BISPHENOL-A

This is a compilation of abstracts of articles identified during the preliminary toxicological evaluation of evidence on the developmental and reproductive toxicology of bisphenol-A. Bisphenol-A (CAS# 80-05-7) is mainly used in the manufacture of polycarbonate plastics and epoxy resins. Consumer exposures to bisphenol-A may result from its use in polycarbonate products (e.g., eyeglass lenses, baby and water bottles, reusable food and drink containers), and epoxy resins (e.g., in dental composites, paints and adhesives, protective coatings in food and beverage containers).

Compiled are abstracts from developmental and reproductive epidemiologic and animal toxicity studies and other relevant investigations. The criterion for passing the epidemiologic screen is the existence of two or more analytical epidemiologic studies judged to be of adequate quality that reported increased risk of adverse developmental or reproductive outcomes. The epidemiologic studies report on developmental and reproductive sequelae related to environmental exposures to bisphenol-A. Based on a review of abstracts of the following studies, the chemical passed the epidemiologic screen.

- Three epidemiologic study of bisphenol-A reporting increased risk of adverse developmental or reproductive outcomes were identified, two of which were analytical studies of adequate quality. One study reporting no increased risk of adverse developmental or reproductive outcomes, one study with unclear findings, and two other related articles were also identified.

- Sixty-three animal studies of bisphenol-A and thirteen meeting abstracts reporting reproductive or developmental toxicity were identified, as well as twenty-six studies and four meeting abstracts reporting no reproductive or developmental toxicity. Ninety-one related articles and meeting abstracts and fifteen studies without abstracts were identified.

Contents

I. Epidemiologic DART Studies

A. Studies reporting increased risk of adverse developmental or reproductive outcomes

[*]Exposure to bisphenol-a is associated with recurrent miscarriage.
Sugiura-Ogasawara M, Ozaki Y, Shin-ichi S, Makino T, Suzumori K.
Human Reproduction. 2005 [Epub ahead of print].

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+/-SD values for bisphenol A in patients were 2.59+/-5.23ng/ml, significantly higher than the 0.77+/-0.38ng/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.

[*]Positive Relationship between Androgen and the Endocrine Disruptor, Bisphenol A, in Normal Women and Women with Ovarian Dysfunction.
Takeuchi T, Tsutsumi O, Ikezuki Y, Takai Y, Taketani Y.
Endocr J. 2004 Apr;51(2):165-9.

This study was performed to investigate the serum levels of bisphenol A (BPA), an endocrine disruptor, in women with ovarian dysfunction and obesity. Fasting serum samples were obtained from 19 non-obese and 7 obese women with normal menstrual cycles: 7 patients with hyperprolactinemia, 21 patients with hypothalamic amenorrhea, and 13 non-obese and 6 obese patients with polycystic ovary syndrome (PCOS). BPA was measured by an enzyme-linked immunosorbent assay. BPA was detected in all human sera. Serum BPA concentrations were significantly higher in both non-obese and obese women with polycystic ovary syndrome (1.05 +/- 0.10 ng/ml, 1.17 +/- 0.16 ng/ml; $p<0.05$, respectively) and obese normal women (1.04 +/- 0.09 ng/ml, $p<0.05$) compared with those in non-obese normal women (0.71 +/- 0.09 ng/ml). There was no difference among women with hyperprolactinemia, women with hypothalamic amenorrhea, and non-obese normal women. There were significant positive correlations between

[*] denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

serum BPA and total testosterone (r = 0.391, p<0.001), free testosterone (r = 0.504, p<0.001), androstenedione (r = 0.684, p<0.001), and DHEAS (r = 0.514, p<0.001) concentrations in all subjects. These findings show that there is a strong relationship between serum BPA and androgen concentrations, speculatively due to the effect of androgen on the metabolism of BPA.

Serum bisphenol a concentrations showed gender differences, possibly linked to androgen levels.
Takeuchi T, Tsutsumi O.
Biochem Biophys Res Commun. 2002 Feb 15;29(1):76-8.

To investigate human exposure to bisphenol A (BPA), a widely used endocrine disruptor, we measured serum BPA concentrations and analyzed the interrelation of BPA with sex-related hormones. BPA was detected in all human sera by a novel enzyme-linked immunosorbent assay. Serum BPA concentrations were significantly higher in normal men (1.49 +/- 0.11 ng/ml; P < 0.01) and in women with polycystic ovary syndrome (1.04 +/- 0.10 ng/ml; P < 0.05) compared with normal women (0.64 +/- 0.10 ng/ml). There were significant positive correlations between serum BPA and total testosterone (r = 0.595, P < 0.001) and free testosterone (r = 0.609, P < 0.001) concentrations in all subjects and likewise between serum BPA and total testosterone (r = 0.559, P < 0.01) and free testosterone (r = 0.598, P < 0.001) concentrations in all female subjects, but not between serum BPA and other sex-related hormone concentrations in any group. These findings showed that there are gender differences in serum BPA concentrations, possibly due to differences in the androgen-related metabolism of BPA. (c)2002 Elsevier Science (USA).

B. Studies reporting no increased risk of adverse developmental or reproductive outcomes

Urinary concentrations of bisphenol A in relation to biomarkers of sensitivity and effect and endocrine-related health effects.
Yang, M., Kim, S. Y., Chang, S. S., Lee, I. S. Kawamoto, T.,
Environ Mol Mutagen. 2006 Oct;47(8): 571-8.

The impact of endocrine-disrupting chemicals (EDCs) on human health is not yet clear because of difficulties in ascertaining their biological effects. In the present study, we evaluated exposure to the EDC, bisphenol A (BPA), in 172 Koreans in relation to biomarkers of susceptibility and effect. The subjects completed questionnaires, which documented occupation, education, lifestyle factors, potential sources of BPA-exposure, and the occurrence of self-diagnosed endocrine disorders. None of the subjects were occupationally exposed to BPA; however, urinary levels of conjugated BPA, determined by HPLC/FD, ranged from 0.03-62.4 microg/l (median, 7.86). The frequencies of potential susceptibility biomarkers, the UGT1A6-Arg184Ser and the SULT1A1-Arg213His polymorphisms, were not associated with urinary BPA levels, either as single genes or in combination. Indirect effects of BPA exposure on the susceptibility to mutagens were evaluated by comparing urinary BPA concentrations with MNNG-induced sister-

chromatid exchange (SCE) in lymphocytes cultured from the subjects. BPA exposure showed marginal or significant associations with theSCEs induced by the low doses of MNNG (0-0.4 mM). However, there was no overall association between urinary BPA levels and MNNG-induced frequency at doses ranging from 0.2-0.6 mM. Finally, we did not detect an association between urinary BPA concentration and endocrine-related disorders. Even though we were unable to find a strong association between BPA exposure and a biological response, possibly because of the limited number of subjects, we observed that most of the subjects were exposed to BPA. Therefore, continuous biological monitoring of BPA is a prudent measure to prevent possible BPA-related health risks.

C. Studies with unclear findings

Differences in serum bisphenol a concentrations in premenopausal normal women and women with endometrial hyperplasia.
Hiroi H, Tsutsumi O, Takeuchi T, Momoeda M, Ikezuki Y, Okamura A, Yokota H, Taketani Y. Endocr J. 2004 Dec;51(6):595-600.

Exposure to endocrine disrupting chemicals (EDCs) has been raised in relation to its potential for adverse health outcomes. Bisphenol A (BPA) is an estrogenic EDC widely found in plastic products. We determined BPA concentrations in premenopausal women by an enzyme-linked immunosorbent assay and evaluated possible linkage between its contamination levels and endometrial hyperplasia, an estrogen-related disorder of the uterus. It has been implied that higher levels of BPA, which binds to estrogen receptor and plays estrogenic roles may, enhance endometrial hyperplasia. Serum BPA was detectable in all subjects and its concentrations in healthy controls with normal endometrium were 2.5 +/- 1.5 ng/ml (mean +/- SD). BPA levels in patients with simple endometrial hyperplasia with benign nature were 2.9 +/- 2.0 ng/ml and were not significantly different from the controls. Unexpectedly, BPA levels in patients with complex endometrial hyperplasia with malignant potential were 1.4 +/- 0.4 ng/ml and significantly lower compared to both control and simple endometrial hyperplasia groups. In addition, we measured the serum BPA levels in postmenopausal endometrial cancer patient (1.4 +/- 0.5 ng/ml), which were also significantly lower than control and simple endometrial hyperplasia groups. These findings suggest the presence of associations between BPA exposure and complex endometrial hyperplasia and endometrial cancer. The mode of action of BPA may be more complex than expected and the contradictory results may serve as a clue to addressing the mechanisms of linkage between occurrence of estrogen-dependent diseases and endocrine disruption.

D. Related articles

Measurement of bisphenol A concentrations in human colostrum
Kuruto-Niwa, R.; Tateoka, Y.; Usuki, Y.; Nozawa, R.
Chemosphere. 2007 66(6):1160-4

Bisphenol A (BPA), an estrogenic endocrine disrupting chemical, has been reported to affect embryos and alter their postnatal development. 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 colostral samples were collected from 101 healthy mothers within three days after delivery. The BPA concentrations of colostral 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+/-0.13 (mean+/-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.

Parent bisphenol A accumulation in the human maternal-fetal-placental unit.
Schonfelder G, Wittfoht W, Hopp H, Talsness CE, Paul M, Chahoud I.
Environ Health Perspect. 2002 Nov;110(11):A703-7

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. We therefore investigated blood samples from mothers (n = 37) between weeks 32 and 41 of gestation. Afer 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 micro g/mL in plasma and tissues. 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. 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 metabolization of parent BPA or variable maternal use of consumer products leaching BPA.

II. Animal DART Studies

A. Studies reporting developmental or reproductive toxicity

Prenatal bisphenol A exposure induces preneoplastic lesions in the mammary gland in Wistar rats.
Durando, M.; Kass, L.; Piva, J.; Sonnenschein, C.; Soto, A. M.; Luque, E. H.; Muntilde, and oz-de-Toro, M.
Environ Health Perspect. 2007 Jan; 115(1):80-6.

Abstract: 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 pg/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.

Bisphenol A exposure in utero disrupts early oogenesis in the mouse

Susiarjo, M; Hassold, TJ; Freeman, E; Hunt, PA
PLoS Genet 2007; 3(1): e5. doi:10.1371/journal. pgen.0030005

Abstract: 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. Pregnant mice were treated with low, environmentally relevant doses of BPA during mid-gestation to assess the effect of BPA on the developing ovary. 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 ERb, one of the two known estrogen receptors. This, coupled with the finding that BPA exposure elicited no additional effects in ERb null females, suggests that BPA exerts its effect on the early oocyte by interfering with the actions of ERb. Together, our 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.

Exposure to environmentally relevant doses of the xenoestrogen bisphenol-A alters development of the fetal mouse mammary gland.
Vandenberg, L. N., Maffini, M. V., Wadia, P. R., Sonnenschein, C., Rubin, B. S., and Soto, A. M.
Endocrinology. 2007 Jan; 148(1):116-27.

Abstract: 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. 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. 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. 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. These results suggest that alterations in mammary gland phenotypes observed at puberty and adulthood in perinatally exposed mice have their origins in fetal development.

Prenatal exposure to bisphenol A impairs sexual differentiation of exploratory behavior and increases depression-like behavior in rats.
Fujimoto, T.; Kubo, K., and Aou, S.
Brain Res. 2006 Jan 12; 1068(1):49-55.

Abstract: Perinatal exposure to bisphenol A (BPA, 0.1 and 1 ppm in drinking water applied to mother rats for 6 weeks) has been shown to impair the sexual differentiation in exploratory behavior, but the exact critical period of this disrupting effect is still unknown. In this study, we examined the effects of prenatal exposure to BPA (0.1 ppm in drinking water applied to dams during the final week of pregnant) on emotional and learning behaviors in addition to exploratory behavior. Estimated daily intake was 15 microg/kg/day, below the reference dose (RfD) in the United States and the daily tolerable intake (TDI) in Japan (50 microg/kg/day). The rats were successively tested in open-field test, elevated plus maze test, passive avoidance test and forced swimming test during development from 6 to 9 weeks of juvenile period. Prenatal exposure to BPA mainly affected male rats and abolished sex differences in rearing behavior in the open-field test and struggling behavior in the forced swimming test. BPA increased the immobility of male rats in the forced swimming test. The avoidance learning and behaviors in the elevated plus maze were not affected. The present study demonstrates that male rats at the final week of prenatal period are sensitive to BPA, which impairs sexual differentiation in rearing and struggling behavior and facilitate depression-like behavior.

Effects of bisphenol A given neonatally on reproductive functions of male rats.
Kato, H., Furuhashi, T., Tanaka, M., Katsu, Y., Watanabe, H., Ohta, Y., and Iguchi, T.
Reprod Toxicol. 2006 Jul; 22(1):20-9.

Abstract: Male Sprague-Dawley rats (Crj:CD (IGS) were treated neonatally with bisphenol A (BPA) to evaluate effects on reproductive parameters. Animals were given BPA subcutaneously in corn oil to dosages of 0.002-97 mg/kg body weight, or 0.9 mg/kg 17beta-estradiol (E2) once a day from postnatal day (PND) 0 to PND 9. Preputial separation, copulatory rate, fertility rate, sperm analysis, serum testosterone levels, and gene expression in the testis were assessed. Males in the E2 group showed a decrease in testis weight and alterations of estrogen-mediated gene expression in the testis on PND 10, and by PND 150 incomplete preputial separation, decreases in the copulatory rate, testicular and accessory organ weights and number of sperm. In contrast, males in all BPA groups showed normal reproductive parameters. These results indicate that in male rats, BPA given during the neonatal period neither affected reproductive function nor evoked estrogen-mediated gene responses in the testis.

[Effect of bisphenol - A on apoptosis of male mice reproductive cells].
Liu, J. F., Liu, Q., Ni, Y. J. and others.
Chung-Kuo Kung Kung Wei Sheng. 2006 May; 22(5):572-3.

Abstract: Objective: To study the effects of bisphenol-A(BPA)on apoptosis of male mice reproductive cells. Methods: Male mice in experiment groups were exposed to BPA by using the intraperitoneal injection for 5d at the dose of 0.250, 500, 1000 umol/kg respectively. Seven mice were randomly sacrificed in each group on the 77th and 14th day after first exposure to BPA. The flow cytometry was used to study the effects of BPA on testicle cells of male mice. The testicular viscera co-efficient were measured and the contents of nitric oxide (NO)and nitric oxide synthase(NOS)were tested. Results: The different dose of BPA could make the testicle cell cycle change. The cells in G0/G1, S phases decreased, the accounts of cells in G2/M increased and Ap part increased with the increase of BPA. The testicular viscera coefficient decreased in the experimental groups compared with that in the blank group. The contents of NO and NOS in the testicles of mice were higher than that in the blank group. There were significant differences between experimental groups and blank control. Conclusion BPA can inhibit DNA synthesis of testicle cells cause the G2 block and delay the mitosis of testice cells, and the cells in M phase decreased with the increase of BPA. It shows that BPA may affect the reproductive cells on apoptosis of male mice. The content of NO and NOS increased to show that the damaged reproductive cells are related with lipid peroxide.

Neonatal genistein or bisphenol-A exposure alters sexual differentiation of the AVPV.
Patisaul, H. B., Fortino, A. E., and Polston, E. K.
Neurotoxicol Teratol. 2006 Jan-2006 Feb 28; 28(1):111-8.

Abstract: There is growing concern that naturally occurring and chemically manufactured endocrine-active compounds (EACs) may disrupt hormone-dependent events during central nervous system development. We examined whether postnatal exposure to the phytoestrogen genistein (GEN) or the plastics component bisphenol-A (BIS) affected sexual differentiation of the anteroventral periventricular nucleus of the hypothalamus (AVPV) in rats. The AVPV is sexually differentiated in rodents. The female AVPV is larger than the male AVPV and contains a higher number of cells expressing tyrosine hydroxylase (TH). Sexual differentiation of the AVPV results from exposure of the male nervous system to estrogen aromatized from testicular testosterone secreted in the first few days after birth. Thus, we hypothesized that exposure to EACs during this critical period could alter the sexually dimorphic expression of TH and the overall expression of estrogen receptor alpha (ERalpha) in the AVPV. Animals were given 4 subcutaneous injections of sesame oil (control), 50 microg 17beta-estradiol (E2), 250 microg GEN, or 250 microg BIS at 12-h intervals over postnatal days (PND) 1 and 2 and sacrificed on PND 19. E2 treatment masculinized TH immunoreactivity (TH-ir) in the female AVPV while exposure to GEN or BIS demasculinized TH-ir in the male AVPV. In addition, we identified a population of neurons co-expressing TH and ERalpha located primarily in the medial region of the AVPV. Normally, females have nearly three times as many double-labeled cells as males, but

their numbers were defeminized by E2, GEN or BIS treatment. These results suggest that acute exposure to EACs during a critical developmental period alters AVPV development.

Evidence of altered brain sexual differentiation in mice exposed perinatally to low, environmentally relevant levels of bisphenol A.
Rubin, B. S., Lenkowski, J. R., Schaeberle, C. M., Vandenberg, L. N., Ronsheim, P. M., and Soto, A. M.
Endocrinology. 2006 Aug; 147(8):3681-91.

Notes: COMMENTS: Comment in: Endocrinology. 2006 Aug;147(8):3679-80 (medline/16847092). Abstract: 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. 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. 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. These data indicate that BPA may be capable of altering important events during critical periods of brain development.

Sexually Dimorphic Lordosis Behavior Is More Sensitive To Disruption By Developmental Exposure To Ethinyl Estradiol Than Is Reproductive Morphology In The Long Evans Female Rat.
Ryan, B. C., Howdeshell, K. L., and Gray, L. E. Jr.
Biol Reprod. 2006; (Spec no):134-5.

Abstract: Anthropogenic estrogens are pervasive in the environment. Although the potential effects of these xenoestrogens are controversial in humans, some fish species are adversely affected in contaminated ecosystems. While studies investigating endocrine disruptors typically focus on reproductive physiology and fertility, exposure to estrogens during development can also alter sexual differentiation of the nervous system and behavior in rodents. As a result, the brain can be imprinted such that it does not respond normally to estrogens in adulthood. Despite this, few studies have rigorously investigated the effect of developmental exposure to

environmental estrogens on adult sexually dimorphic behavior in rats. This study established a framework for using adult sexually dimorphic behaviors as biomarkers for endocrine disruption in rodents. Using this approach, we measured the effects of oral maternal administration (gestational day 7 through postnatal day 18) to ethinyl estradiol (EE) (from 0.5 micro g/kg/day to 50 micro g/kg/day) and bisphenol A (BPA) (from 2 micro g/kg/day to 200 micro g/kg/day) on the female Long Evans rat offspring. At every dose, lordosis response, saccharin preference and running wheel activity in female rats (all sexually dimorphic behaviors) were compared to reproductive tract morphology in female rats. Lordosis behavior in ovariectomized, hormonally primed adults was disrupted by developmental exposure to EE at doses of 0.5 micro g/kg/day and above. At 5 micro g/kg/day and above, EE disrupted organization of saccharin preference, ovarian weight and external reproductive morphology. In contrast, BPA gave inconsistent results and showed no clear dose response throughout the study. These findings show reproductive tract abnormalities after developmental exposure to EE at doses lower than previously published in the rat. In addition, permanent behavioral disruption was seen at developmental doses below those which had an effect on morphology.

Developmental exposure to environmental estrogens alters anxiety and spatial memory in female mice.
Ryan, B. C. and Vandenbergh, J. G.
Horm Behav. 2006 Jun; 50(1):85-93.

Abstract: Humans and wildlife are exposed to numerous anthropogenic drugs and pollutants. Many of these compounds are hormonally active, and recent evidence suggests that the presence of these endocrine disruptors permanently alters normal development and physiology in a variety of vertebrate species. Here, we report on the effects of developmental exposure to two common estrogenic pollutants, bisphenol A and ethinyl estradiol on sexually dimorphic, non-reproductive behavior. Mice (Mus musculus domesticus) were exposed to environmentally relevant levels of these chemicals (2 and 200 microg/kg/day for bisphenol A and 5 microg/kg/day for ethinyl estradiol) throughout prenatal and early postnatal development. As adults, the animals were observed in a variety of tests measuring sexually dimorphic behaviors including short-term spatial memory (in a radial-arm maze and a Barnes maze) and anxiety (in an elevated-plus maze and a light/dark preference chamber). Developmental exposure to ethinyl estradiol was found to masculinize behavior in all of the assays used. Bisphenol A increased anxious behavior in a dose-dependent fashion but had no effect on spatial memory. These results indicate that non-reproductive, sexually dimorphic behavior is sensitive to endocrine disruption. In addition, these experiments suggest that both humans and wildlife are being exposed to levels of these endocrine disrupting compounds that are sufficient to disrupt the development of the nervous system and that may have permanent consequences on sexually dimorphic behaviors.

Developmental programming: differential effects of prenatal exposure to bisphenol-A or methoxychlor on reproductive function.
Savabieasfahani, M., Kannan, K., Astapova, O., Evans, N. P., and Padmanabhan, V.
Endocrinology. 2006 Dec; 147(12):5956-66.

Abstract: Increased occurrence of reproductive disorders has raised concerns regarding the impact of endocrine-disrupting chemicals on reproductive health, especially when such exposure occurs during fetal life. Prenatal testosterone (T) treatment leads to growth retardation, postnatal hypergonadotropism, compromised estradiol-positive feedback, polycystic ovaries, and infertility in the adult. Prenatal dihydrotestosterone treatment failed to affect ovarian morphology or estradiol-positive feedback, suggesting that effects of prenatal T may be facilitated via conversion of T to estradiol, thus raising concerns regarding fetal exposure to estrogenic endocrine-disrupting chemicals. This study tested whether fetal exposure to methoxychlor (MXC) or bisphenol A (BPA) would disrupt cyclicity in the ewe. Suffolk ewes were administered MXC (n=10), BPA (n=10) (5 mg/kg.d sc in cotton seed oil) or the vehicle (C; n=16) from d 30 to 90 of gestation. On d 60 of treatment, maternal MXC concentrations in fat tissue and BPA in blood averaged approximately 200 microg/g fat and 37.4+/-3.3 ng/ml, respectively. Birth weights of BPA offspring were lower ($P < 0.05$) relative to C. There was no difference in the time of puberty between groups. BPA females were hypergonadotropic during early postnatal life and ended their breeding season later, compared with C. Characterization of cyclic changes after synchronization with prostaglandin F2alpha in five C, six MXC, and six BPA females found that the onset of the LH surge was delayed in MXC ($P < 0.05$) and the LH surge magnitude severely dampened ($P < 0.05$) in BPA sheep. These findings suggest that prenatal BPA and MXC exposure have long-term differential effects on a variety of reproductive endocrine parameters that could impact fertility.

Effect of prenatal exposure to bisphenol A on the serum testosterone concentration of rats at birth.
Tanaka, M., Nakaya, S., Katayama, M., Leffers, H., Nozawa, S., Nakazawa, R., Iwamoto, T., and Kobayashi, S.
Hum Exp Toxicol. 2006 Jul; 25(7):369-73.

Abstract: In the rat, testosterone (T) in the neonatal period plays an important role in sexual differentiation and there is a serum T surge in male rats 2 hours after birth. Pregnant female rats were exposed to various doses of bisphenol A (BPA) from gestational day 1 (GD1) through 2 hours after parturition. About half of the BPA-exposed and control dams were subjected to cesarean section on GD22. The male fetuses on GD22 were immediately sacrificed and blood was collected. The other half of the BPA-treated and control dams delivered at GD23 (parturition day). The male pups were sacrificed 2 hours after birth. Serum T concentration was determined by radioimmunoassay (RIA). The BPA concentration in the fetal serum on GD22 increased inversely to the T levels in the serum. The T concentration in the pups' serum 2 hours after birth decreased inversely to the BPA concentration in the serum. However, there were no differences in the serum T concentration among the various doses of BPA. These results suggest that

exposure to BPA in utero inhibits the T surge in the neonatal period and we speculate that exposure to BPA in utero disrupts the endocrine environment in the neonatal male.

Neurobiological effects of bisphenol A may be mediated by somatostatin subtype 3 receptors in some regions of the developing rat brain.
Facciolo, R. M.; Madeo, M.; Alograve, R; Canonaco, M.; Dessigrave, and -Fulgheri, F.
Toxicol Sci. 2005 Dec; 88(2):477-84.

Abstract: Considerable attention has been focused on environmental disruptors such as the xenoestrogen bisphenol A, which influences reproductive, developmental, and cognitive activities through its interaction with specific neuromediating systems in an estrogen-like fashion. In the present study, the effects of this xenoestrogen proved to be preferentially directed toward hypothalamic and extrahypothalamic somatostatin receptor subtype 3, which displayed a higher binding affinity of its specific nonpeptide agonist L-796-778 than that of L-779-976 (subtype 2). One type of action, with respect to animals treated with vehicle alone, consisted of a very strong ($p < 0.001$) decrease of somatostatin receptor subtype 3 mRNA levels in layer V of the frontoparietal cortex of adult rats (Sprague-Dawley) after transplacental and lactational exposure to bisphenol A (400 microg/kg/day). Similarly, such treatment in 7-day-old rats was responsible for a very strong reduction of the subtype 3 mRNA levels in the hypothalamic periventricular nuclei and a strong ($p < 0.01$) increase of the subtype 3 mRNA levels in the ventromedial nuclei. Moreover, even greater upregulated and downregulated activities were reported when subtype 3 mRNA levels were determined in the presence of receptor agonists specific for distinct alpha GABA(A) receptor subunits (alpha(1,5)). The predominant effects of bisphenol A on somatostatin receptor subtype 3 mRNA levels occurring in an alpha GABA(A) subunit-dependent manner tend to suggest the early modulatory importance of this environmental disruptor on cross-talking mechanisms that are implicated in the plasticity of neural circuits, with consequential influence on neuroendocrine/sociosexual behaviors.

Bisphenol-A exposure during pregnancy and lactation affects maternal behavior in rats.
Della Seta, D., Minder, I., Dessi-Fulgheri, F. and Farabollini, F.
Brain Res Bull 2005;65:255-60.

In mammals, endogenous estrogens are crucial for sexual differentiation during the perinatal period, and the modulation in adulthood of many neuroendocrine and behavioral functions involved in reproduction. In rats, the estrogenic environment during pregnancy and lactation affects directly maternal behavior. This experiment was aimed to test whether the exposure to the estrogenic compound bisphenol-A (BPA; 0.040 mg/kg/die, orally) of adult female rats, from mating to weaning of the pups, could alter maternal behavior. An appropriate methodology was applied to reveal differences in the behavior of dams directed to male and female pups, testing the dams on postnatal days 3-4 and 8-9. Results show different maternal behavioral patterns towards male and female pups of control mothers, with more ano-genital licking to males than to females. Exposure of mothers to BPA modified their behavior, reducing specific components of

maternal behavior, both active and passive, irrespective of the sex of pups and the period of observation. This experiment shows that maternal behavior is affected by a prolonged exposure to a low dose of BPA during pregnancy and lactation, thus suggesting an effect on neural circuits in adulthood.

D-amphetamine-related reinforcing effects are reduced in mice exposed prenatally to estrogenic endocrine disruptors.
Laviola, G.; Gioiosa, L.; Adriani, W., and Palanza, P.
Brain Res Bull. 2005; 65(3):235-40.

Estrogenic endocrine disruptors are hormonally active compounds that can bind to estradiol receptors. Central dopamine pathways have been reported to be affected by early developmental exposure to estrogenic endocrine disruptors. In the present study, pregnant female CD-1 mice were allowed to drink spontaneously either oil or environmentally relevant low doses of two estrogenic compounds, methoxychlor (20 microg/kg) or bisphenol-A (10 microg/kg) during gestation days 11-18. Their adult offspring were assessed for conditioned place preference produced by D-amphetamine (0, 1 or 2 mg/kg). Interestingly, prenatal treatment effects were sex-dependent and no changes in conditioned place preference emerged for the male offspring. Conversely, a clear-cut profile of D-amphetamine-induced conditioned place preference was only shown by oil-exposed females, whereas exposure to bisphenol-A or methoxychlor resulted in little or no place conditioning. Locomotor effects of acute d-amphetamine were not affected by prenatal exposure to bisphenol-A or methoxychlor. As a whole, prenatal exposure to estrogenic endocrine disruptors affected some steps in the organization of the brain dopaminergic systems in the female offspring, thus leading to long-term alterations in neurobehavioral function. These data confirm that exposure to weak environmental estrogens in the period of brain sexual differentiation can influence adult behavior.

[Effects of bisphenol A on the placental function and reproduction in rats].
Lee, C. K., Kim, S. H., Moon, D. H., Kim, J. H., Son, B. C., Kim, D. H., Lee, C. H., Kim, H. D., Kim, J. W., Kim, J. E., and Lee, C. U.
J Prev Med Pub Health. 2005 Aug; 38(3):330-6.

Abstract: OBJECTIVES: The aim of this study was to investigate the effects of bisphenol A (BPA), an estrogen-like environmental endocrine disrupter, on the placental function and reproduction in rats. The mRNA levels of the placental prolactin-growth hormone (PRL-GH) gene family, placental trophoblast cell frequency and reproductive data were analyzed. METHODS: The pregnancies of F344 Fisher rats (160 g +/-20 g) were detected by the presence of the copulatory plug or sperm in the vaginal smear, which marked Day 0 of pregnancy. Pregnant rats were divided into three groups. The control group was intraperitoneally injected with a sesame oil vehicle. The two remaining groups were injected with 50 or 500 mg/kg B.W/day of BPA, resuspended in sesame oil, on either days 7 to 11 or 16 to 20 of pregnancy, with the rats sacrificed on either day 11 or 20, respectively. The mRNA levels of PRL-GH and

Pit-1a and b isotype genes were analyzed by Northern blot hybridization and reverse transcription-polymerase chain reaction. The hormone concentrations were analyzed by radioimmunoassay, and the frequency of the placental trophoblast cells observed by a histochemical study. Reproductive data, such as the placental weight and litter size, were surveyed on day 20. The fetal weight was surveyed for 4 weeks after birth. A statistical analysis was carried out using the SAS program (version 8.1). RESULTS: The mRNA levels of the PRL-GH gene family, such as placental lactogen I, Iv and II, prolactin like protein A, C and Cv, and decidual prolactin-related protein were significantly reduced due to BPA exposure. The mRNA levels of the Pit-1a and b isotype genes, which induce the expression of the PRL-GH gene family in the rat placenta, were also reduced due to BPA exposure. The PL-Iv and PL-II concentrations were reduced in the BPA exposed group. During the middle to last stage of pregnancy (Days 11-20), a high dose of BPA exposure reduced the frequency of spongiotrophoblast cells, which are responsible for the secretion of the PRL-GH hormones. Reproductive data, such as the placental and fetal weights and the litter size, were reduced, but that of the pregnancy period was extended in the BPA exposed compared to the control group. CONCLUSIONS: BPA disrupts the placental functions in rats, which leads to reproductive disorders.

Long-term effects of fetal exposure to low doses of the xenoestrogen bisphenol-A in the female mouse genital tract.
Markey, C. M., Wadia, P. R., Rubin, B. S., Sonnenschein, C., and Soto, A. M.
Biol Reprod. 2005 Jun; 72(6):1344-51.

Abstract: 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. 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. 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. 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 mo of age, long after the cessation of BPA exposure.

Perinatal exposure to bisphenol-A alters peripubertal mammary gland development in mice.
Munoz-de-Toro, M., Markey, C. M., Wadia, P. R., Luque, E. H., Rubin, B. S., Sonnenschein, C., and Soto, A. M.
Endocrinology. 2005 Sep; 146(9):4138-47.

Abstract: 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. 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).d] on the peripubertal development of the mammary gland. BPA exposure enhanced the mammary glands' sensitivity to estradiol in ovariectomized CD-1 mice. In their intact 30-d-old littermates, the area and numbers of terminal end buds relative to the gland ductal area increased whereas 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.d. 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, as these two structures are the sites at which cancer arises in humans and rodents.

Effects of in utero exposure to bisphenol A on mRNA expression of arylhydrocarbon and retinoid receptors in murine embryos.
Nishizawa, H., Morita, M., Sugimoto, M., Imanishi, S., and Manabe, N.
J Reprod Dev. 2005 Jun; 51(3):315-24.

Abstract: To evaluate the effects of bisphenol A (BPA), a candidate endocrine disruptor (ED), on embryonic development, we examined the mRNA expression levels of the arylhydrocarbon receptor (AhR), which binds with many EDs and plays crucial roles in xenobiotic metabolism, and of the retinoic acid receptor (RAR) alpha and retinoid X receptor (RXR) alpha, key factors in nuclear receptor-dependent retinoid signal transduction, in murine embryos exposed in utero to BPA (0.02, 2, 200, and 20,000 microg/kg/day) at 6.5-13.5 or 6.5-17.5 days post coitum (dpc), using the real-time reverse transcription-polymerase chain reaction (RT-PCR) method. Extremely low-dose BPA (0.02 microg/kg/day; 1/100 the dose of environmental exposure) remarkably increased AhR mRNA expression in the cerebra, cerebella, and gonads (testes and ovaries) of male and female 14.5- and 18.5-dpc-embryos. In utero exposure to BPA at 2, 200, and 20,000 microg/kg/day also increased levels of AhR mRNA. In gonads of 14.5-dpc-embryos, AhR mRNA levels were elevated and showed diphasic (U) dose-response curves following exposure to BPA, but inverted U dose-response curves were obtained for 18.5-dpc-embryos.

Exposure to BPA increased expression levels of RARalpha and RXRalpha mRNAs in the cerebra, cerebella, and gonads of male and female 14.5- and 18.5-dpc-embryos. Extremely low-dose BPA (0.02 microg/kg/day) increased RARalpha mRNA expression in the cerebella of male and female 14.5- and 18.5-dpc-embryos and in the gonads of female 14.5-dpc-embryos, and significantly increased RXRalpha mRNA expression in the cerebra and cerebella of male and female 14.5-dpc-embryos. The present findings confirm that in utero exposure to an extremely low dose of BPA up-regulates the mRNA expression of AhR, RARalpha, and RXRalpha in murine embryos and disrupts the receptor-dependent signal transducing systems, and will contribute to the assessment of the toxic effects of BPA on xenobiotic metabolism and retinoid signals in embryogenesis.

Early exposure to a low dose of bisphenol A affects socio-sexual behavior of juvenile female rats.
Porrini, S.; Belloni, V.; Della Seta, D.; Farabollini, F.; Giannelli, G., and Dessi-Fulgheri, F.
Brain Res Bull. 2005; 65(3):261-6.

Play behavior is affected by alteration of the hormonal environment during development. In fact, congenital adrenal hyperplasia or early administration of diethylstilbestrol are able to modify female play behavior in mammals. In this research, play behavior of female rats was used to explore the effects of perinatal exposure to low, environmentally relevant dose of bisphenol A (BPA), a xenoestrogen widely diffused in the environment. We used 18 females born to mothers exposed to 40 microg/kg/day BPA during pregnancy and lactation, and 18 control females. The subjects were observed in a heterosexual social situation from 35 to 55 days of age. Six main behaviors were identified by principal component analysis (PCA): exploration, defensive behavior to males, play behavior with males, play behavior with females, low-intensity mating behavior, social grooming. Early administration of BPA was responsible for a significant increase of exploration (including social investigation) ($p<0.001$), as well as a decrease of play with males ($p<0.02$) and social grooming ($p<0.01$) at 45 days of age, indicating a general decrease of playful interactions. In general our results suggest that BPA does not induce a clear masculinization of female behavior, but is able however to defeminize some aspects of female behavior. This result is compatible with the estrogenic properties of BPA, and suggests caution in the use of a chemical that, in the range of human exposure, is able to influence the development of the brain during a critical period, resulting in long-term effects on behavior.

[Exposure to bisphenol-A affects the rewarding system in mice].
Suzuki, T., Mizuo, K., Miyagawa, K., and Narita, M.
Nihon Shinkei Seishin Yakurigaku Zasshi. 2005 Jun; 25(3):125-8.

