United States Department of the Interior
U.S. GEOLOGICAL SURVEY
12201 Sunrise Valley Drive
Reston, VA 20192

In Reply, Refer to:
MS 953
GS12000556

May 15, 2012

Rear Admiral William S. Stokes, Director
National Toxicology Program
Interagency Center for the Evaluation of Alternative Toxicological Methods
National Institutes of Health
National Institute of Environmental Health Sciences
P.O. Box 1223
Research Triangle Park, NC 27709

Dear Rear Admiral Stokes:

In a letter dated February 1, 2012, to Secretary of the Interior, Mr. Kenneth Salazar, Dr. Linda S. Birnbaum of the National Institutes of Health requested Departmental review of the LUMI-Cell® Estrogen Receptor (ER) BG1 Luc Estrogen Receptor (ER) Translational Activiation (TA) test method. As requested by Dr. Birnbaum, the U.S. Geological Survey (USGS) is responding on behalf of the Department of the Interior directly to you.

This document was reviewed and commented upon by several Department of the Interior scientists, and found to be well-written and scientifically-robust. This new method provides a major improvement over the only method that has been validated and currently approved by the U.S. EPA for testing estrogen receptor activation (i.e., Stably Transfected Human Estrogen Receptor-α Transcriptional Activation Assay for the Detection of Estrogenic Agonist-Activity) in that the BG1Luc ER TA provides a means for testing both agonistic and antagonistic effects of putative endocrine disrupting chemicals. Therefore, this method will be able to provide significantly more information on chemicals of interest.

The validation of the assay has ably demonstrated the accuracy and reliability of this new method. However, it is important to keep in mind limitations to the method. Specifically, the assay is currently validated only for chemicals that can be dissolved in dimethyl sulfoxide (DMSO), that do not react with DMSO or the cell culture medium, and that are not directly toxic to the cells. In addition, the usefulness and limitations section of this document should be augmented to emphasize potential ambiguities associated with the assay. For instance, this cell line expresses both ERα and ERβ. This can be viewed as an inherent advantage or disadvantage of this cell line. The current

method does not include a quantitative step to assess the levels or ratios of these receptors which are known to have different affinities for ligand. Variation in measured ER activation could simply be an artifact of variability in the differential expression of these receptor subtypes. This level of variability is likely to differ among agonists and antagonists. Either a step to quantify ER subtype protein should be included in the method, or this limitation should be stated. There should also be more emphasis on the fact that the assay is recommended for testing single, chemically pure compounds. There is a critical need for reporter assays that measure activation by chemical mixtures, and this testing scheme has not been thoroughly evaluated. Moreover, some may see this as a promising platform to screen chemical extracts from environmental samples. This application would be of great benefit, but clearly there are limitations regarding such screening. While the screening of environmental chemical mixtures is not the intent of this assay as currently validated, it would be useful for this approach to be acknowledged and considered for future validation. Although these limitations preclude the testing of all suspected EDCs and mixtures, they are far outweighed by the advantages the method presents, particularly with regard to the dual endpoints screened (agonist and antagonist), cost and throughput, which will greatly advance the screening process.

Please note that our Department conducts ecotoxicological research and monitoring of fish and wildlife, and has very limited regulatory authority for chemicals and pharmaceuticals. The Department of the Interior continues to support new methods and strategies that reduce the number of test animals in such evaluations, and will alert Institutional Animal Care and Use Committees at our research facilities about these new procedures. We are pleased to assist in such reviews, and will gladly provide in depth comments on those test methods that are more closely allied to our mission.

Sincerely,

/s/

David P. Russ
Regional Executive, Northeast Area