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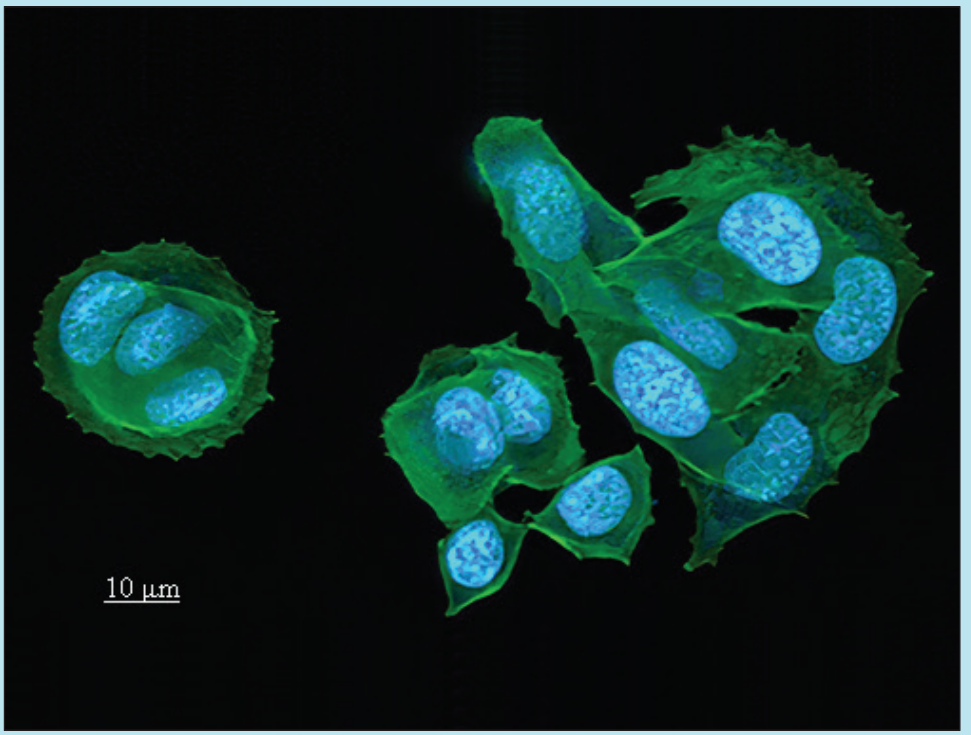

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Cover Photograph: MDA-kb2 cells stained fluorescently for DNA (blue) and F-actin (green) and imaged through confocal microscopy. Scale bar represents 10 µm. Photograph © Amelia K. Rinehart.

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Effect of Plasticizers on Androgen Receptor Activation

Amelia K. Rinehart[1] and Sarah Lundin-Schiller[2,*]

Abstract - Endocrine disrupting chemicals interfere with signaling pathways of the endocrine system and are found in numerous consumer products ranging from cosmetics to water supplies. The compounds bisphenol A (BPA), bisphenol A glycidyl dimethacrylate (bisGMA), bisphenol A dimethacrylate (bisDMA) and bisphenol A diglycidyl ether (BADGE) were screened for androgenic or antiandrogenic properties using an MDA-kb2 transactivation assay. Only BPA displayed antiandrogenic properties in the MDA-kb2 assay ($P \leq 0.0001$). In this study, data from an MDA-kb2 cell assay support that bisGMA, bisDMA, and BADGE do not interact with the androgen receptor.

Introduction

The endocrine system controls most life processes from regulation of embryonic development and initiation of puberty to adult body functions, such as spermatogenesis. Studies have shown that some chemicals are able to mimic endogenous hormones and agonize or antagonize hormonal receptors (La Merrill et al. 2020, Pirard et al. 2012). These chemicals, termed endocrine disrupting chemicals (EDCs), have been found in consumer goods (Jobling et al. 1995, La Merrill et al. 2020, Plotan et al. 2013, Schlumpf et al. 2001) and the environment (La Merrill et al. 2020, Layton et al. 2011, Wang et al. 2013, Werner et al. 2010, Ye et al. 2012). Exposure to EDCs, especially *in utero,* can have deleterious effects. In males, antiandrogens and estrogen-mimicking compounds can lead to severe morphological abnormalities such as hypospadias, decreased prostate weight, or to broader issues such as testicular dysgenesis syndrome.