Abstract: Bisphenol-A has been extensively evaluated for toxicity in a variety of tests as the most common environmental endocrine disruptor. In the present study, we found that prenatal and neonatal exposure to bisphenol-A affects the development of the central dopaminergic system in the mouse limbic area. Additionally, this treatment with bisphenol-A produced a down-

regulation of dopamine D3 receptor and an up-regulation of dopamine D1 receptor function to activate G-protein in the mouse limbic forebrain, which is thought to play a critical role for rewarding effects by drugs of abuse. We next investigated the relationship between the neurobehavioral toxicity and its exposure period. The exposure to bisphenol-A during either organogenesis or lactation, but not implantation and parturition, significantly enhanced the morphine-induced hyperlocomotion and rewarding effect. Furthermore, the exposure to bisphenol-A during either organogenesis or lactation also produced an up-regulation of dopamine D1 receptor function to activate G-protein in the mouse limbic forebrain. These results indicate that either organogenesis or lactation is more sensitive to the bisphenol-A-induced neuronal toxicity than any other periods. In conclusion, we found here that prenatal and neonatal exposure to bisphenol-A can potentiate the central dopaminergic systems, resulting in the supersensitivity of the drugs of abuse-induced rewarding effects and hyperlocomotion in the mouse. Furthermore, the organogenesis and lactation are the most important period to cause the alteration of dopaminergic system by bisphenol-A exposure in the mouse.

Estrogenic chemicals in plastic and oral contraceptives disrupt development of the fetal mouse prostate and urethra.
Timms, B. G.; Howdeshell, K. L.; Barton, L.; Bradley, S.; Richter, C. A., and Vom Saal, F. S. Proc Natl Acad Sci U S A. 2005; 102(19):7014-9.

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

Relief effect of vitamin A on the decreased motility of sperm and the increased incidence of malformed sperm in mice exposed neonatally to bisphenol A.
Aikawa, H., Koyama, S., Matsuda, M., Nakahashi, K., Akazome, Y. and Mori, T.
Cell & Tissue Research 2004;315(1):119-124

Administration of 50 mug of bisphenol A (BPA) for the first 5 days after birth resulted in a decrease in the percentage of moving sperm, and an increase in the incidence of malformed sperm, in the epididymides of mice at 10 weeks of age, although no marked changes were found in the testicular histology between BPA-treated and vehicle-treated control mice. The deteriorating effects of 50 mug of BPA were ameliorated by the concurrent administration of 100 IU of retinol acetate (RA). Neonatal treatment with 0.5 mug of BPA for 5 days resulted in an increase in the incidence of malformed sperm, whereas the BPA effect became more severe in mice nursed by mothers fed a vitamin A-deficient (VAD) diet only a few days before and after parturition. On the other hand, neonatal treatment with 20 mug of estrogen for the first 5 days after birth resulted in an increase in the number of estrogen receptor alpha (ERalpha)-positive cells in the epithelium of the vas deferens, whereas only a few epithelial cells showed weak ERalpha-positive signals in the vehicle-treated control mice at 18 days after birth. This increase, however, was suppressed by the concurrent administration of RA. Although five daily treatments with 50 mug BPA led to no significant increase in the number of ERalpha-positive cells, it may have been due to the weak estrogenic activity of BPA, as discussed. These findings clearly showed that in mice, neonatal exposure to a relatively large dose of BPA causes damage to the motility and morphology of sperm, but the BPA effect is, to some extent, inhibited by a supplement of VA, and enhanced under a VAD condition.

Inhibition of testicular steroidogenesis by the xenoestrogen bisphenol A is associated with reduced pituitary luteinizing hormone secretion and decreased steroidogenic enzyme gene expression in rat Leydig cells.
Akingbemi, B. T.; Sottas, C. M.; Koulova, A. I.; Klinefelter, G. R., and Hardy, M. P.
Endocrinology. 2004 Feb; 145(2):592-603.

Abstract: Exposure of humans to bisphenol A (BPA), a monomer in polycarbonate plastics and a constituent of resins used in food packaging and dentistry, is significant. In this report exposure of rats to 2.4 microg/kg.d (a dose that approximates BPA levels in the environment) from postnatal d 21-35 suppressed serum LH (0.21 +/- 0.05 ng/ml; vs. control, 0.52 +/- 0.04; $P < 0.01$) and testosterone (T) levels (1.62 +/- 0.16 ng/ml; vs. control, 2.52 +/- 0.21; $P < 0.05$), in association with decreased LHbeta and increased estrogen receptor beta pituitary mRNA levels as measured by RT-PCR. Treatment of adult Leydig cells with 0.01 nm BPA decreased T biosynthesis by 25% as a result of decreased expression of the steroidogenic enzyme 17alpha-hydroxylase/17-20 lyase. BPA decreased serum 17beta-estradiol levels from 0.31 +/- 0.02 ng/ml (control) to 0.22 +/- 0.02, 0.19 +/- 0.02, and 0.23 +/- 0.03 ng/ml in rats exposed to 2.4 microg, 10 microg, or 100 mg/kg.d BPA, respectively, from 21-35 d of age ($P < 0.05$) due to its ability to inhibit Leydig cell aromatase activity. Exposures of pregnant and nursing dams, i.e. from gestation d 12 to postnatal d 21, decreased T levels in the testicular interstitial fluid from 420 +/-

34 (control) to 261 +/- 22 ($P < 0.05$) ng/ml in adulthood, implying that the perinatal period is a sensitive window of exposure to BPA. As BPA has been measured in several human populations, further studies are warranted to assess the effects of BPA on male fertility.

Leached components from dental composites and their effects on fertility of female mice.
Al-Hiyasat, A.S., Darmani, H. and Elbetieha, A.M.
Eur J Oral Sci 2004;112:267-72.

This study investigated the effects of leached components from a resin-based dental composite (Z-100) and bisphenol A (BPA) on female mouse fertility. Leached components or BPA (5, 25 and 100 micro g kg(-1)) were administered intragastrically daily to the test and distilled water to the control groups for 28 d. Female mice were then mated with sexually mature untreated males and their fertility was assessed. The results revealed a significant reduction in the number of pregnancies - 54.5% vs. 100% (control) - in mice treated with the leached components from the dental composite, which also showed an increase of 142% in relative ovary weights. Exposure to 25 and 100 micro g kg(-1) BPA resulted in significant increases in the total number of resorptions out of the total number of implantations and significant increases in relative uterine weights. Relative ovarian weights were significantly increased at the highest dose. High performance liquid chromatography analysis showed that tri-(ethylene glycol)-dimethacrylate (TEG-DMA) was the major leached component (total: 5945 micro g ml(-1)) from the composite, followed by bisphenol A glycerolate dimethacrylate (BIS-GMA) (total: 2097 micro g ml(-1)) and BPA (total: 78 micro g ml(-1)). The results suggest that leached components from the dental composite used and commercially purchased BPA have adverse effects on the fertility and reproductive system of female mice.

[Experimental studies on male reproductive toxicity of bisphenol A in vitro and vivo.].
Deng, MX; Wu, DS; Chen, XG; Zhang, LS, and Xu, PY.
Zhonghua Yu Fang Yi Xue Za Zhi. 2004 Nov; 38(6):383-7.

Abstract: OBJECTIVE: To explore the effects of Bisphenol A in adult rats and its possible mechanisms. METHODS: BPA (in corn oil) was administered orally to 9-week-old male Sprague-Dawley rats for 14 days (0, 1 and 5 g/kg bw), and incubated primary Sertoli cells from pubertal SD rats with 0, 10(-7), 10(-6), 10(-5), 10(-4) mol/L BPA. RESULTS: After oral administration, a significant decrease in right testis weight was observed in 5 g/kg dose group, but not in the 1 g/kg bw dose group. Germ cells were detached from basement membrane of seminiferous tubules and Sertoli cells in BPA-treated groups. Administration of BPA at 1 g/kg bw and 5 g/kg bw produced both nucleus pycnosis and vacuolized nucleus in germ cells and Sertoli cells. A marked loss in vimentin staining in Sertoli cells from testis of BPA-treated rats was detected. No change in levels of serum estradiol and testosterone was observed after two-week exposure to BPA. In Sertoli cell primary culture, BPA destroyed the cytoskeleton and cell-cell junctions, and elongated Sertoli cells. CONCLUSION: These results suggest that BPA may

injure reproductive function of male rats by destroying the cytoskeleton and changing the form of Sertoli cells.

Exposure to bisphenol A during gestation and lactation causes loss of sex difference in corticotropin-releasing hormone-immunoreactive neurons in the bed nucleus of the stria terminalis of rats.
Funabashi, T.; Kawaguchi, M.; Furuta, M.; Fukushima, A., and Kimura, F.
Psychoneuroendocrinology. 2004 May; 29(4):475-85.

Abstract: It has been suspected that endocrine disrupters induce abnormal differentiation and development of reproductive organs. In the present study, we examined whether exposure to bisphenol A (BPA), a known endocrine disrupter, during gestation and lactation affects sex difference in the number of corticotropin-releasing hormone-immunoreactive neurons (CRH neurons) in the preoptic area (POA) and the bed nucleus of the stria terminalis (BST). For that purpose, pregnant female Wistar rats (n=8-11 per treatment group) were treated with either 0.1% ethanol (control group) or 10 mg/l BPA (BPA group) dissolved in their drinking water until their offspring were weaned. In the control group, we confirmed a previous report that the POA of female rats contained significantly more CRH neurons than that of male rats ($p < 0.05$). This significant sex difference was also evident in the BPA group, indicating that BPA exposure used in the present study had no effect on the sex difference in CRH neurons in the POA. We also found in the control group that the BST of female rats contained significantly more CRH neurons ($p < 0.05$) than that of male rats. However, this significant sex difference was not observed in the BPA group ($p < 0.05$), suggesting that BPA exposure affected the sex difference in CRH neurons in the BST. Since there was no statistically significant difference in the number of CRH neurons between the control and the BPA group, irrespective of the sex, the results suggested that a loss of sex difference in CRH neurons was due to both an increase in CRH neurons in male rats and a decrease in CRH neurons in female rats. The present study indicates that there is a significant sex difference in the number of CRH neurons in the BST as well as in the POA and that exposure to BPA during gestation and lactation causes a loss of this sex difference in the rat BST, but not in the POA. We suggest that CRH neurons in the BST are more susceptible to endocrine disrupters than those in the POA, irrespective of the sex.

Exposure to bisphenol A during embryonic/fetal life and infancy increases oxidative injury and causes underdevelopment of the brain and testis in mice.
Kabuto, H.; Amakawa, M., and Shishibori, T.
Life Sci. 2004; 74(24):2931-40.

We investigated the modifications in endogenous antioxidant capacity and oxidative damage in the brain, liver, kidney and testis in mice exposed to bisphenol A (BPA), an environmental endocrine disrupter. Mice were exposed to BPA throughout embryonic/fetal life and during lactation by feeding their pregnant/lactating mothers BPA at 5 or 10 microg per milliliter of drinking water. At the age of four weeks, male mice were sacrificed. Exposure to BPA increased

the activity of catalase and glutathione peroxidase in the liver and kidney, respectively. It also increased thiobarbituric acid-reactive substances in the brain, kidney and testis, and decreased the wet weight of the brain, kidney and testis. Our results suggest that exposure to BPA throughout embryonic/fetal life and during infancy induces tissue oxidative stress and peroxidation, ultimately leading to underdevelopment of the brain, kidney and testis.

Dietary Bisphenol A Suppresses the Growth of Newborn Pups by Insufficient Supply of Maternal Milk in Mice.
Matsumoto, C.; Miyaura, C., and Ito, A.
Journal of Health Science. 2004; 50(3):315-8.

Bisphenol A (BPA), a monomer used for the production of polycarbonate, is known to have estrogen activity. In this study, pregnant mice were fed chow diet containing 1% BPA, and we examined the influence of dietary BPA on delivery and growth of newborn pups in mice. When pregnant mice were fed BPA diet, the mice normally delivered pups on day 21 of gestation. The number of offspring pups in maternal mice fed BPA diet was same to those fed normal diet. Therefore, the growth of fetuses and the process of delivery were not influenced by dietary BPA. However, the growth of newborn pups was markedly suppressed when maternal mice were fed BPA diet. The growth of neonatal mice depends on breast milk, and stomach was filled with milk in pups. In newborn pups from maternal mice supplemented with BPA diet, the weight of stomach was lower than that from maternal mice with normal diet. Since the serum level of prolactin was limited in maternal mice supplemented with BPA diet, the suppressed growth of newborn pups by dietary BPA may be due to the insufficient supply of breast milk. These results suggest that the influence of BPA on the growth of newborn pups is related to hormonal condition in maternal mice.

Prenatal and neonatal exposure to bisphenol-A affects the morphine-induced rewarding effect and hyperlocomotion in mice.
Mizuo, K., Narita, M., Miyagawa, K., Narita, M., Okuno, E., and Suzuki, T.
Neurosci Lett. 2004 Feb 12; 356(2):95-8.

Abstract: Bisphenol-A (BPA), one of the most common environmental endocrine disrupters, has been extensively evaluated for toxicity and carcinogenicity. However, little is still known about its action on the CNS. Here we found that prenatal and neonatal exposure to BPA resulted in the enhancement of the rewarding effect and hyperlocomotion induced by morphine in mice. Under these conditions, no change in the G-protein activation by morphine and mu-opioid receptor expression in the lower midbrain was observed by prenatal and neonatal exposure to BPA. These results suggest that chronic exposure to BPA produces the supersensitivity of the morphine-induced rewarding effect and hyperlocomotion without direct changes in mu-opioid receptor function in the lower midbrain. The present data provide further evidence that prenatal and neonatal exposure to BPA can directly influence the development of the central dopaminergic system.

Behavioral alterations in response to fear-provoking stimuli and tranylcypromine induced by perinatal exposure to bisphenol A and nonylphenol in male rats.
Negishi, T.; Kawasaki, K.; Suzaki, S.; Maeda, H.; Ishii, Y.; Kyuwa, S.; Kuroda, Y., and Yoshikawa, Y.
Environ Health Perspect. 2004; 112(11):1159-64.

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. 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. 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.

Effects of maternal xenoestrogen exposure on development of the reproductive tract and mammary gland in female CD-1 mouse offspring.
Nikaido, Y.; Yoshizawa, K.; Danbara, N.; Tsujita-Kyutoku, M.; Yuri, T.; Uehara, N., and Tsubura, A.
Reprod Toxicol. 2004; 18(6):803-11.

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. 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 microg/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. 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 dilatated 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. 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.

Developmental effects of prenatal exposure to bisphenol a on the uterus of rat offspring.
Schonfelder, G.; Friedrich, K.; Paul, M., and Chahoud, I.
Neoplasia. 2004; 6(5):584-94.

Exposure to estrogenic compounds during critical periods of fetal development could result in adverse effects on the development of reproductive organs that are not apparent until later in life. Bisphenol A (BPA), which is employed in the manufacture of a wide range of consumer products, is a prime candidate for endocrine disruption. We examined BPA to address the question of whether in utero exposure affects the uterus of the offspring and studied the expression and distribution of the estrogen receptors alpha (ERalpha) and beta (ERbeta), because estrogens influence the development, growth, and function of the uterus through both receptors. Gravid Sprague-Dawley dams were administered by gavage either 0.1 or 50 mg/kg per day BPA or 0.2 mg/kg per day 17alpha-ethinyl estradiol (EE2) as reference dose on gestation days 6 through 21. Female offspring were killed in estrus. Uterine morphologic changes as well as ERalpha and ERbeta distribution and expression were measured by immunohistochemistry and Western blot analysis. Striking morphologic changes were observed in the uterine epithelium of postpubertal offspring during estrus of the in utero BPA-treated animals (the thickness of the total epithelium was significantly reduced). ERalpha expression was increased in the 50-mg BPA and EE2-treated group. In contrast, we observed significantly decreased ERbeta expression in all BPA- and EE2-treated animals when compared with the control. In summary, these results clearly indicate that in utero exposure of rats to BPA promotes uterine disruption in offspring. We hypothesize that the uterine disruption could possibly be provoked by a dysregulation of ERalpha and ERbeta.

Lack of maternal dietary exposure effects of bisphenol A and nonylphenol during the critical period for brain sexual differentiation on the reproductive/endocrine systems in later life.
Takagi, H.; Shibutani, M.; Masutomi, N.; Uneyama, C.; Takahashi, N.; Mitsumori, K., and Hirose, M. Arch Toxicol. 2004; 78(2):97-105.

Two potential endocrine-disrupting chemicals, bisphenol A (BPA) and nonylphenol (NP), were assessed for their long-lasting effects on endocrine/reproductive systems following transplacental and lactational exposure to rat offspring during a time-window that included the critical period for brain sexual differentiation. Each chemical was mixed with diet at concentrations of 60, 600 and 3000 ppm and was provided to maternal Sprague-Dawley rats from gestational day (GD) 15 to postnatal day (PND) 10. Ethinylestradiol (EE) at 0.5 ppm was used as an estrogenic reference drug. During pregnancy and lactation, including the exposure period, a soy-free rodent diet was provided to eliminate possible modification of the study results by plant-derived phytoestrogens. Effects on endocrine/reproductive systems were evaluated by examining the anogenital distance, organ weights before puberty, onset of puberty, estrous cyclicity, and organ weights and histopathology of adult endocrine organs (at 11 weeks of age), as well as the volume of the sexually dimorphic nucleus of preoptic area. Both NP and BPA, at high doses, caused decreases in maternal body weights and retardation of offspring growth, but neither affected any of the endocrine/reproductive endpoints of offspring, whereas EE induced irreversible changes in estrous cyclicity and histopathology of ovaries and uterus of adult females. The results indicated that maternal dietary exposure to NP or BPA at concentrations up to 3000 ppm from GD 15 through PND 10 do not exert any apparent adverse effects on the endocrine/reproductive systems of offspring.

Adverse effects of bisphenol A to spermiogenesis in mice and rats.
Toyama, Y.; Suzuki-Toyota, F.; Maekawa, M.; Ito, C., and Toshimori, K.
Arch Histol Cytol. 2004; 67(4):373-81.

Either a 20 or 200 microg/kg body weight/injection of bisphenol A (BPA) was subcutaneously administered to adult mice and rats for 6 days, and the effects on the testes were investigated by electron and light microscopy. Abnormalities were observed in the spermatids: acrosomal vesicles, acrosomal caps, acrosomes and nuclei of the spermatids were severely deformed. The ectoplasmic specialization between the Sertoli cell and spermatids were also affected: incomplete specialization, redundant ectopic specialization and aplasia were observed. Rats and mice responded similarly to BPA. There were no dose dependencies between the 20- and 200 microg/kg body weight/injection groups. The ectoplasmic specialization between adjoining Sertoli cells, or blood-testis barrier, was not affected. Since similar adverse effects were observed when adult mice were treated with beta-estradiol 3-benzoate, the effects of BPA reported here seem to reflect the estrogenic effects on the testes. Animals kept for an additional two months after cessation of the administration were shown to be fertile and the testes showed normal histology, indicating that the adverse effects were transitory.

Effects of neonatal administration of 17beta-estradiol, beta-estradiol 3-benzoate, or bisphenol A on mouse and rat spermatogenesis.
Toyama, Y. and Yuasa, S.
Reprod Toxicol. 2004; 19(2):181-8.

Bisphenol A (BPA) is a global environmental contaminant that has been implicated as a potential endocrine disruptor. In the present study, newborn rats and mice were injected subcutaneously with BPA to determine the potential developmental effects on the testis. Testes were examined by light and electron microscopy at 15 weeks of age. Other groups of newborn mice and rats were injected with 17beta-estradiol (E2) or beta-estradiol 3-benzoate (E2B) in a similar manner. BPA, E2, and E2B had similar effects on testes. When treated animals reached puberty and spermiogenesis began, the first sign of the effects was detected in the steps 2-3 spermatids: the acrosomal granule and nucleus were deformed. Henceforth, abnormalities in the acrosome and nucleus were observed in older spermatids and spermatozoa. Ectoplasmic specialization between the Sertoli cell and spermatids was also affected: some specializations were partially or totally deleted. When animals fully matured, the effects of the agents were not found in the testes, and the animals were found to be fertile. The results of the present study show that BPA acts as an estrogen, and causes changes which appear to revert in adults.

Prenatal exposure to bisphenol A up-regulates immune responses, including T helper 1 and T helper 2 responses, in mice.
Yoshino, S.; Yamaki, K.; Li, X.; Sai, T.; Yanagisawa, R.; Takano, H.; Taneda, S.; Hayashi, H., and Mori, Y.
Immunology. 2004; 112(3):489-95.

The effect of prenatal exposure to bisphenol A (BPA) on the immune system in mice was investigated. 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-gamma (IFN-gamma), 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. 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-gamma 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. These results suggest that prenatal exposure to BPA may result in the up-regulation of immune responses, especially Th1 responses, in adulthood.

Induction of oxidative stress by bisphenol A in the epididymal sperm of rats.
Chitra, K.C., Latchoumycandane, C. and Mathur, P.P.
Toxicology 2003;185:119-27.

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. 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. 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 H(2)O(2) and lipid peroxidation increased significantly in the treated rats as compared with the corresponding group of control animals. The results suggested that graded doses of bisphenol A elicit depletion of antioxidant defence 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.

Effect of experimental varicocele on structure and function of epididymis in adolescent rats: a histological and biochemical study.
Chitra, KC, Rao, KR, and Mathur, PP
Asian J Androl. 2003 Sep; 5(3):203-8.

Abstract: AIM: To study the effect of bisphenol A on the epididymis and epididymal sperm of rats and the possible amelioration action of co-administration with vitamin C. METHODS: Male Wistar rats were orally administered bisphenol A (0.2 microg x kg (-1) x day(-1), 2 microg x kg(-1) x day(-1) and 20 microg x kg(-1) x day(-1)) and 0.2 microg, 2 microg and 20 microg bisphenol A + 40 mg vitamin C x kg(-1) x day(-1) for 60 days. On day 61, rats were killed with anesthetic ether and sperm collected from epididymis were used or assessment of sperm count, motility and viability and biochemical studies. A 1 % homogenate of epididymis was prepared and used for biochemical estimations. Caput, corpus and cauda epididymis were fixed in Bouin's fixative for histological studies. RESULTS: Administration of bisphenol A caused a reduction in the epididymal sperm motility and count and the sperm viability remained unchanged. The activities of superoxide dismutase and glutathione peroxidase decreased, while the levels of lipid peroxidation increased in epididymal sperm and epididymis at all doses. Co-administration with vitamin C reversed the effect of bisphenol A-induced oxidative stress in epididymal sperm and

epididymis. A complete degeneration of epididymal epithelium in caput, corpus and cauda regions with reduction in the number of sperms were observed at all doses of bisphenol A-treated rats. CONCLUSION: Bisphenol A induced oxidative stress in epididymis and caused degeneration of the epididymal epithelium of rats. Co-administration with vitamin C had a protective effect against the bisphenol A-induced toxicity in epididymal sperm and epididymis.

Maternal-fetal transfer of endocrine disruptors in the induction of Calbindin-D9k mRNA and protein during pregnancy in rat model.
Hong, E. J.; Choi, K. C., and Jeung, E. B..
Mol Cell Endocrinol. 2003 Dec 30; 212(1-2):63-72.

Abstract: Estrogenic compounds may influence the growth, differentiation and function in many target tissues, especially in the female reproductive tract during pregnancy. The present study was designed to investigate whether CaBP-9k expression in the maternal tissues and fetal uterus is altered following maternal treatment with diethylstilbestrol (DES), 17beta-estradiol (E2), 4-tert-octylphenol (OP), nonylphenol (NP) and bisphenol A (BPA) during late pregnancy. The expression level of CaBP-9k mRNA in maternal uterus significantly increased when treated with a high dose (600 mg/kg BW per day) of OP and NP. Interestingly, the expression level of CaBP-9k mRNA in extra-embryonic membrane decreased in a dose-dependent manner, suggesting that the expression level of CaBP-9k mRNA in the fetal membrane may be differentially regulated when compared to the expression of CaBP-9k in maternal uterus. In parallel with CaBP-9k mRNA level, a high dose (600 mg/kg) of OP and BPA resulted in an increase of CaBP-9k protein in maternal uterus and low dose of OP and NP increased the expression level of CaBP-9k protein in the placenta. High doses of BPA (400 and 600 mg/kg) resulted in an increase of CaBP-9k protein in maternal uterus and placenta, indicating that these estrogenic compounds may affect both maternal uterus and placenta in the induction of CaBP-9k mRNA and/or protein. In parallel with the expression level of CaBP-9k, mRNA decreased in extra-embryonic membrane, treatment with OP (400 and 600 mg/kg) resulted in a significant decrease of CaBP-9k protein in this tissue, suggesting that both CaBP-9k mRNA and protein may be conversely regulated by OP in extra-embryonic membrane when compared to other tissues. Treatment with OP, NP, and BPA induced a significant increase of CaBP-9k mRNA in fetal uterus, indicating that maternally injected estrogenic compounds may transfer directly from placenta to fetus in the induction of fetal uterus CaBP-9k gene. Taken together, we demonstrated for the first time that maternally injected estrogenic compounds resulted in an increase of CaBP-9k mRNA and/or protein in the maternal tissues (uterus and placenta) and fetal uterus during late pregnancy, suggesting that placenta may not be a reliable barrier against these estrogenic compounds for fetal health.

Bisphenol a exposure causes meiotic aneuploidy in the female mouse.
Hunt, PA; Koehler, KE; Susiarjo, M; Hodges, CA; Ilagan, A; Voigt, RC; Thomas, S; Thomas, BF; Hassold, TJ.
Curr Biol. 2003 Apr 1;13(7):546-53.

Abstract: 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.

Change in Sexual Maturation and Estrogen Receptor Expression in Mouse Fetuses Exposed to Bisphenol A.
Iwasaki, T. and Totsukawa, K.
Environmental Sciences 2003;10(4):239-46.

The effects of endocrine-disrupting chemicals are lifelong in fetal or suckling mice, although transient in adult mice. We examined the influence of BPA on the development and the uterus of female pups exposed in utero. BPA was administered to pregnant mice by subcutaneous injection from gestational day 7 until delivery (0.25, 25 and 2500 ug/kg/day). BPA injection increased the rate of weight gain in each pregnant dam. The viability of pups decreased in 0.25 BPA group. The vaginal opening day of pups was delayed in the 0.25 ug/kg/day BPA group, but on the other hand, the day was advanced in the 2500 ug/kg/day BPA group. In uterotrophic assays of 3-week-old pups, the uterine weight of the 25 ug/kg/day BPA group was higher than that of vehicle-exposed pups, but the uterine weight of 0.25 ug/kg/day BPA group was lower. Furthermore, estradiol conditioned uterine estrogen receptor (ER)-alpha expression tended to increase in the 25 ug/kg/day BPA group, but was not detected in the 0.25 ug/kg/day BPA group. The results

suggest this relates to the amount of expression of ER-alpha, although BPA changes the response to estrogen of the uterus.

Aggressive behavior and serum testosterone concentration during the maturation process of male mice: the effects of fetal exposure to bisphenol A.
Kawai, K.; Nozaki, T.; Nishikata, H.; Aou, S.; Takii, M., and Kubo, C.
Environ Health Perspect. 2003 Feb; 111(2):175-8.

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. 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. 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. 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.

Mammalian development in a changing environment: exposure to endocrine disruptors reveals the developmental plasticity of steroid-hormone target organs.
Markey, C. M.; Coombs, M. A.; Sonnenschein, C., and Soto, A. M.
Evol Dev. 2003; 5(1):67-75.

Recent findings in the field of environmental endocrine disruption have revealed that developmental exposure to estrogenic chemicals induces morphological, functional, and behavioral anomalies associated with reproduction. The aim of the present study was to determine the effects of in utero exposure to low doses of the estrogenic chemical bisphenol A (BPA) on the development of the female reproductive tissues and mammary glands in CD-1 mice. Humans are exposed to BPA, which leaches from dental materials and plastic food and beverage containers. Here we report that prenatal exposure to BPA induces alterations in tissue organization within the ovaries and mammary glands and disrupts estrous cyclicity in adulthood. Because estrogen receptors are expressed developmentally in these estrogen-target organs, we propose that BPA may directly affect the expression of genes involved in their morphogenesis. In addition, alterations in the sexual differentiation of the brain, and thus the hypothalamic-pituitary-gonadal axis, may further contribute to the observed phenotype. The emerging field of

endocrine disruptors promises to provide new insights into the mechanisms underlying the development of hormone-target organs and demonstrates that the environment plays important roles in the making of phenotypes.

Expression of the Bcl2 and Bax protein in rat testis exposed prenatally to bisphenol A from birth until adulthood.
Matsuo, T.
Congenit Anom Kyoto. 2003 Sep; 43(3):246-7.

Abstract: Bisphenol A (BPA) is widely used in the production of epoxy, polycarbonate and polyesterstyrene resins: interior coating of cans, artificial teeth, filters and so on. Previously, we reported that prenatal exposure to BPA induced harmful effects on spermatogenesis of rat testes at term in fetal life and 8 weeks of age (Matsuo et al., 1999-2002). The purpose of the present study was to examine the prolonged effect of prenatal exposure to BPA on spermatogenesis in rats. Pregnant rats Gel: Wistar, Clea Inc., Japan) were orally given an olive oil suspension of BPA at 0 and 100 mg/kg body weight once a day during days 13 to 19 of gestation, and allowed to deliver and to suckle their offspring. The testes were removed from the offsprings at 1-,4-, 8- and 12 weeks of age for immunohistochemistry and for Western blotting. Primary antibodies used were anti-Bax (P-19) antigen (Santa Cruz Biotechnology, Inc.) and anti-Bcl-2 antigen (DAKO JAPAN Co., Ltd). We also adapted the sections for terminal deoxynucleotidy transferase (TdT) assay. For Western blotting, testes were homogenized, and the supernatants were used for the determination of Bcl-2 and Bax protein. Light microscopic examinations revealed that testes of l-week-old rats contained significantly more spermatogonia with positive Bax immunostaining in the BPA treated-groups than in the controls ($p < 0.05$). Testes of 8-week-old rats showed more apoptotic spermatogonia in the BPA treated-groups than in the controls ($p < 0.05$). However, no significant differences were detectable in the number of Bcl-2-positive spermatogonia between the BPA-treated rats and the controls. Western blot analysis revealed bands for Bax and Bcl-2 protein of testes of 1-, 4-, 8- and 12-week-old rats. The many Bax-positive spermatogonia in 1-week-old rats suggests that BPA affects young spermatogonia leading to their apoptosis. In conclusion, prenatal exposure to BPA may lead to a reduction in fertility.

Effects of perinatal exposure to bisphenol A on the behavior of offspring in F344 rats.
Negishi, T., Kawasaki, K., Takatori, A., Ishii, Y., Kyuwa, S., Kuroda, Y., and Yoshikawa, Y.
Environmental Toxicology and Pharmacology. 2003 Sep; 14(3): 99-108.

Abstract: The objective of this investigation is to evaluate whether perinatal maternal exposure to bisphenol A (BPA) at 4, 40, and 400 mg/kg per day affects the behavior of offspring in F344 rats. Perinatal BPA exposure inhibited the body weight increases of male and female offspring in a dose-dependent manner, which continued after weaning. Spontaneous activity analyses revealed that BPA elongated immobile time during the dark phase in female offspring. At 4 weeks of age, male offspring exposed to BPA at 40 and 400 mg/kg per day performed avoidance

responses significantly higher in the shuttlebox avoidance test. At 8 weeks of age, however, male offspring only at 4 mg/kg per day showed significantly lower responses. In the open-field behavior test at 8 weeks of age, male offspring exposed to BPA only at 4 mg/kg per day showed a higher percent of grooming than the control male offspring. In conclusion, perinatal exposure to BPA caused the behavioral alterations in the offspring.

Effects of in utero exposure to bisphenol A on expression of RARalpha and RXRalpha mRNAs in murine embryos.
Nishizawa, H., Manabe, N., Morita, M., Sugimoto, M., Imanishi, S., and Miyamoto, H.
J Reprod Dev. 2003 Dec; 49(6):539-45.

Abstract: Retinoic acid receptor (RAR) alpha and retinoid X receptor (RXR) alpha are key factors in a nuclear receptor-dependent signal. To evaluate the effects of bisphenol A (BPA), a candidate endocrine disruptor (ED), on embryonic development, we examined the mRNA levels of RARalpha and RXRalpha in murine embryos, exposed in utero to BPA (2 microg/kg/day) at 6.5-17.5 days post-coitum (dpc), by the real-time reverse transcription-polymerase chain reaction (RT-PCR) method. Higher levels of RARalpha mRNA in cerebra of male and female embryos of control groups were detected at 14.5 dpc. In utero BPA reduced the RARalpha mRNA expression. Higher levels of RXRalpha mRNA in cerebra of male and female embryos were seen at 12.5 dpc. The exposure decreased RXRalpha mRNA expression in male but not female embryos. No remarkable change in the RARalpha mRNA expression level was noted in cerebella of male or female embryos of the control group during embryonic development. Exposure to BPA increased expression levels of RARalpha mRNA in cerebella of male and female embryos at 12.5 dpc. Higher levels of RXRalpha mRNA in cerebella of male and female embryos were seen, but no remarkable changes were noted during embryonic development. BPA significantly decreased the expression levels of RXRalpha mRNA in cerebella of female embryos at 12.5, 14.5 and 18.5 dpc. RARalpha and RXRalpha mRNAs were expressed in gonads (testes and ovaries) of murine embryos from 12.5 to 18.5 dpc. In utero exposure to BPA decreased levels of RARalpha mRNA in testes of 14.5- and 18.5-dpc-embryos, levels of RXRalpha mRNA in testes of 14.5-dpc-embryos, and levels of RXRalpha mRNA in ovaries of 14.5-dpc-embryos. The present findings indicate that RARalpha and RXRalpha play crucial roles in organogenesis, and the growth and development of murine embryos, and will.

Effect of Prenatal Exposure to Dental Composite Resin Monomers on Testosterone Production in the Rat Testis.
Saito, D., Minamida, G., Tani-Ishii, N., Izukuri, K., Ozono, S., Koshika, S., and Teranaka, T.
Environmental Sciences: an International Journal of Environmental Physiology and Toxicology. 2003; 10(6):327-36.

Abstract: The aim of this study was to examine the effect of prenatal exposure to dental composite resin monomers, 2,2-bis(4-hydroxyphenyl)-propane (bisphenol A, BPA), 2,2-bis[4'-(2'hydroxy-3'-methacryloyoxy)phenyl] propane (Bis-GMA), triethylene-glycoldimethacrylate

(TEGDMA) and urethane dimethacrylate (UDMA), on testosterone (T) and cholesterol (Chol) levels in rat plasma. Enzyme activities related to T production in the testis were also examined. Pregnant rats were injected subcutaneously with each of the dental composite resin monomers and estradiol-17beta (E2, 5 ug/day) dissolved in com oil, daily from days 12 to 19 of gestation. Male offspring were sacrificed at 13 weeks old and plasma T and Chol levels were assayed. BPA and E2 decreased T levels, while Bis-GMA, UDMA and TEGDMA did not affect T level in the plasma. For the in vitro study, 14C-progesterone and 14C-delta4-androstenedione were incubated with testicular microsomes, and enzyme activities related to T production from progesterone were assayed. The activity of 17alpha-hydroxylase was enhanced by exposure to E2, Bis-GMA and BPA, and 17beta-hydroxysteroid dehydrogenase activity was enhanced by UDMA, Bis-GMA and BPA. These results demonstrate that prenatal exposure to the dental composite resin monomers, BPA and Bis-GMA, disturbs testicular function.

Testicular toxicity of dietarily or parenterally administered bisphenol A in rats and mice.
Takahashi, O. and Oishi, S.
Food Chem Toxicol. 2003; 41(7):1035-44.

Male Crj:Wistar rats, HsdHot:Holtzman SD rats, Crj:CD-1(ICR) mice and C57BL/6CrSlc mice were administered bisphenol A (BPA) in the diet at a level of 0 (control) and 0.25% for 8 weeks. Daily BPA intake was about 200 and 400 mg/kg for rats and mice, respectively. No conspicuous signs of general or reproductive toxicity were observed after administration in any strain of these animals. Serum testosterone concentrations were not decreased in BPA-fed rats and mice. Successive subcutaneous administration of BPA at a dose of 200 mg/kg/day for 4 weeks significantly decreased the testis, epididymis, prostate and seminal vesicle weights, and the testicular daily sperm production in Jcl:Wistar rats. Successive intraperitoneal administration of BPA at a dose of 20 mg/kg/day for 4 weeks decreased the prostate and seminal vesicle weights but not the testis or epididymis weights. An intraperitoneal dose of 2 mg BPA/kg/day did not cause any toxicity. These results indicate that dietarily administered BPA is less toxic to most strains of rats and mice, and the maximum non-toxic dose and/or minimum toxic dose may be about 200 mg/kg/day. Subcutaneous or intraperitoneal BPA is much more toxic on male reproductive and sex accessory organs than dietary.

Assessment of pubertal development in juvenile male rats after sub-acute exposure to bisphenol A and nonylphenol.
Tan, B. L.; Kassim, N. M., and Mohd, M. A.
Toxicol Lett. 2003; 143(3):261-70.

