Estrogenic Potency of Many Popular Sunscreens and it's "non-Active" Components Detected using the LUMI-CELL ER Bioassay

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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].

The use of sunscreens has increased during the past decades due to growing concern over damage from the sun such as sunburn, photo aging, and skin cancer. Recently there has been a growing concern regarding estrogenic potency of these sunscreens and their components. Recent studies on the "active" (or directly contributing to SPF) components of sunscreens such as 3-(4-methylbenzylidene) camphor (4-MBC), Octyl-Methoxycinnimate, and Benzophenone-3 have shown them to be highly estrogenic in uterine wet weight, cell height, and cell proliferation assays[1,6-9]. Studies by Janjua et al. (2004) have shown these compounds to be present in urine and blood plasma after topical application[6]. Janjua et. al. (2004) also found changes in hormone (estradiol and testosterone) levels of participants after topical application[6]. In this study we looked at several currently marketed sunscreens as well as the "non-active" (or indirectly contributing to SPF) sunscreen components. Here we describe studies in which the LUMI-CELL™ ER estrogenic cell bioassay system was used for high throughput screening (HTPS) evaluation of estrogenic agonist activity of several currently marketed sunscreens as well as some of the "non-active" (indirectly contributing to SPF) sunscreen components. In this study estrogenic potency was measured by activation of an estrogen receptor mediated transcriptional activation luciferase reporter gene. Our results demonstrate the utility of the LUMI-CELL™ ER bioassay HTPS system for screening cosmetics for estrogenic activity.

Methods and Materials

Sunscreens tested were obtained from a department store and include: Coppertone SPF 8; Coppertone SPF 15; Coppertone SPF 30 (Endless Summer); Banana Boat SPF 15; Banana Boat Kids SPF30; Hawaiian Tropic SPF 8; Coppertone Water Babies SPF 45; Banana Boat Baby Magic SPF 50; Hawaiian Tropic Baby Faces SPF 50+; and 3rd Rock Sunblock SPF 20. The "non-active" components are compounds used in sunscreens but do not directly enhance SPF for protection from UV damage and these include: Lexorez 200 (for water resistance); ABIL Wax 9801 (improves SPF response); TEGO care PS (Emulsifier); ABIL WE-09 (Emulsifier - higher SPF); KOBO CM3K40T4 (SPF enhancer); Lanol 84D Dioctyl malate (allows for smooth texture - emollient/emulsifier); Dow Corning 344 (Lubricant); Dow Corning 1401 (Lubricant) were purchased from the Inolex Chemical Co., Goldschmidt Chemical Corp., Kobo Products Inc., and Dow Corning.

Endocrine Extraction Procedure: One gram of each of the lotion components and 0.5 g of each of the sunscreens was placed in MeOH rinsed scintillation vials. Two and 4-gram aliquots of the 3rd Rock Sunblock were also tested. Twenty ml of MeOH was added to each scintillation vial and sonicated for 20 min. Fractions of these extractions, ranging from 1:10 to 1:80,000 were tested. Recoveries were determined using 10ng 17β-estradiol spiked into 3rd

Rock Sunblock prior to extraction with 20 ml MeOH compared to 10ng 17β-estradiol spiked into 20 ml MeOH.

LUMI-CELL™ ER Bioassay. The BG1Luc4E2 cell line was constructed as previously described by Rogers and Denison (2000). Briefly, BG1 cells were transfected with an estrogen-responsive luciferase reporter gene plasmid (pGudLuc7ere) and stable transfectants selected using G418 resistance[5]. For analysis, the BG1Luc4E2 cell clone resulting from this selection was grown in RPMI 1640 medium. The cells were transferred into flasks containing phenol red-free 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 plating. The cells were then plated in 96 well plates and incubated at 37^{0}C for 24-48 hours prior to dosing. The media solution in each well was removed and two hundred microliters of phenol red-free DMEM containing the indicated concentration of the desired chemical to be tested was added to each well. The plate was then incubated for 20 hours before analysis of luciferase activity.

Bioassay Analysis by BertholdLuminometer. After lysing the cells (Promegalysis buffer), the luciferase activity was measured in a Berthold Orion MicroplateLuminometer, with automatic injection of 50 microliters of luciferase enzyme reagent (Promega) into 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 need for a 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.