Bisphenol A (BPA), a plasticizer, and its derivatives, bisphenol A glycidyl dimethacrylate (bisGMA), bisphenol A dimethacrylate (bisDMA), and bisphenol A diglycidyl ether (BADGE) are of concern as potential EDCs. Bisphenol A can interfere with a variety of intracellular pathways and is a known estrogen agonist (Murray et al. 2007), androgen antagonist (Bonefeld-Jørgensen et al. 2007, Lee et al. 2003) and aromatase inhibitor (Bonefeld-Jørgensen et al. 2007). Bisphenol A is commonly found in plastics and has been shown to leech into food from packaging containing BPA (Munguia-Lopez et al. 2005) and from conventional dental resins (Mourouzis et al. 2022). Exposure to this compound has been linked to increased susceptibility to carcinomas (Ho et al. 2006, Murray et al. 2007). Three BPA derivatives, bisGMA, bisDMA, and BADGE, are commonly used in dental sealants (Fleisch et al. 2010). An *in vivo* study performed by Al-Hiyasat and Darmani (2006) found that exposure to bisGMA led to a decrease in testicular and epididymal sperm counts in male mice with a concomitant decrease in fertility. Also, bisGMA has been reported to cause apoptosis, necrosis, and DNA strand breaks in RAW264.7 macrophage cells *in vitro* (Li et al. 2012). BisDMA has the ability to interact via multiple cellular pathways and possesses estrogen agonistic activity, androgen antagonistic activity, and can act as an aromatase inhibitor (Bonefeld-Jørgensen et al. 2007). Boonen et al. (2021) reports bisDMA had sig-

[1] Nashville State Community College, 120 White Bridge Parkway, Nashville, Tennessee, 37209. [2]Austin Peay State University, 601 College Street, Clarksville, Tennessee, 37044. [*]Corresponding author- schillers@apsu.edu

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nificant agonistic estrogenic activity in an estrogen receptor-α assay. As recently reviewed by Wang et al. (2021), BADGE and its derivatives are widespread in the environment and display endocrine disrupting potential *in vitro* and *in vivo*. This compound has been reported to stimulate proliferation in MCF7 breast cancer cells (Olea et al. 1996) and in T47D breast cancer cells (Nakazawa et al. 2002). Yang et al. (2010) found that oral exposure to BADGE led to a disruption in spermatogenesis in Sprague-Dawley rats. This disruption led to a decrease in the number of mature sperm in testis (Yang et al. 2010).

Although detailed mechanisms of action are, in many cases, still under investigation, deleterious health effects correlated with EDC exposure continue to be documented (Diamanti-Kandarakis et al. 2009, Stukenborg et al. 2021). Over the past 50 years, there has been an increase in reproductive abnormalities in males, such as a decline in sperm quality (Andersen et al. 2000, Carlsen et al. 1992, Rolland et al. 2013). Skakkebaek et al. (2001) proposed that reproductive abnormalities, such as declining semen quality, increased incidences of testicular cancer, cryptorchidism, and hypospadias, are part of a larger issue termed testicular dysgenesis syndrome (TDS). This is further supported in a meta-analysis by Bonde et al. (2017) where a small increase in development of male reproductive disorders with prenatal and postnatal exposure to environmental endocrine disruptors was noted.

Antiandrogens disrupt the development and function of the male reproductive system by interfering with the androgen signaling system. In a study performed on Sprague-Dawley rats, Ostby et al. (1999) found that exposure to vinclozolin during gestation caused feminization of male fetuses as indicated by decreased anogenital distance, retained nipples, cleft phallus with hypospadias, small to absent accessory sex glands such as the prostate gland, and cryptorchidism. These effects were also replicated in a study performed by Parks et al. (2000) in which diethylhexyl phthalate, an antiandrogen, was administered to rats during gestation. The offspring in this study were also feminized, recreating the abnormalities induced by exposure to vinclozolin (Ostby et al. 1999). A meta-analysis conducted by Dorman et al. (2018) concluded that increased urinary concentrations of diethylhexyl phthalate metabolites in pregnant women were associated with decreased anogenital distance in male offspring. It is clear that *in utero* exposure to estrogen-mimicking or antiandrogenic chemicals can cause abnormalities of the male fetus.

Endocrine disrupting chemicals are capable of interfering with cell signaling at multiple points (Acconcia et al. 2015). The present study will assess agonistic or antagonistic effects of the four plasticizers (BPA, bisGMA, bisDMA, and BADGE) using the MDA-kb2 cell line assay that tests for binding explicitly at the androgen receptor. MDA-kb2 is a cell line created from the MDA-MB-453 breast cancer cell line that expresses endogenous androgen receptors (Wilson et al. 2002). The MDA-kb2 cell line was transformed with an androgen responsive luciferase reporter gene construct that is activated when exposed to chemicals that act through the androgen receptor (Wilson et al. 2002). Exposure to androgens, and subsequent binding to the androgen receptor, stimulates down-stream synthesis of luciferase. When luciferin is added, luciferase produces luminescence that can be detected spectrophotometrically. The greater the luminescence, the more androgen stimulation occurred. A lack of luminescence following androgen exposure indicates the androgen receptor was not stimulated by an agonist, or an antagonist was present. In this study, MDA-kb2 cells were treated with the vehicle carrier, ethanol (EtOH), a known androgen (DHT), a known antiandrogen, hydroxyflutamide (OHF), a mixture of OHF and DHT, plasticizers at varying concentrations, and plasticizers mixed with either OHF or DHT. The antiandrogen, OHF, competitively inhibits the androgen receptors; therefore, minimal luminescence should be measured in cells exposed to OHF.