The effects of bisphenol A and nonylphenol on pubertal development in the intact juvenile/peripubertal male Sprague-Dawley rats was observed in this study from PND23-52/53. Two groups of rats were administered orally with either 100 mg/kg body weight of nonylphenol or bisphenol A. Another group of rats were administered orally with a mixture of 100 mg/kg body weight of nonylphenol and bisphenol A. Control group was administered with the vehicle

of Tween-80 with corn oil (1:9 v/v). Observations made in this study included growth, age at preputial separation, thyroid, liver, testis and kidney weight and histology, epididymal and seminal vesicle plus coagulation gland weight. Nonylphenol and bisphenol A have been observed to cause delay in puberty onset as well as testicular damage in the treatment groups when compared to the control; spermatogenesis was affected in most treated rats. Bisphenol A also caused the enlargement of the kidney and hydronephrosis. Administration of nonylphenol and bisphenol A as a mixture has caused less than additive effects.

Imbalance of testosterone level in male offspring of rats perinatally exposed to bisphenol A.
Watanabe, S.; Wang, R. S.; Miyagawa, M.; Kobayashi, K.; Suda, M.; Sekiguchi, S., and Honma, T.
Ind Health. 2003; 41(4):338-41.

The purpose of this study was to investigate whether exposure to bisphenol A (BPA) through the placenta and milk has any effect on the reproductive system in male offspring. Pregnant rats were treated with BPA at 0, 4, 40 and 400 mg/kg body weight, from gestation day 6 through lactation day 20 by gavage. Plasma testosterone concentrations in offspring at 9 weeks old were significantly high in BPA groups as compared with those of the control. At the age of 36 weeks the hormone concentrations showed an increase in a dose-dependent manner, although without statistical significance. Testosterone content in testes showed a similar tendency to that in plasma, though statistically insignificant. Little alteration in testes weight was seen in BPA-exposed offspring. There was no remarkable change in plasma concentrations of luteinizing hormone and follicle-stimulating hormone at 9 weeks old. The pathway of E2 (17beta-estradiol) formation from testosterone seemed not to be affected by BPA. The results indicate that exposure to BPA during the perinatal period has a significant effect on testosterone homeostasis in male offspring of rats.

Effects of bisphenol A on adult male mouse fertility
Al-Hiyasat, A.S., Darmani, H. and Elbetieha, A.M.
Eur J Oral Sci 2002;110:163-7.

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. Forty adult male Swiss mice were divided into four groups of 10. BPA (5, 25 and 100 micro g kg(-1) [corrected] 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. Females mated with male mice having ingested 25 and 100 micro g kg(-1) [corrected] 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 micro g kg(-1) [corrected] 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. These results suggest that male fertility and reproduction is impaired by bisphenol A.

Effects of perinatal exposure to bisphenol A on sociosexual behavior of female and male rats.
Farabollini, F., Porrini, S., Della Seta, D., Bianchi, F. and Dessi-Fulgheri, F.
Environmental Health Perspectives Supplements 2002;110(3):409-414.

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 compd. bisphenol A (BPA) could alter the processes affecting sociosexual behavior. To test this hypothesis, the authors studied agonistic and sexual behavior of adult female and male rats whose mothers were administered BPA (40 mg/kg/day) during pregnancy or lactation. An intruder test revealed in males but not in females an increase in defensive behavior due to BPA. The authors 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. Thus, BPA administration, both during pregnancy and during lactation, does not masculinize female behavior or potentiate masculinization processes of males. On the contrary, a potentiation of female behavior in females and a depotentiation of male behavior in males was observed.

Exposure to a low dose of bisphenol A during fetal life or in adulthood alters maternal behavior in mice.
Palanza, P. L.; Howdeshell, K. L.; Parmigiani, S., and vom Saal, F. S.
Environ Health Perspect. 2002; 110 Suppl 3:415-22.

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. Pregnant females were fed daily doses of corn oil (controls) or 10 microg/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. 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. 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.

Effect of an endocrine disruptor on mammalian fertility. Application of monoclonal antibodies against sperm proteins as markers for testing sperm damage.
Peknicova, J.; Kyselova, V.; Buckiova, D., and Boubelik, M.
Am J Reprod Immunol. 2002; 47(5):311-8.

PROBLEM: To determine the influence of an endocrine disruptor [bisphenol-A (BPA)] on the integrated reproductive process as well as on individual reproductive organs and gametes in order to select suitable markers for testing sperm damage. METHOD OF STUDY: The effect of BPA on fertility in vivo in multigenerational studies in an outbred stock of mice was studied. Damage of reproductive organs was assessed by histochemical methods and damage of spermatozoa by means of a panel of monoclonal antibodies (MoAbs) against intra-acrosomal sperm proteins. RESULTS: BPA had a negative influence on offspring born of mice, on reproductive organs, and on acrosome integrity of mice spermatozoa. Selected MoAbs against intra-acrosomal mammalian sperm proteins, cross-reacted with mouse spermatozoa, were used for determination of the acrosome integrity. BPA had no effect on body weight and testicle weight of males. CONCLUSIONS: The present results demonstrate that BPA has a negative effect on in vivo fertility of mice, with impact on spermatogenesis and sperm quality. Monoclonal antibodies against intra-acrosomal sperm proteins can be used for detecting sperm damage.

Evaluation of developmental toxicity in rats exposed to the environmental estrogen bisphenol A during pregnancy.
Kim, J. C.; Shin, H. C.; Cha, S. W.; Koh, W. S.; Chung, M. K., and Han, S. S.
Life Sci. 2001; 69(22):2611-25.

Bisphenol A (BPA) is an essential component of epoxy resins used in the lacquer lining of metal food cans, as a component of polycarbonates, and in dental sealants. The present study was conducted in an attempt to evaluate the adverse effects of the environmental estrogen BPA on initiation and maintenance of pregnancy and embryofetal development after maternal exposure during the entire period of pregnancy in Sprague-Dawley rats. The test chemical was administered by gavage to mated females from days 1 to 20 of gestation (sperm in varginal lavage = day 0) at dose levels of 0, 100, 300, and 1000 mg/kg. All females were subjected to caesarean section on day 21 of gestation and their fetuses were examined for external, visceral and skeletal abnormalities. In the 1000 mg/kg group, significant toxic effects including abnormal clinical signs, decreased maternal body weight and body weight gain, and reduced food consumption were observed in pregnant rats. An increase in pregnancy failure was also found in the successfully mated females. In addition, increased number of embryonal deaths, increased postimplantation loss, reduced litter size and fetal body weight, and decreased number of fetal

ossification centers of several skeletal districts were seen. On the contrary, no significant changes induced by BPA were detected in the number of corpora lutea and implantation sites and by fetal morphological examinations. In the 300 mg/kg group, suppressed maternal body weight and body weight gain, decreased food intake and reduced body weight of male fetuses were seen. There were no adverse signs of either maternal toxicity or developmental toxicity in the 100 mg/kg group. It was concluded that BPA administration during the entire period of pregnancy in rats produced pregnancy failure, pre- and postimplantation loss, fetal developmental delay and severe maternal toxicity, but no embryo-fetal dysmorphogenesis at an oral exposure level of 1000 mg/kg.

The effects of prenatal exposure to ethinyl estradiol and bisphenol-A on the developing brain, reproductive organ and behavior of mouse.
Sato, M.; Shimada, M., and Sato, Y.
Congenital Anomalies. 2001; 41(3):187-93;

This study investigated the effects of ethinyl estradiol (EE) and bisphenol-A (BPA) on the maturation of fetuses, reproductive organ, brain development, and behavior. Twenty-eight Jcl-ICR pregnant mice were divided into 0.2 mg/kg of EE, 0.02 mg/kg of EE, BPA and control groups. Pregnant mice belonging to 0.2 mg/kg of EE and 0.02 mg/kg of EE group were daily injected subcutaneously either 0.2 mg/kg or 0.02 mg/kg of EE dissolved to olive oil from 11 to 19 days of gestation. The BPA group received an injection of 100 mg/kg of BPA dissolved in olive oil while the control group received an injection of olive oil alone subcutaneously on the same days of gestation. Neurological and behavioral development was examined by means of the sensorimotor reflexes until day 10 and openfield test on day 40 after birth. Myelination of the brain and maturation of testis were histologically examined. Obtained results were: 1) Pregnant mice in the 0.2 mg/kg EE group had no live births. 2) The mean litter size in the 0.02 mg/kg EE group was smaller than that in the BPA and control groups. The mean body weight at birth and that at the age of 60 days showed no significant differences among groups. 3) In the openfield test at the age of 40 days, the mean number of grooming and line-crossing in the inner field in the 0.02 mg/kg EE group were significantly higher than those in the control group and the mean number of grooming, rearing and line-crossing in the outer field in 0.02 mg/kg EE group were significantly higher than those in the BPA group. The mean numbers of defecation in both 0.02 mg/kg EE group and BPA group were less than those in the control group. 4) The mean diameter of seminiferous tubules and number of spermatocytes layers in the 0.02 mg/kg EE group and BPA were significantly less than those in the control group. 5) The mean diameter of tractus mamillothalamics in the 0.02 mg/kg EE group and BPA group showed no significant differences compared with that in the control group. These findings suggested that prenatal exposure to EE or BPA adversely affects litter size, openfield behavior and spermatogenesis.

Testicular toxicity of dietary 2,2-bis(4-hydroxyphenyl)propane (bisphenol A) in F344 rats.
Takahashi, O. and Oishi, S.
Arch Toxicol. 2001; 75(1):42-51.

Abstract: Male F344/DuCrj (Fischer) rats were given bisphenol A (BPA) in the diet at levels of 0 (control), 0.25, 0.50 and 1.00%, equivalent to 0, 235, 466 and 950 mg/kg per day, respectively, for 44 days. Body weight gains were depressed dose-dependently in BPA-treated rats, and those of 0.50 and 1.00% groups were significant. Testis and epididymis weights were not significantly decreased. Both absolute and relative weights of dorsolateral prostate and preputial glands were reduced in a dose-related fashion. Absolute weights of seminal vesicles and hypophysis were also decreased. Histopathologically, seminiferous tubule degeneration and loss of elongated spermatids were observed, the severity being related to BPA dose. The disorganization, distortion and degeneration of late spermatids, and the atrophy of seminiferous tubules were found even in the 0.25% BPA group. Serum testosterone concentrations were not decreased in BPA-treated groups. These results indicate that BPA even at a level of 0.25% (235 mg/kg per day) is clearly toxic to male reproductive organs.

Reproductive malformation of the male offspring following maternal exposure to estrogenic chemicals.
Gupta, C.
Proceedings of the Society for Experimental Biology & Medicine 2000;224(2):61-68.

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. 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 mug/kg/day, and 50 mug/kg/day, respectively, during Days 16-18 of gestation. A high dose of DES (200 mug/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. 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. 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 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. 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.

Exposure with the environmental estrogen bisphenol A disrupts the male reproductive tract in young mice.
Takao, T.; Nanamiya, W.; Nagano, I.; Asaba, K.; Kawabata, K., and Hashimoto, K.
Life Sci. 1999; 65(22):2351-7

Environmental estrogens (endocrine disruptive chemicals) have been shown to affect reproduction in wild life and it has been reported that maternal exposure with those chemicals have adverse effects on the male reproductive tract. However, little is known about the potential effects of prepubertal or pubertal exposure with environmental estrogens on the male reproductive tract. Here we examine plasma hormone levels and histology in the testis of mice following either 4- or 8-week oral administration of bisphenol A. Plasma free testosterone levels were dramatically decreased following 8 weeks of bisphenol A treatment compared with control group and morphologically multinucleated giant cells having greater than three nuclei were found in seminiferous tubules in the testis following the 8-week bisphenol A treatment. No differences in plasma corticosterone and luteinizing hormone levels were seen between bisphenol A and control groups. Thus, exposure with bisphenol A around pubertal period may directly disrupt the male reproductive tract. These facts suggest that more detailed studies will warrant the assessment of the risk to the developing human testis from exposure to bisphenol A and other environmental estrogens in prepubertal and pubertal period.

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.
vom Saal, F. S.; Cooke, P. S.; Buchanan, D. L.; Palanza, P.; Thayer, K. A.; Nagel, S. C.; Parmigiani, S., and Welshons, W. V.
Toxicol Ind Health. 1998; 14(1-2):239-60.

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. 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). 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. 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.

Testing of selected workplace chemicals for teratogenic potential.
Hardin, B.D., Bond, G.P., Sikov, M.R., Andrew, F.D., Beliles, R.P. and Niemeier, R.W.
Scand J Work Environ Health 1981;7:66-75.

The reproductive toxicity and teratogenic potential of 19 industrial chemicals have been investigated during the past 3 a. Preliminary studies utilizing intraperitoneal treatments of rats on days 1-15 of gestation have been conducted on the following ten chemicals: allyl chloride, bisphenol A, copper naphthenate, ethylene dibromide, hexachlorobutadiene, 2-mercaptobenzothiazole, methyl styrene, naphthalene, 2-nitropropane, and 1,2,3-trichloropropane. Studies utilizing inhalation exposure of rats and rabbits on days 1-19 and 1-24, respectively, of gestation have been conducted on the following nine chemicals: butylene oxide, carbon disulfide, 2-ethoxyethanol, ethyl benzene, methyl bromide, nitrous oxide, styrene oxide, tetrachloroethylene, and trichloroethylene. In the preliminary studies, evidence of teratogenic potential was seen with allyl chloride and bisphenol A, and fetal toxicity was found in the absence of maternal toxicity with methyl styrene and 2-nitropropane. In the inhalation studies, 2-ethoxyethanol was strongly embryotoxic at the higher exposure levels employed and was teratogenic at the lower concentration.

Testing of selected workplace chemicals for teratogenic potential with attachments, cover sheets and letter dated 022581.
Litton Bionetics Inc.
1981; EPA/OTS; Doc#88-8100188 NTIS/OTS0204918 TSCATS/408269.

Preliminary reproductive and teratogenicity studies with rats (10-15 inseminated females/group) exposed by intraperitoneal injection on gestation days 1 through 15 have been conducted on 10 chemicals: allyl chloride, bisphenol A, copper naphthenate, ethylene dibromide, hexchlorobutadiene, 2- mercaptobenzothiazole, methyl styrene, naphthalene, 2-nitropropane, and 1,2,3-trichloropropane. Evidence of teratogenic potential was seen with allyl chloride (short snout; protruding tongue) and bisphenol A (incomplete skeletal ossification), and fetal toxicity was seen in the absence of maternal toxicity with methyl styrene (increased incidence of resorptions; altered fetal sex ratio, with a deficit of female fetuses) and 2-nitropropane (delayed fetal development). Studies with pregnant rats (30/group) and rabbits (15-20/group) exposed by inhalation, 6 to 7 hours/day, on gestation days 1 through 19 and 1 through 24, respectively, were

conducted on 9 chemicals: butylene oxide, carbon disulfide, 2-ethoxyethanol, ethyl benzene, methyl bromide, nitrous oxide, styrene oxide, tetrachloroethylene, and trichloroethylene. 2-Ethoxyethanol was strongly embryotoxic (all litters were absorbed) to both species at the higher exposure levels, and was teratogenic (increased incidence of cardiovascular and skeletal defects) at the lowest concentration.

B. Meeting abstracts reporting developmental or reproductive toxicity

Developmental Exposure To Environmental Estrogens Alters Adult Behavior And Physiology In The Rat.
Ryan, B. C., Gray, L. E., Crofton, K. M., and Vandenbergh, J. G.
Biol Reprod. 2005 Jul; (Special Issue):227.

Abstract: Anthropogenic estrogens are pervasive in the environment, leading to both human and wildlife exposure. This project focuses on the effects of developmental exposure to two such compounds: ethinyl estradiol, found in contraceptive pills and bisphenol-A, a component of polycarbonate plastic. Studies investigating endocrine disruptors typically focus on reproductive endpoints. Estrogens, however, have the potential to alter a variety of non-reproductive parameters. For this reason, we have chosen to study non-reproductive behaviors that show sexual-dimorphism, namely saccharin preference and activity in a figure-8 maze. For reference, we will also be measuring more traditional reproductive endpoints such as vaginal opening and lordosis response. An initial dose-response study in adult rats indicated no strain difference between Long-Evans and Sprague Dawley rats in response to ethinyl estradiol utilizing both physiological (uterine weight) and behavioral (lordosis) endpoints. Since no strain difference was present, we chose to use Long-Evans rats for this study to maintain consistency with past behavioral work done in the lab. Developmental exposure to ethinyl estradiol (from gestational day 7 to postnatal day 18) causes pup mortality and an earlier vaginal opening at high doses (5 and 50ug/kg/day). These same doses also masculinize saccharin preference in females. Ongoing studies will determine the effects of this developmental exposure to both ethinyl estradiol and bisphenol-A at a range of doses on adult behaviors.

Bisphenol A Increase Steroidal Hormone Secretion Of Testes.
Gharravi, A.; Ghorbani, R.; Khazaei, M.; Al Agha, M.; Ghasemi, J.; Sayadi, P., and Pourmotabbad, A.
Biol Reprod. 2005 Jul; (Special Issue):141.

Abstract: Introduction: Bisphenol A (BPA) is a xenobiotic estrogenic compound. This compound has suspected to have estrogenic effects on reproductive system of male and female. In this present study, we investigated possible low dose effects of BPA on testosterone hormone MATERIAL, METHODS: Male wistar rats (12-13 week old) were administrated a daily intra peritoneal 10ug/kgbw/day, 50ug/kgbw/day, 100ug/kgbw/day dose of BPA for12 days and one

day after last injection serum level of testosterone examined. All data expressed as means and SE. one-way ANOVA performed. RESULTS: Analysis of data showed, in 100ug dose group, serum level of Testosterone Hormone (5.880) significantly increased in compare with control group (2.900). CONCLUSION: The present study showed that BPA at low doses affects steroidgenesis in testicular Leydig cells, and subsequently alters the secretion of Testosterone Hormone, one of main hormone in spermatogenesis in the adult wistar rats.

Effects of environmental endocrine disruptors on sexual differentiation of the brain and behavior.
Aou, S.
Congenit Anom Kyoto. 2004 Dec; 44(4):A21.

Abstract: The effects of exposure to endocrine disruptors during the fetal and suckling periods on the sexual differentiation of behaviors and the brain were examined. We administered bisphenol A (BPA; 0.1, 1, and 5 mg/L) and diethylstilbestrol (DES; 50 ug/L) to mother rats via the drinking water during pregnancy and lactation. In the control group, female offspring were more active in the open field test, and had a larger locus coeruleus than males. BPA exposure abolished sex differences regarding open- field behavior and inverted the volume of the locus coeruleus without any remarkable change in the reproductive system. The rats exposed to DES did not show any sexual dimorphism in the open field test. A reversal of the sex difference in the locus coeruleus volume was also observed, as in the BPA-treated animals. In contrast to BPA, DES inhibited the female reproductive function and reduced the body weight of male offspring. These results suggest that BPA and DES disrupt the sexual differentiation of the brain and non-reproductive behavior in a similar manner, but that of the reproductive function and body weight control in different ways. The locus coeruleus, the major source of noradrenergic neurons, is highly sensitive to low-dose administration of BPAand DES. Noradrenaline has been shown to modulate not only many different kinds of behaviors, but also different types of neuronal responses to sensory, visceral and motor stimuli including reward-related neuronal responses in the lateral hypothalamic area, the amygdala, and the orbitofrontal cortex. Impairments of sexual differentiation of the locus coeruleus may affect these diverse brain functions.

Fetal exposure to bisphenol A impairs sexual differentiation of brain functions in rats.
Fujimoto T, Kubo K, and Aou S.
Neurosci Res 2004;50(Suppl 1):S143.

Perinatal exposure to Bisphenol A (BPA), a well-known endocrine disrupters, has been shown to influence the sexual differentiation of brain and behaviors. In this study, BPA was exposed to the pregnant Wistar rats at a dose of 0.1 ppm during the last week of prenatal period. The offspring were tested using an open field test, an elevated plus maze test, a passive avoidance test and a forced swimming test. Olfactory responses of medial amygdalar neurons were also examined. In the BPA treated group, sex differences of the number of rearing, the duration of struggling, and the pattern of neuronal responses to odors were abolished. The duration of immobility, an index

of depressive behavior, in the forced swimming test was elongated. These findings suggest that fetal exposure to bisphenol A impairs the sexual differentiation of brain functions and enhance depressive response.

Prenatal exposure to bisphenol A may induce apoptosis in gonocytes of rat testis at term.
Matsuo, T. and Yasuda, Y.
Congenit Anom Kyoto. 2004 Dec; 44(4):A49.

Abstract: Bisphenol A (BPA) is widely used in the production of epoxy, polycarbonate and polyesterstyrene resins, for the interior coating of cans, artificial teeth, filters and so on. Previously, we have reported that prenatal exposure to BPA significantly suppresses the fetal development at term. The purpose of the present study was to examine the effect of prenatal exposure to BPA on testicular differentiation at term. Pregnant rats (jcl: Wistar, Clea Inc., Japan) were given orally an olive oil suspension of BPA at 0, 1 or 100 mg/kg body weight once a day during days 6 to 15 of gestation. The pregnant rats in each group were anesthetized with ether and killed by cervical dislocation, and the fetuses were removed from the uteri on day 20 of gestation. At term, the testes were removed from the fetuses, and processed for immunohistochemistry and terminal deoxynucleotidyl transferase (TdT) assay. For Western blotting, lysates of the testes were supplied for detection of Bax and Bcl-2 protein. Light microscopic examinations revealed that 1) many more dying gonocytes were detected significantly in all experimental groups ($p < 0.05$) than in the controls and 2) more apoptotic spermatogonia were seen in the 100 mg/kg-treated group than in the controls ($p < 0.05$). The Bax and Bcl-2 proteins were detectable in the testes at term, but no suggestive effects were recognized in the testicular differentiation. In conclusion, prenatal exposure to BPA appears to suppress spermatogenesis in rats of adult age.

Fetal Programming: Prenatal Exposure To Bisphenol-A But Not Methoxychlor Leads To Growth Retardation.
Savabieasfahani M and Padmanabhan V.
Biol Reprod 2004;Aug(Special Issue):115.

Intrauterine growth retardation predisposes the individual to a number of adulthood diseases. In the female, low birth weight is a risk factor for impaired ovarian development, oligo-ovulation, anovulation and polycystic ovarian syndrome. We had found that prenatal treatment with testosterone produces reproductive anomalies similar to that seen in women with PCOS, which include hypergonadotropism, LH surge defects, multifollicular ovaries and infertility. The defects in the LH surge system and the multi follicular ovaries of the prenatally T-treated sheep appear to be facilitated by conversion of testosterone to estradiol because prenatal dihydrotestosterone, a non-aromatizable androgen, treatment fails to produce such defects. Recently we found that prenatal exposure to bisphenol A (BPA) a plasticizer or methoxychlor (MXC) a pesticide, two environmental estrogen mimics, had differential effects on reproductive function (Biol Reprod 68(Suppl 10): 273). Prenatal BPA treatment resulted in prepubertal

hypergondotropism and reduced LH surge amplitude. Prenatal MXC treatment increased the latency of the LH surge. The deficits in these reproductive attributes of prenatally BPA and MXC- treated females parallel those seen in the prenatal testosterone-treated sheep. We determined if the prenatally BPA and MXC treated sheep, similar to the prenatally testosterone treated females, are growth-retarded. We retrospectively compared the weight, height and chest circumferences of female offspring born to prenatally BPA (n=8) and MXC (n=6) treated (5 mg/kg/day in cotton seed oil) mothers to the offspring of controls (n=9). Results revealed that, controlling for litter size, female offspring of prenatally BPA but not MXC treated sheep were growth-retarded, when compared with those of controls. This growth-retardation was reflected as a reduction in weight (p=0.008), height (p=0.001), and chest circumference (p=0.035) of the prenatally BPA treated female offspring. These findings in conjunction with the reproductive cycle defects of the prenatally BPA-treated sheep suggest that estrogenic environmental pollutants can have deleterious effects on fetal development and differentiation thus predisposing them to adult diseases.

Developmental Effects Of Prenatal Exposure To Bisphenol A On The Uterus Of Rat Offspring.
Schonfelder G, Friedrich K, Wu XD, Paul M, and Chahoud I.
Naunyn Schmiedebergs Arch Pharmacol 2004;369(Suppl 1):R112.

Exposure to estrogenic compounds during critical periods of fetal development could result in adverse effects on the development of reproductive organs later in life. Bisphenol A (BPA), widely discussed as a prime candidate for endocrine disruption, is highly employed in the manufacture of a wide range of consumer products. We examined the influence of BPA to address the question whether in utero exposure affects the uterus of the offspring. Gravid Sprague Dawley dams were administered by gavage either 0.1 (low dose) or 50 mg/kg/d BPA, the currently accepted low observe effect level (LOEL) dose or 0.2 mg/kg/d 17alpha-ethinyl estradiol (E2) on gestation days 6 through 21. We examined uterine morphology and estrogen receptor (ERalpha) expression on mRNA and protein level because BPA binds to the ERalpha which is important for growth of the uterine epithelium. Striking morphological changes, which are similar to DES-specific disruption patterns, were observed in the uterine epithelium of post-pubertal offspring during estrus of the in utero BPA treated animals (the thickness of the total epithelium was significantly reduced). In the 50 mg BPA and E2-treated group the expression of ERalpha was increased at protein level in Western blot analysis and immunohistochemistry when compared to the control and 0.1 mg BPA treated uteri. In addition, within the stroma of the E2-treated group the ERalpha-staining pattern frequently describes a uniform thick mesenchymal cell layer underlying the luminal epithelium. Whereas, within the 50 mg BPA dose-group strongly ERalpha-immunostained stromal cells are not organized in such a uniform cell layer underlying the epithelium. Newly synthesized ERalpha could only be demonstrated by increased ERalpha-mRNA expression in the 50 mg BPA-treated group. In summary, these results clearly indicate that in utero exposure of rats to low-doses of BPA promotes uterine disruption in offsprings. We suggest that this could possibly be due to the disregulation of ERalpha expression connected to a disturbed close epithelial-stromal tissue interaction.

Fetal Programming: Prenatal Exposure To Bisphenol-A And Methoxychlor Disrupts Ovarian Follicular Dynamics.
Steckler TL, Manikkam M, Inskeep EK, and Padmanabhan V.
Biol Reprod 2004;Aug(Special Issue):138.

Exposure of sheep fetuses to testosterone (T) and not dihydrotestosterone disrupts estradiol (E) positive feedback (Rev Reprod 3:130, 1998) and leads to multifollicular ovaries (Mol Cell Endocrinol 185:51, 2001) suggesting that such disruptions are regulated by conversion of T to E. These findings raise concerns as to the threat that unintended exposure to exogenous steroids poses to reproductive health. Recently, we found that in utero exposure to bisphenol-A and methoxychlor (BPA, MXC; estrogenic endocrine disrupting chemicals (EDCs)) had differential effects on the generation of the preovulatory LH surge following induction of luteolysis with PGF2alpha; BPA sheep had reduced latency and/or reduced LH surge amplitude while MXC sheep had increased latency (Biol Rep 68(Suppl 1):293, 2003). Differences in latency of LH surge in BPA/MXC sheep suggest disruptions in follicular dynamics and/or compromised E positive feedback. In this study, we tested the hypothesis that exposure of sheep fetuses to BPA or MXC impairs follicular selection dynamics. Suffolk ewes were treated with MXC (n=6), BPA (n=8) (5 mg/kg/day in cotton seed oil) or vehicle (C; n=8) s.c. from days 30 to 90 of gestation. At ~19 months of age, transrectal ovarian ultrasonography was performed daily for 21 days. Daily changes in appearance and disappearance of ovarian follicles, the follicular sizes and presence of corpora lutea (CL) were monitored in both ovaries. In the MXC group, an increase in total number of follicles (C: 93.7$\pm$2.8; MXC: 112.4 $\pm$7.2; $P < 0.02$) and size of the second largest follicle (C: 5.0$\pm$0.7; MXC: 7.0 $\pm$0.5; $P < 0.05$) was observed, but only a tendency for an increase in mean duration of presence of the second largest follicle (C: 5.5 $\pm$ 0.63 d; MXC: 7.2 $\pm$ 0.5 d; $P < 0.08$). The BPA group was characterized by an increase in the diameter (mm) of the second largest follicle (C: 5.0±0.7; BPA: 7.1$\pm$0.4; $P < 0.02$). The increase in total number of follicles in the MXC group may be a reflection of increased recruitment and/or failure to regress. Altered follicular dynamics of prenatally EDC-treated animals substantiate the concerns regarding the deleterious effects that unintended exposure to EDCs during pregnancy might have on fertility.

Early Programming Of Reproductive Dysfunction: Sisters Influencing Brothers In Utero: A Litter-Bearing Model.
vom Saal FS, Richter CA, Welshons WV, and Timms BG.
Biol Reprod 2004;Aug(Special Issue):82.

Rodent male and female fetuses differ in their serum levels of testosterone and estradiol based on whether they develop between two male or two female fetuses (the intrauterine position phenomenon). These relatively small differences in fetal steroids lead to significant differences in a wide variety of reproductive traits. While some phenotypic differences are mediated by differential fetal exposure to testosterone, experimental studies have shown that very small

changes in serum estradiol also lead to marked changes in development of the male reproductive system. This raised the possibility that environmental chemicals and drugs with estrogenic activity might also alter development. To test this hypothesis we fed pregnant CD-1 mice on gestation day 14-18 a 0.1 micro g/kg/day dose of the estrogenic drug diethylstilbestrol (DES) as a positive control, a 0.1 micro g/kg/day dose of ethinyestradiol (5-fold lower than the dose in most oral contraceptives) or a 10 micro g/kg/day dose of bisphenol A, the estrogenic monomer used to manufacture polycarbonate plastic (this dose results in lower blood levels of bisphenol A in fetal mice than average levels in human fetuses). When examined on GD 19, we found that relative to negative controls, each of these estrogenic chemicals significantly increased the number and size of primary ducts in the dorsolateral prostate and induced a marked increase in proliferation of epithelial cells in the proximal region of the primary prostatic ducts, which preliminary evidence suggests are basal cells. There were also malformations in the urethra that could interfere with the regulation of urine flow. It is possible that acceleration of 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 of 4-tert-octylphenol and bisphenol A on the spermatogenesis in rat testes: induced apoptosis in testicular germ cells.
Kim SK, Kim JM, Lee HJ, and Yoon YD.
Biol Reprod 2003;68(Suppl 1):187.

4-tert-octylphenol (OP) and bisphenol A (BPA) are known to disrupt testicular development and reduce male fertility. However, the precise mechanism for the impairment of reproductive system by OP and BPA treatment are virtually unknown. The purpose of the present study was to investigate the effects of chronic exposure of OP or BPA on the reproductive system of prepubertal male rats. First, we evaluated the expression of Bcl-2 family genes such as bcl-2, bax, bad, and bim regarding to the apoptosis of testicular germ cells induced by OP or BPA treatment. Second, we examined IGF-I and PDGF-A mRNA expression in the testis to elucidate the effect of OP and BPA on the expression of growth factors and measured serum testosterone concentration which play a major role in spermatogenesis. Prepubertal male rats were injected with estradiol valerate (EV; 0.4 ug), OP (0.4, 4 or 40 mg), or BPA (0.1, 1, or 10 mg) s.c. in olive oil three times a week for 1, 2, 3, or 4 weeks. High dosage of OP (OP 40 mg) had the following effects dependent on the exposure time: decreased sizes and weights of testis, epididymis, and seminal vesicle and adversely impaired histological structure of testis. However, all the three dosages of BPA did not change the sizes and weights of testis, epididymis and seminal vesicle. The results on TUNEL and the Bcl-2 family genes expression showed that apoptosis of testicular germ cells was increased by OP treatment and increased expressions of proapoptotic genes, bax and bad were involved in this process. There was no change of the expression of growth factors such as IGF-I and PDGF-A by OP or BPA treatment in the testis. However, serum testosterone concentration was decreased in the rat treated with high dosages of OP and BPA for 4 weeks. Taken together, the present study demonstrated that OP severely reduced the sizes and/or functions of the male reproductive organs and caused the impairment of spermatogenesis leading

to an increase in apoptosis of testicular germ cells and a decrease in amount of testosterone. It is suggested that OP may exert its own toxic effects on the male reproductive organs.

Developmental disruption by bisphenol A in early chick embryos.
Suzuki, K., Saito, K., Oyama, E., Takenaka, M., Yagi, M., and Suzuki, H.
J Toxicol Sci. 2003 Oct; 28(4):335.

Abstract: Recently, public concerns have arisen on the effects of endocrine disrupters, especially estrogenic substances, on reproduction and development of animals including humans. In order to determine the effect of small amount of endocrine disrupters on developmental processes, early stage developing chick embryos at stage 10 were received bisphenol A (BPA) via yolk just beneath the embryo. Fertilized eggs of white Leghorn were obtained within 24 hrs after being laid down. BPA/DMEM solution was administered at 0.0049, 0.0098, 0.0195, 0.039, 0.078, 0.078, 0156, 0.313, 0.625, 1.25, 2.5, 5.0 and 10.0 ug per an embryo. Incubations were done for batches of BPA treated and control eggs, 10 eggs for a batch. Eggs were kept at 37 +/- 0.5 degrees C and more than 80% relative humidity with one rotation per an hour for 48 hrs. Then chick embryos were excised and fixed in 70% ethanol. The embryos were observed under a stereomicroscope on morphological abnormalities and number of somites. There were no significant difference in the average number of somites of BPA injection groups and control. The abnormal embryos were found in BPA treated groups at more than 0.0098u/ embryo but not in 0.0049u BPA embryo group. It appeared to be a dose related increase in the ratio of abnormal embryogenesis caused by BPA. The abnormal embryogenesis included dysmorphology of the neural tube, duplication anomalies, heteroplasia of the head, and neural tube formation alone.

Developmental disruption by bisphenol A in early chick embryos (No.3).
Suzuki, K., Saito, K., Oyama, E., Takenaka, M., Yagi, M., and Suzuki, H.
Congenit Anom Kyoto. 2003 Sep; 43(3):244.

Abstract: Recently, public concern has arisen over the effects of endocrine disrupters, especially estrogenic substances, on reproduction and development of animals including humans. In order to determine the effect of small amounts of endocrine disrupters on developmental processes, early stage developing chick embryos at stage 10 were treated with bisphenol A (BPA) via the yolk just beneath the embryo. Fertilized eggs of white Leghorn were obtained within 24 hrs after being laid down. A BPAIDMEM solution was administered at 0.0049, 0.0098, 0.0195, 0.039, 0.078, 0.078, 0156, 0.313, 0.625, 1.25, 2.5, 5.0 and 10.0 micro g per embryo. Incubations were done for batches of BPA-treated and control eggs, 10 eggs for a batch. Eggs were kept at 37 plus/minus 0.5 degrees C and more than 80% relative humidity with one rotation per hour for 48 hrs. Then chick embryos were excised and fixed in 70% ethanol. The embryos were observed under a stereo microscope for morphological abnormalities and numbers of somites. There were no significant differences in the average number of somites between BPA injection groups and the control. Abnormal embryos were found in BPA-treated groups at more than 0.0098 micro g/embryo but not in the 0.0049 micro g BPA group. There appeared to be a dose-related increase

in the ratio of abnormal embryogenesis caused by BPA. The abnormal embryogenesis included dysmorphology of the neural tube, duplication anomalies, heteroplasia of the head, and neural tube formation alone.

Prenatal and neonatal exposure to bisphenol-A enhances the central dopamine D1 receptor-mediated action in mice.
Suzuki, T., Mizuo, K., Sakata, M., and Narita, M.
J Pharmacol Sci. 2003; 91(Suppl):34P.

Abstract: Recently, the general public has received alarming reports regarding the reproductive and health hazards of endocrine-disrupting chemicals in the environment. Bisphenol-A (BPA), one of the most common environmental endocrine disrupters, has been extensively evaluated for toxicity in a variety of tests in rodents, including developmental and reproductive toxicity, and carcinogenicity. However, little is known about its action on the central nervous system. In the present study, we found that prenatal and neonatal exposure to BPA markedly enhanced the rewarding effect and hyperlocomotion induced by methamphetamine, which reflect extensive abuse associated with sociological and psychiatric problems. We also demonstrated that chronic exposure to BPA produced an up-regulation of dopamine D1 receptor function to activate G-protein in the mouse limbic forebrain, which is thought to be a critical site for the expression of rewarding effects by abuse drugs. Additionally, chronic BPA exposure produced a significant increase in levels of the dopamine D1 receptor mRNA in the whole brain. The present data provide first evidence that prenatal and neonatal exposure to BPA can potentiate the central dopamine D1 receptor-dependent neurotransmission, resulting in supersensitivity of methamphetamine-induced pharmacological actions related to psychological dependence on psychostimulants.

