

## **Neonatal and Prepubertal Exposure to the Estrogenic Xenobiotic N-Butyl Benzyl Phthalate (BBP) Alters the Gene Expression Pattern of the Rat Mammary Gland.**

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Xenoestrogens like n-butyl benzyl phthalate (BBP), a phthalic ester that is widely used as a plasticizer, act as endocrine disruptors, playing an important potential role in the development and physiology of the mammary gland. To further define the effects of exposure of this xenobiotic on mammary gland development and gene expression pattern during critical stages of development and differentiation we analyzed the influence of neonatal and prepubertal exposure to BBP on those parameters. Pregnant Sprague Dawley rats were bred and maintained on a phytoestrogen-free diet. After delivery the nursing dams were daily gavaged with sesame oil (controls) or 500 mg BBP/kg body weight for exposing the offspring during the lactational period, from birth, day 1, to weaning, 21 days of life. Ten female litters per group were euthanized when they reached the ages of 21, 35, 50 or 100 days. Abdominal mammary glands were excised for RNA extraction, quality verification and pooling for reduction to three replicas per group. Samples were fluorescently labeled for hybridization to Agilent 60-mer oligo microarrays containing 22,000 features. Image analysis of the scanned microarrays was carried out using Feature Extraction and ImaGene softwares, and data were analyzed using GeneSight software and normalized by Lowess method. To determine the genes with significant expression changes, confidence analyses at  $p < 0.05$  were performed. At 21 days of age, mammary glands of rats exposed to BBP showed downregulation of GAD1 and upregulation of 515 genes, 133 of which were known and included hormone related proteins (hydroxysteroid dehydrogenase, lutropin, parathyroid-like peptide, GHRH); adhesion molecules (rCdh8, tenascin R), and many regulating cell proliferation and differentiation genes, such as FABP3, growth factors (EGF, Insl6, PDGF), the tumor suppressors WT1, or other transcription factors (FKHR, AHR, HFH1, HNF3a, TCF2). In the 35 day-old rats there were 2 downregulated and 4 upregulated genes that were unknown. In the 50 day-old rat the mammary gland had 14 genes that were downregulated (8 known and 6 unknown) and 25 genes were upregulated (9 known and 16 unknown). At 100 days of age the mRNA expression of 3 genes, including the whey acidic protein gene (WAP), was upregulated. The expression of FABP3, WT1, FKHR, AHR, HFH1, HNF3a, WAP and GAD1 was validated by concordance with quantitative real-time reverse transcription PCR. Our results showed that neonatal and prepubertal exposure to BBP induce significant upregulation in gene expression in the mammary gland at 21 days of age. In older animals the number of upregulated genes becomes less prominent, as the number of downregulated genes increases. Future studies will provide insights into the influence of these changes on the susceptibility of the mammary gland to carcinogenesis. (Work supported by NIEHS Grant U01 ES012771)