In Vitro Thyroid Hormone Metabolism

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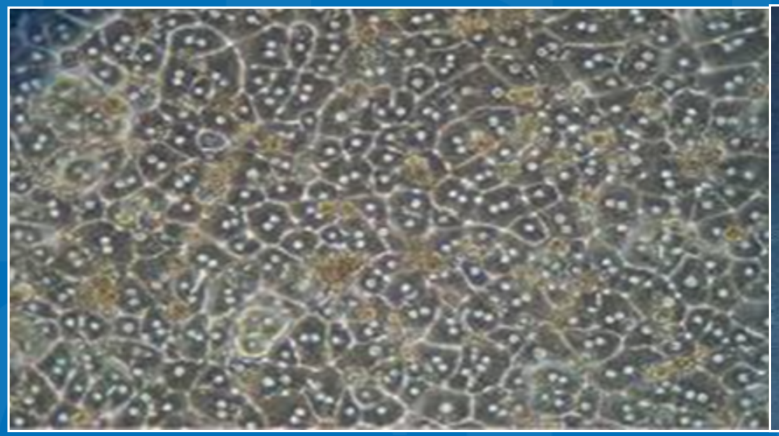

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November 15, 2018

Why the Concern Over Thyroid Hormone Disrupting Chemicals?

Thyroid hormones play a critical role in the developing nervous system.

Lack of THs result in adverse neurological development (sensory, motor, cognitive)

- Amphibians, birds, fish, and mammals
- Significant evolutionary conservation of thyroid hormones and neurodevelopment

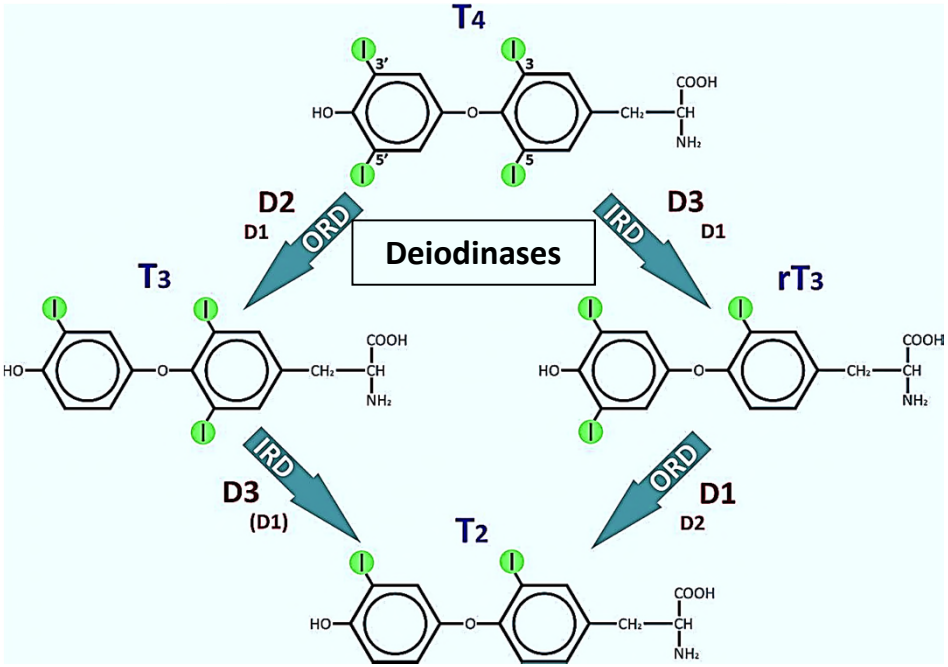
Major Pathways of Thyroid Hormone Disruption

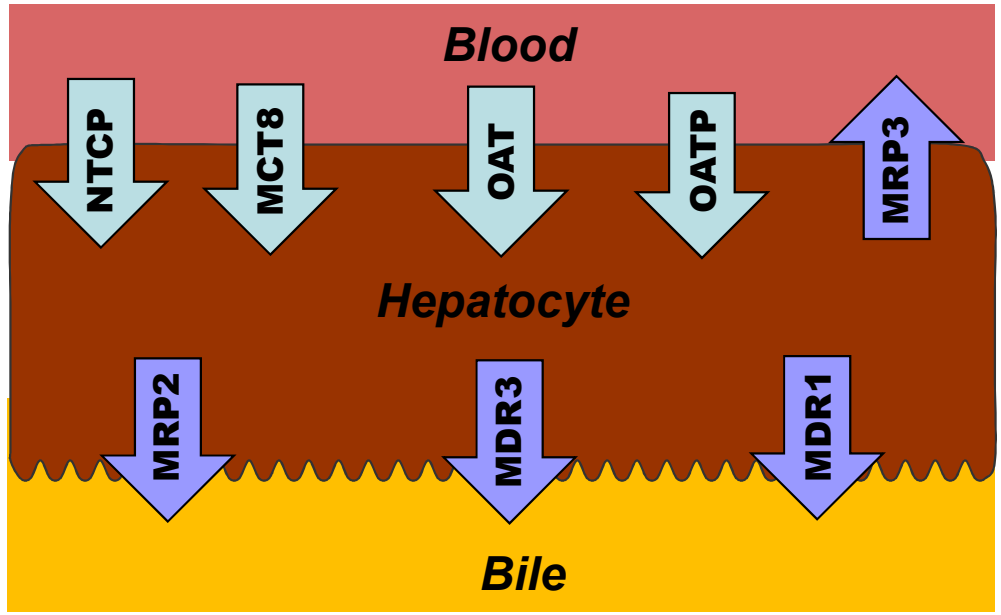
- Thyroid hormone synthesis
  - Iodide uptake
  - Thyroid Peroxidase
- Serum binding proteins
  - Transthyretin (TTR)
  - Thyroid Binding Globulin (TBG)
- Metabolism
  - Hepatic
  - Nuclear receptor activation

The Liver: A Site For Thyroid Hormone Metabolism

Major site of xenobiotic and thyroid hormone metabolism

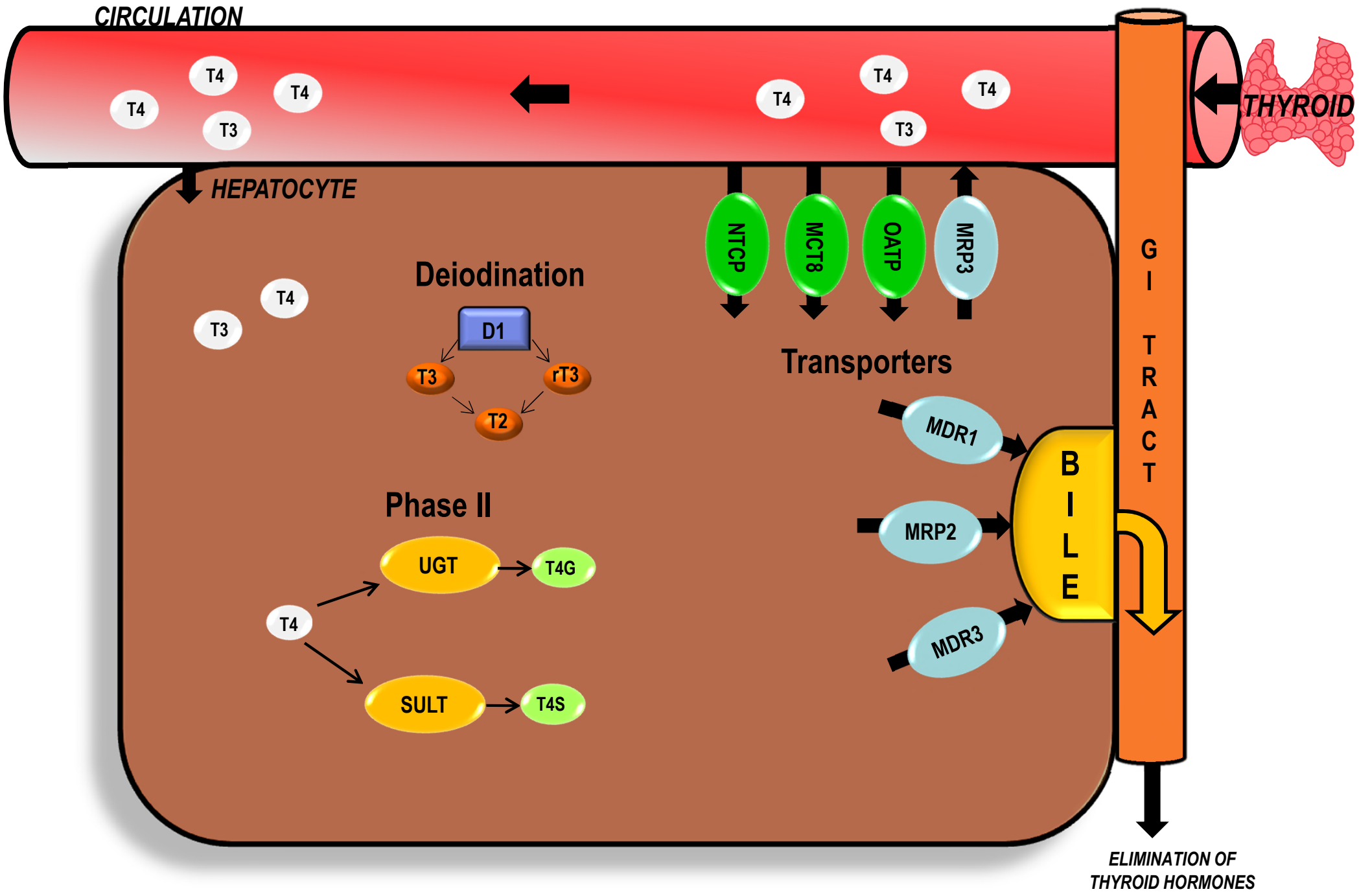
- Glucuronidation
- Sulfation
- Deiodination (D1)
- Transporters



