

ABSTRACTS OF SELECTED BISPHENOL A (BPA) STUDIES

INTRODUCTION

This document provides selected references from published scientific literature concerning the effects of low-dose exposure to bisphenol A (BPA) on cancer causation and treatment, and on the developmental, reproductive, immune and neurobehavioral systems in humans and animals. It also identifies common sources of BPA exposure. This selection of abstracts should not be considered to be comprehensive.

BPA is one of the most pervasive chemicals in modern life. More than 2 million pounds are produced in the United States each year. It is a building block of polycarbonate plastic. Everyone in the industrialized world is exposed to BPA, primarily through food and food packaging, but also through dental sealants, plastic water pipes, air and dust.

BPA is commonly found in the epoxy resin lining of metal food cans and in some types of plastic food containers, including some baby bottles, water bottles, microwave ovenware and eating utensils. Because BPA is an unstable polymer and is also lipophilic (fat-seeking), it can leach into infant formula and other food products, especially when heated. Once in food, BPA can move quickly into people—a particular concern for women of childbearing age and for young children.

Nearly 200 scientific studies show that exposures to low doses of BPA, particularly during prenatal development and early infancy, are associated with a wide range of adverse health effects in later life. These effects include increased risk of breast and prostate cancer, genital abnormalities in male babies, infertility in men, early puberty in girls, metabolic disorders such as insulin resistant (Type 2) diabetes and obesity, and neurobehavioral problems such as attention deficit hyperactivity disorder (ADHD). Exposures that occur before birth are particularly troubling, as the effects on the developing fetus are irreversible.

Despite mounting evidence of its adverse effects on human health, BPA is virtually unregulated in the United States. For the most part, the chemical industry dismisses evidence of harm as inconclusive or biased, citing their own studies which show no adverse effects. An analysis of the literature follows.

Human Exposure Studies

Summary: *These studies make clear that human exposure to BPA is chronic and widespread among people of all ages. Levels in people are comparable to levels that have been shown to cause adverse effects in laboratory animals. CDC scientists found that concentrations of BPA were highest among non-Hispanic blacks and non-Hispanic whites. Concentrations were higher in women than in men, and higher still in children. The presence of BPA in women of childbearing age is the most worrisome. Women are the first environment for their developing babies, which is why levels of BPA are highest among the youngest: fetuses, premature newborns, and young infants. BPA crosses the placenta, enters the fetal bloodstream and is excreted by the fetus into the amniotic fluid. Scientists have detected BPA in human urine and blood (including the cord blood of newborns), in placental tissue and in breast milk.*

One study found that levels of BPA in the urine of premature infants in intensive care were an order of magnitude higher than in the general population. In another study using a computer model, scientists estimated that concentrations of BPA in the blood of 3- and 6-month-olds were 5 times higher than in adults.

The most recent study (below) shows that BPA is not rapidly or completely cleared from the body as previously thought. This finding suggests that food and drink are not the only sources of exposure to BPA, and because BPA is a fat-seeking (lipophilic) chemical, it accumulates in body tissue.

The following excerpts are taken directly from the scientific abstracts.

Stahlhut RW, Welshons WV, Swan SH. “Bisphenol A Data in NHANES Suggest Longer Than Expected Half-Life, Substantial Non-Food Exposure, or Both.” *Environmental Health Perspectives*, doi: 10.1289/ehp.0800376 [Online 28 January 2009].

BACKGROUND: It is commonly stated in the human bisphenol A (BPA) literature that food is the predominant BPA exposure source, and that BPA is rapidly and completely cleared from the body. If correct, BPA levels in fasting individuals should decrease with increased fasting time.

OBJECTIVES: To investigate the relationship between urine BPA concentration and fasting time in a population-based sample.

METHODS: The authors modeled log BPA urine concentration as a function of fasting time, adjusted for urine creatinine and other confounders, in 1469 adult participants in the 2003-2004 National Health and Nutrition Examination Survey. We estimated the BPA “population-based half-life” (pop $\frac{1}{2}$) for a fasting time of 0-24 hours, <4.5 hours, 4.5-8.5 hours and >8.5 hours.

RESULTS: The overall pop $\frac{1}{2}$ for the 0-24 hr interval was 43 hrs (95% CI 26-119 hrs). Among those reporting fasting times of 4.5-8.5 hrs (n = 441), BPA declined significantly with fasting time, with a pop $\frac{1}{2}$ of 4.1 hrs (95% CI 2.6-10.6 hrs). However, within the fasting time intervals of 0-4.5 hrs (n = 129) and 8.5-24 hrs (n = 899), we saw no appreciable decline. Fasting time did not significantly predict highest (> 12 ng/ml) or lowest (below limit of detection) BPA levels.

CONCLUSIONS: Overall, BPA levels did not decline rapidly with fasting time in this sample. This suggests substantial non-food exposure, accumulation in body tissues such as fat, or both. Explaining these findings may require experimental pharmacokinetic studies of chronic BPA exposure, further examination of BPA levels and effects in fat, and a search for important non-food sources.

Calafat AM, Weuve J, Ye X, Jia LT, Hu H, Ringer S, Huttner K, Hauser R. “Exposure to Bisphenol A and other Phenols in Neonatal Intensive Care Unit Premature Infants.” *Environmental Health Perspectives*, doi:10.1289/ehp.0800265 [Online 10 December 2008].

OBJECTIVE: In a previous study, the authors demonstrated that exposure to polyvinyl chloride plastic medical devices containing di(2-ethylhexyl) phthalate (DEHP) was associated with higher urinary concentrations of several DEHP metabolites in 54 premature infants in two neonatal intensive care units than in the general population.

METHODS: For 42 of these infants, the urinary concentrations of several phenols were evaluated, including bisphenol A (BPA), in association with the use of the same medical devices. The authors measured the urinary concentrations of free and total (free plus conjugated) species of BPA, triclosan, benzophenone-3, methyl paraben, and propyl paraben.

RESULTS: The percentage of BPA present as its conjugated species was greater than 90% in more than three-quarters of the premature infants. Intensity of use of products containing DEHP was strongly associated with BPA total concentrations but not with any other phenol. Adjusting for institution and

sex, BPA total concentrations among infants in the group of high use of DEHP-containing products were 8.75 times as high as among infants in the low use group ($p < 0.0001$). Similarly, after adjusting for sex and DEHP containing product use category, BPA total concentrations among infants in Institution A were 16.6 times as high as those among infants in Institution B ($p < 0.0001$).

CONCLUSION: BPA geometric mean urinary concentration (30.3 $\mu\text{g/L}$) among premature infants undergoing intensive therapeutic medical interventions was one order of magnitude higher than that among the general population. Conjugated species were the primary urinary metabolites of BPA suggesting that premature infants have some capacity to metabolize BPA. The differences in exposure to BPA by intensity of use of DEHP-containing medical products highlight the need for further studies to determine the specific source(s) of exposure to BPA.

Edginton AN and Ritter L. "Predicting Plasma Concentrations of Bisphenol A in Young Children (< Two Years) Following Typical Feeding Schedules using a Physiologically-based Toxicokinetic Model." *Environmental Health Perspectives*, doi:10.1289/ehp.0800073 [Online 14 November 2008].

BACKGROUND: Concerns have recently been raised regarding the safety of potential human exposure to bisphenol A (BPA), an industrial chemical found in some polycarbonate plastics and epoxy resins. Of particular interest is the exposure of BPA to young children via food stored in BPA-containing packaging.

OBJECTIVES: To assess the age-dependence of the toxicokinetics of BPA and its glucuronidated metabolite, BPA-Glu, using a coupled BPA_BPA-Glu physiologically based toxicokinetic (PBTK) model.

METHODS: Using information gathered from toxicokinetic studies in adults, a PBTK model was built. The model was then scaled to young children under the age of 2 based on the age-dependence of physiological parameters relevant for absorption, distribution, metabolism and excretion.

RESULTS: The average steady state BPA plasma concentration in newborns is estimated to be 11 times greater than that in adults when given the same weight-normalized dose. Due to the rapid development of the glucuronidation process, this ratio is reduced to 2 by 3 months of age. Simulation of typical feeding exposures, as estimated by regulatory authorities, showed a 5 times greater steady state BPA plasma concentration in 3 and 6 month olds when compared to adults. This was due to both a reduced capacity for BPA metabolism as well as a greater weight-normalized BPA exposure. Due to an uncertainty in defining the hepatic BPA intrinsic clearance in adults, these values represent preliminary estimates.

CONCLUSIONS: Simulation of the differential BPA dosimetry between adults and young children point to the need for more sensitive analytical methods for BPA to define, with greater certainty, the adult hepatic BPA intrinsic clearance, as well as, a need for external exposure data in young children.

Calfat AM, Ye X, Wong L, Reidy JA, Needham LL. "Exposure of the U.S. Population to Bisphenol A and 4-tertiary-Octylphenol: 2003–2004." *Environmental Health Perspectives*, 116(1):39-44 (2008).

BACKGROUND: Bisphenol A (BPA) and 4-tertiary-octylphenol (tOP) are industrial chemicals used in the manufacture of polycarbonate plastics and epoxy resins (BPA) and nonionic surfactants (tOP). These products are in widespread use in the United States.

OBJECTIVES: The authors aimed to assess exposure to BPA and tOP in the U.S. general population.

METHODS: They measured the total (free plus conjugated) urinary concentrations of BPA and tOP in 2,517 participants ≥ 6 years of age in the 2003–2004 National Health and Nutrition Examination Survey using automated solid-phase extraction coupled to isotope dilution–high-performance liquid chromatography–tandem mass spectrometry.

RESULTS: BPA and tOP were detected in 92.6% and 57.4% of the persons, respectively. Least square geometric mean (LSGM) concentrations of BPA were significantly lower in Mexican Americans than in non-Hispanic blacks ($p = 0.006$) and non-Hispanic whites ($p = 0.007$); LSGM concentrations for non-Hispanic blacks and non-Hispanic whites were not statistically different

($p = 0.21$). Females had statistically higher BPA LSGM concentrations than males ($p = 0.043$). Children had higher concentrations than adolescents ($p < 0.001$), who in turn had higher concentrations than adults ($p = 0.003$). LSGM concentrations were lowest for participants in the high household income category ($> \$45,000/\text{year}$).

CONCLUSIONS: Urine concentrations of total BPA differed by race/ethnicity, age, sex, and household income. These first U.S. population representative concentration data for urinary BPA and tOP should help guide public health research priorities, including studies of exposure pathways, potential health effects, and risk assessment.

Kuruto-Niwa R, Tateoka Y, Usuki Y, Nozawa R. "Measurement of Bisphenol A Concentrations in Human Colostrum." *Chemosphere*, 66:1160-1164 (2006).

BACKGROUND: Bisphenol A (BPA), an estrogenic endocrine disrupting chemical, has been reported to affect embryos and alter their postnatal development.

METHOD: In the present study, we measured the concentrations of BPA in human colostrum by a competitive enzyme-linked immunosorbent assay (ELISA) with the aim of understanding the present status of BPA burden in human breast milk in Shizuoka, Japan. Human colostrum samples were collected from 101 healthy mothers within three days after delivery.

RESULTS: The BPA concentrations of colostrum samples were estimated by ELISA after the acetonitrile extraction and solid phase extraction column purification. BPA in 101 samples was detected in the concentration range of 1-7 ng ml⁻¹. The mean concentration of BPA was 3.41 \pm 0.13 (mean \pm SD) ng ml⁻¹. This is the first demonstration as to what BPA concentrations are in human colostrum. The BPA concentrations in colostrum were higher than those in blood sera samples obtained from healthy women in a previous study. In our study, there was no significant correlation between the concentrations of BPA in colostrum and the age and parity of mothers.

CONCLUSION: BPA was found in human breast milk.

Ikezuki Y, Tsutsumi O, Takai Y, Kamei Y, Taketani Y. "Human Biological Fluids Reveals Significant Early Prenatal Exposure." *Human Reproduction*, 17:2839-2841 (2002).

BACKGROUND: There is broad human exposure to bisphenol A (BPA), an estrogenic endocrine-disrupting chemical widely used for the production of plastic products. BPA is reported to affect preimplantation embryos or fetuses and alter their postnatal development at doses typically found in the environment. We measured contamination of BPA in various kinds of human biological fluids by a novel enzyme-linked immunosorbent assay.

METHODS: Blood samples were obtained from healthy premenopausal women, women with early and full-term pregnancy, and umbilical cord at full-term delivery. Ovarian follicular fluids obtained during IVF procedures and amniotic fluids obtained at mid-term and full-term pregnancy were also subject to BPA measurements.

RESULTS: BPA was present in serum and follicular fluid at approximately 1-2 ng/ml, as well as in fetal serum and full-term amniotic fluid, confirming passage through the placenta. Surprisingly, an approximately 5-fold higher concentration, 8.3 \pm 8.7 ng/ml, was revealed in amniotic fluid at 15-18 weeks gestation, compared with other fluids.

CONCLUSION: These results suggest accumulation of BPA in early fetuses and significant exposure during the prenatal period, which must be considered in evaluating the potential for human exposure to endocrine-disrupting chemicals.

Schönfelder G, Wittfoht W, Hopp H, Talsness CE, Paul M, Chahoud I. "Parent Bisphenol A Accumulation in the Human Maternal-Fetal-Placental Unit." *Environmental Health Perspectives*, 110:A703-A707 (2002).

BACKGROUND: Bisphenol A (BPA), an endocrine disruptor, is employed in the manufacture of a wide range of consumer products. The suggestion that BPA, at amounts to which we are exposed, alters the reproductive organs of developing rodents has caused concern. At present, no information exists concerning the exposure of human pregnant women and their fetuses to BPA.

METHODS: We therefore investigated blood samples from mothers ($n = 37$) between weeks 32 and 41 of gestation. After the births, we also analyzed placental tissue and umbilical cord blood from the same subjects. We developed a novel chemical derivatization–gas chromatography/mass spectrometry method to analyze parent BPA at concentrations $< 1 \mu\text{g/mL}$ in plasma and tissues.

RESULTS: Concentrations of BPA ranged from 0.3 to 18.9 ng/mL (median = 3.1 ng/mL) in maternal plasma, from 0.2 to 9.2 ng/mL (median = 2.3 ng/mL) in fetal plasma, and from 1.0 to 104.9 ng/g (median = 12.7 ng/g) in placental tissue. BPA blood concentrations were higher in male than in female fetuses. Here we demonstrate parent BPA in pregnant women and their fetuses.

CONCLUSIONS: Exposure levels of parent BPA were found within a range typical of those used in recent animal studies and were shown to be toxic to reproductive organs of male and female offspring. We suggest that the range of BPA concentrations we measured may be related to sex differences in metabolism of parent BPA or variable maternal use of consumer products leaching BPA.

Routes of Exposure

Summary: *The following studies show that BPA is an unstable chemical that can leach from polycarbonate plastic products into food or beverages. For example, when polycarbonate baby bottles are heated, BPA leaches into formula or water. Ordinary wear and tear—washing, boiling and brushing—increases leaching.*

Scientists working with laboratory animals also found that BPA can leach from polycarbonate cages into water at room temperature. Both new and used cages leached the chemical, but leaching increased in used cages. Female mice exposed to BPA through polycarbonate cages developed a reproductive abnormality called meiotic aneuploidy. Meiosis is a multi-stage process of cell division and aneuploidy means that the egg cells have the wrong number of chromosomes. In humans, aneuploidy is a major cause of birth defects, including Down syndrome.

The final study in this section refutes industry arguments claiming that data from any study in which newborn animals were injected with BPA should be discounted because injection would result in a higher level of exposure than oral feeding of BPA. This following study shows that exposing newborn laboratory animals to BPA through feeding or by injection results in the same levels of the chemical in the blood. In fact, when compared with studies of adult mice, oral feeding resulted in noticeably higher levels of BPA in the blood.

The following excerpts are taken directly from the scientific abstracts.

Brede C, Fjeldal P, Skjevraak I, Herikstad H. “Increased Migration Levels of Bisphenol A From Polycarbonate Baby Bottles After Dishwashing, Boiling and Brushing.” *Food Additives and Contaminants*, 20(7):684-689 (2003).

BACKGROUND: Baby bottles are often made of polycarbonate plastic. Impurities remaining in the bottle from the monomer bisphenol A can migrate from the plastic bottles into baby food, thereby causing a health concern. Previous migration testing of new baby bottles showed only trace migration levels of the substance. In the present work, polycarbonate baby bottles were subjected to simulated use by

dishwashing, boiling and brushing. *Migration testing performed with both new and used bottles revealed a significant increase in migration of bisphenol A due to use. This finding might be explained by polymer degradation.*

METHODS: Bisphenol A was determined in 200-ml samples of water food simulant by a method based on solid-phase extraction followed by gas chromatography coupled with mass spectrometry. The detection limit was 0.1 microg l(-1). Twelve different polycarbonate baby bottles were tested by filling them with hot water (100 degrees C) for 1 h.

RESULTS: The mean bisphenol A level from new bottles was 0.23 + -0.12 microg l(-1), while the mean levels from bottles subjected to simulated use were 8.4 + -4 microg l(-1) (dishwashed 51 times) and 6.7 + -4 microg l(-1) (dishwashed 169 times), respectively.

CONCLUSION: None of the bottles released bisphenol A at levels that exceed the recently established provisional tolerable daily intake (0.01 mg kg(-1) body weight/day) in the European Union.

