



April 11, 2002

Ms. Jane Smith  
EDMVS Designated Federal Official  
Office of Science Policy and Coordination  
US EPA Ariel Rios Building  
1200 Pennsylvania Avenue, NW  
Room ET 809A MC 7203M  
Washington, DC 20460

Dear Ms. Smith

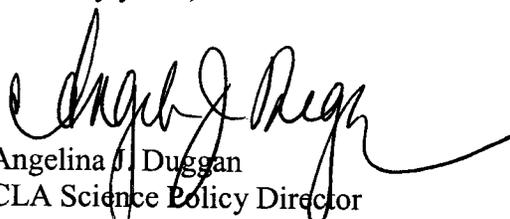
CLA supports the Agency's establishment of the Endocrine Disruptor Methods Validation Subcommittee (EDMVS), and EPA's efforts to seek technical advice and peer-review through multi-stakeholder involvement on the Subcommittee and public comments. In the spirit of our commitment, CLA would like to offer the following written versions of the following CLA public comments.

- Avian Dosing Study, March 25, 2002
- Fish Reproduction Study Plan, March 25, 2002
- In Utero Lactation Assay, March 26, 2002

CLA represents the manufacturers and formulators of conventional chemical and biotechnology derived crop protection products in the United States. We have long supported EPA in the development and implementation of a validated endocrine screening and testing program in accordance with the mandates in the 1996 Food Quality Protection Act. Our staff and member company representatives actively participated in the previous EDSTAC process. We believe that FQPA directs EPA to ensure that an endocrine evaluation program has a strong scientific foundation in the design of methodology and protocols to support a robust validation process. We also believe that EPA should ensure a transparent and systematic priority setting process for chemicals and pesticides.

Questions regarding these comments should be directed to me.

Sincerely yours,



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**Crop Life America (CLA)  
Public Comments Avian Dosing Study**

**Endocrine Disruption Methods Validation Subcommittee (EDMVS) Meeting  
March 25, 2002**

**Presented by Kristin Brugger, Ph.D.  
DuPont Crop Protection Products**

Crop Life America is submitting comments on the USEPA draft document entitled "Draft Study Plan on Avian Dosing Study, dated 26 February 2002. The document is a proposal for developing background data for establishing an agreed method for exposing birds to test substances. The study is proposed to address the need for experimental data regarding timing of onset of treatment for detecting reproductive and developmental effects over two generations and whether the first filial generation should receive dietary treatment of the test substance.

There are 7 main points to be made in these comments. First is a policy issue: one must ask if the dosing study is needed or if a reasonable worst case exposure can be agreed at the start of research. Second is a question regarding the framework in which physiological data are needed in a Tier 2 study. Assuming the work will go forward in an effort to develop science-based decision making within USEPA, then the remaining comments focus on conduct of the proposed study. The third comment is a precautionary note about the capacity required by a lab to successfully complete the proposal in its current form, with suggestions to reduce the scale and narrow the focus of research. Fourth is a recommendation that USEPA develop an expert team familiar with details of the procedures to assist in design, protocol development and implementation, data interpretation and analyses of feasibility of each research activity. Fifth is a comment that the test substances proposed for evaluation appear to be reasonable. Sixth is a comment that the proposed procedures to evaluate dosing and mating behavior appear to be reasonable.

Is the approach appropriate? The proposed study correctly identifies the OECD expert group's concerns about dosing in a 2-generation protocol. However the need for the protocol and design features are policy issues. Given that other protocols for long term exposure include a full-life exposure (e.g., fish full life cycle protocol), would it not be prudent to use expert judgment to define dosing methods in the avian two-generation protocol? A reasonable worst-case exposure scenario could be defined as exposure at every life stage (i.e., exposure begins prior to sexual maturation in P1 and continues through F1) since this likely will occur in practice and since many products are used prior to birds coming into breeding condition. Also, even if a product is not intended for use until after birds come into breeding conditions, business cases often change and new uses developed. A company would not want to put itself in a situation where it had to redo this type of study due to a new use. This also will reduce a large number of birds to be used in this dosing study. Then the study design would be comparable to existing higher tier protocols. At point is the need for  $\pm 19,440$  animals per chemical at a potential cost of  $> \$1$  million to develop data to support a science-based decision. Although the laboratory will gain valuable experience with SOPs, there are likely other study designs that would use fewer animals and return greater information to the scientific community.

