Endocrine panel tackles assays and obstacles

The first substantive meeting of an advisory panel assisting EPA on the development of a screening program for endocrine-disrupting pesticides ran headlong into major issues while debating the first group of assays proposed for the initiative.

The Endocrine Disruptor Methods Validation Subcommittee met Dec. 10–13 in Washington, D.C. The program being developed by EPA’s Office of Science Coordination and Policy is required by the Food Quality Protection Act.

It also is strongly encouraged by an agency settlement with the Natural Resources Defense Council on FQPA implementation. Although the endocrine-disrupter language in the consent decree is not legally enforceable, the NRDC has warned that it might sue EPA if the screening assays are not validated by 2004.

Obstacles could hinder deadlines

If the opinions expressed by some of the EDMVS panelists are any indication, however, there could be more hurdles standing in the way of the deadline than EPA will have time to surmount. Nevertheless, EDMVS facilitator Paul DeMorgan, a senior mediator with the Washington, D.C., office of Resolve Inc., said “people see the position EPA is in with the court-mandated deadlines, but it’s also important to get this right.”

One of the major issues raised by the panelists arose from debate on the In Utero/Lactational assay. The assay will measure as many as 20 endpoints in animals exposed to dibutyl phthalate, methoxychlor, nonylphenol, bisphenol-A, flutamide, linuron, vinclozolin and other chemicals used during the pre-validation stage.

Several panelists argued, however, that In Utero/Lactation does not qualify as a screen because it involves a great number of individual animal tests. Some panelists maintained that In Utero/Lactation should be used as a Tier 2 test. “There’s no way it fits the criteria for Tier 1 screening assays,” said Ron Miller, a senior toxicologist with Dow Chemical Company.

Miller also argued that, until the method of administration was known, there was no point in discussing this particular assay any further. He added that gavage, which administers doses through a stomach tube, might be fine for screening but not for a test.

More information needed

“Most people feel this assay is more like a Tier 1.5 or Tier 2,” said Gary Timm, a senior technical advisor at EPA’s Office of Science Coordination and Policy. However, Timm was not willing to let this screening assay die, particularly in light of pre-validation tests that have been tentatively scheduled for January. He suggested more information was needed for the debate.

“Let’s use the expansive test and challenge it with three or four chemicals,” Timm suggested. “We’ll see what the data say and perform a statistical analysis, which could tell us if this assay could work as a screen. But let’s run it as a broader study, first.”

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Another issue is the possibility that dosing by gavage might obscure the relevance of test findings with respect to estrogen/androgen/thyroid screens. “My concern is that dosing by gavage is fine for general toxicology, but for separate effects, I don’t think it’s appropriate,” said James Yager, senior associate dean of the Johns Hopkins Bloomberg School of Public Health. “The doses might build up and I don’t know how you’ll interpret the results when they do.”

Debate on low-dose effects held off

The issue of dose size is an issue of vital significance to the program. Although some chemicals are endocrine-disruptors at acute doses, their effects at low doses are disputed. During the panel’s plenary session (see PTCN, Nov. 5, Page 3), it was decided to discuss low-dose effects during the December meeting. The discussion, however, has been postponed.

“EPA was unable to formulate its position on low-dose effects in time for this meeting,” said OSPC Director Vanessa Vu, “so it has been tabled until the March meeting.”
Differences noted in rat strains

Another important issue was that of sensitivity differences among the different strains of rat used for testing. The issue was raised during discussion of single- and multi-dose puberty-onset screens, which involve measuring organ weights after a period of dosing.

James Stevens, who heads Global Human Risk Assessment for Syngenta, said he was particularly interested in strain differences because studies on blood/brain barriers and blood/testes barriers in the neonatal rat were incomplete.

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“The MDR1 gene develops differently in the Sprague-Dawley rat than it does in the Wistar rat. There are unique differences in these strains and this could have a significant impact on what happens in the endocrine-effects argument.”

“The literature indicates that the Long-Evans rat can work quite well in these tests,” added Ralph Cooper, from the Endocrinology Branch of the Reproductive Toxicology Division of EPA’s Office of Research and Development. “The problems [in variability] start in other ways. It’s got nothing to do with sensitivities across strains. I think the problems we see are strain-dependent.”

