Corrections to:

Independent Scientific Peer Review Panel Report
Evaluation of the LUMI-CELL® ER (BG1Luc ER TA) Test Method

The following changes made by the peer review Panel members were inadvertently omitted in the printed version of the report.

Executive Summary (page ix)

- Paragraph 1: Add "and proposed performance standards." to the end of the last sentence in the paragraph.
- Paragraph 3: Add "The Panel recommends that a potency endpoint, such as the half-maximal effective and/or inhibitory concentration (EC/IC_{50}), be included in each study report and that the uncertainty associated with these estimates should also be reported. The Panel considered the descriptive approach for evaluating test method reliability acceptable but suggested additional statistical analyses that could be performed to better characterize and understand variability." to the end of the paragraph.
- Paragraph 5: Delete "The Panel considered the descriptive approach for evaluating test method reliability acceptable but suggested additional statistical analyses that could be performed to better characterize and understand variability." from the paragraph.

Overview (page 1)

- Paragraph 3: Delete "and the rat uterine cytosol (RUC) ER binding assay" from the penultimate sentence.
- Bulleted list: Delete the second bullet: "The concordance of BG1Luc ER TA test method with the RUC ER binding assay suggests that the BG1Luc ER TA test method and the RUC ER binding assay produce similar results. Additional analysis of existing data could help to further support this recommendation."
- Paragraph 4: Delete the paragraph "Nevertheless, the artificial nature of any cell-based reporter construct will require confirmation of results from *in vivo* tests and traditional competitive ER binding assays to determine whether a substance is truly an estrogen agonist or antagonist."

Appendix B: Peer Review Panel Member Biosketches (page 38)

- Add biosketches for Dr. Sherry Ward, Dr. Marc Weimer, Dr. James Witliff, and Dr. James Yager, Jr.

The electronic version of the report available on the NICEATM-ICCVAM website reflects these corrections.

If you have a printed copy of the document, please print this summary as well as the following replacement pages:

- pages ix and x (Executive Summary)
- page 1 (Overview)
- pages 39 and 40 (Appendix B)

Executive Summary

This report describes the conclusions and recommendations of an international independent scientific peer review panel (Panel). The Panel was charged by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) with evaluating the validation status of the BG1Luc estrogen receptor (ER) transcriptional activation (TA) test method according to established Federal and international criteria (ICCVAM 1997). The Panel also commented on ICCVAM draft recommendations regarding the usefulness and limitations of the test method and proposed performance standards.

The Panel considered the results of an international interlaboratory validation study that included laboratories in the United States, Italy, and Japan. Based on their evaluation of these data, the Panel agreed with ICCVAM's draft test method recommendation that the BG1Luc ER TA test method can be used to identify substances with *in vitro* estrogenic and anti-estrogenic activity. Based on results of concordance analyses for a limited number of substances, the Panel further concluded that the BG1Luc ER TA test method could be considered as a replacement for other *in vitro* assays that may provide substantially similar information, specifically the Chemicals Evaluation and Research Institute stably transfected transactivation assay (CERI STTA) and the rat uterine cytosol (RUC) ER binding assay. The Panel noted that additional analysis could further support this recommendation, particularly regarding the RUC ER binding assays.

The Panel endorsed the draft ICCVAM-recommended test method protocols and noted several advantages provided by this assay over the currently accepted test method for this endpoint, including the robust test method protocol, the validated testing range, and the ability to detect substances with *in vitro* anti-estrogenic activity. However, the Panel also noted that careful analysis of cytotoxicity is critical for correctly interpreting results. The Panel expressed a preference for using quantitative approaches for such a measurement. The Panel recommends that a potency endpoint, such as the half-maximal effective and/or inhibitory concentration (EC/IC_{50}), be included in each study report and that the uncertainty associated with these estimates should also be reported. The Panel considered the descriptive approach for evaluating test method reliability acceptable but suggested additional statistical analyses that could be performed to better characterize and understand variability.

The Panel agreed with the draft ICCVAM-recommended future studies and suggested additional studies that should be conducted to expand the usefulness of the BG1Luc ER TA test method. The Panel recommended additional evaluations of the utility of the current categorical assessment of cytotoxicity and advocated for the implementation of a quantitative method for its replacement. The Panel also recommended studies to add *in vitro* metabolism (compound activation or inactivation) to the test method. This addition could expand the utility of this and other ER TA test methods. The Panel recommended that additional efforts focus on expanding the reference substance list, and subsequently the BG1Luc ER TA test results, with additional negative agonist and positive antagonist test substances.