Howdeshell KL, Peterman PH, Judy BM, Taylor JA, Orazio CE, Ruhlen RL, vom Saal FS, Welshons WV. "Bisphenol A is released from used polycarbonate animal cages into water at room temperature." *Environmental Health Perspectives*, 111:1180-1187 (2003).

BACKGROUND: Bisphenol A (BPA) is a monomer with estrogenic activity that is used in the production of food packaging, dental sealants, polycarbonate plastic, and many other products. The monomer has previously been reported to hydrolyze and leach from these products under high heat and alkaline conditions, and the amount of leaching increases as a function of use. We examined whether new and used polycarbonate animal cages passively release bioactive levels of BPA into water at room temperature and neutral pH.

METHODS: Purified water was incubated at room temperature in new polycarbonate and polysulfone cages and used (discolored) polycarbonate cages, as well as control (glass and used polypropylene) containers. The resulting water samples were characterized with gas chromatography/mass spectrometry (GC/MS) and tested for estrogenic activity using an MCF-7 human breast cancer cell proliferation assay.

RESULTS: Significant estrogenic activity, identifiable as BPA by GC/MS (up to 310 micro g/L), was released from used polycarbonate animal cages. Detectable levels of BPA were released from new polycarbonate cages (up to 0.3 micro g/L) as well as new polysulfone cages (1.5 micro g/L), whereas no BPA was detected in water incubated in glass and used polypropylene cages. Finally, BPA exposure as a result of being housed in used polycarbonate cages produced a 16% increase in uterine weight in prepubertal female mice relative to females housed in used polypropylene cages, although the difference was not statistically significant.

CONCLUSION: The study's findings suggest that laboratory animals maintained in polycarbonate and polysulfone cages are exposed to BPA via leaching, with exposure reaching the highest levels in old cages.

Hunt PA, Koehler KE, Susiarjo M, Hodges CA, Ilagan A, Voigt RC, Thomas S, Thomas BF, Hassold TJ. "Bisphenol A causes meiotic aneuploidy in the female mouse." *Current Biology*, 13:543-553 (2003).

BACKGROUND: There is increasing concern that exposure to man-made substances that mimic endogenous hormones may adversely affect mammalian reproduction. Although a variety of reproductive complications have been ascribed to compounds with androgenic or estrogenic properties, little attention has been directed at the potential consequences of such exposures to the genetic quality of the gamete.

RESULTS: A sudden, spontaneous increase in meiotic disturbances, including aneuploidy, in studies of oocytes from control female mice in our laboratory coincided with the accidental exposure of our animals to an environmental source of bisphenol A (BPA). BPA is an estrogenic compound widely used in

the production of polycarbonate plastics and epoxy resins. We identified damaged caging material as the source of the exposure, as we were able to recapitulate the meiotic abnormalities by intentionally damaging cages and water bottles. In subsequent studies of female mice, we administered daily oral doses of BPA to directly test the hypothesis that low levels of BPA disrupt female meiosis. Our results demonstrated that the meiotic effects were dose dependent and could be induced by environmentally relevant doses of BPA.

CONCLUSIONS: Both the initial inadvertent exposure and subsequent experimental studies suggest that BPA is a potent meiotic aneugen. Specifically, in the female mouse, short-term, low-dose exposure during the final stages of oocyte growth is sufficient to elicit detectable meiotic effects. These results provide the first unequivocal link between mammalian meiotic aneuploidy and an accidental environmental exposure and suggest that the oocyte and its meiotic spindle will provide a sensitive assay system for the study of reproductive toxins.

Taylor JA, Welshons WV, vom Saal FS. "No effect of route of exposure (oral; subcutaneous injection) on plasma bisphenol A throughout 24 h after administration in neonatal female mice." *Reproductive Toxicology*, 25:169-176 (2008).

BACKGROUND: Route of administration of chemicals in adults is an important factor in pharmacokinetics of chemicals such as bisphenol A (BPA), the monomer with estrogenic activity used to make polycarbonate plastic products and to line food and beverage cans. Based on findings in adults it has been proposed (CERHR, 2007) that non-oral routes of administration in newborn rodents would also lead to high exposure relative to oral administration. However, in fetuses and neonates, the enzyme that conjugates BPA (UDP-glucuronosyltransferase) is expressed at low levels, suggesting that there may be no differences in pharmacokinetics between oral and non-oral dosing.

METHODS: We thus conducted an analysis of plasma concentrations of unconjugated 3H-BPA after HPLC separation in postnatal day 3 female mice throughout the 24 h after administering 3H-BPA orally or via subcutaneous injection at doses above and below the current EPA reference dose.

RESULTS: We found no significant difference in plasma BPA based on route of administration in neonatal mice at either dose. However, compared to data from other studies conducted with adults, there was a markedly higher plasma BPA level after oral administration of BPA in newborn mice.

CONCLUSIONS: This finding sets aside the belief that non-oral administration of BPA renders data as not suitable for consideration of the hazard posed by low-dose exposure to BPA during neonatal life. Therefore the large numbers of BPA studies that used non-oral administration at very low doses during the neonatal period should not be dismissed by scientists or the regulatory community based on route of administration.

Breast Cancer Susceptibility (Cells in culture)

Summary: *These two studies show that exposing breast cancer cells in culture to BPA causes the cells to proliferate (grow and divide). The first study showed that BPA changed the expression of more than 300 genes, activating the estrogen receptor.*

The following excerpts are taken directly from the scientific abstracts.

Singleton DW, Feng Y, Yang J, Puga A, Lee AV, Khan SA. "Gene Expression Profiling Reveals Novel Regulation by Bisphenol A in Estrogen Receptor-Alpha-Positive Human Cells." *Environmental Research* 100:86-92, (2006).

BACKGROUND: Bisphenol-A (BPA) shows proliferative actions in uterus and mammary glands and may influence the development of male and female reproductive tracts in utero or during early postnatal life. Because of its ability to function as an estrogen receptor (ER) agonist, BPA has the potential to disrupt normal endocrine signaling through regulation of ER target genes. Some genes are regulated by both estradiol (E2) and BPA, but those exclusive to either agent have not been described.

METHODS: Using a yeast strain incorporating a vitellogenin A2 ERE-LacZ reporter gene into the genome, we found that BPA induced expression of the reporter in colonies transformed with the ERalpha expression plasmid, illustrating BPA-mediated regulation within a chromatin context. Additionally, a reporter gene transiently transfected into the endometrial cancer (Ishikawa) cell line also showed BPA activity, although at 100-fold less potency than E2. To compare global gene expression in response to BPA and E2, we used a variant of the MCF-7 breast cancer cell line stably expressing HA-tagged ERalpha. Cultures were treated for 3h with an ethanol vehicle, E2 (10⁻⁸M), or BPA (10⁻⁶M), followed by isolation of RNA and microarray analysis with the human U95A probe array (Affymetrix, Santa Clara, CA, USA).

RESULTS: More than 300 genes were changed 2-fold or more by either or both agents, with roughly half being up-regulated and half down-regulated. A number of growth- and development-related genes, such as HOXC1 and C6, Wnt5A, Frizzled, TGFbeta-2, and STAT inhibitor 2, were found to be affected exclusively by BPA. We used quantitative real-time PCR to verify regulation of the HOXC6 gene, which showed decreased expression of approximately 2.5-fold by BPA.

CONCLUSIONS: These results reveal novel effects by BPA and E2, raising interesting possibilities regarding the role of endocrine disruptors in sexual development.

Recchia AG, Vivacqua A, Gabriele S, Carpino A, Fasanella G, Rago V, Bonofiglio D, Maggiolini M.

“Xenoestrogens and the induction of proliferative effects in breast cancer cells via direct activation of oestrogen receptor alpha.” *Food Additives and Contaminants* 21:134-144, (2004).

BACKGROUND: Environmental contamination with a variety of industrial products has been associated with developmental and reproductive abnormalities in wildlife species. Increasing evidence has suggested that bisphenol A (BPA) and 4-nonylphenol (NPH), two major endocrine-disrupting chemicals, might be responsible for adverse effects on humans as a consequence of ubiquitous use together with potential oestrogen-like activity.

METHODS AND RESULTS: To provide insight into the oestrogen-like nature of BPA and NPH, their ability to activate a reporter gene construct via an oestrogen response element in the hormone-dependent breast cancer cell lines MCF7 and T47D was ascertained. Both compounds transactivated the endogenous oestrogen receptor (ER) alpha in a direct fashion since the anti-oestrogen 4-hydroxytamoxifen abolished the response. In addition, using steroid-receptor-negative HeLa cells engineered to express ERalpha and ERbeta and the hormone-binding domains of both ERalpha and ERbeta, BPA and NPH confirmed the direct transcriptional activity. Interestingly these properties were supported in MCF7 cells by the ability to autoregulate ERalpha expression as well as to induce its nuclear compartmentalization. We therefore evaluated by reverse transcriptase polymerase chain reaction the expression of oestrogen-controlled genes such as cathepsin D and TFF1 (formerly pS2), which were increased by both chemicals tested. The agonistic effects exhibited in all assays performed prompted the evaluation of a more complex biological response such as the proliferation of MCF7 and T47D cells. The same concentration of xenoestrogens eliciting substantial transcriptional activity significantly stimulated the proliferation of both breast cancer cell lines, although with a reduced effectiveness with respect to the natural hormone 17beta-oestradiol.

CONCLUSIONS: The results indicate that the biological action of environmental oestrogen such as BPA and NPH should be taken into account for the potential impact on human disease-like hormone-dependent breast cancer. However, further studies are needed to clarify their bioavailability and

metabolism as well as whether compound mixtures could produce noticeable effects by synergistic activity.

Breast Cancer Susceptibility (animals)

Summary: *This section includes studies showing effects of BPA exposure on the mammary glands of rodents. As a group, they offer evidence that BPA mimics the biological effects of other estrogens. Increased exposure to estrogens has long been associated with increased risk of breast cancer.*

The most recent study is the first to show that newborn rats exposed to BPA through nursing have increased mammary cancer as adults. The dams (mother rats) were treated orally with BPA before nursing began. After being exposed to a chemical carcinogen as adults, the BPA-exposed animals developed more cancers than animals nursed by unexposed dams. BPA-related changes in the mammary gland included increased cell proliferation (growth) and decreased apoptosis (cell death).

Earlier studies showed similar precancerous changes in the structure of the mammary gland of both rats and mice exposed before birth or during the newborn period. In one study, rats exposed to low-dose BPA during fetal development had abnormal cell growth (ductal hyperplasia) and carcinoma in situ (cancer that has not broken through the cell membrane) even without later exposure to a chemical carcinogen. Changing the internal structure of the mammary gland also changes how cells communicate with each other to grow and develop.

The following excerpts are taken directly from the scientific abstracts.

Jenkins S, Raghuraman N, Eltoum I, Carpenter M, Russo C, Lamartiniere CA. "Oral Exposure to Bisphenol A Increases Dimethylbenzanthracene-Induced Mammary Cancer in Rats." *Environmental Health Perspectives*, doi:10.1289/ehp.11751 [Online 7 January 2009].

BACKGROUND: Bisphenol A (BPA) is widely used in the manufacture of polycarbonate plastics, including infant formula bottles.

OBJECTIVES: Based on the reported endocrine disruptor activity of this polyphenol, the study's authors hypothesized that exposure to BPA early in life would elicit developmental changes in the mammary tissue and predispose for mammary cancer.

METHODS: Neonatal/prepubertal rats were exposed to BPA *via* lactation from nursing dams treated orally with 0, 25, and 250 µg BPA/kg body weight/day. For tumorigenesis studies, female offspring were exposed to 30 mg dimethylbenzanthracene (DMBA)/kg body weight at 50 days postpartum.

RESULTS: The combination of DMBA treatment after lactational exposure to BPA demonstrated a dose-dependent increase in mammary tumor multiplicity and reduced tumor latency compared to control. In the absence of DMBA treatment, lactational BPA exposure resulted in increased cell proliferation and decreased apoptosis at 50, but not 21, days postpartum (shortly after last BPA treatment). Using western blot analysis, steroid receptor co-activators (SRCs) 1-3, Akt, phospho-Akt, progesterone receptor A (PR-A), and erbB3 proteins were determined to be significantly up-regulated at 50 days.

CONCLUSIONS: The data presented here provide the first evidence that maternal exposure to BPA during lactation increases mammary carcinogenesis in a DMBA-induced model of rodent mammary cancer. Changes in PR-A, SRC proteins, erbB3, and Akt activity are consistent with increased cell proliferation and decreased apoptosis playing a role in mammary cancer susceptibility. These

alternations provide an explanation of enhanced mammary carcinogenesis following lactational BPA exposure.

Vandenberg LN, Maffini MV, Schaeberle CM, Ucci AA, Sonnenschein C, Rubin BS, Soto AM. "Perinatal Exposure to the Xenoestrogen Bisphenol-A Induces Mammary Intraductal Hyperplasias in Adult CD-1 Mice." *Reproductive Toxicology*, 26(3-4):210-219 (2008).

BACKGROUND: Humans are routinely exposed to bisphenol-A (BPA), an estrogenic compound that leaches from consumer products. Given the sensitivity of the developing organism to hormones, exposure of fetuses and infants is a concern.

METHODS: CD-1 mice were exposed to environmentally relevant doses of BPA during gestation and the lactational period (gestational day 8 through postnatal day 16). At 3, 9 and 12-15 months of age, mammary glands from exposed offspring were examined for structural changes.

RESULTS: BPA-exposed females demonstrated altered mammary phenotypes including the appearance of alveolar buds. Additionally, intraductal hyperplasias were observed exclusively in BPA-exposed females. These lesions had the appearance of "beaded" ducts, with epithelial cells present inside the ductal lumen and increased proliferation indexes compared to normal ducts. Similar structures have also been observed following exposure to other estrogens.

CONCLUSION: These results are further evidence that perinatal BPA exposure can alter the morphology of the rodent mammary gland in adulthood.

Durando M, Kass L, Piva J, Sonnenschein C, Soto AM, Luque EH, Muñoz-de-Toro M. "Prenatal Bisphenol A Exposure Induces Preneoplastic Lesions in the Mammary Gland in Wistar Rats." *Environmental Health Perspectives*, 115:80-86 (2007).

BACKGROUND: Humans are routinely exposed to bisphenol A (BPA), an estrogenic compound that leaches from dental materials, food and beverage containers, and other consumer products. Prenatal exposure to BPA has produced long-lasting and profound effects on rodent hormone-dependent tissues that are manifested 1–6 months after the end of exposure.

OBJECTIVE: The aim of the present work was to examine whether *in utero* exposure to BPA alters mammary gland development and increases its susceptibility to the carcinogen *N*-nitroso-*N*-methylurea (NMU).

METHODS: Pregnant Wistar rats were exposed to BPA (25 µg/kg body weight per day) or to vehicle. Female offspring were sacrificed on postnatal day (PND) 30, 50, 110, or 180. On PND50 a group of rats received a single subcarcinogenic dose of NMU (25 mg/kg) and they were sacrificed on either PND110 or PND180.

RESULTS: At puberty, animals exposed prenatally to BPA showed an increased proliferation/apoptosis ratio in both the epithelial and stromal compartments. During adulthood (PND110 and PND180), BPA-exposed animals showed an increased number of hyperplastic ducts and augmented stromal nuclear density. Moreover, the stroma associated with hyperplastic ducts showed signs of desmoplasia and contained an increased number of mast cells, suggesting a heightened risk of neoplastic transformation. Administration of a subcarcinogenic dose of NMU to animals exposed prenatally to BPA increased the percentage of hyperplastic ducts and induced the development of neoplastic lesions.

CONCLUSIONS: Our results demonstrate that the prenatal exposure to low doses of BPA perturbs mammary gland histoarchitecture and increases the carcinogenic susceptibility to a chemical challenge administered 50 days after the end of BPA exposure.

Murray TJ, Maffini MV, Ucci AA, Sonnenschein C, Soto AM. "Induction of Mammary Gland Ductal Hyperplasias and Carcinoma *in situ* Following Fetal Bisphenol A Exposure." *Reproductive Toxicology*, 23:383-390 (2007).

BACKGROUND: Exposure of the fetus to excess estrogen is believed to increase the risk of developing breast cancer during adult life.

RESULTS: Fetal exposure to low doses of the xenoestrogen bisphenol A resulted in long-lasting effects in the mouse mammary gland that were manifested during adult life. It enhanced sensitivity to estradiol, decreased apoptosis, increased the number of progesterone receptor-positive epithelial cells at puberty and increased lateral branching at 4 months of age. We now report that fetal exposure to 2.5, 25, 250 and 1000µg bisphenol A/kg body weight/day induces the development of ductal hyperplasias and carcinoma *in situ* at postnatal day 50 and 95 in rats. These highly proliferative lesions have an increased number of estrogen receptor-α positive cells.

CONCLUSION: Thus, fetal bisphenol A exposure is sufficient to induce the development of preneoplastic and neoplastic lesions in the mammary gland in the absence of any additional treatment aimed at increasing tumor development.

Vandenberg LN, Maffini MV, Wadia PR, Sonnenschein C, Rubin BS, Soto AM. “Exposure to Environmentally Relevant Doses of the Xenoestrogen Bisphenol A Alters Development of the Fetal Mouse Mammary Gland.” *Endocrinology*, 148(1):116-127 (2007).

