AGENDA

Meeting of the Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel (FIFRA SAP) to Consider and Review Scientific Issues Associated with the Proposed Endocrine Disruptor Screening Program (EDSP) Tier Ecotoxicity Tests

June 25-28, 2013

Docket Number: EPA-HQ-OPP-2013-0182 **OPP Docket Tel: 703-305-5805**

Please note that all times are approximate
(See note at the end of the Agenda)

Tuesday, June 25, 2013

9:00 A.M. **Opening of Meeting and Administrative Procedures** – Sharlene Matten, Ph.D., Designated Federal Official, Office of Science Coordination and Policy, EPA

9:05 A.M. **Introduction and Identification of Panel Members** – Martha Sandy, Ph.D., Session Chair, FIFRA Scientific Advisory Panel

9:15 A.M. **Opening Remarks** – Steven Knott, Deputy Director, Office of Science Coordination and Policy and Steven Bradbury, Ph.D. Director, Office of Pesticide Programs, EPA

9:25 A.M. **Overview of EDSP and Proposed Tier 2 Ecotoxicity Tests** – Mary Manibusan, Director, Exposure Assessment Coordination and Policy Division, Office of Science Coordination and Policy, EPA

10:00 A.M. **Break**

10:10 A.M. **Japanese Quail Two-generation Toxicity Test –** Leslie Touart, Ph.D., Exposure Assessment Coordination and Policy Division, Office of Science Coordination and Policy, EPA

11:10 A.M. **Medaka Multigeneration Toxicity Test –** Rodney Johnson, Ph.D., Mid-Continent Ecology Division, National Health and Environmental Effects Research Laboratory – Duluth, Office of Research and Development, EPA

12:10 P.M. **Lunch**

1:10 P.M. **Larval Amphibian Growth and Development Assay –**Sigmund Degitz, Ph.D., Mid-Continent Ecology Division, National Health and Environmental Effects Research Laboratory – Duluth, Office of Research and Development, EPA

2:30 P.M. **Break**

2:40 P.M. **Mysid Two-generation Toxicity Test and Harpacticoid Copepod Development & Reproduction Test –** Leslie Touart, Ph.D., Exposure Assessment Coordination and Policy Division, Office of Science Coordination and Policy, EPA

3:40 P.M. **Break**

3:45 P.M. **Public Comments**

5:45 P.M. **Adjournment**

AGENDA

Wednesday, June 26, 2013

9:00 A.M. **Opening of Meeting and Administrative Procedures** – Sharlene Matten, Ph.D., Designated Federal Official, Office of Science Coordination and Policy, EPA

9:05 A.M. **Introduction and Identification of Panel Members** – Martha Sandy, Ph.D., Session Chair, FIFRA Scientific Advisory Panel

9:10 A.M. **Follow-up from Previous Day's Meeting** – Mary Manibusan, Director, Exposure Assessment Coordination and Policy Division, Office of Science Coordination and Policy, EPA

9:20 A.M. **Additional Public Comments (if needed)**

10:20 A.M. **Break**

10:30 A.M. **Panel Discussion of Charge Questions**

JAPANESE QUAIL TWO-GENERATION TEST (JQTT)

JQTT Question 1

A rationale for the test method should be available, including a clear statement of scientific basis and the regulatory purpose and need for the test method. The EDSTAC described the Tier 2 tests as having the purpose to "*characterize the nature, likelihood, and dose-response relationship of endocrine disruption of estrogen, androgen, and thyroid in humans and wildlife*." Tier 2 tests were designed to be definitive tests that generate sufficient data to characterize the specific hazard of the substance and provide sufficient information on dose-response and adverse effects to permit risk decisions.

Please comment on the rationale and purpose of the assay as part of the Tier 2 testing in the EDSP, as described in Sections 2.5 and 2.6 of the JQTT ISR.

JQTT Question 2

Test methods and their associated endpoint(s) should be scientifically relevant to the biological processes of interest and should be demonstrated to be responsive to the specific type of effect/toxicity of interest. Each species presents unique characteristics from a biological perspective and allows for specialized endpoints to address a specific toxicological mode of action.