C. Studies reporting no developmental or reproductive toxicity

Effects Of Gestational And Lactational Exposure To Ethinyl Estradiol And Bisphenol A On Reproductive Physiology And Serum Steroid Hormones In The Male Long Evans Hooded Rat.
Howdeshell, K. L.; Furr, J.; Lambright, C. R.; Wilson, V. S.; Ryan, B. C.; Vandenbergh, J. G., and Gray, L. E. Jr.
Biol Reprod. 2006; (Spec no):133-4.

Abstract: Many chemicals released into the environment are capable of disrupting normal sex steroid balance, including ethinylestradiol (EE) and bisphenol A (BPA). Ethinylestradiol is a synthetic estrogen used principally in female oral contraceptives and is present in aquatic systems at sufficient concentrations to adversely affect fish reproduction. Bisphenol A is an estrogenic monomer used in the manufacture of polycarbonate products and is known to leach from them with use. Concerns have been expressed about potential low dose effects of BPA on human development because it has been found in human amniotic fluid and in baby products. Both EE and BPA are reported to impair reproductive organ development when administered in

utero in laboratory animals; however, lower dose effects of these chemicals have been debated. The goal of this study was to determine whether relatively low oral doses of EE or BPA would alter male reproductive morphology (gross and histological) and associated hormone levels in the Long Evans rat. The Long Evans rat dams were exposed to EE (0.05, 0.5, 5 and 50 microg/kg/day) or BPA (2, 20 and 200 microg/kg/day) during pregnancy through lactation from gestational day 7 to postnatal day 18. AGD was measured at birth and nipple retention was measured at PND13 in male pups. Male offspring were euthanized beginning at PND150, and sera and organs were collected for analysis. Body weight was significantly decreased in males developmentally-exposed to EE50. Treatment with EE5 and EE50 reduced the weight of the testes and epididymides. Epididymal sperm counts were also significantly lower in EE50 with a trend in the EE5 males. In the EE50 group, relative and absolute androgen-dependent organ weights were reduced even though serum testosterone was not affected, implying abnormal imprinting. No other effects were noted with EE treatment and BPA treatment did not significantly affect any endpoint in the current study. In conclusion, we found adverse effects on the male reproductive system at lower doses of EE than previous reported with the Sprague Dawley rat. These results demonstrate that developmental exposure to oral micromolar doses of EE can permanently demasculinize the reproductive tract of the male rat.

Effects of in utero and lactational exposure to bisphenol A on thyroid status in F1 rat offspring.
Kobayashi, K., Miyagawa, M., Wang, R. S., Suda, M., Sekiguchi, S., and Honma, T.
Ind Health. 2005 Oct; 43(4):685-90.

Abstract: Bisphenol A (BPA), a xenoestrogen, has been reported to mimic the actions of estrogen or to affect the endocrine glands in vivo and in vitro. In this study, we examined whether in utero and lactational exposure to BPA alters thyroid status in rat F1 offspring. Dams were orally administered various doses of BPA (0, 4 or 40 mg/kg body weight per day) from gestation day (GD) 6 through postnatal day (PND) 20. The BPA and control groups did not differ significantly with respect to plasma thyroxine (T4) concentration. The thyroid glands from the BPA groups had normal T4 responses to exogenous thyroid-stimulating hormone in vivo. These results suggest that in utero and lactational exposure (indirect exposure) to BPA (4-40 mg/kg/day, GD 6 - PND 20) does not affect thyroid functions in the F1 generation of male and female rats.

[Effect of Bisphenol A Administration on Reproductive Toxicant of Dam and Sex Ratio of Pups in Pregnant Mice].
Park, D. H., Jang, H. Y., Kim, C. I., Cheong, H. T., Park, C. K., and Yang, B. K.
Journal of Toxicology and Public Health : an Official Journal of the Korean Society of Toxicology. 2005 Jun; 21(2):161-5.

Abstract: Bisphenol A (BPA), a environmental endocrine disruptor, is considered to bind to estrogen receptors and to regulate the expressions of estrogen responsive genes. This study was to evaluate the effect of BPA administration on body weight sex ratio and litter size on 18 days

in prenatal periods, the effect of reproductive organ weight and blood hematological values on 24 days postpartum in pregnant mice. The female mice was administrated to low doses of BPA (0, 0.05, 0.5 and 5.0 mg/kg B.W.) by intraperitoneal injection in gestation days 0-15 with 5 times at 3 days interval. The maternal body weight, litter size and sex ratios were similar to in all experimental groups, but body weights of male and female offspring was significantly lower in 5.0 mg BPA group when compared to any other groups ($P < 0.05$). No treatment-related effects on body weight, ovary weight and blood hematological values were observed in dams on 24 days after delivery. The uterine weight in 5.0 mg BPA group was slightly higher than those of any other groups, but not significantly difference. The histological evaluation of ovary in dam mice on 24 days after delivery was not difference in all experimental groups, but the endometriosis of uterus in dam mice were significantly increased in 0.5 mg BPA group when compared to control group. These results indicates that low concentration of BPA should not be considered as a selective reproductive toxicant.

[Studies on the Reproductive Toxicant and Blood Metabolite in Pups Born After Bisphenol A Administration in Pregnant Mice].
Park, D. H., Jang, H. Y., Kim, C. I., Cheong, H. T., Park, C. K., and Yang, B. K..
Journal of Toxicology and Public Health: an Official Journal of the Korean Society of Toxicology. 2005 Jun; 21(2):167-73.

Abstract: Bisphenol A (BPA) is a monomer used in the manufacture of a multitude of chemical products, including epoxy resins and polycarbonate. The objective of this study was to evaluate the effects of BPA administration on reproductive characteristics and blood hematological and chemical values in offspring of pregnant dams treated with BPA. BPA was administrated to pregnant mice by intraperitoneally injection with 0, 0.05, 0.5 and 5.0 mg/kg B.W. for 5 times at 3 days interval on gestation days 1-16. There were no treatment-related effects of BPA on reproductive organ weight in male offsprings at 45 days-of-age, but body weight was the lowest in 5.0 mg BPA group when compared to other groups ($P < 0.05$). No differences in semen characteristics (sperm concentration, viability, motility and abnormality) were observed between the control and BPA treatment groups. The WBC, HB, HT, MCV, MCH, MCHC, albumin, BUN and total protein of blood hematological and chemical values in male offsprings were not difference for any treatment groups, but RBC value in BPA groups was significantly increased comparing to the control group ($P < 0.05$). The PLT value was slightly higher in 5.0 mg BPA groups than in any other group, but not significantly difference among the experimental groups. In female offsprings, the effects of BPA didn't affect to the body and ovary weight. but the uterus weight in 5.0 mg BPA group was slightly heavier than that of control group ($P < 0.05$). No statically significant difference in blood hematological values in female offsprings were observed between the control group and BPA groups, but the concentration of albumin and BUN were significantly higher in 0.5 mg BPA group when compared to control and other BPA treatment groups ($P < 0.05$). The histological evaluation of testis and ovary in growing offspring at 45 days-of-age was not difference between the control group and BPA groups, but endometriosis of the uterus in female offspring was dramatically increased in 0.5 and 5.0 mg

BPA groups. These founding suggest that low concentration of BPA might not have an important role on reproductive ability or blood metabolite in offspring of pregnant dams treated with BPA.

Toxicologic/carcinogenic Effects of Endocrine Disrupting Chemicals on the Female Genital Organs of Rodents.
Maekawa, A., Yoshida, M., Katsuda, S. I., and Imai, K.
Journal of Toxicologic Pathology. 2004 Summer; 17(2):69-83.

Abstract: Toxicologic/carcinogenic effects of some representative endocrine disrupting chemicals (EDCs) having estrogenic activity, such as alkylphenols, on the female genital organs of rodents, especially rats, are reviewed and discussed, focusing on our recent research. Neonatal treatment of high-dose p-tert octylphenol (t-OP, 100 mg/kg s.c. injection every other day from postnatal day 1 (PND 1) to PND 15) induced various long-term persistent irreversible effects on the female reproductive system of Donryu rats, such as lower gonadotropin levels at prepuberty, inhibition of uterine gland genesis, persistent estrus and polycystic ovaries. The result indicates that neonatal high-dose treatment of estrogenic EDCs can affect gonadotropin secretion during the developmental period of sexual maturation with direct masculinization of the hypothalamic function. Exposure limited to the first 5 days after birth (PNDs 1-5) to 100 mg/kg t-OP, however, caused delayed influence which was characterized by accerelated appearance of atrophic ovary, manifested by early-occurring and long-term continuing persistent estrus after puberty, whereas no abnormalities could be found with regard to growth and differentiation of the reproductive organs and the hypothalamo-pituitary-gonadal control system up to maturation, the influence being caused by delayed modulation of the hypothalamo-pituitary-gonadal control system. The most notable effect on the female reproductive system when normal cycling rats were exposed to high-doses t-OP for 28 days, was disappearance of the estrous cycle, but no clear changes were detected in other parameters such as uterine weight and morphology. These results indicate that the most serious issue with EDCs is the potential effects of prenatal and/or neonatal exposure on rodents. Well or moderately differentiated adenocarcinomas increased in Donryu rats initiated with N-ethyl-N-nitro-N-nitrosoguanidine, when high-dose t-OP was given subcutaneously during adulthood. Neonatal exposure for PNDs 1-5 to high-dose t-OP also showed promoting effects on uterine adenocarcinoma development. However, in rats given t-OP for PNDs 1-15, uterine tumor malignancy was clearly increased, although there was no significant alteration in the total incidence of adenocarcinomas. The results are very interesting in consideration of the histogenesis of uterine adenocarcinomas. However, maternal exposure to low doses of EDCs such as nonylphenol and bisphenol A at actual human exposure levels by the oral route showed no effects on growth and development of the female reproductive system or uterine carcinogenesis. These results indicate that dietary exposure to low doses of EDCs might not induce any adverse effects on the female genital system in mammals including humans.

Maternal exposure to low doses of bisphenol a has no effects on development of female reproductive tract and uterine carcinogenesis in Donryu rats.
Yoshida M, Shimomoto T, Katashima S, Watanabe G, Taya K, and Maekawa A.
J Reprod Dev 2004;50(3):349-60.

Effects of maternal exposure to low doses of bisphenol A (BPA), including those comparable with human exposure levels, on growth and development of the female reproductive system and uterine carcinogenesis in Donryu rats were investigated. Dams were administered BPA (0, 0.006 and 6 mg/kg/day) daily by gavage from gestation day 2 up to the day before weaning (postnatal day 21 at offspring). The serum levels of BPA were significantly elevated in the dams receiving 6 mg/kg/day, however, BPA levels in the milk of dams, and those in the serum and liver of offspring were similar between control and treated groups. The treatment did not exert any influences on uterine development including weight, gland genesis and estrogen receptor alpha expression, vaginal opening and gonadotropin secretion in the female offspring up to puberty. After maturation, no effects were evident with regard to estrous cyclicity in female offspring treated with BPA. In addition, the treatment had no effects on age-related morphological changes of the reproductive and endocrine organs and uterine carcinogenesis until 15 months of age. The results demonstrate that maternal exposure to BPA at levels comparable to human exposure did not have any effects on the female reproductive system of offspring in rats. In addition, BPA was also found in the serum, milk and liver of control dams and pups, and low levels of BPA were detected in drinking water and pellet diet. The present study showed that the experimental animals were also exposed to environmental BPA in the animal room.

The effect on sperm production in adult Sprague-Dawley rats exposed by gavage to bisphenol A between postnatal days 91-97.
Ashby J, Tinwell H, Lefevre PA, Joiner R, and Haseman J.
Toxicol Sci 2003;74(1):129-38.

M. Sakaue et al. (2001,J. Occup. Health vol. 43, pp. 185-190) have described how oral exposure of sexually mature male rats to bisphenol A (BPA) between postnatal days (PND) 91-97 led to a reduction in daily sperm production (DSP) 5 weeks later (18 weeks of age). Activity was observed over the dose range 20 microgram/kg-200 mg/kg BPA, with an absence of activity over the dose range 2 ng/kg-2 microgram/kg BPA. There was no evidence of a dose response relationship over the active dose range (five orders of magnitude range). The observation of endocrine disruption (ED) effects for BPA at such low doses, and in sexually mature animals, was unexpected. It was therefore decided to mount an independent repeat of their study. A total of four independent studies were conducted according to the protocol used by Sakaue et al. Doses of 20 microgram/kg, 2 mg/kg, or 200 mg/kg BPA were administered to adult Sprague-Dawley (SD) rats over PND 91-97, and the studies were terminated when the rats reached the age of 18 weeks. Three different rodent diets were employed (RM3, Purina 5002, and CE2), the last of which had been used by Sakaue et al. BPA failed to give any evidence of ED activities, including the changes in DSP reported by Sakaue et al. 2001. During the course of these studies, the test protocol was adapted to coincide more precisely with that used by Sakaue et al.; this

included restricting the number of animals per cage, removing bedding from the cages, and changing to the use of glass water bottles in the cages. The only thing of interest to emerge from our studies was the observation of a significant difference in DSP between the control groups of our first and second study. As the change in diet from RM3 to Purina 5002 was the major difference between those two studies, we conducted a repeat of the second study, but we were unable to confirm the differences seen between the first and second study. The probability that those differences arose either by chance, or as the result of intrinsic study-to-study variability, was strengthened by the absence of significant differences in the sperm parameters in a final (fifth) study where the sperm parameters for control animals maintained on the three different diets were compared under the conditions of the main experiments. No explanation for our failure to replicate the effects reported by Sakaue et al. is evident. A review of DSP values reported in the recent literature is provided and discussed, and it is concluded that use of the term DSP/g testis rather than DSP/testis could increase the sensitivity of DSP assessments.

Lack of carcinogenic risk in the prostate with transplacental and lactational exposure to bisphenol A in rats.
Ichihara T, Yoshino H, Imai N, Tsutsumi T, Kawabe M, Tamano S, Inaguma S, Suzuki S, and Shirai T.
J Toxicol Sci 2003;28(3):165-71.

The current study was designed to examine the modulating effects of bisphenol A (BPA) on prostate cancer risk in male offspring exposed transplacentally and lactationally. BPA was administered to F344 female rats by gavage at 0, 0.05, 7.5, 30, 120 mg/kg/day during pregnancy and lactation periods. When F1 males reached 5 weeks old, they were given 10 subcutaneous injections of 3,2'-dimethyl-4-aminobiphenyl (DMAB) or corn oil vehicle and rats were then sacrificed under ether anesthesia at week 60. There were no observable effects on the accessory sex organ weights of male offspring. Transplacental and lactational exposure to BPA did not affect the incidences of preneoplastic and neoplastic lesions in the accessory sex organs (prostate and seminal vesicle) of F1 rats and did not induce any proliferating lesions without DMAB. Our data suggest that maternal exposure to BPA during the period of pregnancy and lactation does not affect the risk of prostate carcinogenesis in male offspring.

Intrauterine bisphenol A exposure leads to stimulatory effects on Sertoli cell number in rats.
Wistuba J, Brinkworth MH, Schlatt S, Chahoud I, and Nieschlag E.
Environ Res 2003;91(2):95-103.

Using the optical disector for quantifying cell numbers, we investigated whether oral treatment of rats on days 6-21 of gestation with the weakly estrogenic bisphenol A (BPA, 0.1 or 50 mg/kg) or the highly estrogenic ethinyl estradiol (EE, 0.02 mg/kg) alters testicular histology, in those offspring 9-12 month of age. Since production of male germ cells depends on Sertoli cell number, possible changes in that parameter were investigated using unbiased stereology.

Spermatogenesis was qualitatively normal in all groups. BPA increases Sertoli cell number per organ but not when expressed as per gram testis. EE did not affect cell number per organ but did affect numbers on a per gram testis basis due to a lowered testis weight. In contrast to the lowering of Sertoli cell numbers that might have been expected according to the estrogen hypothesis, intrauterine administration of these xenoestrogens in fact resulted in minor increases in Sertoli cell numbers and had no qualitative effect on spermatogenesis.

Low dose effect of in utero exposure to bisphenol A and diethylstilbestrol on female mouse reproduction.
Honma S, Suzuki A, Buchanan DL, Katsu Y, Watanabe H , and Iguchi T.
Reprod Toxicol 2002;16(2):117-22.

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. 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. 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. 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.

Effects of in utero and lactational exposure to bisphenol A on somatic growth and anogenital distance in F1 rat offspring.
Kobayashi K, Miyagawa M, Wang RS, Sekiguchi S, Suda M, and Honma T.
Ind Health 2002;40(4):375-81.

Bisphenol A (BPA), a xenoestrogen, has been reported to mimic the actions of estrogen or to affect the endocrine glands in vivo and in vitro. In this study, we examined whether in utero and lactational exposure to BPA altered the somatic growth and anogenital distance (AGD) of F1 offspring (1, 3, and 9 weeks of age) in vivo in rats. Dams were orally administered with various doses of BPA (0, 4, or 40 mg/kg body weight (BW)/day) from gestation day (GD) 6 through postnatal day (PND) 20. There were no significant changes in body weight, liver weight, kidneys

weight, testes weight, AGD, the ratio of AGD to BW, or the ratio of AGD to the cube root of BW in BPA exposed pups compared to the vehicle-exposed control. This suggests that prenatal and postnatal exposure (indirect exposure) to BPA (4-40 mg/kg/day, GD 6-PND 20) does not affect on somatic growth or AGD of F1 generation of male and female rats.

Low-dose bisphenol A does not affect reproductive organs in estrogen-sensitive C57BL/6N mice exposed at the sexually mature, juvenile, or embryonic stage.
Nagao T, Saito Y, Usumi K, Yoshimura S, and Ono H.
Reprod Toxicol 2002;16(2):123-30.

Bisphenol A (BPA) is used on a large scale in the manufacture of polycarbonate plastics. BPA has been shown to bind weakly to both estrogen receptor (ER) alpha and ER beta. The objective of this study was to evaluate the effects of low-dose BPA on male sexual development after exposure at various stages of development. Mice of the estrogen-sensitive strain C57BL/6N were exposed to BPA orally at doses of 2, 20, or 200 microg/kg at various stages, i.e. adulthood, the immature stage just after weaning, or the embryonic/fetal stage, to evaluate the effects of low-dose BPA on male reproductive organs. Body weight changes, weights of reproductive organs (testes, epididymides, seminal vesicles), cauda epididymal sperm density, and histology of reproductive organs including the ventral prostate were not affected by exposure to BPA at any dose examined. The results of this study indicate that exposure of estrogen-sensitive C57BL/6N mice to low-dose BPA did not reduce sperm density or disrupt development of the male reproductive organs.

Normal sexual development of two strains of rat exposed in utero to low doses of bisphenol A.
Tinwell H, Haseman J, Lefevre PA, Wallis N, and Ashby J.
Toxicol Sci 2002;68(2):339-48.

Pregnant Sprague-Dawley (SD) and Alderley Park (Wistar derived) rats were exposed by gavage during gestation days 6-21 to 20 microg/kg, 100 microg/kg, or 50 mg/kg body weight of BPA with ethinylestradiol (EE; 200 microg/kg) acting as a positive control agent. The sexual development of the derived pups was monitored until termination at postnatal day 90-98. The endpoints evaluated were litter size and weight, anogenital distance at birth, days of vaginal opening, first estrus and prepuce separation, weights of the liver, seminal vesicles, epididimydes, testes, ventral prostate, uterus, vagina, cervix and ovaries, and daily sperm production. Males were terminated at postnatal day 90 and females at postnatal day 98. The only statistically significant effects observed for any dose of BPA were a decrease in daily sperm production and an increase in the age of vaginal opening for the Alderley Park animals at the highest dose evaluated (50 mg/kg). The dose of EE evaluated proved to be maternally toxic in our laboratory, but provided gross evidence of endocrine disruption in the treated dams. These results diverge from those of Chahoud and his colleagues who indicated disturbances to the sexual development of both male and female SD rat pups administered the same 3 doses of BPA. This failure to

confirm low dose endocrine effects for BPA is discussed within the context of similar divergent conclusions derived from other assessments of the endocrine toxicity of this agent to rats.

Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats.
Tyl RW, Myers CB, Marr MC, Thomas BF, Keimowitz AR, Brine DR, Veselica MM, Fail PA, Chang TY, Seely JC, Joiner RL, Butala JH, Dimond SS, Cagen SZ, Shiotsuka RN, Stropp GD, and Waechter JM.
Toxicol Sci 2002;68(1):121-46.

Bisphenol A (BPA) was evaluated at concentrations of 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm (approximately 0.001, 0.02, 0.3, 5, 50, and 500 mg/kg/day of BPA) administered in the diet ad libitum to 30 CD((R)) Sprague-Dawley rats/sex/dose for 3 offspring generations, 1 litter/generation, through F3 adults. Adult systemic toxicity at 750 and 7500 ppm in all generations included: reduced body weights and body weight gains, reduced absolute and increased relative weanling and adult organ weights (liver, kidneys, adrenals, spleen, pituitary, and brain), and female slight/mild renal and hepatic pathology at 7500 ppm. Reproductive organ histopathology and function were unaffected. Ovarian weights as well as total pups and live pups/litter on postnatal day (PND) 0 were decreased at 7500 ppm, which exceeded the adult maximum tolerated dose (MTD). Mating, fertility, gestational indices; ovarian primordial follicle counts; estrous cyclicity; precoital interval; gestational length; offspring sex ratios; postnatal survival; nipple/areolae retention in preweanling males; epididymal sperm number, motility, morphology; daily sperm production (DSP), and efficiency of DSP were all unaffected. At 7500 ppm, vaginal patency (VP) and preputial separation (PPS) were delayed in F1, F2, and F3 offspring, associated with reduced body weights. Anogenital distance (AGD) on PND 0 was unaffected for F2 and F3 males and F3 females (F2 female AGD was increased at some doses, not at 7500 ppm, and was considered not biologically or toxicologically relevant). Adult systemic no observed adverse effect level (NOAEL) = 75 ppm (5 mg/kg/day); reproductive and postnatal NOAELs = 750 ppm (50 mg/kg/day). There were no treatment-related effects in the low-dose region (0.001-5 mg/kg/day) on any parameters and no evidence of nonmonotonic dose-response curves across generations for either sex. BPA should not be considered a selective reproductive toxicant, based on the results of this study.

Lack of significant alteration in the prostate or testis of F344 rat offspring after transplacental and lactational exposure to bisphenol A.
Yoshino H, Ichihara T, Kawabe M, Imai N, Hagiwara A, Asamoto M, and Shirai T.
J Toxicol Sci 2002;27(5):433-9.

Bisphenol A (BPA), a compound of great concern as an estrogenic xenobiotic, was assessed for its ability to cause alteration in the accessory sex organs and spermatogenesis in male offspring exposed preneonatally and neonatally. In a series of experiments focusing on rat sensitivity to gestational and lactational exposure to BPA, we investigated its effects on gestation period and

reproductive organs in male offspring. In the first instance, BPA was administered to F344 female rats by gavage at 0, 7.5, 120 mg/kg/day during pregnancy and lactation period. There were no observable adverse effects in pregnant rats and the treatment did not induce any morphological abnormalities in the accessory sex organs of male offspring. However, lowered numbers of sperm in the testis were found with a dose of 120 mg/kg/day. In the second study, the same protocol with a higher number of male offspring was applied, but no reduction in the sperm count was apparent. We conclude that transplacental and lactational exposure to BPA dose not exert any adverse effects on morphogenesis of rat accessory sex organs or spermatogenesis.

Rat two-generation reproductive toxicity study of bisphenol A.
Ema M, Fujii S, Furukawa M, Kiguchi M, Ikka T, and Harazono A.
Reprod Toxicol 2001;15(5):505-23.

This study was conducted to determine the low-dose effects of bisphenol A (BPA) in a rat two-generation reproduction study. Groups of 25 male and 25 female Crj: CD (SD) IGS rats were given BPA at 0.2, 2, 20, or 200 microg/kg/day by gastric intubation throughout the study beginning at the onset of a 10- and 2-week premating period, in F0 males and females, respectively, and continuing through the mating, gestation, and lactation periods, for two generations. There were adult (F0, F1, F2) and postnatal day (PND) 22 (F1, F2) necropsies: the oldest F2 males and females being killed at postnatal weeks 7 and 14, respectively. No compound-related clinical signs or effects on body weight or food consumption were observed in any generation. There were no compound-related changes in surface righting reflex, negative geotaxis reflex, mid-air righting reflex, pinna detachment, incisor eruption, eye opening, testes descent, preputial separation, or vaginal opening in F1 and F2 generations, or behavior in the open field or water filled multiple T-maze in the F1 generation. No test compound-related changes in estrous cyclicity, copulation index, fertility index, number of implantations, gestation length, litter size, pup weight, pup sex ratio, pup viability, or other functional reproductive measures were noted in any generation. A few significant changes in the anogenital distance (AGD) per cube root of body weight ratio were found at 0.2 and 20 microg/kg in F1 males, at 2, 20, and 200 microg/kg in F1 females, and at 20 and 200 microg/kg in F2 females. However, the changes in the AGD were consistently small (within 5% of control values), and no continuous changes in the AGD or AGD/cube root of body weight ratio were detected. There were no compound-related changes in epididymal sperm counts or motility in F0 and F1 males. No compound-related necropsy findings or effects on organ weight including the reproductive organs were found in any generation. Histopathologic examinations revealed no evidence of compound-related changes in any organs including the reproductive organs of both sexes. The data indicate that oral doses of BPA of between 0.2 and 200 microg/kg over 2 generations did not cause significant compound-related changes in reproductive or developmental parameters in rats.

Lack of effects of bisphenol A in maternal rats or treatment on response of their offspring to N-nitrosobis(2-hydroxypropyl)amine.
Takashima Y, Tsutsumi M, Sasaki Y, Tsujiuchi T, Kusuoka O, and Konishi Y.
Journal of Toxicologic Pathology 2001;14(2):87-98.

Safety assessment of endocrine-disrupting chemicals (EDCs) is now an important issue because of fear of human exposure. Bisphenol A (BPA), a compound initially synthesized as a chemical oestrogen, is now used as a monomer for the production of polycarbonate plastic products such as baby bottles. In the present experiment, toxicity and effects of BPA administration to maternal rats and response of their offspring to N-nitrosobis(2-hydroxypropyl)amine (BHP) treatment were studied. Growth retardation was observed in maternal rats throughout the period of BPA exposure (total dose of 21 +/- 3 grams per rat). Serum TSH was elevated in maternal rats receiving a soybean-devoid diet and offspring of maternal rats with or without BPA but this was not thought to be caused by BPA. Histopathologically, no significant organ toxicity was observed in BPA-treated maternal rats. Examination of cleavage of the balanopreputial gland in males and opening of the vagina in females performed on 5 to 7-week-aged offspring, demonstrated no abnormal differentiation or growth retardation. BPA exposure did not affect BHP-induced carcinogenesis in the thyroid, lung, thymus, esophagus, and liver, location, incidences, and numbers of tumors not differing in offspring born from maternal rats with or without BPA administration. These results indicate that BPA does not induce any tissue injury for maternal or offspring rats, with no effects on target organ carcinogenesis of BHP in offspring.

Pubertal development and reproductive functions of Crl:CD BR Sprague-Dawley rats exposed to bisphenol A during prenatal and postnatal development.
Kwon S, Stedman DB, Elswick BA, Cattley RC, and Welsch F.
Toxicol Sci 2000;55(2):399-406.

Bisphenol A (BPA) is used on a large scale in the manufacture of polycarbonate plastics. BPA has been shown to bind weakly to both estrogen receptor (ER)alpha and ERbeta, and to transactivate reporter genes in vitro. The purpose of the present study was to determine whether exposure of rats to BPA during pre- and postnatal development affects estrogen-mediated end points related to pubertal development and reproductive functions. BPA was administered to pregnant Crl:CD BR Sprague-Dawley rats by gavage at 0, 3.2, 32, or 320 mg/kg/day from gestation day (GD) 11 through postnatal day (PND) 20. Diethylstilbestrol (DES) at 15 microg/kg/day was used as a reference chemical with known estrogenic effects. Female pubertal development was not affected by indirect BPA exposure of the offspring at any of the dose levels. Treatment with this chemical also did not produce detectable effects on the volume of the sexually dimorphic nucleus of the preoptic area (SDN-POA), estrous cyclicity, sexual behavior, or male reproductive organ weights of F(1) offspring. However, DES at 15 microg/kg/day increased the volume of the SDN-POA of female offspring and affected their normal estrous cyclicity following puberty. In this study, pre- and postnatal exposure of rats to BPA at 3.2, 32, or 320 mg/kg/day from GD 11 through PND 20 did not have any apparent adverse effects on female rat pubertal development and reproductive functions.

Lack of effects for low dose levels of bisphenol A and diethylstilbestrol on the prostate gland of CF1 mice exposed in utero.
Ashby J, Tinwell H, and Haseman J.
Regul Toxicol Pharmacol 1999;30(2 Pt 1):156-66.

vom Saal et al. (Proc. Natl. Acad. Sci. 94, 2056-2061, 1997) have reported that low dose exposure (0.02-2 microg/kg/day) of CF1 mice to diethylstilbestrol (DES) in utero led to increases in the prostate gland weight when the pups reached 8 months of age. Nagel et al. (Environ. Health Perspect. 105, 70-76, 1997) reported similar effects in CF1 mice at 6 months of age after exposure in utero to low dose levels (2 and 20 microg/kg/day) of bisphenol A (BPA). vom Saal et al. (Toxicol. Indust. Health 14(1/2) 239-260, 1998) subsequently reported reduced sperm efficiency (daily sperm production per gram testes) in a subset of the BPA animals for which enlarged prostates had been observed. These three experiments have been repeated in a single experiment that was terminated when the offspring reached 6 months of age. No statistically significant effects on prostate weight or sperm efficiency were recorded for offspring of animals exposed to either DES (0.2 microg/kg/day) or BPA (2 and 20 microg/kg/day) in utero. Significant dam effects were seen for several of the assay parameters indicating that the litter, as opposed to the individual, should be considered as the statistical unit in such experiments. A statistically significant increase in body weight was recorded for the low dose BPA male offspring. Females from the study underwent normal sexual maturation and showed no significant differences in reproductive tissue weights at termination and the mean day of vaginal opening. The possible reasons for this failure to confirm the earlier reported effects for DES and BPA at these low doses are discussed.

Normal reproductive organ development in Wistar rats exposed to bisphenol A in the drinking water.
Cagen SZ, Waechter JM Jr, Dimond SS, Breslin WJ, Butala JH, Jekat FW, Joiner RL, Shiotsuka RN, Veenstra GE, and Harris LR.
Regul Toxicol Pharmacol 1999;30(2 Pt 1):130-9.

Bisphenol A (BPA) is a chemical used primarily as a monomer in the manufacture of numerous chemical products, such as epoxy resins and polycarbonate. The objective of this study was to evaluate potential effects of BPA on sexual development of male rats and was designed to clarify low-dose observations reported as preliminary results by Sharpe et al. (1996). The protocol for the present study followed the same treatment schedule as reported by Sharpe et al. (1995, 1996), but included more treatment groups, a greater number of animals per group, and a more comprehensive number of reproductive endpoints. Groups of 28 female Han-Wistar albino rats were exposed to drinking water that contained 0, 0.01, 0.1, 1.0, or 10 ppm BPA or 0.1 ppm diethylstilbestrol (DES), 7 days per week, for a total of 10 weeks. Treatment of the females began at 10 weeks of age and continued throughout a 2-week premating period, 2 weeks of mating (to untreated males), 21-22 days of gestation, and 22 days of lactation. Offspring weanling males were given untreated drinking water and maintained until 90 days of age when evaluations were made of various reproductive organs. Consistent with Sharpe et al. (1996) the

female offspring were not evaluated. No treatment-related effects on growth or reproductive endpoints were observed in adult females exposed to any concentration of BPA. Similarly, no treatment-related effects were observed on the growth, survival, or reproductive parameters (including testes, prostate and preputial gland weights, sperm count, daily sperm production, or testes histopathology) of male offspring from dams exposed to BPA during gestation and lactation. DES administered in the drinking water at 0. 1 ppm resulted in decreased body weight, body weight change, and food consumption in adult females. In addition, an increase in the duration of gestation and a decrease in the number of pups delivered and number of live pups were also observed in animals exposed to DES. In conclusion, these results do not confirm the previous findings of Sharpe et al. (1996) and show that low doses of BPA had no effects on male sexual development in the rat.

Normal reproductive organ development in CF-1 mice following prenatal exposure to bisphenol A.
Cagen SZ, Waechter JM Jr, Dimond SS, Breslin WJ, Butala JH, Jekat FW, Joiner RL, Shiotsuka RN, Veenstra GE, and Harris LR.
Toxicol Sci 1999;50(1):36-44.

Bisphenol A (BPA) is a monomer used in the manufacture of a multitude of chemical products, including epoxy resins and polycarbonate. The objective of this study was to evaluate the effects of BPA on male sexual development. This study, performed in CF-1 mice, was limited to the measurement of sex-organ weights, daily sperm production (DSP), epididymal sperm count, and testis histopathology in the offspring of female mice exposed to low doses of BPA (0, 0.2, 2, 20, or 200 microg/kg/day), by deposition in the mouth on gestation days 11-17. Male sexual development determinations were made in offspring at 90 days-of-age. Since this study was conducted to investigate and clarify low-dose effects reported by S. C. Nagel et al., 1997, Environ. Health Perspect. 105, 70-76, and F. S. vom Saal et al., 1998, Toxicol. Indust. Health 14, 239-260, our study protocol purposely duplicated the referenced studies for all factors indicated as critical by those investigators. An additional group was dosed orally with 0.2 microg/kg/day of diethylstilbestrol (DES), which was selected based on the maternal dose reported to have maximum effect on the prostate of developing offspring, by F. S. vom Saal (1996, personal communication), vom Saal et al. (1997, Proc. Natl. Acad. Sci. U S A 94, 2056-2061). Tocopherol-stripped corn oil was used as the vehicle for BPA and DES, and was administered alone to control animals. No treatment-related effects on clinical observations, body weight, or food consumption were observed in adult females administered any dose of BPA or DES. Similarly, no treatment-related effects on growth or survival of offspring from dams treated with BPA or DES were observed. The total number of pups born per litter was slightly lower in the 200-microg/kg/day BPA group when compared to controls, but this change was not considered treatment-related since the litter size was within the normal range of historical controls. There were no treatment-related effects of BPA or DES on testes histopathology, daily sperm production, or sperm count, or on prostate, preputial gland, seminal vesicle, or epididymis weights at doses previously reported to affect these organs or at doses an order of magnitude higher or lower. In conclusion, under the conditions of this study, the effects of low doses of

BPA reported by S. C. Nagel et al., 1997 (see above) and F. S. vom Saal et al., 1998 (see above), or of DES reported by F. S. vom Saal et al., 1997 (see above) were not observed. The absence of adverse findings in the offspring of dams treated orally with DES challenges the "low-dose hypothesis" of a special susceptibility of mammals exposed perinatally to ultra-low doses of even potent estrogenic chemicals. Based on the data in the present study and the considerable body of literature on effects of BPA at similar and much higher doses, BPA should not be considered as a selective reproductive or developmental toxicant.

Reproductive function in rats exposed neonatally to bisphenol A and estradiol benzoate.
Nagao T, Saito Y, Usumi K, Kuwagata M, and Imai K.
Reprod Toxicol 1999;13(4):303-11.

The reproductive function in rats treated subcutaneously (s.c.) with 300 microg/g bisphenol A or 2 microg/g estradiol benzoate from postnatal Day 1 to 5 was examined after puberty as well as histolopathogic changes in reproductive organs. All male and female rats treated postnatally with estradiol benzoate showed poor reproductive capability, including adverse effects on masculine sexual behavior, and marked histopathologic alterations of the reproductive organs. In addition, estradiol benzoate markedly reduced the volume of the sexually dimorphic nucleus of the preoptic area (SDN-POA) in males. On the other hand, all male and female rats treated postnatally with bisphenol A showed normal reproductive function and no histopathologic abnormalities of reproductive organs. Bisphenol A did not affect the volume of the SDN-POA. These results indicated that neonatal exposure to estradiol benzoate affects reproductive function in male and female rats, and treatment with bisphenol A at a fairly high dose was ineffective if given postnatally to male and female rats.

The developmental toxicity of bisphenol A in rats and mice.
Morrissey RE, George JD, Price CJ, Tyl RW, Marr MC, and Kimmel CA.
Fundam Appl Toxicol 1987;8(4):571-82.

