

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 $_2$ . 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 $_2$  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 gene and steroid receptors.

## Endocrine Disruption

### ENDOCRINE DISRUPTING COMPOUNDS: MECHANISMS OF ACTION AND CLINICAL IMPLICATIONS

#### *Thyroid Gland and Male Reproductive Anomalies Among Fuel Handlers in Gampaha District, Sri Lanka*

Dulani Chathurika Kottahachchi,  
Consultant Endocrinologist MD(Col), MRCP(UK)<sup>1</sup>,  
Tania Warnakularuriya, MPhil<sup>1</sup>, Deepthi De Silva, MRCP<sup>1</sup>,  
Kushan Madagoda, MD<sup>1</sup>, Dilesha Wadasinghe, MD<sup>1</sup>,  
BKPTI Dayanath, MD<sup>2</sup>, Sajith Siyambalapitiya, MD<sup>2</sup>,  
Niranga Devanarayana, PhD<sup>1</sup>.

<sup>1</sup>University of Kelaniya, Sri Lanka, Kelaniya, Sri Lanka, <sup>2</sup>Colombo North Teaching Hospital, Sri Lanka, Ragama, Sri Lanka.

**Introduction:** Fuel handlers at petrol stations are continuously exposed to organic solvents from fuel and vehicle emissions. Endocrine disrupting chemicals (EDC) are present in fuel, which are harmful to endocrine organs. Thyroiditis and hypogonadism are reported among fuel handlers. Thyroid gland and male reproductive function anomalies were investigated among fuel handlers in the Gampaha district of Sri Lanka. **Method:** 43 were recruited from 6 fuel stations in the Gampaha district for the study and 28 age matched male workers who were not exposed to fuel in an occupational setting were recruited as controls. Thyroid gland was examined clinically and TSH, free T4, FSH, LH and Testosterone were done on all the participants. TPO antibody and a thyroid scan was done on the fuel handlers. **Results:** Median (IQR) age was 38 years (27-46 years). The mean TSH value was 1.62 IU/mL (1.15-2.35) vs 1.33 IU/mL (0.83-1.79) respectively in study and control populations with significantly higher levels in the study population ( $p=0.023$ ). The median (IQR) TSH value above the reference range was identified in 7% of fuel handlers and all controls were within the normal range, while 16.9% of fuel handlers had a derangement in the TPO levels. On examination, only one control had a small goiter but his T4 and TSH levels were normal. On ultrasound thyroid scans, benign nodules were seen in 2 fuel handlers. TPO levels did not correlate with the TSH levels among the fuel handlers ( $r=-0.078$ ,  $p=0.652$ ). Inability to sustain an erection was reported by 35.5% fuel handlers which was significantly higher than controls who reported 5.6% ( $p=0.019$ ). Premature ejaculation was reported by 27.9% of fuel handlers which was significantly higher than controls ( $p=0.023$ ). The testosterone levels were significantly higher among fuel handlers compared to controls ( $p=0.048$ ). The FSH and LH levels positively correlated with each other as expected in each subgroup and the total population ( $p<0.005$ ). The TSH levels significantly negatively correlated with the testosterone levels among the fuel handlers. ( $r=-0.338$ ,  $p=0.023$ ). When the fuel handlers with premature ejaculation was considered the FSH, LH, Testosterone levels were not significantly different between the two groups, however the duration of employment was significantly longer among those reporting premature ejaculation. ( $p=0.024$ ). **Conclusion:** There are thyroid and reproductive abnormalities among those exposed to fuel in an occupational setting. Disturbances to sexual functions may also be related to alteration of autonomic functions. Limiting exposure to fuel vapor will eliminate these detrimental effects and we propose self-service fuel pumps to be the best alternative to avoid occupational health hazards among fuel handlers.

## Endocrine Disruption

### ENDOCRINE DISRUPTING COMPOUNDS: MECHANISMS OF ACTION AND CLINICAL IMPLICATIONS

#### *Tildacerfont for the Treatment of Patients With Classic Congenital Adrenal Hyperplasia: Results From a 12-Week Phase 2 Clinical Trial in Adults With Classic CAH*