BACKGROUND: Humans are routinely exposed to bisphenol-A (BPA), an estrogenic compound that leaches from dental materials, food and beverage containers, and other plastic consumer products. Effects of perinatal BPA exposure on the mouse mammary gland have been observed in puberty and adulthood, long after the period of exposure has ended.

OBJECTIVE: The aim of this study was to examine fetal mammary gland development at embryonic day (E)18 and assess changes in the tissue organization and histoarchitecture after exposure to an environmentally relevant dose of BPA. In unexposed fetuses, the relative position of the fetus with respect to its female and male siblings in the uterus influenced growth of the ductal tree, which was more developed in females placed between two males than in females placed between two females.

METHODS and RESULTS: Exposure of dams to 250 ng BPA per kilogram body weight per day from E8 to E18 significantly increased ductal area and ductal extension in exposed fetuses and obliterated positional differences. In the stroma, BPA exposure promoted maturation of the fat pad and altered the localization of collagen. Within the epithelium, BPA exposure led to a decrease in cell size and delayed lumen formation.

CONCLUSION: Because mammary gland development is dependent on reciprocal interactions between these compartments, the advanced maturation of the fat pad and changes in the extracellular matrix may be responsible for the altered growth, cell size, and lumen formation observed in the epithelium.

Muñoz-de-Toro M, Markey C, Wadia PR, Luque EH, Rubin BS, Sonnenschein C, Soto AM. “Perinatal Exposure to Bisphenol A Alters Peripubertal Mammary Gland Development in Mice.” *Endocrinology*, 146:4138-4147 (2005).

BACKGROUND: Developmental exposure to estrogenic chemicals induces morphological, functional and behavioral anomalies associated with reproduction. Humans are exposed to bisphenol-A (BPA), an estrogenic compound that leaches from dental materials and plastic food and beverage containers.

METHODS: The aim of the present study was to determine the effects of perinatal exposure to low, environmentally relevant doses of BPA (25 and 250 ng BPA/kg body weight (bw)/day) on the peripubertal development of the mammary gland.

RESULTS: BPA exposure enhanced the mammary glands' sensitivity to estradiol in ovariectomized CD-1 mice. In their intact 30-day-old littermates, the area and numbers of terminal end buds relative to the gland ductal area increased while their apoptotic activity decreased. There was a positive correlation between ductal length and the age at first proestrus; that was reduced as the BPA dose increased, suggesting that BPA exposure slows down ductal invasion of the stroma. There was also a significant

increase of progesterone receptor-positive ductal epithelial cells that were localized in clusters, suggesting future branching points. Indeed, lateral branching was significantly enhanced at 4 months of age in mice exposed to 25 ng BPA /kg bw/day.

CONCLUSION: In conclusion, perinatal exposure to environmentally relevant BPA doses results in persistent alterations in mammary gland morphogenesis. Of special concern is the increased terminal end bud density at puberty as well as the increased number of terminal ends reported previously in adult animals, since these two structures are the sites where cancer arises in humans and rodents.

Markey CM, Luque EH, Muñoz de Toro M, Sonnenschein C, Soto AM. "In Utero Exposure to Bisphenol A Alters the Development and Tissue Organization of the Mouse Mammary Gland." *Biology of Reproduction*, 65:1215-1223 (2001).

BACKGROUND: Exposure to estrogens throughout a woman's life, including the period of intrauterine development, is a risk factor for the development of breast cancer. The increased incidence of breast cancer noted during the last 50 years may have been caused, in part, by exposure of women to estrogen-mimicking chemicals that are released into the environment. Here, we investigated the effects of fetal exposure to one such chemical, bisphenol A (BPA), on development of the mammary gland.

METHODS: CD-1 mice were exposed in utero to low, presumably environmentally relevant doses of BPA (25 and 250 µg/kg body weight), and their mammary glands were assessed at 10 days, 1 mo, and 6 mo of age.

RESULTS: Mammary glands of BPA-exposed mice showed differences in the rate of ductal migration into the stroma at 1 mo of age and a significant increase in the percentage of ducts, terminal ducts, terminal end buds, and alveolar buds at 6 mo of age. The percentage of cells that incorporated BrdU was significantly decreased within the epithelium at 10 days of age and increased within the stroma at 6 mo of age.

CONCLUSION: These changes in histoarchitecture, coupled with an increased presence of secretory product within alveoli, resemble those of early pregnancy, and they suggest a disruption of the hypothalamic-pituitary-ovarian axis and/or misexpression of developmental genes. The altered relationship in DNA synthesis between the epithelium and stroma and the increase in terminal ducts and terminal end buds are striking, because these changes are associated with carcinogenesis in both rodents and humans. These results suggest that alterations in mammary gland phenotypes observed at puberty and adulthood in perinatally exposed mice have their origins in fetal development.

Effects on Cancer Treatment

Summary: *Women with estrogen receptor positive (ER-positive) breast cancer are treated with drugs to shut down their bodies' production of estrogen, thereby killing the cancer cells and preventing recurrence. In this study, scientists treated cancer cells (both ER-positive and ER-negative cells) in culture with anti-cancer chemotherapy drugs and low-dose BPA. They found that BPA interfered with the chemotherapy's ability to kill cancer cells, creating what is known as chemoresistance in both ER-positive and ER-negative cells.*

The following excerpt is taken directly from the scientific abstract.

LaPensee EW, Tuttle TR, Fox SR, Ben-Jonathan N. "Bisphenol A at Low Nanomolar Doses Confers Chemoresistance in Estrogen Receptor Alpha Positive and Negative Breast Cancer Cells." *Environmental Health Perspectives*, doi:10.1289/ehp.11788, [Online 8 October 2008].

BACKGROUND: Resistance to chemotherapy is a major problem facing breast cancer patients, and identifying potential contributors to chemoresistance is a critical area of research. Bisphenol A (BPA) has long been suspected to promote carcinogenesis, but the high doses of BPA used in many studies generated conflicting results. In addition, the mechanism by which BPA exerts its biological actions is unclear. While estrogen has been shown to antagonize anti-cancer drugs, the role of BPA in chemoresistance has not been examined.

OBJECTIVE: The objective was to determine whether BPA at low nanomolar concentrations opposes the action of doxorubicin, cisplatin and vinblastine in the ER α positive T47D and the ER α negative MDA-MB-468 breast cancer cells.

METHODS: The responsiveness of cells to anti-cancer drugs and BPA was determined by the MTT cytotoxicity assay. Specific ER α and ER β inhibitors and real-time PCR were used to identify potential receptor(s) that mediate the actions of BPA. Expression of anti-apoptotic proteins was assessed by Western blotting.

RESULTS: BPA antagonizes the cytotoxicity of multiple chemotherapeutic agents in both ER α positive and negative breast cancer cells independent of the classical ERs. Both cell types express alternative ER receptors, including GRP30 and members of the estrogen related receptor (ERR) family. Increased expression of anti-apoptotic proteins is a potential mechanism by which BPA exerts its anti-cytotoxic effects.

CONCLUSION: BPA at environmentally relevant doses reduces the efficacy of chemotherapeutic agents. These data provide considerable support to the accumulating evidence that BPA is hazardous to human health.

Studies Related to Prostate Cancer (animals)

Summary: *The following studies show that early life exposure (before birth or immediately after birth) of male rodents to low doses of BPA or estradiol (the most powerful female hormone) interferes with normal development of the prostate, making it more susceptible to cancer in later life. The developmental interference occurs through changes in gene signaling, resulting in accelerated cell growth, inflammation and other precancerous changes in the prostate.*

The following excerpts are taken directly from the scientific abstracts.

Prins GS, Tang WY, Belmonte J, Ho SM. "Perinatal Exposure to Oestradiol and Bisphenol A Alters the Prostate Epigenome and Increases Susceptibility to Carcinogenesis." *Basic and Clinical Pharmacology and Toxicology*, 102(2):134-138 (2008).

BACKGROUND: An important and controversial health concern is whether low-dose exposures to hormonally active environmental oestrogens such as bisphenol A can promote human diseases including prostate cancer. The authors' studies in rats have shown that pharmacological doses of oestradiol administered during the critical window of prostate development result in marked prostate pathology in adulthood that progress to neoplastic lesions with ageing. Their recent studies have also demonstrated that transient developmental exposure of rats to low, environmentally relevant doses of bisphenol A or oestradiol increases prostate gland susceptibility to adult-onset precancerous lesions and hormonal carcinogenesis. These findings indicate that a wide range of oestrogenic exposures during development can predispose to prostatic neoplasia that suggests a potential developmental basis for this adult disease.

METHODS: To identify a molecular basis for oestrogen imprinting, we screened for DNA methylation changes over time in the exposed prostate glands.

RESULTS: We found permanent alterations in DNA methylation patterns of multiple cell signalling genes suggesting an epigenetic mechanism of action. For phosphodiesterase type 4 variant 4 (PDE4D4), an enzyme responsible for intracellular cyclic adenosine monophosphate breakdown, a specific methylation cluster was identified in the 5'-flanking CpG island that was gradually hypermethylated with ageing in normal prostates resulting in loss of gene expression. However, in prostates exposed to neonatal oestradiol or bisphenol A, this region became hypomethylated with ageing resulting in persistent and elevated PDE4D4 expression.

CONCLUSIONS: In total, these findings indicate that low-dose exposures to ubiquitous environmental oestrogens impact the prostate epigenome during development and in so doing, promote prostate disease with ageing.

Prins GS, Birch L, Tang WY, Ho SM. "Developmental Estrogen Exposures Predispose to Prostate Carcinogenesis with Aging." *Reproductive Toxicology*, 23(3):374-382 (2007).

BACKGROUND: Prostate morphogenesis occurs in utero in humans and during the perinatal period in rodents. While largely driven by androgens, there is compelling evidence for a permanent influence of estrogens on prostatic development. If estrogenic exposures are abnormally high during the critical developmental period, permanent alterations in prostate morphology and function are observed, a process referred to as developmental estrogenization. Using the neonatal rodent as an animal model, it has been shown that early exposure to high doses of estradiol results in an increased incidence of prostatic lesions with aging which include hyperplasia, inflammatory cell infiltration and prostatic intraepithelial neoplasia or PIN, believed to be the precursor lesion for prostatic adenocarcinoma.

RESULTS: The present review summarizes research performed in the authors' laboratory to characterize developmental estrogenization and identify the molecular pathways involved in mediating this response. Furthermore, recent studies performed with low-dose estradiol exposures during development as well as exposures to environmentally relevant doses of the endocrine disruptor bisphenol A show increased susceptibility to PIN lesions with aging following additional adult exposure to estradiol. Gene methylation analysis revealed a potential epigenetic basis for the estrogen imprinting of the prostate gland.

CONCLUSION: Taken together, the authors' results suggest that a full range of estrogenic exposures during the postnatal critical period - from environmentally relevant bisphenol A exposure to low-dose and pharmacologic estradiol exposures - results in an increased incidence and susceptibility to neoplastic transformation of the prostate gland in the aging male which may provide a fetal basis for this adult disease.

Ho S, Tang W, Belmonte de Frausto J, Prins GS. "Developmental Exposure to Estradiol and Bisphenol A Increases Susceptibility to Prostate Carcinogenesis and Epigenetically Regulates Phosphodiesterase Type 4 Variant 4." *Cancer Research*, 66(11):5624-5632 (2006).

BACKGROUND: Early developmental perturbations have been linked to adult-onset prostate pathology, including excessive exposure to estrogenic compounds; however, the molecular basis for this imprinting event is not known. An important and controversial health concern is whether low-dose exposures to hormonally active environmental estrogens, such as bisphenol A, can promote human diseases, including prostate cancer. Here, we show that transient developmental exposure of rats to low, environmentally relevant doses of bisphenol A or estradiol increases prostate gland susceptibility to adult-onset precancerous lesions and hormonal carcinogenesis.

RESULTS: We found permanent alterations in the DNA methylation patterns of multiple cell signaling genes, suggesting an epigenetic basis for estrogen imprinting. For phosphodiesterase type 4 variant 4

(PDE4D4), an enzyme responsible for cyclic AMP breakdown, a specific methylation cluster was identified in the 5'-flanking CpG island that was gradually hypermethylated with aging in normal prostates, resulting in loss of gene expression. Early and prolonged hypomethylation at this site following neonatal estradiol or bisphenol A exposure resulted in continued, elevated PDE4D4 expression. Cell line studies confirmed that site-specific methylation is involved in transcriptional silencing of the *PDE4D4* gene and showed hypomethylation of this gene in prostate cancer cells. Importantly, the *PDE4D4* alterations in the estrogen-exposed prostates were distinguishable before histopathologic changes of the gland, making *PDE4D4* a candidate molecular marker for prostate cancer risk assessment as a result of endocrine disruptors.

CONCLUSION: In total, these findings indicate that low-dose exposures to ubiquitous environmental estrogens affect the prostate epigenome during development and, in so doing, promote prostate disease with aging.

Timms BG, Howdeshell KL, Barton L, Bradley S, Richter CA, vom Saal FS. "Estrogenic chemicals in plastic and oral contraceptives disrupt development of the fetal mouse prostate and urethra." *Proceedings of the National Academy of Sciences*, 102:7014-7019 (2005).

BACKGROUND: Exposure of human fetuses to man-made estrogenic chemicals can occur through several sources. For example, fetal exposure to ethinylestradiol occurs because each year approximately 3% of women taking oral contraceptives become pregnant. Exposure to the estrogenic chemical bisphenol A occurs through food and beverages because of significant leaching from polycarbonate plastic products and the lining of cans.

METHOD: We fed pregnant CD-1 mice ethinylestradiol (0.1 microg/kg per day) and bisphenol A (10 microg/kg per day), which are doses below the range of exposure by pregnant women.

RESULTS: In male mouse fetuses, both ethinylestradiol and bisphenol A produced an increase in the number and size of dorsolateral prostate ducts and an overall increase in prostate duct volume. Histochemical staining of sections with antibodies to proliferating cell nuclear antigen and mouse keratin 5 indicated that these increases were due to a marked increase in proliferation of basal epithelial cells located in the primary ducts. The urethra was malformed in the colliculus region and was significantly constricted where it enters the bladder, which could contribute to urine flow disorders. These effects were identical to those caused by a similar dose (0.1 microg/kg per day) of the estrogenic drug diethylstilbestrol (DES), a known human developmental teratogen and carcinogen. In contrast, a 2,000-fold higher DES dose completely inhibited dorsolateral prostate duct formation, revealing opposite effects of high and low doses of estrogen.

CONCLUSION: Acceleration in the rate of proliferation of prostate epithelium during fetal life by small amounts of estrogenic chemicals could permanently disrupt cellular control systems and predispose the prostate to disease in adulthood.

Effects on Prostate Cancer Treatment

Summary: *The goal of prostate cancer treatment is to shut down production of androgens that promote cancer growth. These two studies show that BPA interferes with the effectiveness of prostate cancer therapies by independently activating androgen receptors that promote tumor cell growth.*

The following excerpts are taken directly from the scientific abstracts.

Wetherill YB, Hess-Wilson JK, Comstock CES, Shah SA, Buncher CR, Sallans L, Limbach PA, Schwemberger S, Babcock GF, Knudsen KE. "Bisphenol A facilitates bypass of androgen ablation therapy in prostate cancer." *Molecular Cancer Therapy*, 5(12):3181-3190 (2006).

BACKGROUND: Prostatic adenocarcinomas depend on androgen for growth and survival. First line treatment of disseminated disease exploits this dependence by specifically targeting androgen receptor function. Clinical evidence has shown that androgen receptor is reactivated in recurrent tumors despite the continuance of androgen deprivation therapy. Several factors have been shown to restore androgen receptor activity under these conditions, including somatic mutation of the androgen receptor ligand-binding domain. We have shown previously that select tumor-derived mutants of the androgen receptor are receptive to activation by bisphenol A (BPA), an endocrine-disrupting compound that is leached from polycarbonate plastics and epoxy resins into the human food supply. Moreover, we have shown that BPA can promote cell cycle progression in cultured prostate cancer cells under conditions of androgen deprivation.

METHODS: Here, we challenged the effect of BPA on the therapeutic response in a xenograft model system of prostate cancer containing the endogenous BPA-responsive AR-T877A mutant protein.

RESULTS: We show that after androgen deprivation, BPA enhanced both cellular proliferation rates and tumor growth. These effects were mediated, at least in part, through androgen receptor activity, as prostate-specific antigen levels rose with accelerated kinetics in BPA-exposed animals.

CONCLUSION: Thus, at levels relevant to human exposure, BPA can modulate tumor cell growth and advance biochemical recurrence in tumors expressing the AR-T877A mutation.

Wetherill YB, Petre CE, Monk KR, Puga A, Knudsen KE. "The Xenoestrogen Bisphenol A Induces Inappropriate Androgen Receptor Activation and Mitogenesis in Prostatic Adenocarcinoma Cells." *Molecular Cancer Therapeutics*, 1:515-524 (2002).

BACKGROUND: Treatment for prostatic adenocarcinoma is reliant on the initial androgen dependence of this tumor type. The goal of therapy is to eliminate androgen receptor activity, either through direct inhibition of the receptor or through inhibition of androgen synthesis. Although this course of therapy is initially effective, androgen-refractory tumors ultimately arise and lead to patient morbidity. Factors contributing to the transition from a state of androgen dependence to the androgen-refractory state are poorly understood, but clinical evidence in androgen-refractory tumors suggests that the androgen receptor is inappropriately activated in these cells. Thus, the mechanisms that contribute to inappropriate (androgen-independent) activation of the androgen receptor (AR) is an area of intensive research. Here we demonstrate that bisphenol A (BPA), a polycarbonate plastic monomer and established xenoestrogen, initiates androgen-independent proliferation in human prostatic adenocarcinoma (LNCaP) cells.

