

# Determining the Effect of Triclosan on the Growth of Cancer Cells

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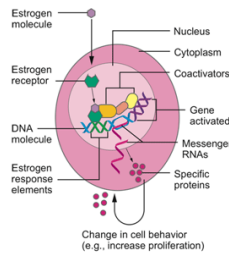
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## INTRODUCTION

17  $\beta$ -estradiol ( $E_2$ ) is an ovarian steroid and regulates the differentiation, growth, and function of various body tissues.  $E_2$  affects target tissues by binding to an estrogen receptor (ER), a protein found in the nucleus of target cells. Such interaction influences gene transcription and the expression of proteins that change the behavior of a target cell.



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$E_2$  effects are generally beneficial, however, estrogen exposure is correlated with higher incidence of cancer in tissues where estrogens stimulates growth (e.g. breast and uterus). Moreover in cancers where ER is expressed,  $E_2$  can stimulate the growth of tumor cells.

**Environmental estrogens** may be naturally occurring (e.g. phytoestrogens) or man-made (e.g., xenoestrogens). They mimic the effects of estrogen by binding to ERs and modeling similar mechanisms of action. **Triclosan** is an antibacterial and antifungal agent included as an active ingredient in many products such as antibacterial soaps and personal care products. Triclosan has also been recently identified as a xenoestrogen. **The hypothesis to be tested is that triclosan stimulates proliferation of tumor cells that express ERs.**



## MATERIALS AND METHODS

✓ **Proliferation assay**—Breast cancer cells (MCF-7) were cultured in nutritive media at 95%  $O_2$ /5%  $CO_2$  at 37C. On the day of the experiment, cells were plated to a density of 1.25 million cells/mL and treated for an hour with a range of doses of triclosan. Tetrazolium MTT, a salt, was added to the culture media. The reduction of this salt by the coenzymes NADH and NADPH will turn the yellow salt purple in color. The expression and subsequent activity of these coenzymes will increase when cells grow. Light absorption at 570 $\lambda$  was measured using a standard spectrophotometer. Thus, color intensity represents an accurate index of cellular proliferation.

✓ **Western Blot**—MCF-7 cells were treated for two hours with 10<sup>-6</sup>M triclosan. Cellular proteins were extracted, solubilized, and separated using gel electrophoresis. The proteins were transferred to a nitrocellulose membrane. Expression of estrogen receptor subtypes  $\alpha$  and  $\beta$  was visualized following incubation with antibodies specific to ER protein.

## CURRENT RESULTS

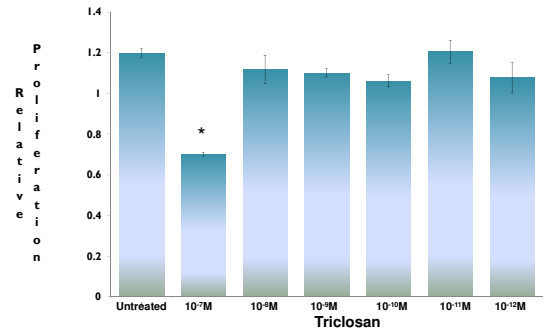


Figure 1. Immortalized MCF-7 breast cancer cells were treated with triclosan for 16 hours. Cell growth was determined using a commercially available colorimetric assay. A representative graph is shown.

• **In response to triclosan treatment, the proliferation of cancer cells decreased.** The highest concentration of triclosan exhibited the most robust suppression of growth as compared to untreated cells, but not vehicle treated cells (not shown). Triclosan has been shown to have stimulatory effects on uterine growth (Toxicol Sci, 117(1):43-53), so these results were surprising. This leads us to believe that the timing and dosage of estrogenic compounds is important in assessing affects of these chemicals in cancer cells. We may need to expose cells to triclosan in a more cyclic pattern, rather than an acute one. The highest dose of triclosan used fell within the "in use" range. That is the highest dose of triclosan used in these studies approached those concentrations of what are typically found in the commercial products that contain triclosan as an active ingredient. In future studies, pulse the cells with additional doses of triclosan (as opposed to an acute treatment).

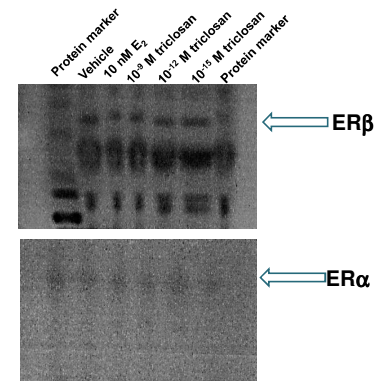


Figure 2. The expression of the two isoforms of the estrogen receptor were measured in MCF-7 cells that were treated with increasing doses of triclosan for 16 hours. Cell lysates were collected and proteins separated by gel electrophoresis. Blots were probed with MC-20 antibody for ER $\alpha$ . The same blot was re-probed with 063-629 antibody for ER $\beta$ .

• **There was no change in ER $\alpha$  expression, however there an increase in ER $\beta$  expression of the protein when cells were treated with lower doses of triclosan.** The ER $\alpha$  antagonist ICI 132,780 did not reverse the triclosan-induced decrease in cellular proliferation (not shown). This indicates ER $\alpha$ 's lack of involvement in triclosan-induced decreases in cellular proliferation. Thus, triclosan's effects may be mediated by ER $\beta$ . Future studies will examine the ability of triclosan to regulate transcriptional responses in ER $\beta$  sensitive genes.

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