May 22, 2008

Meeting of the NTP Board of Scientific Counselors
Dr. Gail McCarver, Chairwoman

We would like to acknowledge the conclusions drawn by the NTP in their Brief on bisphenol A draft dated April 14, 2008. We are very pleased to see the addition of "*some* concern for bisphenol A exposure" in fetuses, infants and children based on effects observed in the rodent mammary gland. Although it is very important that the NTP recognize the long-term consequences of gestational and perinatal exposure to bisphenol A on the development of the mammary gland in rodents, we feel that the level of concern should be higher.

Based on the results obtained using laboratory animals perinatally exposed to various estrogenic endocrine disruptors, we are confident that the conclusions drawn by us and others are relevant to the human population at large. For example, between 1979 and 1987 it was first reported that rodents exposed to the synthetic estrogen diethylstilbestrol (DES) had an increased propensity to develop mammary tumors compared to unexposed control animals *(Boylan, E. S. and R. E. Calhoon. Journal of Toxicology and Environmental Health 5, 1979; Boylan, E. S. and R. E. Calhoon. Cancer Research,1983;; Rothschild, T. C., et al. Cancer Research, 1987)*. In 2006, epidemiological data indicated an increase in the risk of developing breast cancer in women exposed to DES during gestation *(Palmer JR et al. Cancer Epidemiol Biomarkers Prev. 2006)*. Additionally, the overwhelming majority of DES-induced defects found in humans were also observed in rodent models *(Newbold, R. R. and J. A. McLachlan. Estrogens in the Environment II: Influences on Development. Ed. J. A. McLachlan. New York: Elsevier Science Publishing Co.,Inc., 1985)*.

Regarding our experimental work on bisphenol A exposure, we have described both mammary ductal hyperplasias and carcinoma *in situ* developing in adult rats and mice exposed during the perinatal period. Intraductal hyperplasias are considered pre-cancerous lesions based on the criterion that when these lesions are transplanted into normal animals, they produce palpable tumors. These lesions are precursors of carcinoma *in situ* and adenocarcinomas (*Haslam and Bern. Proc.Nat.Acad.Sci.USA 1977)*.

The histopathological analysis of the lesions and tumors in our tissues was performed by Dr. Angelo Ucci (MD, Ph.D), a renowned pathologist at Tufts Medical Center with vast experience in animal pathology gained during his postdoctoral training at NIH. In his analyses, he followed the criteria used by other investigators with multiple years of experience in rat mammary gland pathologies. Thus, we have classified the anomalies found in the rat mammary glands according to:

1- Singh M et al. A comparison of the histopathology of premalignant and malignant mammary gland lesions induced in sexually immature rats with those occurring in the human. Lab.Invest. 2000; 80:221-31.

2- Thompson HJ et al. Temporal sequence of mammary intraductal proliferations, ductal carcinomas *in situ* and adenocarcinomas induced by 1-methyl-1-nitrosourea in rats. Carcinogenesis 1998;19:2181-5.

3- Russo J, Russo IH, Rogers AE, Van Zwieten MJ, Gusterson BA. Tumours of the mammary gland. In: Turusov VS, Mohr U, eds. Pathology of tumours in laboratory animals. Vol I. Tumors of the rat. Lyon: IARC Scientific Publication N99; 1990:47-78.

The main criterion used to diagnose a ductal hyperplasia was an increase in the number of epithelial cells lining the ducts (from 1-2 in normal ducts to 3-4 cells thick). In Murray et al. (*Murray et al. Reproductive Toxicology, 2007*), the incidence of preneoplastic lesions was expressed in comparison to the total number of ductal structures present in a $4mm^2$ area analyzed. This area did not include terminal end buds (these structures are present at the tips of the ducts during puberty and contained several layers of epithelial cells, that will differentiate into luminal epithelial and myoepithelial cells) and was selected starting 400µm from the most proximal terminal end bud. Three 5µm sections separated by 50µm were used to assess the presence of pre-neoplastic lesions in the mammary glands. This was done to increase the sampling area of the tissue. All ducts present within the $4mm^2$ area were counted.

The structures classified as carcinoma *in situ* exhibited the hallmarks of these structures according to Russo et al., namely (i) an increased ductal size due to proliferation of the luminal epithelial cells, (ii) enlargement of the luminal epithelial cells along with the presence of nucleoli and a variability in chromatin pattern and (iii) rounded luminal spaces (secondary lumina) formed by trabecular rods of cells aligned perpendicular to the longer axis of the duct.

Data obtained from animal models are essential before any vaccines, therapeutic drugs or treatments are tested in humans. As mentioned above, the rodent animal model for DES exposure accurately predicted the outcomes observed in humans over 20 years later. We should learn from the experience gained with rodent and human DES exposures and consider the data obtained in the laboratory using environmentally relevant doses of bisphenol A. Both should help in reducing exposures of vulnerable populations and ultimately minimize risks and incidence of devastating diseases in the future.

However, it is also acknowledged that after successful results are observed in animals, those same results may not be reproduced in the human population. Nonetheless, ethical considerations strictly limit the use of humans for testing of deleterious effects, even more so when fetal exposure is being assessed, as fetuses cannot provide an informed consent. The field of endocrine disruptor research is one of the most scrutinized, in which every aspect of a particular animal model is pored over and often the results are dismissed based on arguable and/or flawed reasoning. Ignoring the data collected using animal models will not stop endocrine disruptors from causing harm to human and wildlife populations.

We appreciate your time and the consideration to our comments.

Sincerely yours,

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