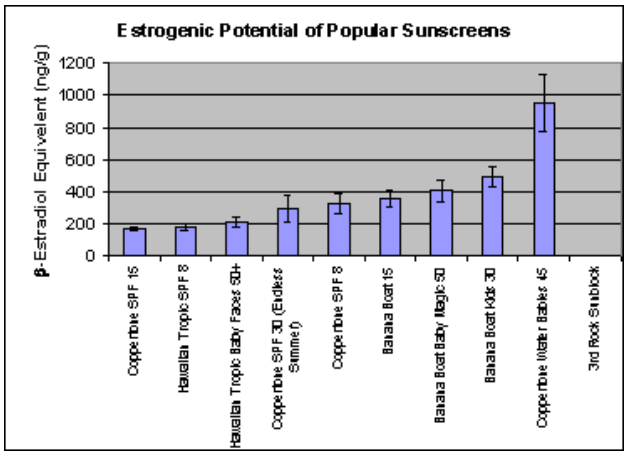

Figure 1: Estrogenic potential of popular sunscreens.

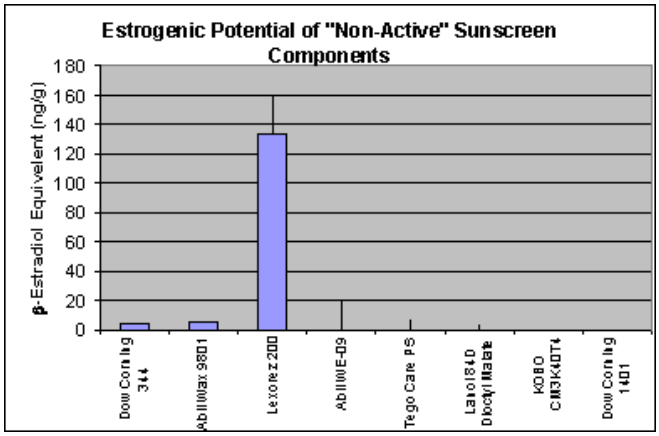

Figure 2: Estrogenic potential of “non-active” sunscreen lotion components.

In this study 10 sunscreen products and 8 “non-active” lotion components were tested for estrogenic potency. The samples were tested at 4g, 2g, 1g, 0.5g, and 0.1g equivalents. The 0.5g aliquot was selected for sunscreens and 1g for “non-active” components due to it showing the most activity with the least toxicity. The 3rd Rock Sunblock SPF 20 was used as a negative control due to it previously testing as a non-detect. The 3rd Rock Sunblock SPF 20 was also used in recovery determinations. This was done by dividing the average RLU for the 10ng 17β-estradiol spiked 3rd Rock Sunblock SPF 20 by the 10ng 17β-estradiol spiked into 20 ml MeOH. The average recovery was found to be 77.4%.

All of the sunscreens detected positive for estrogenic activity with the exception of 3rd Rock Sunblock, which was shown as a non-detect at less than 0.308 pg/g 17β-estradiol equivalent. The sunscreen with the highest estrogenic potential was Coppertone Water Babies SPF45 at 948.66 ± 176.62 ng/g 17β-estradiol equivalent. Based on our test results, the order of estrogenic potency appears to be: Coppertone Water Babies 45 > Banana Boat Kids 30 > Banana Boat Baby Magic 50 > Banana Boat 15 > Coppertone SPF 8 > Coppertone SPF 30 (Endless Summer) > Hawaiian Tropic Baby Faces 50+ > Hawaiian Tropic SPF 8 > Coppertone SPF 15 > 3rd Rock Sunblock SPF 20. While three of the “non-active” components showed any estrogenic activity, only Lexorez 200 exhibited significant estrogenic potency.

This study demonstrates that the only “non-active” (or indirectly contributing to SPF) component tested which contributes any significant estrogenic potency to sunscreen is Lexorez 200. Both Dow Corning 344 and Abil Wax 9801 showed only slight estrogenic potency, while the remaining components were classified as non-detects. Thus, the vast majority of the estrogenic potency remains attributed to the “active” (or directly contributing to SPF) components of the same formulations and/or other “non-active” components not yet tested. Given the results of previous studies the bulk of the activity is likely associated with the “active” sunscreen components although whether interactions occur remain to be examined. Further investigations will include testing “active” and “non-active” components for more detailed analysis regarding estrogenic potency ratios.

Acknowledgements

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