RESULTS: The mitogenic capacity of BPA occurred in the nanomolar range, indicating that little BPA is required to stimulate proliferation. We show that BPA stimulated nuclear translocation of the tumor-derived receptor (AR-T877A), albeit with delayed kinetics compared with dihydrotestosterone. This translocation event was followed by specific DNA binding at androgen response elements, as shown by electrophoretic mobility shift assays. Moreover, the ability of BPA to stimulate AR-T877A activity was demonstrated by reporter assays and by analysis of an endogenous AR target gene, prostate-specific antigen. Thus, BPA is able to activate AR-T877A in the absence of androgens. Lastly, full mitogenic function of BPA is dependent on activation of the tumor-derived AR-T877A.

CONCLUSION: These data implicate BPA as an inappropriate mitogen for prostatic adenocarcinoma cells and provide the impetus to study the consequence of BPA exposure on prostate cancer.

Reproductive Effects in Females (Humans)

Summary: *This study links low-dose BPA exposure to recurrent miscarriage in women.*

The following excerpt is taken directly from the scientific abstract.

Sugiura-Ogasawara M, Ozaki Y, Sonta S, Makino T, Suzumori K. "Exposure to Bisphenol A is Associated with Recurrent Miscarriage." *Human Reproduction*, 20:2325-2329 (2005).

BACKGROUND: Little is known about the influence of high exposure to bisphenol A on recurrent miscarriage and immunoendocrine abnormalities.

METHODS: Serum bisphenol A, antiphospholipid antibodies (aPLs), antinuclear antibodies (ANAs), natural killer cell (NK) activity, prolactin, progesterone, thyroid-stimulating hormone (TSH) and free T4 were examined in 45 patients with a history of three or more (3-11) consecutive first-trimester miscarriages and 32 healthy women with no history of live birth and infertility. Subsequent pregnancy outcome and embryonic karyotype of abortuses were examined prospectively.

RESULTS: The mean \pm SD values for bisphenol A in patients were 2.59 \pm 5.23 ng/ml, significantly higher than the 0.77 \pm 0.38 ng/ml found for control women (P=0.024). High exposure to bisphenol A was associated with the presence of ANAs but not hypothyroidism, hyperprolactinaemia, luteal phase defects, NK cell activity or aPLs. A high level of bisphenol A in itself did not predict subsequent miscarriage.

CONCLUSION: Exposure to bisphenol A is associated with recurrent miscarriage.

Reproductive Effects in Females (animals)

Summary: *These studies explore the effects of low-dose BPA exposure on the reproductive system of rats and mice during fetal and neonatal development. As indicated earlier, BPA easily crosses the placenta from mother to fetus. The structural effects seen in exposed animals include ovarian cysts, uterine polyps, vaginal abnormalities, cervical cancer and, in some cases, mammary cancer. Functional changes include changes in hormone signaling, early onset of puberty and interference with estrus (comparable with menstrual cycle in humans). The effects of BPA-induced aneuploidy in female rodents appear to be permanent and multigenerational. Exposure to BPA during pregnancy disturbs egg cell development in unborn female fetuses. When these fetuses reach adulthood, the disturbances manifest as chromosomally abnormal eggs and embryos. As one author (Susiarjo et al) explained, "low-dose BPA exposure during pregnancy...increases the likelihood of chromosomally abnormal grandchildren."*

The following excerpts are taken directly from the scientific abstracts.

Newbold RR, Jefferson WN, Padilla-Banks E. "Prenatal Exposure to Bisphenol A at Environmentally-Relevant Doses Adversely Affects the Murine Female Reproductive Tract Later in Life." *Environmental Health Perspectives*, doi: 10.1289/ehp.0800045 [Online 15 January 2009].

BACKGROUND: Exposure to endocrine disrupting chemicals during critical developmental periods causes adverse consequences later in life; an example is prenatal exposure to the pharmaceutical diethylstilbestrol (DES). Bisphenol A (BPA), an environmental estrogen used in the synthesis of plastics, is of concern because its chemical structure resembles DES and it's a "high volume production" chemical with widespread human exposure.

OBJECTIVES: This study investigates whether prenatal BPA causes long-term adverse effects in female reproductive tissues by using an experimental animal model previously shown useful in studying effects of prenatal DES.

METHODS: Timed pregnant CD-1 mice were treated on days 9-16 of gestation with BPA (0.1, 1, 10, 100 or 1000 Hg/kg/day). After delivery, pups were held for 18 months when reproductive tissues were evaluated.

RESULTS: Ovarian cysts were significantly increased in the BPA-1 group; ovarian cystadenomas were seen in the BPA-10, 100, and 1000 groups but not Controls. Progressive proliferative lesion (PPL) of the oviduct was increased following BPA similar to that described following DES. Further, although not statistically different from Controls, prominent mesonephric (Wolffian) remnants and squamous metaplasia of the uterus, as well as, vaginal adenosis were observed in BPA mice similar to lesions reported following DES treatment. More severe pathologies were observed in some BPA animals included atypical hyperplasia and stromal polyps of the uterus, sarcoma of the uterine cervix, and mammary adenocarcinoma; these lesions were not observed in Controls.

CONCLUSION: These data suggest BPA causes long-term adverse reproductive and carcinogenic effects if exposure occurs during critical periods of differentiation.

Fernández M, Bianchi M, Lux-Lantos V, Libertun C. “Neonatal Exposure to Bisphenol A Alters Reproductive Parameters and Gonadotropin Releasing Hormone Signaling in Female Rats.” *Environmental Health Perspectives*, doi:10.1289/ehp.0800267 [Online 7 January 2009].

BACKGROUND: Bisphenol A (BPA) is a component of polycarbonate plastics, epoxy resins and polystyrene found in many products. Several reports revealed potent *in vivo* effects, as BPA acts like an estrogen agonist and/or antagonist and androgen and thyroid hormone antagonist.

Objectives: We analyzed the effects of neonatal exposure to BPA on the reproductive axis of female Sprague-Dawley rats.

METHODS: Females were injected subcutaneously, daily, from postnatal day 1 (PND1) to PND10 with BPA, 500 µg/50µl (high), 50 µg/50µl (low) in castor oil, or vehicle. We studied: body weight and age at vaginal opening, estrous cycles, pituitary hormone release *in vivo* and *in vitro*, as well as gonadotropin releasing hormone (GnRH) pulsatility, at PND13 and in adults. We also analyzed GnRH-activated signaling pathways in adults: inositol-triphosphate (IP3), and extracellular signal-regulated kinase 1/2 (ERK1/2).

RESULTS: Exposure to BPA altered pituitary function in infantile rats, lowering basal and GnRH-induced luteinizing hormone (LH), and increasing GnRH pulsatility. BPA dose dependently accelerated puberty onset and altered estrous cyclicity, the high dose causing permanent estrus. In adults, neonatal BPA decreased GnRH-induced LH secretion *in vivo*, and GnRH pulsatility remained disrupted. *In vitro*, pituitary cells from BPA-animals showed lower basal LH, dose-dependently affected GnRH-induced IP3 formation; the higher dose also impaired GnRH-induced LH secretion. In addition, both doses altered ERK1/2 activation.

CONCLUSIONS: Neonatal exposure to BPA altered reproductive parameters and hypothalamic-pituitary function in female rats. To our knowledge, these results demonstrate for the first time that neonatal *in vivo* BPA permanently affects GnRH pulsatility and pituitary GnRH signaling.

Susiarjo M, Hassold TJ, Freeman E, Hunt PA. “Bisphenol A Exposure In Utero Disrupts Early Oogenesis in the Mouse.” *PLoS Genetics*, 3(1): e5. doi:10.1371/journal.pgen.0030005 (2007).

BACKGROUND: Estrogen plays an essential role in the growth and maturation of the mammalian oocyte, and recent studies suggest that it also influences follicle formation in the neonatal ovary. In the course of studies designed to assess the effect of the estrogenic chemical bisphenol A (BPA) on mammalian

oogenesis, we uncovered an estrogenic effect at an even earlier stage of oocyte development—at the onset of meiosis in the fetal ovary.

METHODS: Pregnant mice were treated with low, environmentally relevant doses of BPA during mid-gestation to assess the effect of BPA on the developing ovary.

RESULTS: Oocytes from exposed female fetuses displayed gross aberrations in meiotic prophase, including synaptic defects and increased levels of recombination. In the mature female, these aberrations were translated into an increase in aneuploid eggs and embryos. Surprisingly, we observed the same constellation of meiotic defects in fetal ovaries of mice homozygous for a targeted disruption of ER β , one of the two known estrogen receptors. This, coupled with the finding that BPA exposure elicited no additional effects in ER β null females, suggests that BPA exerts its effect on the early oocyte by interfering with the actions of ER β .

CONCLUSIONS: Together, the results show that BPA can influence early meiotic events and, importantly, indicate that the oocyte itself may be directly responsive to estrogen during early oogenesis. This raises concern that brief exposures during fetal development to substances that mimic or antagonize the effects of estrogen may adversely influence oocyte development in the exposed female fetus.

Newbold RR, Jefferson WR, Banks EP. “Long-term Adverse Effects of Neonatal Exposure to Bisphenol A on the Murine Female Reproductive Tract.” *Reproductive Toxicology* 24:253-258, (2007).

BACKGROUND: The developing fetus is uniquely sensitive to perturbation by chemicals with hormone-like activity. The adverse effects of prenatal diethylstilbestrol (DES) exposure are a classic example.

OBJECTIVE: Since concern has been mounting regarding the human health and environmental effects of bisphenol A (BPA), a high-production-volume chemical with estrogenic activity used in the synthesis of plastics, we investigated its long-term effects in an experimental animal model that was previously shown useful in studying the adverse effects of developmental exposure to DES.

METHOD: Outbred female CD-1 mice were treated on days 1-5 with subcutaneous injections of BPA (10, 100 or 1000 microg/kg/day) dissolved in corn oil or corn oil alone (Control). At 18 months, ovaries and reproductive tract tissues were examined.

RESULTS: There was a statistically significant increase in cystic ovaries and cystic endometrial hyperplasia (CEH) in the BPA-100 group as compared to Controls. Progressive proliferative lesion (PPL) of the oviduct and cystic mesonephric (Wolffian) duct remnants were also seen in all of the BPA groups. More severe pathologies of the uterus following neonatal BPA treatment included adenomyosis, leiomyomas, atypical hyperplasia, and stromal polyps.

CONCLUSION: These data suggest that BPA causes long-term adverse effects if exposure occurs during critical periods of differentiation.

Markey CM, Wadia PR, Rubin BS, Sonnenschein C, Soto AM. “Long-term Effects of Fetal Exposure to Low Doses of the Xenoestrogen Bisphenol-A in the Female Mouse Genital Tract.” *Biology of Reproduction* 72: 1344-1351, (2005).

BACKGROUND: Developmental exposure to estrogenic chemicals induces morphological, functional, and behavioral anomalies associated with reproduction. Humans are routinely exposed to bisphenol-A (BPA), an estrogenic compound that leaches from dental materials and plastic food and beverage containers.

OBJECTIVE: The aim of the present study was to determine the effects of in utero exposure to low, environmentally relevant doses of BPA on the development of female reproductive tissues in CD-1 mice. In previous publications, we have shown that this treatment alters the morphology of the mammary gland and affects estrous cyclicity.

METHOD/RESULTS: Here we report that in utero exposure to 25 and 250 ng BPA/ kg of body weight per day via osmotic pumps implanted into pregnant dams at Gestational Day 9 induces alterations in the

genital tract of female offspring that are revealed during adulthood. They include decreased wet weight of the vagina, decreased volume of the endometrial lamina propria, increased incorporation of bromodeoxyuridine into the DNA of endometrial gland epithelial cells, and increased expression of estrogen receptor-alpha (ERalpha) and progesterone receptor in the luminal epithelium of the endometrium and subepithelial stroma.

CONCLUSIONS: Because ERalpha is known to be expressed in these estrogen-target organs at the time of BPA exposure, it is plausible that BPA may directly affect the expression of ER-controlled genes involved in the morphogenesis of these organs. In addition, BPA-induced alterations that specifically affect hypothalamic-pituitary-gonadal axis function may further contribute to the anomalies observed at 3 months of age, long after the cessation of BPA exposure.

Nikaido Y, Yoshizawa K, Danbara N, Tsujita-Kyutoku M, Yuri T, Uehara N, Tsubura A. "Effects of maternal xenoestrogen exposure on development on the reproductive tract and mammary gland in female CD-1 mouse offspring." *Reproductive Toxicology*, 18:803-811 (2004).

OBJECTIVE: The objective of this study was to examine the effects of maternal exposure to xenoestrogen, at levels comparable to or greater than human exposure, on development of the reproductive tract and mammary glands in female CD-1 mouse offspring. Effects of genistein (GEN), resveratrol (RES), zearalenone (ZEA), bisphenol A (BPA) and diethylstilbestrol (DES) were examined.

METHODS: Beginning on gestational day 15, pregnant CD-1 mice were administered four daily subcutaneous injections with 0.5 or 10 mg/kg/day of GEN, RES, ZEA or BPA, 0.5 or 10 µg/kg/day of DES dissolved in dimethylsulfoxide (DMSO), or DMSO vehicle (n = 6). Vaginal opening was monitored, 6 animals per group were autopsied at 4, 8, 12 and 16 weeks of age and estrous cyclicity was monitored from 9 to 11 weeks of age.

RESULTS: Maternal exposure to xenoestrogen accelerated puberty onset (vaginal opening) and increased the length of the estrous cycle; mice treated with GEN, RES, BPA or DES spent more time in diestrus, and ZEA-treated mice spent more time in estrus. Lack of corpora lutea and vaginal cornification were observed at 4 weeks of age in the high-dose GEN (33%) and RES (17%) groups, and in the high- and low-dose BPA groups (33 and 50%, respectively) and DES groups (83 and 100%, respectively). Lack of corpora lutea and vaginal cornification was observed in the high-dose ZEA group at 4, 8, 12 and 16 weeks of age (83, 100, 83 and 33%, respectively). Mammary gland differentiation was accelerated in ZEA- and BPA-treated mice with corpora lutea at 4 weeks of age. ZEA-treated mice without corpora lutea showed mammary growth arrest at 8, 12 and 16 weeks of age; their mammary glands consisted only of a dilated duct filled with secreted fluid. Mammary gland growth was similar with xenoestrogens other than ZEA or BPA to that of the controls at all time points.

CONCLUSION: High-dose GEN and RES and high- and low-dose BPA and DES exerted transient effects on the reproductive tract and mammary glands, whereas ZEA exerted prolonged effects.

Hunt, PA, Koehler KE, Susiarjo M, Hodges CA, Ilagan A, Voigt RC, Thomas S, Thomas BF, Hassold TJ. "Bisphenol A Exposure Causes Meiotic Aneuploidy in the Female Mouse." *Current Biology*, 13: 546-553 (2003).

BACKGROUND: There is increasing concern that exposure to man-made substances that mimic endogenous hormones may adversely affect mammalian reproduction. Although a variety of reproductive complications have been ascribed to compounds with androgenic or estrogenic properties, little attention has been directed at the potential consequences of such exposures to the genetic quality of the gamete.

RESULTS: A sudden, spontaneous increase in meiotic disturbances, including aneuploidy, in studies of oocytes from control female mice in our laboratory coincided with the accidental exposure of our animals to an environmental source of bisphenol A (BPA). BPA is an estrogenic compound widely used in

the production of polycarbonate plastics and epoxy resins. We identified damaged caging material as the source of the exposure, as we were able to recapitulate the meiotic abnormalities by intentionally damaging cages and water bottles. In subsequent studies of female mice, we administered daily oral doses of BPA to directly test the hypothesis that low levels of BPA disrupt female meiosis. Our results demonstrated that the meiotic effects were dose dependent and could be induced by environmentally relevant doses of BPA.

CONCLUSIONS: Both the initial inadvertent exposure and subsequent experimental studies suggest that BPA is a potent meiotic aneugen. Specifically, in the female mouse, short-term, low-dose exposure during the final stages of oocyte growth is sufficient to elicit detectable meiotic effects. These results provide the first unequivocal link between mammalian meiotic aneuploidy and an accidental environmental exposure and suggest that the oocyte and its meiotic spindle will provide a sensitive assay system for the study of reproductive toxins.

Honma S, Suzuki A, Buchanan DL, Katsu Y, Watanabe H and Iguchi T. "Low Dose Effect of *in utero* Exposure to Bisphenol A and Diethylstilbestrol on Female Mouse Reproduction." *Reproductive Toxicology*, 16:117-122 (2002).

BACKGROUND: In utero exposure to bisphenol-A (BPA) at doses relevant to human consumption has been reported to accelerate weight gain and puberty in female mice, but the effect of low dose BPA on female reproduction has not been described. In this study, we investigated low dose effects of BPA on sexual maturation and reproduction in female ICR/Jcl mice.