Please comment on the biological and toxicological relevance of the assay in regards to the stated purpose of characterizing endocrine disruptors, as described in Sections 3 and 4 of the JQTT ISR.

JQTT Question 3

JQTT Question 3.1 The test protocol should be sufficiently detailed and should include a description of what is measured and how it is measured. The selection of endpoints within the assay should be reflective of the biological processes of interest and the endpoints should be intrinsically relevant and have established sensitivity. The test protocol should demonstrate the ability to measure the endpoints and provide adequate performance criteria for evaluation.

Please comment on the selection, optimization and demonstration of the assay endpoints, as outlined in Sections 4 and 5 of the JQTT ISR.

JQTT Question 3.2 Pathological evaluation of histological tissue preparations is an established, sensitive and integral endpoint in the assessment of effects in long term *in vivo* assays. The tissues targeted for histopathological evaluation should be shown to be sensitive to exposure and relevant to a mode of action or pathway determination. Tissue samples from several organs or glands (*e.g.*, kidney, liver, thyroid, gonads) underwent histopathological examination in the

JQTT inter-laboratory validation and a discussion of histopathology as an endpoint is provided in Section 4.1.13 of the JQTT ISR.

Please comment on the value of histopathological analyses in the JQTT assay for each of the tissue types examined and what (if any) critical information is gained from their inclusion or would be lost if histology were not examined.

12:00 P.M. Lunch

1:00 P.M. JQTT Charge Questions (cont'd)

JQTT Question 4

Demonstration of the test method performance should be based on the testing of reference chemicals representative of the types of substances for which the test method will be used. Test substances should adequately represent an appropriate range of responses and physical/chemical properties for which the test method is proposed to be appropriate. The selection of the most appropriate statistical approaches depends in part on the nature of the data and also on the design of the validation study. Statistical and non-statistical methods used to analyze should be described.

Please comment on the selection of test substances and methods (analytical and statistical where appropriate) chosen for the demonstration and validation of the JQTT assay.

JQTT Question 5

Considering the variability inherent in biological and chemical test methods, a test method needs to be repeatable and reproducible. A test is robust and reliable if the results are repeatable and reproducible within a laboratory and between different laboratories, respectively. A test protocol should provide sufficient guidance to ensure proper and consistent performance across labs and chemicals.

Please comment on the test method robustness and reliability and the repeatability and reproducibility of the results obtained with the JQTT assay.

JQTT Question 6

The test protocol should be descriptive enough to be fully transferable to a competent laboratory. The protocol should describe the methodology of the assay in a clear and concise manner so that a laboratory could comprehend the objective, conduct the assay, observe and measure prescribed endpoints, compile and prepare data for statistical analyses, and report the results. Section 6 of the JQTT ISR outlines the process of and challenges experienced in the inter-laboratory studies.

Please comment on the transferability across labs and provide any suggestions or recommendations for improvement of the JQTT assay.

2:45 P.M. Break

3:00 P.M. JQTT Charge Questions (cont'd)

JQTT Question 7

The purpose of the validation process is to determine the readiness of a test for inclusion in a testing program. A component of readiness of a test is the evaluation of the usefulness and limitations of the test, including the classes and types of test substances that can and cannot be tested.

Please comment on the strengths and/or limitations of the JQTT assay, as described in Section 7 of the JQTT ISR.

JQTT Question 8

There is sufficient evidence to indicate that EDCs can disrupt normal development and reproductive success, however the sensitivity of the F2 generation compared to the P0 or F1 is less clearly defined. In the JQTT, there is an increase in endpoint CVs with each subsequent generation (JQTT ISR Table 6-4), which indicates decreased power of discrimination.

Please comment from a scientific and risk assessment perspective on the value added of multiple generations in the JQTT assay.

3:55 P.M. **Break**

4:00 P.M. **Panel Discussion of Charge Questions (cont'd)**

MEDAKA MULTIGENERATION TOXICITY TEST (MMT)

Section 5 of the MMT ISR outlines the EPA proposed Medaka Reproduction Test (MRT) assay which utilizes the culmination of knowledge and experience gained from many successive U.S. and Japanese studies over multiple years.