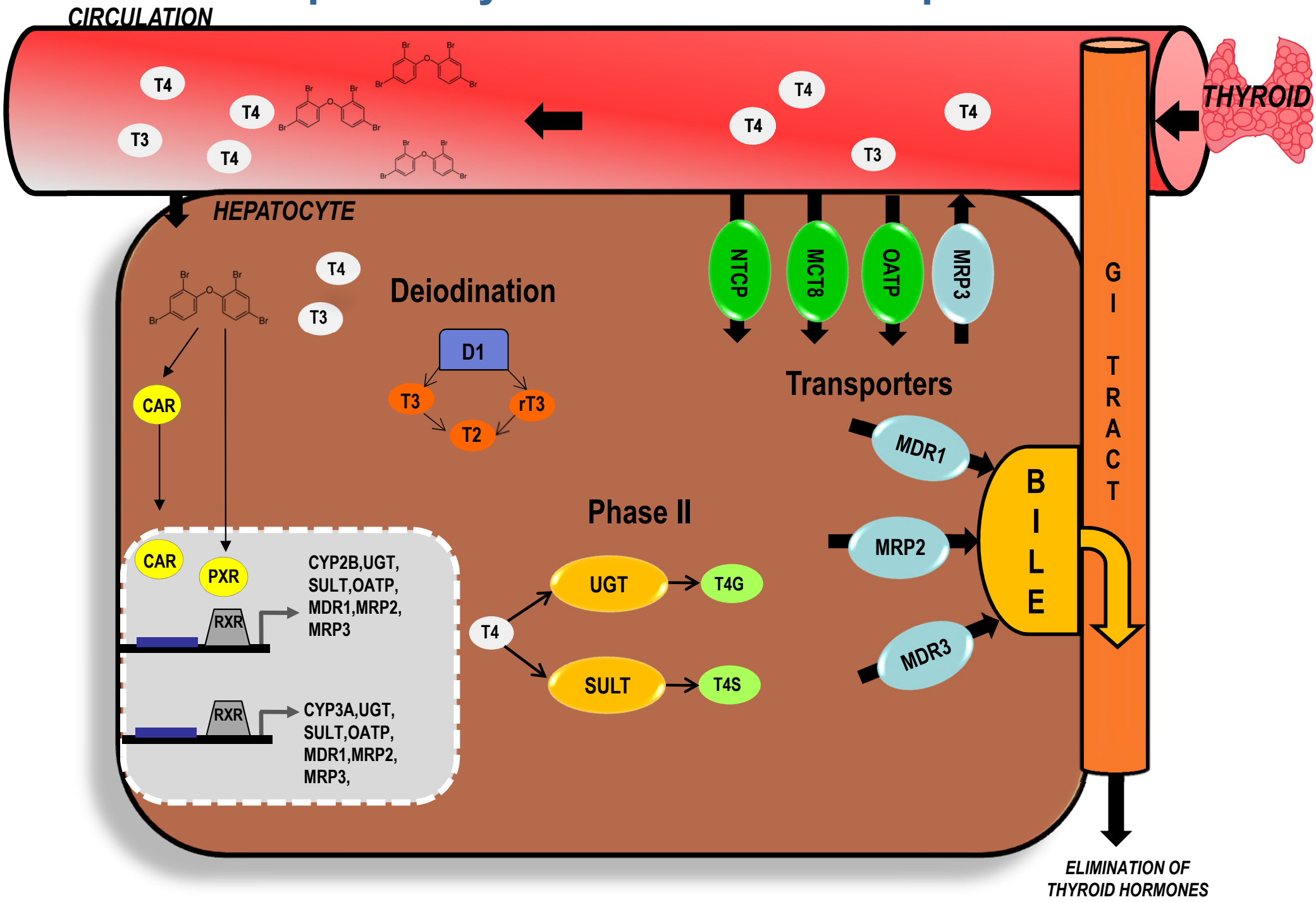


Darras, V. M. and Van Herck S. L. J Endocrinol. 2012 Nov;215(2):189-206.

Thyroid Hormone Metabolism in the Liver



Hepatic Thyroid Hormone Disruption



Physiological Differences in Human and Rat Thyroid Hormone Parameters

	Human	Rat	Reference
TTR	0.2mg/ml	0.5mg/ml	Benvenga and Robbins 1998
TBG	0.02mg/ml	Undetectable >PND 60	Wade *et al*., 1988
T_4 $t_{1/2}$ (days)	5-9	0.5-1	Capan, 2001 Bianco *et al*., 2002
T_3 $t_{1/2}$ (days)	1	0.25	Bianco *et al*., 2002

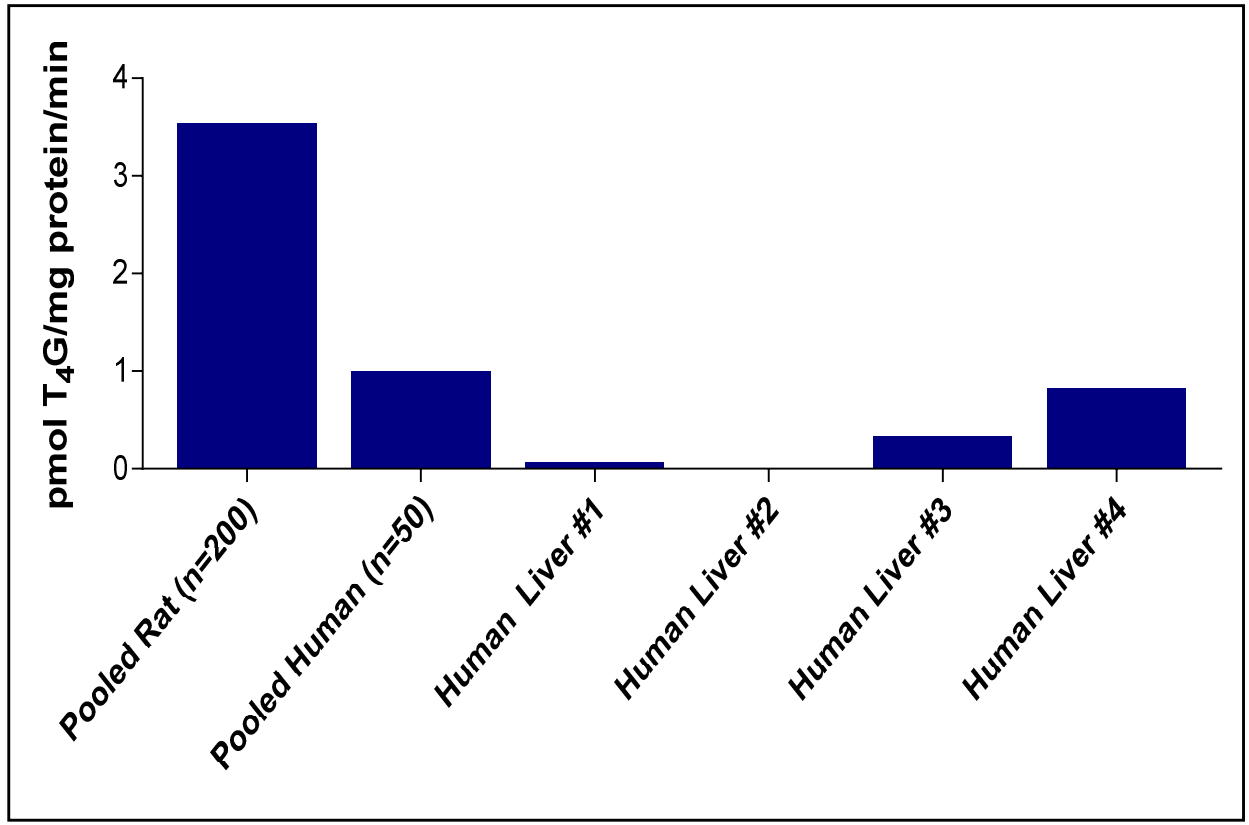
Binding Affinity (Ka)

