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Jane Smith
Designated Federal Officer
Endocrine Disruption Methods Validation Subcommittee
Office of Science Coordination and Policy/OPPTS
Room 4106-M
U.S. Environmental Protection Agency Ariel Rios Building
1200 Pennsylvania Ave, NW
Washington, DC 20460

Dear Ms. Smith:

The following are answers to some of the questions posed at the December 10-12, 2003 EDMVS meeting in Washington, DC. with additional comments.

Does the EDMVS agree that the pubertal assays show adequate sensitivity over a range of chemicals for use as a Tier 1 assay? That is, are the pubertal assays ready for an inter-laboratory validation study? If not, what specific areas need further prevalidation work?
Yes, the male and female pubertal protocols show adequate sensitivity and are ready for inter-laboratory validation studies. Laboratories should be selected based on past history where there are already experienced supervisors and/or technicians who have demonstrated they can do the precision histology, excisions, biochemistry, etc associated with the seven specific protocols listed on page1 slide 2 of Dr. Stoker's presentation. The use of the EPA NHEERL laboratory as one of the three labs should be considered seriously. Dose setting should be required and require at least a range of 5 orders of magnitude. EPA should do the dose setting as the lead laboratory. However, here again, laboratories that have already demonstrated their ability to set doses using a range of five orders of magnitude should get preferential consideration. It would be best to have each lab do its own dose setting following instructions from the EPA lead lab.

Positive and negative controls should be used in every case. Feed, water, strain of animal and caging should be described and rigidly followed. Most important, the diet of the maternal animals and their offspring, even if the offspring are purchased after they are born, should be as rigidly controlled as it would be during the testing period. Some sort of agreement is going to have to be worked out with animal suppliers for these and future studies. Outliers should not be thrown out, instead they should be given special consideration.

Is there a better set or sequence of studies to perform for validation?
Nothing that has been presented to date surpasses the pubertal assay. However, as validation moves forward there must be flexibility for the labs involved to incorporate into the list of

endpoints something new, relevant, and feasible that might appear in the open literature -- or similarly, expand on something that surfaces during the course of carrying out the assay that would enrich the results -- rather than reporting that it could have been done in their final report after the experiment is over.

Should lab personnel be trained as part of the validation exercise?
Training should be an integral part of the overall plan. Training has to be more than observing. It has to be hands on, and for more than one day. It should be written into the contracts with the labs that the technicians must be certified and that there will be spot checks at labs by observers to see that the technicians can really do what is expected.

This is in response to your request for the names of chemicals to add to the list of reference chemicals.
4-tert octylphenol should be considered for addition to the list. Much has already been published about 4-tert octylphenol and its metabolites and its ubiquity in the environment, its lethal threshold in several economically important species, its toxicokinetics in various species and in specific organs, as well as its effects at the cellular level on hormone secretion in the reproductive system.

The following is in response to any questions raised about choice of moving ahead with the Adult Intact-Male Assay vs the Male and Female Pubertal Assays.
The December 11-12, 2003 presentation and discussion about the results of the pubertal assays represented a major step forward for the EPA's EDSP for several reasons. It shifted the focus to animals that were still developing. And most important, the results proved that the number of endpoints and their sensitivity can be increased significantly in a single assay. It demonstrated that the effect of chemicals on more than one endocrine-specific system can be monitored in a single study. The use of multiple measurements of biochemical, morphological, and physiological changes in a single assay, also provides a better perspective of the hazard of the exposure posed by a specific chemical.

The approach taken in these preliminary pubertal assays demonstrates that a fingerprint of disrupted development for each chemical can be developed. This is very important, because a single chemical can through several different mechanisms cause multiple, unexpected changes depending on stage of development and sex of the tissue it contacts -- and with different outcomes between high and low doses. Consequently, the end points (and hopefully more) provided in a design such as the pubertals can have far more significance for animal and human health than the traditionally used endpoints, such as organ weight and hormone levels, assays that most often use mature, fully developed animals.

The trend that NHEERL took in the pubertals toward probing earlier in development should continue as well as broadening the number of endpoints in a single assay. Future research effort with whole animals should merge with efforts to develop rapid, inexpensive "in vitro" screens, some of which will surely replace whole animal testing. Ultimately, this trend will reduce the number of animals required for testing significantly.

Just as a reminder. Why would anyone move ahead with an assay that has been demonstrated to produce false negatives? The EDSP should not move forward with the Adult Intact-Male Assay.

Thank you for the opportunity to submit the above comments.

Sincerely,

Theo Colborn, PhD
Senior Fellow, World Wildlife Fund