Xenobiotic Detection Systems, Inc.
"Dioxin Bioassays"

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January 22, 2004

Re: Request for Cross-Laboratory Validation Studies of Xenobiotic Detection Systems LUMI-CELL™ ER bioassay for chemicals with estrogen disruptor activity, by NICEATM and ICCVAM.

Dr. William S. Stokes:
Director
NTP Interagency Center for the Evaluation
of Alternative Toxicological Methods (NICEATM)
NIEHS
P.O. Box 12233
MD EC-17
Research Triangle Park, NC 27709

Dear Dr. Stokes,

Xenobiotic Detection Systems (XDS) has developed a cell based transcriptional method to evaluate the endocrine disruptor activity of chemicals for the estrogen receptor (ER). XDS has termed this test method the LUMI-CELL™ ER bioassay. We have developed a standardized test procedure in a stably transfected recombinant cell line that is sensitive, robust, and reproducible in detecting estrogen active chemicals. We nominate this test method for validation by NICEATM and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) as a regulatory method for evaluating estrogen agonists and antagonists. I will briefly summarize the extent to which this *in vitro* test method meets the ICCVAM prioritization criteria:

- **Applicable to regulatory testing needs:** The LUMI-CELL™ ER bioassay will meet the need for a high throughput screening (HTPS) system of chemicals for their potential estrogen disruptor activity. The US Environmental Protection Agency (EPA) identified a need for this technology in the Endocrine Disruptor Steering and Testing Advisory Committee (EDSTAC) recommendations in order to meet a mandate of the Food Quality Protection Act of 1996 and the Safe Drinking Water Act of 1996. This test method is also in response to Federal Register Notice (Vol. 66, No. 57/Friday, March 23, 2001) as a HTPS method for estrogen active compounds.

- **Applicable to multiple agencies/programs:** The LUMI-CELL™ ER bioassay technology may also be applicable to the US Food and Drug Administration,, Department of Agriculture, Department of Defense, and Department of Homeland Security, since methodologies are being developed to screen feed and

food for potential estrogen disruptor chemicals. Both food and feed are a potential source for exposure to EDCs.

- **Warrant, based on the extent of expected use or application and impact on human, animal, or ecological health:** The association of exposure to EDCs and adverse health effects in human and wildlife populations has led to worldwide concern. Some of the health effects that have led to this concern include global increases in testicular cancer, regional declines in sperm counts, altered sex ratios in wildlife populations, increases in the incidence of breast cancer and endometriosis, and accelerated puberty in females that are expected to result from exposure to chemicals that adversely affect steroid hormone action(*1-3*).

- **The potential for the proposed test method, compared to current test methods accepted by regulatory agencies, to:** There are no currently accepted methods that are being used to screen for EDCs but some have been proposed and are in the process of validation by the EPA. Most of these methods require substantial use of animals to evaluate endocrine disruptor activity. The LUMI-CELL™ ER bioassay method would allow for a rapid process to screen and set priorities on testing chemicals for disruption of estrogenic activity in other animal models. This would consequently result in a significant reduction in animal use in the screening process.

- **The potential for the proposed test method to provide improved prediction of adverse health or environmental effects, compared to current test methods accepted by regulatory agencies:** There are no current methods approved for the detection of ECDs by any federal agency. However, the LUMI-CELL™ ER bioassay shows tremendous potential for prediction of adverse health and environmental effects. This is shown by the very high correlation between agonist response data collected using our test method and the historical data available in the database developed by NICEATM on these compounds. The LUMI-CELL™ ER bioassay is sensitive enough to allow for an extremely low detection limit (ppq), which should be lower than federal regulations are likely to mandate. Unlike ELISA detection limits which have a lower limit of >1 ppb. The LUMI-CELL™ ER bioassay will give a measure of bioavailability, being a biological system itself.

- **The extent to which the test method provides other advantages (e.g., reduced cost and time to perform) compared to current methods:** LUMI-CELL™ ER bioassay is an extremely rapid in vitro method that can evaluate the estrogenic activity of chemicals within two days. The method also provides relative activity of a chemical to the standard, beta-estradiol, and provides dose response activity of the chemical. The standardized protocol developed allows for a very robust system with low variability and high sensitivity. The cost of the LUMI-CELL™ ER bioassay is a few hundred dollars per chemical, which is substantially less than any animal base method. The LUMI-CELL™ ER

bioassay is a transcriptionally based assay capable of testing for antagonistic responses of EDCs, which is not possible using binding assays.

XDS is requesting that NICEATM and ICCVAM aid in and manage the cross-laboratory validation studies needed to formally evaluate the reliability and accuracy of the LUMI-CELL™ ER bioassay and its use as a regulatory test method for detecting chemicals with estrogenic agonist and antagonist activity. The pre-validation and method development steps for this test method are essentially complete and data on the screening of 120 chemicals for estrogenic agonist activity can be made available to NICEATM and ICCVAM. XDS proposes that it act as the primary laboratory providing training and technical support to other participating laboratories (i.e. Hiyoshi Corp. in Japan and Scientific Institute of Public Health in Belgium, both using our CALUX® system).

We hope that ICCVAM and NICEATM will look upon this request favorably.

Regards,

George C. Clark, Dr. P.H.
President

John D. Gordon, PhD.
Director of Research

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2. Okoumassoun LE, Averill-Bates D, Gagne F, Marion M, Denizeau F. Assessing the estrogenic potential of organochlorine pesticides in primary cultures of male rainbow trout (Oncorhynchus mykiss) hepatocytes using vitellogenin as a biomarker. Toxicology 178:193-207(2002).
3. Safe SH, Pallaroni L, Yoon K, Gaido K, Ross S, McDonnell D. Problems for risk assessment of endocrine-active estrogenic compounds. Environ Health Perspect 110 Suppl 6:925-9(2002).