Materials and Methods

Chemicals

L-15 Leibovitz media + 2.05 mM L-Glutamine (L-15) was purchased from HyClone Laboratories, Inc., Logan, UT. RPMI media + L-Glutamine – Phenol Red (RPMI), antibiotic/antimycotic (AbAm), and phosphate buffered saline pH 7.4 (PBS) were purchased from Life Technologies, Grand Island, NY. Charcoal stripped fetal bovine serum (CS FBS) was purchased from Atlanta Biologicals, Lawrenceville, GA. Trypsin-EDTA solution, hydroxyflutamide (OHF), bisphenol A, bisphenol A dimethacrylate, bisphenol A glycidyl dimethacrylate, and bisphenol A diglycidyl ether were purchased from Sigma-Aldrich, St. Louis, MO. Luciferase assay systems were purchased from Promega, Madison, WI.

Culture of MDA-kb2 cells

MDA-kb2 cells were purchased from American Type Culture Collection, Manassas, VA. MDA-kb2 cells were cultured in L-15 media supplemented with 10% heat-inactivated CS FBS (HI CS FBS) and 2% AbAm (complete L-15 media) in a tissue-treated 75 cm^2 flask. The complete L-15 media was changed every 48 hours. MDA-kb2 cells were incubated at 37°C without CO_2 at 89% humidity. At 100% confluence, the flask was split. Cells were removed from the seed flask using trypsin (0.25% w/v)-EDTA (0.03% w/v) solution, resuspended in complete L-15 media, and plated at $1x10^4$ cells/cm^2 in a fresh 75 cm^2 flask or in 96-well plates prior to experimentation.

Working solutions

All chemicals were dissolved in 95% EtOH and stored at 4°C for later use. The compounds, DHT, OHF, and BPA, were dissolved in 95% EtOH and vortexed to create a 100 mM stock solution. In order to obtain a workable consistency, bisGMA was warmed to 56°C in a water bath. After equilibrating to 56°C and obtaining a working consistency, bisGMA was maintained at 37°C in water bath. bisGMA was then added to 95% EtOH and vortexed to create a 100 mM stock solution. For subsequent working dilutions, and before experimental treatments, bisGMA was warmed to 37°C to ensure that the compound was completely dissolved. Bisphenol A dimethacrylate and BADGE were added to 95% EtOH to create 100 mM stock solutions. Stock solutions were then warmed to 56°C to allow crystals to dissolve. After all crystals were dissolved, stock solutions were maintained at 37°C during creation of subsequent dilutions. Prior to treatments, bisDMA and BADGE solutions were warmed to 37°C to ensure compounds were completely dissolved.

Transactivation assay using MDA-kb2 cells

MDA-kb2 cell transactivation assay was performed following protocol determined by Wilson et al. (2002). MDA-kb2 cells were cultured in 96-well plates (Costar 3610). Cells were treated with 7 treatments as follows: 1. vehicle only (0.01% and 0.02% EtOH, negative control), 2. DHT (androgen receptor agonist, positive control at 0.01 nM, 0.1 nM, 1 nM, 10 nM, and 100 nM), 3. OHF (androgen receptor antagonist at 1 μM) 4. DHT (1 nM) + OHF (1 μM), 5. plasticizer (10 nM, 100 nM, 1 μM, and 10 μM), 6. plasticizer (10 μM) + OHF (1 μM), or 7. plasticizer (10 μM) + DHT (1 nM). Each chemical or chemical combination was tested in replicates of 4 and the entire screening assay was conducted twice for each compound. Treatments 1–4 above represent assay controls that are included every time a plasticizer was tested in the assay. For the assay to be valid, DHT must be shown

to stimulate luciferase activity and OHF must be shown to inhibit the ability of DHT to stimulate luciferase activity. Noted above as treatment 5, each plasticizer was tested at concentrations of 10 nM, 100 nM, 1 µM, and 10 µM to detect potential androgenic activity. The combination of plasticizer and OHF, treatment 6 above, would further test if any potential androgenic activity displayed by a plasticizer was occurring through androgen receptor activation specifically. Plasticizers were tested for anti-androgenic activity by combination of plasticizer and DHT, treatment 7 above. If a plasticizer had androgen receptor antagonist activity, then the DHT effect in the assay would be inhibited. During experimentation, complete L-15 media was removed from each well and each well subsequently received 100 µl of appropriate treatment. After 24 hours, treatment media were removed and 100 µl of treatment media were re-administered. After 24 hours, second treatment media were removed, and cell lysates were created.

To create cell lysates, all treatment media were removed from each well. Cells were then washed once with 50 µl of room temperature PBS. All PBS was removed, and plates were incubated on the lab bench for an additional minute at 45°C to allow any remaining PBS to pool at the bottom. Any residual PBS was removed and 25 µl of room temperature cell culture lysis buffer (CCLB) at 1X, diluted according to manufacturer instructions from 5X CCLB (Promega E1500), was added. After addition of CCLB, plates were incubated on the lab bench for 30 min. and visually inspected to ensure complete lysis. After complete lysis, plates were sealed with Parafilm and stored at -80°C until luminescence could be quantified.