**Public Comments Avian Dosing Study**

How did we get here? At the stage of a Tier 2 test, there is already an indication of endocrine effects from studies at the lower tier. In the Tier 2 test, we are looking for relevant reproductive effects, not mode of action. Are physiological or endocrine endpoints necessary at Tier 2? We believe that physiological endpoints can be important at Tier 2 as long as the results are used to identify further research needs for protocol development. It may be best to separate effects (fitness) data from future research (physiological) data in a pre-validation study. Fitness and physiological endpoints can be part of the same study, but a framework is needed for data interpretation. However, evaluation of all of the physiological endpoints should not occur at the expense of the study as a whole. Therefore, there must be a clear statement of priorities, division of labor, and resources. It may be necessary to focus on fitness endpoints with fewer, more important physiological endpoints that address effects, not mode of action.

Assuming the work will go forward in an effort to develop science-based decision making within USEPA, the remaining comments are provided as suggestions to improve the plan.

The proposed research with two chemicals will be a very large undertaking by any laboratory. Thus, capacity needs to be well planned and pragmatic limits should be recognized. Because this is a first attempt to undertake a two-generation study with two chemicals, the USEPA should consider reducing endpoints to a few clear ones and consider reducing sample sizes to statistically acceptable sizes that will allow critical interpretation. Clear focus on reproductive and developmental endpoints would ensure that the protocol addresses Tier 2 testing objectives. The number of pairs could be reduced by 50% to 8 or even to 6 and still meet statistical power requirements. Available evidence suggests that reproductive parameters of Japanese quail are less variable than those of mallards or bobwhite, thus 16 pairs per treatment level may not be needed. The number of test levels could be reduced from 5 to 4, with an increase in the range of test concentrations to elicit a greater range of responses. Alternatively, several focused research projects could be established to develop pilot data on endpoints.

Because of the exploratory nature of the study and the critical need to focus research on empirical endpoints that assist the decision making process, the chemical industry strongly recommends establishment of an expert team for technical oversight and collaboration with the laboratory and USEPA. There is a strong need to establish *a priori* hypotheses for every protocol within the larger proposal and develop peer reviewed standard operating procedures for each protocol. The research lends itself to a feasibility analysis of each method to evaluate cost, time, teams of staff, equipment required to complete each assay.

Management of large-scale blood collections in chronic toxicity testing is a familiar activity to industry toxicologists. Pragmatic suggestions to the laboratory that will conduct this work are to consider staggering blood collections over 2-4 days. It is expected that the hormonal responses to both proposed test substances are likely to be stable after a 6-week exposure duration. As the proposal states, it is necessary to sample at same time of day (6-9:30 am). Consideration should be given to what is analyzed (e.g., plasma v. serum). In DuPont's experience, it is possible to see lower basal estradiol and T4 in plasma as compared to serum. It is pragmatic to bleed across dose groups if the collection is staggered across days to reduce temporal bias. Based on the current proposal, there would be a need for at least 5 teams for blood collection at necropsy.

**Public Comments Avian Dosing Study**

The proposal is not clear on inclusion of histopathology. The background section suggests that expert consultation agreed to not include histopath, but a statement is made that organs will be collected and analyzed. It may be worth preserving the tissues in case there are questions regarding certain endpoint that need clarification. For higher tier (i.e., mode of action) research, methods need to be developed and validated prior to the introduction of histopathology as a standard requirement.

The section on statistical analyses needs greater effort, possibly by the proposed expert team, to ensure clarity of hypotheses and appropriateness of study designs to meet the needs of each analysis. For example, the use of ANOVAs needs clarification. Is this Bonferonni adjusted? How will uneven samples and unbalanced designs be handled? There is a statement that time series analyses will be used, however the study design appears to be a repeated measures ANOVA.

Comments were requested on the selection of test substances. Based on experience at DuPont with mammalian testing, 17B estradiol is appropriate. It is a test substance recommended by OECD and would enable global comparison of results with other endocrine research, possibly with other species and test protocols. In DuPont's experience the test substance may be solubilized in acetone and mixed in the diet. Usual personal protective equipment is required. Additionally, methyl parathion is appropriate for birds as a test substance. Several studies exist from the USA and Germany with methyl parathion and northern bobwhite and Japanese quail. Data from the proposed research will enable comparison of species sensitivities and protocol outcomes.

The USEPA asked whether proposed methods to develop dosing methods of the F1 and P1 generations are appropriate. The proposals appear to be appropriate. Additionally, the proposal to include male mating behavior seems to be appropriate. Previous research supports the behavioral correlate to plasma testosterone. Additionally a feasibility analysis will be helpful here as well.