MMT Question 1
A rationale for the test method should be available, including a clear statement of scientific basis and the regulatory purpose and need for the test method. The EDSTAC introduced the Tier 2 tests as having the purpose to "*characterize the nature, likelihood, and dose-response relationship of endocrine disruption of estrogen, androgen, and thyroid in humans and wildlife.*" Tier 2 tests were designed to be definitive tests which generate sufficient data to characterize the specific hazard of the substance and provide sufficient information on dose-response and adverse effects to permit risk decisions.
Please comment on the rationale and purpose of the assay as part of the Tier 2 testing in the EDSP, as described in Sections 2.1 and 2.2 of the MMT ISR.

MMT Question 2
Test methods and their associated endpoint(s) should be scientifically relevant to the biological processes of interest and should be demonstrated to be responsive to the specific type of effect/toxicity of interest. Each species presents unique characteristics from a biological perspective and allows for specialized endpoints to address a specific toxicological mode of action.
Please comment on the biological and toxicological relevance of the assay in regards to the stated purpose of characterizing endocrine disruptors, as described in Section 3 of the MMT.

MMTQuestion 3
The test protocol should be sufficiently detailed and should include a description of what is measured and how it is measured. The selection of endpoints within the assay should be reflective of the biological processes of interest and the endpoints should be intrinsically relevant and have established sensitivity. The test protocol should demonstrate the ability to measure the endpoints and provide adequate performance criteria for evaluation.
Please comment on the selection, optimization and demonstration of the assay endpoints, as outlined in Section 3 of the MMT ISR.

5:30 P.M. **Adjournment**

Thursday, June 27, 2013

9:00 A.M. **Opening of Meeting and Administrative Procedures** – Sharlene Matten, Ph.D., Designated Federal Official, Office of Science Coordination and Policy, EPA

9:05 A.M. **Introduction and Identification of Panel Members** – Martha Sandy, Ph.D., Session Chair, FIFRA Scientific Advisory Panel

9:10 A.M. **Follow-up from Previous Day's Meeting** – Mary Manibusan, Director, Exposure Assessment Coordination and Policy Division, Office of Science Coordination and Policy, EPA

9:20 A.M. **MMT Test Charge Questions (cont'd)**

MMT Question 4

Demonstration of the test method performance should be based on the testing of reference chemicals representative of the types of substances for which the test method will be used. Test substances should adequately represent an appropriate range of responses and physical/chemical properties for which the test method is proposed to be appropriate. The selection of the most appropriate statistical approaches depends in part on the nature of the data and also on the design of the validation study. Statistical and non-statistical methods used to analyze should be described.

Please comment on the selection of test substances and methods (analytical and statistical where appropriate) chosen for the demonstration and validation of the MMT and MRT assays.

MMT Question 5

Considering the variability inherent in biological and chemical test methods, a test method needs to be repeatable and reproducible. A test is robust and reliable if the results are repeatable and reproducible within a laboratory and between different laboratories, respectively. A test protocol should provide sufficient guidance to ensure proper and consistent performance across labs and chemicals.

Please comment on the test method robustness and reliability and the repeatability and reproducibility of the results obtained with the MTT and MRT assays.

10:20 A.M. Break

10:30 A.M. MMT Test Charge Questions (cont'd)

MMT Question 6

The test protocol should be descriptive enough to be fully transferable to a functional laboratory. The protocol should describe the methodology of the assay in a clear and concise manner so that a laboratory could comprehend the objective, conduct the assay, observe and measure prescribed endpoints, compile and prepare data for statistical analyses, and report the results. Section 4 of the MMT ISR outlines the process of and challenges experienced in the inter-laboratory studies and Section 5 presents the optimization and proposed Medaka Reproduction Test (MRT) protocol.

Please comment on the transferability across labs and provide any suggestions or recommendations for improvement of the.MMT and MRT assays.

MMT Question 7

The purpose of the validation process is to determine the readiness of a test for inclusion in a testing program. A component of readiness of a test is the evaluation of the usefulness and limitations of the test, including the classes and types of test substances that can and cannot be tested.

Please comment on the strengths and/or limitations of the MMT and MRT assays.