Bisphenol A (BPA) was evaluated for developmental toxicity in CD rats (0, 160, 320, or 640 mg/kg/day) and CD-1 mice (0, 500, 750, 1000, or 1250 mg/kg/day) dosed daily by gastric intubation on Gestational Days 6 through 15. Timed-pregnant dams were sacrificed 1 day prior to parturition, the uterine contents were examined, and all fetuses were examined for external, visceral, and skeletal malformations. In rats, maternal weight gain during gestation, weight gain corrected for gravid uterine weight, and weight gain during treatment were significantly reduced at all BPA doses. Gravid uterine weight and average fetal body weight per litter were not affected by BPA. No increase in percentage resorptions per litter or percentage fetuses malformed per litter was detected. In mice, maternal mortality occurred at all BPA doses, reaching 18% at the high dose, which also produced a significant decrease in maternal body weight gain during gestation and treatment. Weight gain corrected for gravid uterine weight was not affected by BPA. Reductions in gravid uterine weight and average fetal body weight were observed with the 1250 mg/kg dose of BPA. Relative maternal liver weight was increased at all

doses of BPA. There was a significant increase in the percentage of resorptions per litter with 1250 mg BPA/kg/day. Malformation incidence was not altered by BPA. Thus, BPA treatment at maternally toxic dose levels during organogenesis produced fetal toxicity in mice but not in rats and did not alter fetal morphologic development in either species.

Ninety day oral toxicity study in dogs.
International Research & Development Corp.
1984; EPA/OTS Doc #878214682; NTIS/OTS0206618; TSCATS/021963

Subchronic toxicity was evaluated in groups of 8 beagle dogs (4 male and 4 female) ingesting bisphenol-A via the diet at levels of 1000, 3000, or 9000 ppm for 90 days. Mortality was not observed at any dose level and animals were sacrificed after 90 days. The test article did not induce changes in general behavior and appearance, ophthalmological parameters, body weight, food consumption, hematological and biochemical parameters, or urinalysis values at any dose level. Bisphenol-A did not induce compound related gross pathologic lesions at any dose level, and lesions were not observed on microscopic analysis of the following tis. sues from high dose group animals: adrenals, aorta, brain, esophagus, eye (+ optic nerve), gallbladder, duodenum, jejunum, ileum, cecum, colon, rectum, urinary bladder, kidneys, liver, lungs, mesenteric lymph node, skin, nerve, skeletal muscle, spleen, pancreas, pituitary, prostrate/uterus, bone marrow, salivary gland, spinal cord, stomach, testis/ovary, thymus, thyroid/parathyroid, and tongue. A group mean relative liver weight increase observed at the 9000 ppm dose level was considered to be compound related. Statistical analyses were not reported.

Reproduction and ninety day feeding study in rats.
International Research & Development Corp.
1984; EPA/OTS Doc #40+8486013; NTIS/OTS0509954; TSCATS/202898

Subchronic inhalation toxicity was evaluated in groups of male and female Charles River CD rats (10/sex/concn level) ingesting bisphenol-A via the diet for 90 days at nominal concentrations of 100, 250, 500, 750 and 1000 ppm. In the male group given 1000 ppm there was a slight decrease in body weight when compared to the control group. Food consumption values were slightly higher for treated female rats than for female control rats. Other parameters of general behavior, appearance and survival of the treated F0 parents were not consistently affected by the treatment. At 100 days of age F0 parents were mated to produce F1 offsprin. g. 15 Male and 15 female F1 rats were treated via the diet with bisphenol-A for 90 days. Body weight gains of the F1 male rats were slightly lower at the 750 ppm concn level. Other parameters such as appearance, survival, male and female fertility indices, female estrous cycles, length of gestation period, number of pups/litter, and pup body weights were not consistently affected by the treatment.

Reproduction and ninety day oral toxicity study in rats.
International Research & Development Corp.
1984; EPA/OTS; Doc #40+8486013; NTIS/OTS0509954; TSCATS/202900

Subchronic toxicity was evaluated in weanling rats (F1) obtained from the mating of Charles River CD rats fed bisphenol-A in the diet during their growth and reproductive period. Groups of male and female rats (10/sex/concn level) ingested bisphenol-A via the diet for 90-days at nominal concentrations of 1000, 3000 and 9000 ppm. In the F0 generation, body weight gain was slightly decreased at the 3000 ppm concn level, and moderately decreased at the 9000 ppm level. Other parameters such as, general behavior, food consumption, appearance, ophthalmoscopy or hematological, biochemical, urinalysis studies, fertility indices, number o. f pups/litter and pup survival were not consistently affected by the treatment. At the age of 100 days, the F0 rats were mated 1 to 1, and subsequently the offspring (F1) rats were initiated on the 90-day feeding study of bisphenol-A. Reproductive effects were evaluated using 15 male and 15 female rats per concn level (1000,3000 or 9000 ppm). in the F1 generation, slight reductions in body weight (at 21 days of age) were noted for pups at the 3000 and 9000 ppm levels. Body weight gain was moderately decreased for female rats at 1000 ppm, and for male and female rats at the 3000 and 9000 ppm levels. food consumption also decreased. for male rats at the 9000 ppm level and for all treated females. Other parameters as mentioned above were not consistently affected by the treatment.

D. Meeting abstracts reporting no developmental or reproductive toxicity

Monotonic Low-Dose Response to Estrogens in the Fetal Rat Testis as Evaluated by Microarray.
Daston, GP; Hess, KA; Overmann, GJ; Foertsch, LM; Tiesman, JP; Torontali, S; Carr, G, and Naciff, JM.
Birth Defects Res A Clin Mol Teratol. 2005 May; 73(5):318.

Abstract: One of the most important controversies in developmental toxicology over the past several years has been the possibility of non-monotonic dose-response curves for the effects of estrogens on the developing male reproductive system. The most publicized example has been a purported increase in prostate weight in mice prenatally exposed to sub-NOAEL dosages of estrogens, with either no effect or a decrease in prostate weight at higher dose levels. Others have been unable to replicate these results. However, a NTP panel concluded that it is impossible to dismiss the former result, even though the preponderance of evidence favors the latter. Their reasoning is that the magnitude of the effect is small and subject to many sources of variability that may obscure the ability to detect a small effect. The purpose of our study was to evaluate whether a more sensitive endpoint, gene expression, could be used to evaluate the shape of the dose-response curve in a developing male reproductive tissue. Pregnant SD rats were treated sc daily on GD 11-20 with ethynyl estradiol (EE), genistein (GES) or bisphenol A (BPA), with dosages ranging from 0.001-10 ug/kg/day, 0.001-100 mg/kg/day, or 0.002-400 mg/kg/day, respectively. The top dose levels are approximately equipotent in the rat uterotrophic assay. Fetal testes with epididymides were removed on GD 20; mRNA was isolated and evaluated on

Affymetrix microarrays. All three treatments produced significant changes from control in gene expression, although even at the top dose this represented less than 1% of the genes evaluated. Independent analyses of the three compounds identified 141 genes modified by the highest dose of EE, 46 for Ges, and 67 for BPA (out of 8740). Global analysis to detect genes modified by all three chemicals identified a profile of 52 genes consistently, dose-responsively changed. The dose-response curves for all three compounds were monotonic, with numbers of genes and magnitude of change decreasing with decreasing dose. There was no evidence of non-monotonicity or of unique sets of genes for which expression changed at very low doses. (Microarrays with 15,000 genes were used for low doses.) This study demonstrates that gene expression analysis is a sensitive tool capable of detecting small responses, and that under these experimental conditions there is no indication of unusual dose-response to estrogens.

Ovarian Function In Rats Neontally Exposed To Estrogen Disrupters.
Campbell PS and Gates AM.
Biol Reprod 2004;Aug (Special Issue):111-2

A single sc injection of one of several putative estrogen disrupting chemicals (synthetic estrogen, phytoestrogen, pesticide, an industrial byproduct and plastic) on day 2 of life in the neonatal rat impaired later ovarian function. Precocious puberty was induced in the treated animals by sc injection of 30 IU PMSG on day 26 of life. Superovulation produced by a sc injection of 10 IU hCG at 56 hrs. after PMSG was used to assess the effects of the neonatal exposure to such compounds upon ovulation. Ovarian and uterine growth and post-ovulatory serum progesterone titer were also evaluated. The true estrogens genistein and DES reduced ovarian and uterine weights but daidzein, a weaker estrogen, did not. No significant differences from control (peanut oil) were observed with DDT exposure. However, the pesticide dieldrin lowered ova counts. The industrial byproduct dioxin reduced uterine weight, while the plastic component bisphenol A had no significant effects. PMSG-treated control, dioxin- and dieldrin-exposed females paired overnight at 56-58 hrs. after PMSG injection resulted in 100% of control but only 50% of dioxin and 20% of dieldrin females mating as evidenced by the presence of a copulatory plug the following morning. Furthermore, plasma progesteone levels at 20-22 hrs. after hCG injection tended to be lower in rats previously exposed to estrogen disrupters than that observed in the controls. These results suggest that genistein, DES, dieldrin, and dioxin, in particular, can impair later reproductive function as a result of a one-time neonatal exposure. Therefore, we suggest that environmenal exposure to these chemicals may have deleterious effects on adult female fertility.

Pituitary-Thyroid Axis In The Postnatal Rat Offspring Following Gestational and Lactational Exposure To Bisphenol A.
Kobayashi, K., Miyagawa, M., Wang, R. S., Suda, M., Sekiguchi, S., and Honma, T..
Toxicol Lett. 2003 Sep; 144 (Suppl 1):S175-S176.

Abstract: Bisphenol A (BPA), a xenoestrogen, is very widely used in the manufacture of polycarbonate and epoxy resins. Although BPA has been reported to mimic the actions of estrogen or to affect the reproductive organs and accessory genital glands, the effects of maternal exposure to BPA on the offspring of rats still remain unclear. In the present study, we examined whether gestational and lactational exposure to BPA altered the postnatal growth and thyroid function of male and female offspring in vivo in rats. Pregnant Sprague-Dawley rats were exposed to BPA (0, 4, or 40 mg/kg/day) in corn oil once daily via oral gavage from gestation day 6 through postnatal day 20, and the control group was given the same amount of corn oil during the same period. There were no significant changes in body weight, liver weight, kidneys weight, testes weight (male), anogenital distance (AGD), or AGD indices in the BPA-exposed groups compared to the control group. Plasma concentrations of thyroid hormone (T4) and thyroid-stimulating hormone (TSH) were unaffected. No differences in the plasma T4 response to exogenous TSH stimulation occurred in all exposed groups compared to the control group. These results suggest that BPA did not produce any severe impairment in the postnatal growth and pituitary-thyroid axis of the Fl generation in rats under the present experimental conditions wherein the exposure levels were relatively high. The effects of BPA exposure are, however, still incompletely understood and further study should be carried out to confirm the toxicity of BPA during gestational and lactational period in rats.

Effects of perinatal exposure of five putative endocrine disrupting chemicals (EDCs), methoxychlor, genistein, diisononylphthalate, 4-nonylphenol and bisphenol A, on endocrine/reproductive systems in rats.
Takagi H, Shibutani M, Masutomi N, Uneyama C, Mitsumori K, and Hirose M.
Toxicologist 2003;72(S-1):77.

Methoxychlor (MXC, 24, 240, 1200 ppm), genistein (GEN, 20, 200, 1000 ppm), diisononylphthalate (DINP, 400, 4000, 20,000 ppm), 4-nonylphenol (NP, 60, 600, 3000 ppm) or bisphenol A (BA, 60, 600, 3000 ppm) were given to maternal rats from gestational day 15 to postnatal day (PND) 10 to assess their perinatal exposure effects on offsprings. Soybean-free diet was used as a basal diet. Organ weights at PND21, onset of puberty, estrous cyclicity, gonadotrophin-immunopositive index (IPI) in pituitary at PND21 and 77, and histological changes at PND77 were assessed as well as the size of sexually dimorphic nucleus of preoptic area (SDN-POA). In terms of MXC, DINP and GEN studies, expression of GABA transporter-1 (GAT-1), an estrogen responsive gene, was analyzed in medial preoptic area (MPOA) at PND10 using microdissection and real-time RT-PCR techniques. Females exposed to 1200 ppm MXC showed accelerated onset of puberty, irregular estrous cyclicity, histological changes such as multifollicular ovaries, hyperplasia in endometrium, vaginal mucosa and anterior pituitary at PND77, and decrease in LH-IPI at PND21 and increase in FSH- and PRL-IPIs at PND77.

Females of 240 ppm MXC also showed increased PRL-IPI at PND77. Males of 1200 ppm MXC showed delayed onset of puberty and decreased LH-, FSH- and PRL-IPIs at PND21. DINP at 20,000 ppm caused very slight degeneration of spermatocytes and Sertoli cells at PND77. The sizes of SDN-POA did not alter at any doses of chemicals examined. GAT-1 levels in male MPOAs decreased with DINP at 20,000 ppm, and also showed a dose-related decreasing tendency with MXC. GEN, BA and NP did not affect any endocrine parameter examined. Results suggest that maternal exposure to MXC and DINP affects reproductive system of offsprings by disrupting brain sexual differentiation.

E. Related articles and meeting abstracts

Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development.
Dolinoy DC, Huang D, and Jirtle RL.
Proc Natl Acad Sci U S A. 2007 Aug 1; [Epub ahead of print]

The hypothesis of fetal origins of adult disease posits that early developmental exposures involve epigenetic modifications, such as DNA methylation, that influence adult disease susceptibility. In utero or neonatal exposure to bisphenol A (BPA), a high-production-volume chemical used in the manufacture of polycarbonate plastic, is associated with higher body weight, increased breast and prostate cancer, and altered reproductive function. This study shows that maternal exposure to this endocrine-active compound shifted the coat color distribution of viable yellow agouti (A(vy)) mouse offspring toward yellow by decreasing CpG (cytosine-guanine dinucleotide) methylation in an intracisternal A particle retrotransposon upstream of the Agouti gene. CpG methylation also was decreased at another metastable locus, the CDK5 activator-binding protein (Cabp(IAP)). DNA methylation at the A(vy) locus was similar in tissues from the three germ layers, providing evidence that epigenetic patterning during early stem cell development is sensitive to BPA exposure. Moreover, maternal dietary supplementation, with either methyl donors like folic acid or the phytoestrogen genistein, negated the DNA hypomethylating effect of BPA. Thus, we present compelling evidence that early developmental exposure to BPA can change offspring phenotype by stably altering the epigenome, an effect that can be counteracted by maternal dietary supplements.

Developmental Toxicity by Exposure to Bisphenol A Diglycidyl Ether during Gestation and Lactation Period in Sprague-dawley Male Rats.
Hyoung, U. U.; Yang, Y. J.; Kwon, S. K.; Yoo, J. H.; Myoung, S. C.; Kim, S. C., and Hong, Y. P.
J Prev Med Pub Health. 2007 Mar; 40(2):155-61.

Abstract: OBJECTIVES: Bisphenol A diglycidyl ether (BADGE) is the major component in commercial liquid epoxy resins, which are manufactured by co-reacting bisphenol A with epichlorohydrin. This study was performed to show the developmental effects of prenatal and postnatal exposures to BADGE in male rat offspring. METHODS: Mated female rats were

divided into four groups, each containing 12 rats. The dosing solutions were prepared by thoroughly mixing BADGE in corn oil at the 0, 375, 1500 and 3000 mg/kg/day concentrations. Mated females were dosed once daily by oral gavage on gestation day (GD) 6 - 20 and postnatal day (PND) 0 - 21. Pregnant female dams were observed general symptoms and body weight. Also, male pups were observed the general symptoms, body weight, developmental parameters (e.g. anogenital distance, pina detachment, incisor eruption, nipple retention, eye opening, testis descent), organ pathologic changes and hormone levels of plasma. RESULTS: Pregnant rats treated with BADGE died at a rate of about 70% in the 1500 mg/kg/day group and all rats treated with 3000 mg/kg/day died. Body weight, for male pups treated with doses of 375 mg/kg/day, was significantly lower than in the control group at PND 42, 56, and 63 ($p < 0.05$). Evaluation of body characteristics including; separation of auricle, eruption of incisor, separation of eyelid, nipple retention, descent of testis, and separation of the prepuce in the BADGE treated group showed no difference in comparisons with the control group. AGD and adjusted AGD (mm/kg) for general developmental items in BADGE 375 mg/kg/day treated pups tended to be longer than in controls, however, these differences were not statistically significant. Relative weights of adrenal gland, lung ($p < 0.05$), brain, epididymis, prostate, and testis ($p < 0.01$) were heavier than in control in measures at PND 9 weeks. There were no significant changes in comparisons of histological findings of these organs. Loss of spermatids was observed in the seminiferous tubule at PND 9 weeks, but no weight changes were observed. The plasma estrogen levels were similar in the control and treatment groups at PND 3, 6 and 9 weeks. The plasma testosterone levels in the control group tended to increase with age. However, in the BADGE 375 mg/kg/day treated male pups it did not tend to increase. CONCLUSIONS: These findings suggest that BADGE is a chemical that has developmental effects consistent with it being an endocrine disruptor.

Bisphenol A causes malformation of the head region in embryos of Xenopus laevis and decreases the expression of the ESR-1 gene mediated by Notch signaling.
Imaoka, S.; Mori, T., and Kinoshita, T.
Biol Pharm Bull. 2007 Feb; 30(2):371-4.

Abstract: Bisphenol A (BpA) is widely used in industry and dentistry. Its effects on the embryonic development of Xenopus laevis were investigated. Xenopus embryos at stage 10.5 were treated with BpA. Developmental abnormalities were observed at stage 35; malformation of the head region including eyes and scoliosis. The expression of several markers of embryonic development was investigated by reverse transcription-polymerase chain reaction (RT-PCR). The pan-neural marker SOX-2, the neural stem cell marker nrp-1, the mesodermal marker MyoD, and the endodermal marker sox17alpha, were used. Although the expression of marker genes was not changed by treatment with BpA, that of Pax-6, a key regulator of the morphogenesis of the eyes, was decreased by BpA. Pax-6 is a downstream factor of Notch signaling. So, the expression of a typical Notch-dependent factor, ESR-1, was investigated in the presence of BpA. The expression of ESR-1 was efficiently suppressed by BpA. In whole mount in situ hybridization (WISH), Pax-6 was expressed in the central nervous system and eyes. The expression was lost completely on treatment with BpA. The expression of ESR-1 in the central nervous system and eyes also disappeared with BpA treatment. Injection of the intracellular

domain of Notch efficiently recovered ESR-1 expression in the presence of BpA although injection of a ligand for notch, Delta, did not. These results suggest that BpA decreased the expression of ESR-1 by disrupting the Notch signal.

Changes in estrogen receptors alpha and beta expression in the brain of mice exposed prenatally to bisphenol A.
Kawai, K., Murakami, S., Senba, E., Yamanaka, T., Fujiwara, Y., Arimura, C., Nozaki, T., Takii, M., and Kubo, C.
Regul Toxicol Pharmacol. 2007 Mar; 47(2):166-70.

Abstract: The expression of ERs alpha and beta and serotonergic neurons were evaluated in the brains of mice prenatally exposed to Bisphenol A, a known endocrine disrupting chemical (EDc). Bisphenol A was administered orally at a dose of 2ng/g body weight on gestinational days 11-17 to pregnant ICR mice. Newborn male offspring (Bis-A mice) were evaluated for the immunoreactivity of ERs alpha and beta, serotonin, and serotonin transporter positive cells in the dorsal raphe nucleus (DRN). The serum testosterone level was also evaluated. In the Bis-A mice, the expression of ERs alpha and beta at 5 and 13 weeks was increased compared with the controls ($P < 0.04$), but this difference disappeared by the 9th week. The serotonin, serotonin transporter, and testosterone level differences between two groups did not reach significance. Exposure to bisphenol A may have changed the expression of ERs in the brain, but did not directly affect serotonin neurons in the DRN.

In vivo effects of BISGMA-a component of dental composite-on male mouse reproduction and fertility.
Al-Hiyasat, AS, and Darmani, H
J Biomed Mater Res A. 2006 Jul; 78(1):66-72.

Abstract: The aim of this study is to investigate the effects of the resin monomer bisphenol A glycerolate dimethacrylate (BISGMA) on adult male mouse fertility. Male Swiss mice were administered various concentrations of BISGMA (0, 25, and 100 microg/kg) for a period of 28 days, and the effects on fertility was assessed by breeding these males with untreated female mice after the exposure periods. The results showed that fertility was significantly reduced when male mice were exposed to BISGMA, in comparison with their control counterparts. In females mated with males exposed to BISGMA, there was a significant reduction in the pregnancy rates as well as the number of viable fetuses. The number of resorptions out of the total number of implantations was significantly increased in females mated with males that had been exposed to BISGMA. Furthermore, the number of females with resorptions was also significantly increased. Significant reductions in bodyweight and weights of the testis and preputial glands were also observed. The weights of the seminal vesicles were significantly increased in males exposed to BISGMA in comparison with their control counterparts. There were significant reductions in testicular sperm counts, epididymal sperm counts and in the efficiency of sperm production. In

conclusion, exposure of male mice to BISGMA results in an impairment of the reproductive system and fertility.

Estrogen agonists, 17beta-estradiol, bisphenol A, and diethylstilbestrol, decrease cortactin expression in the mouse testis.
Anahara, R.; Yoshida, M.; Toyama, Y.; Maekawa, M.; Kai, M.; Ishino, F.; Toshimori, K., and Mori, C.
Arch Histol Cytol. 2006 Jun; 69(2):101-7.

Abstract: Previous reports have revealed that estrogen agonists or anti-androgenic chemicals induce abnormal spermiogenesis in rodents. In the seminiferous epithelium, the apical ectoplasmic specialization (ES) is an actin-based (cell-cell) junctional structure developing between the Sertoli cells and spermatids as is the basal ES also--although it is located between adjoining Sertoli cells. In the apical and basal ES are several adhesion complex proteins that control the spermatid developing process. Cortactin, an actin-binding protein, is one of the ES adhesion proteins, combining with several cell-cell adhesions associating proteins. In the present study, 17beta-estradiol (E2, 1.2 microg/kg), bisphenol A (BPA, 2.4 microg/kg), and diethylstilbestrol (DES, 2.5 microg/kg) were subcutaneously injected in ICR 12-week-old male mice. Mice testes were observed for the expression of cortactin protein after E2, BPA, and DES treatments by Western blot analysis, immunohistochemical analysis, and immunoelectron microscopic analysis. Observations showed that the immunoreactivity of the treated testes was significantly decreased. The immunohistochemical reactivity of cortactin in the apical ES was decreased in the treated testis. In immunoelectron microscopic observations, ultrastructural immunolocalizations of cortactin protein in the apical ES by both E2 and BPA were decreased, and the immuno-gold particles of apical and basal ES by DES were much less than the control. In the toxicological field, cortactin may be considered to be one of the indicator proteins of abnormal spermiogenesis which is affected by exogenous chemicals, such as endocrine disrupting chemicals. In summary, this study helps toward understanding the cortactin protein expression underlying the histological abnormalities of spermatogenesis induced by exogenous hormonal chemical treatment.

The effects of BIS-GMA and TEG-DMA on female mouse fertility.
Darmani, H and Al-Hiyasat, AS
Dent Mater. 2006 Apr; 22(4):353-8.

Abstract: OBJECTIVES: The current study evaluated the effect of bisphenol A glycerolate dimethacrylate (BIS-GMA) and triethyleneglycol dimethacrylate (TEG-DMA) on female mouse fertility. METHODS: Adult female mice were exposed to BIS-GMA or TEG-DMA (0, 25 and 100 microg/kg) intragastrically daily for 28 d and then mated with sexually mature untreated male mice and after mating their fertility was assessed. RESULTS: In females exposed to BIS-GMA at both doses significant increases in the total number of resorptions out of the total number of implantations were observed, with a significant increase in the number of animals

with resorptions at the higher dose. Significant reductions in body weights and significant increases in ovary weights were also observed. Exposure to TEG-DMA at a dose of 100 microg/kg resulted in significant reductions in pregnancy rates and a significant increase in the total number of embryonal resorptions. Significant reductions in body and uterine weights were also observed in females exposed to TEG-DMA. SIGNIFICANCE: The results suggest that both BIS-GMA and TEG-DMA have reproductive toxic effects in female mice.

[Effects of perinatal exposure to bisphenol A inducing dopaminergic neuronal cell to apoptosis happening in midbrain of male rat offspring].
Lin, Y., Zhang, H., Wang, W. D., Wu, D. S., Jiang, S. H., and Qu, W. D
Sichuan Da Xue Xue Bao Yi Xue Ban. 2006 Jul; 37(4):570-3.

Abstract: OBJECTIVE: To investigate the mechanism and effect of rat perinatal exposure to bisphenol A (BPA) resulting in midbrain dopaminergic neuronal cell apoptosis and tyrosine hydroxylase expression of male offspring. METHODS: Rat dams were randomLy divided into 4 groups on gestational day(GD) 10 and given orally the bisphenol A doses as 0, 0.5, 5, 50 mg/kg x d from GD10 to weaning. The brains of male offspring were obtained for detecting, with immunohistochemistry protocol, the Caspase-3, Bcl-2 and tyrosine hydroxylase expression in the midbrain on postnatal day 21 or 30 respectively, and the midbrain apoptotic neuronal cell were detected by TUNEL on PND21. RESULTS: The expression of Caspase-3 in the midbrain of rat male offspring were increased but bcl-2 were decreased on PND21 and 30, respectively. On PND21, apoptotic neuronal cell were found in the midbrain of high and medium doses groups. TH protein expression was decreased. CONCLUSION: Perinatal exposure to bisphenol A can induce the apoptosis of midbrain dopaminergic neuron in the male rat offspring even after weaning, and concomitantly decrease the midbrain TH immunoreactivity, this may cause the abnormal function of dopaminergic pathway of rat male offspring.

Dynamic changes in dopaminergic neurotransmission induced by a low concentration of bisphenol-A in neurones and astrocytes.
Miyatake, M., Miyagawa, K., Mizuo, K., Narita, M., and Suzuki, T.
J Neuroendocrinol. 2006 Jun; 18(6):434-44.

Abstract: One of the most common chemicals that behaves as an endocrine disruptor is the compound 4,4'-isopronylidenediphenol, called bisphenol-A (BPA). We previously reported that prenatal and postnatal exposure to BPA potentiated central dopaminergic neurotransmission, resulting in supersensitivity to psychostimulant-induced pharmacological actions. Many recent findings have supported the idea that astrocytes, which are a subpopulation of glial cells, play a critical role in neuronal transmission in the central nervous system. The present study aimed to investigate the role of neurone-astrocyte communication in the enhancement of dopaminergic neurotransmission induced by BPA. We found that treatment of mouse purified astrocytes and neurone/glia cocultures with BPA in vitro caused the activation of astrocytes, as detected by a stellate morphology and an increase in levels of glial fibrillary acidic protein. A low

concentration of BPA significantly enhanced the Ca2+ responses to dopamine in both neurones and astrocytes. Furthermore, a high concentration of BPA markedly induced the activation of caspase-3, which is a marker of neuronal apoptotic cell death in mouse midbrain neurone/glia cocultures. By contrast, treatment with 17beta-oestradiol (E2) had no such effects. Prenatal and neonatal exposure to BPA led to an enhancement of the dopamine-dependent rewarding effect induced by morphine. These findings provide evidence that BPA alters dopamine responsiveness in neurones and astrocytes and that, at least in part, it may contribute to potentiate the development of psychological dependence on drugs of abuse.

Toxicokinetics of bisphenol A in pregnant DA/Han rats after single i.v. application.
Moors, S., Diel, P., and Degen, G. H.
Arch Toxicol. 2006 Oct; 80(10):647-55.

Abstract: Bisphenol A (BPA) is an important chemical in the production of epoxy resins and polycarbonate plastics, and basic monomers which are used for a variety of applications. Consumer exposure to BPA may be possible from migration of BPA from dental sealants or from polycarbonate or epoxy-lined food and drink containers. BPA is known to act as weak estrogen mimic in rodents, and there is a concern of adverse endocrine effects, especially from prenatal exposure to this potential 'endocrine disruptor'. To address this concern, we have studied the disposition and transplacental transfer of BPA in pregnant DA/Han rats on day 18 of gestation. The BPA concentrations were determined by GC/MS analysis in maternal blood, maternal organs (liver, kidney, uterus), placenta and fetuses (fetal liver and residual tissues) at different time-points (5-360 min) after intravenous administration of 10 mg BPA/kg body weight. Total BPA (aglycone and conjugates) was analyzed in all tissue samples after enzymatic hydrolysis and liquid/liquid extraction; in maternal plasma, total BPA and BPA aglycone were analyzed in parallel samples (with/without hydrolysis). Soon (5 min) after the i.v. injection a mean total BPA concentration of 3.8 microg/ml was found in maternal plasma; it declined in the first 2 h to 0.7 microg/ml. Early after injection, the majority of circulating BPA (almost 80%) was still in the aglycone form, but, metabolism by phase II enzymes decreased the BPA aglycone concentration to 0.3 microg/ml after 2 h. Despite this efficient conjugation, BPA was rapidly distributed in the organism: In well perfused organs peak concentrations for total BPA were attained 20-30 min after intravenous administration, with mean values of about 9.7 microg/g in maternal liver, 8.6 microg/g in kidneys, and 6.2 microg/g in the uterus. The peak values in other tissues were lower, with 4.0 microg/g for placenta, 3.3 microg/g for fetal liver, and 2.4 microg/g for residual fetus homogenate. The BPA levels in all tissues thereafter declined more or less in parallel with those in maternal blood. The rather similar concentration time course in placenta and fetal liver indicates that BPA is readily transferred across the placenta of DA/Han rats to the fetus. Our data on BPA disposition in DA/Han rats are discussed in the context of other kinetic studies with BPA in pregnant rats, and in relation to the previous results from our laboratory (Degen et al. Arch Toxicol 76:23-29, 2002a, b, c) demonstrating comparable transplacental transfer of daidzein, a phytoestrogen that accounts for a significant portion of total human exposure to potential endocrine disruptors.

Murine neocortical histogenesis is perturbed by prenatal exposure to low doses of Bisphenol A.
Nakamura, K., Itoh, K., Yaoi, T., Fujiwara, Y., Sugimoto, T., and Fushiki, S.
J Neurosci Res. 2006 Nov 1; 84(6):1197-205.

Abstract: Bisphenol A (BPA) has been shown to disrupt thyroid hormone function. We therefore studied whether prenatal exposure to low-doses of BPA affects the morphology and the expression of some genes related to brain development in the murine fetal neocortex. Pregnant mice were injected subcutaneously with 20 microg/kg of BPA daily from embryonic day 0 (E0). Control animals received vehicle alone. For evaluating cell proliferation, neuronal differentiation and migration, bromodeoxyuridine (BrdU) was injected intraperitoneally into pregnant mice with various regimens and the brains were processed for immunohistochemistry. The total RNA was extracted from the embryonic telencephalon at various embryonic stages. The BrdU-labeled cells examined 1 hour after BrdU injection showed no differences between the BPA-treated and control groups (n = 10, each), which indicated that the proliferation of precursor cells was not affected. The BrdU-labeled cells, analysed 2 days after BrdU injection, were decreased in the ventricular zone of BPA-treated mice at E14.5 and E16.5, whereas they were increased in the cortical plate at E14.5 as compared with those in control mice (n = 10, each). Furthermore, the expression of Math3, Ngn2, Hes1, LICAM, and THRalpha was significantly upregulated at E14.5 in the BPA-treated group. These results suggested that BPA might disrupt normal neocortical development by accelerating neuronal differentiation/migration.

Prenatal and neonatal exposure to low-dose of bisphenol-A enhance the morphine-induced hyperlocomotion and rewarding effect.
Narita, M., Miyagawa, K., Mizuo, K., Yoshida, T., and Suzuki, T.
Neurosci Lett. 2006 Jul 24; 402(3):249-52.

Abstract: Bisphenol-A has been extensively evaluated for toxicity in a variety of tests as the most common environmental endocrine disruptors. In the previous study, we reported that prenatal and neonatal exposure to high-dose of bisphenol-A affects the development of central dopaminergic system in the mouse limbic area. The present study was then undertaken to investigate whether prenatal and neonatal exposure to lower dose of bisphenol-A could change the morphine-induced several pharmacological actions such as rewarding effect and hyperlocomotion in mice. Prenatal and neonatal exposure to low-dose of bisphenol-A enhanced the morphine-induced hyperlocomotion and rewarding effect. Additionally, the treatment with bisphenol-A produced an up-regulation of dopamine receptor function to activate G-protein in the mouse limbic forebrain, which is thought to play a critical role for hyperlocomotion and rewarding effects by drugs of abuse. These findings suggest that prenatal and neonatal exposure to low-dose of bisphenol-A can potentiate the central dopamine receptor-dependent neurotransmission, resulting in the supersensitivity of the morphine-induced hyperlocomotion and rewarding effects in the mouse.

Gene expression profiling reveals novel regulation by bisphenol-A in estrogen receptor-alpha-positive human cells.
Singleton, D. W., Feng, Y., Yang, J., Puga, A., Lee, A. V., and Khan, S. A.
Environ Res. 2006 Jan; 100(1):86-92.

Abstract: 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. 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(-8)M), or BPA (10(-6)M), followed by isolation of RNA and microarray analysis with the human U95A probe array (Affymetrix, Santa Clara, CA, USA). 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. These results reveal novel effects by BPA and E2, raising interesting possibilities regarding the role of endocrine disruptors in sexual development.

Male reproductive toxicity of four bisphenol antioxidants in mice and rats and their estrogenic effect.
Takahashi, O. and Oishi, S.
Arch Toxicol. 2006 Apr; 80(4):225-41.

Abstract: Male mice and rats were fed a diet containing four bisphenol antioxidants, 2,2'-methylenebis(4-ethyl-6-tert-butylphenol) (ME), 2,2'-methylenebis(4-methyl -6-tert-butylphenol) (MM), 4,4'-butylidenebis(3-methyl-6-tert-butylphenol) (BM), or 4,4'-thiobis(3-methyl-6-tert-butylphenol) (TM) at levels of 0.06-0.25% for 2 months. BM and TM decreased epididymal, seminal vesicular, prostate and preputial weights, and injured seminiferous tubules in mice in a dose-dependent fashion. BM and TM also reduced sex accessory organ weights and sperm production capacity in rats, but MM and ME were more toxic to rats than BM and TM. ME and MM did not bind ERalpha up to 10(-3) M, while BM and TM competitively bound ERalpha against beta-estradiol (E2). Fifty percent inhibitory concentrations (IC50 s) of BM, TM, and bisphenol A (positive control) against E2-binding were 7.3 x 10(-6) M, 1.8 x 10(-5) M, and 1.4 x 10(-5) M, respectively. When ovariectomized (OVX) mice were sc administered TM at doses of

60 and 300 mg/kg/day for 4 days, or when OVX mice were fed BM in the diet at a level of 0.25% for 2 months, uterine weight was significantly increased. These results suggest that BM and TM are weakly toxic, possibly through an estrogenic mechanism to male reproductive organs in mice as well as rats, while MM and ME may be the direct testicular toxins in rats but not mice.

Neonatal exposure to bisphenol A affects the exploratory and emotional behaviors in rats: Comparison with prenatal exposure.
Fujimoto, T.; Kubo, K., and Aou, S..
Neurosci Res. 2005; 52(Suppl):S109;

Abstract: We have demonstrated that perinatal exposure to Bisphenol A (BPA) impairs the sexual differentiation of brain and behavior (Kubo et al., 2003). In addition, we reported recently that a low dose of BPA during the last 1 week of prenatal period abolished the sex difference of the exploratory behavior and enhanced depressive behavior. In this study, 0.1 ppm of BPA was exposed to mother rats just after delivery until postnatal day 7. In the exploratory behavior, sex difference was shown in both groups. BPA exposure decreased the anxiety in female rats but sex difference (female; male) was also shown in both groups. In addition, BPA enhanced the depressive behavior in male rats. These findings suggest that a low dose of BPA during a neonatal period was less effective on sexual differentiation of exploratory behavior, but this chemical enhanced depressive behavior in a similar manner as the case of prenatal exposure.

Maternal exposure to bisphenol a during late pregnancy resulted in an increase of Calbindin-D9k mRNA and protein in maternal and postnatal rat uteri.
Hong, E. J.; Choi, K. C., and Jeung, E. B.
J Reprod Dev. 2005 Aug; 51(4):499-508.