METHODS: Pregnant ICR mice (F0) were injected (s.c.) with BPA (2 and 20 microg/kg), diethylstilbestrol (DES; 0.02, 0.2, and 2 microg/kg) or oil vehicle once per day from gestational days 11-17. For both female and male offspring (F1), body weights were measured on postnatal day (PND) 0 (the day of birth), 11, 22, and 60, and anogenital distance (AGD) was measured on PNDs 22 and 60. Pups were weaned at PND 22 and males were caged separately from females. Vaginal smears were taken daily beginning the day of vaginal opening for 30 days.

RESULTS: The age at vaginal opening was significantly earlier in all exposed females except for 2 microg/kg BPA females compared to oil controls. Body weight at vaginal opening was lower than controls in all exposed females. The first vaginal estrus was earlier in all exposed females except for the 2 microg/kg BPA group females compared to controls. From PND 90 to 120, gestationally exposed F1 female mice were mated with unexposed males. Total numbers of pups and sex ratio in F1 mice exposed to BPA or DES, and those of their offspring (F2) were not different from controls in any treatment group.

CONCLUSION: The present results indicate that prenatal exposure to low doses of BPA and DES induces early vaginal opening, but does not affect reproductive functioning at the first breeding.

Schonfelder G, Flick B, Mayr E, Talsness C, Paul M, Chahoud I. "In Utero Exposure to Low Doses of Bisphenol A Lead to Long-Term Deleterious Effects in the Vagina." *Neoplasia*, 4:99-102 (2002).

BACKGROUND: The origins of the "endocrine disrupter hypothesis" may be traced to reports on adolescent daughters born to women who had taken the highly potent synthetic estrogen, diethylstilbestrol, while pregnant, and who developed a rare form of vaginal cancer and adenocarcinoma. Bisphenol A (BPA) is an estrogenic chemical that is highly employed in the manufacture of a wide range of consumer products. Some observational studies have suggested that the amounts of BPA to which we are exposed could alter the reproductive organs of developing rodents. We examined the influence of BPA at low doses to address the questions of (a) whether in utero exposure affects the vagina of the offspring and (b) which mechanisms cause the toxic effects.

METHODS: Gravid Sprague-Dawley dams were administered either 0.1 (low dose) or 50 mg/kg per day BPA, the no observed effect level, or 0.2 mg/kg per day 17 alpha-ethinyl estradiol by gavage.

RESULTS: Striking morphological changes were observed in the vagina of postpubertal offspring leading us to examine vaginal estrogen receptor (ER) expression because BPA binds to the ER alpha, which is important for growth of the vaginal epithelium. We show that the full-length ER alpha is not expressed during estrus in the vagina of female offspring exposed to either dose of BPA when compared to the control group, whereas ER alpha expression does not differ from the control group during the diestrus stage.

CONCLUSION: ER alpha downregulation seems to be responsible for the observed altered vaginal morphology.

Rubin BS, Murray MK, Damassa DA, King JC, Soto AM. "Perinatal Exposure to Low Doses of Bisphenol A Affects Body Weight, Patterns of Estrous Cyclicity, and Plasma LH Levels." *Environmental Health Perspectives*, 109:675-680 (2001).

BACKGROUND: The nonsteroidal estrogenic compound bisphenol A (BPA) is a monomer used in the manufacture of polycarbonate plastics and resins. BPA may be ingested by humans as it reportedly leaches from the lining of tin cans into foods, from dental sealants into saliva, and from polycarbonate bottles into their contents. Because BPA is weakly estrogenic--approximately 10,000-fold less potent than 17 β -estradiol--current environmental exposure levels have been considered orders of magnitude below the dose required for adverse effects on health.

METHODS: Herein we demonstrate measurable effects on the offspring of Sprague-Dawley female rats that were exposed, via their drinking water, to approximately 0.1 mg BPA/kg body weight (bw) /day (low dose) or 1.2 mg BPA/kg bw/day (high dose) from day 6 of pregnancy through the period of lactation.

RESULTS: Offspring exposed to BPA exhibited an increase in body weight that was apparent soon after birth and continued into adulthood. In addition, female offspring exposed perinatally to the high dose of BPA exhibited altered patterns of estrous cyclicity and decreased levels of plasma luteinizing hormone (LH) in adulthood. Administration of neither the doses of BPA that caused effects during perinatal exposure nor a 10-fold higher dose was able to evoke a uterotrophic response in ovariectomized postpubertal females.

CONCLUSION: These data indicate an increased sensitivity to BPA during the perinatal period and suggest the need for careful evaluation of the current levels of exposure to this compound.

Takahashi O and Oishi S. "Disposition of Orally Administered 2,2-Bis(4-hydroxyphenyl)propane (Bisphenol A) in Pregnant Rats and the Placental Transfer to Fetuses." *Environmental Health Perspectives*, 108:931-935 (2000).

BACKGROUND: We studied the disposition of bisphenol A (BPA) in pregnant female F344/DuCrj(Fischer) rats and its placental transfer to fetuses after a single oral administration of 1 g/kg BPA dissolved in propylene glycol.

RESULTS: BPA in maternal blood, liver, and kidney reached maximal concentrations (14.7, 171, and 36 μ g/g) 20 min after the administration and gradually decreased. The levels were 2-5% of the maximum 6 hr after the administration. The maximal concentration of BPA in fetuses (9 μ g/g) was also attained 20 min after the administration. BPA levels then gradually reduced in a similar manner to maternal blood.

CONCLUSION: These results suggest that the absorption and distribution of BPA in maternal organs and fetuses are extremely rapid and that the placenta does not act as a barrier to BPA.

Takai Y, Tsutsumi O, Ikezuki Y, Kamei Y, Osuga Y, Yano T, Taketan Y. "Preimplantation Exposure to Bisphenol A Advances Postnatal Development." *Reproductive Toxicology*, 15:71-74 (2000).

BACKGROUND: Prenatal exposure to bisphenol A (BPA), an estrogenic compound, has been shown to alter postnatal development at an environmentally relevant exposure level.

METHOD: To elucidate these low dose effects of preimplantation exposure to BPA, two-cell mouse embryos were cultured with 1 nM BPA.

RESULTS: More embryos exposed to 1 nM BPA than controls reached the blastocyst stage. When the blastocysts with or without BPA exposure were transferred to uterine horns of pseudopregnant recipient mice not treated with BPA, the number of pups per litter and body weight at birth did not differ. At weaning on postnatal day 21, however, pups treated with 1 nM BPA during the preimplantation period were significantly heavier than controls.

CONCLUSION: These findings suggest that BPA may not only affect early embryonic development even at low, environmentally relevant doses, but also may exert late effects on postnatal development.

Reproductive Effects in Males (cell studies)

Summary: *This new study shows that low-dose BPA increases the proliferation of testicular seminoma cells in culture without activating the estrogen receptor. This suggests that BPA could increase the risk of testicular cancer.*

The following excerpt is taken directly from the scientific abstract.

Bouskine A, Nebout M, Brücker-Davis, Benahmed M, Fenichel P. “Low Doses of Bisphenol A Promote Human Seminoma Cell Proliferation by Activating PKA and PKG via a Membrane G protein-coupled Estrogen Receptor.” *Environmental Health Perspectives* doi:10.1289/ehp.0800367 Online 11 February (2009).

BACKGROUND: Fetal exposure to environmental estrogens may contribute to hypofertility and/or to testicular germ cell cancer. However, many of these xenoestrogens have only a weak affinity for the classical ERs, which is 1000 fold less potent than the affinity of E2. Thus several mechanisms have been suggested in order to explain how they could affect male germ cell proliferation at low environmental relevant concentrations.

OBJECTIVES: In this study we aimed to explore the possible promoting effect of Bisphenol A (BPA), a well recognized estrogenic endocrine disruptor, used as monomer to manufacture polycarbonate plastic, released from resin lining canned food or beverages or from dental sealants, on human testicular seminoma cells.

METHODS and RESULTS: BPA at very low concentrations (10⁻⁹ to 10⁻¹²M) similar to that found in human fluids was able to stimulate JKT-1 cell proliferation in vitro. BPA activated both PKA and PKG pathways and triggered a rapid (15min) phosphorylation of the transcription factor CREB and the cell cycle regulator Rb. This non genomic activation, did not involve classical ERs since it could not be reversed by ICI182780, an ER antagonist, nor reproduced either by E2 or by DES a potent synthetic estrogen which triggered instead, a suppressive effect. It was only reproduced by E2 coupled to BSA, unable to enter the cell. As E2-BSA, BPA promoted JKT-1 cell proliferation through a G protein coupled non classical membrane estrogen receptor (GPCR) involving a Gas and a Gai/Gaq subunit as shown by the reversible effect observed by the corresponding inhibitors NF449 and PertussisToxin.

CONCLUSION: This GPCR-mediated non genomic action represents in addition to the classical ER-mediated effect, a new basis for evaluating xenoestrogens as BPA, which could at low doses and with a high affinity for this GPCR, interfere, when crossing the placenta, with the developmental programming of fetal germ cell proliferation and/or differentiation.

Summary: In this study, scientist used a special assay to predict how BPA and another estrogenic chemical, octylphenol, would affect the reproductive system of male and female mice. They found that exposure to BPA at low doses (parts per billion—PPB) comparable to average human exposure would alter the reproductive system in adult mice.

The following excerpt is taken directly from the scientific abstract.

Nagel SC, vom Saal FS, Thayer KA, Dhar MG, Boechler M, Welshons WV. “Relative Binding Affinity-Serum Modified Access (RBA-SMA) Assay Predicts the Relative *in vivo* Bioactivity of the Xenoestrogens Bisphenol A and Octylphenol.” *Environmental Health Perspectives*, 105(1):70-76 (1997).

METHODS: We have developed a relative binding affinity-serum modified access (RBA-SMA) assay to determine the effect of serum on the access of xenoestrogens to estrogen receptors within intact cultured MCF-7 human breast cancer cells. We used this assay to predict low dose activity of two xenoestrogens in mice.

RESULTS: In serum-free medium, bisphenol A, a component of polycarbonates and of resins used to line metal food cans, showed a lower relative binding affinity (RBA; 0.006%) than octylphenol (0.072%) and nonylphenol (0.026%), which are used as surfactants in many commercial products (all RBAs are relative to estradiol, which is equal to 100%). In 100% serum from adult men, bisphenol A showed a higher RBA (0.01%) than in serum-free medium and thus enhanced access to estrogen receptors relative to estradiol. In contrast, octylphenol showed a 22-fold decrease in RBA (0.0029%) and nonylphenol showed a 5-fold decrease in RBA (0.0039%) when measured in adult serum. This indicates that, relative to estradiol, serum had less of an inhibitory effect on the cell uptake and binding in MCF-7 cells of bisphenol A, while serum had a greater inhibitory effect on octylphenol and nonylphenol relative to estradiol. Extrapolation of these relative activities in adult serum predicted that the estrogenic bioactivity of bisphenol A would be over 500-fold greater than that of octylphenol in fetal mouse serum. Bisphenol A and octylphenol were fed to pregnant mice at 2 and 20 micrograms/kg/day. Exposure of male mouse fetuses to either dose of bisphenol A, but to neither dose of octylphenol, significantly increased their adult prostate weight relative to control males, which is consistent with the higher predicted bioactivity of bisphenol A than octylphenol in the RBA-SMA assay.

CONCLUSION: In addition, our findings show for the first time that fetal exposure to environmentally relevant parts-per-billion (ppb) doses of bisphenol A, in the range currently being consumed by people, can alter the adult reproductive system in mice.

Reproductive Effects in Males (animals)

Summary: The following studies show that exposure to BPA at various times across the lifespan can affect reproductive structure and function of male rodents. The study that immediately follows was the first to show that fetal exposure to estrogenic chemicals such as BPA directly affect fetal reproductive organs without interfering with the endocrine system. These chemicals can cause malformations even without fetal testosterone but are more effective when testosterone is present.

Other studies show that BPA exposure decreases sperm production, sperm quality and fertility.

The following excerpts are taken directly from the scientific abstracts.

Chhanda Gupta. "Reproductive Malformation of the Male Offspring Following Maternal Exposure to Estrogenic Chemicals." *Proceedings of the Society of Experimental Biology and Medicine*, 224:61-68 (2000).

BACKGROUND: Recently, significant concerns have been placed on the widespread use of chemicals with persistent estrogenic activity for their long-term effects on human health. In this communication, we investigated whether fetal exposure to some of these chemicals at doses consumed by people, has any long-term effect on the reproductive functions of the male offspring.

METHODS: Thus, time-pregnant CD-1 mice were fed diethylstilbestrol (DES), bisphenol A (BPA), and aroclor (aroclor 1016) at an average concentration of 100 ng/kg/day, 50 µg/kg/day, and 50 µg/kg/day, respectively, during Days 16–18 of gestation. A high dose of DES (200 µg/kg/day) was also tested to compare the results of the current study with those of others using the high dose only. The offspring were examined at Day 3, Day 21, and Day 60 following birth.

RESULTS: We demonstrated that BPA, aroclor, and the lower dose of DES enhanced anogenital distance, increased prostate size, and decreased epididymal weight. No effect was found on the testicular weight or size. The chemicals also permanently increased androgen receptor (AR) binding activity of the prostate at this dosage. This is the first demonstration that environmental chemicals program AR function permanently at the dosage consumed by the general population. The higher dosage of DES, on the other hand, produced an opposite effect, decreasing prostate weight, prostate AR binding, and anogenital distance, thus confirming the previous reports.

METHODS: To investigate whether the above mentioned effects of the chemicals represent direct or indirect effects, we also tested the effect of the chemicals on prostate development *in vitro*. Thus fetal urogenital sinus (UGS), isolated at the 17th day of gestation was cultured with the chemicals in the presence and absence of testosterone (10 ng/ml) for 6 days, and prostate growth was monitored by determining the size and branching of the specimen following histology.

RESULTS: Results showed that these chemicals induced prostate growth in the presence and absence of testosterone. They also increased androgen-binding activity. Thus, the results of the *in vivo* studies were reproduced in the *in vitro* experiments, suggesting a direct effect of these chemicals on the development of fetal reproductive organs.

CONCLUSION: This is the first demonstration that estrogenic chemicals induce reproductive malformation by direct interference with the fetal reproductive organs and not by interfering with the maternal or fetal endocrine system. The chemicals are able to induce malformation even in the absence of fetal testosterone; however, they are more effective in the presence of testosterone.

Chitra KC, Latchoumycandane C, Mathur PP. "Induction of oxidative stress by bisphenol A in the epididymal sperm of rats." *Toxicology*, 185(1-2):119-127 (2003).

BACKGROUND: Bisphenol A has been shown to affect the reproduction of male rats and mice. However, the mechanism of action of bisphenol A on the epididymal sperm is not elucidated. The present study was undertaken to evaluate the effect of bisphenol A on the antioxidant system of rat epididymal sperm.

METHODS: Bisphenol A was administered orally to male rats at the dose levels of 0.2, 2 and 20 microg/Kg body weight per day for 45 days. After 24 h of the last treatment, rats were weighed and killed using anesthetic ether.

RESULTS: The body weight of treated rats did not show significant change as compared with the corresponding control groups. In bisphenol A-treated rats there was a significant decrease in the weight of the testis and epididymis; the weight of ventral prostate increased significantly whereas there was no significant change in the weight of seminal vesicles as compared with the corresponding group of control animals. Sperm collected from the epididymis were used for sperm count and biochemical estimations. Administration of bisphenol A caused a reduction in the epididymal sperm motility and

sperm count in a dose-dependent manner. The activities of superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase were decreased while the levels of H₂O₂ and lipid peroxidation increased significantly in the treated rats as compared with the corresponding group of control animals. **CONCLUSION:** The results suggested that graded doses of bisphenol A elicit depletion of antioxidant defense system and induce oxidative stress in epididymal sperm of rats. In conclusion, the adverse effect of bisphenol A on male reproduction may be due to induction of oxidative stress in sperm.

Al-Hiyasat AS, Darmani H, Elbetieha AM. "Effects of Bisphenol A on adult male mouse fertility." *European Journal of Oral Sciences*, 110:163-167 (2002).

BACKGROUND: The aim of this investigation was to evaluate the effect of bisphenol A (BPA), a contaminant of resin-based dental composites and sealants, on the fertility of male mice.

METHODS: Forty adult male Swiss mice were divided into four groups of 10. BPA (5, 25 and 100 ng/kg⁻¹) was administered intragastrically daily to the mice in the test groups and distilled water to the control group for 28 d. Male fertility was assessed by mating each mouse with two untreated females.

RESULTS: Females mated with male mice having ingested 25 and 100 ng/kg⁻¹ BPA showed a significant reduction in pregnancy rates. Furthermore, the total number of resorptions out of the total number of implantations was significantly increased in females impregnated with males having ingested all three doses of BPA. Males having ingested 25 and 100 ng/kg⁻¹ BPA showed a significant reduction in testicular sperm counts and in the efficiency of sperm production. Epididymal sperm counts were also significantly reduced in males that had ingested BPA. There were significant reductions in the absolute weights of the testes and seminal vesicles.

CONCLUSION: These results suggest that male fertility and reproduction is impaired by bisphenol A.

Sakaue M, Ohsako S, Ishimura R, Kuroawa S, Kurohmaru M, Hayashi Y, Aoki Y, Yonemoto J, Tohyama C. "Bisphenol-A Affects Spermatogenesis in the Adult Rat Even at a Low Dose." *Journal of Occupational Health*, 43:185-190 (2001).

