In Vitro to *In Vivo* (IVIVE) and Species Extrapolation for the Disruption of Thyroid Hormone Synthesis by Oxyfluorfen Using Physiologically Based Pharmacokinetic (PBPK) and Thyroid Hormones Kinetics Models

Rhylee Decrane[1], Tammy Stoker[2], Ashley Murr[2], Jermaine Ford[3] and Hisham El-Masri[3]

[1]US DOE, ORAU, Oak Ridge, TN, [2]US EPA, ORD, CPHEA, RTP, NC, [3]US EPA, ORD, CCTE, RTP, NC.

Abstract

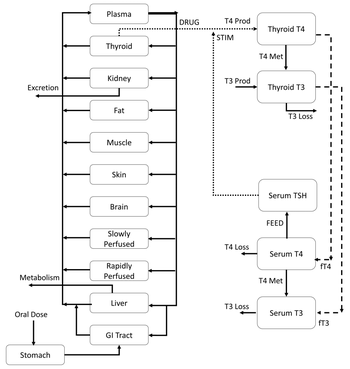
The thyroid hormones play key roles in physiological processes such as regulation of the metabolic and cardiac systems as well as the development of the brain and surrounding sympathetic nervous system. Recent efforts to screen environmental chemicals for their ability to alter thyroid hormone synthesis, transport, metabolism and/or function have identified novel chemicals that target key processes in the thyroid pathway. One newly identified chemical, oxyfluorfen, is a diphenyl-ether herbicide used for control of annual broadleaf and grassy weeds in a variety of tree fruit, nut, vine, and field crops. Using *in vitro* high-throughput screening (HTS) assays, oxyfluorofen was identified to be a potent inhibitor of the thyroidal sodium-iodide symporter (NIS). To quantitatively assess this inhibition mechanism in vivo, we extrapolated *in vitro* NIS inhibition data to *in vivo* disruption of thyroid hormones synthesis in rats using physiologically based pharmacokinetic (PBPK) and thyroid hormone kinetics models. The overall computational model was calibrated against *in vivo* data for the levels of oxyfluorfen in thyroid tissue and serum and against levels of thyroid hormones triiodothyronine (T3) and thyroxine (T4) in serum in rats. The calibrated rat model simulations were within a factor of 3-fold from experimental data. The rat thyroid model was then extrapolated to humans using human *in vitro* HTS data for NIS inhibition and the chemical specific hepatic clearance rate in humans. The overall species extrapolated PBPK-thyroid kinetics model can be used to predict dose-response (% drop in thyroid serum levels compared to homeostasis) relationships in humans. These relationships can be used to estimate points of departure for health risks related to a drop in serum levels of TH hormones based on HTS assays IVIVE, toxicokinetics, and physiological principles.

Introduction

- T3 and T4 *in vivo* serum levels are affected by exposure to environmental chemicals.
- Disruption of these hormones in the serum has been associated with adverse outcomes including abnormal brain development and heart defects in experimental animals.
- Oxyfluorfen is an environmental chemical that has recently been identified as a potent inhibitor of NIS, the first key step of thyroid hormone (TH) synthesis in the thyroid gland.
- Using two *in vitro* screening assays (human hNIS and FRTL-5), oxyfluorfen was found to inhibit NIS with an IC50 of 2 µM.
- *In vivo* follow-up studies in the juvenile rat have confirmed that Oxyfluorfen inhibits T4 and T3 serum levels.
- IVIVE was performed by coupling chemical concentration in the thyroid to TH kinetics *in vivo* using a rat or human PBPK model.

Materials and Methods

- Both rat and human modeling were created by combining a species-specific PBPK model using ADME (Absorption, Distribution, Metabolism, Excretion) biological and physiological parameters with thyroid hormone kinetics.
- The PBPK model consisted of tissue compartments for: thyroid, GI tract, liver, muscle, slowly perfused, rapidly perfused, kidney, skin, plasma, stomach and brain.
- TH hormone kinetics was mathematically described similar to previous published models.
- *In vitro* and an *in vivo* literature and experimental data were used for model development and calibration.



Results

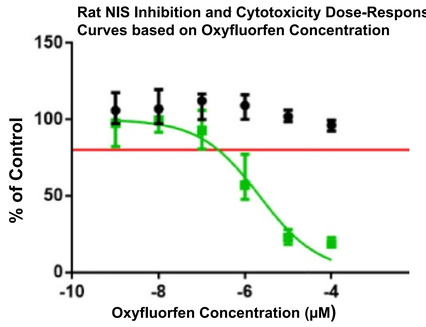


Figure 1: Dose- Response Curves of rat NIS (FRTL- 5) *in vitro* assay where the green and black markers represent inhibition and cytotoxicity; respectively

