

March 2023

## The Effects of Tert-butyl Hydroquinone (TBHQ) on Estrogen Receptor Alpha (ER $\alpha$ ) and Tumor Suppressor Protein p53 in T-47D and MCF-7 Breast Cancer Cell Lines

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### Recommended Citation

Pfiffner, Samantha E.; Farrar, Katelyn; Hallman, Erin; Whittaker, Kaylin; Zanib, Aisha; and Dinda, Sumi Ph.D., "The Effects of Tert-butyl Hydroquinone (TBHQ) on Estrogen Receptor Alpha (ER $\alpha$ ) and Tumor Suppressor Protein p53 in T-47D and MCF-7 Breast Cancer Cell Lines" (2023). *Medical Student Research Symposium*. 270.

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## **The Effects of Tert-butyl Hydroquinone (TBHQ) on Estrogen Receptor Alpha (ER $\alpha$ ) and Tumor Suppressor Protein p53 in T-47D and MCF-7 Breast Cancer Cell Lines**

Tert-butyl hydroquinone (TBHQ) is an aromatic compound that is commonly used as a preservative in processed food to prevent rancidity and lengthen shelf life. TBHQ is known to act as an antioxidant by protecting cells from radical oxygen species and thus preventing DNA damage. Although previous studies have found TBHQ to cause cancer cell death at high concentrations, they have also contrastingly found TBHQ, when studied in animal models, to enhance carcinogenic effects. However, the effect of TBHQ on breast cancer has not been thoroughly explored. With the prevalence of breast cancer and the wide use of TBHQ in processed food items, it is imperative that we explore their possible relationship. This study examined the effects of TBHQ, alone and in combination with hormones and anti-hormones, on ER $\alpha$  and p53 expression in both MCF-7 and T-47D breast cancer cell lines. To ensure treatment conditions without the presence of endogenous steroids or growth factors, the cells were cultured with a 5% charcoal-stripped fetal bovine serum (FBS) for six days. Western blot analysis revealed alterations in the expression of ER $\alpha$  and p53 protein levels after 24 hours of treatment with varying concentrations of TBHQ (0.005 to 1 mM). A concentration-dependent decrease of ER $\alpha$  protein levels was observed in both cell lines, with a 49% reduction occurring with 100  $\mu$ M TBHQ as compared to the control. P53 levels portray a continued increase of expression through concentrations of TBHQ (0.005 to 1 mM), found similarly in both cell lines. To gain further insight into possible similarities between BPS and other known effectors of ER $\alpha$ , the optimal concentration of TBHQ (100  $\mu$ M) was used in combination with hormones and anti-hormones. Down-regulation of ER $\alpha$  protein levels was observed after 24-hour co-treatment of T-47D & MCF-7 cells with a combination of TBHQ and E<sub>2</sub>. Antiestrogen ICI with TBHQ showed a significant down-regulation as compared to TBHQ alone, and TBHQ with TAM portrayed no significant differences. A similar trend in the effects on p53 expression was depicted in T-47D and MCF-7 cells. Image cytometric analysis with propidium iodide staining was utilized to quantify cell values and viability changes to further portray the effects of TBHQ on T-47D and MCF-7 cellular growth. The viability assay shows a biphasic effect with increasing concentrations of TBHQ, with a maximum decrease in proliferation seen at a concentration of 100  $\mu$ M TBHQ. TBHQ alone and in combination with E<sub>2</sub> and antiestrogens showed a decreased proliferation compared to the control in T-47D cells. However, cytolocalization of ER $\alpha$  upon treatment with estradiol and TBHQ remained unaltered. Our studies offer a unique perspective on the effects of TBHQ on two different breast cancer cell lines and provide valuable insight for further exploration of the mechanism of action of TBHQ on tumor suppressor proteins and steroid receptors.