BACKGROUND: Bisphenol-A (BPA), a xenobiotic estrogenic compound widely used as a plastics monomer, has been suspected to have a so-called low dose effect on the reproductive system when administered transplacentally. In the present study, we investigated possible low-dose effects of BPA on spermatogenesis in adult rats.

METHODS: Male rats (13 weeks old; W13) were administered a daily oral dose of BPA, ranging from 2 ng to 200 mg/kg, for 6 days and examined for testicular weight (TW) and daily sperm production (DSP) at W14 and W18.

RESULTS: A BPA dose as low as 20 µg/kg tended to decrease TW and significantly reduced both DSP and the efficiency of spermatogenesis (DSP per gram testis) at W18, showing that BPA suppressed a normal increase in DSP and TW from W13 to W18. A single administration of 20 µg BPA/kg to W13 rats affected the intensity or mobility of several protein spots in the testicular cytosol fraction as shown by two-dimensional gel electrophoresis analysis.

CONCLUSION: The present study showed that BPA at a low dose affects spermatogenesis in the adult rat.

Vom Saal FS, Cooke PS, Buchanan DL, Palanza P, Thayer KA, Nagel SC, Parmigiani S, Welshons WV. "A Physiologically Based Approach to the Study of Bisphenol A and Other Estrogenic Chemicals on the Size of Reproductive Organs, Daily Sperm Production, and Behavior." *Toxicology and Industrial Health*, 14: 239-260 (1998).

BACKGROUND: Two chemicals previously shown to have estrogenic activity, bisphenol A and octylphenol, were examined for their effects on accessory reproductive organs and daily sperm

production in male offspring of mice fed these chemicals during pregnancy. These chemicals are used in the manufacture of plastics and other products, and have been detected in food and water consumed by animals and people.

METHODS: From gestation day 11-17 female mice were fed an average concentration (dissolved in oil) of bisphenol A or octylphenol of 2 ng/g body weight (2 ppb) and 20 ng/g (20 ppb). The 2 ppb dose of bisphenol A is lower than the amount reported to be swallowed during the first hour after application of a plastic dental sealant (up to 931 micrograms; 13.3 ppb in a 70 kg adult).

RESULTS: We found that the 2 ng/g dose of bisphenol A permanently increased the size of the preputial glands, but reduced the size of the epididymides; these organs develop from different embryonic tissues. At 20 ng/g, bisphenol A significantly decreased efficiency of sperm production (daily sperm production per g testis) by 20% relative to control males. The only significant effect of octylphenol was a reduction in daily sperm production and efficiency of sperm production at the 2 ng/g dose.

CONCLUSION: A new approach to studying physiologically relevant doses of environmental endocrine disruptors is discussed, particularly with regard to the development of the reproductive organs, the brain, and behavior.

Metabolic effects (humans)

Summary: *In this study of nearly 1,500 U.S. adults, scientists found that higher concentrations of BPA in urine were associated with cardiovascular problems, diabetes and abnormalities in liver enzymes. These findings suggest that higher exposure to BPA may contribute to chronic health problems in adults. This study is the first human study with **RESULTS:** similar to findings in laboratory animals (see next section).*

The following excerpt is taken directly from the scientific abstract.

Lang IA, Galloway TS, Scarlett A, Henley WE, Depledge M, Wallace RB, Melzer D. "Association of Urinary Bisphenol A Concentration with Medical Disorders and Laboratory Abnormalities in Adults." *JAMA*, 300(11):1303-1310 (2008).

BACKGROUND: Bisphenol A (BPA) is widely used in epoxy resins lining food and beverage containers. Evidence of effects in animals has generated concern over low-level chronic exposures in humans.

OBJECTIVE: To examine associations between urinary BPA concentrations and adult health status.

METHODS: Cross-sectional analysis of BPA concentrations and health status in the general adult population of the United States, using data from the National Health and Nutrition Examination Survey 2003-2004. Participants were 1455 adults aged 18 through 74 years with measured urinary BPA and urine creatinine concentrations. Regression models were adjusted for age, sex, race/ethnicity, education, income, smoking, body mass index, waist circumference, and urinary creatinine concentration. The sample provided 80% power to detect unadjusted odds ratios (ORs) of 1.4 for diagnoses of 5% prevalence per 1-SD change in BPA concentration, or standardized regression coefficients of 0.075 for liver enzyme concentrations, at a significance level of $P < .05$.

MAIN OUTCOME MEASURE: Chronic disease diagnoses plus blood markers of liver function, glucose homeostasis, inflammation, and lipid changes.

RESULTS: Higher urinary BPA concentrations were associated with cardiovascular diagnoses in age-, sex-, and fully adjusted models (OR per 1-SD increase in BPA concentration, 1.39; 95% confidence interval [CI], 1.18-1.63; $P = .001$ with full adjustment). Higher BPA concentrations were also associated with diabetes (OR per 1-SD increase in BPA concentration, 1.39; 95% confidence interval [CI], 1.21-1.60; $P < .001$) but not with other studied common diseases. In addition, higher BPA concentrations were associated with

clinically abnormal concentrations of the liver enzymes γ -glutamyltransferase (OR per 1-SD increase in BPA concentration, 1.29; 95% CI, 1.14-1.46; $P < .001$) and alkaline phosphatase (OR per 1-SD increase in BPA concentration, 1.48; 95% CI, 1.18-1.85; $P = .002$).

CONCLUSION: Higher BPA exposure, reflected in higher urinary concentrations of BPA, may be associated with avoidable morbidity in the community-dwelling adult population.

Metabolic effects (animals)

Summary: *These studies show that exposure to BPA caused insulin resistance, high cholesterol (hyperlipidemia), high blood pressure (hypertension) and increased body weight in mice. These effects in humans all increase the risk of developing type II diabetes. In two of the studies, adult mice were exposed to BPA. In the third study (Miyawaki, et al), female mice were exposed to BPA during pregnancy and lactation, and effects of BPA exposure were seen in the offspring. This study reaffirms that early-life exposure can have lifelong effects.*

The following excerpts are taken directly from the scientific abstracts.

Ropero AB, Alonso-Magdalena P, García-García E, Ripoll C, Fuentes E, Nadal A. "Bisphenol-A Disruption of the Endocrine Pancreas and Blood Glucose Homeostasis." *International Journal of Andrology*, 31(2):194-200 (2008).

BACKGROUND: The link between endocrine disruptors and altered blood glucose homeostasis has been recently suggested. Epidemiological studies have correlated levels of phthalates, dioxins and persistent organic pollutants with alterations of blood glucose homeostasis in humans. Environmentally relevant doses of the ubiquitous endocrine disruptor bisphenol-A (BPA) have profound effects on mice endocrine pancreas—an essential tissue involved in glucose metabolism. BPA exerts rapid non-genomic effects on insulin releasing beta-cells and glucagon releasing alpha-cells within freshly isolated islets of Langerhans. In vivo, a single BPA injection of 10 microg/kg rapidly increases plasma insulin and concomitantly decreases glycaemia.

METHODS: When mice were treated with BPA 100 microg/kg/day for 4 days, the environmental oestrogen produced an increase in beta-cell insulin content along with a post-prandial hyperinsulinaemia and insulin resistance.

RESULTS: The results reviewed here demonstrate that doses well below the current lowest observed adverse effect level considered by the US-EPA, disrupt pancreatic beta-cell function producing insulin resistance in male mice.

CONCLUSION: Altered blood glucose homeostasis by BPA exposure may enhance the risk of developing type II diabetes.

Miyawaki J, Sakayama K, Kato H, Yamamoto H, Masuno H. "Perinatal and Postnatal Exposure to Bisphenol A Increases Adipose Tissue Mass and Serum Cholesterol Level in Mice." *Journal of Atherosclerosis and Thrombosis*, 14:245-252 (2007).

OBJECTIVE: To investigate whether the perinatal and postnatal exposure of mice to bisphenol A (BPA) caused the development of obesity and/or hyperlipidemia.

METHODS: Pregnant mice were exposed to BPA in drinking water at concentrations of either 1 microg/mL (LD group) or 10 microg/mL (HD group) from gestation day 10 and throughout the lactating period. After weaning, the pups were allowed free access to drinking water containing the appropriate concentrations of BPA. The body weight, adipose tissue weight, and serum lipid levels were measured in the offspring at postnatal day 31.

RESULTS: In females, the mean body weight increased by 13% in the LD group ($p < 0.05$) and 11% in the HD group ($p < 0.05$) compared with the control group. The mean adipose tissue weight increased by 132% in the LD group ($p < 0.01$). The mean total cholesterol level increased by 33% in the LD group ($p < 0.01$) and 17% in the HD group ($p < 0.05$). In males, the mean body weight and mean adipose tissue weight increased by 22% ($p < 0.01$) and 59% ($p < 0.01$), respectively, in the HD group compared with the control group. The mean triacylglycerol level increased by 34% in the LD group ($p < 0.05$).

CONCLUSION: The continuous exposure of mice to BPA during the perinatal and postnatal periods caused the development of obesity and hyperlipidemia.

Alonso-Magdalena P, Morimoto S, Ripoll C, Fuentes E, Nadal A. "The Estrogenic Effect of Bisphenol A Disrupts the Pancreatic β -Cell Function *In Vivo* and Induces Insulin Resistance." *Environmental Health Perspectives*, 114:106-112 (2006).

BACKGROUND: The function of the pancreatic β -cell is the storage and release of insulin, the main hormone involved in blood glucose homeostasis. The results in this article show that the widespread environmental contaminant bisphenol-A (BPA) imitates 17β -estradiol (E_2) effects *in vivo* on blood glucose homeostasis through genomic and nongenomic pathways.

METHODS and RESULTS: The exposure of adult mice to a single low dose (10 $\mu\text{g}/\text{kg}$) of either E_2 or BPA induces a rapid decrease in glycemia that correlates with a rise of plasma insulin. Longer exposures to E_2 and BPA induce an increase in pancreatic β -cell insulin content in an estrogen-receptor-dependent manner. This effect is visible after 2 days of treatment and starting at doses as low as 10 $\mu\text{g}/\text{kg}/\text{day}$. After 4 days of treatment with either E_2 or BPA, these mice developed chronic hyperinsulinemia, and their glucose and insulin tolerance tests were altered.

CONCLUSION: These experiments unveil the link between environmental estrogens and insulin resistance. Therefore, either abnormal levels of endogenous estrogens or environmental estrogen exposure enhances the risk of developing type 2 diabetes mellitus, hypertension, and dyslipidemia.

Metabolic effects (animals)

Summary: *These two studies show that BPA exposure interferes with normal glucose metabolism in fat cells (adipocytes) and increases the number of fat cells in the body. Both of these effects increase the risk of type II diabetes and obesity.*

The following excerpts are taken directly from the scientific abstracts.

Sakurai K, Kawazuma M, Adachi T, Harigaya T, Saito Y, Hashimoto N, Mori C. "Bisphenol A Affects Glucose Transport in Mouse 3T3-F442A Adipocytes." *British Journal of Pharmacology*, 141:209-214 (2004).

BACKGROUND: Recently, environmental chemicals have appeared in daily human life, and these chemicals have been incidentally taken in by humans. The serum concentrations of some of these chemicals have been found to be associated with the onset and incidence rate of diabetes mellitus. It has been suggested that one of the environmental chemicals, bisphenol A (BPA), has hormone-like activity. It has also been demonstrated that some hormones affect insulin resistance and fat distribution in the body. To study the effects of these environmental chemicals on glucose metabolism, the effect of BPA on glucose transport in mouse 3T3-F442A adipocytes was investigated.

METHODS: The 3T3-F442A adipocytes were incubated with various concentrations of BPA in a medium. Deoxyglucose uptake assay was performed with and without insulin. Immunoblot analysis was performed with a glucose transporter (GLUT) 4-specific antibody and antiphosphotyrosine antibody. **RESULTS:** The BPA treatment enhanced basal and insulin-stimulated glucose uptake, and caused an increased amount of GLUT4 protein. Thus, the enhanced glucose uptake resulting from the BPA treatment was at least partially due to the increased amount of GLUT4. Tyrosine phosphorylation of insulin receptor substrate-1 with insulin stimulation was not significantly affected. **CONCLUSION:** In conclusion, it was demonstrated that BPA, one of the chemicals that we intake incidentally, affects the glucose transport in adipocytes, and also that the environmental chemicals may be identified as one of the environmental factors that affect diabetes and obesity.

Masuno H, Kidani T, Sekiya K, Sakayama K, Shiosaka T, Yamamoto H, Honda K. "Bisphenol A in Combinations with Insulin Can Accelerate the Conversion of 3T3-L1 Fibroblasts to Adipocytes." *Journal of Lipid Research*, 43:676-684 (2002).

METHOD: The confluent cultures of 3T3-L1 fibroblasts were treated with or without bisphenol A (BPA) for 2 days and subsequently treated with insulin (INS) alone for 9 days.

RESULTS: When BPA was absent during the first 2-day treatment period, the cultures contained 1.6 µg/µg DNA of triacylglycerol (TG), 202 mU/mg DNA of lipoprotein lipase (LPL) activity, and 462 nmol/min/mg DNA of glycerol-3-phosphate dehydrogenase (GPDH) activity. The presence of BPA during the same period caused a 150% increase in the TG content, a 60% increase in the LPL activity, and a 500% increase in the GPDH activity. Thus, BPA by itself can trigger 3T3-L1 fibroblasts to differentiate into adipocytes.

METHOD: Next, the confluent cultures were treated with BPA for 2 days and subsequently treated with a combination of INS and BPA for 9 days.

RESULTS: The simultaneous presence of BPA with INS caused a 370% increase in the TG content, a 200% increase in the LPL activity, and a 225% increase in the GPDH activity compared with the cultures treated with INS alone. The amount of [³H]thymidine incorporated into DNA was lower in the cultures treated with INS in the presence of BPA than in those treated with INS alone, indicating that BPA has an anti-proliferative activity on 3T3-L1 cells.

CONCLUSION: Taken together, our results indicate that BPA in combination with INS can accelerate the adipocyte conversion.

Effects on Brain and Behavior (animals)

Summary: *These studies explore how BPA exposure during critical windows of development—the fetal and newborn (perinatal) period and puberty—affect the brain and behavior later in life. Scientists used both mice and rats to study these effects.*

Estrogen is one of the most important hormones in brain development in both men and women. It normally promotes the growth and health of developing neurons in the brain. As an artificial estrogen, BPA both mimics the action of estrogen (primarily estradiol) in neurons and at low doses—in the parts per trillion (PPT) range—totally inhibits the action of estrogen. These studies suggest that BPA exposure can do permanent harm to developing brain cells.

The first article (Palanza et al) reviews a series of studies on the effects of maternal BPA exposure on the behavior of the mouse offspring. The most consistent finding among all the studies was the lack of

sexual differences in behavior between males and females whose mothers had been exposed to BPA. In contrast, there were significant sexual differences in behavior among animals in the unexposed (control) group. In addition, BPA exposure of female mice during fetal life or during adulthood changed their mothering behaviors. The exposed females spent less time nursing and more time out of the nest than the unexposed females.

The remaining studies in this section show other brain and behavioral effects of BPA exposure. These effects include hyperactivity; impaired spatial learning, memory and cognitive function; increased aggressive behavior in males even without increased testosterone production; and masculinization of female behavior. One study (Xu, et al) also suggests that BPA exposure affects thyroid function, which can also interfere with normal brain development and lead to mental retardation.

The following excerpts are taken directly from the scientific abstracts.

Palanza P, Gioiso L, vom Saal FS, Parmigiani S. "Effects of Developmental Exposure to Bisphenol A on Brain and Behavior in Mice." *Environmental Research*, 108(20):150-157 (2008).

BACKGROUND: Bisphenol A (BPA) is a widespread estrogenic chemical used in the production of polycarbonate, and epoxy resins lining food and beverage cans and in dental sealants. During fetal life the intrauterine environment is critical for the normal development, and even small changes in the levels of hormones, such as estradiol or estrogen-mimicking chemicals, can lead to changes in brain function and consequently in behavior.

METHODS: The authors reviewed a series of ethological studies on the effects of maternal oral exposure during the last part of gestation (prenatal exposure) or from gestation day 11 to postnatal day 7 (perinatal exposure) to a low, environmentally relevant dose of BPA (10 microg/kg bw/day) on behavioral responses of CD-1 mouse offspring.

RESULTS: The authors examined both male and female offspring and found that maternal exposure to BPA affected: (1) behavioral responses to novelty before puberty and, as adults; (2) exploration and activity in a free-exploratory open field; (3) exploration in the elevated plus maze and (4) sensitivity to amphetamine-induced reward in the conditioned place preference test.

A consistent effect of the maternal exposure to BPA is that in all these different experimental settings, while a significant sex difference was observed in the control group, exposure to BPA decreased or eliminated the sex difference in behavior. In addition, exposure of female mice to BPA in both adulthood or during fetal life altered subsequent maternal behavior.

CONCLUSION: These findings, together with those from other laboratories, are evidence of long-term consequences of maternal exposure to low-dose BPA at the level of neurobehavioral development.

Alyea R, Watson C. "Differential Regulation of Dopamine Transporter Function and Location by Low Concentrations of Environmental Estrogens and 17 β -estradiol" *Environmental Health Perspectives*, doi:10.1289/ehp.0800026 (2009)

BACKGROUND: The effects of 17 β -estradiol (E2) and xenoestrogens (XEs) on dopamine transport may have important implications for the increased incidence of neurological disorders, especially in women during life stages characterized by frequent hormonal fluctuations.

