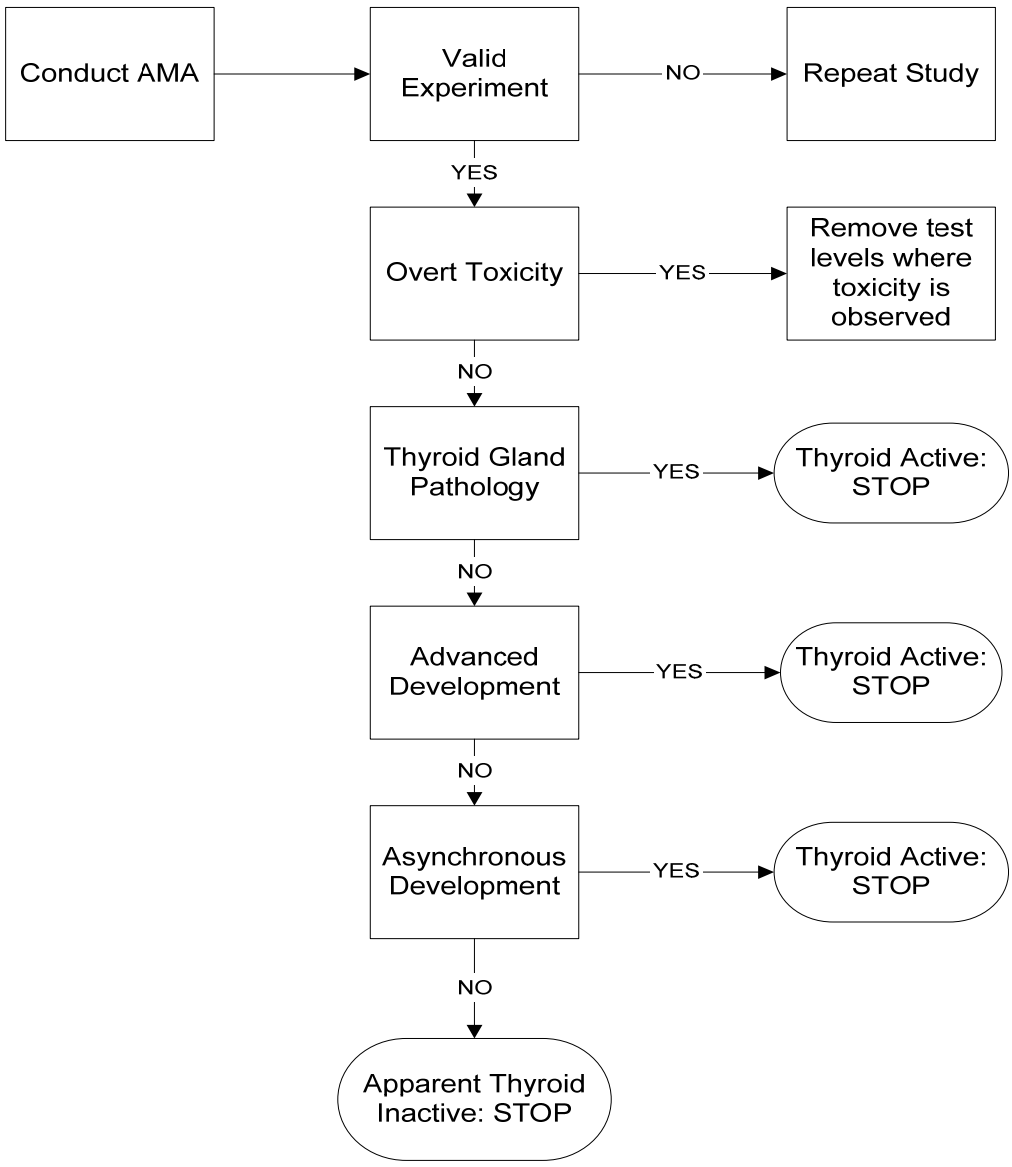
Appendix A1 – Amphibian Metamorphosis

Amphibian Metamorphosis	
Purpose	The AMA is a screening assay intended to empirically identify substances which may interfere with the normal function of the hypothalamic-pituitary-thyroid (HPT) axis. The AMA represents a generalized vertebrate model to the extent that it is based on the conserved structure and functions of thyroid systems. It is not intended to quantify or confirm endocrine disruption, or to provide a quantitative assessment of risk, but only provide evidence that thyroid regulated processes may be sufficiently perturbed to warrant more definitive testing.
Design	The general experimental design entails exposing *Xenopus laevis* tadpoles at NF stage 51 to a minimum of three different aqueous concentrations of a test chemical and a dilution water control for 21 days. There are four replicate tanks at each test substance concentration or treatment. Larval density at test initiation is 20 tadpoles per test tank for all treatment groups.
Endpoints	Daily mortality Morphological endpoints Whole body length/snout-vent length (d 7 and 21) Hind limb length (d 7 and 21) Wet weight (d 7 and 21) Developmental stage (d 7 and 21) Histology Thyroid gland (d 21)
Interpretation	Results are evaluated for evidence of interaction of the test chemical with the HPT axis as follows. Data values and the study report are evaluated for deviations from the test method or performance criteria to evaluate the validity of the study. If necessary, test concentrations with overt toxicities are removed from the data set. Significant histological findings in thyroid tissue deem the assay positive. If no thyroid gland histopathology is observed, then developmental landmarks are evaluated. If development is accelerated or asynchronous, the test is deemed positive. The assay is considered negative if no effects are detected in thyroid gland histology or morphological landmarks of development. The following decision flow chart diagrams the interpretation logic.

Amphibian Metamorphosis

Decision Flow Chart



Amphibian Metamorphosis	
Main peer review comments	• Assay is relevant to its purpose. • Data interpretation needs to be better described. • Protocol is generally clear and appropriate, but notable changes and clarifications are needed to improve the assay. [EPA accepts the recommendations of the peer review panel and will revise the protocol guidance accordingly.]
Strengths (within the context of the proposed battery)	• intact *in vivo* system on an animal undergoing morphological development allowing for evaluation of parent compounds and degradates • amphibian metamorphosis is a well-studied developmental process that is dependent on thyroid hormone, thus effects on metamorphic development are relatively specific indicators of HPT axis perturbation • conserved nature of the components and functions of the amphibian HPT axis are relevant for other vertebrate classes • apical assay covering several modes of HPT axis interaction, including central homeostatic mechanisms and peripheral mechanisms • redundant endpoints, maximizing chance for detection while minimizing false negatives • provides toxicological data in a taxon (amphibians) underrepresented in available Agency protocols • well-established relationship between endpoints and endocrine system • endpoints easy to measure
Limitations (within the context of the proposed battery)	• inherent difficulties in testing some substances not amenable to aquatic systems • sensitivity of the assay has not been fully characterized • non-thyroidal toxicities have the potential to affect some of the morphological endpoints of the assay