	T4 (M)	T3 (M)
TTR	10^{-7}	10^{-7}
TBG	10^{-10}	10^{-8}

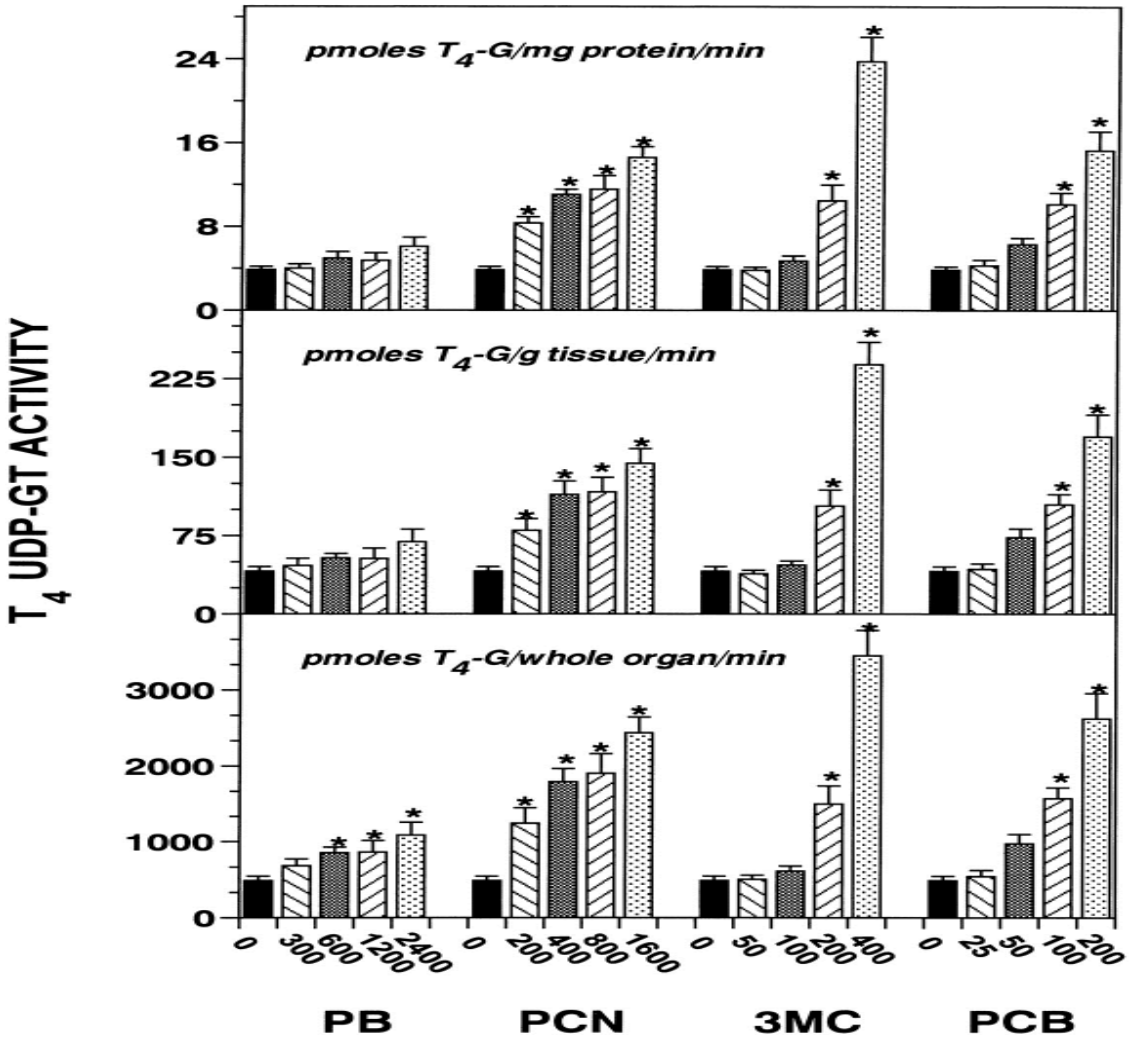
Key Events in Thyroid Hormone Disruption and Relevance to Humans

Key Event	Evidence in Rats	Evidence in Humans	Reference
Serum TH Decrease	**Yes**	**Yes**	Cavlieri *et al.* 1973 Brucker-Davis 1998
Nuclear Receptor Activation	**Yes** *In vivo* and *in vitro*	**Yes** *In vitro*	Barter and Klaassen 1994 Hood and Klaassen 2000
Hepatic UGT Induction	**Yes** *In vivo* and *in vitro*	**Yes** *In vitro*	Barter and Klaassen 1994 Hood and Klaassen 2000
TTR Binding	**Yes** *Ex vivo*	**Yes** *In vitro*	Cheek *et al.,* 1999 Hallgren and Darnerud 2002 Meerts *et al.* 2002
TBG Binding	**No Data** **(TBG not present)**	**Yes** *In vitro*	Cheek *et al.* 1999
Hepatic Transporter Induction	**Yes** *In vivo*	**Limited** *In vitro*	Ribeiro *et al.* 1996; Mitchell *et al.* 2005; Wong *et al.* 2005; *Richardson et al*., 2014
Increased TH or Conjugated TH Biliary Elimination	**Yes** *In vivo* and *in vitro*	**No Data**	Kato *et al.* 2005 Wong *et al.* 2005
Increased Hepatic Uptake/Accumulation of TH	**Yes** *In vivo*	**Limited** *In vitro*	Cheek *et al.,* 1999 Richardson *et al*., 2014

T4-UGT Activity in Liver Microsomes



Effect of Microsomal Enzyme Inducers on T4-UGT Activity in Rat Liver



Hood, A. *et al*. Toxicol. Sci. 2000 55:78-84

Inconsistencies in Serum T4 Decreases and Hepatic T4-UGT Activity

Chemical	Nuclear Receptor	T4-UGT	Serum T4	Reference
β-NF	AhR	↑ ↑ ↑	↓ ↓ ↓	Hood and Klaassen, 2000
3-MC	AhR	↑ ↑ ↑	↓ ↓	Hood and Klaassen, 2000
PCB	AhR/PXR	↑ ↑	↓ ↓ ↓	Hood and Klaassen, 2000
PCN	PXR	↑ ↑	↓ ↓	Hood and Klaassen, 2000
PB	CAR	↑	↓ ↓ ↓	Hood and Klaassen, 2000
DE 71	AhR/CAR/PXR	↑↑	↓ ↓	Zhou *et al*., 2002
BDE 47 (mouse)	CAR	↔	↓	Richardson *et al*., 2008
PB/PCB (Gunn Rat)	AhR/CAR/PXR	↔	↓ ↓ ↓	Kato *et al*., 2007; Richardson and Klaassen, 2010

↑ = increase
↓ = decrease
↔ = no change

Primary Hepatocytes: The Gold Standard

- Their origin in native liver, they reflect the complete functionality of the human organ *in vivo* and thus provide highly predictive results in pharmacological and toxicological *in vitro* research

- Directly reflect the specific metabolism and functionality of the liver.
  - Nuclear receptor activation well studied in PHHs.

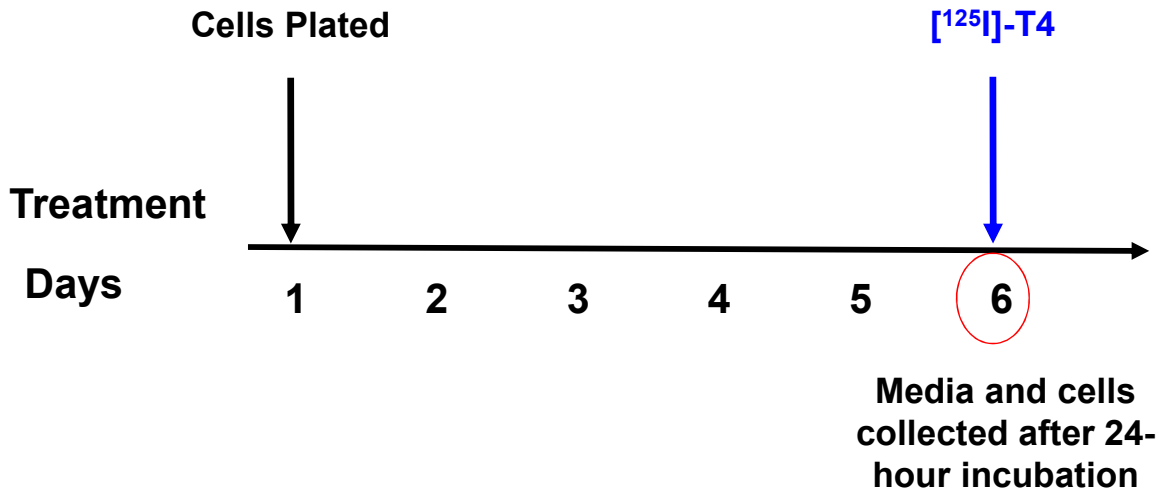
QUESTION?

- Can nuclear receptor activators increase TH metabolism in primary hepatocyte cultures?

- Can we use rat and human hepatocytes to aid in species extrapolation?

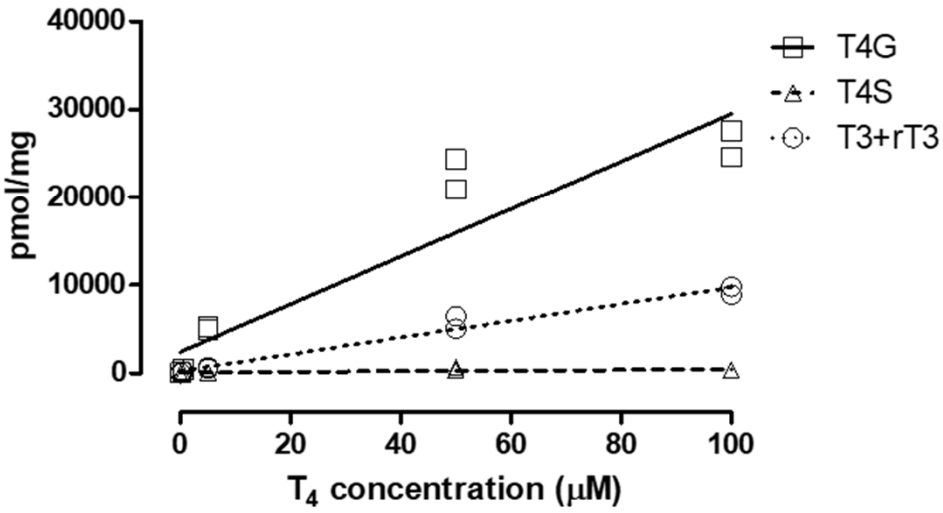
Thyroid Hormone Metabolism in Hepatocytes
Experimental Methods

- Fresh male rat or human hepatocytes (Life Technologies)
- Sandwich-culture plated in 24-well plates
- Up to 24- hour incubation with [^{125}I]-T_4 (Perkin –Elmer)
  - 0.05uM – 100uM
- Media and cells collected for metabolite analysis with UPLC and gamma counter