METHODS: Activity of the dopamine transporter (DAT) was measured by the efflux of 3Hdopamine in non-transfected NGF-differentiated PC-12 rat pheochromocytoma cells expressing membrane DAT, ER α , ER β , and GPR30. We used a plate immuno-assay to monitor trafficking of these proteins.

RESULTS: All compounds at a 1nM either caused efflux or inhibited efflux, or both, each compound evoking a distinct oscillatory pattern. At optimal times for each effect, we examined different concentrations of XEs. All XEs were active at some concentration below 10 nM and doseresponses were

all nonmonotonic. For example, 10-14-10-11M DDE caused significant efflux inhibition, while nonylphenol and BPA enhanced or inhibited efflux at several concentrations. We also measured the effects of E2-XE combinations; DDE potentiated E2-mediated dopamine efflux while BPA inhibited it. In E2-induced efflux, 15% more ER α trafficked to the membrane while ER β waned; during BPA-induced efflux, 20% more DAT was trafficked to the plasma membrane.

CONCLUSION: Low levels of environmental estrogen contaminants acting as endocrine disruptors via membrane ERs can alter dopamine efflux temporal patterning and the trafficking of DAT and membrane ERs, providing a cellular mechanism which could explain the disruption of physiological neurotransmitter function.

Gioiosa L, Fissore E, Ghiradelli G, Parmigiani S, Palanza P. "Developmental Exposure to Low-dose Estrogenic Endocrine Disruptors Alters Sex Differences in Exploration and Emotional Responses in Mice." *Hormones and Behavior*, 52:307-316 (2007).

BACKGROUND: Estrogenic endocrine disruptors (EEDs) are naturally occurring or man-made compounds present in the environment that are able to bind to estrogen receptors and interfere with normal cellular development in target organs and tissues. There is mounting evidence that EEDs can interfere with the processes of sexual differentiation of brain and behavior in different animal models. We investigated the effects of maternal exposure to EEDs, at concentrations within the range of human exposure and not patently teratogenic, on behavioral responses of male and female house mice (*Mus musculus domesticus*) before and after puberty.

METHODS: Pregnant dams were trained to spontaneously drink daily doses of corn oil with or without the estrogenic plastic derivative, bisphenol A (BPA 10 $\mu\text{g}/\text{kg}$), or the estrogenic insecticide methoxychlor (MXC 20 $\mu\text{g}/\text{kg}$) from gestation day 11 to postpartum day 8. Their male and female offspring were examined at different ages to examine several components of explorative and emotional behaviors in 3 experimental paradigms: a novelty test before puberty and, as adults, a free-exploratory open-field test and the elevated plus maze test.

RESULTS: The main results are sex differences in control mice on a number of behavioral responses at both ages and in all experimental paradigms, while perinatal exposure to BPA or MXC decreased or eliminated such sex differences.

CONCLUSION: The present findings are evidence of long-term consequences of developmental exposure to BPA and MXC on neurobehavioral development and suggest a differential effect of low-dose exposure to these estrogenic chemicals in males and females.

Xu X, Liu Y, Sadamatsu M, Tsutsumi S, Akaike M, Ushijima H, Kato N. "Perinatal Bisphenol A Affects the Behavior and SRC-1 expression of male pups but does not influence on the thyroid hormone receptors and its responsive gene." *Neuroscience Research* 58:149-155 (2007).

BACKGROUND: Bisphenol A (BPA) has been shown to interfere with thyroid hormone receptors (THR) and to influence the expression of THR-responsive elements in vivo and in vitro, while some studies reported hyperactivity induced by BPA treatment.

OBJECTIVE and METHOD: In the present study, our purpose was to investigate the effect of BPA exposure on behavioral alteration and its mechanism of action, especially focusing on the thyroid hormone pathway.

RESULTS: Significant sexual difference on behaviors was observed in perinatal BPA exposure, as manifested by hyperactivity and impaired spatial learning/memory in male pups after matured. Dams treated with 0.1mg/l BPA showed transient hypothyroidism, while male pups were found to exhibit a transient hyperthyroidism followed by hypothyroidism. Furthermore, significant up-regulated expression levels of mRNA and protein of SRC-1 in the hippocampus were observed in male pups by

0.1mg/l BPA treatment. However the expression of THRA/beta and RC3/neurogranin were not affected by BPA treatment.

CONCLUSIONS: These results indicate that perinatal BPA exposure at a very low level may influence thyroid function and then consequently affect brain development, but at the same time, suggest that thyroid hormone receptor may not be a direct target of BPA action, but instead, another factor may be involved in this action.

Ceccarelli I, Seta DD, Fiorenzani P, Farabollini F, Aloisi AM. "Estrogenic chemicals at puberty change ER α in the hypothalamus of male and female rats." *Neurotoxicology and Teratology*, 29(1):108-115 (2007).

BACKGROUND: The effects of two environmental endocrine disruptors, the synthetic pharmaceutical estrogen 17-ethinylestradiol (EE) and bisphenol-A (BPA), were analysed in male and female rats in a very sensitive developmental period, puberty.

METHODS: Immunohistochemistry was used to evaluate changes in the number of cells expressing estrogen receptors (ER- α) in the arcuate nucleus (ARC), ventromedial nucleus (VMH) and medial preoptic area (MPA) of the hypothalamus. Animals were treated during early puberty, from PND 23 to PND 30, with EE and BPA given orally every day. They were then sacrificed and perfused on PND 37 or PND 90, and blood and brains were collected for hormonal determination (testosterone and estradiol) and immunohistochemistry (estrogen receptors, ER).

RESULTS: At PND 37, ER-labelled neurons were higher in males than in females in the ARC and MPA. EE and BPA increased ER-labelled neurons in the ARC and MPA. At PND 90, females showed higher ER-labelled neurons in the VMH. EE and BPA increased ER-labelled neurons in the MPA in females. EE increased testosterone in males at PND 37 and estradiol in females at PND 90.

CONCLUSION: These results indicate the ability of estrogenic chemicals to change the reproductive neural circuits during puberty in male and female rats.

Seta DD, Minder I, Belloni V, Aloisi AM, Dessi-Fulgheri F, Farabollini F. "Pubertal Exposure to Estrogenic Chemicals Affects Behavior in Juvenile and Adult Male Rats." *Hormones and Behavior*, 50:301-307 (2006).

BACKGROUND: In this paper, we tested the hypothesis that exposure to estrogens of different source and estrogenic potency at early puberty could affect the development of socio-sexual behavior in the male rat. Puberty is regarded as a second stage of the ontogenetic period, in the sexual maturation of mammals, particularly sensitive to gonadal hormone milieu.

METHODS: We treated animals orally, from postnatal day 23 to 30, with an environmentally compatible dose of bisphenol A (BPA, 40 μ g/kg/day) and with a dosage of ethinylestradiol (EE, 0.4 μ g/kg/day) comparable to the human oral contraceptives.

RESULTS: Exposure to EE altered the temporal pattern of male sexual activity, reducing performance, in the adult animals; slight modifications, in the same direction, were observed with BPA. Short-term behavioral effects were observed in the treated animals, both with BPA and EE: the exploratory drive, directed to a stimulus object and to the environment, as well as to conspecifics, was reduced in the juveniles. Modifications in the circulating T levels were observed after treatments: T was reduced in the juveniles, both with BPA and EE. The decrement persisted in the adult animals but reached significance only in the BPA group.

CONCLUSION: On the whole, effects of pubertal exposure on behavior are more marked with EE than BPA. This can be due to the much higher estrogenic potency of EE; the direction of the behavioral effects of BPA, compared with EE, is however indicative of an estrogenic mechanism.

Rubin BS, Lenkowski JR, Schaeberle CH, Vandenberg LN, Ronsheim PM, Soto AM. "Evidence of Altered Brain Sexual Differentiation in Mice Exposed Perinatally to Low, Environmentally Relevant Levels of Bisphenol A." *Endocrinology*, 147(8):3681-3691 (2006).

BACKGROUND: Humans are routinely exposed to bisphenol A (BPA), an estrogenic chemical present in food and beverage containers, dental composites, and many products in the home and workplace. BPA binds both classical nuclear estrogen receptors and facilitates membrane-initiated estrogenic effects. Here we explore the ability of environmentally relevant exposure to BPA to affect anatomical and functional measures of brain development and sexual differentiation.

METHODS: Anatomical evidence of alterations in brain sexual differentiation were examined in male and female offspring born to mouse dams exposed to 0, 25, or 250 ng BPA/kg body weight per day from the evening of d 8 of gestation through d 16 of lactation. These studies examined the sexually dimorphic population of tyrosine hydroxylase (TH) neurons in the rostral periventricular preoptic area, an important brain region for estrous cyclicity and estrogen-positive feedback.

RESULTS: The significant sex differences in TH neuron number observed in control offspring were diminished or obliterated in offspring exposed to BPA primarily because of a decline in TH neuron number in BPA-exposed females. As a functional endpoint of BPA action on brain sexual differentiation, we examined the effects of perinatal BPA exposure on sexually dimorphic behaviors in the open field. Data from these studies revealed significant sex differences in the vehicle-exposed offspring that were not observed in the BPA-exposed offspring.

CONCLUSION: These data indicate that BPA may be capable of altering important events during critical periods of brain development.

MacLusky NJ, Hajszan T, Leranath C. "The Environmental Estrogen Bisphenol A Inhibits Estradiol-Induced Hippocampal Synaptogenesis." *Environmental Health Perspectives*, 113:675-679 (2005).

BACKGROUND: Bisphenol A (BPA) is an estrogenic chemical that is widely used in the manufacture of plastics and epoxy resins. Because BPA leaches out of plastic food and drink containers, as well as the BPA-containing plastics used in dental prostheses and sealants, considerable potential exists for human exposure to this compound.

RESULTS: In this article we show that treatment of ovariectomized rats with BPA dose-dependently inhibits the estrogen-induced formation of dendritic spine synapses on pyramidal neurons in the CA1 area of the hippocampus. Significant inhibitory effects of BPA were observed at a dose of only 40 µg/kg, below the current U.S. Environmental Protection Agency reference daily limit for human exposure.

CONCLUSION: Because synaptic remodeling has been postulated to contribute to the rapid effects of estrogen on hippocampus-dependent memory, these data suggest that environmental BPA exposure may interfere with the development and expression of normal sex differences in cognitive function, via inhibition of estrogen-dependent hippocampal synapse formation. It may also exacerbate the impairment of hippocampal function observed during normal aging, as endogenous estrogen production declines.

Zsarnovszky A, Le HH, Wang HS, Belcher SM. "Ontogeny of Rapid Estrogen-mediated Extracellular Signal-regulated Kinase Signaling in the Rat Cerebellar Cortex: Potent Nongenomic Agonist and Endocrine Disrupting Activity of the Xenoestrogen Bisphenol A." *Endocrinology*, 146:5388-5396 (2005).

BACKGROUND: In addition to regulating estrogen receptor-dependent gene expression, 17β-estradiol (E(2)) can directly influence intracellular signaling. In primary cultured cerebellar neurons, E(2) was previously shown to regulate growth and oncotic cell death via rapid stimulation of ERK1/2 signaling. Here we show that ERK1/2 signaling in the cerebellum of neonatal and mature rats was rapidly responsive to E(2) and during development to the environmental estrogen bisphenol A (BPA).

METHODS: In vivo dose-response analysis for each estrogenic compound was performed by brief (6-min) intracerebellar injection, followed by rapid fixation and phosphorylation-state-specific immunohistochemistry to quantitatively characterize changes in activated ERK1/2 (pERK) immunopositive cell numbers.

RESULTS: Beginning on postnatal d 8, E(2) significantly influenced the number of pERK-positive cells in a cell-specific manner that was dependent on concentration and age but not sex. In cerebellar granule cells on postnatal d 10, E(2) or BPA increased pERK-positive cell numbers at low doses (10^{-12} to 10^{-10} M) and at higher (10^{-7} to 10^{-6} M) concentrations. Intermediate concentrations of either estrogenic compound did not modify basal ERK signaling. Rapid E(2)-induced increases in pERK immunoreactivity were specific to the ERK1/2 pathway as demonstrated by coinjection of the mitogen-activated, ERK-activating kinase (MEK)1/2 inhibitor U0126. Coadministration of BPA (10^{-12} to 10^{-10} M) with 10^{-10} M E(2) dose-dependently inhibited rapid E(2)-induced ERK1/2 activation in developing cerebellar neurons.

CONCLUSION: The ability of BPA to act as a highly potent E(2) mimetic and to also disrupt the rapid actions of E(2) at very low concentrations during cerebellar development highlights the potential low-dose impact of xenoestrogens on the developing brain.

Ishido M, Masuo Y, Kunimoto M, Oka S, Morita M. "Bisphenol A Causes Hyperactivity in the Rat Concomitantly with Impairment of Tyrosine Hydroxylase Immunoreactivity." *Journal of Neuroscience Research*, 76:423-433 (2004).

BACKGROUND: We examined the effects of bisphenol A, an endocrine disruptor, on rat behavioral and cellular responses.

METHODS and RESULTS: Single intracisternal administration of bisphenol A (0.2-20 microg) into 5-day-old male Wistar rats caused significant hyperactivity at 4-5 weeks of age. Rats were about 1.6-fold more active in the nocturnal phase after administration of both 2 and 20 microg of bisphenol A than were control rats. The response was dose-dependent. Based on DNA microarray analyses of the midbrain, bisphenol A decreased by more than twofold gene expression levels of the dopamine D4 receptor at 4 weeks of age and the dopamine transporter at 8 weeks of age. Furthermore, bisphenol A decreased by more than twofold gene expression levels of the dopamine D4 receptor at 4 weeks of age and the dopamine transporter at 8 weeks of age.

CONCLUSION: We conclude that bisphenol A affected central dopaminergic system activity, resulting in hyperactivity due most likely to a large reduction of tyrosine hydroxylase activity in the midbrain.

Negishi T, Kawasaki K, Suzaki S, Maeda H, Ishii Y, Kyuwa S, Kuroda Y, Yoshikawa Y. "Behavioral Alterations in Response to Fear-Provoking Stimuli and Tranylcypromine Induced by Perinatal Exposure to Bisphenol A and Nonylphenol in Male Rats." *Environmental Health Perspectives*, 112:1159-1164 (2004).

BACKGROUND and METHODS: The purpose of this study was to examine whether perinatal exposure to two major environmental endocrine-disrupting chemicals, bisphenol A (BPA; 0.1 mg/kg/day orally) and nonylphenol [NP; 0.1 mg/kg/day (low dose) and 10 mg/kg/day (high dose) orally] daily from gestational day 3 to postnatal day 20 (transplacental and lactational exposures) would lead to behavioral alterations in the male offspring of F344 rats.

RESULTS: Neither BPA nor NP exposure affected behavioral characteristics in an open-field test (8 weeks of age), in a measurement of spontaneous motor activity (12 weeks of age), or in an elevated plus-maze test (14 weeks of age). A passive avoidance test (13 weeks of age) showed that both BPA- and NP-treated offspring tended to delay entry into a dark compartment. An active avoidance test at 15 weeks of age revealed that BPA-treated offspring showed significantly fewer avoidance responses and low-dose NP-treated offspring exhibited slightly fewer avoidance responses. Furthermore, BPA-treated

offspring significantly increased the number of failures to avoid electrical unconditioned stimuli within 5-sec electrical shock presentation compared with the control offspring. In a monoamine-disruption test using 5 mg/kg (intraperitoneal) tranylcypromine (Tcy), a monoamine oxidase inhibitor, both BPA-treated and low-dose NP-treated offspring at 22-24 weeks of age failed to show a significant increment in locomotion in response to Tcy, whereas control and high-dose NP-treated offspring significantly increased locomotion behavior after Tcy injection. In addition, when only saline was injected during a monoamine-disruption test, low-dose NP-treated offspring showed frequent rearing compared with the control offspring.

CONCLUSION: The present results indicate that perinatal low-dose BPA or NP exposure irreversibly influenced the reception of fear-provoking stimuli (e.g., electrical shock), as well as monoaminergic neural pathways.

Kubo K, Arai O, Omura M, Watanabe R, Ogata R, Aou S. "Low Dose Effects of Bisphenol A on Sexual Differentiation of the Brain and Behavior in Rats." *Neuroscience Research*, 45:345-356 (2003).

BACKGROUND: There is an endocrinological concern that environmental endocrine disruptors (EEDs) may influence sexual differentiation. Bisphenol A (BPA), one of EEDs, is released from polycarbonate plastics, and has been detected in the human umbilical cord. In this study, we examined the effect of BPA on the sexual differentiation of open-field behavior and the sexually dimorphic nuclei in the brain in the offspring of rats exposed to BPA during the fetal and suckling periods at a dosage below the human tolerable daily intake (TDI) level.

RESULTS: In the control group, females were more active in the open field and had a larger locus coeruleus (LC) volume than males. BPA abolished and inverted the sex differences of the open-field behavior and the LC volume, respectively, without affecting the reproductive system. We also compared the effects of estrogenic compounds, diethylstilbestrol (DES) and resveratrol (RVT), to that of BPA because of their structural similarities. DES affected the open-field behavior, LC volume and reproductive system, while RVT affected the LC volume and the reproductive system.

CONCLUSION: These results suggest that the brain is highly sensitive to BPA at a dosage below TDI and that the disrupting effects of BPA on sexual differentiation may vary from those of RVT and DES.

