VALIDATION OF THE LUMI-CELL ä ER RECOMBINANT BIOASSAY FOR RAPID EVALUATION OF CHEMICALS FOR POTENTIAL ESTROGENIC ACTIVITY

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Introduction

The association between the exposure and bioaccumulation of endocrine disruptor chemicals (EDCs) and their adverse effects on human and wild life populations has raised concern worldwide[1,2]. Due to the detrimental effects of environmental exposure to EDCs, there is an obvious need to develop a relevant bioassay, which can both detect these chemicals, as well as provide a relevant estimate of their endocrine disrupting potency. Some examples of the effects of EDCs are: decreased reproductive success and feminization of males in several wildlife species; increased hypospadias along with reductions in sperm counts in men; increase in the incidence of human breast and prostate cancers; and endometriosis [3-5]. Because these chemicals are ubiquitous, highly lipophilic, and often chlorinated, ensures their persistent presence in the environment resulting in their bioaccumulation in the food chain.

In fact, these concerns over the effects of environmental EDCs, lead to the passage of Congressional legislation (the Food Quality Act of August 1996 and the Safe Water Reauthorization Act and Amendments of 1996), which mandated the EPA to investigate the effects of exposure to environmental EDCs [6,7]. Based on this mandate, the EPA established the Endocrine Disruptor Steering and Testing Advisory Committee (EDSTAC). The EPA also established the Endocrine Disruptor Screening Program (EDSP) within the agency. Therefore, there is a growing need for a fast reliable high-throughput system for the screening of known and potential environmental contaminants, which act to disrupt normal endocrine homeostasis.

In order to detect EDCs using a high-throughput bioassay system, Xenobiotics Detection System (XDS) Inc. in collaboration with Dr. Michael S. Denison (Univ. California-Davis), have developed the LUMI-CELL™ ER bioassay. BG-1 cells were stably transfected with an estrogen-responsive luciferase reporter gene plasmid (pGudLuc7ere)[5]. The resulting cell line ($BG1Luc4E_2$), responds to estrogenic chemicals in a time-, dose dependent- and chemical-specific manner with the induction of luciferase gene expression[5]. In initial studies, $BG1Luc4E_2$ cells, using the LUMI-CELL™ ER bioassay, were able to detect as little as 0.1pM of 17β-estradiol[5].

In May of 2002 XDS submitted preliminary data to ICCVAM for review as a validated regulatory method in response to the Federal Register Notice (Vol. 66, No. 57/Friday, March 23, 2001) as a

HTPS method for estrogen active compounds[8]. Here we describe studies in which XDS's LUMI-CELL™ ER estrogenic cell bioassay system was used for high throughput screening (HTPS) analysis of the ICCVAM EDWG proposed 78 substances for validation of estrogen receptor (ER) and androgen receptor (AR) binding and transcriptional activation assays[8]. Our results demonstrate the utility of XDS's BG1Luc4E$_2$ LUMI-CELL™ ER bioassay HTPS system for screening chemicals for estrogenic/antiestrogenic activity.

Methods and Materials

Chemicals were purchased from the Aldrich Chemical Co. Sigma Chemical Corporation, and Chem Service Inc.

LUMI-CELLä ER Bioassay. The BG1Luc4E$_2$ cell line was constructed as previously described by Rodgers and Dennison (2000). Briefly, BG1 cells were stably transfected with an estrogen-responsive luciferase reporter gene plasmid (pGudLuc7ere) and selected for using G418 resistance[5].

Cell Culture and Bioassay Plates. BG1Luc4E$_2$ cells were grown in RPMI 1640. The cells were transferred into flasks containing DMEM media (supplemented with 5% carbon stripped fetal calf serum and G418 sulfate solution), and incubated for four days before harvesting for BG1Luc4E$_2$ bioassay plates. The cells were then plated in 96 well plates and incubated at 37^o C for 24-48 hours prior to dosing.

Bioassay Dosing Process. Once the assay plate completed its incubation, the media solution in each well was removed and two hundred microliters of DMEM containing the indicated concentration of the desired chemical to be tested was added to each well. The plate was then incubated for 24 hours before analysis of luciferase activity.

Bioassay Analysis by Berthold Luminometer. After lysing the cells (Promega lysis buffer), the luciferase activity was measured in a Berthold Orion Microplate Luminometer, with automatic injection of 50 microliters of luciferase enzyme reagent (Promega) to each well. The relative light units (RLUs) measured were compared to that induced by the 17beta-estradiol standard after subtraction of the background activity. Each compound was tested at least three times on three different sets of plates and the EC50 value in mmol/ml was determined using the Microsoft Excel Forecast function.

Results and Discussion

There is a growing concern for a need for a system fast, reliable, inexpensive method to detect EDCs in the environment. This concern arises from the detrimental effects of EDCs on human and wildlife populations resulting from its bioaccumulation in the food chain. Here we report a fast, reliable, relatively inexpensive high throughput cell based recombinant bioassay screening method (LUMI-CELL™ ER bioassay) for xenoestrogenic EDCs.

