Appendix B – Expert Interviews

The following experts were contacted and asked to answer a questionnaire on the development of amphibian metamorphosis assays to detect endocrine disrupting chemicals that potentially target the thyroid axis:

	Expertise
Dr. James G. Burkhart National Institute of Environmental Health Sciences 111 Alexander Dr., C446 Research Triangle Park, NC 27709	Amphibian thyroid, molecular and organismal biology
Dr. Robert J. Denver Department of Biology 3077 Natural Science Building University of Michigan Ann Arbor, MI 48109-1048	Amphibian develop and metamorphosis, molecular biology
Dr. Robert Grainger Department of Biology Gilmer Hall Charlottesville, VA 22901	*Xenopus* development and reproduction, *X. tropicalis* transgenic lines, molecular cytogenetics
Dr. Tyrone B. Hayes Department of Integrative Biology University of California Berkeley, CA 94720-3140	Amphibian endocrinology, developmental biology, molecular biology

A blank questionnaire and completed questionnaires for each expert interviewed are presented in this Appendix.

Questionnaire sent via email:

Questionnaire: Development of Amphibian Metamorphosis Assays to detect endocrine disrupting chemicals that potentially target the thyroid axis.

From: Dr. Doug Fort, Fort Environmental Laboratories (email: djfort@hotmail.com)

The United States Environmental Protection Agency (EPA) is implementing an Endocrine Disruptor Screening Program (EDSP). In the EDSP, toxicological and ecotoxicological screens and tests are being developed for identifying and characterizing the endocrine effects of various contaminants. One potential target for endocrine disrupting chemicals is the thyroid axis that includes the CNS, hypothalamus, pituitary, thyroid gland, thyroid hormone transport proteins, and thyroid hormone receptors. Since thyroid hormone controls metamorphosis in most amphibians, the EPA is considering using Amphibian Metamorphosis Assays as a Tier 1 Screen in the EDSP. Battelle Memorial Institute, Columbus, Ohio, was awarded the prime EPA contract for review, development, and validation of the screen and test methods. I serve as consultant to Battelle because of my expertise in amphibian metamorphosis, the thyroid axis, and amphibian ecotoxicology.

The Amphibian Metamorphosis Assays consist of a battery of whole organism morphological tests, biochemical measures of thyroid status, and molecular techniques that could be used to evaluate thyroid axis status.

Morphological Tests Under Consideration:

- 14-d metamorphic climax assay using *Xenopus sp.* (includes *X. laevis* or *X. tropicalis*)
- 28-d Full metamorphosis Assay using *Xenopus sp.*
- *Hyperolius argus* endocrine screen (HAES) using the sexual dichromatic reed frog

Complementary Biochemical Measures:

- Thyroid Stimulating Hormone (TSH), thyroxin (T4), triiodothyronine (T3), DIT, MIT, and deiodinase using RIA, ELISA, or LC/GC-MS.

Molecular Techniques Potentially Useful for Evaluating Thyroid Axis Activity:

- Transgenesis
- Differential display – gene arrays
- RNase Protection Assay (RPA)

A component of reviewing relevant literature includes contacting experts in thyroid endocrinology, amphibian metamorphosis, and amphibian ecotoxicology. We would greatly appreciate you comments to the following questions:

1. What are the strengths and weaknesses of the study designs under consideration? Do you have alternative recommendations?

2. What endpoints do you feel will be most appropriate?

3. What changes would you recommend to the study approaches and why?

4. What statistical methods would you suggest and why?

5. Based on your experience, what test substances, routes, durations, developmental periods, and doses should be used to validate the proposed assays.

6. Can you suggest published references (yours and others) to aid us in our study and endpoint selection? If so, which?

7. Do you have an unpublished data relevant to these assays that you are willing to share? If so, are there any restrictions?

8. Would you be willing/like to be involved in the study progress, results, and interpretations?

9. Is there anyone else you think we should contact? If so, whom? Can we mention your name when we contact this person?

   On behalf of the project team, we would like to thank you for your participation.

