

Xenoestrogens from Household Plastics Bind Estrogen Receptors and Affect Cell Proliferation

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Xenoestrogens are “foreign” chemicals or compounds that interact with estrogen receptors as either agonists or antagonists to interfere with endocrine system function. Xenoestrogens include phytoestrogens, pesticides, industrial by-products and synthetic estrogens. In this study, consumer plasticware, including baby bottles and food storage containers, were found to release xenoestrogenic compounds that bound to ER α and ER β and affected cell proliferation.

Introduction

Environmental endocrine-disrupting chemicals (EDC) affect cellular function by targeting receptors, drug metabolizing enzymes and transcriptional and translational machinery¹⁻³. Xenoestrogens, a type of EDC, include phytoestrogens, mycoestrogens, pesticides, industrial by-products and synthetic estrogens¹⁻³. These compounds may directly or indirectly affect estrogen receptor alpha and beta (ER α and ER β) signaling¹⁻⁵. Indirect mechanisms of activation or inactivation may involve coregulators or components of signal transduction cascades that crosstalk with estrogen receptor pathways⁴⁻⁶. Estrogen signaling pathways play an important role in reproduction, bone formation and maintenance, and cerebrovascular function, therefore identifying sources of EDC exposure is important to understanding epidemiological trends in endocrine-related diseases¹⁻⁴. Several xenoestrogenic compounds have been identified in plastics manufacturing, including bisphenol A (BPA), phthalates, biphenyls and several alkylphenols¹⁻⁴. In this study, receptor- and cell-based screening methods were used to determine if microwaving plastic food and laboratory containers, conditions typical of everyday use, causes the release of xenoestrogens.

Methods

Polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyvinylchloride (PVC) plastic containers were purchased from local retailers in South Carolina (baby bottles and foods storage containers). Glass Quorpak bottles and all other supplies were obtained from VWR (West Chester, PA). The containers were grouped into non-microwaved (NM) and microwaved (MW) treatments (see Figure 1). Deionized H₂O (dH₂O) was placed into each container to cover approximately ¼ of the surface area of each container. NM containers were the negative control. MW containers were microwaved at a maximum power (1200 watts) for 90 sec every other day for 30 days. Every 6 days, the water was transferred into glass containers and fresh dH₂O water was placed into each MW container. After 30 days, pooled water samples from each container were extracted for xenoestrogens using C18 column chromatography and then run in the ER α and ER β binding assays and a MTS cell proliferation assay². For the MTS assay, a 96-well plate was seeded with 5000 T47D cells per well in RPMI media containing serum. 24 hours later, the media was replaced with RPMI media without serum. After 24 hours, water samples from the treatment groups were added to cell wells with 200 μ l of RPMI without serum. The negative control was 1 μ l DMSO and the positive control was 2 μ l of 1 x 10⁻⁸ M estradiol-17 β (E₂). After 48 hours, the media was removed and



Figure 1. Photo of containers. Container 1- PE storage container; 2- PP storage container; 3- PP food storage container; 4- unknown baby bottle; 5- unknown baby bottle; 6- PC baby bottle; 7- unknown baby bottle liner; 8- PE food storage bag; 9- PP food storage container; 10- polyethylene dish; 11- polyethylene dish; 12- PE drink container.

100 μ l of RPMI was added to each well plus 25 μ l of MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfo-phenyl)-H-tetrazolium). Plates were incubated at 37 $^{\circ}$ C for 4 hr and read on a dual-wavelength spectrophotometer at 490 nm and 655 nm to assess cell proliferation. The data were analyzed by ANOVA using SAS (Cary, NC).

Results

Results from receptor binding and cell proliferation assays are presented in Table 1 and Figure 2. In the ER β assay, seven MW and three NM containers leached xenoestrogens. The three NM containers that leached xenoestrogenic chemicals were a plastic baby bottle (container 4), a PE bag (container 8) and a PE food storage dish (container 9). Leachate from containers 8 and 9 in both NM and MW groups displayed estrogen receptor binding activity. Only one MW container (container 1) a PE food storage dish, had activity in the ER α assay and also had the greatest level of binding in the ER β assay. None of the NM samples registered quantifiable activity in the ER α assay. A significant difference was found between individual containers as well as pools of all MW and NM groups (2.2 ng/ml vs. 1.0 ng/ml, P < 0.05, n=22). Results from the MTS assay indicate that xenoestrogenic compounds released from plastics after microwaving can increase breast cancer cell proliferation.