There are minor errors in the draft document. These include but are not limited to spelling errors, missing words, designation of P1 and F1/F2 to treatment scenarios, and a cut and paste from a University of Maryland protocol to the Battelle protocol. On page A-6, birds of different ages appear to be breeding in the P1 vs. F1a and F1b. An unbalanced study design is suggested for egg laying in P1 v. F1 (28 vs. 42 days of egg laying). Appendix B is missing ZM189,154 as a rat uterotrophic estrogen agonist and p,p' prefix to DDE and CGS 183 in the rat sub-chronic. Last, the proposal implies use of Japanese quail, but never specifically states so. A clarification would be helpful.

As an industry we are in agreement with the direction of USEPA in developing a two-generation study with birds for detection of endocrine effects, but we believe some of the details need more attention and the study needs to be put in a risk assessment context.

**Crop Life America (CLA)  
Public Comments for Fish Reproduction Study Plan**

**Endocrine Disruption Methods Validation Subcommittee (EDMVS) Meeting  
March 25, 2002**

**Presented by Katie Holmes, Ph.D.  
BASF AgroResearch**

Tomorrow you will be asked to comment on the fish reproduction study plan. I ask that you consider the following thoughts during your discussion.

First and foremost, the USEPA proposed fish reproduction method is neither a "short term" nor a "screening assay". A 14-21 day (28-35 day, with the mandatory pre-exposure period) reproduction assay is not a feasible Tier I screen. EDSTAC clearly defines the goals Tier I screening. First, they should be relatively inexpensive, quick, and technically easy to perform. Second they should be sensitive and specific, capture multiple endpoints, and be predictive across species, gender, and age. And third, they should be validated and standardized before they are used routinely by testing laboratories. While Crop Life America applauds efforts to move forward with the third goal of validation and standardization, we believe that the current proposed fish reproduction draft study plan misses the mark on the first two stated goals.

(It should also be noted that the OECD Eco Validation Management Group specifically asked that this study be reevaluated. The overall impression was that the proposed study was too complex and long to be a screen and a bit light to be a higher tier definitive study.)

In the USEPA proposed plan, only three of the nine endpoints are relevant for identifying specific modes of action. These three include vitellogenin levels, observation of secondary sex characteristics, and the measurement of plasma steroids. The remaining six, including fecundity, fertility, hatchability and survival, gonad weight and GSI, behavior, and gonad histopathology are apical and are affected by multiple biochemical and physiochemical pathways.

In terms of improving the proposed study, Crop Life America believes that it should be clearly stated which endpoints are indicative of a specific mode of action. Analysis should be designed to specifically link an endpoint with a mode of action. There is a need to examine which modes of action are not currently linked with a specific endpoint. Finally, given the large number of chemicals involved, a feasibility assessment needs to be completed.

Crop Life America believes that the proposed OECD 14-day screening assay more closely follows the definition of a screen. The proposed study allows the determination of mode of action with fewer extraneous endpoints. We do not believe that histopathology should be a routine screening assay requirement. We support further testing to determine the feasibility of a 7-day assay.

Finally, some brief technical comments:

- Vitellogenin measurement should use the optimized assay selected in the vitellogenin study.
- The proposed hatchability and survival criteria are unusually stringent and do not reflect the current guidelines.
- Two of the proposed model compounds may not be suitable:

- 1) Methyltestosterone (androgen)- produces estrogenic metabolite causing vitellogenin induction in both male and female fish
- 2) Flutamide (mammalian anti-androgen)- reportedly does not bind to fish androgen receptor (Wells and Van Der Kraak, 2000).

**Crop Life America (CLA)  
Public Comments In Utero Lactation Assay**

**Endocrine Disruption Methods Validation Subcommittee (EDMVS) Meeting  
March 26, 2002**

**Presented by Angelina J. Duggan, Ph.D.  
Director of Science Policy**

CropLife America (CLA) thanks EPA for the opportunity to address the Endocrine Disruptor Methods Validation Subcommittee (EDMVS) and the public about the in utero lactation assay.

