SOT 2014 Poster Abstract

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Comparison of *in vitro and ex vivo* thyroid hormone synthesis inhibition results and *in vivo* outcomes for a series of benzothiazoles

Michael Hornung, Jonathan Haselman, Joseph Korte, Patricia Kosian, Katie Challis, Stephanie Hall, Sigmund Degitz

US EPA, ORD, NHEERL, Mid-Continent Ecology Division, Duluth, MN 55803

Assessing how *in vitro* data may be used to predict adverse effects *in vivo* is critical as efforts are advanced to incorporate *in vitro* assays into a risk assessment framework. Within the context of a thyroid hormone (TH) synthesis inhibition adverse outcome pathway (AOP), *in vitro*, *ex vivo* and *in vivo* assays were used to study the TH disrupting potential for a series of benzothiazoles: benzothiazole (BTZ), 2-mercaptobenzothiazole (MBT), 5-chloro-2-mercaptobenzothiazole (CMBT), 2-aminobenzothiazole (ABT), 2-hydroxybenzothiazole (HBT), and 2-(methylthio)benzothiazole (MTBT). A thyroid peroxidase (TPO) inhibition assay was used to determine the activity of these chemicals *in vitro*. The rank order potency for TPO inhibition was MBT=CMBT>ABT>BTZ>HBT. MTBT did not inhibit TPO activity. The benzothiazoles were tested further in *Xenopus laevis* thyroid gland explant culture with inhibition of TH release as the endpoint. Toxicity was assessed as decreased glandular ATP. MBT inhibited TH release at non-cytotoxic concentrations and with similar potency to methimazole. The benzothiazoles with greatest potency for T4 release inhibition were MBT, CMBT, and HBT, with IC50s of 3, 30, and 133 µM, respectively, but all benzothiazoles showed some inhibitory activity. Benzothiazoles were further assessed *in vivo* in a 7-d *X. laevis* tadpole assay. MBT and CMBT were the most potent for affecting endpoints of thyroid hormone synthesis inhibition, whereas others showed little or no effect. Both MBT and CMBT significantly increased sodium iodide symporter (NIS) mRNA indicative of compensatory TSH stimulation in response to decreased circulating TH. Taken as a whole, these results indicate the utility of *in vitro* assays for queuing chemicals for further testing, but illustrate the need for caution in interpreting results of *in vitro* or *ex vivo* inhibition assays especially where toxicity may be a confounding factor. *This abstract does not necessarily reflect U.S. EPA policy.*

Impact: This abstract presents the results of a study that addresses how assays conducted along different levels along an **Adverse Outcome Pathway** can provide information on **extrapolating** in vitro assay data to in vivo outcomes. It further supports efforts to develop in vitro assays for **thyroid** hormone disruption that are essential to populating a battery of in vitro assays that may be used to support efforts in the **EDSP21** program to prioritize chemicals with potential to disrupt thyroid hormone pathways.

Keywords: Thyroid, AOP, amphibian, extrapolation, in vitro, ex vivo, in vivo, endocrine, extrapolation, Adverse Outcome Pathway