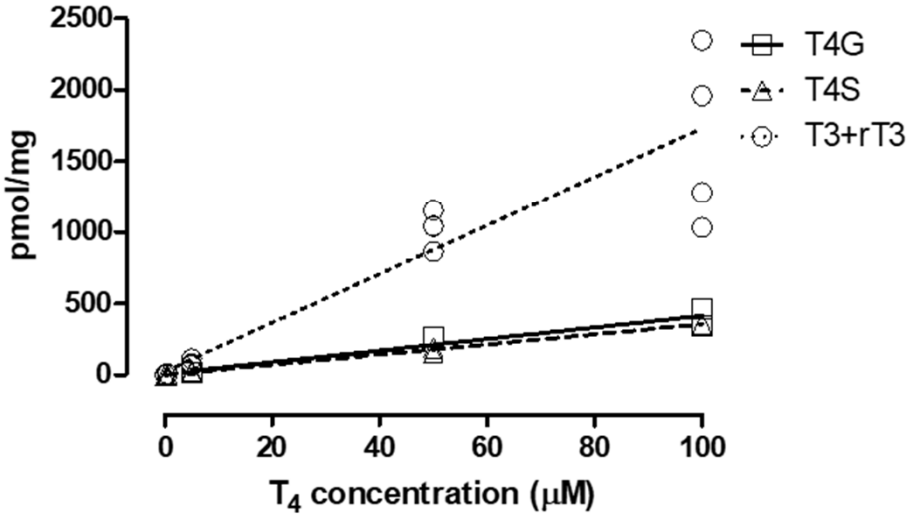
Metabolite Levels in Rat and Human Hepatocyte Media

Rat



$T_4G > T_3 + rT_3 > T_4S$

Human



$T_3 + rT_3 > T_4G \approx T_4S$

Richardson, V. M., *et al*. (2014). Xenobiotica 44(5): 391-403.

Metabolite Levels in Rat and Human Hepatocyte Media

Metabolite Summary

All Metabolites

	Rat	Human
T_4G	91.6%	5.3%
T_4S	3.6%	4.4%
T_3+rT_3	4.8%	90.3%

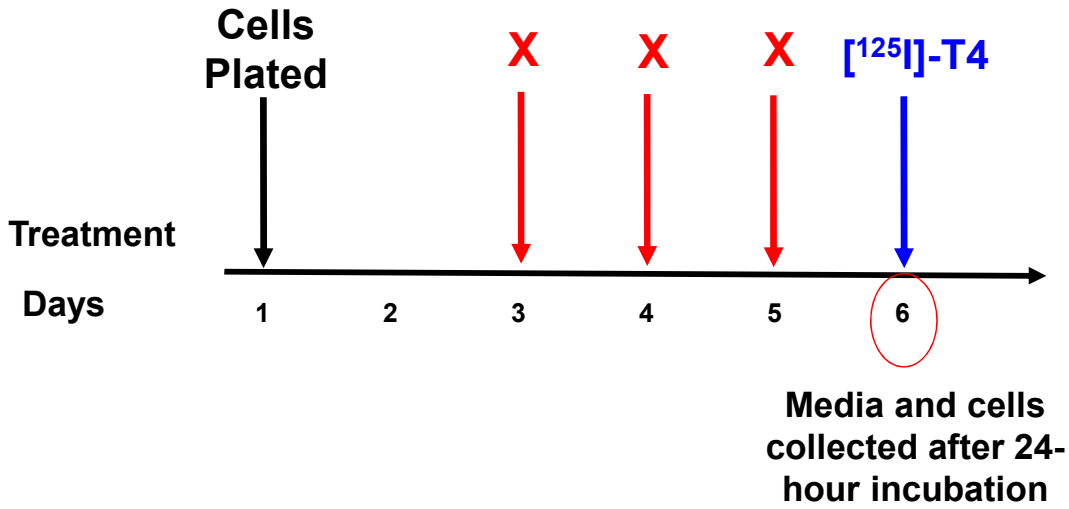
Conjugated Metabolites

	Rat	Human
T_4G	96.2%	54.6%
T_4S	3.8%	45.4%

T4 Metabolic Profiles Following Exposure to Prototypical Nuclear Receptor Activators

Experimental Methods

- Fresh male rat or human hepatocytes (Life Technologies).
- Sandwich-culture plated in 24-well plates.
- 72-hour incubation with PB (10, 100 or 1000μM), PCN (0.1, 1, or 10μM), Rif (0.1, 1, or 10μM) , 3-MC (0.05, 0.5, 5μM), or PCB 153 (0.3, 3, 30μM).
- 24- hour incubation with 0.05μM (rat) or 0.1μM (human) [^{125}I]-T4 (Perkin –Elmer).
- Media and cells collected for metabolite analysis with UPLC.

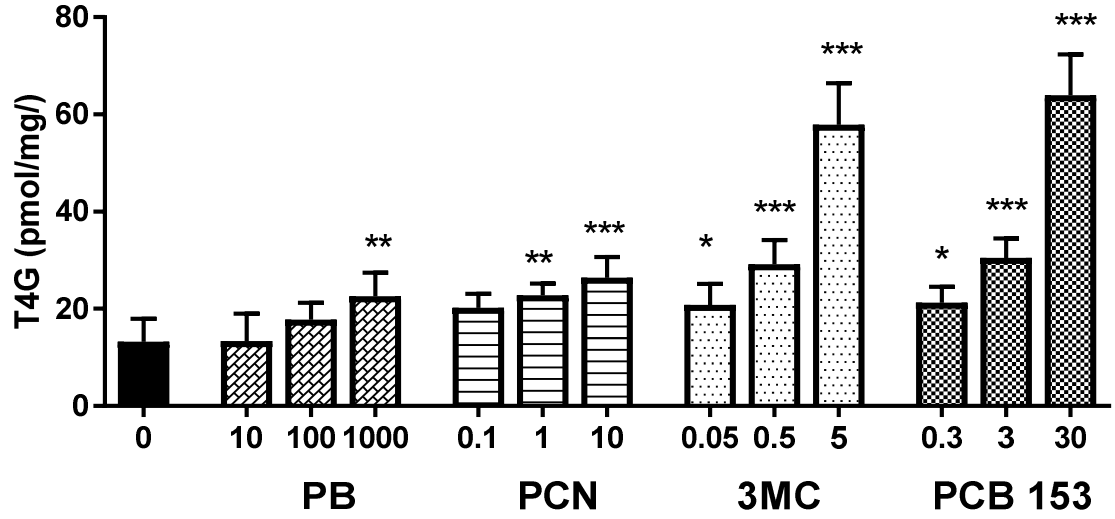


Prototypical Nuclear Receptor Activators

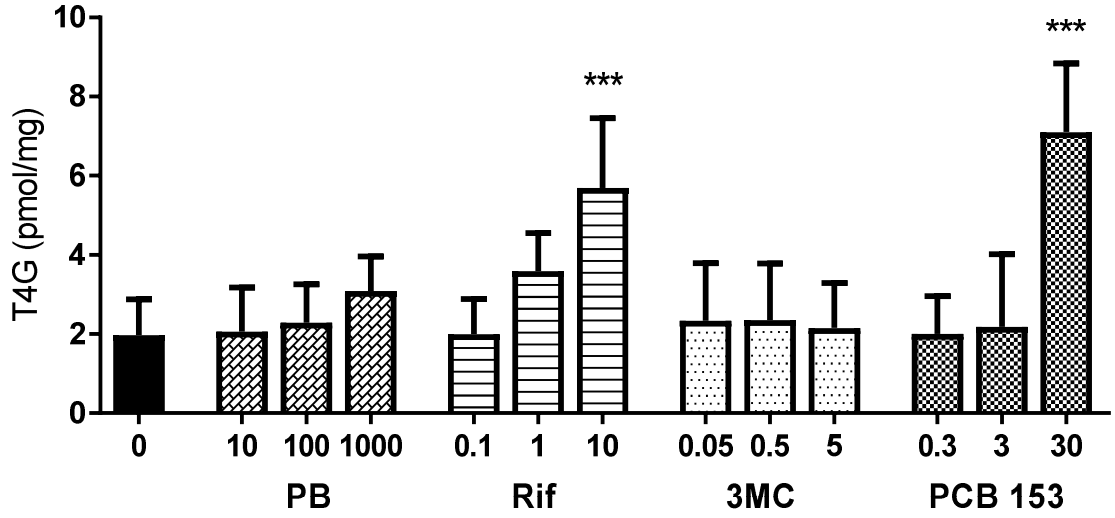
- **PB**= Phenobarbital
  - CAR activator in rat and human
- **PCN**= Pregnenolone-16 α-carbonitrile
  - PXR activator in rat
- **Rif**= Rifampicin
  - PXR activator in human
- **3MC**= 3-Methylcholanthrene
  - AhR activator in rat and human
- **PCB 153**= 2,2',4,4',5,5'-Hexachlorobiphenyl
  - PB-like PCB
  - Possible CAR activator in rat and human

T4G in Media of Rat and Human Hepatocytes

Rat



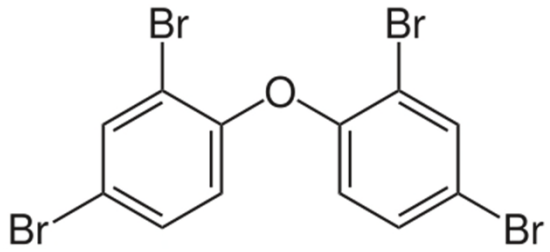
Human



*Significantly different from control group ($p<0.05$).

Richardson *et. al.*, (In preparation)

2,2',4,4'-Tetrabromodiphenyl Ether (BDE-47)



- Fire retardant in consumer products.
- Predominant congener found in wildlife and human samples.
- Decreases serum thyroid hormone concentrations in rodents.
  - Induction in hepatic T4 glucuronidation resulting in decreases in circulating T4 concentrations in rats.
  - CAR activator in mice (Richardson *et al.*, 2008).

