DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
National Institute of
Environmental Health Sciences
P. O. Box 12233
Research Triangle Park, NC 27709

February 1, 2012

The Honorable Lisa P. Jackson
Administrator
U.S. Environmental Protection Agency
Ariel Rios Building, Mail Code 1101A
1200 Pennsylvania Avenue, NW
Washington, DC 20201

Dear Ms. Jackson:

I am pleased to forward toxicological test method recommendations for the LUMI-CELL® Estrogen Receptor (ER) BG1Luc Estrogen Receptor (ER) Transcriptional Activation (TA) test method from the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) for your consideration. These test method recommendations are being sent to you for action pursuant to Sections 3(e)(4) and 4(a)-(e) of the ICCVAM Authorization Act of 2000 (42 U.S.C. 285*l*-3).

Xenobiotic Detection Systems, Inc. (XDS, Durham, NC) nominated the BG1Luc ER TA test method to ICCVAM for an interlaboratory validation study. ICCVAM and the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) recommended the study as a high priority, and NICEATM subsequently coordinated an international validation study with counterparts in Japan (JaCVAM) and Europe (ECVAM).

ICCVAM's Interagency Endocrine Disruptor Working Group (EDWG), composed of scientists from ICCVAM member agencies, worked with NICEATM to carry out relevant evaluation activities following completion of the international validation study. A draft background review document, draft test method performance standards, and draft ICCVAM test method recommendations were reviewed by an international independent scientific peer review panel ("the Panel"). ICCVAM considered the Panel report and comments from the public, the EDWG, and SACATM in preparing the final test method recommendations provided in NIH Publication No. 11- 7850, ICCVAM *Test Method Evaluation Report: The LUMI-CELL® ER (BG1Luc ER TA) Test Method, An In Vitro Assay for Identifying Human Estrogen Receptor Agonist and Antagonist Activity of Chemicals* (enclosed).

Based on this evaluation, ICCVAM recommends that the accuracy and reliability of the BG1Luc ER TA test method support its use as a screening test to identify substances with *in vitro* ER agonist or antagonist activity, and concludes that the accuracy of this assay is at least equivalent to US EPA's OPPTS 890.1300: Estrogen Receptor Transcriptional Activation (Human Cell Line (HeLa- 9903)). In addition, the BG1Luc ER TA test method was found to offer several advantages over the existing EPA method:

- BG1Luc ER TA test method is validated for use up to the 1 mM limit required by EPA for EDSP Tier 1 screening, whereas OPPTS 890.1300 was only validated up to 10 µM
- BG1 cells endogenously express both ER-α and ER- ß, whereas HeLa-9903 cells only express transfected ER-α. BG1 cells therefore have the potential to detect a wider range of ER-active substances

- Cells used for the BG1Luc assay are available from two domestic sources (XDS Inc., RTP, NC and UC Davis, Davis, CA), whereas HeLa-9903 cells required for OPPTS 890.1300 must be purchased from a foreign (Japanese) sole source provider

The only ER TA test method included in national regulatory testing guidelines is EPA's OPPTS 890.1300. The BG1Luc ER TA test method is another *in vitro* ER TA method that could be considered for regulatory use to assess ER agonist activity. A direct comparison of these two methods showed identical accuracy when the same reference chemicals were tested. In addition, there are no currently approved ER antagonist assays, and the BG1Luc ER TA test method was determined to be valid for assessing ER antagonist activity. Accordingly, the BG1Luc ER TA test method appears applicable to the ER TA component of EPA's EDSP Tier 1 screening, and may offer a valid alternative to the HeLa-9903 test method.

Pursuant to Sections 4(a)-(e), of the ICCVAM Authorization Act, Federal agencies are required to review ICCVAM test method recommendations and notify ICCVAM in writing of the agency's findings no later than 180 days after receipt of this letter. In accordance with these requirements, we ask that you please state whether your agency will adopt the ICCVAM test method recommendation that the BG1Luc ER TA test method can be used as a screening test to identify substances with *in vitro* ER agonist or antagonist activity and that the accuracy of this assay is at least equivalent to OPPTS 890.1300, or whether your agency has determined that one or more of the criteria in Section 4(e)(1) to (4) for not adopting the recommendations are met.

Please send your agency's response regarding each of the requirements to RADM William S. Stokes, Director, NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (contact information, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709, telephone: 919-541-2384, facsimile: 919-541-0947, email: Stokes@niehs.nih.gov. ICCVAM is required to make the final ICCVAM test method recommendations and corresponding agency responses available to the public per Section 3(e)(6) of the Act. Accordingly, your response will be made available on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov.

I appreciate your agency's participation on ICCVAM. This committee serves an important role in facilitating the scientific evaluation and adoption of test methods that will help protect human health and the environment while providing for improved animal welfare.

Sincerely,

/s/

Linda S. Birnbaum, Ph.D., D.A.B.T., A.T.S.
Director

Enclosure

cc:
Anna Lowit, Ph.D., EPA ICCVAM Principal Agency Representative