Seventy-Eight chemicals were tested using XDS's LUMI-CELL™ ER bioassay system. Twenty-four of these chemicals were recommended by ICCVAM for validation of ER binding and

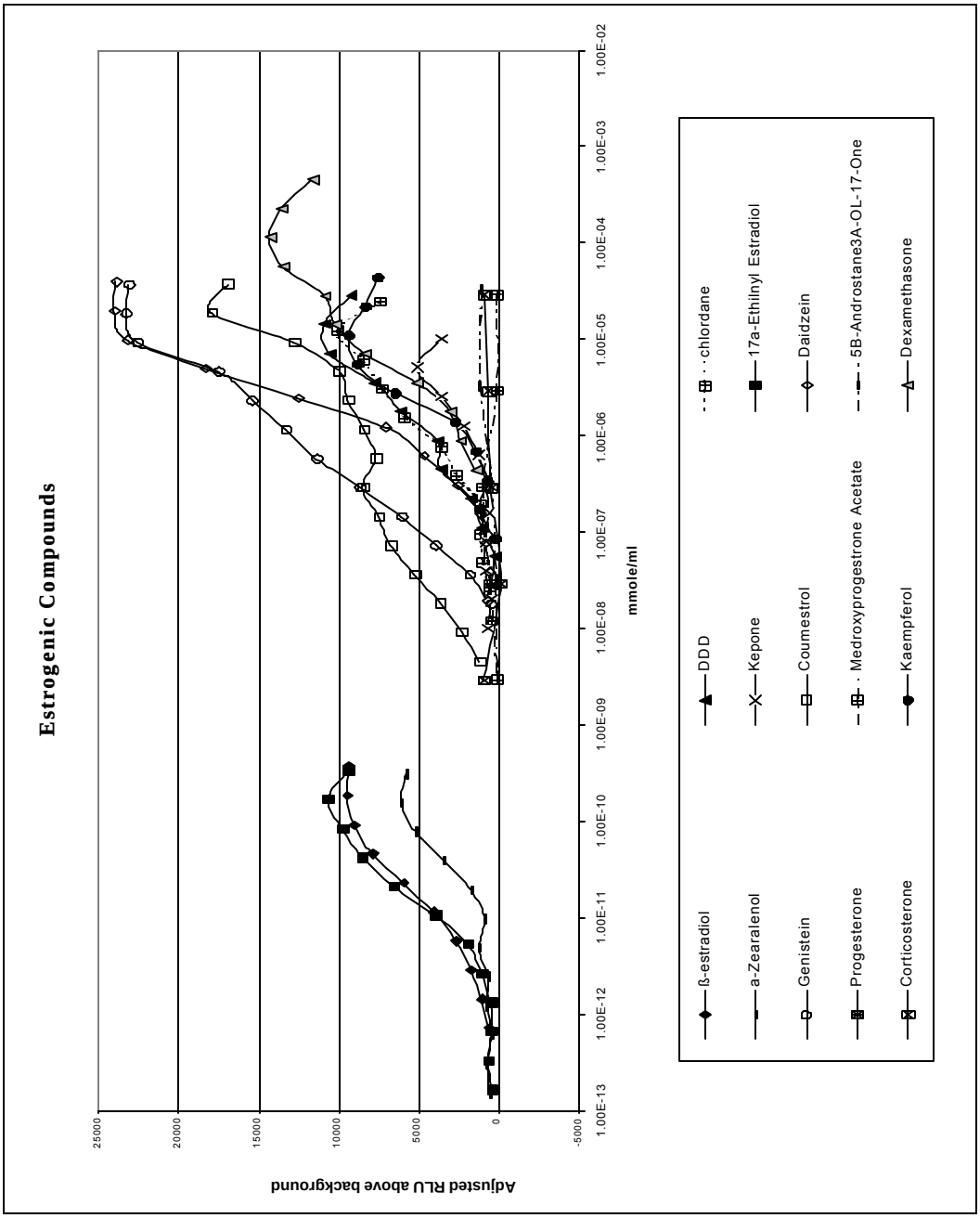

Figure 1. Selected compounds tested to demonstrate dose dependent response relationships using XDS's BG1Luc4E$_2$ LUMI-CELL™ ER HTPS bioassay system for known and potential estrogenic EDCs.

transcriptional activation assays and are known to posses estrogenic activity, thereby making them potential endocrine disruptors[8]. Fifty-four of these chemicals are environmental contaminants, and were tested to determine their estrogenic activity as unknowns and their potential to be endocrine disruptors. Five other compounds on the ICCVAM list of chemicals to test for ER and AR validation, which have previously been shown to not posses' estrogenic activity, and are therefore not potential endocrine disruptors, were also tested[8].

Of the Seventh-Eight chemicals tested by XDS's LUMI-CELL™ ER bioassay system, 61 demonstrated estrogenic activity, while 17 showed no activity. All of the 24 chemicals tested, which were recommended by ICCVAM for validation, with the exception of Progesterone, which has both agonist and antagonist activity, demonstrated estrogenic activity. Of the Fifty-four chemicals tested, which were not included in the ICCVAM requirements for validation, 27 were found to have estrogenic activity and 17 showed no activity. Five of the 17 chemicals not showing any activity, had been previously shown to not have estrogenic activity[8]. 17β-Estradiol proved to have the highest estrogenic activity, having an EC50 detection level of 1.43×10^{-11} mmol/ml.

Figure 1 shows the results of an analysis of 15 of the chemicals tested in this study via the LUMI-CELL™ ER bioassay. Eleven of these significantly induced luciferase reporter gene activity at or below the 1 µM level and 4 were essentially inactive. Of the eleven chemicals showing activity, nine were on the list of chemicals suggested by ICCVAM for validation of ER binding and transcriptional activation assays and two (DDD and Chlordane) had not been previously shown to posses estrogenic activity[8]. Three of the four chemicals showing no activity were on the ICCVAM list for validation and according to their report had no estrogenic activity[8]. The fourth chemical (5B-Androstane3A-OL-17-One) was tested as an unknown. This data clearly demonstrates that XDS's LUMI-CELL™ ER high-throughput bioassay system is a fast, reliable, and relatively inexpensive method for detection of environmental EDCs, meeting the requirements mandated by the EPA and ICCVAMs Tier I (screening) requirements for EDC detection assays.

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