DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

National Institutes of Health
National Institute of
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MEMORANDUM

DATE: April 18, 2012

TO: The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

FROM: Director, National Institute of Environmental Health Sciences (NIEHS) and the National Toxicology Program (NTP)

SUBJECT: NIEHS Response to ICCVAM Test Method Recommendations on an Endocrine Disruptor Screening Test Method

On February 1, 2012, at the request of the Secretary of the Department of Health and Human Services, I forwarded toxicological test recommendations from ICCVAM to 14 Federal agencies for their consideration. The recommendations were developed and transmitted pursuant to Section 3(e)(4) of the ICCVAM Authorization Act of 2000 (42 U.S.C. 285*l*-3). Pursuant to Sections 4(a) and 4(d) of the ICCVAM Authorization Act, agencies are required to review ICCVAM test recommendations and notify ICCVAM in writing of their findings, including identification of relevant test methods for which the ICCVAM test recommendations may be added or substituted. This memorandum provides the NIEHS response to ICCVAM regarding the test recommendations.

ICCVAM provided test method recommendations relevant to identifying human estrogen receptor (ER) agonist and antagonist activity of chemicals using the *in vitro* LUMI-CELL® ER (BG1Luc ER Transactivation [TA]) test method. The recommendations are 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*.

NIEHS agrees with ICCVAM that the BG1Luc ER TA test method, which uses a human ovarian adenocarcinoma cell line expressing endogenous ERα and ERβ, can be used as a screening test to identify substances with *in vitro* ER agonist or antagonist activity. This use is based on an evaluation of available validation study data and corresponding accuracy and reliability. ICCVAM concluded, and NIEHS agrees, that the accuracy of this assay is at least equivalent to the current ER TA assay included in regulatory testing guidance (EPA OPPTS 890.1300). In addition, the BG1Luc ER TA test method is currently under evaluation by OECD as an international test guideline for detecting ER agonist and antagonist activity of chemicals, and once adopted, all 33-member countries, including the United States, will accept testing data generated in accordance with the BG1Luc ER TA Test Guideline. These test methods can also be used as part of a weight-of-evidence approach as described in the OECD's conceptual framework, which may further reduce animal use for screening chemicals for endocrine activity.

NIEHS also agrees with ICCVAM on the recommended protocols for the BG1Luc ER TA agonist and antagonist test methods. Future studies with the intent of characterizing the usefulness and/or limitations of the BG1Luc ER TA test methods should be conducted using the recommended protocols. The performance standards developed by ICCVAM for evaluation of mechanistically and functionally

similar test methods as well as the studies suggested by ICCVAM for future consideration are also supported by NIEHS.

The rat uterine cytosol ER binding assay, while considered "*in vitro*", requires the use of animals as a source of ER. The BG1Luc ER TA test method offers advantages over such receptor binding assays. The BG1Luc ER TA does not require animals; it performs at physiologically relevant temperatures, measures the biological response to receptor binding, distinguishes between receptor agonists and antagonists, and detects substances that initiate transcription by an indirect mechanism. The high concordance (97% [33/34]) between the BG1Luc ER TA and ER binding test methods suggests that the BG1Luc ER TA appears to be a promising alternative to the ER binding assay.

NIEHS is not a regulatory agency and therefore does not promulgate regulatory testing requirements or guidelines for which the ICCVAM recommendations may be applicable. However, NIEHS does conduct toxicity testing as part of its National Toxicology Program activities, and supports environmental health research on endocrine active substances to determine if and how such substances may cause or contribute to adverse health effects. NIEHS and the NTP will therefore promote and encourage the consideration and use of the BG1Luc ER TA for research and testing where determined appropriate.

NIEHS also recognizes that the BG1Luc ER TA agonist and antagonist assays, as *in vitro* alternative testing methods, could potentially help further reduce and refine the use of animals for assessing the endocrine disrupting potential of chemicals and products. In addition to potentially replacing the rat uterine cytosol ER binding assay, using the BG1Luc ER TA in a mechanism-based screening strategy that can accurately identify substances that are not likely to have endocrine disrupting effects could avoid the need for further testing of such substances in multi-generational testing that uses large numbers of animals. Accordingly, NIEHS and NTP scientists and the NIEHS Institutional Animal Care and Use Committee (IACUC) have been informed about the availability of the BG1Luc ER TA and its potential usefulness for reducing animal use. They have also been advised that if endocrine disruptor studies are proposed, then these alternative test methods should be routinely considered and used where appropriate in order to avoid or minimize animal use. To comply with the Public Health Service Policy on Humane Care and Use of Laboratory Animals and applicable USDA Animal Welfare Act Regulations, the NIEHS IACUC has also been asked to ensure that these alternative methods are always considered whenever applicable and to ensure that the methods are used when determined appropriate.

NIEHS appreciates ICCVAM's comprehensive evaluation of the BG1Luc ER TA test method. NIEHS remains highly committed to the development, validation, and regulatory acceptance of scientifically sound safety testing methods that will support improved protection of people, animals, and the environment while providing for improved animal welfare.

/s/

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cc:
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