To quantify luminescence, plates and reagents were brought to room temperature. Luciferase assay reagent was prepared by adding luciferase assay buffer to luciferase assay reagent and vortexing briefly. After mixing, 100 µl of luciferase reagent was then added to each well. Luminescence was quantified in relative light units using Biotek Synergy HT spectrophotometer following manufacturer instructions. Luminescence was measured from the bottom of each well using a 2 sec delay before the start of each measurement. Luciferin was added to 6 wells simultaneously and luminescence was quantified within one minute after addition of substrate to ensure maximal luminescence was measured. To ensure that anti-androgenic activity was not due to cell death, a trypan blue exclusion cytotoxicity test was performed. Cells were grown in 35 mm dishes and then treated for 48 hours with either 0.01% EtOH, DHT, or BPA in triplicate. Following incubation, 100 µl of 0.4% trypan blue was added directly to media (1.5 ml). Cells with intact cell membranes (viable cells) will exclude trypan blue and those without intact cell membranes (nonviable) cells, will take up trypan blue. Cells incubated for 2 min. Viable and nonviable (blue) cells were counted in three randomly selected fields of view for each 35 mm plate using the 40X objective on an inverted phase contrast microscope (Table 1).

Table 1. Viability test on MDA-kb2 cells treated with BPA as compared to positive and negative controls. There was no effect of cytotoxicity ($P = 0.61$).

Treatment	Mean % dead	Stdev % dead
0.01% EtOH	4%	1.40%
1nM DHT	3.5%	1.5%
10µM BPA	4.60%	2%

Statistical analysis for MDA-kb2 assay

Each plasticizer compound was tested twice; one additional experimental replicate was conducted for BPA. Luminescence data were analyzed using a two-way analysis of variance (ANOVA) with treatment and replicates (plates) as main effects using JMP Pro 10 software (SAS Institute, Inc., Cary, NC). Relative light units were used during analysis. A $P \leq 0.05$ indicated statistically significant effects. Data were compared using LSMEANS Tukey Kramer HSD connecting letters report. To determine androgenic activity, 1 µM OHF + 10 µM plasticizer treatment was compared to 1 µM OHF treatment. To determine anti-androgenic activity, 1 nM DHT + 10 µM plasticizer was compared to 1 nM DHT treatment. For cytotoxicity test, data were analyzed using one-way ANOVA where $P \leq 0.05$ represented statistically significant effects.

Results

Luciferase activity measured in transactivation assay using MDA-kb2 cells

MDA-kb2 cells respond in a dose dependent manner when exposed to increasing concentrations of the agonist, DHT (Fig. 1). At 0.1 nM DHT, there is a significant increase in luminescence when compared to vehicle control (EtOH). At 1 nM DHT, there is a significant increase in luminescence compared to the 0.1 nM DHT and vehicle control. The response plateaus and there is no significant increase in luminescence noted at higher concentrations of DHT (Fig. 1). These results confirm the integrity of the stably transfected cell line and are consistent with Wilson et al. (2002) who reported that 1 nM DHT gives a 5–6 fold increase in luciferase activity in the MDA-kb2 cells. The 1 nM DHT was used in the combination treatments, OHF + DHT, and plasticizer + DHT, in the screening assays as it was the lowest concentration giving a maximal response. The androgen antagonist, 1 µM OHF, does not induce

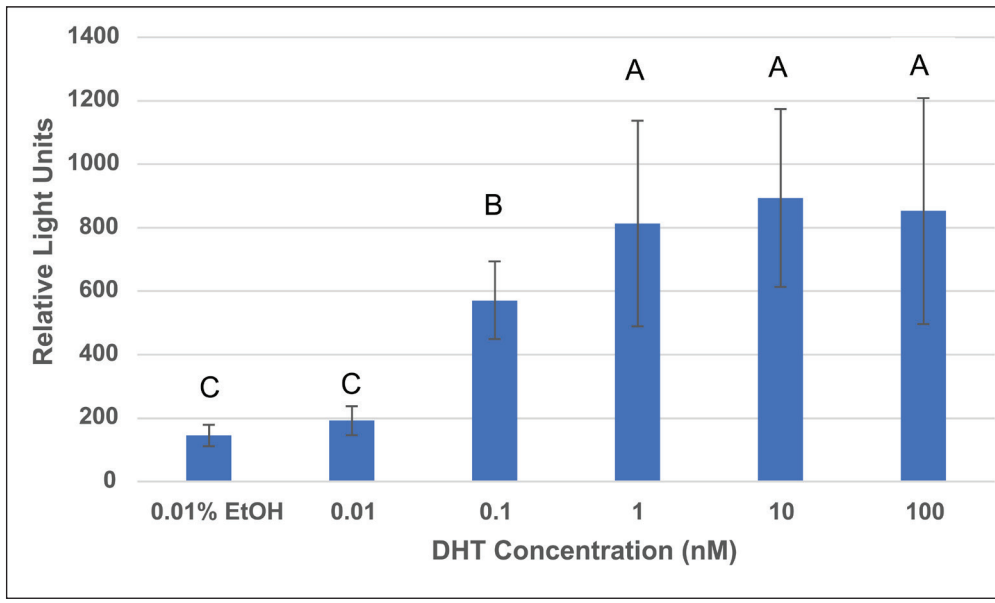

Figure 1. The effect of increasing concentrations of dihydrotestosterone (DHT) on MDA-kb2 luciferase expression in relative light units (mean ± std, n = 8). Treatments marked with different letters are statistically different *(P ≤ 0.05)*.