MMT Question 8

There is sufficient evidence to indicate that EDCs can disrupt normal development and reproductive success, however the value added of the F2 generation compared to the P0 or F1 is less clearly defined. The EPA is proposing the Medaka Reproduction Test (MRT) as an EDSP Tier 2 assay. The MRT test terminates after the F2 embryo hatch, and a rationale for the proposed protocol is provided in Section 5 of the MMT ISR.

Please comment on the Agency's rationale that the value added by the F2 generation is not sufficient to warrant its inclusion in the Tier 2 fish test protocol.

MMT Question 9

It is the Agency's opinion that the outcomes of the various MMT trials have provided enough information to recommend a medaka reproduction test (MRT) for use as the fish test in Tier 2 of the EDSP. Two major changes from the MMT are proposed, *i.e.,* an increase in the number of replicates per treatment for evaluating effects on reproduction, and terminating the test after the embryos hatch in F2. Other proposed changes include minimizing the collection of endpoint data from F0, and evaluating pathology in only the F1 adults sampled after the assessment of reproduction.

Overall, the authors conclude that both the MMT and the MRT are transferable methods and are capable of adequately characterizing potential disruption of the endocrine system by putative endocrine disrupting chemicals. However, the MRT is recommended as the preferred EDSP Tier 2 test method for fish because it is less resource intensive with improved statistical power, appears to be as sensitive, and is better able to ensure consistent findings when performed routinely by testing laboratories.

Please comment on the scientific rationale of the Agency's proposed Tier 2 fish test, the MRT, with respect to statistical power, sensitivity, and consistency in performance across laboratories. In addition, please comment on the adequacy of the MRT to characterize potential endocrine disruption, a requirement of Tier 2 of the EDSP.

12:15 P.M. Lunch

1:15 P.M. Panel Discussion of Charge Questions

LARVAL AMPHIBIAN GROWTH AND DEVELOPMENT ASSAY (LAGDA)

LAGDA Question 1

A rationale for the test method should be available, including a clear statement of scientific basis and the regulatory purpose and need for the test method. The EDSTAC introduced the Tier 2 tests as having the purpose to "*characterize the nature, likelihood, and dose-response relationship of endocrine disruption of estrogen, androgen, and thyroid in humans and wildlife*." Tier 2 tests were designed to be definitive tests which generate sufficient data to characterize the specific hazard of the substance and provide sufficient information on dose-response and adverse effects to permit risk decisions.

Please comment on the rationale and purpose of the assay as part of the Tier 2 testing in the EDSP, as described in Section 3 of the LAGDA ISR.

LAGDA Question 2

Test methods and their associated endpoint(s) should be scientifically relevant to the biological processes of interest and should be demonstrated to be responsive to the specific type of effect/toxicity of interest. Each species presents unique characteristics from a biological perspective and allows for specialized endpoints to address a specific toxicological mode of action. **Please comment on the biological and toxicological relevance of the assay in regards to the stated purpose of characterizing endocrine disruptors, as described in Sections 3 and 4 of the LAGDA ISR.**

LAGDA Question 3

LAGDA Question 3.1

The test protocol should be sufficiently detailed and should include a description of what is measured and how it is measured. The selection of endpoints within the assay should be reflective of the biological processes of interest and the endpoints should be intrinsically relevant and have established sensitivity. The test protocol should demonstrate the ability to measure the endpoints and provide adequate performance criteria for evaluation.

Please comment on the selection, optimization and demonstration of the assay endpoints, as outlined in Sections 3 and 4 of the LAGDA ISR.

LAGDA Question 3.2

Three of the four labs in the inter-laboratory validation of the LAGDA were unsuccessful in properly performing the thyroxine (T4) analyses using commercially available enzyme-linked Immunosorbent assay (ELISA) kits with antibodies specific for human or canine T4. Subsequently, the EPA developed an extraction method (Section 5.3.2) and presents a revised ELISA method for measuring T4 in amphibian samples in Appendix 7 of the LAGDA ISR.

Please comment on the technical feasibility, reproducibility, and accuracy of the revised ELISA T4 measurement method. Provide any recommendations regarding additional guidance to ensure the reproducibility of T4 measurements.