Effects of BDE-47 on Thyroxine Metabolism
Experimental Methods
(*In vivo*)

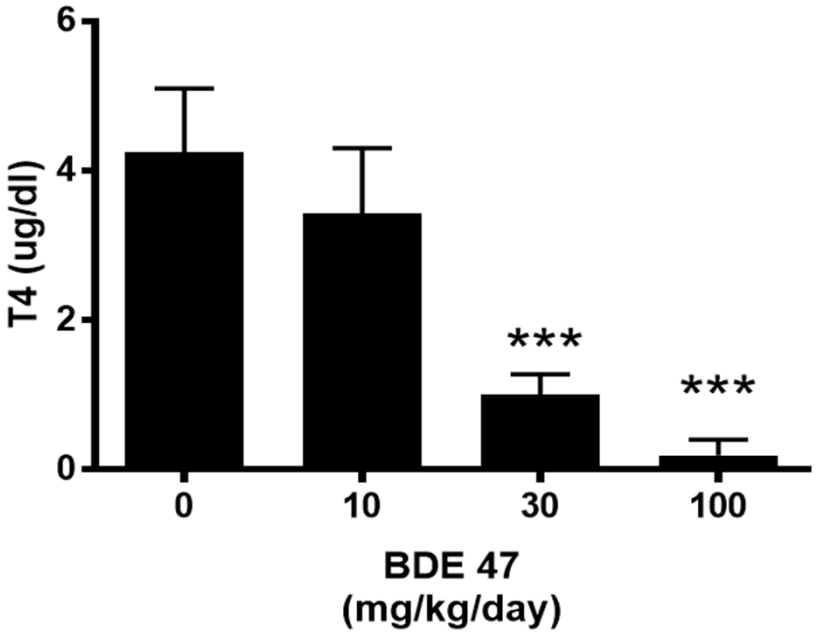
- 60 day old female Sprague-Dawley rats.
- Treatment with a corn oil vehicle, 0, 10, 30 or 100 mg/kg BDE-47 for 4 consecutive days via oral gavage.
- Serum and liver collected on day 5.
- Serum total T4, liver enzymes (UGT and SULT), mRNA expression by real-time RT-PCR.

Effects of BDE-47 on Thyroxine Metabolism
Experimental Methods
(*In vitro*)

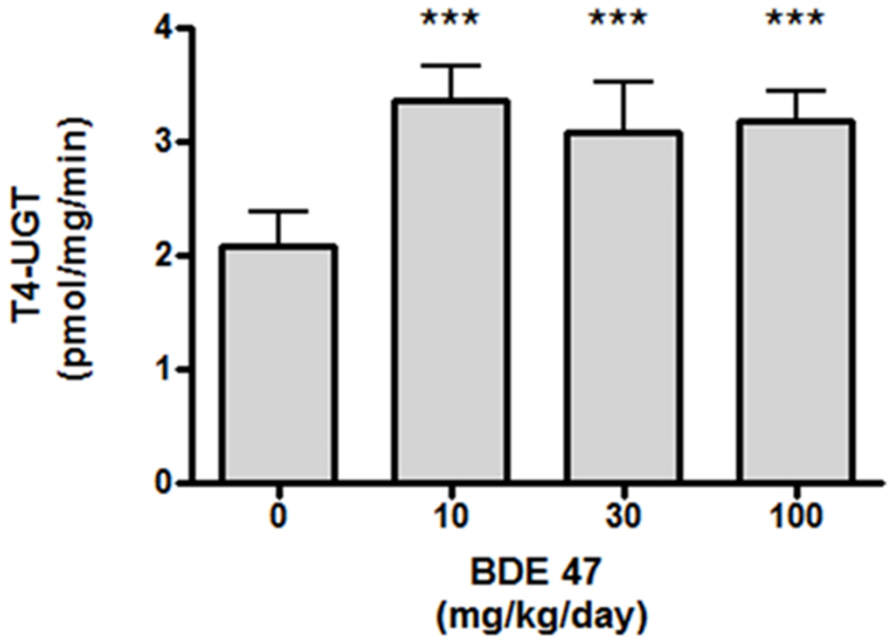
- Fresh male rat or human hepatocytes (Life Technologies).
- Sandwich-culture plated in 24-well plates.
- 72-hour incubation with BDE-47 (0, 0.3, 3 or 30μM).
- 24-hour incubation with 0.05μM (rat) or 0.1μM (human) [^{125}I]-T4 (Perkin –Elmer).
- Media and cells collected for metabolite analysis with UPLC.
- Cells collected for mRNA expression analysis.

Effects of BDE-47 on Serum T4 and Hepatic T4-UGT Activity in Rats

Serum T4



T4-UGT Activity

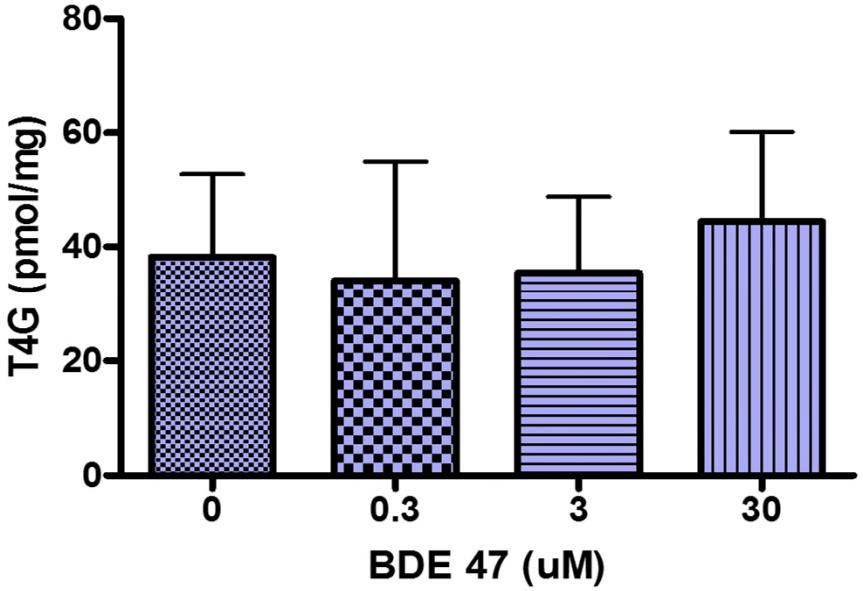


*****Significantly different from control group ($p<0.05$).**
N=6/group

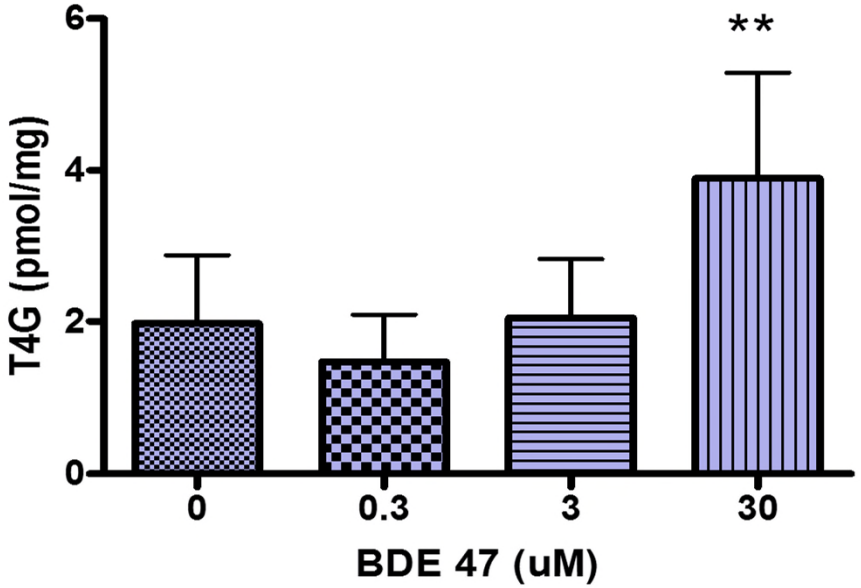
Richardson *et. al.*, (In preparation)

