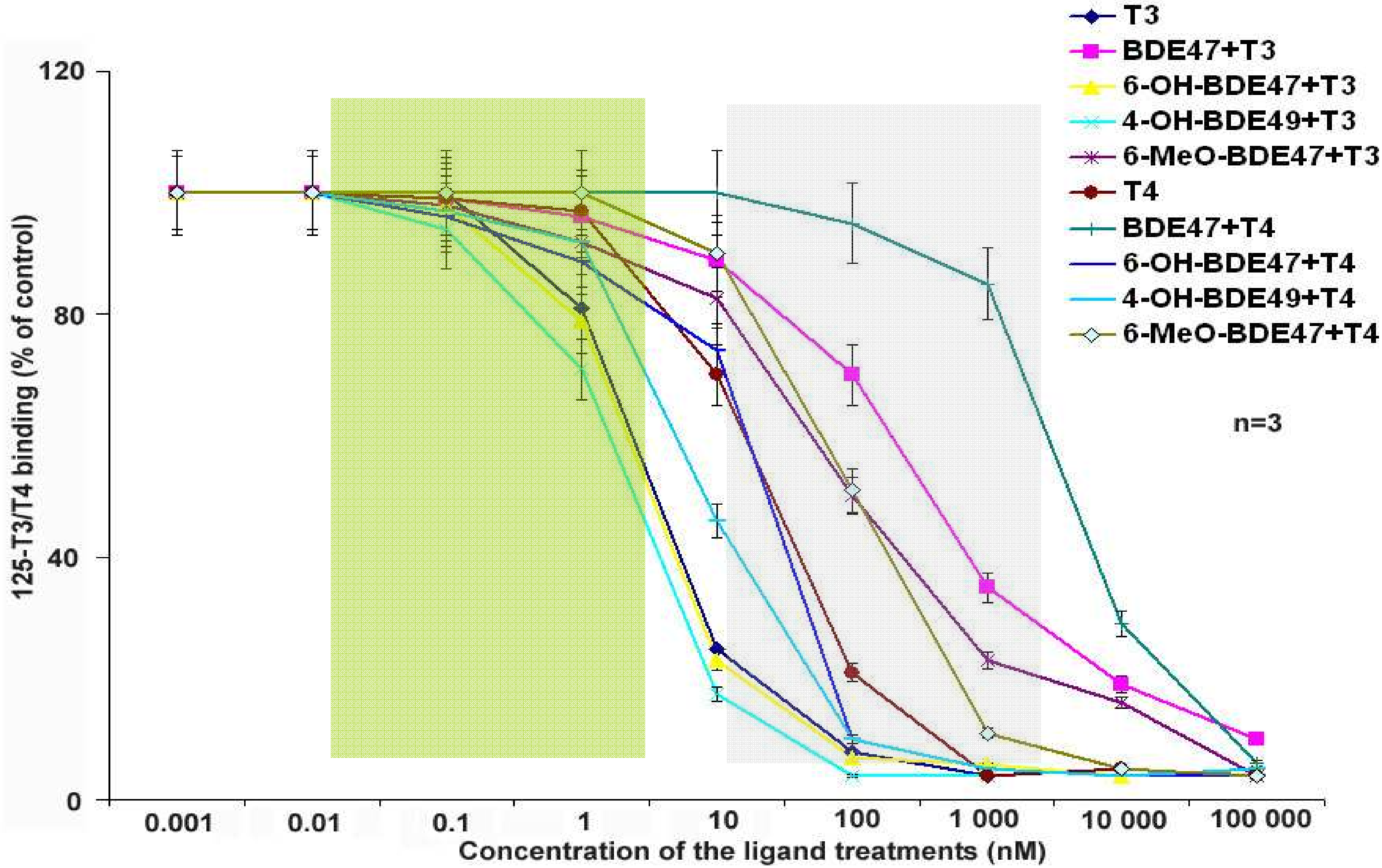



| | 1<br>IC50 (nM) | 2<br>Relative Potency | 3<br>Ka ($\times 10^{7}$ $M^{-1}$) | 4<br>Maximum competition (%) |
|---|---|---|---|---|
| Avian TTR | | | | |
| T3 | 7.9±1.3 | 1 | 4.38±0.6 | 96±4.3 |
| T4 | 60.3±3.5 | 1 | 3.9±0.4 | 96±4.3 |
| Human TTR | | | | |
| T4 | 85.1±1.3 | 1 | 2.09±0.53 | 98±2.1 |
| T3 | 1.05±17.3x$10^{3}$ | 0.08104 | 0.19±1.13 | 77.3±3.7 |

# Concentration-dependent competition binding curves of T3 or T4 displacement from gTTR (±SD, n=3, PCB. )



# Concentration-dependent competition binding curves of T3 or T4 displacement from gTTR (±SD, n=3, PBDE. )



## Discussion

- **Organohalogenated compounds, arising from either the industrial, medical or agricultural sector have been reported to interact strongly with plasma and thyroid hormones**

- **Gull TTR sequences and comparison to other species**

- **Despite the location and ecosystem differences between the two gull species and populations, the nucleotide and amino acid sequences for TTR in the liver and brain for both gull species were 100% identical.**

- **The homologous herring gull/glaucous gull TTR sequence is highly similar to other birds whose TTR sequence is known, e.g., Mallard Duck (*Anas platyrhynchos*) and Common Chicken (*Gallus gallus*).**

## Discussion

- **THHs bound to TTR is likely profoundly different between birds and mammals, and that such interactions would likely affect TH homeostasis differently. Furthermore, competitive binding studies with mammalian (human) TTR cannot be used as a surrogate to assess the effects on circulating THs in birds (or at least gull species).**
- **From the binding curves the calculated *K*a values demonstrated that binding affinity of T4 and T3 for gull TTR was higher than for human TTR. The IC50 concentrations for T4 and T3 also illustrated that binding potency was also greater for gull TTR relative to human TTR.**

# Conclusions

- Differences?

  Conservation of the TTR nucleotide sequence among brain and liver, and inter-avian (99%). No divergence among 2 predator avian models were found. Differences were found with other birds and human TTR.

- Relation?

  OH's, Me-O had significant differences binding TTR in competition with T3 / T4. A probably effect in avian homeostasis

- Differences?

  T3 / T4 had significant differences binding human & avian TTR. Cloned avian TTR is recommended for a more proper assessment of toxicological risks.

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# Participants and Acknowledgments


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