Discussion

The results from ER binding assays indicated that xenoestrogenic compounds were released from some plastic containers and that ER β displayed greater affinity for these xenoestrogens than ER α .

Table 1. ER α and ER β Binding Assays and MTS Cell Proliferation Assay

Container	ER α		ER β		MTS (%)
	MW	NM	MW	NM	
1	140	0	630	0	23
2	0	0	0	0	0
3	0	0	40	0	38
4	0	0	2	140	4
5	0	0	0	0	1
6	0	0	0	0	5
7	0	0	0	0	0
8	0	0	40	50	0
9	0	0	30	40	34
10	0	0	280	0	87
11	0	0	20	0	0
12	0	0	10	0	0
Controls					
Glass	0		0		0
Negative	0		0		0
Positive	1290		2160		100

Note: Results for ER α and ER β are expressed as estrogen binding equivalents (EBE). EBE is determined by calculating the displacement of labeled E₂ by extracts and standards from a E₂ standard curve. The positive control in the receptor assays was 5 ng diethylstilbestrol. Results from the MTS cell proliferation assay are expressed as a percentage of the positive control (1 x 10⁻⁸ M E₂).

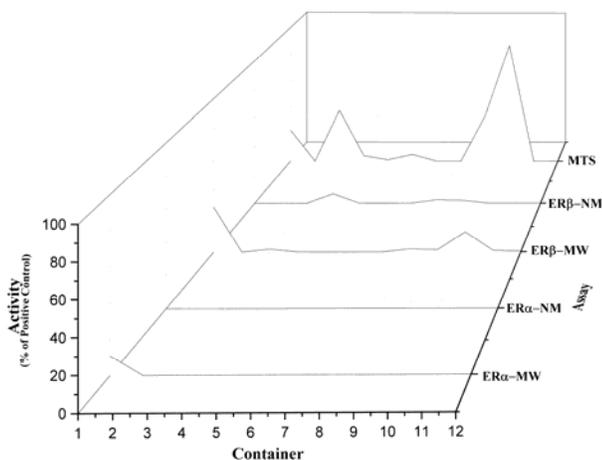


Figure 2. Results for microwaved (MW) and nonmicrowaved (NM) containers in MTS, ER α and ER β receptor assays expressed as a percentage of estrogen positive control (1 x 10⁻⁸ M E₂).

Container 1 released chemicals capable of binding to ER α and ER β and increasing breast cancer cell proliferation. Interestingly, some containers released xenoestrogenic compounds from both MW and NM groups; however, only MW samples increased breast cancer cell proliferation. A purple leachate was visible from containers 5 and 10 after column concentration. Container 10 had the highest amount of cell proliferation among plastic containers and it is not known whether the purple leachate interfered with quantification in the ER binding assays or stimulated cell proliferation through an ER-independent mechanism. In summary, some consumer plastics do release

xenoestrogenic compounds that bind to both ER α and ER β , display a greater affinity for ER β than ER α and increase breast cancer cell proliferation. Further studies are warranted to investigate whether phthalate- and BPA-free products have reduced xenoestrogenic and cancer-stimulating activity⁵.

Notes and References

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1. Sonnenschein C, Soto AM: An updated review of environmental estrogen and androgen mimics and antagonists. *J Steroid Biochem Mol Biol* **65**: 143-150, 1998.
2. Gray SL, Lackey BR, Tate PL, Riley MB, Camper ND: Mycotoxins in root extracts of American and Asian ginseng bind estrogen receptors alpha and beta. *Exp Biol Med* **229**: 560-568, 2004.
3. Vom Saal FS, Hughes C: An extensive new literature concerning low-dose effects of bisphenol A shows the need for a new risk assessment. *Environ Health Perspect* **113**: 926-933, 2005.
4. Sultan C, Balaguer P, Terouanne B, Georget V, Paris F, Jeandel C, Lumbroso S, Nicolas J: Environmental xenoestrogens, antiandrogens and disorders of male sexual differentiation. *Mol Cell Endocrinol* **178**: 99-105, 2001.
5. Roy P, Pereira BM: A treatise on hazards of endocrine disruptors and tool to evaluate them. *Indian J Exp Biol* **43**: 975-992, 2005.
6. Tsai KS, Yang RS, Liu SH: Benzo[a]pyrene regulates osteoblast proliferation through an estrogen receptor-related cyclooxygenase-2 pathway. *Chem Res Toxicol* **17**: 679-684, 2004.