Effects of BDE-47 on T4G in Media of Rat and Human Hepatocytes

Rat Hepatocytes



Human Hepatocytes

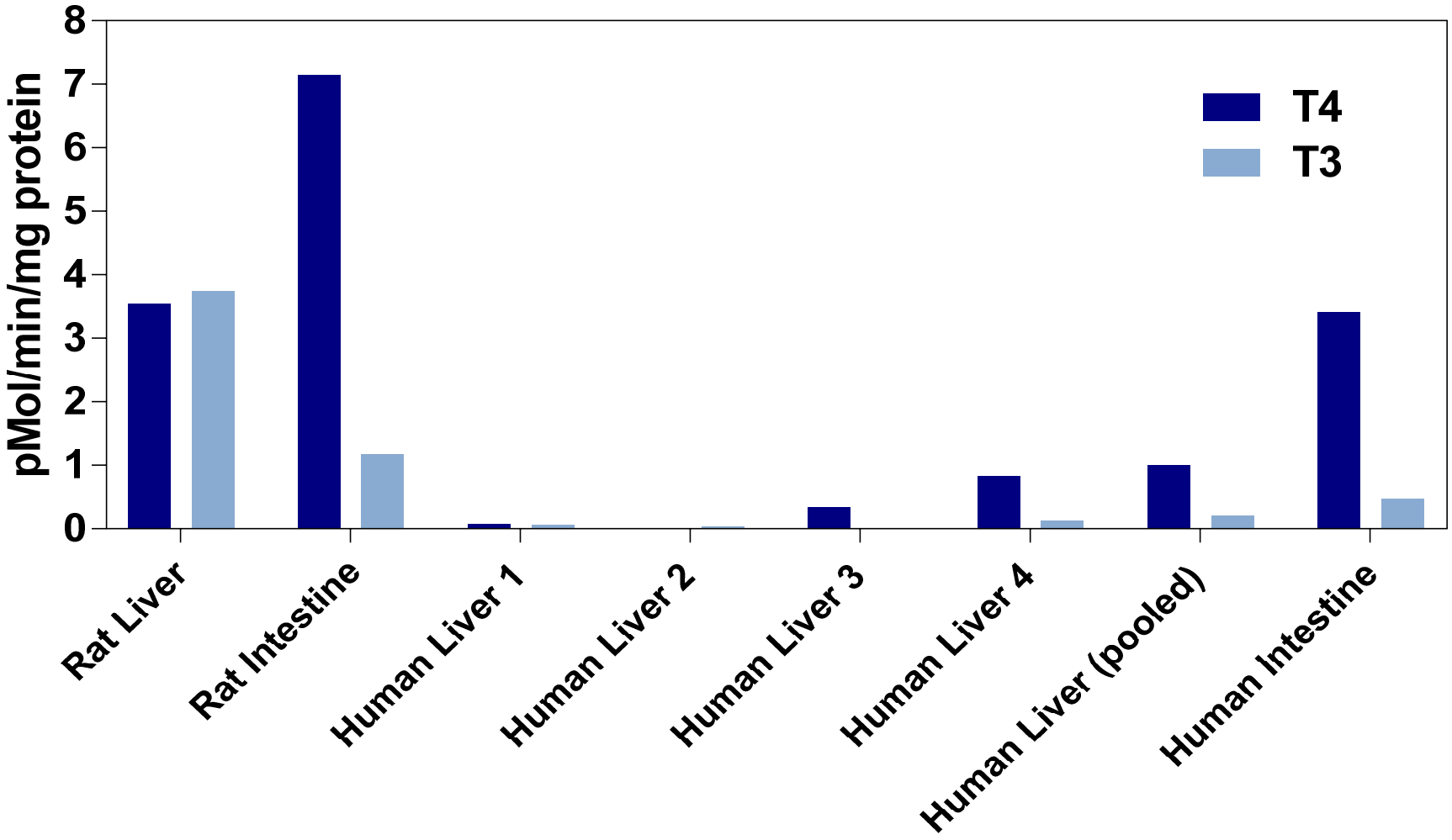


**Significantly different from control group ($p<0.05$).

Richardson *et. al.*, (In preparation)

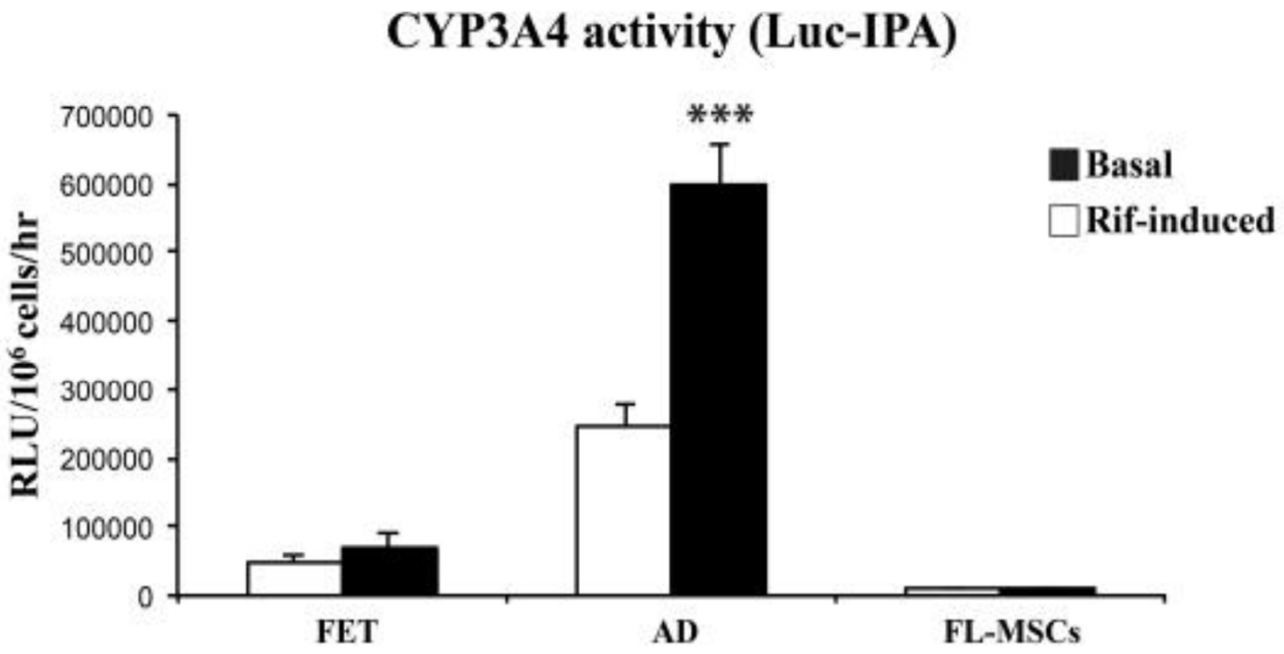
What We Don't Know

Influence of Intestinal UGT Activity on Thyroid Hormone Metabolism



What We Don't Know

Ontogeny of Metabolizing Enzymes

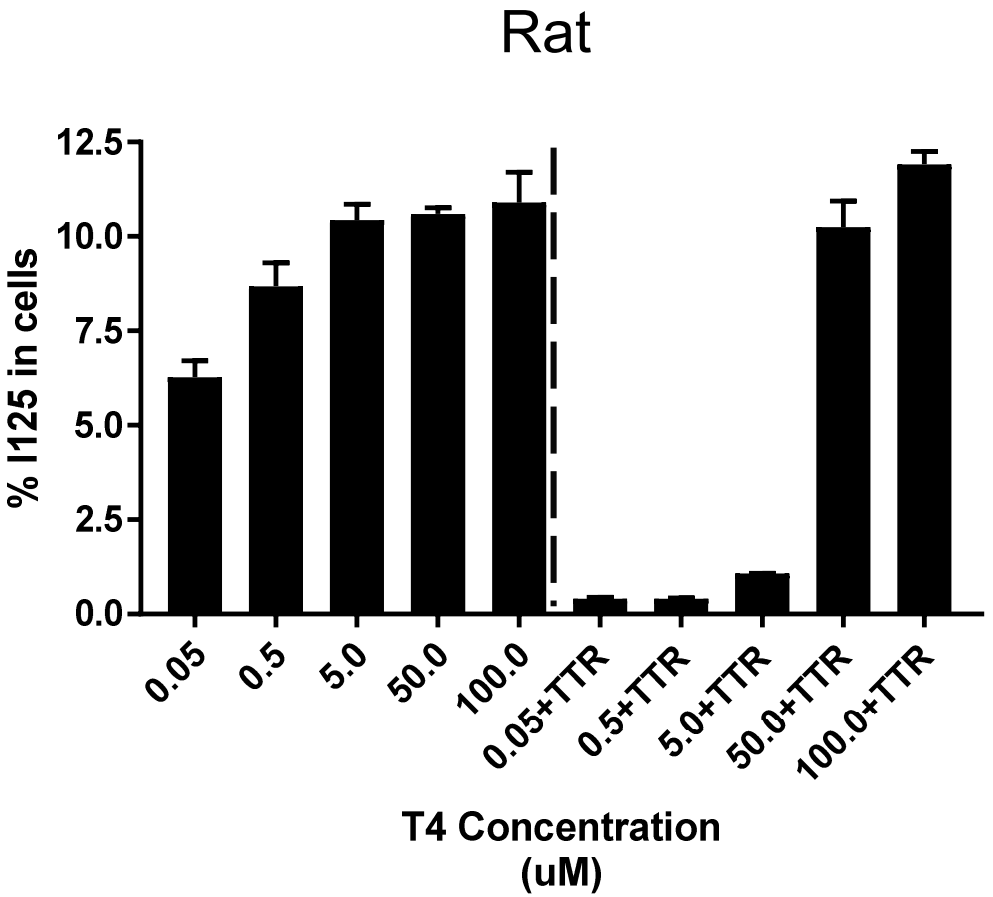


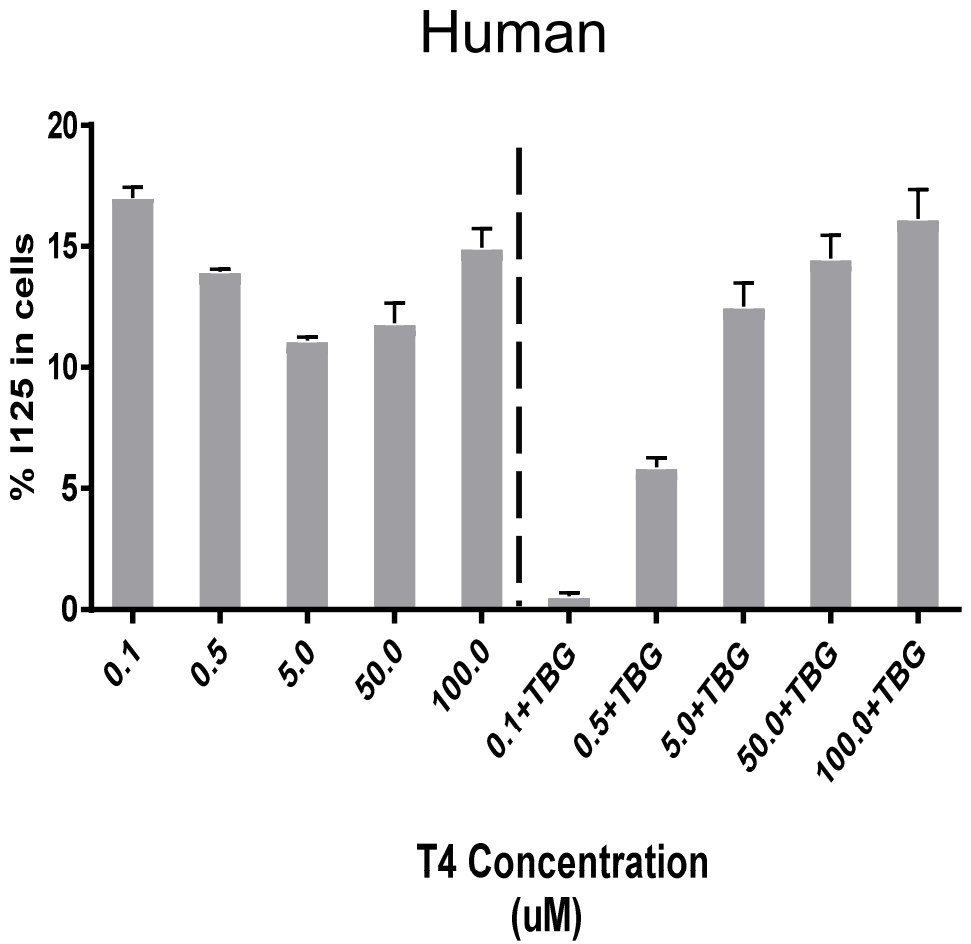
FET = Fetal hepatocytes (16-22 weeks gestation)
AD = Adult hepatocytes
FL-MSCs = human fetal liver mesenchymal-stromal cells