Questionnaire sent via email:

Questionnaire: Development of Amphibian Metamorphosis Assays to detect endocrine disrupting chemicals that potentially target the thyroid axis.

From: Dr. Doug Fort, Fort Environmental Laboratories (email: djfort@hotmail.com)

To: Dr. James G. Burkhart, NIEHS, Research Triangle Park, NC

Response: via telephone

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  A component of reviewing relevant literature includes contacting experts in thyroid endocrinology, amphibian metamorphosis, and amphibian ecotoxicology. We would greatly appreciate you comments to the following questions:

1. What are the strengths and weaknesses of the study designs under consideration? Do you have alternative recommendations? ***The study design needs to consider morphological, biochemical, and molecular assays. This represents a well-rounded approach. For the morphological assays the 28-d test would be better since it encompasses more of metamorphosis. The Hyperolius assay is not truly a metamorphosis assay. It evaluates sexual differentiation, which can be affected by altering metamorphosis. More work will need to be done here to establish it for the intended purpose of evaluating thyroid disruption.***

2. What endpoints do you feel will be most appropriate? ***The morphological endpoints should include tail resorption, skin development, and limb emergence at a minimum. The biochemical parameters and methods are appropriate. Of the three molecular techniques evaluated, evaluation of transgenic response elements that respond to thyroid disruption would be valuable. Differential display and the PRA assays are similar, although the RPA could be used more quantitatively and differential display is often difficult to interpret.***

3. What changes would you recommend to the study approaches and why? ***None at this point.***

4. What statistical methods would you suggest and why? *No comment beyond what has been recommended. Evaluation of accumulative data over the duration of an exposure using specific data transform measures would be a novel approach.*

5. Based on your experience, what test substances, routes, durations, developmental periods, and doses should be used to validate the proposed assays. ***Dosing via the culture water and feed would probably be best at this point. Sublethal doses should be considered primarily. A representative selection of thyroid inhibitors, stimulators, non-actives, and unknowns should be compiled and reviewed for validation.***

6. Can you suggest published references (yours and others) to aid us in our study and endpoint selection? If so, which? ***None beyond those already included in your reference database.***

7. Do you have an unpublished data relevant to these assays that you are willing to share? If so, are there any restrictions? *I am willing to share information, but none*

of it is at the point yet that it should be released. When it reaches that point I would be willing to share it.

8. Would you be willing/like to be involved in the study progress, results, and interpretations? ***Yes.***

9. Is there anyone else you think we should contact? If so, whom? Can we mention your name when we contact this person? ***Not beyond those listed already.***

   On behalf of the project team, we would like to thank you for your participation.

Questionnaire sent via email:

Questionnaire: Development of Amphibian Metamorphosis Assays to detect endocrine disrupting chemicals that potentially target the thyroid axis.

From: Dr. Doug Fort, Fort Environmental Laboratories (email: djfort@hotmail.com)

To: Dr. Robert J. Denver, Department of Biology, University of Michigan, Ann Arbor, MI

Response: via telephone

The United States Environmental Protection Agency (EPA) is implementing an Endocrine Disruptor Screening Program (EDSP). In the EDSP, toxicological and ecotoxicological screens and tests are being developed for identifying and characterizing the endocrine effects of various contaminants. One potential target for endocrine disrupting chemicals is the thyroid axis that includes the CNS, hypothalamus, pituitary, thyroid gland, thyroid hormone transport proteins, and thyroid hormone receptors. Since thyroid hormone controls metamorphosis in most amphibians, the EPA is considering using Amphibian Metamorphosis Assays as a Tier 1 Screen in the EDSP. Battelle Memorial Institute, Columbus, Ohio, was awarded the prime EPA contract for review, development, and validation of the screen and test methods. I serve as consultant to Battelle because of my expertise in amphibian metamorphosis, the thyroid axis, and amphibian ecotoxicology.

