



December 18, 2003

Dr. Joseph J. Merenda
Director, Office Science Coordination and Policy
US Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Dear Dr. Merenda:

RE: CLA comments intact adult male assay and pubertal rat female and male assays.

Crop Life America (CLA) appreciated the opportunity to participate in the December 10-12, 2003 Endocrine Disruption Methods Validation Subcommittee (EDMVS.) CLA represents the manufacturers, formulators, and distributors of plant science solutions for agriculture and pest management in the United States. We are strongly committed to providing scientifically sound advice to EPA on the development of a fully validated endocrine screening and testing program and the opportunity for broad stakeholders involvement through the Federal Advisory Committee Act process.

CLA supports EPA's intent to conduct a side-by-side comparison of the performance of the intact adult male study and pubertal assays. Such a comparison may also warrant side-by-side testing of the two assays with a similar set of test chemicals and in the same testing facilities and include one or more independent laboratories in addition to those previously associated with the evaluation of these studies. Moreover, CLA urges EPA to include negative control chemicals in the assays in order to assess the reliability and reproducibility of the assays to identify and differentiate systemic (non-hormonal) toxicity from hormonal effects.

Following are CLA's comments regarding the side-by-side comparison:

- **CLA supports the inclusion of the intact adult male assay in the validation of the Tier I screening battery as a potential alternative to the female and male pubertal assays and as an assay to supplement data from existing rat reproduction studies.**
 - The intact male is capable of providing mechanism of action data (e.g., receptor agonists/antagonists, biosynthesis inhibitors etc.), addressing a broad array of EAT hormonally mediated endpoints.

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- As a short, in vivo 15 day test (vs. the longer pubertal assays), the assay meets the requirements for a “screen”, having sufficient sensitivity and specificity for identifying the following endocrine activities:
 - Agonists or antagonists -- ER, AR, & dopamine receptor
 - 5 α -reductase inhibitors
 - Steroid biosynthesis inhibitors (aromatase activity and testosterone biosynthesis)
 - Compounds that alter thyroid function
 - Steroidogenesis and aromatase inhibitors in vivo thereby avoiding of the use of in vitro assays that are confounded by issues of cytotoxicity

- The assay has the potential to replace of number of assays in the proposed EDSTAC Tier I battery thereby providing an opportunity to streamline testing requirements and reduce overall numbers of test animals such as:
 - Steroidogenesis (and alternative aromatase assay)
 - Hershberger assay
 - Female (or male) pubertal assays

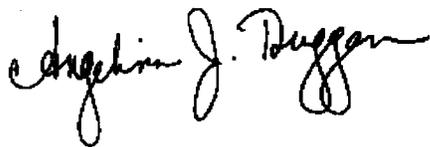
- **CLA underscores serious technical issues regarding the male and female pubertal assays that justifies the need for further evaluations and consideration of an alternative assay, the adult intact male.**
 - CLA emphasizes that including the pubertal female assay in the Tier 1 battery will necessitate the inclusion of an *in vitro* steroidogenesis assay. As we commented in August 2003, the major limitation of the proposed steroidogenesis methodology (minced testes) was the difficulty of assessing cytotoxicity, which will confound the observations of positive and negative results. We recommended the use of isolated Leydig cells from minced testes preparation as one alternative but as the preferred alternative, EPA should devote additional resources to evaluating the 15-Day Intact Male assay.
 - Establishing criteria to designate positives in the pubertal assays will be difficult. For example, low sensitivity in the female pubertal and the inherent high variability of vaginal opening measurements makes it difficult to determine whether a 2-day change in vaginal opening is biologically significant. So, only differences >2 days should be considered treatment related.
 - Specificity is also a concern for the male and female pubertal assays, since puberty is influenced by body weight, diet, etc. The results of the recent studies conducted by RTI International point out the difficulty of distinguishing changes in vaginal opening or preputial separation resulting from hormonally mediated mechanisms vs. delays in onset due to significantly lower body weight and delayed development since the changes observed for at least one of the test substances (PTU and possibly atrazine) in these studies occurred only at doses where there were significant depressions in body weight.

- CLA recommends that EPA undertake the following actions in completing validation of the male and female pubertal assays:
 - Include non-specific systemic toxicants in order to demonstrate the specificity of the assays for assessing hormonal effects.
 - Establish clear criteria on dose selection and the interpretation of results above maximum tolerated doses defined by either body weight reductions of >10% or other indications of systemic toxicity.
 - Broaden the validation to additional laboratories to assess the reliability and reproducibility of the assays.

In closing, CLA also points out that it is not premature for EPA to develop and communicate weight-of-evidence criteria to trigger the necessity of Tier 2 studies based on the results of Tier I screens. These criteria should be clearly communicated to all stakeholders in future EDSP Federal Register Notices. We strongly advocate that decisions to move to Tier 2 studies should not be based on the results of a single positive study, especially those from the pubertal assays where changes in apical endpoints (onset of puberty, organ weight changes) may have been obtained only at doses above the MTD.

Once again, CLA thanks EPA for the opportunity to provide technical advice to the Agency and the EDMVS. We urge EPA to expeditiously initiate the comparative evaluation of the intact adult male and male/female pubertal assays.

Sincerely yours,



Angelina Duggan, Ph.D.

cc: Jim Jones, EPA
Bill Jordan, EPA
Isi Siddiqui, CLA