

**The Story of the Amphibian Metamorphosis Assay
in EPA' Endocrine Disruptor Screening Program
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Background

Frog metamorphosis is a well studied phenomena which is driven by the thyroid and offers a representative vertebrate model for the thyroid hormone axis. On this basis, an amphibian metamorphosis assay was proposed for the Endocrine Disruptor Screening Program (EDSP) Tier 1 screening battery. The proposed screening battery includes several assays which address estrogen and androgen effects, but only two which are relevant to thyroid hormone disruption, and only one, the frog assay, specific to thyroid.

Protocol Development

The Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) recommended the use of a frog tail resorption protocol – a type of metamorphic climax assay. There was some question regarding whether this type of assay would be sufficiently sensitive to thyroid-active compounds; therefore, an EPA Office of Research and Development (ORD) laboratory (MED, Duluth, MN) evaluated the protocol, both empirically and conceptually. The ORD laboratory concluded that the tail resorption endpoint was not ideal because this metamorphic stage was too insensitive. The laboratory then suggested that EPA examine the earlier *prometamorphosis* stage, when the larvae become responsive to the thyroid hormone and production of the thyroid hormone is initiated.

The ORD laboratory developed a 14-day version of the prometamorphosis protocol with *Xenopus laevis* for the amphibian metamorphosis assay and recommended Nieuwkoop and Faber (NF) developmental stage 54 as the initial stage. Other protocols were discussed in a Detailed Review Paper (DRP), including a full metamorphosis protocol, where embryos are exposed through metamorphic climax; a 21-day version of the prometamorphosis protocol, in which the assay is initiated at an earlier stage of development and exposure occurs over a longer time period; assays using other species; and assays based on contexts outside of an EDSP screening battery.

Prevalidation

EPA decided to proceed with validating the 14-day prometamorphosis protocol, and the U.S. presented this protocol to the Organization of Economic Cooperation and Development (OECD). However, the Germans recommended a 28-day version beginning at NF stage 51. EPA felt the 14-day NF stage 54 version was more practical within the context of an EDSP screening battery. The German and U.S. protocols not only differed in duration and the initial stage of metamorphosis, but also in the preferred exposure method. The Germans used a static renewal method, while the U.S. preferred a flow-through exposure method.

An international workshop on amphibian methods preceded an OECD expert group meeting where the two amphibian metamorphosis protocols were discussed. It was decided that both should be further evaluated. The Germans agreed to shorten their protocol to 21 days but held that the assay should still be initiated at NF stage 51 of metamorphosis. A Phase 1 validation trial was undertaken using a thyroid agonist and a thyroid antagonist as test chemicals. One cohort was evaluated beginning at NF stage 51 for 21 days and another at NF stage 54 for 14 days for both chemicals. Three labs (one in Germany, one in Japan, and one in the U.S.) tested both protocols and found that, while the results for the two compounds and the two protocols differed slightly, they were generally comparable.

Validation

After reviewing the results from Phase 1, the OECD expert group recommended continuing with the 21-day protocol and decided to develop a Phase 2 validation study. This study would utilize the 21-day version of the protocol, beginning at NF stage 51. The endpoints to be evaluated are hind limb length, body length, developmental stage, and thyroid histology.

To better compare the 14 and 21 day versions, EPA undertook a multi-chemical study using six compounds to evaluate both versions. Both compounds used in the Phase 1 validation study were included; therefore, the results of this study can be examined alongside the data generated in Phase 1. EPA hopes that the multi-chemical study will shed light on whether NF stage 51 can provide more information than NF stage 54, but results from the study will not be available until May 2005. Thus, this study will not influence the design of the OECD Phase 2 experiments.

An OECD validation management group decided to proceed with Phase 2 validation as designed. Once Phase 2 is completed, OECD will then decide whether sufficient data have been generated to prepare a validation package for peer review or whether additional interlaboratory tests will be needed to validate the decided-upon protocol.

The Endocrine Disruptor Methods Validation Subcommittee (EDMVS) was presented with the amphibian metamorphosis DRP and they offered advice with how to present the preferred protocol to OECD. However, an advisory committee has not met since Phase 1 results were available to provide additional input. At this time, EPA will rely on the Endocrine Disruptor Methods Validation Advisory Committee (EDMVAC) to advise the Agency on the next steps in completing a validation report and preparing for peer review. EPA hopes that the results from the multi-chemical study and eventually Phase 2 will support the recommended next steps.