2:45 P.M. Break

3:00 P.M. LAGDA Charge Questions (cont'd)

LAGDA Question 3.3

Vitellogenin (VTG) is an established biomarker of estrogenic exposure and is used as a key endpoint in endocrine disruptor testing. There is currently not a standardized commercial source for *Xenopus laevis* VTG antibodies for an ELISA.

Please comment on the protocol for measuring and reporting VTG levels. Provide any recommendations regarding additional guidance to ensure the consistency, repeatability and reproducibility of VTG measurements.

LAGDA Question 4

Demonstration of the test method performance should be based on the testing of reference chemicals representative of the types of substances for which the test method will be used. Test substances should adequately represent an appropriate range of responses and physical/chemical properties for which the test method is proposed to be appropriate. The selection of the most appropriate statistical approaches depends in part on the nature of the data and also on the design of the validation study. Statistical and non-statistical methods used to analyze should be described.

Please comment on the selection of test substances and methods (analytical and statistical where appropriate) chosen for the demonstration and validation of the LAGDA assay.

LAGDA Question 5

Considering the variability inherent in biological and chemical test methods, a test method needs to be repeatable and reproducible. A test is robust and reliable if the results are repeatable and reproducible within a laboratory and between different laboratories, respectively. A test protocol should provide sufficient guidance to ensure proper and consistent performance across labs and chemicals.

Please comment on the test method robustness and reliability and the repeatability and reproducibility of the results obtained with the LAGDA assay.

LAGDA Question 6

The test protocol should be descriptive enough to be fully transferable to a functional laboratory. The protocol should describe the methodology of the assay in a clear and concise manner so that a laboratory could comprehend the objective, conduct the assay, observe and measure prescribed endpoints, compile and prepare data for statistical analyses, and report the results. Sections 5 and 6 of the LAGDA ISR outline the process of and challenges experienced in the inter-laboratory studies.

Please comment on the transferability across labs and provide any suggestions or recommendations for improvement of the LAGDA assay.

LAGDA Question 7

The purpose of the validation process is to determine the readiness of a test for inclusion in a testing program. A component of readiness of a test is the evaluation of the usefulness and limitations of the test, including the classes and types of test substances that can and cannot be tested.

Please comment on the strengths and/or limitations of the LAGDA assay.

5:30 P.M. Adjournment

AGENDA

Friday, June 28, 2013

9:00 A.M. **Opening of Meeting and Administrative Procedures** – Sharlene Matten, Ph.D., Designated Federal Official, Office of Science Coordination and Policy, EPA

9:05 A.M. **Introduction and Identification of Panel Members** – Martha Sandy, Ph.D., Session Chair, FIFRA Scientific Advisory Panel

9:10 A.M. **Follow-up from Previous Day's Meeting** – Mary Manibusan, Director, Exposure Assessment Coordination and Policy Division, Office of Science Coordination and Policy, EPA

9:20 A.M. **Panel Discussion of Charge Questions (cont'd)**

MYSID TWO-GENERATION TOXICITY TEST (MTTT) AND HARPACTICOID COPEPOD DEVELOPMENT & REPRODUCTION TEST (HCDRT)

MTTT Question 1

A rationale for the test method should be available, including a clear statement of scientific basis and the regulatory purpose and need for the test method. The EDSTAC introduced the Tier 2 tests as having the purpose to "*characterize the nature, likelihood, and dose-response relationship of endocrine disruption of estrogen, androgen, and thyroid in humans and wildlife.*" Although the hormones produced and used by invertebrates are not directly analogous to those of vertebrates (e.g., estrogen, androgen, and thyroid), growth, reproduction, development, and other aspects of invertebrate physiology and life cycle are known to be under endocrine control. EDSTAC went on to note that "*chemicals that affect these vertebrate hormones may also affect invertebrate hormones resulting in altered reproduction, development, and growth*." Tier 2 tests were designed to be definitive tests which generate sufficient data to characterize the specific hazard of the substance and provide sufficient information on dose-response and adverse effects to permit risk decisions.

Please comment on the rationale and purpose of the assay as part of the Tier 2 testing in the EDSP, as described in Sections 2.1 and 2.2 of the MTTT ISR.