Abstract: It has been reported that Calbindin-D9k (CaBP-9k) is rapidly and strongly induced by environmental estrogenic compounds, possibly through estrogen receptors (ERalpha) in the uterus of mammals. CaBP-9k can be evaluated as an early gene marker for assaying estrogenic effects of putative environmental chemicals in the rat uterus. This study was undertaken to investigate CaBP-9k mRNA and protein expression in the postnatal rat uterus following maternal exposure to 17beta-estradiol (E2) and bisphenol A (BPA) during the neonatal period. Treatment with a high dose of BPA (600 mg/kg body weight (BW) per day) resulted in a 3-fold increase in CaBP-9k mRNA expression for 3 days, while a single dose of E2 (40 microg/kg BW per day) induced 2-fold increase of this gene in the maternal uterus. In an agreement with maternal CaBP-9k mRNA, postnatal CaBP-9k mRNA in the uterus increased 4-fold when treated with BPA (600 mg/kg BW per day). In addition, treatment with increasing concentrations of BPA resulted in significant increases in CaBP-9k protein in the maternal rat uterus. It is of interest that increasing doses of BPA induced a significant ERalpha mRNA increase in the postnatal uterus. Furthermore, immunohistochemistry revealed that treatment with BPA induced CaBP-9k protein in the maternal uterus. We demonstrated that maternal exposure to BPA during late pregnancy

induced CaBP-9k mRNA and protein in maternal and postnatal rat uteri. These results suggest that rapid absorption and distribution of environmental estrogenic compounds occurs in maternal and neonatal rat uteri and these chemicals can easily pass though the placenta during pregnancy to affect postnatal reproductive functions.

Alterations in steroid hormone production by porcine ovarian granulosa cells caused by bisphenol A and bisphenol A dimethacrylate.
Mlynarcikova, A, Kolena, J., Fickova, M, Scsukova, S.
Mol Cell Endocrinol. 2005 Dec 1; 244(1-2):57-62.

Abstract: We have investigated the effects of bisphenol A (BPA) and BPA-dimethacrylate (BPA-DMA), endocrine disruptors used as plasticizers, on steroid hormone production by porcine ovarian granulosa cells after 72 h incubation. BPA at 10(-8) M to 10(-5) M increased basal progesterone levels, while the same concentration range of BPA-DMA did not cause any changes. After FSH-stimulation of the cells, BPA-DMA showed a tendency to inhibit progesterone production. BPA, however, at 10(-7) M and 10(-6) M concentrations was even able to amplify FSH-stimulated progesterone synthesis. BPA as well as BPA-DMA inhibited FSH-induced estradiol production in the whole concentration range. LH-stimulated progesterone production was not altered by BPA in 10(-8) M to 10(-5) M, while BPA-DMA decreased progesterone levels in the cultured media. Significant inhibitory effect of both tested agents at 10(-4) M concentrations was observed specifically on progesterone production, basal as well as gonadotropin-stimulated. The results indicate that ovarian steroidogenesis might be one of the possible sites afflicted by the endocrine disrupting action of BPA and BPA-DMA.

Estrogen and bisphenol A disrupt spontaneous [Ca(2+)](i) oscillations in mouse oocytes.
Mohri T and Yoshida S.
Biochem Biophys Res Commun 2005; 326(1):166-73.

The present work aims to study the effects of estrogen or endocrine disrupters (EDs) on the dynamic changes in intracellular Ca(2+) concentration of mouse immature oocytes (IOs) loaded with Ca(2+)-sensitive dye Fura-2 using an image analyzer. The majority of IOs isolated from the ovary exhibited spontaneous Ca(2+) oscillations at regular intervals. Entry of external Ca(2+), probably through gap junctions, contributes to Ca(2+) oscillations since they were reversibly inhibited by removing Ca(2+) from the bathing medium or by the application of a gap-junction inhibitor carbenoxolone (CBX, 30 microM). Both 17beta-estradiol (E2) and E2-BSA, a membrane impermeable estrogen, shortened the duration of Ca(2+) oscillations in a dose-dependent manner (1-1000 nM), and produced an irregular pattern of the oscillations, strongly suggesting that E2 acts on the plasma membrane of the oocyte. For bisphenol A (BPA), one of the estrogen-mimicking EDs, a 10,000-fold higher concentration (100 microM) was necessary to exert similar inhibitory action to that of E2.

Gene expression changes induced in the testis by transplacental exposure to high and low doses of 17{alpha}-ethynyl estradiol, genistein, or bisphenol A.
Naciff, J. M., Hess, K. A., Overmann, G. J., Torontali, S. M., Carr, G. J., Tiesman, J. P., Foertsch, L. M., Richardson, B. D., Martinez, J. E., and Daston, G. P.
Toxicol Sci. 2005 Aug; 86(2):396-416.

Abstract: The purpose of this study was to determine (1) the transcriptional program elicited by exposure to three estrogen receptor (ER) agonists: 17 alpha-ethynyl estradiol (EE), genistein (Ges), and bisphenol A (BPA) during fetal development of the rat testis and epididymis; and (2) whether very low dosages of estrogens (evaluated over five orders of magnitude of dosage) produce unexpected changes in gene expression (i.e., a non-monotonic dose-response curve). In three independently conducted experiments, Sprague-Dawley rats were dosed (sc) with 0.001-10 microg EE/kg/day, 0.001-100 mg Ges/kg/day, or 0.002-400 mg BPA/kg/day. While morphological changes in the developing reproductive system were not observed, the gene expression profile of target tissues were modified in a dose-responsive manner. Independent dose-response analyses of the three studies identified 59 genes that are significantly modified by EE, 23 genes by Ges, and 15 genes by BPA (out of 8740), by at least 1.5 fold (up- or down-regulated). Even more genes were observed to be significantly changed when only the high dose is compared with all lower doses: 141, 46, and 67 genes, respectively. Global analyses aimed at detecting genes consistently modified by all of the chemicals identified 50 genes whose expression changed in the same direction across the three chemicals. The dose-response curve for gene expression changes was monotonic for each chemical, with both the number of genes significantly changed and the magnitude of change, for each gene, decreasing with decreasing dose. Using the available annotation of the gene expression changes induced by ER-agonist, our data suggest that a variety of cellular pathways are affected by estrogen exposure. These results indicate that gene expression data are diagnostic of mode of action and, if they are evaluated in the context of traditional toxicological end-points, can be used to elucidate dose-response characteristics.

Effects of exposure in utero to bisphenol a on the expression of aryl hydrocarbon receptor, related factors, and xenobiotic metabolizing enzymes in murine embryos.
Nishizawa, H.; Imanishi, S., and Manabe, N.
J Reprod Dev. 2005 Oct; 51(5):593-605.

Abstract: To evaluate the effects of bisphenol A (BPA), a candidate endocrine disruptor (ED), on embryonic development, we examined the mRNA expression levels of the aryl hydrocarbon receptor (AhR; which binds with many EDs and plays crucial roles in their metabolism) and related factors [aryl hydrocarbon receptor repressor (AhRR) and AhR nuclear translocator (Arnt)], xenobiotic metabolizing enzymes [XMEs; cytochrome P450 1A1 (CYP1A1) and UDP-glucuronosyltransferase, and the glutathione S-transferase Ya subunit (GST)], in murine embryos exposed in utero to BPA (0.02, 2, 200, and 20,000 microg/kg/day) and 17beta-estradiol (E2; 5 microg/kg/day, used as a positive control) at 6.5-13.5 or 6.5-17.5 days post coitum (dpc) using the quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) method.

Protein levels of CYP1A1 and GST in embryonic livers were estimated by Western immunoblotting. Exposure in utero to BPA [0.02 (1/100 dose of environmental exposure), 2, 200, and 20,000 microg/kg/day] increased AhR mRNA expression in the cerebra, cerebella, and gonads (testes and ovaries) of male and female mid-and late-developmental stage (14.5- and 18.5-dpc, respectively) embryos. BPA dose-independently up-regulated the expression of AhRR and Arnt in mid- and late-stage embryos. BPA had no remarkable effect on the mRNA levels of XMEs in mid-stage embryos, but dose-dependently up-regulated the expression in late-stage embryos. Moreover, the protein levels of these enzymes in the livers of late-stage embryos were increased. The present findings revealed that exposure to BPA in utero disrupts the expression of AhR and related factors and of xenobiotic metabolizing enzymes, and that mid-stage embryos, in the organogenic stage, are sensitive to BPA.

Induction of reactive oxygen species by bisphenol A and abrogation of bisphenol A-induced cell injury by DJ-1.
Ooe, H., Taira, T., Iguchi-Ariga, S. M., and Ariga, H.
Toxicol Sci. 2005 Nov; 88(1):114-26.

Abstract: DJ-1 was first identified as an activated ras-dependent oncogene. DJ-1 is related to male fertility, and its expression in sperm decreases in response to exposure to a number of reproductive toxicants. DJ-1 has been associated with the onset of familial Parkinson's disease (PD) in humans, and has been found to have activity against oxidative damage by eliminating reactive oxygen species (ROS). In this study, we investigated the role of DJ-1 in oxidative stresses by administration of bisphenol A (BPA), which has been reported to induce oxidative stress in rodents, to male mice and cultured cells. In male mice, we found that BPA significantly increased the expression level of DJ-1 in the sperm and brain. In cultured Neuro2a and GC1 cells, we found that BPA induced ROS production and significantly compromised mitochondrial function concomitant with elevated expression and oxidization of DJ-1. DJ-1 was found to maintain the complex I activity against BPA-induced oxidative stress after the localization in mitochondria. The results showed that DJ-1 plays a role in the prevention of mitochondrial injury-induced cell death.

Early embryonic administration of xenoestrogens alters vasotocin system and male sexual behavior of the Japanese quail.
Panzica, G., Mura, E., Pessatti, M., and Viglietti-Panzica, C.
Domest Anim Endocrinol. 2005 Aug; 29(2):436-45.

Abstract: The copulatory behavior and the parvocellular vasotocin (VT) system of the nucleus of the stria terminalis (BST) are sexually dimorphic in the Japanese quail. Embryonic administration of estradiol benzoate (EB) induces an organizational effect determining the disappearance of such a dimorphism (male shows behavior and cerebral phenotype of the female). The VT parvocellular system can therefore be considered an accurate marker of the sexual differentiation of brain circuits and a very sensitive indicator of the activity of estrogen-

like substances on neural circuits. To test this hypothesis we administered diethylstilbestrol (DES), a powerful synthetic xenoestrogen, genistein (GEN), a phytoestrogen produced by soy, and bisphenol A (BPA). After 3 days of incubation, quail eggs were injected with vehicle, EB, DES, GEN or BPA. Administration of BPA caused an early blockage of development and no further analyses were done on the BPA groups. At puberty, the copulatory behavior of EB- or DES-treated male quail was totally abolished, whereas only the highest doses of GEN determined a significant decrease of the behavior. After the tests, the animals were sacrificed and perfused. The fractional area (FA) covered by VT immunoreactivity was analyzed in BST, medial preoptic nucleus, and lateral septum by computerized image analysis. The FA was significantly reduced after treatment with EB, DES and GEN at high doses. These results confirm that the sexually dimorphic VT system of the Japanese quail is a sensible indicator of the effects of xenoestrogens at the level of the central nervous system.

In vitro embryotoxicity assessment with dental restorative materials.
Schwengberg, S., Bohlen, H., Kleinsasser, N., Kehe, K., Seiss, M., Walther, U. I., Hickel, R., and Reichl, F. X.
J Dent. 2005 Jan; 33(1):49-55.

Abstract: OBJECTIVES: Resin (co)monomers may be released from restorative dental materials and can diffuse into the tooth pulp or the gingiva, and can reach the saliva and the circulating blood. Genotoxic potential of some dental composite components has been clearly documented. The genotoxic effects of xenobiotics can represent a possible step in tumor initiation and/or embryotoxicity/teratogenesis. A modified fluorescent mouse embryonic stem cell test (R.E.Tox) was used to test the embryotoxic potential of following dental restorative materials: Bisphenol A glycidylmethacrylate (BisGMA), urethanedimethacrylate (UDMA), hydroxyethylmethacrylate (HEMA), and triethyleneglycoldimethacrylate (TEGDMA), as well as some of their metabolic intermediates 2,3-epoxy-2-methyl-propionicacid-methylester (EMPME), methacrylic acid (MA), and 2,3-epoxy-2-methylpropionic acid (EMPA). METHODS: Mouse embryonic stem (ES) cells stably transfected with a vector containing the gene for the green fluorescent protein under control of the cardiac alpha-myosin heavy chain promoter were differentiated in the presence of various concentrations of the test compounds for 12 days. Fluorescence was measured using the TECAN Safire and values were expressed as percent of control values. To distinguish between cytotoxic and embryotoxic effects, all compounds were tested in a standard MTT assay. RESULTS: HEMA, TEGDMA and EMPME did not influence the differentiation process of ES cells towards cardiac myocytes. No cytotoxic effects were observed at any of the concentration levels tested. Exposure to BisGMA resulted in a 50% decrease in cell survival and a very strong inhibition of cell differentiation at 10(-5)M ($p < 0.01$). Embryotoxic effects were also present at 10(-6) and 10(-7)M ($p < 0.05$). EMPA induced a decrease in ES cell differentiation at 10(-5)M ($p < 0.01$) without cytotoxic effects. No embryotoxic effects were induced at lower concentrations. Exposure to UDMA resulted in a slight decrease of cell differentiation at 10(-5)M ($p < 0.05$). Exposure of cells to MA resulted in an increase of cardiac differentiation up to 150% ($p < 0.05$) at 10(-5)M without cytotoxic effects. CONCLUSIONS: BisGMA induced a significant high

embryotoxic/teratogenic effect over a large range of concentration. Therefore attention should be focused on this dental monomer, which should be investigated further by in vivo experiments.

Mixtures Of Exogenous Estrogens Cause Additive Uterotrophic Effects In Prepubertal Rats.
van Meeuwen, J., van den Berg, M., Sanderson, T., and Piersma, A. H.
Reprod Toxicol. 2005 Sep-2005 Oct 31; 20(3):472.

Abstract: The human diet contains numerous compounds, of which a minority has shown (anti)-estrogenic effects in vitro and in vivo. The origin of these compounds varies from plantderived (phytochemicals; PC) to industrial pollutants and pesticides (synthetic estrogens; SE). It has been suggested that these compounds may act in a non-additive way in combination with endogenous estrogens (e.g. 17beta-estradiol = E2). To investigate potential non-additive effects, two mixtures were composed that reflected concentrations reported in serum from humans consuming a regular diet. One mixture (PCM) contained coumestrol, genistein, naringenin, (+,-)catechin, (-,-)epicatechin and quercetin. The other (SEM) contained, nonyl-, and octylphenol, beta-hexachlorocyclohexane, methoxychlor, bisphenol A and dibutylphthalate. Our previous in vitro studies, applying cell proliferation and pS2 gene expression in MCF7 (ERalpha positive) cells as estrogenicity markers, demonstrated that within the mixtures, these compounds followed additive models (estrogen equivalence and concentration-addition calculations). Also combinations of E2 with one or both of the mixtures showed no departure from additivity based on cell proliferative and pS2 expression level response. To confirm this additivity in an in vivo model, E2, PCM and SEM were tested alone or at various combinations in the rat uterotrophic assay. Pre-pubertal female rats were dosed subcutaneously once every 24 h for 3 days and sacrificed on day 4. The uterine weight served as a measure of estrogenicity. Tested doses for PCs and SEs were within the range reported in plasma of humans consuming a regular diet. ED50 for E2 was found at 2.3 ug/kg bw. ED50s for PCM was found at a dose level 12 times lower and for SEM at a dose level at least 20 times higher than reported in human plasma. Average uterine weight increase (n = 5/group) of animals dosed with different combinations of PCMor SEM with E2 again supported the additivity model. Based on our results from these uterotrophic assays we conclude that a possible contribution of estrogenic effect caused by PCs from the diet could be more significant than that of SEs and that (plantderived or synthetic) estrogenic compounds in human diet probably interact with E2 in an additive way.

The importance of appropriate controls, animal feed, and animal models in interpreting results from low-dose studies of bisphenol A.
Vom Saal, F. S., Richter, C. A., Ruhlen, R. R., Nagel, S. C., Timms, B. G., and Welshons, W. V.
Birth Defects Res A Clin Mol Teratol. 2005 Mar; 73(3):140-5.

Abstract: Interpreting results of studies that report only negative effects is problematic. A number of published studies to determine whether chemicals with estrogenic activity can cause effects at low doses have not taken into account the possibility that the commercial animal feed

being used can mask effects of even potent estrogenic drugs such as diethylstilbestrol (DES). In addition, the sensitivity of the strain of animal being used for the specific category of chemical being tested has not always been described. For environmental chemicals, such as the estrogenic polycarbonate plastic monomer bisphenol A, DES is an appropriate positive control for estrogenic effects, and using an appropriate low dose of DES can eliminate the possibility of false-negative conclusions of safety when the above or other variables contribute to the negative outcome. Only when simultaneous positive effects of low doses of a positive control chemical such as DES and negative effects of environmentally relevant low doses of the test chemical are demonstrated within the same experiment are conclusions of no effect of the test chemical warranted, and this has not been reported for bisphenol A in any study. Instead, more than 90 refereed journal publications have reported effects due to exposure to low doses of bisphenol A in a wide variety of animals (for references see: http://rcp.missouri.edu/endocrinedisruptors/vomsaal/vomsaal.html). However, due to lack of attention to the importance of appropriate positive controls, a small number of studies reporting negative effects of bisphenol A have created a false sense of controversy regarding low-dose effects of bisphenol A.

[Experimental behavioral tests using monkey and rat offspring born from mothers exposed perinatally to EDCs].
Yoshikawa, Y.
Nihon Shinkei Seishin Yakurigaku Zasshi. 2005 Jun; 25(3):115-24.

Abstract: Purpose of this study is to conduct risk assessment of EDCs for the development of CNS in humans by extrapolation from the results of behavioral tests in rats and monkeys. Our hypotheses on the mechanism which gives an adverse effect of EDCs to the developing neural systems are as follows. Thyroid hormone (TH) disrupting chemicals induce deterioration of neural development and estrogen (E2) agonistic chemicals may disturb apoptosis of fetal neural cells resulting in injury of normal neural circuit. The strategy of this study is a bottom up system; for example, basic information was obtained by an experiment using rats and then an experiment using monkey was designed to adapt the results from rats. The monkey experiment data will be assessed in comparison with human behavior. The tactics of this study are, however, a top down system. It is neural behaviors which are an end point evaluation that are primarily performed. They are mother-infant interactions, social behaviors, open field test, memory and learning tests, etc. As for analysis of the mechanism of EDCs' adverse effect, we tried two methods: one is an in vivo drug biased test which interferes with the monoamine oxidase (MAO) system and the other is an in vitro primary neural cell culture. EDCs including BPA, NP, propylthiouracil (PTU) and PCB-OH are injected orally to pregnant rats from gestation day 3 (GD3) to post natal day 21 (PND21) at weaning and their offspring were tested. On the other hand TCDD, BPA and PCB effect are assessed in rhesus monkey or cynomolgus monkey offspring. The study is still continuing and we will present the results obtained to date.

Bisphenol-A, an environmental contaminant that acts as a thyroid hormone receptor antagonist in vitro, increases serum thyroxine, and alters RC3/neurogranin expression in the developing rat brain.
Zoeller RT, Bansal R, and Parris C.
Endocrinology 2005;146(2):607-12.

Considering the importance of thyroid hormone (TH) in brain development, it is of potential concern that a wide variety of environmental chemicals can interfere with thyroid function or, perhaps of greater concern, with TH action at its receptor (TR). Recently bisphenol-A (BPA, 4,4' isopropylidenediphenol) was reported to bind to the rat TR and act as an antagonist in vitro. BPA is a high production volume chemical, with more than 800 million kg of BPA produced annually in the United States alone. It is detectable in serum of pregnant women and cord serum taken at birth; is 5-fold higher in amniotic fluid at 15-18 wk gestation, compared with maternal serum; and was found in concentrations of up to 100 ng/g in placenta. Thus, the human population is widely exposed to BPA and it appears to accumulate in the fetus. We now report that dietary exposure to BPA of Sprague Dawley rats during pregnancy and lactation causes an increase in serum total T4 in pups on postnatal d 15, but serum TSH was not different from controls. The expression of the TH-responsive gene RC3/neurogranin, measured by in situ hybridization, was significantly up-regulated by BPA in the dentate gyrus. These findings suggest that BPA acts as a TH antagonist on the beta-TR, which mediates the negative feedback effect of TH on the pituitary gland, but that BPA is less effective at antagonizing TH on the alpha-TR, leaving TRalpha-mediated events to respond to elevated T4.

Sertoli cell junctional proteins as early targets for different classes of reproductive toxicants.
Fiorini, C.; Tilloy-Ellul, A.; Chevalier, S.; Charuel, C., and Pointis, G.
Reprod Toxicol. 2004 May; 18(3):413-21.

Abstract: In the testis, Sertoli cells establish intercellular junctions that are essential for spermatogenesis. The SerW3 Sertoli cell line displays some features of native Sertoli cells. Western blot and immunofluorescence analyses showed that SerW3 Sertoli cells expressed typical components of tight (occludin and zonula occludens-1), anchoring (N-cadherin) and gap (connexin 43) junctions. Testicular toxicants (DDT, pentachlorophenol, dieldrin, dinitrobenzene, cadmium chloride, cisplatin, gossypol, bisphenol A and tert-octylphenol) affected intercellular junctions by either reducing the amount or inducing aberrant intracellular localization of these membranous proteins. Phosphodiesterase inhibitors (isobutyl methylxantine, rolipram, zaprinast, zardaverine) did not alter junctional-complex component levels but caused a rapid and reversible redistribution of these proteins to the cytoplasmic compartment. The present study showed that occludin, ZO-1, N-cadherin and specifically Cx43 could be early targets for testicular toxicants. The SerW3 cell line therefore appears as a useful in vitro model to evaluate molecules with potential anti-reproductive effects.

Temporal and Spatial Distribution of Steroidogenic Acute Regulatory Protein in Developing Male Sprague-Dawley Rats Following Transplacental Exposure to 17-alpha - Ethynyl Estradiol or Bisphenol A.
Hess KA, Naciff JM, Overmann GJ, Richardson BD, Foertsch LM, and Daston GP.
Birth Defects Res Part A Clin Mol Teratol 2004;70(5):262.

Our laboratory has determined the transcript profiles for developing rat female and male reproductive tracts following transplacental exposure to estrogenic compounds (17-alpha - ethynyl estradiol (EE) and bisphenol A (BPA)) at low dosages (0.001, 0.1, 10 g/kg/day and 0.02, 5, 400mg/kg/day, respectively). The purpose of this study was to establish the spatial distribution of one of the key genes involved in steroidogenesis, steroidogenic acute regulatory protein (StAR) which is significantly affected following transplacental exposure of EE and BPA in male embryos. Thus, fetal male reproductive tissues were examined using immunohistochemical analyses specific for StAR. On gestation days (GD) 11 through 20, pregnant Sprague-Dawley rats were exposed to EE or BPA at the above concentrations. On GD20, fetal pelvic halves containing the male reproductive tissues (testis and epididymis) were harvested and fixed for histological analyses. Results demonstrate that transplacental exposure EE or BPA had no significant morphological consequences on the developing seminiferous tubules, Sertoli cells, gonocytes or the interstitial cells including the Leydig cells. However, immunohistochemistry assays demonstrated that at this time point, the level of StAR protein in the interstitial cells was significantly decreased only at the highest doses of EE and BPA compared to controls. These protein level results are consistent with the established transcript profile for StAR, which exhibits a significant decrease at the highest doses of these two compounds. Furthermore, to explore the temporal distribution of StAR following estrogenic exposure, animals were exposed to only the highest EE dose and the male fetal pelvic halves containing the reproductive tissues were harvested at GD16 and GD18. Immunohistochemistry results demonstrated that transplacental exposure to EE at 10 g/kg/day, results in an absence of StAR protein in the developing testes of the GD16 male embryos. Additionally, at this EE dose, StAR protein was significantly decreased in the interstitial cells of GD18 male embryos compared to controls. Thus, results presented here have aided in determining the molecular effect of estrogens during specific developmental time points in a rodent model.

Transfer of maternally injected endocrine disruptors through breast milk during lactation induces neonatal Calbindin-D9k in the rat model.
Hong EJ, Choi KC, Jung YW, Leung PC, and Jeung EB.
Reprod Toxicol 2004;18(5):661-8.

The uterus is a highly estrogen-responsive tissue, which can be measured through changes in CaBP-9k expression. In this study, we investigated the potential for estrogenic compounds 4-tert-octylphenol (OP), nonylphenol (NP), bisphenol A (BPA), diethylstilbestrol (DES) and 17beta-estradiol (E2) to be transferred through breast milk from dam to neonate during lactation using the induction of CaBP-9k in uterine tissue as a biomarker. Dams were treated with OP, NP and BPA, dissolved in corn oil, at doses of 200, 400 and 600 mg/kg body weight per day l for 5 days

after delivery. Dams and neonates were euthanized after 24h. Treatment with these estrogenic compounds increased the expression of CaBP-9k mRNA in the maternal uterus, in a dose-dependent manner. All doses of estrogenic compounds resulted in an increase in CaBP-9k protein levels. These compounds have an estrogenic effect on the maternal uterus during the lactation period as shown by the induction of both CaBP-9k mRNA and protein. In the neonatal uterus, the expression of CaBP-9k mRNA and protein significantly increased with DES exposure. There was a significant increase in CaBP-9k mRNA in neonatal uterus when the dams were treated with high doses of estrogenic compounds, but protein levels of CaBP-9k were undetectable. Taken together, these findings suggest that maternally injected estrogenic compounds may be transferred to neonates through breast milk and thus affecting uterine function, as shown by the induction of CaBP-9k gene expression in the neonatal uterus.

Exposure To Bisphenol A During Late Pregnancy Resulted In An Increase Of Calbindin-D9k mRNA And Protein In Maternal And Postnatal Rat Uteri.
Hong EJ, Choi KC, Leung PC, and Jeung EB.
Biol Reprod 2004;Aug(Special Issue):282.

The expression level of CaBP-9k mRNA is rapidly and strongly induced by environmental estrogenic compounds, possibly through a classical pathway of estrogen receptor (ERalpha), in which can be evaluated as an early gene marker for assaying an estrogenic effect of putative environmental chemicals in rat uterus. Based on the previous results, the present study was further performed to investigate the expression levels of CaBP-9k mRNA and protein in postnatal rat uterus following maternal exposures to diethylstilbestrol (DES), 17beta-estradiol (E2) and bisphenol A (BPA) during neonatal period. Thus, we investigated the effect of BPA on the expression levels of CaBP-9k mRNA and protein in the maternal and postnatal uterus on day 5 of delivery. Treatment with a high dose of BPA (600 mg/kg body weight (BW) per day) resulted in an increase of CaBP-9k mRNA (3-fold) for 3 days, and a single dose of DES (50 ug/kg BW) and E2 (40 ug/kg BW) induced an increase of CaBP-9k mRNA (5-fold and 2-fold, respectively) in maternal uterus. In parallel with maternal expression of CaBP-9k mRNA, the expression level of CaBP-9k mRNA was significantly increased when treated with BPA (4-fold, 600 mg/kg BW per day) and DES (41-fold, 50 ug/kg BW per day) in postnatal uterus. Treatment with increasing concentrations of BPA and a single dose of DES resulted in a significant increase in the expression level of CaBP-9k protein in maternal uterus. In addition, increasing doses of BPA and a single dose of DES induced a significant increase of ERalpha mRNA in postnatal uterus. In addition, treatment with BPA resulted in the induction of CaBP-9k protein in maternal uterus by immunohistochemistry. In summary, we demonstrated that maternal exposure to BPA during late pregnancy induced the expression levels of CaBP-9k mRNA and protein in maternal and postnatal uteri. These results suggest that the absorption and distribution of environmental estrogenic compounds in maternal and neonatal uteri are extremely rapid and these chemicals can easily pass though placenta during pregnancy to affect postnatal reproductive tissues.

[Study on cell cycle of testis cells in mouse treated by B(a)P].
Jin MH, Shi L, Liu XM and others.
Chung-Kuo Kung Kung Wei Sheng (China Public Health) 2004;20(2):153-4.

Objective: Effects of B(a)P on the cell cycle of testicle cells of male mice were studied. Methods: The flow cytometry was used to study the effects of B(a)P on testicle cells of male mice. Results: The different doses of B(a)P could make the testicle cell cycle change. The cells in G0/G1, S phase decreased with the increase of B(a)P. There were significant differences among the 5, 10, 20 mg/kg and negative control ($P < 0.05$). The accounts of cells in G2/M increased and there were significant differences between experimental groups and negative control ($P < 0.05$). Conclusion: B(a)P can inhibit DNA synthesis of testicle cells, cause the G2 block and delay the mitosis of testicle cells.

Endocrine disrupters as disrupters of brain function: a neurosteroid viewpoint.
Kawato S.
Environ Sci 2004;11(1):1-14.

The mechanisms of neurosteroid synthesis in the rat hippocampus were investigated. Metabolism assay demonstrated the pathway of "cholesterol alpha pregnenolone --> dehydroepiandrosterone --> androstenedione --> testosterone --> estradiol." Upon exposure of pups to bisphenol A (BPA) from the embryonic stage until 3 week-old stage, a significant facilitation of the synthesis of estradiol was observed in the hippocampus. The localization of cytochrome P450s (P450scc, P45017alpha, and P450arom) as well as estrogen receptor alpha (ER(alpha)) was observed in pyramidal and granule neurons, using immunohistochemical staining. Furthermore, the synaptic localization of P45017alpha, P450arom and ER(alpha) was demonstrated with immuno-electron microscopic analysis. The acute action of estradiol and endocrine disrupters were then analyzed with an electrophysiological measurement of hippocampal pyramidal neurons. A 30 min preperfusion of diethylstylbesterol (DES) enhanced the induction of long-term potentiation (LTP) by almost an identical magnitude to that obtained by estradiol perfusion. On the other hand, although the application of BPA alone did not affect LTP-induction, the co-perfusion of BPA with estradiol completely suppressed the enhancement effect of LTP by estradiol. The current investigations demonstrate in the hippocampus (1) that locally synthesized estrogen rapidly enhances the synaptic plasticity of neurons, and (2) that BPA and DES modulate the synaptic plasticity as well as the synthesis of estradiol. The probable targets of BPA and DES are ER(alpha) and steroidogenic proteins.

Analysis of differentially regulated proteins in TM4 cells treated with bisphenol A.
Lee DY, Lee SS, Joo WA, Lee EJ, and Kim CW.
Biosci Biotechnol Biochem 2004;68(6):1201-8.

BPA, bisphenol A, a monomer of epoxy resins and polycarbonate plastic, is used in many consumer products including the plastic linings of cans for food and babies' bottles. BPA has

been reported to cause reproductive toxicity and affects cells in rats and mice at high doses. In this study, the effect of BPA on protein expression in TM4 cells (a mouse Sertoli cell line) known to play an essential role in Spermatogenesis was investigated by two-dimensional electrophoresis (2-DE). After 16 h exposure to 50, 100, 150, 200, and 250 microM of BPA, the viability of TM4 cells decreased to about 90, 85, 78, 55, and 30% of control respectively. Approximately 800 protein spots in TM4 cells were analyzed by 2-DE with pH 4-7 linear immobilized pH gradient (IPG) Dry Strip, and 11 proteins which showed significantly different expression levels were identified by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS). Among these, HSP 27 and placental calcium binding protein may be proteins differentially expressed by BPA exposure.

In vivo imaging of activated estrogen receptors in utero by estrogens and bisphenol A.
Lemmen JG, Arends RJ, van der Saag PT, and van der Burg B.
Environ Health Perspect 2004;112(15):1544-9.

Environmental estrogens are of particular concern when exposure occurs during embryonic development. Although there are good models to study estrogenic activity of chemicals in adult animals, developmental exposure is much more difficult to test. The weak estrogenic activity of the environmental estrogen bisphenol A (BPA) in embryos is controversial. We have recently generated transgenic mice that carry a reporter construct with estrogen-responsive elements coupled to luciferase. We show that, using this in vivo model in combination with the IVIS imaging system, activation of estrogen receptors (ERs) by maternally applied BPA and other estrogens can be detected in living embryos in utero. Eight hours after exposure to 1 mg/kg BPA, ER transactivation could be significantly induced in the embryos. This was more potent than would be estimated from in vitro assays, although its intrinsic activity is still lower than that of diethylstilbestrol and 17beta-estradiol dipropionate. On the basis of these results, we conclude that the estrogenic potency of BPA estimated using in vitro assays might underestimate its estrogenic potential in embryos.

Alteration of pituitary hormone-immunoreactive cell populations in rat offspring after maternal dietary exposure to endocrine-active chemicals.
Masutomi, N., Shibutani, M., Takagi, H., Uneyama, C., Lee, K. Y., and Hirose, M.
Arch Toxicol. 2004 Apr; 78(4):232-40.

Abstract: We previously performed dose-response studies of genistein, diisononyl phthalate, 4-nonylphenol, methoxychlor (MXC), and bisphenol A to examine the impact of maternal dietary exposure from gestational day 15 to postnatal day 10 on the development of rat reproductive system in later life. Among the chemicals MXC alone showed typical estrogenic effects only at the maternally toxic 1200 ppm. The present study was performed to examine the sensitivity of immunohistochemical analysis of pituitary cells of offspring similarly exposed to each chemical for detection of endocrine-disrupting effects. For this purpose, ratios of pituitary cells expressing luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin (PRL), were

measured at 3 and 11 weeks of age. Ethinylestradiol (EE) at 0.5 ppm was used as a reference chemical. At week 3, decrease in the relative proportions of LH, FSH, and PRL cells in males and LH cells in females was evident with MXC at 1200 ppm. At week 11, increase was found for PRL cells from 240 ppm MXC, and FSH cells at 1200 ppm in females. On the other hand, EE increased the PRL cell percentage in females at week 3 but no effects were apparent at week 11. The other chemicals were without influence at either time point. The results suggest that the assessment of the pituitary cell populations might be a more sensitive approach to detect perinatal endocrine-disrupting effects than other methods. The difference in the pituitary effect between MXC and EE is discussed.

Effects of endocrine-disrupting chemicals on expression of ubiquitin C-terminal hydrolase mRNA in testis and brain of the Japanese common goby.
Mochida, K., Ohkubo, N., Matsubara, T., Ito, K., Kakuno, A., and Fujii, K.
Aquat Toxicol. 2004 Nov 18; 70(2):123-36.

Abstract: We investigated the effects of endocrine-disrupting chemicals (EDCs) on the expression of ubiquitin C-terminal hydrolase (UCH) mRNA in the testis and brain of the Japanese common goby, Acanthogobius flavimanus. The cDNA sequence of goby UCH contained an open reading frame encoding 220 amino acid residues (M(r)=24,223) with 51.3% overall sequence identity with human and mouse UCHL1. A competitive PCR assay was used to quantify the levels of UCH mRNA in the testis and brain of male gobies after exposure to bisphenol A, nonylphenol, or estradiol-17beta for 3 weeks. Exposure to estradiol-17beta at a nominal concentration of 100 ng/L induced significant increase in UCH mRNA levels in both testis and brain ($P < 0.05$), whereas exposure to nonylphenol induced a significant decrease in UCH mRNA levels in the testis ($P < 0.01$). These results suggest that EDCs can either positively or negatively regulate UCH mRNA levels.

Functional changes in dopamine D3 receptors by prenatal and neonatal exposure to an endocrine disruptor bisphenol-A in mice.
Mizuo K, Narita M, Yoshida T, Narita M, and Suzuki T.
Addict Biol 2004;9(1):19-25.

Bisphenol-A (BPA), one of the most common environmental endocrine disrupters, has been evaluated extensively for toxicity and carcinogenicity. However, little is still known about its action on the central nervous system (CNS). In the previous study, we found that prenatal and neonatal exposure to BPA markedly enhanced the rewarding effect induced by morphine. Here we found that prenatal and neonatal exposure to BPA resulted in the attenuation of dopamine D3 receptor-mediated G-protein activation by 7-OH-DPAT in the mouse limbic forebrain. This treatment also caused a significant decrease in the B(max) value of [(3)H]PD128907, a dopamine D3 receptor ligand, in this area. Under these conditions, no change in dopamine D3 receptor mRNA expression in the limbic forebrain and lower midbrain was observed by prenatal and neonatal exposure to BPA. The present data provide further evidence that prenatal and neonatal

exposure to BPA leads to the reduction of functional dopamine D3 receptors without affecting the new synthesis of dopamine D3 receptors in the mouse limbic forebrain.

Toxicokinetics Of The Xenoestrogen Bisphenol A In Pregnant And Non-Pregnant DA/Han Rats.
Moors S, Selinski S, and Degen GH.
Naunyn Schmiedebergs Arch Pharmacol 2004;369(Suppl 1):R109.