Kawai K, Nozaki T, Nishikata H, Aou S, Takii M, Kubo C. "Aggressive Behavior and Serum Testosterone Concentration During the Maturation Process of Male Mice: The Effects of Fetal Exposure to Bisphenol A." *Environmental Health Perspectives*, 111:175-178 (2003).

BACKGROUND: The relationship between exposure to endocrine-disrupting chemicals (EDs) and risk to reproductive organs is well documented, but the influence of EDs on behavioral development has not been studied. In this study we evaluated the effect of fetal exposure to bisphenol A, which mimics estrogenic activity, on aggressive behavior and hormonal change in male mice.

METHODS: On gestation days 11-17, female mice were fed bisphenol A at 2 ng/g or 20 ng/g of body weight (environmentally relevant concentration). Aggression rating and blood sampling of the offspring were done at 8, 12, and 16 weeks of age.

RESULTS: Aggression scores increased significantly ($p < 0.01$) at 8 weeks of age in male mice exposed to bisphenol A at both the 2 ng/g and 20 ng/g concentrations compared with a control group, but no difference was found after 12 weeks. Relative testis weight (per gram of body weight) was significantly lower at 8 and 12 weeks in mice treated with 2 ng/g than in controls ($p < 0.05$) and was significantly lower at 12 weeks in mice treated with 20 ng/g than in controls ($p < 0.01$). The serum testosterone concentration in treated mice was not significantly different from that in controls.

CONCLUSION: These results demonstrate that bisphenol A temporarily activated aggressive behavior in mice at 8 weeks of age and that low doses of bisphenol A interfered with the normal development of

reproductive organs. The mechanism activating this aggressive behavior was not elevated testosterone concentration.

Farabollini F, Porrini S, Seta DD, Bianchi F, Dessi-Fulgheri F. "Effects of Perinatal Exposure to Bisphenol A on Sociosexual Behavior of Female and Male Rats." *Environmental Health Perspectives*, 110 Suppl 3:409-401 (2002).

BACKGROUND: Perinatal action of estrogens or aromatizable steroids at the central nervous system level is responsible for brain sexual differentiation. Through early contact with the central nervous system, the estrogenic compound bisphenol A (BPA) could alter the processes affecting sociosexual behavior.

METHOD: To test this hypothesis, we studied agonistic and sexual behavior of adult female and male rats whose mothers were administered BPA (40 microg/kg/day) during pregnancy or lactation.

RESULTS: An intruder test revealed in males but not in females an increase in defensive behavior due to BPA. We studied the effect of BPA on sexual behavior by testing sexual orientation and sexual activity. Male sexual orientation toward a stimulus female was not affected by BPA, whereas the sexual activity test revealed a slight impairment of sexual performance due to BPA in terms of latency and frequency of intromissions. In females, BPA produced a small increase in sexual motivation and receptive behavior.

CONCLUSION: In conclusion, BPA administration, both during pregnancy and during lactation, does not masculinize female behavior or potentiate masculinization processes of males. On the contrary, we observed a potentiation of female behavior in females and a depotentiation of male behavior in males.

Aloisi AM, Seta DD, Rendo C, Ceccarelli I, Scaramuzzino A, Farabollini F. "Exposure to the Estrogenic Pollutant Bisphenol A Affects Pain Behavior Induced by Subcutaneous Formalin Injections in Male and Female Rats." *Brain Research*, 937:1-7 (2002).

BACKGROUND: We investigated the effects of perinatally administered bisphenol A (BPA), an environmental contaminant with estrogenic activity, on formalin-induced nociceptive responses.

METHODS: Male and female offspring of mother rats treated with BPA or oil were cross-fostered after birth to obtain three homogeneous groups: BPA-prenatal, receiving BPA via the placenta; BPA-postnatal, receiving BPA through suckling; OIL, control, from mothers receiving only peanut oil (vehicle). All groups underwent a pain test with s.c. formalin injection (50 microl, 10%) or were sham injected (pricking with a syringe needle) in the dorsal hind paw. They were immediately placed in an open field apparatus where pain responses (licking, flexing and paw-jerk) were recorded for 60 min. Corticosterone, testosterone and estradiol serum levels were determined in blood obtained at the end of the experiment.

RESULTS: BPA-prenatal treatment induced an increase in licking duration in females and in flexing duration in both sexes in the first half of the test (0-30 min after formalin injection). BPA-postnatal treatment induced a decrease in paw-jerk frequency in males and females during the second part of the test (30-60 min after formalin injection). Plasma concentrations of corticosterone, estradiol and testosterone did not differ significantly between groups.

CONCLUSION: These results indicate that exposure to BPA modified the activity of neural pathways and/or centers involved in nociception and pain in a sex-related and exposure-related manner.

Palanza P, Howdeshell KL, Parmigiani S, vom Saal FS. "Exposure to a Low Dose of Bisphenol A during Fetal Life or in Adulthood Alters Maternal Behavior in Mice." *Environmental Health Perspectives*, 110:415-422 (2002).

BACKGROUND: Maternal behavior in mammals is the result of a complex interaction between the lactating dam and her developing offspring. Slight perturbations of any of the components of the mother-infant interaction may result in alterations of the behavior of the mother and/or of the

offspring. We studied the effects of exposure of female CD-1 mice to the estrogenic chemical bisphenol A (BPA) during fetal life and/or in adulthood during the last part of pregnancy on subsequent maternal behavior.

METHODS: Pregnant females were fed daily doses of corn oil (controls) or 10 µg/kg body weight BPA during gestation days 14–18. As adults, the prenatally treated female offspring were time-mated and again fed either corn oil (controls) or the same doses of BPA on gestation days 14–18, resulting in four treatment groups: controls, prenatal BPA exposure, adult BPA exposure, and both prenatal and adult BPA exposure. Maternal behavior was then observed on postnatal days 2–15 and reflex responses were examined in the offspring.

RESULTS: Dams exposed to BPA either as fetuses or in adulthood spent less time nursing their pups and more time out of the nest compared with the control group. Females exposed to BPA both as fetuses and in adulthood did not significantly differ from controls. No alterations in postnatal reflex development were observed in the offspring of the females exposed to BPA.

CONCLUSION: The changes seen in maternal behavior may be the result of a direct effect of BPA on the neuroendocrine substrates underlying the initiation of maternal behavior.

Dessi-Fulgheri F, Porrini S, Farabollini F. “Effects of Perinatal Exposure to Bisphenol A on Play Behavior of Female and Male Juvenile Rats.” *Environmental Health Perspectives*, 110 Suppl 3:403-407 (2002).

BACKGROUND: In higher vertebrates, estrogen can exert an organizational effect on sexually dimorphic areas of the central nervous system (CNS) during the perinatal phase of development. The possibility that estrogenic pollutants may mimic estrogen action on the CNS during development and produce long-lasting or irreversible effects is an issue of great concern. Bisphenol A (BPA), a compound widely used in the food industry and in dentistry, has proven estrogenic actions.

METHODS: To study its potential developmental effects on behavior, we gave female Sprague-Dawley rats 40 microg/kg/day BPA from conception to weaning postnatal day 21 and 400 microg/kg/day BPA from gestation day 14 to postnatal day 6. After exposure, we studied social behavior in a play situation in juvenile male and female offspring. The attempt to use play behavior to study the effects of BPA yielded some interesting results.

RESULTS: We observed an early action of BPA on several behavioral categories in both males and females. In particular we observed a masculinization of female behavior in two behavioral categories (play with females and sociosexual exploration), an effect probably mediated by the estrogenic activity of BPA in the CNS.

CONCLUSION: The research suggests that very important mechanisms underlying certain behaviors are involved, and thus, in the long run, even small changes can have consequences on individual fitness and on population structure. The mechanisms underlying the observed effects need to be clarified by further research.

Effects on Brain and Behavior (non-human primate)

Summary: *This study shows that low-dose BPA exposure inhibits the action of estrogen on the developing brain, particularly the hippocampus and prefrontal cortex. These two structures play a critical role in cognition and mood. The dose of BPA used in this study was equal to that of what the U.S. Environmental Protection Agency says is safe.*

The following excerpt is taken directly from the scientific abstract.

Leranth C, Hajszan T, Szigeti-Buck K, Bober J, Maclusky NJ. "Bisphenol A prevents the synaptogenic response to estradiol in hippocampus and prefrontal cortex of ovariectomized nonhuman primates." *Proceedings of the National Academy of Sciences of the United States of America*, 105(37):14187-14191 (2008).

BACKGROUND: Exposure measurements from several countries indicate that humans are routinely exposed to low levels of bisphenol A (BPA), a synthetic xenoestrogen widely used in the production of polycarbonate plastics. There is considerable debate about whether this exposure represents an environmental risk, based on reports that BPA interferes with the development of many organs and that it may alter cognitive functions and mood. Consistent with these reports, we have previously demonstrated that BPA antagonizes spine synapse formation induced by estrogens and testosterone in limbic brain areas of gonadectomized female and male rats. An important limitation of these studies, however, is that they were based on rodent animal models, which may not be representative of the effects of human BPA exposure.

METHODS: To address this issue, we examined the influence of continuous BPA administration, at a daily dose equal to the current U.S. Environmental Protection Agency's reference safe daily limit, on estradiol-induced spine synapse formation in the hippocampus and prefrontal cortex of a nonhuman primate model.

RESULTS: Our data indicate that even at this relatively low exposure level, BPA completely abolishes the synaptogenic response to estradiol. Because remodeling of spine synapses may play a critical role in cognition and mood, the ability of BPA to interfere with spine synapse formation has profound implications.

CONCLUSION: This study is the first to demonstrate an adverse effect of BPA on the brain in a nonhuman primate model and further amplifies concerns about the widespread use of BPA in medical equipment, and in food preparation and storage.

Immune System Effects

Summary: Immune system function is complex and incompletely understood. These studies show that exposure to BPA modulates the immune system in mice. Effects on the immune response vary according to timing of exposure and sex of the animal exposed. Altered immune system response could affect the development and progression of allergy and autoimmune diseases such as lupus, rheumatoid arthritis, scleroderma and multiple sclerosis.

The following excerpts are taken directly from the scientific abstracts.

Yurino H, Ishikawa S, Sato T, Akadegawa K, Ito T, Ueha S, Inadera H, Matsushima K. "Endocrine disruptors (Environmental Estrogens) Enhance Autoantibody production by B1 Cells." *Toxicological Sciences* 81:139-147 (2004).

BACKGROUND: Accumulating data suggest that endocrine disruptors affect not only the reproductive system, but also the immune system. We demonstrate here that endocrine disruptors including diethylstilbestrol (DES) and bisphenol-A (BPA) enhance autoantibody production by B1 cells both in vitro and in vivo.

METHOD AND RESULTS: BWF1 mice, a murine model for systemic lupus erythematosus (SLE), implanted with Silastic tubes containing DES after orchidectomy developed murine lupus characterized by immunoglobulin G (IgG) anti-DNA antibody production and IgG deposition in the glomeruli in the kidney as well as those implanted with 17 β -estradiol (E2). Plaque-forming cells (PFC) producing autoantibodies specific for bromelain-treated red blood cells were significantly increased in mice implanted with DES

and BPA. IgM antibody production by B1 cells in vitro was also enhanced in the presence of endocrine disruptors including DES and BPA. Estrogen receptor (ER) expression was upregulated in B1 cells in aged BWF1 mice that developed lupus nephritis.

CONCLUSIONS: These results suggest that endocrine disruptors are involved in autoantibody production by B1 cells and maybe an etiologic factor in the development of autoimmune diseases.

Yoshino S, Yamaki K, Xiaojuan L, Sai T, Yanagisawa R, Takano H, Taneda S, Hayashi H, Mori Y.

“Prenatal Exposure to Bisphenol A Up-regulates Immune Responses, Including T helper 1 and T helper 2 Responses, in Mice.” *Immunology*, 112:489-495 (2004).

BACKGROUND: The effect of prenatal exposure to bisphenol A (BPA) on the immune system in mice was investigated.

METHODS: Virgin female mice were fed varying doses of BPA, on a daily basis, over a period of 18 days commencing on the day of pairing with stud males (day 0). On day 77, their male offspring of 8 weeks were immunized with hen egg lysozyme (HEL). Three weeks later, anti-HEL immunoglobulin G (IgG) in sera, and proliferative responses of spleen cells to the antigen, were measured. Anti-HEL IgG2a and interferon- γ (IFN- γ), secreted from splenic lymphocytes, were measured as indicators of T helper 1 (Th1) immune responses, while anti-HEL IgG1 and interleukin-4 (IL-4) were measured as indicators of Th2 responses.

RESULTS: The results showed that fetal exposure to BPA was followed by significant increases in anti-HEL IgG as well as antigen-specific cell proliferation. Both Th1 responses (including anti-HEL IgG2a and IFN- γ production) and Th2 responses (including anti-HEL IgG1 and IL-4 production) were augmented by prenatal exposure to BPA, although the augmentation of Th1 responses appeared to be greater than that of Th2 responses. Two-colour flow cytometric analysis showed that mice exposed prenatally to BPA had 29% and 100% more splenic CD3⁺ CD4⁺ and CD3⁺ CD8⁺ cells, respectively, than control animals. Similar results were obtained from females whose mothers had consumed BPA during pregnancy.

CONCLUSION: These results suggest that prenatal exposure to BPA may result in the up-regulation of immune responses, especially Th1 responses, in adulthood.

Sawai C, Anderson K, Walser-Kuntz D. “Effect of Bisphenol A on Murine Immune Function: Modulation of Interferon-Gamma, IgG2a, and Disease Dymptoms in NZB X NZW F1 Mice.” *Environmental Health Perspectives*, 111:1883-1887 (2003).

BACKGROUND and METHODS: To investigate the effects of the estrogen receptor-binding molecule bisphenol A (BPA) on murine immune function in vivo, we fed a low dose of 2.5 micro g BPA/kg body weight/day to both normal C57BL/6 and lupus-prone NZB X NZW F(1) (NZB/NZW) 5-week-old mice for 1 week.

RESULTS: Analysis of concanavalin A (ConA)-stimulated splenic mononuclear cells by ELISA demonstrated that BPA-fed C57BL/6 males produced, on average, 40% less interferon-gamma (IFN-gamma; $p < 0.01$) and C57BL/6 females 28% less IFN-gamma ($p < 0.05$) compared with controls. Treated female NZB/NZW mice were monitored for lupus disease symptoms, defined as proteinuria (> 100 mg/dL albumin in urine for 2 consecutive weeks). Before the development of proteinuria, BPA-fed NZB/NZW mice produced significantly less ConA-stimulated IFN-gamma than did controls and displayed an average reduction of 50% in immunoglobulin G2a (IgG2a) antibody production from lipopolysaccharide (LPS)-stimulated splenocytes ($p < 0.05$). It is striking that 5-week-old female NZB/NZW mice fed a 7-day low-dose course of BPA developed proteinuria an average of 7 weeks later than did controls. Once proteinuria developed, splenocytes were stimulated with ConA for cytokine analysis. The BPA-fed mice showed a dramatic reduction of 64% in IFN-gamma production and a 32% reduction in ConA-stimulated interleukin-10 ($p < 0.05$).

CONCLUSION: The long-lasting effects of BPA on IFN-gamma and IgG2a production likely contributed to the increased symptom-free period of the NZB/NZW mice. The studies suggest that a low-dose, short-term exposure to the ER-binding molecule BPA affects murine immune function *in vivo* and may have important implications for modulating autoimmunity.

Yoshino S, Yamaki K, Yanagisawa R, Takano H, Hayashi H, Mori Y. "Effects of Bisphenol A on Antigen-specific Antibody Production, Proliferative Responses of Lymphoid Cells, and TH1 and TH2 Immune Responses in Mice." *British Journal of Pharmacology*, 138:1271-1276 (2003).

BACKGROUND: We investigated the effect of bisphenol A (BPA), which binds estrogen receptors, on immune responses including production of antigen-specific antibodies, proliferative responses of lymphoid cells, and Th 1 and Th2 responses.

METHODS: For this investigation, mice were p.o. [orally] given varying doses including 3, 30, 300, and 3000 $\mu\text{g kg}^{-1}$ of BPA immediately after immunization with hen egg lysozyme (HEL) (day 0) and then daily by day 20. On day 21, anti-HEL IgG antibodies in sera and proliferative responses of spleen cells to the antigen were measured. Anti-HEL IgG2a antibodies and IFN- γ secreted from splenic lymphocytes were also measured as indicators of Th1 immune responses, while anti-HEL IgG1 antibodies and IL-4, as those of Th2 responses.

RESULTS: The results showed that treatment with 3000 $\mu\text{g kg}^{-1}$ of BPA was followed by a significant increase in anti-HEL IgG as well as the antigen-specific cell proliferation. Anti-HEL IgG2a production and IFN- γ secretion were significantly enhanced in mice treated with 300 and 30 $\mu\text{g kg}^{-1}$ of BPA, respectively, while anti-HEL IgG1 production and IL-4 secretion were augmented in animals given 3000 and 300 $\mu\text{g kg}^{-1}$ of the chemical, respectively. Augmentation of these immune responses was also observed in mice exposed to 0.3 - 30 $\mu\text{g kg}^{-1}$ of estradiol, although Th1 responses appeared to be more sensitive to the sex hormone than Th2 responses.

CONCLUSION: These results suggest that BPA may play a role in augmenting immune responses, especially Th1 responses.