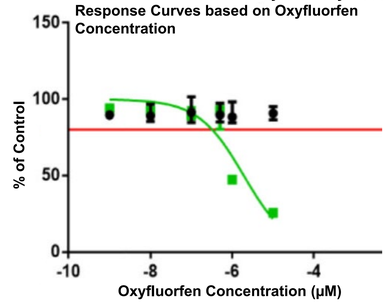
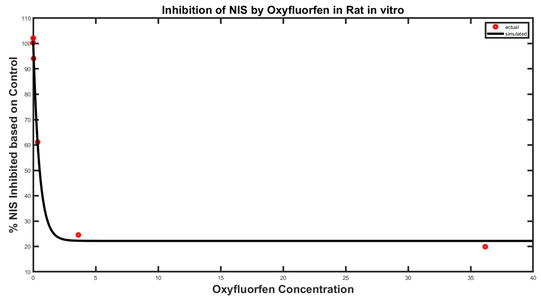
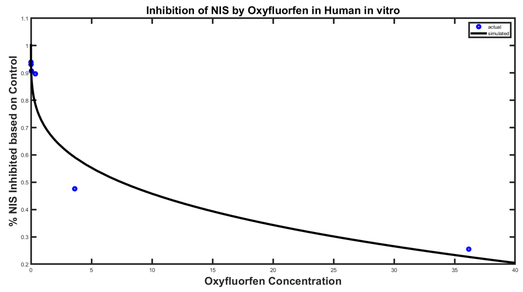


Figure 2: Dose- Response Curves of human NIS (hNIS) *in vitro* assay where the green and black markers represent inhibition and cytotoxicity; respectively.



$$\%Inhibition_{rat} = .788*exp(-2.006*(Oxyfluorfen\ Concentration\_Thyroid\ blood))+.2218$$

Figure 3: A mathematical fit to *in vitro* data of the percent inhibition of the rat NIS compared to control based on Oxyfluorfen Concentration (mg/kg)



$$\%Inhibtion_{human} = -.2917*(Oxyfluorfen\ Concentration\_Thyroid\ blood)^{-.2739}+1.006$$

Figure 4: A mathematical fit to *in vitro* data of the percent inhibition of the human NIS compared to control based on Oxyfluorfen Concentration (mg/kg).

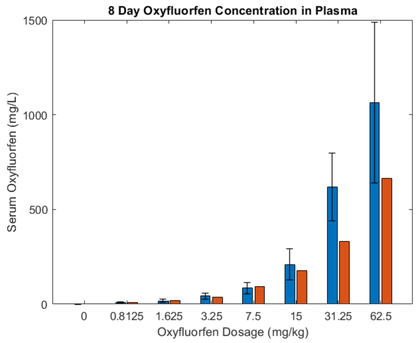


Figure 5: Concentration of Oxyfluorfen in rat plasma. The blue bars with standard deviation and red bars are experimental data and overall model predicted levels for concentration of Oxyfluorfen, respectively.

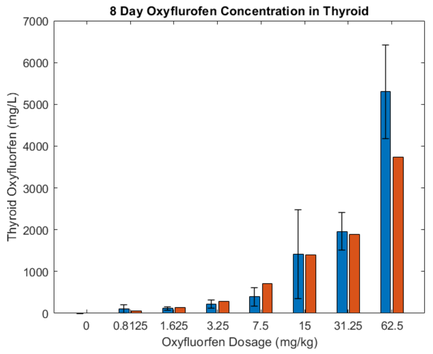


Figure 6: Concentration of Oxyfluorfen in rat thyroid. The blue bars with standard deviation and red bars are experimental data and overall model predicted level for concentration of Oxyfluorfen, respectively.

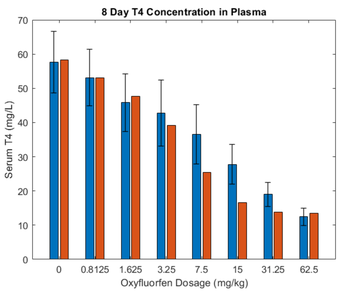


Figure 7: Concentration of T4 in the rat plasma. The blue bars with standard deviation and red bars are experimental data and overall model predicted level for serum T4, respectively.

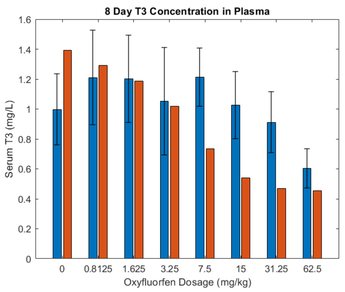


Figure 8: Concentration of T3 in the rat plasma. The blue bars with standard deviation and red bars are experimental data and overall model predicted level for serum T3, respectively.

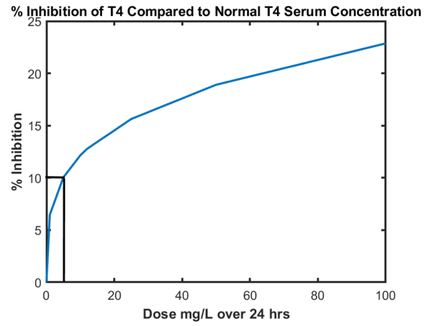


Figure 9: Dose-response model prediction for T4 % inhibited based on normal serum values in humans exposed to Oxyfluorfen orally.

Summary and Conclusion

- Computational methods are useful in assessing the integrative impact of chemical and TH kinetics on circulating serum levels of the hormones.
- Dose-response information from high throughput screening *in vitro* assays along the hypothalamic-pituitary-thyroid (HPT) axis can be used as inputs in an integrative computational framework to estimate *in vivo* TH serum levels in response to chemical exposure.
- This integrative quantitative approach can be generalized across many chemicals and exposure scenarios to screen chemicals for their potential disruption of TH serum levels.