Chinnici, C.M., *et. al*. (2015) Cell Transplant. 24(6):1139-53.

What We Don't Know

Influence of Serum Binding Proteins on T4 Uptake

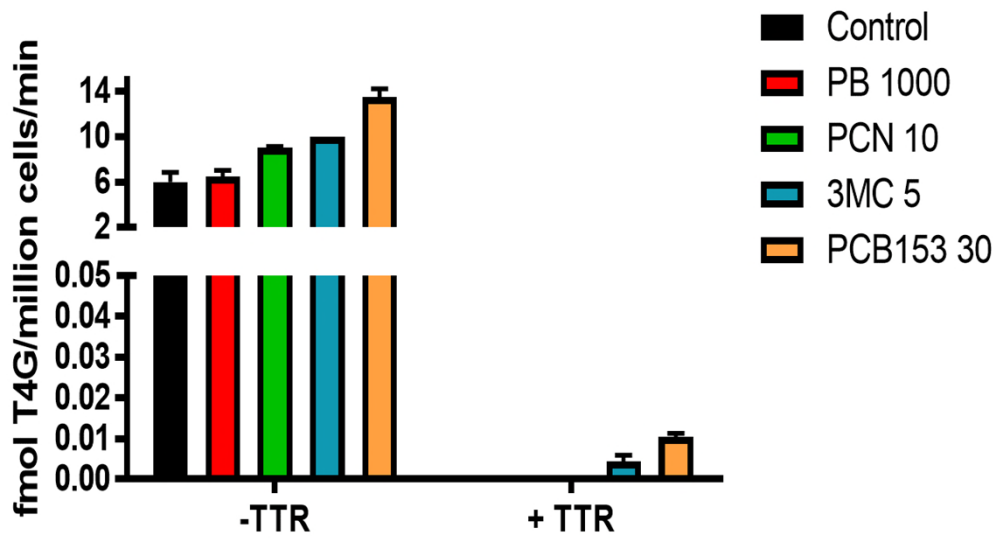




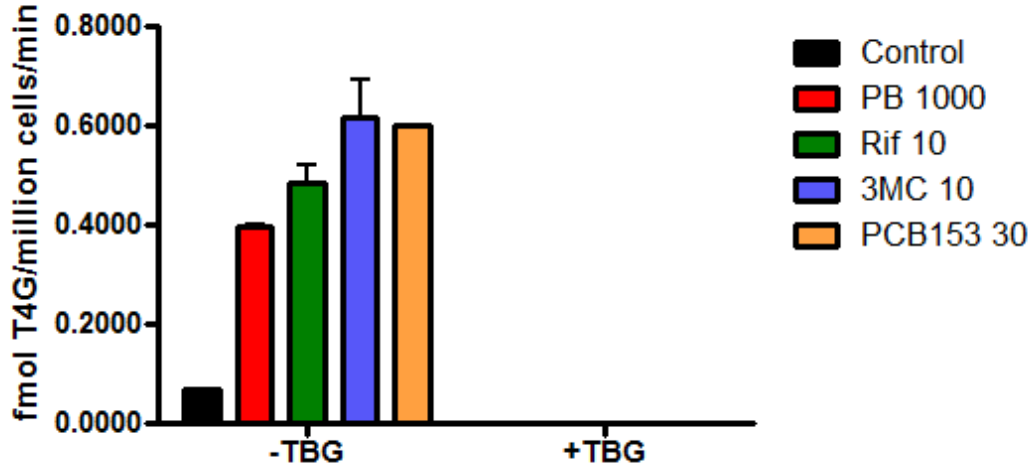
What We Don't Know

Effects of Binding Proteins on Hepatic T4 Metabolism

Rat

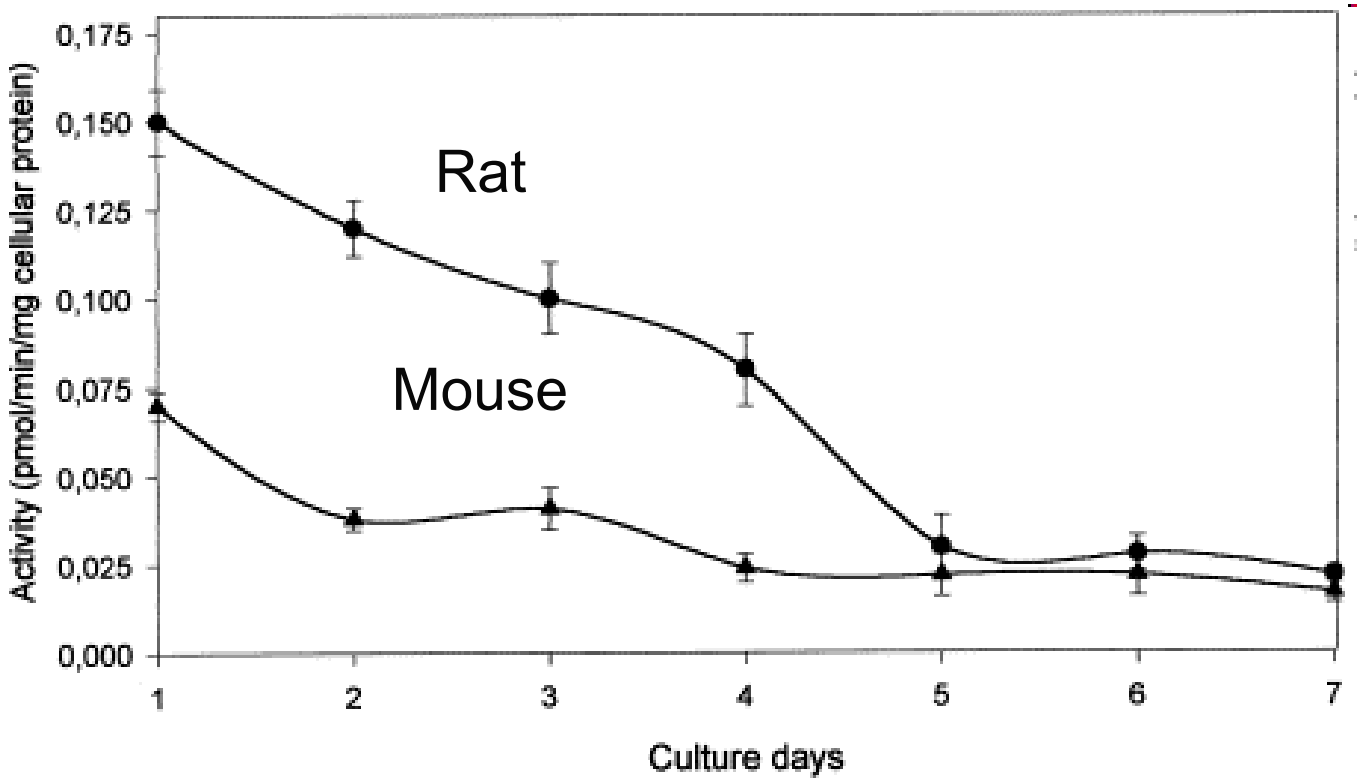


Human



Limitations of Using Primary Hepatocytes

T4-UGT Activities in Rat and Mouse Hepatocytes

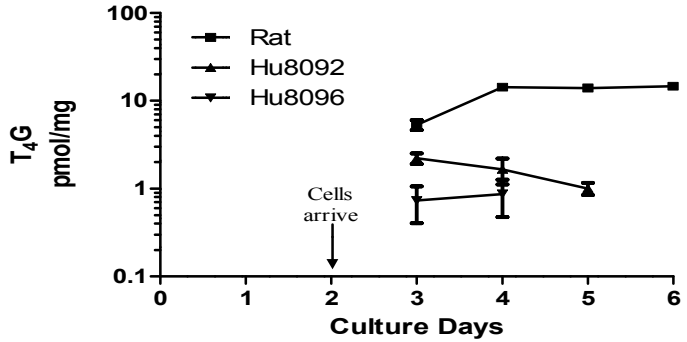


Viollon-Abadie *et al.*,Toxicol In Vitro. 2000 Dec;14(6):505-12

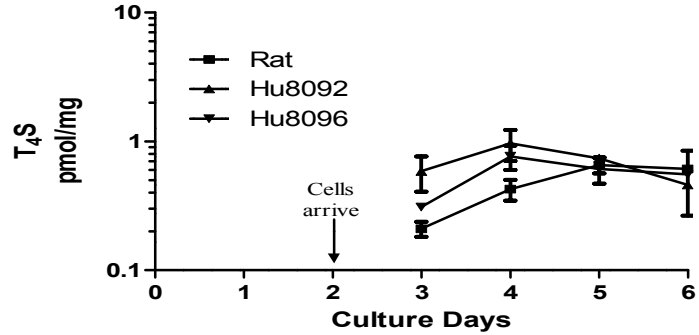
Limitations of Using Primary Hepatocytes