Other panelists disagreed. “I’d like to see a breakout where we demonstrate repeatability among strains and blocks,” said Willie Owens, principal scientist at the Procter & Gamble Company. “These are potent reference compounds,” he added, referring to the chemicals that will be used in pre-validation. “When I see the variability in previous studies I become a little concerned.”

White paper to be developed

Vu asked if EPA should devote additional resources to studies on strain differences before protocol research and pre-validation tests proceed. Although there was no consensus on the issue of strains, panelists agreed that EPA would develop a “white paper” on the subject for further discussion.

Exposure effects themselves were raised as an issue. “What are the effects we’ll see in screening that trigger Tier 2 testing?” asked Ted Schettler, science director for the Boston-based Science and Environmental Health Network. “The question is: are we seeing effects or adverse effects? If we carry out In Utero/Lactation further than planned, we could end up with findings you’d expect to see in Tier 2 testing. We don’t want to have this conversation on adverse effects one or two years from now. We might say, ‘If only we’d carried out these tests a little further it would have been very useful for regulatory purposes.’”

— Phil Zahodiakin

Animal welfare issues affect endocrine debate

The fate of animals subjected to screening tests for endocrine disruption was a significant factor during debate on the first group of validation tests discussed by an EPA advisory panel.

To confirm the endocrine-disrupting potential of a chemical, the sexual and hormonal organs of test-animals will be removed for a variety of measurements. Moreover, only a certain number of animals from each test-litter will be used. The others must be killed.

One potential alternative to in vivo animal tests is Quantitative Structure-Activity Relationship modeling. QSAR models describe the chemical and/or biological properties of chemicals relative to their molecular structure. During a Dec. 10 meeting of the Endocrine Disruptor Methods Validation Subcommittee, EPA officials said QSAR validation would not be part of the panel’s work.

Panelist requests QSAR discussions

“I’m not persuaded that QSAR doesn’t fit into our mission statement,” said Robert Combes, president of the European Society of Toxicology in Vitro. “I think we should have some discussion of QSAR in our work, and I hope this isn’t set in stone.”

EPA officials assured the panelists that they would be updated on QSAR developments. They were also advised and reminded that that in vitro methods were not within their purview. Nevertheless, the large number of animals that would be required for some of the validation tests remained an issue during panel discussions.

Willie Owens, a principal scientist with the Procter & Gamble Co., said the Detailed Review Paper on the
In Utero/Lactation assay indicated that the potential number of animals that might be tested with this assay ranged from 430 to 1,300 per chemical. However, Owens noted, a screen is supposed to flag suspected endocrine-disruptors for Tier 2 tests, which would involve many more animals than the screens.

“In Utero/Lactation assay indicated that the potential number of animals that might be tested with this assay ranged from 430 to 1,300 per chemical. However, Owens noted, a screen is supposed to flag suspected endocrine-disruptors for Tier 2 tests, which would involve many more animals than the screens.”

“Biasing this to where this assay has feathers, a yellow bill and webbed feet, I’d say this was a test, not a screen,” Owens said.

**Predictive accuracy questioned**

Seidle also questioned how the EDMVS intended to evaluate the relevance of the proposed endocrine assays to humans. “The predictive accuracy of even such mainstay tests as the [Lethal Dose/50] is pitiful,” Seidle said. “It has been found to predict toxicity in humans with 65% accuracy at best.”

To place more emphasis on in vitro approaches to validation, Seidle argued, EPA should allow the Interagency Coordinating Committee on Validation of Alternative Toxicological Methods to make the final determination of whether or not an assay has been properly validated for regulatory purposes. Seidle added that the National Toxicology Program’s Advisory Committee on Alternative Toxicological Methods, and an earlier EPA work group — the Endocrine Disruptor Standardization and Validation Task Force — also advised EPA to allow ICCVAM to play a major role in protocol evaluation.

During a recent Pesticide Program Dialogue Committee meeting, Office of Pesticide Programs Director Marcia Mulkey stated that the agency is committed to reducing the number of animals sacrificed for its toxicity-testing programs.

— Phil Zahodiakin