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A PATHWAY APPROACH TO PREDICTING THYROID HORMONE DISRUPTING ACTIVITY OF CHEMICALS USING IN VITRO, EX VIVO AND IN VIVO ASSAYS

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The potential for commercial and industrial chemicals that may be released into the environment to have endocrine disrupting activity is of concern for human health and wildlife. Most initial endocrine disruptor research has focused on estrogen- or androgen-mediated pathways. In contrast, research on the capacity of chemicals to alter thyroid hormone (TH) pathways has been relatively limited. Disruption of thyroid hormone function may occur via multiple pathways including altered TH receptor binding, increased TH metabolism and elimination, and altered TH synthesis. The aim of this research was to assess chemicals for their ability to affect TH synthesis and downstream pathways using assays that span biological levels of organization from in vitro to in vivo. The activity of TPO, which is the enzyme that catalyzes iodination and coupling of tyrosines to produce TH, was measured in vitro using porcine thyroid microsomes. Inhibition of TPO activity was determined initially for the two model TH synthesis inhibitors, methimazole and propylthiouracil (PTU). Other chemicals were selected for testing based upon their structural similarity to methimazole or other known TPO inhibitors. Most chemicals were inactive for inhibiting TPO activity; however, mercaptobenzothiazole (MBT) was found to inhibit TPO activity, with an IC50 near 1 µM. This chemical was tested further in a *Xenopus laevis* thyroid gland explant culture assay in which inhibition of thyroxine (T4) release was the measured endpoint. MBT inhibited T4 release from thyroid glands at non-cytotoxic concentrations and with potency similar to methimazole. The activity of MBT for disrupting thyroid hormone production was confirmed in vivo in *X. laevis* tadpoles where it produced effects consistent with TH synthesis inhibition. Using a suite of assays across levels of biological organization is a promising approach to identifying endocrine disrupting chemicals. This abstract does not necessarily reflect U.S. EPA policy.

Impact Statement:
This abstract presents research being conducted at MED under the Safe Pesticides/Safe Products research area. The research described herein is in response to the mandate to the Agency to develop a research program to evaluate the potential adverse effects of chemicals on vertebrate endocrine systems including thyroid hormones. The presentation demonstrates how in vitro and ex vivo assays can help to queue chemicals for evaluation in vivo assays and how the development of shorter term in vitro and ex vivo assays can be used to help define structure activity relationships that can be the basis for predicting thyroid hormone disrupting activity of chemicals.

Potential Program Office Interest: Some of the chemicals that will be shown in this presentation are on EPA inventories. The 2-mercaptobenzothiazole that was positive in the in vitro TPO inhibition assay, was positive in the thyroid gland explant culture, and inhibited amphibian metamorphosis is on the EPA Antimicrobial inventory.