MTTT Question 2

Test methods and their associated endpoint(s) should be scientifically relevant to the biological processes of interest and should be demonstrated to be responsive to the specific type of effect/toxicity of interest. Each species presents unique characteristics from a biological perspective and allows for specialized endpoints to address a specific toxicological mode of action.

Please comment on the biological and toxicological relevance of the assay in regards to the stated purpose of characterizing endocrine disruptors, as described in Section 2 of the MTTT ISR.

10:20 A.M. **Break**

10:30 A.M. **MTTT and HCDRT Charge Questions (cont'd)**

MTTT Question 3

The test protocol should be sufficiently detailed and should include a description of what is measured and how it is measured. The selection of endpoints within the assay should be reflective of the biological processes of interest and the endpoints should be intrinsically relevant and have established sensitivity. The test protocol should demonstrate the ability to measure the endpoints and provide adequate performance criteria for evaluation.

Please comment on the selection, optimization and demonstration of the assay endpoints, as outlined in Sections 3 and 4 of the MTTT ISR.

MTTT Question 4

MTTT Question 4.1

Demonstration of the test method performance should be based on the testing of reference chemicals representative of the types of substances for which the test method will be used. Test substances should adequately represent an appropriate range of responses and physical/chemical properties for which the test method is proposed to be appropriate. The selection of the most appropriate statistical approaches depends in part on the nature of the data and also on the design of the validation study. Statistical and non-statistical methods used to analyze should be described. **Please comment on the selection of test substances and methods (analytical and statistical where appropriate) chosen for the demonstration and validation of the MTTT assay.**

MTTT Question 4.2

The proposed MTTT study design uses three replicates per control and treatment group and additional replicates require greater resources.

Please comment on the proposed level of replication and the subsequent statistical analysis of the data.

MTTT Question 5

Considering the variability inherent in biological and chemical test methods, a test method needs to be repeatable and reproducible. A test is robust and reliable if the results are repeatable and reproducible within a laboratory and between different laboratories, respectively. A test protocol should provide sufficient guidance to ensure proper and consistent performance across labs and chemicals.

Please comment on the test method robustness and reliability and the repeatability and reproducibility of the results obtained with the MTTT assay.

12:00 P.M. Lunch

1:00 P.M MTTT and HCDRT Charge Questions (cont'd)

MTTT Question 6

MTTT Question 6.1

The test protocol should be descriptive enough to be fully transferable to a functional laboratory. The protocol should describe the methodology of the assay in a clear and concise manner so that a laboratory could comprehend the objective, conduct the assay, observe and measure prescribed endpoints, compile and prepare data for statistical analyses, and report the results. Section 5 of the MTTT ISR outlines the process of and challenges experienced in the inter-laboratory studies. **Please comment on the transferability across labs and provide any suggestions or recommendations for improvement of the MTTT assay.**

MTTT Question 6.2

Based on the validation results using the two different invertebrate species, mysid (MTTT) and copepod (HCDRT), the EPA is proposing the mysid protocol as the preferred Tier 2 assay.

Please comment on the rationale to recommend the mysid protocol as the preferred Tier 2 invertebrate assay, as described in Section 6.4 of the MTTT ISR.

MTTT Question 7

The purpose of the validation process is to determine the readiness of a test for inclusion in a testing program. A component of readiness of a test is the evaluation of the usefulness and limitations of the test, including the classes and types of test substances that can and cannot be tested.

Please comment on the strengths and/or limitations of the assay, as described in Section 6 of the MTTT ISR.

MTTT Question 8

There is sufficient evidence to indicate that EDCs can disrupt normal development and reproductive success, however the sensitivity of the F1 generation compared to the F0 is less clearly defined.

Please comment from a scientific and risk assessment perspective on the value added of multiple generations in the MTTT assay.

3:00 P.M. Adjournment

Please be advised that agenda times are approximate; when the discussion for one topic is completed, discussions for the next topic will begin. For further information, please contact the Designated Federal Official for this meeting, Dr. Sharlene Matten, telephone: (202)-564-0130, fax: (202) 564-8382, or email: matten.sharlene@epa.gov.