Bisphenol A (BPA), an environmental estrogen, can be found as a contaminant in the human diet. Hormonally active compounds can alter signalling processes of the endocrine system leading to a range of effects during fetal and postnatal development. The possibility of adverse effects due to BPA exposure is a matter of current concern. We have studied the disposition of BPA in a rodent model, the female DA/Han rat. Pregnant and non-pregnant rats received a single dose of BPA (10 mg/kg b.w.) by intravenous injection, and the xenoestrogen was also given orally by gavage (10 or 100 mg/kg b.w.) to non-pregnant females. Blood samples and tissues were collected at defined times after i.v. or p.o. administration and frozen for GC/MS analysis. Blood concentration-time curves of BPA were fitted to a tri-exponential model. A population based statistical approach was used to calculate individual AUCs and to estimate an average AUC for each dose-group of non-pregnant rats. Oral bioavailability of 10 mg/kg BPA was 20.3% and 5.0% at 100 mg/kg. Thus, the high dose was absorbed to a lesser extent than the lower. The comparison of plasma concentrations of BPA aglycone in pregnant and non-pregnant rats shows slightly lower plasma concentrations for the pregnant rats within the first half hour after i.v. dosing. A Mann-Whitney U-test indicated significant differences between pregnant rats in two early time-groups, but no significant differences in the later time-groups. In all maternal and fetal tissues analysed, peak concentrations of BPA (aglycone and conjugates) were found 20-30 min after i.v. administration. BPA levels were usually two- to three-fold higher in maternal liver and kidney than in plasma. This shows an effective uptake of BPA and metabolites in maternal tissues. The tissue distribution indicates also an efficient hepatic extraction of BPA from the maternal blood and only a small part of a given dose is transferred to the fetus. Consequences of exposures to xenoestrogens early in life should be examined along with toxicokinetics as a relevant subject to be integrated into assessments of toxicological data.

Transplacental Exposure to 17alpha- Ethynyl Estradiol or Bisphenol A Induces a Specific Gene Expression Profile in the Developing Male Rat Reproductive System.
Naciff J, Hess K, Richardson B, Overmann G, Foertsch L, Torontali S, Carr G, Tiesman J, and Daston G.
Birth Defects Res Part A Clin Mol Teratol 2004;70(5):262.

Exposure to estrogenic compounds during fetal development, at relatively high doses, induces abnormalities in the male reproductive system and causes a predisposition to abnormal function during adulthood. We hypothesize that these latent developmental effects are preceded by immediate changes in the fetal gene expression. Thus, an approach to address the potential

influence on the physiology of the male reproductive system to estrogenic exposure is the evaluation of the gene expression changes induced in this system. In this study, high-density oligonucleotide arrays were used to determine the transcriptional program elicited by transplacental exposure to graded doses (s.c.) of 17alpha -ethynyl estradiol (EE; 0.001, 0.01, 0.1, 1, and 10 g/kg/day), or bisphenol A (BPA; 0.002, 0.02, 0.5, 50, and 400 mg/kg/day), in the developing rat testis and epididymis on gestation day (GD) 20. Our results demonstrate that exposure to EE or BPA, from GD11 to GD20, does not induce evident morphological changes in the developing reproductive system of the male. However, the gene expression profile of testis and epididymis is modified by exposure to these chemicals. At the highest dose (10 g EE and 400 mg BPA/kg/day) we identified that 141 and 67 genes (out of 8740), respectively, showed a statistically significant change in their expression levels. Comparing the effect of EE versus BPA exposure, we identified that the expression of 35 genes changes in the same direction, although at a different magnitude. Among those are down-regulated genes whose products are involved in the biosynthesis of androgens, such as: scavenger receptor class B type I, steroidogenic acute regulatory protein, P450 side-chain cleavage enzyme, 3-beta-hydroxysteroid dehydrogenase, and 17-alpha -hydoxylase cytochrome P450. Analysis of the highest three dose levels indicated that the expression of 56 genes is significantly modified in a dose-dependent manner by EE, while only 15 genes are dose responsive to BPA. There are very few gene expression changes induced by exposure to low dosages, and no transcripts not identified at the higher dose levels were observed. Thus, our data demonstrate that transplacental exposure to ER agonists changes the gene expression profile of estrogen-sensitive tissues of the male, only at relatively high dosages, affecting various molecular pathways, and that these transcripts could be valuable markers of estrogen exposure during fetal development.

Evaluation Of The (Anti-) Androgenic Potential Of The Xenoestrogen BPA On The Reproductive Tract In Wistar Rats.
Nishino T, Wedel T, Schmitt O, Buhlmeyer K, Schonfelder M, Schulz T , Kuhnel W, and Michna H.
Naunyn Schmiedebergs Arch Pharmacol 2004;369(Suppl 1):R116.

Recently, endocrine disrupting effects of xenoestrogens like SPA have been intensively discussed (Bolt et aI., Arch. Toxicol., 74: 649-662, 2001]. Therefore, we evaluated androgen-like effects of SPA on the reproductive tract using orchiectomized Wistar rats (n=13). Animals were treated p.o. either with vehicle or with 3, 50, 200, 500 mg/kg/day BPA for seven days. One group was treated s.c. with 1 mg/kg/day testosterone propionate (TP). Flutamide (FL, 3 mg/kg/day, p.o.) was used to antagonize androgen effects. Morphometry and densitometry were performed by using the Axiophot from Zeiss-Vision. Proliferating activity was determined using two different primary antibodies (Ki-67 Antigen, Clone MIB-5, DakoCytomation) and (PCNA, Clone PC10, Novocastra). For the densitometric evaluation of the androgen receptor (AR) staining, a polyclonal antibody (sc-815, Santa Cruz) as well as a monoclonal antibody (No. 554224, BD PharMingen) were used. Results showed that BPA at huge doses of 200 and 500 mg caused a significant increase in relative weights of anterior prostate and seminal vesicle, possibly due to a decrease in body weights and general side effects. Morphometric data showed a

significant increase in epithelial height and luminal area of prostate and seminal vesicle at lower doses of BPA, whereas BPA induced no stimulation of the cell proliferating activity in prostate at all doses tested. In contrast, BPA (3 and 50 mg) like TP significantly increased the intensity of staining by the monoclonal antibody for AR in prostate. Finally, at the suprapharmacological dose of 500 mg/kg bw, staining intensity of the AR was similar to the castrated control. In conclusion, these findings suggest that both at ultra low doses as well as (even high) pharmacological doses of SPA no statistical significant (anti-) androgenic effects could be detected on rat reproductive organs.

Effects Of In Utero Exposure To Bisphenol A On mRNA Expression Of Arylhydrocarbon And Retinoid Receptors In Murine Embryos.
Nishizawa H, Imanishi S, Sugimoto M, and Manabe N.
Biol Reprod 2004;Aug;(Special Issue)(114.

To evaluate the effects of low-dose exposure of bisphenol A (BPA), a candidate endocrine disruptor (ED), on embryonic development, we examined the mRNA expression levels of the arylhydrocarbon receptor (AhR), which binds with many EDs and plays crucial roles in xenobiotic metabolism, and of retinoic acid receptor (RAR)alpha and retinoid X receptor (RXR)alpha, key factors in a nuclear receptor-dependent retinoids signal transduction, in murine embryos exposed in utero to BPA (0.02, 2, 200 and 20,000 microg/kg/day) at 6.5-13.5 or -17.5 days post coitum (dpc), using quantitative reverse transcription-polymerase chain reaction (RT-PCR) method. Extremely low-dose BPA (0.02 microg/kg/day; 1/100 the dose of environmental exposure) remarkably increased AhR mRNA expression in the cerebra, cerebella and gonads (both testes and ovaries) of male and female 14.5- and 18.5-dpc-embryos. In utero exposure to BPA at 2, 200 and 20,000 microg/kg/day also increased levels of AhR mRNA. In gonads of 14.5-dpc-embryos, AhR mRNA levels were elevated and showed diphasic (U) dose-response curves following exposure to BPA, but inverted U dose-response curves were obtained for 18.5-dpc-embryos. Exposure to BPA increased expression levels of both RARa and RXRa mRNAs in the cerebra, cerebella and gonads of male and female 14.5- and 18.5-dpc-embryos. Extremely low-dose BPA (0.02 microg/kg/day) increased RARa mRNA expression levels in the cerebella of male and female 14.5- and 18.5-dpc-embryos and in gonads of female 14.5-dpc-embryos, and significantly increased RXRa mRNA expression levels in the cerebra and cerebella of male and female 14.5-dpc-embryos. The present findings confirm that in utero exposure to an extremely low-dose exposure of BPA up-regulates the mRNA expression of AhR, RARa and RXRa in murine embryos, indicating that BPA disrupts the receptor-dependent signal transducing systems. Our data will contribute to the assessment of the toxic effects of BPA on xenobiotic metabolism and retinoid signals in embryogenesis.

Effects of 17beta-estradiol, nonylphenol, and bisphenol-A on developing Xenopus laevis embryos.
Sone, K., Hinago, M., Kitayama, A., Morokuma, J., Ueno, N., Watanabe, H., and Iguchi, T.
Gen Comp Endocrinol. 2004 Sep 15; 138(3):228-36.

Abstract: Many chemicals released into the environment have the capacity to disrupt the normal development of aquatic animals. We investigated the influence of nonylphenol (NP), bisphenol-A (BPA), and 17beta-estradiol (E2) on developing Xenopus laevis embryos, as a model animal in the aquatic environment. Embryos were exposed to eight different concentrations of NP, BPA or E2 between 3 and 96 h post-fertilization (p.f.). Short body length, microcephaly, flexure, edema, and abnormal gut coiling were induced by 20 microM NP, BPA or 10 microM E2 by 96 h p.f. To clarify sensitive stages to these compounds, embryos were exposed to chemicals for 45 or 48 h starting at different developmental stages and experiments were terminated 96 h p.f. BPA and NP induced abnormalities in developing X. laevis, though the sensitive stages of embryos to these chemicals are different, BPA affecting earlier stages and NP affecting at later stages. To analyze the functional mechanisms of BPA and NP in induction of morphological changes, we adapted a DNA array technology and identified 6 X. laevis genes, XIRG, alpha skeletal tropomyosin, cyclin G1, HGF, troponin C2, and ribosomal protein L9. These findings may provide important clues to elucidate common mechanisms underlying teratogenic effects of these chemicals.

Prenatal estrogen exposure differentially affects estrogen receptor-associated proteins in rat testis gonocytes.
Wang Y, Thuillier R, and Culty M.
Biol Reprod 2004; 71(5):1652-64.

We previously reported that gonocytes from 3-day-old rat testes proliferate in response to estradiol. In the present study, we found that purified gonocytes contained the mRNAs of estrogen receptor beta (ERbeta) and the chaperones Hsp90, p23, and Cyp40, but no inducible Hsp70. Immunoblot analysis showed high levels of ERbeta, Hsp90, p23, Cyp40, and the constitutive Hsc70 in gonocytes. Prenatal exposure to the estrogenic compounds diethylstilbestrol, bisphenol A, genistein, and coumestrol led to significantly increased Hsp90 mRNA levels in testis, but not p23 and Cyp40. In situ hybridization analysis indicated that Hsp90 mRNA was prominent in gonocytes, where it was increased following phytoestrogen exposure, whereas bisphenol A induced a more generalized increase throughout the testis. Immunoblot analysis of testicular extracts demonstrated that Hsp90 protein levels were significantly increased following estrogen exposure, and immunohistochemical analysis indicated that this increase occurred predominantly in gonocytes. By contrast, no change was observed in the expression of Cyp40, p23, and ERbeta, whereas Hsc70 was increased by bisphenol A only. Using an antibody and reverse transcriptase-polymerase chain reaction probes specific for Hsp90alpha, we subsequently confirmed that Hsp90alpha was primarily expressed in gonocytes, and that it was increased following estrogen exposure. Hsp90 immunolocalization in fetal and prepubertal testes showed an increased expression in fetal gonocytes upon estrogen

exposure, but no difference in the subsets of Hsp90-positive germ cells in prepubertal testes. These results demonstrate that prenatal estrogen exposure specifically affects Hsp90 expression in gonocytes. Considering the interaction of Hsp90 with several signaling molecules, changes in its expression levels may lead to subsequent changes in gonocyte development.

Perinatal exposure to SPA affects ER expression in rat hippocampus.
Xu X, Liu Y, Ushijima H, and Kato N.
Neurosci Res 2004;50(Suppl 1):S143.

We have reported the rats perinatally exposed to low dose bisphenol A (BPA) expressed hyperactivity and impaired memory. Estrogen receptor alpha (ER alpha) expression in the hippocampus was investigated to further clarify the mechanism. Pregnant SD rats were treated with BPA and/or ICI from the pregnant 11th day to the 21st day after childbirth. The male pups were tested in open field and Morris water maze. ER alpha of hippocampus was studied with the methods of immunohistochemistry, Western blot and real-time PCR. The male pups from BPA+ICI showed no difference from control group, while hyperactivity and impaired spatial memory was observed in BPA group. In CA1, CA3 and DG, the control and BPA+ICI groups showed highest immunoactivity for ER alpha on 7day, but in BPA group the peak appeared on 11day. The results of Western blot and real-time PCR for ER alpha indicated same tendency as above. These findings suggest that ER alpha is involved in the effect induced by perinatal exposure to BPA.

Altered profiles of spontaneous novelty seeking, impulsive behavior, and response to D-amphetamine in rats perinatally exposed to bisphenol A.
Adriani W, Seta DD, Dessi-Fulgheri F, Farabollini F, and Laviola G.
Environ Health Perspect 2003; 111(4):395-401.

Bisphenol A (BPA) is an environmental estrogen with potentially averse effects on public health. We studied the long-term effects of perinatal exposure to BPA on later behavior in adult rats of both sexes. BPA or vehicle was administered orally to mother rats from mating to pups' weaning, at a concentration (0.040 mg/kg) within the range of human exposure. The offspring of both sexes were tested at adolescence (postnatal days 35-45) for novelty preference (experiment 1). After a 3-day familiarization to one side of a two-chamber apparatus, on day 4 rats were allowed to freely explore the whole apparatus. BPA-exposed females spent significantly less time than did controls in exploration of the novel side (i.e., increased neophobia), whereas no effect was found in the male group. At adulthood, the same animals were food deprived and tested for profiles of impulsive behavior (experiment 2), in operant chambers provided with two nose-poking holes (delivering either five or one food pellet). After the establishment of a baseline preference for the large reinforcer, a delay was introduced before the delivery of the five food pellets, which was progressively increased each day (10, 20, 30, 45, 60, 80, 100 sec). As expected, all animals exhibited a progressive shift toward the immediate but smaller reinforcer. A reduced level of impulsive behavior (i.e., a shift to the right in the intolerance-delay curve)

was evidenced in BPA-treated rats. The frequency of inadequate responding (during the length of the delay) also provided a measure of restless behavior. Interestingly, the profile of BPA-treated males was feminized, strongly resembling that of control females. Animals were then tested (experiment 3) for the response to an amphetamine challenge (1 mg/kg, subcutaneously). The drug-induced increment activity was significantly less marked in BPA-treated male rats compared with controls. These findings provide clear indirect evidence of long-term alterations in brain monoaminergic function after perinatal BPA exposure. This may be a cause for concern for public health, confirming that exposure to a weak environmental estrogen in the period of sexual differentiation of the brain can influence adult behavior.

Low dose exposures to bisphenol A decrease prolifertative capacity in developing rat leydig cells.
Akingbemi BT and Hardy MP.
Biol Reprod 2003;68(Suppl 1):309.

Exposures of human populations to pesticides and industrial pollutants and chemicals present in foods and plastics - collectively referred to as endocrine disruptors (EDs) - have raised concern that these compounds may affect reproductive health. Bisphenol A (BPA) is widely used in the manufacture of food packaging products and dental sealants. The early phase of Leydig cell development involves proliferative activity and estrogen modulation of this process has implications for androgen biosynthesis in the adult. BPA is known to bind the estrogen receptor (ER), and Leydig cells possess ERs and are subject to estrogen action. There is growing evidence that low dose exposures to EDs may alter reproductive function. Therefore, pregnant Long-Evans rats were gavaged with 0, 2.5 and 25 ug/kg/day BPA from gestation days 12 to 21. Leydig cells were obtained from male offspring at 21 and 35 days of age and evaluated for proliferative capacity by measuring tritiated (3H)thymidine incorporation. At 21 days, 2.5 ug/kg/day BPA decreased thymidine incorporation by Leydig cells (1.94 +/- 0.05 CPM/103 cells) compared to control (2.32 +/- 0.04; P < 0.01). However, Leydig cells exposed to either 2.5 or 25 ug/kg/day BPA exhibited decreased proliferative capacity at 35 days: 2.84 +/- 0.08 versus 1.89 +/- 0.10 and 1.78 +/- 0.05; CPM/10(3) cells, P < 0.01). As the pituitary is a major target organ for BPA, Leydig cells were incubated with this agent in order to remove its confounding effects on pituitary LH secretion in vivo. Leydig cells, obtained from 35-day old Long-Evans rats, were incubated with 10 pM BPA (a dose known to inhibit steroidogenesis) for 18 h and assessed for proliferative capacity. Exposure to BPA in vitro caused a decrease in (3H)thymidine incorporation compared to control (0.96 +/- 0.02 versus 0.71 +/- 0.04 CPM/103 cells, P < 0.01). Since exposures to high levels of estrogen stimulate Leydig cell proliferation, these data indicate that the effect of ER agonists on Leydig cell proliferation is dose-dependent. The results also suggest that low dose exposures to BPA and environmental estrogens may affect male fertility because the establishment of normal numbers of Leydig cells in the adult testis relies on initial proliferative activity.

Metabolism and pharmacokinetics of bisphenol A (BPA) and the embryo-fetal distribution of BPA and BPA-monoglucuronide in CD Sprague-Dawley rats at three gestational stages.
Domoradzki, JY, Pottenger, LH, Thornton, CM, Hansen, SC, Card, TL, Markham, DA, Dryzga, MD, Shiotsuka, RN, and Waechter, JM Jr.
Toxicol Sci. 2003 Nov; 76(1):21-34.

Abstract: The pharmacokinetics of bisphenol A (BPA), including the quantification of the major BPA metabolite BPA-monoglucuronide conjugate (BPA-glucuronide) was studied in Sprague-Dawley rats at different stages of gestation. 14C-BPA was administered orally at 10 mg BPA/kg body weight (0.2 mCi/rat) to nongravid rats and to other groups on gestation days (GD) 6, 14, and 17. GD 0 was when the vaginal smear was sperm positive or a copulatory plug was observed. Radioactivity derived from 14C-BPA was quantified in the maternal blood, selected tissues, and the embryo or fetus. BPA and BPA-glucuronide were quantified in maternal plasma and excreta. Additional rats were dosed orally at 10 mg 14C-BPA/kg (0.2 mCi/rat or 0.5 mCi/rat) on GD 11, 13, and 16 to further study the distribution of BPA and BPA-glucuronide to the embryo/fetal tissue. The tissue distribution, metabolism, or the rates or routes of excretion of BPA, or the plasma concentration-time profiles of BPA-glucuronide did not appear to be altered at any stage of gestation as compared to nonpregnant rats. In the GD 11 group, neither BPA nor BPA-glucuronide was detected in the yolk sacs or embryos, except for trace concentrations of BPA-glucuronide in the yolk sacs at 15 min postdosing. In the GD 13 group, both BPA and BPA-glucuronide were detected in the yolk sacs of the conceptus but not in the embryos/fetuses, except for BPA at 15 min. For the animals dosed with 0.2 mCi/rat on GD 16, both analytes were detected in the placentae at 15 min and 12 h, but not at 96 h. Traces of both analytes were detected in fetal tissue in two of five specimens at 15 min only. In rats dosed on GD 16 with 0.5 mCi/rat, the BPA-glucuronide and BPA concentrations in maternal plasma at 15 min were 1.7 and 0.06 mug equivalents (eq)/g plasma, respectively. At the same time postdosing in these animals, the placental BPA-glucuronide concentrations were lower (0.34 mug eq BPA [as glucuronide]/g), and the BPA concentrations were about equivalent (0.095 mug/g). Fetal BPA-glucuronide and BPA concentrations were markedly lower, 0.013 and 0.018 mug eq/g, respectively. Therefore, no selective affinity of either yolk sac/placenta or embryo/fetus for BPA or BPA metabolites relative to maternal plasma or tissues was observed in this study.

Microarray and in situ analysis of developing Sprague-Dawley fetal reproductive tissues following in utero exposure to estrogenic compounds.
Hess, K. A.; Naciff, J. M., and Daston, G. P.
Biol Reprod. 2003; 68(Suppl 1):310.

Abstract: Various naturally occurring and synthesized chemicals have been shown to alter hormonally regulated processes in mammals. These endocrine-disrupting substances can affect not only the growth and development of fetuses but have also been linked to reproductive dysfunction and cancers in reproductive organs during adulthood. Using a rodent model, the specific genetic consequences following exposure to estrogenic compounds during fetal development was examined in reproductive tissues using microarray technology followed by

histological and in situ hybridization analyses. On gestation days (GD) 11 through 20, pregnant Sprague-Dawley rats were exposed to three different estrogenic compounds (17alpha-ethynyl estradiol, genistein or bisphenol A) at various concentrations. Following the dosing regimen, testes or uterus and ovaries were harvested on GD20 and analyzed using oligonucleotide arrays to establish transcription expression profiles for both developing male and female animals. Of the approximately 8,000 rat genes and 1,000 expressed sequence tags analyzed, the expression of a subset of regulatory and developmental genes were found to be consistently affected following in utero estrogenic compound exposure. Additionally, there were sex-specific differences in the profile of gene expression. Following the microarray screening, male and female fetal reproductive tissues as well as placentas harvested on GD16, 18 and 20 from pregnant Sprague-Dawley rats exposed to 17alpha-ethynyl estradiol daily from GD11 were analyzed histologically. Compared to controls, no significant morphological differences were found in the embryonic reproductive tracts from the three different time points. The placentas from the same time points exhibited only slight cellular differences compared to controls. Finally, to establish the temporal and spatial distribution of several key regulatory genes that were affected by endocrine disruption, in situ hybridization analyses were performed on developing male and female rat reproductive tissues from the three different time points. Results presented here have aided in determining the molecular effect of endocrine disruption during development in a rodent model.

Bisphenol A-induced apoptosis of cultured rat Sertoli cells.
Iida H, Maehara K, Doiguchi M, Mori T, and Yamada F.
Reproductive Toxicology 2003;17(4):457-464.

Bisphenol A (BPA) was examined for its effects on cultured Sertoli cells established from 18-day-old rat testes. We demonstrated that exposure of cultured Sertoli cells to BPA decreased the cell viability in a dose- and a time-dependent manner and that exposure to BPA brought about morphologic changes of the cells, such as membrane blebs, cell rounding, cytoskeletal collapse, and chromatin condensation or fragmentation, all of which conform to the morphologic criteria for apoptosis. Immunocytochemistry showed that active caspase-3, a major execution caspase, was expressed in round Sertoli cells positively labeled by the TUNEL method. Co-localization of active caspase-3 and aggregated actin fragments was also observed in the round Sertoli cells. Theses results suggest that BPA induces cell death of Sertoli cells by promoting apoptosis. Apoptosis-inducing cell death was observed in cells exposed to 150-200 muM BPA, while BPA at <100 muM had only slight cytotoxic effects on the cells.

Effects of Oral Exposure of Bisphenol A on mRNA Expression of Nuclear Receptors in Murine Placentae Assessed by DNA Microarray.
Imanishi S, Manabe N, Nishizawa H, Morita M, Sugimoto M, Iwahori M, and Miyamoto H.
Journal of Reproduction and Development 2003;49(4):329-36.

Bisphenol A (BPA), a candidate endocrine disruptor (ED), is considered to bind to estrogen receptors and to regulate expressions of estrogen responsive genes. It has also shown evidence of

affecting the reproductive, immunological and nervous systems of mammalian embryos. However, the effects of BPA on placentae, a central organ of feto-maternal interlocution, are still unclear. To reveal the mechanisms of BPA effects on placentae in mammals, we compared the mRNA expression of 20 nuclear receptors between placentae of vehicle controls and those of orally BPA exposed pregnant mice by a DNA microarray technique. In murine placentae, mRNAs of 11 nuclear receptors were not detected. However, greater than 1.5 fold changes in mRNA expression of nine nuclear receptors between vehicle control and BPA treated mice were noted. Moreover, remarkable changes in mRNA expression of six non-nuclear receptor proteins were induced by BPA exposure. There were various differences in the effects of BPA on the expression of these mRNAs between the placentae with male embryos and those with female embryos. Such embryo-sex dependent differences are interesting and important pointers to understanding of the endocrine disrupting effect of BPA. The present data indicate that BPA affects the expression of nuclear receptor mRNAs in placentae and may disrupt the physiological functions of placentae.

Teratogenic and anti-metamorphic effects of bisphenol A on embryonic and larval Xenopus laevis.
Iwamuro, S., Sakakibara, M., Terao, M.; Ozawa, A, Kurobe, C., Shigeura, T., Kato, M., and Kikuyama, S.
Gen Comp Endocrinol. 2003 Sep; 133(2):189-98.

Abstract: Effects of bisphenol A (BPA) on embryonic and larval development were investigated. In Xenopus laevis blastulae treated with 2.5-3.0 x 10(-5) M BPA or with 10(-5) M 17beta estradiol (E2), malformation of the head region, scoliosis (curved vertebrate), and suppression of organogenesis were observed. In addition, 10(-5)-10(-4) M BPA blocked tri-iodothyronine (T3)-inducible resorption of the tail segments from premetamorphic (stage 52-54) larvae in vitro. When stage 52 tadpoles were immersed in 1.0-2.5 x 10(-5) M BPA, deceleration of both spontaneous and thyroxin (T4)-induced metamorphic changes occurred. Furthermore, BPA suppressed thyroid hormone receptor (TR) beta gene expression both in vivo and in vitro. Thus, we concluded that BPA at the concentrations examined affects both embryonic development and larval metamorphosis.

Low dose effects of bisphenol A on sexual differentiation of the brain and behavior in rats.
Kubo K, Arai O, Omura M, Watanabe R, Ogata R, and Aou S.
Neurosci Res 2003;45(3):345-56.

There is an endocrinological concern that environmental endocrine disrupters (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. 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. 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.

Novel openers of Ca2+-dependent large-conductance potassium channels: symmetrical pharmacophore and electrophysiological evaluation of bisphenols.
Li, Y., Johnson, G., Romine, J. L., Meanwell, N. A., Martin, S. W., Dworetzky, S. I., Boissard, C. G., Gribkoff, V. K., and Starrett, J. E. Jr.
Bioorg Med Chem Lett. 2003 Apr 17; 13(8):1437-9.

Abstract: Electrophysiological evaluation of symmetrical analogues of the known maxi-K opener NS-004 (1) led to the discovery of bisphenols 2a, 3a and 4a as openers of cloned maxi-K channels expressed in oocytes.

[Teratogenicity of bisphenol A on post-implanted rat and mouse embryos: an in vitro study].
Li, Y., Pei, X., Long, D., and Chen, X.
Wei Sheng Yan Jiu. 2003 Mar; 32(2):89-92.

Abstract: In order to evaluate the developmental toxicity of BPA and to investigate the possible mechanism of it in vitro, whole embryo culture was used for the study. The embryos that removed from mother mice on gestational 8.5d (GD8.5) and mother rats on gestational 9.5d (GD9.5) were cultured immediately in centrifugal serum (ICS) with a series of concentrations of 0, 40, 60, 80, 100 mg/L of BPA for 48 h to evaluate the embryo development and morphological differentiation. The results showed that the BPA inhibited the development of embryos in vitro and there was a dose-response relationship between concentrations of BPA. At high level ($\leq$ or = 60 mg/L), BPA could delayed the growth and cardiac tube differentiation of visceral yolk sac (VYS), and induced various embryo defects, including abnormal neural system, arch, optic, flexion and small limb bud, etc. It is concluded that at high level, BPA had toxicity to mice and rat embryos in vitro. The damage on structure and function of VYS might be part of teratogenic mechanisms of BPA.

Toxicokinetics of bisphenol A and daidzein in pregnant DA/HAN rats: a comparison.
Moors S, Janning P, Diel P, Bolt HM, and Degen GH.
Naunyn Schmiedebergs Arch Pharmacol 2003; 367(Suppl 1):R129.

Bisphenol A (BPA) and daidzein (DAI), two environmental estrogens, are found in the human diet: BPA as a contaminant, DAI as a natural ingredient, e.g. of soy products. There is concern that endocrine active compounds might reach biologically significant levels in human and animal tissues upon prenatal exposure. We have studied the disposition of BPA and of DAI in pregnant DA/Han rats and their placental transfer to fetuses, with the aim to compare their toxicokinetics. DAI levels were analysed by HPLC in maternal blood, liver, kidney, placenta, and in fetuses between 5 min and 2 h after i.v. administration of a 10 mg/kg b.w. dose (Janning et al., 2002, Arch Toxicol 76:23-29); BPA levels were determined by GC/MS analysis between 5 min and 6 h after an i.v. dose of 10 mg/kg b.w., also on day 18 of gestation, in maternal and fetal samples from another group of rats. Concentration-time-curves for BPA and DAI in maternal plasma showed a rapid decline within an hour. The analysis of aglycone and total BPA or DAI indicates a faster conjugation of DAI (at 40 min 15% more conjugate than BPA). In maternal liver and all other tissues analysed DAI levels peaked at 10 min after dosing, yet the placental and fetal concentrations were considerably lower than in maternal liver. BPA peak levels were measured in placenta and fetuses after 20-30 min, again with lower levels than in maternal liver at all times. BPA levels in the maternal and fetal tissues decreased more slowly in comparison to DAI. The data indicate the following: Both compounds undergo efficient hepatic extraction from the maternal blood, and parts of a given administered dose are transferred to the rat fetus. At given maternal doses, BPA could result in a higher prenatal exposure than DAI. It is important to point out that human dietary exposures to estrogenic isoflavones are far (1000-fold) higher than those to BPA.

Bisphenol A induces both transient and permanent histofunctional alterations of the hypothalamic-pituitary-gonadal axis in prenatally exposed male rats.
Ramos JG, Varayoud J, Kass L, Rodriguez H, Costabel L, Munoz-de-Toro M, and Luque EH.
Endocrinology 2003;144(7):3206-3215.

Exposure to bisphenol A (BPA) in utero has been shown to induce alterations in the prostate of 30-d-old Wistar rats. Herein, we examine both the time course of BPA action on the rat prostate and the effects of BPA on the male hypothalamic-pituitary-gonadal axis. This was achieved by exposing rats to BPA in utero, followed by immunohistochemistry and morphometric analysis of prostatic tissue, evaluation of estrogen receptor-alpha (ERalpha) and ERbeta mRNA expression in both the preoptic area (POA) and medial basal hypothalamus, and determination of PRL, LH, and testosterone serum levels. On d 30 (peripubertal period), the prostatic periductal stroma of BPA-exposed rats demonstrated a significantly larger layer of fibroblasts than that of controls, whereas on d 120 (adulthood) no significant differences were observed. Moreover, BPA-exposed rats on d 15 exhibited an increase in stromal cellular proliferation compared with controls. Decreased expression of both androgen receptor in prostatic stromal cells and prostatic acid phosphatase in epithelial cells was observed only on d 30 in BPA-exposed males. BPA did not

alter POA ERalpha mRNA expression, whereas a 4-fold increase in POA ERbeta mRNA expression was observed on both d 30 and 120. No alterations were observed in either ERalpha or ERbeta expression in the medial basal hypothalamus. BPA-exposed males exhibited increased PRL levels only on d 30, whereas a transient increase in serum testosterone levels was observed on d 15. These results support the hypothesis that prenatal exposure to environmental doses of BPA induces both transient and permanent age-dependent alterations in the male reproductive axis at different levels.

Effects of Pubertal Treatment with Bisphenol A and Bis-GMA on Sex Hormone Level in Male Rats.
Saito D, Minamida G, Izukuri K, Tani-Ishii N, Kato Y, Ozono S, Kawase T, Teranaka T, and Koshika S.
Environmental Sciences 2003; 10(1):55-61.

Environmental estrogens (xenoestrogens) are a diverse group of chemicals that mimic estrogenic actions. In the present study, we reported the in vivo evaluation of the effects of the environmental estrogenic chemicals, 2,2-bis-(4-hydroxyphenyl)-propane (bisphenol A, BPA) and 2,2-bis(4'-(2'-hydroxy-3'-methacryloyoxy) phenyl) propane (Bis-GMA) on the levels of testosterone (T) and estradiol-17beta (E2) in plasma of pubertal male rats. The chemicals, BPA (5 ug or 5 mg/day), Bis-GMA (5 ug or 5 mg/day), E2(5 ug/day), and diethylstilbestrol (DES, 5 ug/day) were injected subcutaneously into male rats every two days from 3 to 11 weeks of age. The rats were sacrificed at 13 weeks of age and their plasma T and E2 levels were analyzed. Neither BPA nor Bis-GMA injections changed the body weight, while E, and DES reduced the gain in body weight. E2, DES and low-dose (5 ug) Bis-GMA reduced the testis weight. Although the T levels in plasma were not affected by high-dose treatments (5 mg) with BPA and Bis-GMA, treatment with E2, DES and low-dose BPA and Bis-GMA reduced the T levels. Significant increases in plasma E2 levels were observed in animals treated with E2, DES and high-dose BPA compared with those treated with the vehicle control. Bis-GMA and BPA at a low dose had no effects on the E2 level in plasma. BPA and Bis-GMA were found to disturbed the sex hormone production in male rats during puberty. BPA and Bis-GMA have similar potency in reducing T production, while BPA was found to more actively increase the plasma E, level than Bis-GMA. We postulate that chemicals at low doses exhibit multiple endocrine activities via complex mechanisms.

Fetal programming: differential effects of prenatal exposure to bisphenol-A and methoxychlor on postnatal reproductive function.
Savabieasfahani M, Evans NP, Herkimer C, Kannan K, Vazquez M, and Padmanabhan V.
Biol Reprod 2003; 68(Suppl 1):273-4.

Increases in the sheer volume of reproductive toxicants and the increased occurrence of reproductive disorders raise concerns regarding the impact of endocrine disrupting chemicals (EDCs) on reproductive health especially when such exposure occurs during prenatal life, when

the fetus is very vulnerable. Studies in sheep have found that fetal exposure to testosterone (T) lead to growth retardation, hypergonadotropism, and compromised estradiol (E) positive feedback, multifollicular ovaries and anovulation in the adult. Exposure of fetuses to dihydrostestosterone, a non aromatizable androgen, did not alter ovarian morphology or E positive feedback suggesting that such disruptions may be facilitated via estrogenic actions of T. In this study we tested the hypothesis that fetal exposure to estrogenic EDCs, MXC and BPA, like T, would also lead to hypergonadotropism and disrupt cyclicity in the ewe. Suffolk ewes were administered MXC (n = 10), BPA (n = 10) (5 mg/kg/day in cotton seed oil) or the vehicle (C; n = 8) s.c. from days 30 to 90 of gestation. Maternal BPA levels measured by HPLC thrice during the exposure period averaged < 10 in C and 49 ng/mL in BPA ewes. MXC and BPA offsprings were similar to C in their weight, height and chest circumferences. Progestogenic cycles began at 26.2 +/- 0.4, 24.6 +/- 1.1, and 26.4 +/- 0.6 weeks, respectively, in C, BPA and MXC sheep. Characterization of LH in blood samples collected twice/week revealed that BPA (n = 9) and not MXC-treated females (n = 8) were hypergonadotropic when compared with C (n = 8) (mean LH +/- SE at 2 weeks: C: 2.3 +/- 0.7, MXC: 1.9 +/- 0.3, BPA: 6.5 +/- 1.8 ng/mL; C vs. BPA: P < 0.05; 3-13 weeks: C: 2.8 +/- 0.2: MXC: 3.2 +/- 0.4; BPA: 4.2 +/- 0.4 ng/mL; C vs. BPA: p < 0.05) suggesting that LH sensitivity to E negative feedback may be compromised. Characterization of cyclic changes in LH in 2h samples taken for 120h following synchronization with PGF2a revealed that the onset of LH surge from luteolysis was delayed in MXC but not BPA-treated females (C 34.8 +/- 2.9h, BPA 33.5 +/- 3.5h, MXC 50.0 +/- 3.8h; C vs. MXC: P < 0.05). This delay suggests disruption of preovulatory sequence of events due to either compromised follicular development and/or compromised E positive feedback. Together these findings suggest that prenatal exposure to BPA and MXC have differential effects on reproductive function.

Effect of neonatal treatment of rats with potent or weak (environmental) oestrogens, or with a GnRH antagonist, on Leydig cell development and function through puberty into adulthood.
Sharpe RM, Rivas A, Walker M, McKinnell C, and Fisher JS.
Int J Androl 2003; 26(1):26-36.