CLA represents the manufacturers and formulators of conventional chemical and biotechnology derived crop protection products in the United States. We have long supported EPA in the development and implementation of a validated endocrine screening and testing program in accordance with the mandates in the 1996 Food Quality Protection Act. In the spirit of our commitment, CLA would like to offer the following comments regarding the proposed in-utero lactation assay. We hope that our comments will spark a thorough consideration of important issues that we believe need to be considered by EPA and the Subcommittee before EPA invests significant resources to validate this assay. CropLife America does not support the in utero lactation assay as a Tier 1 screen or as a Tier 2 test. In our opinion, EPA has not provided convincing scientific rationale about the benefits and need to proceed with the lengthy time and extensive resources that would be required to develop and validate an in utero lactation assay.

The proposed Tier 1 screens currently include in vitro systems for receptor binding/gene transcriptional activity to assess activity at the molecular level and the uterotrophic and Hershberger assays that use immature and/or castrated animals to heighten sensitivity. These screens have been purposely designed to maximize the identification of chemical substances that may interact with the endocrine system and to provide mechanistic information on potential estrogen, androgen and thyroid effects.

The in utero lactation assay is not a Tier-1 screen. The proposed study design, that includes histopathology, is too complex to serve as a screen. Moreover, the sensitivity of this assay, in comparison to other Tier 1 screens, has not been demonstrated or supported by the available scientific data. The assay is also resource intensive, more than 1000 rats will be used to provide 800 off-spring from 60 pregnant dams. The assay is also inefficient since it will take at least 13 weeks to complete the study – much too long for a screen.

Additionally, there is no information to support that the assay is an economical replacement for other screens or tests, as examples, the Hershberger or uterotrophic assay. To examine this, we recommend that EPA conduct a resource analysis (time, number of animals, and cost) that compares the in-utero lactation assay with the other EDSTAC recommended screens and tests.

We are also concerned that the in utero lactation assay could provide confounding results that would not be explained by subsequent Tier 2 endocrine tests since the assay measures apical endpoints that may be altered via non-endocrine mechanisms of toxicity. Additionally, the route of exposure, gavage (“forced feeding” by a tube), does not reflect real world dietary exposure to either the mothers or their offspring.

**Public Comments In Utero Lactation Assay**

The in utero lactation assay is more appropriately aligned with the objectives of a Tier 2 test since the assay utilizes whole animals and includes gross and histopathology. However, as a regulatory test, the in-utero lactation assay is redundant with the other regulatory tests that have a long history of use under either the Federal Insecticide Fungicide and Rodenticide Act (FIFRA) or the Toxic Substances Control Act (TSCA). Under FIFRA food use pesticides undergo at least 120 separate tests. This testing includes the rat multi-generation reproduction test, developmental toxicity studies in rats and rabbits, sub-chronic and chronic testing in rats, mice, and dogs (with evaluation of oncogenic potential), and a battery of neurological toxicology testing with optional Tier 2 developmental testing. Many industrial chemicals are also undergoing similar evaluation in the Voluntary Children's Chemical Safety Program, and the Voluntary High Production Volume initiative will also provide SIDS data packages that will include reproduction and developmental endpoints.

Under the auspices of EDMVS, additional estrogen, androgen, and thyroid endpoints to the 1998 rat 2-generation reproduction study are also undergoing validation. Additionally, a research program is currently underway at EPA ORD to add additional androgenic endpoints beyond those already recommended by EDSTAC. CLA supports the validation and research for the rat 2-generation reproduction test, and we suggest that this effort and the validation of other screens and tests recommended by EDSTAC continue without any undue delay or diversion of EPA resources.

The in utero lactation assay is still very much an ORD research endeavor. The validation timeframe for this assay is beyond that outlined for the Tier 1 screens and the majority of Tier 2 tests. Development and validation of this assay will require a significant effort to meet the present timing for the proposed EDMVS validation schedule. Therefore, CLA does not support a delay in the development of the EDSTAC recommended screens and tests in deference to this assay.

In our opinion EPA's efforts, time, and budget are better spent completing the timely validation of the EDSTAC screens and tests and coordinating international validation efforts within the OECD framework. Adding the in-utero lactation assay can only serve to foster international harmonization issues since this type assay is not under consideration as an endocrine test by either the OECD or within the EU.

CLA stresses that an in utero lactation assay is not ready for regulatory use without thorough research and validation. However, we do not discourage EPA ORD to continue research in the evaluation of in utero lactation exposures. A comprehensive in-utero lactation exposure test may have potential regulatory use if it were evaluated as part of a broader effort to streamline existing regulatory tests or as a more cost effective alternative to other EDMVS screens and endocrine tests, but EPA must first demonstrate that this is possible.