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Molecular Techniques Potentially Useful for Evaluating Thyroid Axis Activity:

- Transgenesis
- Differential display – gene arrays

- RNase Protection Assay (RPA)
  A component of reviewing relevant literature includes contacting experts in thyroid endocrinology, amphibian metamorphosis, and amphibian ecotoxicology. We would greatly appreciate you comments to the following questions:

1. What are the strengths and weaknesses of the study designs under consideration? Do you have alternative recommendations? ***The study design needs to consider morphological, biochemical, and molecular assays. However, many factors affect metamorphosis beyond the thyroid axis that need to be considered. Molecular test methods would be better suited for evaluating thyroid disruption initially. Morphological tests could then be used to determine the consequence to the whole organism. Shorter-term morphological tests would be better suited as the complexities of development during the entire metamorphic process can dilute out the capacity to observe morphological changes associated with thyroid disruption. Biochemical analysis will probably be the least useful and TSH analysis should probably be dropped. Corticosteroid monitoring might be more useful. Overall, the intended purpose of the amphibian metamorphosis assays will dictate which approach will be the most appropriate. Is it a test for metamorphosis or a test for thyroid dysfunction, or both? If it is a test of metamorphosis, an organism culture assay with biochemical analysis followed by a molecular test to establish potential mechanisms of action. If you are after the latter two, a rapid molecular screening test followed by a morphological test to demonstrate activity in the whole organism would be more appropriate.***

2. What endpoints do you feel will be most appropriate? ***The morphological endpoints could include tail resorption, skin development, and limb emergence. The biochemical parameters and methods are probably appropriate, but analysis is difficult since whole tissue must be measured. This analysis is only useful in the context of molecular and whole organism tests. Of the three molecular techniques evaluated, evaluation of transgenic response elements that respond to thyroid disruption is valuable. For example, we are working with T3/T4 target gene activities. Levels of induction are not great and promotor selection will require some work, but the use of transgenic lines should prove useful in this area. Differential display and the PRA assays also have potential. Two potential areas to consider are the use of TH transport protein expression (i.e., transthyretin), and transfected amphibian cell culture lines.***

3. What changes would you recommend to the study approaches and why? ***None, other than those addressed above.***

4. What statistical methods would you suggest and why? ***No comment beyond what has been recommended.***

5. Based on your experience, what test substances, routes, durations, developmental periods, and doses should be used to validate the proposed assays. *No comment as this point.*

6. Can you suggest published references (yours and others) to aid us in our study and endpoint selection? If so, which**?** ***None beyond those already sent to you (note: these are included in the literature cited).***

7. Do you have an unpublished data relevant to these assays that you are willing to share? If so, are there any restrictions? ***I am willing to share information on some new RT-PCR techniques evaluating brain TSH expression transfected cell culture lines with TH responsive reporter elements. I would be willing to share it when the work is further along.***

8. Would you be willing/like to be involved in the study progress, results, and interpretations? ***Yes.***

9. Is there anyone else you think we should contact? If so, whom? Can we mention your name when we contact this person? ***Not beyond those listed already.***

   On behalf of the project team, we would like to thank you for your participation.

Questionnaire sent via email:

Questionnaire: Development of Amphibian Metamorphosis Assays to detect endocrine disrupting chemicals that potentially target the thyroid axis.

From: Dr. Doug Fort, Fort Environmental Laboratories (email: djfort@hotmail.com)

To: Dr. Robert Grainger, Department of Biology, University of Virginia, Charlottesville, VA

Response: Dr. Grainger had not responded to the questionnaire at the time of publication.

Questionnaire sent via email:

Questionnaire: Development of Amphibian Metamorphosis Assays to detect endocrine disrupting chemicals that potentially target the thyroid axis.

From: Dr. Doug Fort, Fort Environmental Laboratories (email: djfort@hotmail.com)

To: Dr. Tyrone B. Hayes, Department of Integrative Biology, University of California, Berkeley, CA

Response: Dr. Hayes had not responded to the questionnaire at the time of publication.