luciferase expression, but it does significantly inhibit DHT (1 nM) induced expression (Fig. 2). Furthermore, luciferase expression in OHF only treated cells is not significantly different from vehicle control luciferase expression indicating no background androgenic activity in the assay (Fig. 2–6). These results establish the basis for the screening assay. If a plasticizer increases the luciferase expression in the assay similarly to DHT, and the effect can be inhibited by OHF, then one can conclude that the plasticizer acts as an androgen receptor agonist. If, however, the plasticizer does not increase luciferase activity in the assay but rather inhibits the effect of DHT, then one can conclude the plasticizer acts as an androgen receptor antagonist.

Figures 3–6 show the screening assay results for the four plasticizers with the goal of determining whether any of the plasticizers have androgen receptor agonist or antagonist activity. Figure 3 shows the effect of BPA (10 µM) alone and in combination with OHF and DHT. BPA did not stimulate luciferase activity in this assay at any concentration tested. Thus, BPA does not display androgen receptor agonistic activity in the assay. This is illustrated by the lack of statistically significant difference in luminescence generated by vehicle control and BPA. However, compared to the 1 nM DHT treatment, the BPA (10 µM) + DHT (1 nM) treatment has a statistically significant decrease in luciferase activity. Therefore, BPA does possess anti-androgenic properties in this assay (Fig. 3).

Figure 4 shows bisGMA (10 µM) does not possess androgen receptor agonistic activity as there was no significant increase in luminescence over vehicle control. Comparison of the androgen receptor antagonist (1 µM OHF) and 1 µM OHF + 10 µM BisGMA show no statistically significant difference in luminescence production, further supporting that bisGMA does not possess agonistic capabilities. Comparison of androgen receptor agonist (1 nM DHT) and 1 nM DHT + 10 µM BisGMA show no statistically significant reduction in luminescence; therefore, bisGMA does not possess any anti-androgenic properties in this assay (Fig. 4).

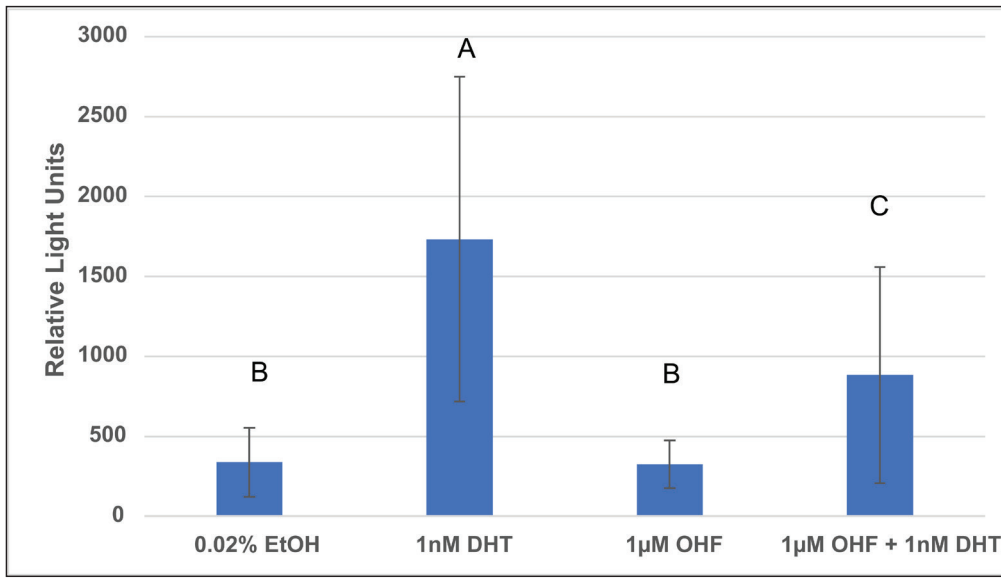

Figure 2. The effect of vehicle only (0.02% EtOH), androgen receptor agonist positive control (1 nM DHT), androgen receptor antagonist alone (1 µM OHF), and androgen receptor antagonist plus agonist (1 µM OHF + 1 nM DHT) on luciferase activity of the MDA-kb2 cells measured in Relative Light Units (mean ± std, n = 8). Treatments marked with different letters are statistically different *(P* ≤ 0.05*)*.