Finally, the Panel concurred that the draft ICCVAM performance standards could be used to evaluate the validation status of test methods that are functionally and mechanistically similar to the BG1Luc ER TA test method. The Panel considered the list of performance standards reference substances to be adequate. The Panel noted that ideally more negatives should be included but recognized that data on such substances are not currently available. When evaluating test method accuracy, the Panel strongly supported quantification of relative agonist and antagonist activity in addition to the dichotomous call of positive or negative. In addition, the Panel concluded that the potent estrogens on the reference list should not be misclassified, but there could be some tolerance for discordance for the weakly active reference substances. Discordant results need to be discussed in terms of the ability of the test method to detect a similar range of potencies and intrinsic activities compared to current

validated test methods. Discordant results for particular chemicals or product classes also need to be discussed.

Overview

Use of the BG1Luc ER TA Test Method to Identify Substances as Potential *In Vitro* Estrogen Receptor Agonists or Antagonists

The overall question that the international independent scientific peer review panel (Panel) considered is whether the validation status of the BG1Luc estrogen receptor (ER) transcriptional activation (TA) test method has been adequately characterized for its intended purpose and whether it is sufficiently accurate and reliable to be used to identify substances with estrogen agonist and/or antagonist activity.

The Panel discussed the intended use of this assay and the potential for its inclusion in a regulatory testing battery. Panel members agreed that the BG1Luc ER TA test method and the Chemicals Evaluation and Research Institute stably transfected transactivation assay (CERI STTA) are similarly capable of assessing *in vitro* estrogen receptor (ER) agonist activity. In addition, the BG1Luc ER TA test method is capable of detecting *in vitro* estrogen antagonists. Clarification of the intended use of these assays in regulatory decision making, particularly in the context of the U.S. EPA's Endocrine Disruptor Screening Program (EDSP), would enable a better understanding of the relative merits of the various screening assays for their intended purpose.

In the absence of clear regulatory guidance, the Panel recommends that the BG1Luc ER TA test method be endorsed as a scientifically valid method for assessing the *in vitro* estrogen agonist and antagonist activity of compounds within a test battery or tiered testing scheme. The Panel recommends that the BG1Luc ER TA method be considered as a replacement for other *in vitro* assays that, in combination, may provide substantially similar information, specifically the CERI STTA assay. This is supported by the following findings:

- The concordance of the BG1Luc ER TA test method with the CERI STTA assay suggests that the BG1Luc ER TA test method and the CERI assay produce similar results.
- The thoroughness and transparency of the BG1Luc ER TA method validation process compare favorably with other *in vitro* assays.
- The detailed BG1Luc ER TA agonist and antagonist protocols permit ease of use.
- The detailed and publically available BG1Luc ER TA data permits thorough evaluation of the performance of the method.
- The endogenous expression of both ERα and ERβ in BG1Luc4E2 cells allows *in vitro* activity through both receptors to be assessed in the BG1Luc ER TA test method. Endogenous expression of the receptor and its related endogenous cellular machinery may be an advantage over receptors that are stably transformed into an immortal cell line and constitutively expressed at high levels.

Sherry Ward, PhD, MBA
Dr. Ward received her PhD in Biochemistry from Michigan State University, an MBA from the University of Maryland University College (UMUC), and an executive MS in Technology Management from UMUC. She is currently a consultant with BioTred Solutions in New Market, Maryland. Dr. Ward has expertise in *in vitro* toxicology, scientific writing and project management, grant proposal review, and grant writing. She also has experience in biotechnology market research, commercialization, and strategy development. Dr. Ward is a contributing editor to AltTox.org. She is an adjunct faculty member at UMUC in Biotechnology & Project Management. She has animal welfare experience and has served since 2006 on the board of the International Foundation for Ethical Research. As a Staff Scientist at the Gillette Company, she developed, characterized, and drafted patent applications for the first human conjunctival epithelial cell lines and gained experience in bioassay development and validation. Dr. Ward has served on numerous scientific panels and committees and was a panel member and presenter at the ICCVAM symposia on mechanisms of ocular injury and recovery and minimizing pain and distress in ocular toxicity testing held at the NIH in May 2005. She has been actively involved with trade organizations and served on the European Cosmetic, Toiletry and Perfumery Association Eye Irritation Task Force and the ILSI-HESI Alternatives to Animals Task Force. Dr. Ward's experience in models of eye irritation and mechanisms of injury is reflected in 19 publications in peer-reviewed journals, 4 unpublished validation or prevalidation documents related to ICCVAM activities, 17 presentations, 28 abstracts, and a patent. She is a member of the Hopkins Medical and Surgical Association and the Washington Academy of Sciences.

