The effect of pre-pubertal exposure of Benzyl Butyl Phthalate (BBP) on the rat mammary gland.

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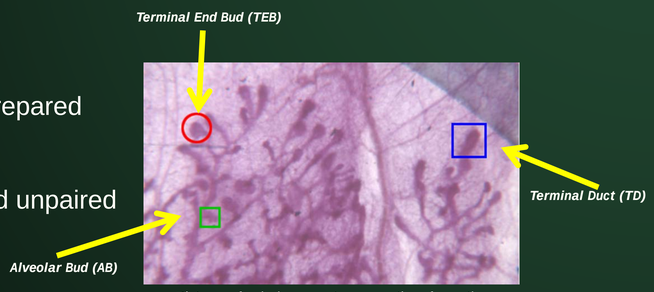
Abstract

Benzyl Butyl Phthalate (BBP) is an identified carcinogen that has been linked to breast cancer through studies conducted on Sprague Dawley rats. BBP is a commonly used plasticizer found in toys, PVC, cosmetics, and carpeting. This phthalate is absorbed into the body through inhalation, dermal and oral exposure and accumulates in the fatty tissues of the body. BBP can also be passed from mother to offspring through the placenta and during lactation. BBP is capable of binding to estrogen hormone receptors. The purpose of this study was to examine the effects of BBP on the mammary glands of Sprague Dawley rats. The animals were exposed to BBP through mother's lactation at a concentration of 500mg/kg of body weight or equivalent volume of sesame oil (control group). At 50 days of age the animals were sacrificed and the mammary gland submitted to morphological study through whole mount preparation. The number of terminal end buds (TEBs) were counted in the abdominal mammary glands for both groups (exposed to BBP and control). The effects of the BBP were further analyzed by using cDNA-microarrays to compare the gene expression profile between the experimental and the control rats. There were not morphological differences between the control and experimental group. However, there were 80 genes significantly different in the mammary gland of BBP exposed animals compared with the matching control. The genes differentially expressed are involved in controlling the circadian rhythm such as Dopa decarboxylase (Ddc), organ development, androgen and estrogen receptors and apoptosis. These genes potentially play a role in the development of cancer cells. In conclusion, pre-pubertal exposure to BBP did not alter the mammary gland structure, but it modifies their genomic profile.

Methodology

Structural Analysis Procedures

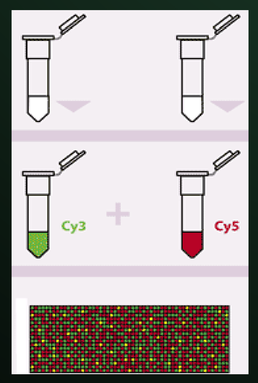
–Whole mounts of the rat mammary gland were prepared using Alum Carmine staining
–Terminal End Buds (TEBs) were counted
–The resulting data were analyzed using two-tailed unpaired t-test for statistical comparison



Picture of whole mount preparation from the mammary gland of the female 50 days old rat.

Microarray Analysis Procedures

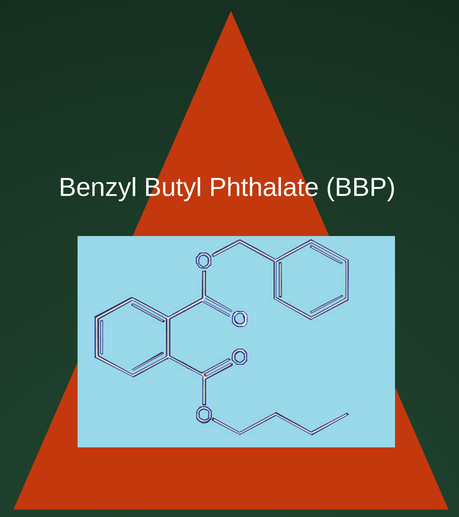
–Comparison of BBP experimental group to control group (SO) showed whether a gene was over-expressed or under-expressed by BBP.
–Genes with p<0.001 were considered differentially expressed
–A literature search was done to determine which of these genes have documented links to mammary carcinogenesis



Picture of Two Color Agilent Microarray

Results

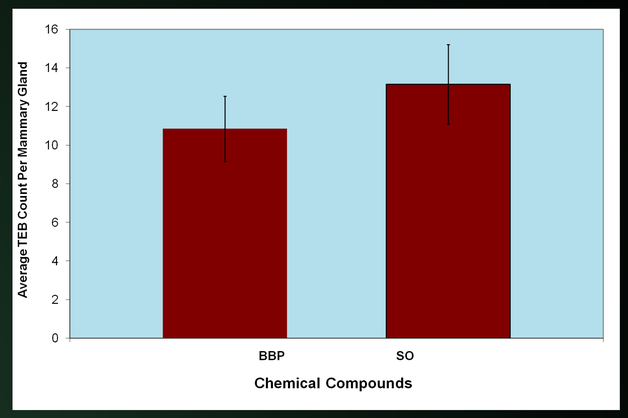
Structural Analysis



BBP Group: 500 mg of BBP/kg of body weight

Control Group (SO): equivalent volume of sesame oil

Graph shows the average number of terminal end buds per group, the error bars represent the standard error.



The BBP cohort did not exhibit notable structural differences between the experimental and control groups.

Cohort	BBP Cohort	
Group	BBP	SO
Mean TEB Count	10.9	13.2
Standard Deviation	5.4	6.5
Standard Error	1.7	2.0
Range	2-18	5-24
p-value (t-test)	0.42	

Genetic Expression Analysis

Statistical analysis was used to identify genes deregulated by BBP treatment

BBP
Tjp1: cell growth
Nrg1: inactivates tumor suppressor activity
Nrip1: estrogen receptor binding
Ddc : clock gene
Ddr1 , Htr1f : hormone receptor activity
Tap1 : androgen expression and tumor response
Pawr : apoptosis
CBL : androgen-dependent apoptosis

Microarray Analysis Genetic Expression Results

		BBP
Number of Genes		79
Number of Identified Genes		36
Number Upregulated		40
Number Downregulated		39
Genes Studied Further	**Gene**	**Log Ratio**
	Nrip1	-2.5
	Tap1	-1.8
	CBL	-1.5
	Htr1f	-1.4
	Pawr	-1.0
	Nrg1	1.0
	Ddr	1.2
	Tjp1	1.5
	Ddc	1.9
Most Upregulated	*Hcfc1*	1.9
Most Downregulated	*Gad1*	-3.6

Conclusions

Morphological analysis in our study found that the mammary glands of rats treated with BBP did not show substantial changes in overall TEB count.

In the cDNA microarray analysis, there were several differentially expressed genes in BBP-treated rats that may encourage an hospitable environment to the development of breast cancer. Some of these genes were Nrg1, that was upregulated and it inactivates tumor suppressor activity, Dcd, that is a clock gene, and other genes involved in apoptosis (Pawr and CBL).

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