In Figure 5, when comparing vehicle control to bisDMA (10 µM) alone, no statistically significant difference in luminescence was observed indicating that bisDMA does not possess androgenic activity. Lack of androgen receptor agonistic activity is further supported by lack of statistically significant difference between OHF (1 µM) and 1 µM OHF + 10 µM BisDMA treatment. Comparison of treatments DHT (1 nM) and 1 nM DHT + 10 µM

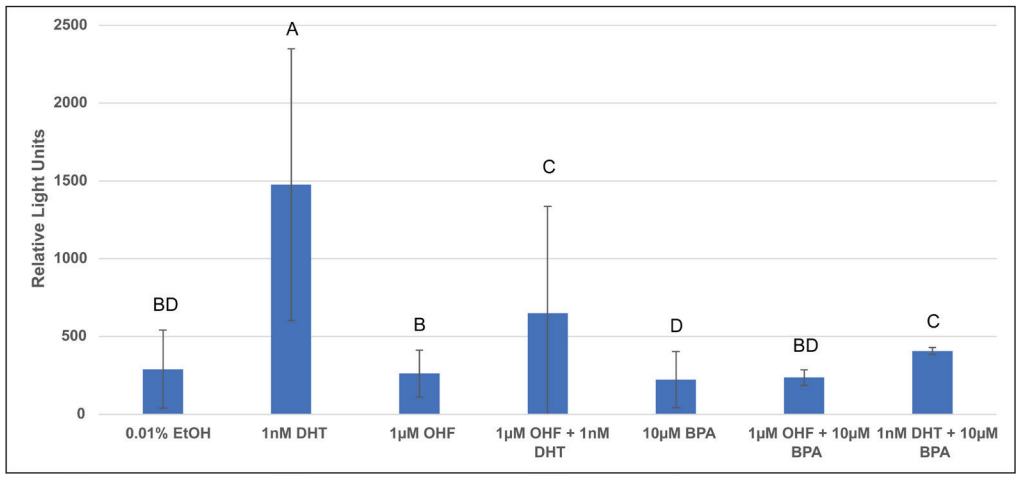

Figure 3. The effect of BPA alone (10 µM), BPA plus androgen receptor antagonist (1 µM OHF + 10 µM BPA) and BPA plus androgen receptor agonist (1 nM DHT + 10 µM BPA) on luciferase activity of MDA-kb2 cells measured in Relative Light Units (mean ± std, n = 12) as compared to the assay controls: vehicle only (0.01% EtOH), androgen receptor agonist positive control (1 nM DHT), androgen receptor antagonist alone (1 µM OHF), and androgen receptor antagonist plus agonist (1 µM OHF + 1 nM DHT). Treatments marked with different letters are statistically different ($P \leq 0.0001$).

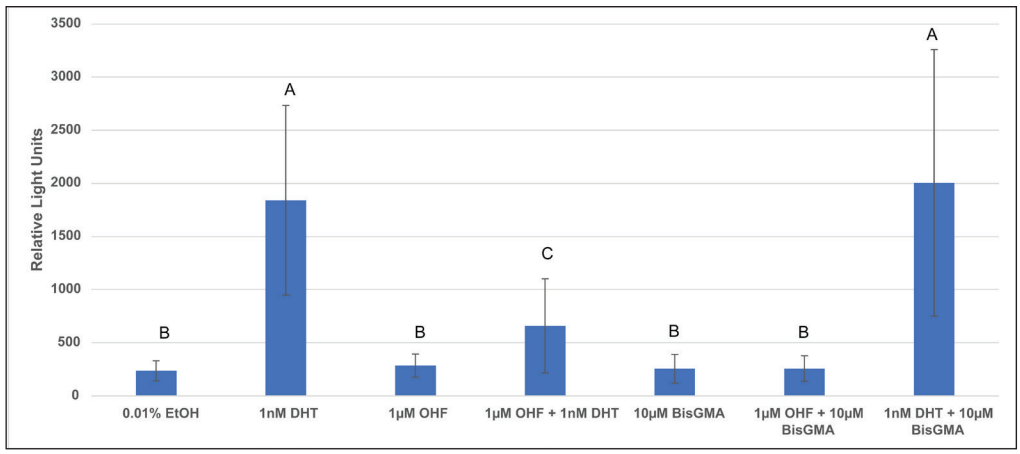

Figure 4. The effects of BisGMA on luciferase activity of MDA-kb2 cells measured in Relative Light Units (mean ± std, n = 8) as compared to vehicle only (0.01% EtOH), androgen receptor agonist positive control (1 nM DHT), androgen receptor antagonist alone (1 µM OHF), and androgen receptor antagonist plus agonist (1 µM OHF + 1 nM DHT). Treatments marked with different letters are statistically significantly different ($P \leq 0.0001$).

BisDMA show no statistically significant difference in luminescence. Therefore, bisDMA does not possess any anti-androgenic properties in this assay (Fig. 5).

Similar to results seen with BPA, bisGMA, and bisDMA, BADGE (10 µM) showed no agonistic activity when compared to vehicle control. This is further supported by a lack of statistically significant difference in luminescence production when comparing OHF (1 µM) and 1 µM OHF + 10 µM BADGE. Comparison of treatments 1 nM DHT and 1 nM DHT + 10 µM BADGE show no statistically significant difference. Therefore, BADGE does not possess any anti-androgenic properties in this assay (Fig. 6).