T4 Metabolism Across Culture Days

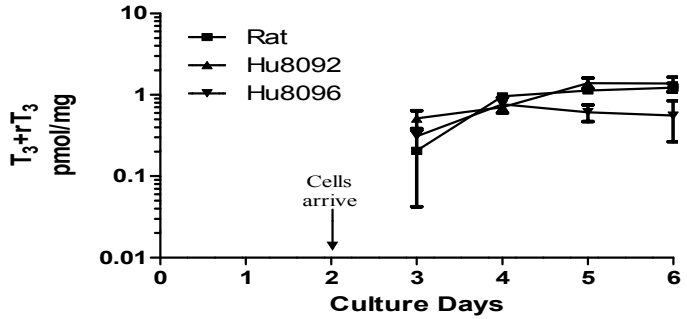
T4G



T4S



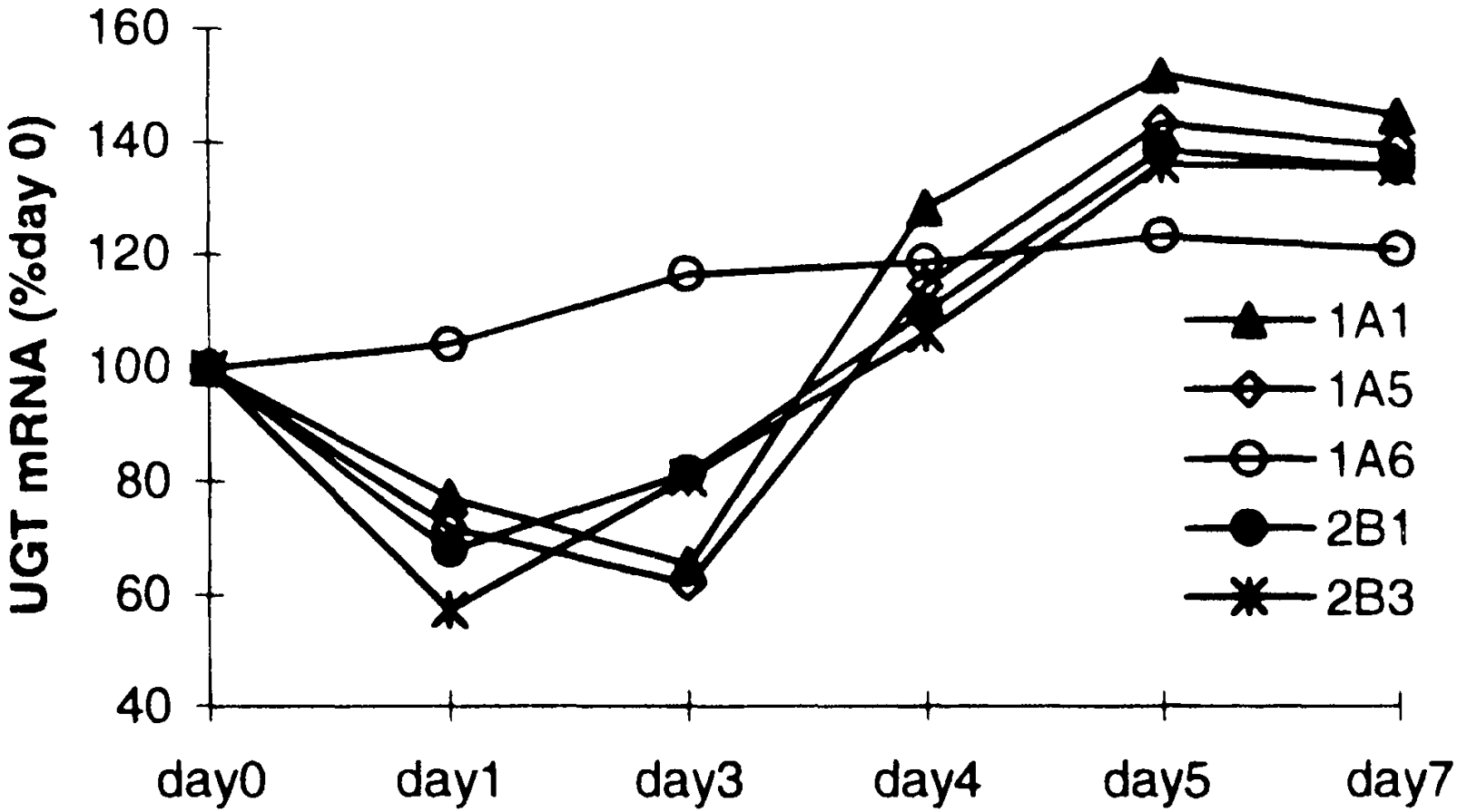
T3 + rT3



Richardson, V. M., *et al*. (2014). Xenobiotica 44(5): 391-403.

Limitations of Using Primary Hepatocytes

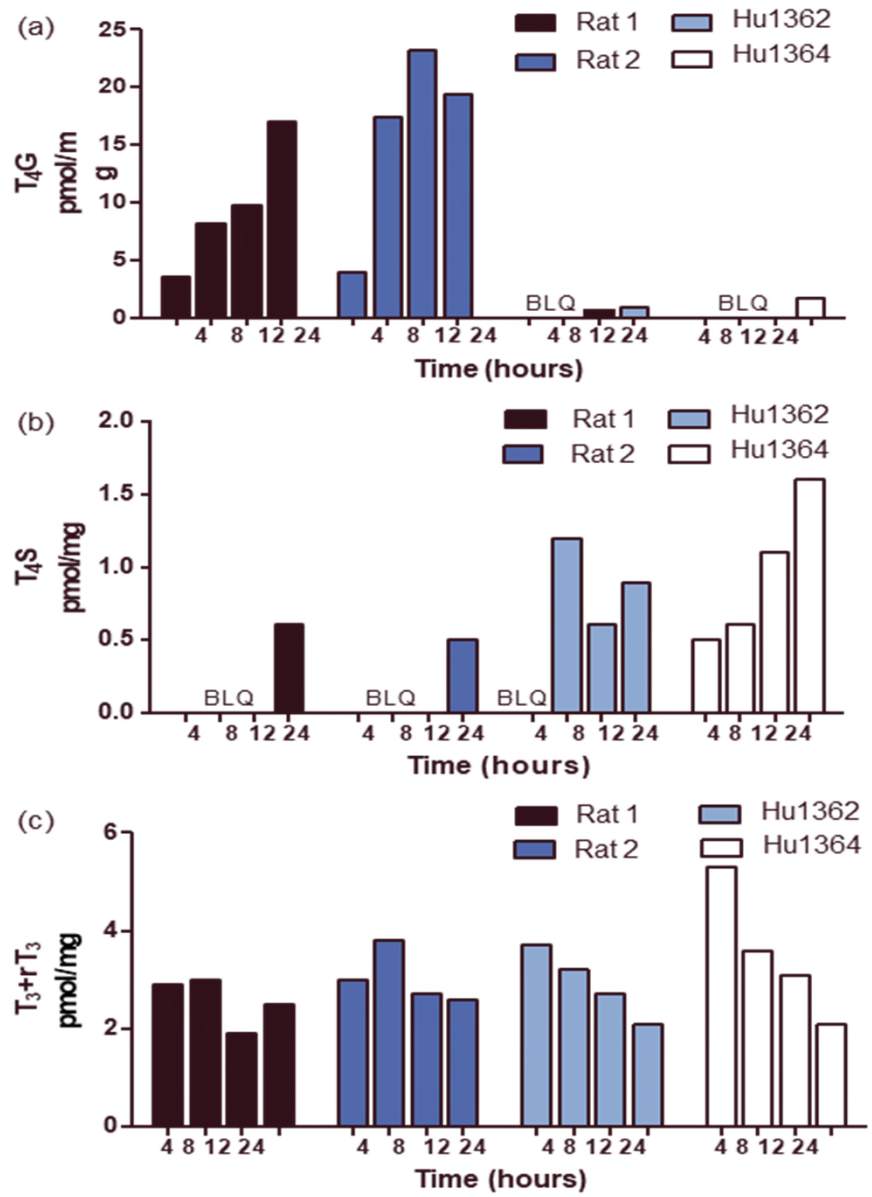
Daily Ugt mRNA Expression in Rat Hepatocytes



Li *et al.*, Pharm Res.1999 Feb;16(2):191-7

Limitations of Using Primary Hepatocytes

T4 Metabolite Levels in Media During Incubation Time



Richardson, V. M., *et al*. (2014). Xenobiotica 44(5): 391-403.

Summary and Conclusions

- New discoveries in nuclear receptor signaling biology are uncovering additional mechanisms for regulating hepatic metabolism and disposition.

- Hepatocyte cultures can be used to evaluate species differences in thyroid hormone metabolism and the impact of nuclear receptor activation on thyroid metabolism
  - Determine methods for assessing transporter interactions and enzyme induction (Adult and pediatric hepatocytes).
  - Focus on conditions to include the binding protein (TTR, TBG).
  - Validate cell culture and experimental conditions.
  - Standardize experiments to provide reproducible results from *in vitro* hepatic cultures.
  - Define the impact of cell quality metrics to outcomes.

Thank You

US Environmental Protection Agency

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