SCIENTIFIC ADVISORY COMMITTEE ON ALTERNATIVE TOXICOLOGICAL METHODS (SACATM)

Sep 5-6, 2012 Meeting

Oral Comments on Adaptation and Performance of the BG1Luc ER TA Agonist and Antagonist Assays

My name is Patricia Bishop and I am submitting these comments on behalf of the People for the Ethical Treatment of Animals and the Physicians Committee for Responsible Medicine.

At the time the Lumi-Cell® BG1Luc ERTA assay was nominated for an inter-laboratory validation study in January 2004, there were no *in vitro* ER TA methods considered adequately validated for regulatory use. In the eight years it has taken for the Lumi-Cell to be validated by ICCVAM, the *in vitro* Stably Transfected Human Estrogen Receptor-α Transcriptional Activation (STTA) method was validated by the Chemicals Evaluation and Research Institute (CERI, Japan). By 2009, the CERI STTA had been described in an OECD Chemicals Test Guideline (TG 455) and adopted by USEPA as OPPTS method 890.1300 for use in EPA's Endocrine Disruptor Screening Program (EDSP). The excessive length of time it took to validate Lumi-cell underscores the fact that current processes for validation of regulatory test methods are inappropriate for many of the scientific tools being rapidly developed as a part of the 21st Century Toxicity Testing Vision. We must have timely and appropriate validation procedures that keep pace with changing science and meet agency needs, a requirement that ICCVAM's approach to validation does not appear capable of fulfilling.

ICCVAM states that the Lumi-Cell method is at least as accurate as the CERI STTA assay, and offers some advantages over the latter, including the abilities to detect both agonist and antagonist substances and endogenously express both hERα and hERβ. However, the fact remains that despite these improvements, Lumi-Cell is an *in vitro* test that is an alternative to another *in vitro* test, thereby offering absolutely no reduction in animal use.

ICCVAM's mission, as stated on its website, "…is to facilitate development, validation and regulatory acceptance of new and revised regulatory test methods that reduce, refine, and replace the use of animals in testing while maintaining and promoting scientific quality and the protection of human health, animal health, and the environment." As it stands today, the considerable amount of work that has gone into validating the Lumi-cell method has not resulted in achievement of any of the "3 Rs".

There is, however, still potential for the Lumi-cell assay to save animals. Based on a 97% concordance (33/34 reference substances) with the ER rat cytosol binding assay (OSCPP 890.1250) used by EPA in the Tier 1 EDSP screening battery, the Lumi-cell assay can be considered a replacement for the latter, which though billed as an *in vitro* test actually consumes large numbers of animals – as many as 18 per assay – through harvesting of uterine tissues to collect cytosol. In the Lumi-cell BG1Luc Test Method Evaluation Report, ICCVAM states that "In light of the excellent degree of agreement between ER binding and BG1Luc ER TA data, it appears that evaluating results from BG1Luc ER TA agonist and antagonist testing may provide a viable alternative to conducting ER binding studies." The report goes on to say "ICCVAM recommends that additional validation studies could be performed to determine whether or not the BG1Luc ER TA method could replace the rat uterine cytosol ER binding assay." Instead of performing a lengthy, full validation study, it seems possible that performing a more rigorous side-by-side comparison

of the two methods might be enough to determine whether or not the Lumi-cell can successfully replace the rat cytosol ER binding assay.

Similarly, the Lumi-cell assay produced 92% concordance (12/13 reference substances) when compared to results of the rat uterotrophic assay, an *in vivo* EDSP Tier 1 assay, suggesting it as a replacement for the uterotrophic test, particularly if *in vitro* metabolizing systems were added. Again, ICCVAM recommended in the Test Evaluation Report that "…further work be carried out to determine if the BG1Luc ER TA test method could be used in combination with other methods (to include in vitro metabolic activation) in a weight-of-evidence approach to replace the uterotrophic bioassay."

There is no evidence that NICEATM or ICCVAM plans to actively pursue investigation of either of these two potential animal-saving possibilities. In its five-year draft plan, the only planned work with the Lumi-cell test, as stated on pp. 28-29, is to evaluate it in the context of a Tox21 high throughput environment. There is absolutely no mention of evaluating it as a means of reducing animal use in the EDSP. ICCVAM states on its own website that it should play a leading role: "in promoting and facilitating the development of priority alternative test methods, and; in identifying key alternative test methods and strategies and facilitating their validation and acceptance." Given that charge, we urge ICCVAM to immediately initiate and complete further investigation of the Lumi-cell assay as a priority replacement for both the rat cytosol ER binding assay and the rat uterotrophic assay.

Thank you for the opportunity to comment.