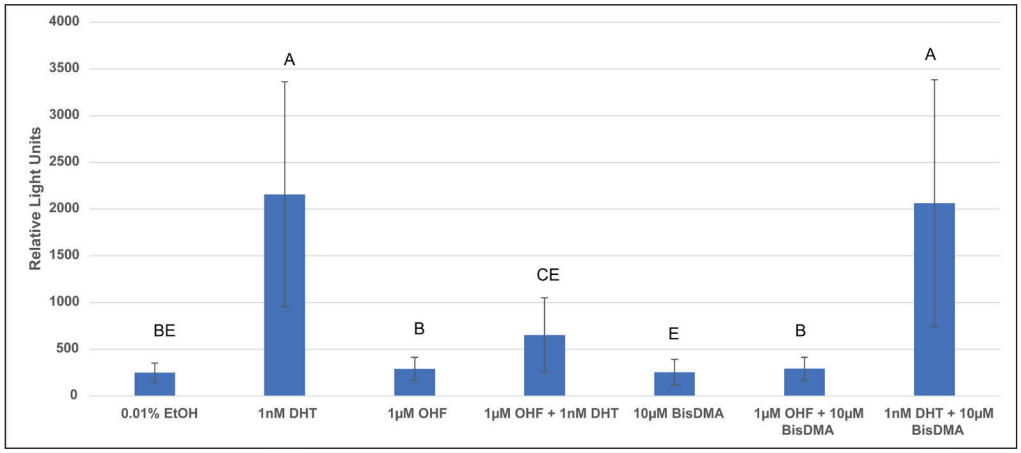

Figure 5. The effects of BisDMA on luciferase activity of MDA-kb2 cells measured in Relative Light Units (mean ± std, n = 8) as compared to vehicle only (0.01% EtOH), androgen receptor agonist (1 nM DHT), androgen receptor antagonist alone (1 µM OHF), and androgen receptor antagonist plus agonist (1 µM OHF + 1 nM DHT). Treatments marked with different letters are statistically significantly different ($P \leq 0.0001$).

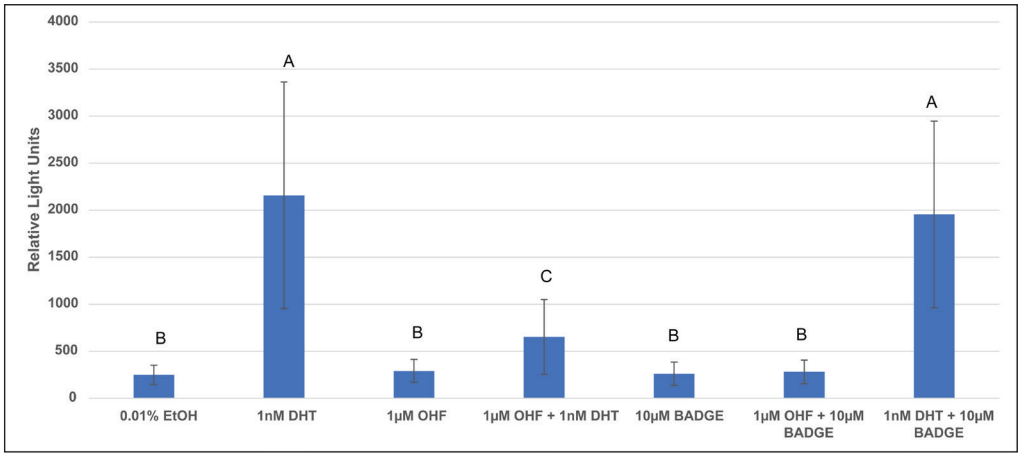

Figure 6. The effects of BADGE on luciferase activity of MDA-kb2 cells measured in Relative Light Units (mean ± std, n = 8) as compared to vehicle only (0.01% EtOH), androgen receptor agonist positive control (1 nM DHT), androgen receptor antagonist alone (1 µM OHF), and androgen receptor antagonist plus agonist (1 µM OHF + 1 nM DHT). Treatments marked with different letters are statistically significantly different ($P \leq 0.0001$).

Discussion

The goal of this research was to evaluate commonly used chemicals for androgenic or anti-androgenic properties. Bisphenol A, bisGMA, bisDMA, and BADGE were screened with a stably transfected breast cancer cell line, MDA-kb2. The results of this study show that bisGMA, bisDMA, and BADGE do not possess androgenic or anti-androgenic properties in the MDA-kb2 assay. Bisphenol A does show statistically significant anti-androgenic properties.

Bisphenol A is produced in quantities greater than one million pounds per year classifying it as a high production volume (HPV) chemical used in manufacturing of plastics (EPA 2010). Yearly environmental release exceeds one million pounds per year with most human exposure attributed to food packaging (EPA 2010). Bisphenol A is a known endocrine disruptor whose potentially deleterious effects may be produced through binding at receptors such as estrogen receptors (Murray et al. 2007), thyroid hormone receptors, and androgen receptors (Acconcia et al. 2015). Bisphenol A did show strong antiandrogenic properties in MDA-kb2 cells. Our results show that BPA will bind to the AR and act as an AR antagonist. These results support studies performed by Bonefeld-Jørgensen et al. (2007) where BPA bound to the AR in the Chinese hamster ovary (CHO) cell line, and further support Lee et al. (2003) where BPA bound to the AR in a yeast two-hybrid system and in a transfected HeLa cell line. Bisphenol A can competitively bind to the AR and thus inhibits binding of endogenous hormones and expression of androgen regulated genes (Lee et al. 2003).