Marc Weimer, PhD
Dr. Weimer received a PhD in Neurophysiology from the University of Hohenheim, Germany, and an MS in Methods and Models from FernUniversität in Hagen, Germany. He joined the Department of Biostatistics, German Cancer Research Center (DKFZ), in Heidelberg in 2006 as a biostatistician. Dr. Weimer's primary areas of work are toxicogenomics and development and validation of alternative methods to animal experiments. As a statistical consultant, he has been involved in national and international research projects aimed at reducing, refining, and replacing animal testing in toxicology. His main interests include dose–response modeling, agreement statistics, and toxicogenomics. Funded by ECVAM, he has been responsible for the statistical evaluation of the quality of *in vitro* assays developed within ReProTect, a project of the European Union advancing alternative methods in reproductive toxicity. Dr. Weimer has authored or coauthored 19 peer-reviewed journal articles.

James Wittliff, PhD, MD *hc*, FACB
Dr. Wittliff received his PhD in Molecular Biology from the University of Texas at Austin and completed postdoctoral studies in the Biology Division of Oak Ridge National Laboratory in Tennessee. He is currently a Professor of Biochemistry and Molecular Biology in the Graham Brown Cancer Center in the School of Medicine at the University of Louisville with additional appointments as Research Professor of Surgery and Director of the Institute for Molecular Diversity & Drug Design (IMD). He is also the Director of the Hormone Receptor Laboratory at the University and has held numerous professorships at universities in Europe, Asia, and Africa. Dr. Wittliff's research interests include mechanisms and applications of steroid and peptide hormone action in disease, biochemical techniques and concepts for detection and treatment of cancer, and laser capture microdissection and its use in proteomics and genomics. He was among the first to prove that the appearance of estrogen receptors in breast cancer predicted a patient's response to hormone therapy. Dr. Wittliff has researched the biological properties and cellular roles of estrogen and progestin receptors in human cancers and the actions of estrogen mimics acting as endocrine disruptor compounds (EDC). Formerly at NEN/DuPont, Dr. Wittliff developed the original FDA-approved kits for assessing receptors in biopsies, celebrated as a major contribution to laboratory medicine. He was the Principal Investigator or the Investigator for a number of funded studies on genomic approaches to disease,

including a Genomic Approach for Assessing Clinical Outcome of Breast Cancer using Cells Isolated by Laser Capture Microdissection. Dr. Wittliff has served on numerous panels and committees, including the ICCVAM Endocrine Disruptor Peer Review Panel (2002). He is a member of several professional societies including the Endocrine Society, the American Association for Cancer Research, and the American Association for the Advancement of Science. Dr. Wittliff has authored or coauthored over 250 peer-reviewed publications and holds patents on methods and apparatus for measurement of the effect of test compounds on signal transduction at the receptor level, quantitative immunohistochemistry, breast cancer signatures, and gene expression profiles.

James Yager, Jr., PhD

Dr. Yager received his PhD from the University of Connecticut, Storrs Campus, in Cell and Developmental Biology and conducted postdoctoral studies at the McArdle Laboratory for Cancer Research at the University of Wisconsin. He is currently a Professor in Preventive Medicine and Toxicology in the Department of Environmental Health Sciences at the Johns Hopkins Bloomberg School of Public Health with a joint appointment in the Department of Oncology. He has administrative responsibility as the Senior Associate Dean for Academic Affairs. He was formerly a Professor of Anatomy and an Adjunct Professor in the Biochemistry Program at Dartmouth College. Dr. Yager has served as the Program Director and Principal Investigator for the Training Program in Environmental Health Sciences, Director of the Division of Toxicological Sciences, and Director of the Molecular Toxicology Program of the NIEHS-supported Center in Urban Environmental Health. His research interests include mechanisms of promotion of hepatocarcinogenesis by estrogenic xenobiotics, mechanisms of estrogen-induced oxidative DNA damage in liver and human breast epithelium, and the role of genetic susceptibility in human cancer through polymorphisms in biotransformation enzymes involved in estrogen oxidative metabolism. Dr. Yager serves on various committees and task forces for several professional societies, including the American Association of Cancer Research (AACR), the American Society for Investigative Pathology (ASIP, FASEB), and the Society of Toxicology. Dr. Yager serves or has served on various advisory boards and chartered review panels, including the EPA Endocrine Disruptor Methods Validation Subcommittee and the ICCVAM Scientific Review Panel to evaluate the validation status of *in vitro* estrogen and androgen receptor binding and transcriptional activation assays (2002). He is a peer reviewer for numerous journals, including Biochemical Pharmacology, Cancer Research, Chemical–Biological Interactions, Molecular Carcinogenesis, Science, and the Journal of the National Cancer Institute. Dr. Yager is on the editorial board for the Journal of Environmental Pathology, Toxicology and Oncology; In Vitro-Cell & Developmental Biology; Toxicology Sciences; and Chemical Research in Toxicology. He has authored or coauthored 84 peer-reviewed journal articles, 15 book chapters, 66 abstracts or presentations at national and international meetings; and he and has given over 50 invited presentations.