Bisphenol A glycidyl dimethacrylate based resins are most commonly used to make dental sealants (Fleisch et al. 2010). However, to our knowledge, there are few studies that have elucidated the ability of bisGMA to act as an endocrine disruptor. Bisphenol A glycidyl dimethacrylate did not stimulate the AR in our study. Therefore, it does not appear to possess any androgenic or antiandrogenic properties in MDA-kb2 cells. It is worth noting that, in a previous *in vivo* study, exposure to bisGMA led to a decrease in fertility in male mice (Al-Hiyasat and Darmani 2005). However, this could be caused through contamination with the parent compound, BPA (Al-Hiyasat and Darmani 2005). Interestingly, bisGMA has been identified by the EPA as possessing potential endocrine disrupting capabilities when screened by their ToxCast/Tox21 *in vitro* screening program by testing positive in 3 out of 13 estrogen receptors assays and positive in 6 out of 9 androgen receptor assays (EPA 2023b) warranting future studies.

Bisphenol A dimethacrylate is capable of converting to BPA via hydrolysis when exposed to high pH and saliva (Schmalz et al. 1999). It has been shown to act as an endocrine disruptor through multiple pathways and exert estrogenic activity, androgen antagonist activity and act as an aromatase inhibitor (Bonefeld-Jørgensen et al. 2007). Bonefeld-Jørgensen et al. (2007) tested for agonist and antagonist activity in a CHO cell line that had been stably transfected with human AR and the MMTV-LUC reporter plasmid (Bonefeld-Jørgensen et al. 2007). Their results found the lowest effect concentration was 2.5×10^{-6} M and maximum effect concentration was 2×10^{-5} M (Bonefeld-Jørgensen et al. 2007). While our results do not show bisDMA as possessing androgenic or antiandrogenic properties, this result could be due to differences in transfected cell lines or due to differences in treatment conditions tested. While bisDMA is used less frequently in dental materials compared to bisGMA (Löfroth et al. 2019), Schmalz et al. (1999) found one hundred percent conversion of bisDMA to BPA when exposed to high pH (11) and exposure to esterases from oral microbes (Nakamura and Slots 1983), making it a compound of interest. Our tested concentration was 1 x 10^{-5} M, within range illustrated by Bonefeld-Jørgensen et al. (2007). Therefore,

it is unlikely that the difference is due to concentration. However, treatment conditions in our study had lower pH (~7) and were without esterases, possibly accounting for the lack of antagonistic activity. Bisphenol A dimethacrylate's potential to convert to BPA potentially eliciting endocrine disrupting effects (Schmalz et al. 1999) warrants future studies.

Similar to BPA, BADGE is also an HPV chemical used in manufacturing of epoxy resins, paints, and as a coating on food containers (Chamorro-García et al. 2012). Our results show that BADGE did not bind to AR and thus stimulate luciferase production. This leads to the conclusion that BADGE does not possess androgenic or antiandrogenic properties in the MDA-kb2 assay. This result is supported by Satoh et al. (2004) where BADGE did not bind to AR in a stably transfected CHO cell line. However, Satoh et al. (2004) did show that chlorinated derivates of BADGE had androgen antagonist activity in their assay. BADGE is known to be a reactive chemical in the environment resulting in numerous derivatives including BPA (Wang et al. 2021). In our assay, however, the concentration of BADGE used, and the conditions of treatment did not result in any detectable agonistic or antagonistic activity suggesting that extensive chemical change did not occur. Kleinstreuer et al. (2017) integrated 11 high throughput screening assays from the EPA's ToxCast/Tox21 database into a computational model to identify potential androgen receptor agonists or antagonists. BADGE was found to be a potential androgen antagonist in this analysis in contrast to the present study. The analysis by Kleinstreuer et al. (2017) did not indicate significant androgen agonist potential for BADGE, which is in agreement with our data. EPA reports that BADGE is a potential endocrine disruptor as it showed positive interaction in 3 out of 21 estrogen receptor assays and 5 out of 15 androgen receptor assays (EPA 2023a). Thus, it is not surprising that we did not see a positive result in our assay for BADGE but does not eliminate it as a chemical of endocrine concern.

Endocrine disrupting compounds are found in consumer goods (Jobling et al. 1995, La Merrill et al. 2020, Plotan et al. 2013, Schlumpf et al. 2001) and in the environment (Jobling et al. 1995, Wang et al. 2013, Werner et al. 2010). It has been shown in numerous studies that these compounds can enter the body and cause detrimental effects (Diamanti-Kandarakis et al. 2009, La Merrill et al. 2020). Very little data is available on bisGMA as an endocrine disruptor and while it did not bind to the AR receptor in this assay, further studies are warranted. To our knowledge, this transactivation assay has never been used to screen these plasticizers and results of this research support that BPA is able to bind to the androgen receptor and has the potential to inhibit downstream effects.

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