This study addressed whether reduced Sertoli cell number or manipulation of the neonatal hormone environment has an influence on final Leydig cell number per testis in the rat, by applying neonatal treatments known to affect these parameters, namely administration of a GnRH antagonist (GnRHa) or diethylstilboestrol (DES, in doses of 10, 1 or 0.1 microg per injection). The effect of treatment with either of two 'environmental oestrogens', bisphenol-A (Bis-A) or octylphenol (OP), was also evaluated. Leydig (3beta-hydroxysteroid dehydrogenase immunopositive) cell development and function (plasma testosterone levels) were studied through puberty into adulthood. Treatment with GnRHa impaired testis growth, Leydig cell (nuclear) volume per testis and testosterone levels during puberty, when compared with controls, but final Leydig cell volume/number in adulthood was comparable with controls. As adult testis weight was reduced by 45% in GnRHa-treated rats, the percentage Leydig cell volume per testis was approximately double ($p < 0.01$) that in controls, and also at day 35. Testosterone levels in

adulthood in GnRHa-treated rats were lower ($p < 0.01$) than in controls but were within the lower end of the normal range. Treatment with DES caused largely dose-dependent suppression of testis growth, Leydig cell (nuclear) volume per testis and testosterone levels up to day 35. Although by adulthood, Leydig cell volume/number per testis was comparable with controls in DES-treated rats, testosterone levels remained grossly subnormal. Neonatal treatment with either Bis-A or OP had little consistent effect on any of the parameters studied except that both treatments significantly elevated testosterone levels on day 18, as did treatment with DES-0.1 microg. The present findings are interpreted in the context of what is known about the hormonal regulation of Leydig cell development. These lead to the conclusion that final Leydig cell number per testis is not determined by the number of Sertoli cells per testis and appears not to be influenced in any major way by gonadotrophins, androgens or oestrogens in the first 2 weeks of postnatal life. This implies that adult Leydig cell number may be determined prior to birth.

Prenatal and neonatal exposure to bisphenol-A enhances the central dopamine D1 receptor-mediated action in mice: enhancement of the methamphetamine-induced abuse state.
Suzuki T, Mizuo K, Nakazawa H, Funae Y, Fushiki S, Fukushima S, Shirai T, and Narita M. Neuroscience 2003; 117(3):639-44.

Bisphenol-A (BPA), one of the most common environmental endocrine disrupters, has been extensively evaluated for toxicity in a variety of tests in rodents, including developmental and reproductive toxicity, and carcinogenicity. However, little is known about its action on the CNS. In this report, we show that prenatal and neonatal exposure to BPA in mice leads to the enhancement of the dopamine D1 receptor-dependent rewarding effect induced by a psychostimulant methamphetamine. Furthermore, this treatment with BPA markedly enhanced hyperlocomotion and its sensitization induced by methamphetamine, which reflects extensive abuse associated with sociological and psychiatric problems. We also demonstrated that chronic exposure to BPA produced an up-regulation of dopamine D1 receptor function to activate G-protein in the mouse limbic forebrain, which is thought to be a critical site for the expression of rewarding effects by abuse drugs. Additionally, chronic BPA exposure produced a significant increase in levels of the dopamine D1 receptor mRNA in the whole brain. In contrast, no change in protein levels of methamphetamine-targeted proteins, dopamine transporter or the type 2 vesicle monoamine transporter in the brain was observed by prenatal and neonatal exposure to BPA. The present data provide the first evidence that prenatal and neonatal exposure to BPA can potentiate the central dopamine D1 receptor-dependent neurotransmission, resulting in supersensitivity of methamphetamine-induced pharmacological actions related to psychological dependence on psychostimulants.

cDNA microarray analysis reveals chop-10 plays a key role in Sertoli cell injury induced by bisphenol A.
Tabuchi, Y. and Kondo, T.
Biochem Biophys Res Commun. 2003 May 23; 305(1):54-61.

Abstract: We examined the time course of changes in gene expression in detail using cDNA microarray analysis of mouse Sertoli TTE3 cells treated with bisphenol A (BPA). A subtoxic dose of BPA (200 microM) transiently increased intracellular Ca(2+) concentration and time-dependently induced an increase in mRNA level of 78-kDa glucose-regulated protein, indicating that BPA induces endoplasmic reticulum stress. Of the 865 genes analyzed, 31 genes showed increased levels of expression. TaqMan analysis confirmed that the mRNA levels of chop-10, fra-2, c-myc, and ornithine decarboxylase were increased, and showed that chop-10 is the most sensitive gene. The expression level of chop-10 protein and cell injury induced by BPA were significantly reduced in stable TTE3 cells overexpressing full-length chop-10 antisense RNA. We conclude that chop-10 plays a key role in Sertoli cell injury induced by BPA.

Exposure to the environmental estrogen bisphenol A differentially modulated estrogen receptor-alpha and -beta immunoreactivity and mRNA in male mouse testis.
Takao T, Nanamiya W, Nazarloo HP, Matsumoto R, Asaba K, and Hashimoto K.
Life Sci 2003;72(10):1159-69.

We examined the effects of bisphenol A (0.5 microg/ml or 50 microg/ml) in the drinking water on estrogen receptor (ER) alpha and beta proteins and mRNA in the testis of young mice following 8-weeks of oral administration of bisphenol A utilizing immunohistochemistry and semiquantitative reverse transcription polymerase chain reaction amplification (RT-PCR). ER beta was clearly localized in the nuclei of spermatogonia and/or spermatocytes. ER beta immunopositive cell numbers per testis section were significantly decreased in the 50 microg/ml bisphenol A-treated group compared with control and the 0.5 microg/ml bisphenol A-treated group. The number of ER alpha positive cells in the testis was significantly lower than ER beta positive cells in control group. ER alpha immunopositive cell numbers per testis section were markedly increased in the 50 microg/ml bisphenol A-treated group compared with the control and the 0.5 microg/ml bisphenol A-treated group. ER beta mRNA expression was significantly decreased in the 50 microg/ml bisphenol A-treated group compared with the control and the 0.5 microg/ml bisphenol A-treated group. In contrast, ER alpha mRNA expression was markedly increased in the 50 microg/ml bisphenol A-treated group compared with the control and the 0.5 microg/ml bisphenol A-treated group. The existence of ER alpha and beta in the testis suggests that estrogens directly affect germ cells during testicular development and spermatogenesis, and differential modulation of ER alpha and beta in the testis could be involved in the effects of bisphenol A.

Prenatal exposure to estrogenic compounds alters the expression pattern of platelet-derived growth factor receptors alpha and beta in neonatal rat testis: identification of gonocytes as targets of estrogen exposure.
Thuillier R, Wang Y, and Culty M.
Biol Reprod 2003; 68(3):867-80.

We examined the effects of maternal exposure to estrogens on platelet-derived growth factor (PDGF) receptor (PDGFR) expression in newborn rat testis. Pregnant rats were treated from gestation Day 14 to birth with corn oil containing diethylstilbestrol, bisphenol A, genistein, or coumestrol by gavage or subcutaneous injection. These treatments induced a dose-dependent increase in the expression of PDGFR alpha and beta mRNAs, determined by semiquantitative reverse transcription polymerase chain reaction, though diethylstilbestrol had a biphasic effect on both mRNAs. In situ hybridization analysis showed that PDGFRalpha mRNA increased mostly in the interstitium, while PDGFRbeta mRNA increased both in the interstitium and seminiferous cords. Immunohistochemical studies of PDGFRalpha and beta proteins revealed that both receptors were present in testis before and after birth and that they were upregulated upon treatment with estrogens in 3-day-old rats, with PDGFRbeta increasing dramatically in gonocytes. PDGFRalpha and beta mRNAs and proteins were also found in purified gonocytes. Our previous finding that PDGF and 17beta-estradiol induce gonocyte proliferation in vitro, together with the present finding that in vivo exposure to estrogens upregulates PDGF receptors in testis, suggest that PDGF pathway is a target of estrogens in testis. In addition, these data identify PDGFRbeta in gonocytes as a major target of gestational estrogen exposure, suggesting that estrogen may have a physiological interaction with PDGF during gonocyte development. These results, however, do not exclude the possibility that the effects of the compounds examined in this study might be due to estrogen receptor-independent action(s).

The influence of dietary isoflavone on the uterotrophic response in juvenile rats.
Wade, M. G., Lee, A., McMahon, A., Cooke, G., and Curran, I.
Food Chem Toxicol. 2003 Nov; 41(11):1517-25.

Abstract: Current in vivo methods to identify and assess reproductive hazards of endocrine disrupting substances are often confounded by the presence of isoflavones (genistein, diadzein, glycitein), strongly hormonally-active substances, in the diet of laboratory rodents. However, studies that have attempted to study the influence of dietary isoflavone on qualitative and quantitative uterotrophic responses have been limited by the few doses of isoflavone tested, stress to the animals due to changing of the diet immediately prior to testing and/or comparing effects of diets of very different composition. The current study examined the effects of isoflavone on uterotrophic response by using immature female rats reared from conception on diets varying only in the amount of isoflavone concentrate (Novasoy) added to a virtually isoflavone-free soya-based diet. The effects of these diets, and a soya-free semipurified diet (AIN 93G) on uterotrophic responses to treatment with a strong (Ethinyl Estradiol, EE) or a weak (bisphenol A, BPA) estrogenic substance were examined. The pups were treated with subcutaneous injections of either EE (1 microg/kg/day), BPA (600 mg/kg/day) or corn oil

(vehicle) control for 3 days starting at weaning on post natal day (PND) 21. On the morning of PND 24 pups were sacrificed and uterus weight, epithelium labeling index (Bromo deoxyuridine incorporation), uterine epithelium thickness, and peroxidase activity were determined. Diet did not influence unstimulated uterine weight, epithelial height or peroxidase activity except at the highest isoflavone diet where animals had significantly increases in all three endpoints. Uterine weight, epithelial thickness and peroxidase were all significantly increased by EE or BPA treatment. There was no evidence of diet-induced potentiation or inhibition of the stimulatory actions of either EE or BPA on either uterine weight or epithelial thickness while EE-induced increase in uterine peroxidase activity was increased synergistically by the highest dose of isoflavone. A similar response to the latter effect was seen in BPA treated animals although this response was not significantly different from that of BPA treated rats fed the isoflavone-free soy diet. The rate of endometrial epithelium labeling with BrdU was not altered by any treatment. These results indicate that dietary isoflavone content can directly influence uterine weight and other estrogen-dependent endpoints demonstrating the potential of these to reduce the active range of the uterotrophic assay. However, there is no indication that isoflavones impair or potentiate the stimulatory action of either strong (EE) or weaker (BPA) estrogen agonists on uterine weight or epithelial morphology although the data do suggest the potential for synergy between high isoflavone content and estrogen agonist in inducing uterine peroxidase.

Evaluation of hazardous effects of chemicals by transcriptome analysis.
Watanabe, H.
Congenit Anom Kyoto. 2003 Sep; 43(3):216.

Abstract: The chemicals that affect reproduction have been reported to have hazardous effects at much lower concentrations than the doses causing acute toxicity. These chemicals are called endocrine disruptors and are considered to mimic or disturb the action of the endocrine systems. Many endocrine disruptors, have been reported to have estrogenic activities. The estrogenic effects of DDT and nonylphenol have been demonstrated not only in laboratories but also in the field. Nonylphenol and Bisphenol A are among the chemicals considered endocrine disruptors and known to have weak estrogenic activity. They have long been considered weak estrogens, but weak estrogenic activity alone is not sufficient to explain the various effects of these chemicals. In order to examine identity and differences between weak estrogens and estradiol, we examined changes in gene expression caused by these chemicals compared with estradiol. Dose-dependent gene activation profiling revealed that a subset of genes were not affected by estrogen but by weak estrogens. This result suggests the presence of a gene-activation mechanism specific to weak estrogens, which is different from activation by estrogen. An analysis of their gene functions and understanding of activation mechanisms will facilitate understanding of the complex effect of endocrine disruptors.

Biotransformations of bisphenol A in a mammalian model: answers and new questions raised by low-dose metabolic fate studies in pregnant CD1 mice.
Zalko, D., Soto, A. M., Dolo, L., Dorio, C., Rathahao, E., Debrauwer, L., Faure, R., and Cravedi, J. P.
Environ Health Perspect. 2003 Mar; 111(3):309-19.

Abstract: We investigated the metabolic fate of a low dose (25 micro g/kg) of bisphenol A [2,2-bis(4-hydroxy-phenyl)propane] (BPA) injected subcutaneously in CD1 pregnant mice using a tritium-labeled molecule. Analytic methods were developed to allow a radio-chromatographic profiling of BPA residues in excreta and tissues, as well as in mothers' reproductive tracts and fetuses, that contained more than 4% of the administered radioactivity. BPA was extensively metabolized by CD1 mice. Identified metabolite structures included the glucuronic acid conjugate of BPA, several double conjugates, and conjugated methoxylated compounds, demonstrating the formation of potentially reactive intermediates. Fetal radioactivity was associated with unchanged BPA, BPA glucuronide, and a disaccharide conjugate. The latter structure, as well as that of a dehydrated glucuronide conjugate of BPA (a major metabolite isolated from the digestive tract), showed that BPA metabolic routes were far more complex than previously thought. The estrogenicity of the metabolites that were identified but not tested for hormonal activity cannot be ruled out; however, in general, conjugated BPA metabolites have significantly lower potency than that of the parent compound. Thus, these data suggest the parental compound is responsible for the estrogenic effects observed in fetuses exposed to BPA during gestation in this mammalian model.

Exposure to the estrogenic pollutant bisphenol A affects pain behavior induced by subcutaneous formalin injection in male and female rats.
Aloisi AM, Della Seta D, Rendo C, Ceccarelli I, Scaramuzzino A, and Farabollini F.
Brain Res 2002;937(1-2):1-7.

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

[Study on the developmental toxicity of Bisphenol A by using micromass culture in vitro].
Li Y, Long D, and Pei X.
Wei Sheng Yan Jiu 2002;31(3):178-9, 183.

The micromass culture of Wistar rat embryo limb bud cells was used to investigate the characteristic of developmental toxicity of Bisphenol A (BPA) and its mechanism in vitro. The results showed that BPA inhibited both proliferation and differentiation of rat limb bud cells in vitro. The high level of BPA appeared to be cytotoxic to Wistar rat embryo limb bud cells in culture and inhibited the clone formation with dose-response relationship. The concentration of BPA for IP50 and ID50 were 38.74 mg/L and 27.93 mg/L respectively. According to Renaults' teratogenic criteria, BPA belonged to a positive teratogen. And the data suggested that the specific inhibition of cell proliferation and differentiation might be one of the mechanisms of high level BPA.

Gene expression profile induced by 17alpha-ethynyl estradiol, bisphenol A, and genistein in the developing female reproductive system of the rat.
Naciff JM, Jump ML, Torontali SM, Carr GJ, Tiesman JP, Overmann GJ, and Daston GP.
Toxicol Sci 2002;68(1):184-99.

Exposure to some compounds with estrogenic activity, during fetal development, has been shown to alter development of reproductive organs, leading to abnormal function and disease either after birth or during adulthood. In order to understand the molecular events associated with the estrogenicity of different chemicals and to determine whether common sets of gene expression changes can be predictive of estrogenic activity, we have used microarray technology to determine the transcriptional program influenced by exposure to this class of compounds during organogenesis and development. Changes in patterns of gene expression were determined in the developing uterus and ovaries of Sprague-Dawley rats on GD 20, exposed to graded dosages (sc) of 17alpha-ethynyl estradiol (EE), genistein, or bisphenol A (BPA) from GD 11 to GD 20. Dose levels were roughly equipotent in estrogenic activity. We compared the transcript profiles between treatment groups and controls, using oligonucleotide arrays to determine the expression level of approximately 7000 rat genes and over 1000 expressed squence tags (ESTs). At the highest tested doses of EE, BPA, or genistein, we determined that less than 2% of the mRNA detected by the array showed a 2-fold or greater change in their expression level (increase or decrease). A dose-dependent analysis of the transcript profile revealed a common set of genes whose expression is significantly and reproducibly modified in the same way by each of the 3 chemicals tested. Additionally, each compound induces changes in the expression of other transcripts that are not in common with the others, which indicated not all compounds with estrogenic activity act alike. The results of this study demonstrate that transplacental exposure to chemicals with estrogenic activity changes the gene expression profile of estrogen-sensitive

tissues, and that the analysis of the transcript profile of these tissues could be a valuable approach to determining the estrogenicity of different compounds.

Maternal-fetal disposition of bisphenol a in pregnant Sprague-Dawley rats.
Shin BS, Yoo SD, Cho CY, Jung JH, Lee BM, Kim JH, Lee KC, Han SY, Kim HS, and Park KL.
J Toxicol Environ Health A 2002;65(5-6):395-406.

This study describes the maternal-fetal disposition of bisphenol A and its distribution into the placenta and amniotic fluid after iv injection (2 mg/kg) to pregnant Sprague-Dawley rats. Bisphenol A was distributed extensively to the placenta and fetus, with their respective AUC values 4.4- and 2.2-fold greater than AUC for the maternal serum. In contrast, the distribution of bisphenol A into the amniotic fluid was low, with the mean amniotic fluid-to-maternal serum AUC ratio of 0.2. The decay curves of bisphenol A in the placenta, fetus, and amniotic fluid paralleled that of the maternal serum during the terminal elimination phase. A five-compartment open model consisting of the maternal central, maternal peripheral, placental, fetal, and amniotic fluid compartments was used to describe the disposition of bisphenol A in pregnant rats, with the elimination occurring from the maternal central and fetal compartments. Based on this model, bisphenol A delivered to the placenta was transferred primarily to the fetus [kpf/(kpf + kpc + kpa) = 65.4 %], with the remaining fraction transported to the maternal central (33.2%) and amniotic fluid (1.4%) compartments. Bisphenol A was eliminated from the amniotic fluid by the fetal (63.9%) and placental (36.1%) routes. On the other hand, bisphenol A was eliminated from the fetus primarily by the placental route back to mother [kfp/(kfp + kfa + kfo) = 100%], with the amniotic route playing an insignificant role in fetal elimination. The percent contribution of the fetal elimination to the total elimination in the maternal-fetal unit was 0.0% [CLfoAUCfetus/(CLcoAUCmaternal serum + CLfoAUCfetus)]. The pharmacokinetic model used in this study provides insights into the routes of elimination of bisphenol A in the maternal-fetal rat upon maternal administration.

Developmental effects of perinatal exposure to bisphenol-A and diethylstilbestrol on reproductive organs in female mice.
Suzuki A, Sugihara A, Uchida K, Sato T, Ohta Y, Katsu Y, Watanabe H, and Iguchi T.
Reprod Toxicol 2002;16(2):107-16.

Reproductive tract development is influenced by estrogen. The aim of this study was to determine the effects of an environmental estrogenic chemical bisphenol-A (BPA) on prenatal and postnatal development of female mouse reproductive organs. In the prenatal treatment group, BPA or the synthetic estrogen diethylstilbestrol (DES) were given by subcutaneous (s.c.) injections to pregnant mice during gestational days 10-18. Some offspring treated prenatally with 10 and 100 mg/kg bw BPA or 0.67 and 67 microg/kg bw DES were ovariectomized at 30 days and sacrificed at 40 days of age. Vaginal smears were examined in the remaining offspring, then these offspring were mated with normal males. Prenatal exposure to 10 mg/kg BPA reduced the

number of mice with corpora lutea compared to sesame oil controls at 30 days, but more than 80% of mice from either prenatally exposed BPA group were fertile at 90 days. Mice exposed prenatally to maternal doses of 67 microg/kg DES were sterile and showed ovary-independent vaginal and uterine epithelial stratification; however, mice exposed prenatally to BPA did not show ovary-independent vaginal and uterine changes. The number of offspring and litter sex ratio from mice exposed prenatally to BPA (10 or 100 mg/kg) or 0.67 microg/kg DES were not different compared to controls.In postnatal treatment group, female mice were given s.c. injections of BPA (15 or 150 microg/pup) or DES (0.3 or 3 microg/pup) for 5 days from the day of birth, then some mice were ovariectomized at 30 days and examined at 40 and 90 days. In the remaining mice, vaginal smears were examined from 61 to 90 days and ovarian histology was evaluated at 90 days. Mice exposed postnatally to 150 microg BPA exhibited ovary-independent vaginal epithelial stratification. Postnatal DES (0.3 and 3 microg) treatment also induced ovary-independent vaginal stratification. Polyovular follicles having more than one oocyte in a follicle were induced by postnatal injections of BPA (150 microg) or DES (0.3 or 3 microg) at 30 days. These findings indicate for the first time that a large dose of BPA can induce ovary-independent vaginal epithelial changes when given postnatally but not prenatally.

Dietary bisphenol A prevents ovarian degeneration and bone loss in female mice lacking the aromatase gene (Cyp19).
Toda K, Miyaura C, Okada T, and Shizuta Y.
Eur J Biochem 2002;269(8):2214-22.

We previously generated mice lacking aromatase activity by targeted disruption of Cyp19 (ArKO mice), and reported phenotypes of the female mice, showing hemorrhage formation and follicular depletion in the ovary, diminution in uterine size, and bone loss. In the present study, we examined the influence of dietary bisphenol A (BPA), a monomer used for the production of polycarbonate and known to have estrogenic activity, on these phenotypes of the ArKO mice. When ArKO mice were fed chow diets supplemented with 0.1% or 1% (w/w) BPA for 5 months, they were protected from ovarian degeneration, uterine diminution and bone loss in a dose-dependent manner. Northern blot analyses of ovarian RNA of ArKO mice showed differences in the expression levels of insulin-like growth factor (IGF)-I, IGF-I receptor, growth differentiation factor 9 and bone morphogenetic protein 15 as compared with those in the ovaries of wild-type mice. The differences in the expression levels were restored by dietary BPA. In the ArKO uteri, expression of progesterone receptor and vascular endothelial growth factor mRNAs was diminished, and was restored by BPA to the levels in wild-type mice. In contrast, BPA had little effect on the ovarian, uterine and skeletal structures of wild-type mice. In conclusion, estrogenic effects of BPA on the reproductive tract as well as skeletal tissue were evident in adult female ArKO mice. These results suggest that the ArKO mouse is an animal model suitable for studying effects of estrogenic chemicals as well as estrogen in vivo.

Bisphenol-A administration during pregnancy results in fetal exposure in mice and monkeys.
Uchida K, Suzuki A, Kobayashi Y, Buchanan DL, Sato T, Watanabe H, Katsu Y, Suzuki J, Asaoka K, Mori C and others.
Journal of Health Science 2002;48(6):579-82.

Placental transfer of bisphenol-A (BPA) was studied in mice and Japanese monkeys (Macaca fuscata). BPA was found in maternal and fetal sera, liver, brain, uteri, testes and placenta as early as 30 min after a single subcutaneous (s.c.) injection to 17 days of pregnancy in mice. BPA was also found in fetal liver, kidney, and brain of Japanese monkeys 1 hr after a single s.c. injection to 150 days of pregnancy. These results clearly indicate that the maternal placental barrier can not protect the fetus from the consequences of BPA exposure in these species.

Bisphenol A induces apoptosis and G2-to-M arrest of ovarian granulosa cells.
Xu J, Osuga Y, Yano T, Morita Y, Tang X, Fujiwara T, Takai Y, Matsumi H, Koga K, Taketani Y, and Tsutsumi O.
Biochemical & Biophysical Research Communications 2002;292(2):456-462.

We investigated the impact of bisphenol A (BPA) on murine ovarian granulosa cells. Ovarian granulosa cells were cultured with 100 fM to 100 muM BPA for 24 h to 72 h. BPA decreased granulosa cell viability in a dose- and time-dependent manner. The lowest concentration that induced a significant decrease was 100 pM (89.2+-4.0% of the control). TUNEL analysis demonstrated that treatment with BPA increased apoptosis of granulosa cells in a dose- and time-dependent manner. In addition, flow cytometry analyses revealed that treatment with BPA resulted in G2-to-M arrest, which was most prominent at 48 h. BPA increased the expression of Bax and concomitantly decreased the expression of Bcl2 at both protein and mRNA levels of granulosa cells. These findings suggest that low, presumably environmentally relevant doses of BPA, decrease the viability of granulosa cells by inducing apoptosis and G2-to-M arrest. Up-regulation of Bax and down-regulation of Bcl2 were suggested to be involved in this apoptotic effect.

Bisphenol-A differently affects estrogen receptors-alpha in estrous-cycling and lactating female rats.
Aloisi AM, Della Seta D, Ceccarelli I, and Farabollini F.
Neurosci Lett 2001;310(1):49-52.

The effect of long-term exposure to bisphenol-A (BPA) on estrogen receptor-alpha (ER) immunoreactivity was studied in the medial preoptic area, arcuate nucleus and the ventromedial nucleus of the hypothalamus of estrous cycling and lactating female rats. Pregnant/lactating or estrous cycling rats were exposed to BPA (40 mg/Kg/day) or peanut oil. Lactating females showed fewer ER-immunoreactive cells than non-lactating females in the medial preoptic area and ventromedial nucleus of the hypothalamus. BPA induced an increase in ER-immunoreactive

cells in the medial preoptic nucleus irrespective of lactation. BPA only induced a decrease in ER-immunoreactive cells in the arcuate nucleus of the lactating group; oil induced an increase in ER-immunoreactive cells in the lactating with respect to non-lactating group. The results demonstrate that exposure of adult females to BPA modifies the number of ERs.

Exposure to bisphenol A during the fetal and suckling periods disrupts sexual differentiation of the locus coeruleus and of behavior in the rat.
Kubo K, Arai O, Ogata R, Omura M, Hori T, and Aou S.
Neurosci Lett 2001;304(1-2):73-6.

This study tested the effect of exposure to bisphenol A (BPA) early in life on the sexual differentiation in the brain and behavior in Wistar rats. We administered BPA only to mother rats during pregnancy and lactation at a dosage of approximately 1.5 mg/kg per day far less than the no-observed-adverse-effect level (NOAEL; 50 mg/kg per day). Control female offspring showed a higher activity, a lower avoidance memory, and larger locus coeruleus than the male controls, while the BPA-exposed group did not show any sexual dimorphism. BPA did not affect the reproductive organs or sex hormones. Our results suggest that the current methods to determine the NOAEL of artificial industrial chemicals may not be sufficient to detect a disruption of the sexual differentiation in the brain.

Perinatal exposure to low doses of bisphenol A affects body weight, patterns of estrous cyclicity, and plasma LH levels.
Rubin BS, Murray MK, Damassa DA, King JC, and Soto AM.
Environ Health Perspect 2001;109(7):675-80.

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 17beta-estradiol--current environmental exposure levels have been considered orders of magnitude below the dose required for adverse effects on health. 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. 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 uterotropic response in ovariectomized postpubertal females. 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.

Bisphenol A inhibits testicular functions and increases luteinizing hormone secretion in adult male rats.
Tohei A, Suda S, Taya K, Hashimoto T, and Kogo H.
Exp Biol Med (Maywood) 2001;226(3):216-21.

Effects of a xenobiotic estrogen, bisphenol A (BPA), on reproductive functions were investigated using adult male rats. BPA was dissolved into sesame oil and injected s.c. every day (1 mg/rat) for 14 days. Animals were killed by decapitation after the final administration of BPA, and the trunk blood, pituitary, and testes were collected. Plasma concentrations of prolactin were dramatically increased and pituitary contents of prolactin were slightly increased in the BPA group compared to the control group. Plasma concentrations of testosterone were decreased and plasma concentrations of LH were increased in BPA-treated rats compared to control rats. Testicular contents of inhibin were decreased in BPA-treated rats compared to control rats, although plasma concentrations of inhibin were not changed after administration of BPA. The testicular response to hCG for progesterone and testosterone release was decreased in BPA-treated rats. Administration of BPA did not change the pituitary response to luteinizing hormone-releasing hormone (LH-RH) in castrated male rats treated with testosterone. Male sexual behavior also was not changed as a result of BPA treatment. These results suggest that BPA directly inhibits testicular functions and the increased level of plasma LH is probably due to a reduction in the negative feedback regulation by testosterone. The testis is probably a more sensitive site for BPA action than the hypothalamus-pituitary axis.

Comparative effects of neonatal exposure of male rats to potent and weak (environmental) estrogens on spermatogenesis at puberty and the relationship to adult testis size and fertility: evidence for stimulatory effects of low estrogen levels.
Atanassova N, McKinnell C, Turner KJ, Walker M, Fisher JS, Morley M, Millar MR, Groome NP, and Sharpe RM.
Endocrinology 2000;141(10):3898-3907.

This study investigated whether neonatal exposure of male rats to oestrogenic compounds altered pubertal spermatogenesis (days 18 and 25) and whether the changes observed resulted in long-term changes in testis size, mating, or fertility (days 90-100). Rats were treated neonatally with a range of doses (0.01-10 µg) of diethylstilbestrol (DES; administered on alternate days from days 2-12), a high dose of octylphenol (OP; 2 mg administered daily from days 2-12) or bisphenol A (Bis-A; 0.5 mg administered daily from days 2-12), or vehicle, while maintained on a standard soy-containing diet. The effect on the same parameters of rearing control animals on a soy-free diet was also assessed as was the effect of administering such animals genistein (4 mg/kg/day daily from days 2-18). Testis weight, seminiferous tubule lumen formation, the germ cell apoptotic index (apoptotic/viable germ cell nuclear volume), and spermatocyte nuclear volume per unit Sertoli cell nuclear volume were used to characterize pubertal spermatogenesis. Compared with (soy-fed) controls, DES administration caused dose-dependent retardation of

pubertal spermatogenesis on day 18, as evidenced by decreases in testis weight, lumen formation, and spermatocyte nuclear volume per unit Sertoli cell and elevation of the germ cell apoptotic index. However, the two lowest doses of DES (0.1 and 0.01 µg) significantly increased spermatocyte nuclear volume per unit Sertoli cell. Similarly, treatment with either OP or Bis-A significantly advanced this and some of the other aspects of pubertal spermatogenesis. Maintenance of control animals on a soy-free diet also significantly advanced lumen formation and spermatocyte nuclear volume per unit Sertoli cell compared with controls fed a soy-containing diet. Administration of genistein reversed the stimulatory effects of a soy-free diet and significantly retarded most measures of pubertal spermatogenesis. In general, plasma FSH levels in the treatment groups changed in parallel to the spermatogenic changes (reduced when pubertal spermatogenesis retarded, increased when pubertal spermatogenesis advanced). By day 25, although the changes in FSH levels largely persisted, all of the stimulatory effects on spermatogenesis seen on day 18 in the various treatment groups were no longer evident. In adulthood, testis weight was decreased dose dependently in rats treated neonatally with DES, but only the lowest dose group (0.01 µg) showed evidence of mating (3 of 6) and normal fertility (3 litters). Animals treated neonatally with OP or Bis-A had normal or increased (Bis-A) testis weights and exhibited reasonably normal mating/fertility. Animals fed a soy-free diet had significantly larger testes than controls fed a soy-containing diet, and this difference was confirmed in a much larger study of more than 24 litters, which also showed a significant decrease in plasma FSH levels and a significant increase in body weight in the males kept on a soy-free diet. Neonatal treatment with genistein did not alter adult testis weight, and although most males exhibited normal mating and fertility, a minority did not mate or were infertile. It is concluded that (1) neonatal exposure of rats to low levels of oestrogens can advance the first wave of spermatogenesis at puberty, although it is unclear whether this is due to direct effects of the oestrogen or to associated elevation of FSH levels; (2) the effect of high doses of OP and Bis-A on these processes is essentially benign; and (3) the presence or absence of soy or genistein in the diet has significant short-term (pubertal spermatogenesis) and long-term (body weight, testis size, FSH levels, and possibly mating) effects on males.

Assessment of estrogenicity by using the delayed implanting rat model and examples.
Cummings AM and Laws SC.
Reprod Toxicol 2000;14(2):111-7.

Endocrine disrupting chemicals have recently drawn increased interest. The delayed implanting rat model is a method that can identify and quantify the estrogenic activity of a chemical. In rats hypophysectomized after breeding, the administration of progesterone delays embryo implantation, and exposure to one dose of an estrogenic substance initiates implantation. Although methoxychlor was ineffective at dosages below 400 mg/kg when given by injection, the administration of the chemical by gavage resulted in an increase in the percent of fertilized rats exhibiting implantation sites. These results were statistically significant at dosages of 50, 100, 200, and 300 mg methoxychlor/kg. When bisphenol A was administered, by subcutaneous injection, dosages of 50, 100, and 200 mg/kg induced implantation. Only the 400 mg/kg dose of 4-tert-octylphenol was effective. Doses of beta-sitosterol up to 30 mg/kg failed to initiate

implantation. These data confirm previous evidence of the availability of this model for evaluating estrogenic activity and provide estimates of the estrogenic potencies of several environmentally important chemicals.

Developmental exposure to bisphenol A: Interaction with endogenous estradiol during pregnancy in mice.
Howdeshell KLA and vom Sal FS.
American Zoologist 2000;40(3):429-437.

Individual variability in endogenous hormones can confound the interpretation of effects of developmental exposure to endocrine disrupting chemicals. In single-birth species, such as humans, there are many sources of variability in fetal sex hormone levels, such as birth order or race. In litter-bearing species a source of fetal variability in serum levels of estradiol and testosterone is the sex of adjacent fetuses due to fetus-to-fetus steroid transport (called the intrauterine position phenomenon or IUP). Distinct phenotypes of reproductive physiology and behavior are due to IUP in house mice and other litter-bearing animals. We review here the effects of background levels of sex steroids in fetuses due to IUP in an experiment in which pregnant mice were exposed to an environmentally relevant low dose of the estrogen-mimicking chemical, bisphenol A. Bisphenol A is the monomer used to make polycarbonate plastic products (such as baby bottles), the resin lining of food and beverage cans, dental sealants, and a host of other products. Fetal exposure via the mother to bisphenol A increased the rate of postnatal growth in males and females and also advanced the timing of puberty in females. However, the greatest response to bisphenol A occurred in males and females with the highest background levels of endogenous estradiol during fetal life due to their IUP, while fetuses with the lowest endogenous levels of estradiol showed no response to maternal bisphenol A treatment. This finding suggests that estrogen-mimicking chemicals interact with endogenous estrogen in altering the course of development.

Estrogenic alkylphenols induce cell death by inhibiting testis endoplasmic reticulum Ca2+ pumps.
Hughes PJ, McLellan H, Lowes DA, Khan SZ, Bilmen JG, Tovey SC, Godfrey RE, Michell RH, Kirk CJ, and Michelangeli F.
Biochemical & Biophysical Research Communications 2000;277(3):568-574.

Industrial alkylphenols in the environment may act as "xenoestrogens" to disrupt testicular development and decrease male fertility. Amongst possible targets for these compounds are testicular Sertoli cells, which nurture the developing sperm cells. We demonstrate that SERCA 2 and 3 Ca2+ pumps are relatively abundant in rat testis microsomal membranes, and also in Sertoli, myoid, and TM4 cells (a Sertoli cell line). A number of estrogenic alkylphenols such as nonylphenol, octylphenol, bisphenol A, and butylated hydroxytoluene all inhibit testicular Ca2+ ATPase in the low micromolar concentration range. These agents also mobilize intracellular Ca2+ in intact TM4 cells in a manner consistent with the inhibition of ER Ca2+ pumps.

Alkylphenols dramatically decrease the viability of TM4 cells, an effect that is reversed by either a caspase inhibitor or by BAPTA, and is therefore consistent with Ca2+-dependent cell death via apoptosis. We postulate that alkylphenols disrupt testicular development by inhibiting ER Ca2+ pumps, thus disturbing testicular Ca2+ homeostasis.

Disposition of orally administered 2,2-Bis(4-hydroxyphenyl)propane (Bisphenol A) in pregnant rats and the placental transfer to fetuses.
Takahashi O and Oishi S.
Environ Health Perspect 2000;108(10):931-5.

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. BPA in maternal blood, liver, and kidney reached maximal concentrations (14.7, 171, and 36 microg/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 microg/g) was also attained 20 min after the administration. BPA levels then gradually reduced in a similar manner to maternal blood. 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.

Estrogen receptor-mediated effects of a xenoestrogen, bisphenol A, on preimplantation mouse embryos.
Takai Y, Tsutsumi O, Ikezuki Y, Hiroi H, Osuga Y, Momoeda M, Yano T, and Taketani Y.
Biochem Biophys Res Commun 2000;270(3):918-21.

The effects of bisphenol A, a xenoestrogen widely used in industry and dentistry, were studied in early preimplantation mouse embryos. Two-cell mouse embryos were cultured with 100 pM to 100 microM bisphenol A with or without 100 nM tamoxifen and evaluated at 24-h intervals for their development to eight-cell and blastocyst stages. At 72 h, blastocysts were cultured for another 48 h without bisphenol A, and surface areas of trophoblast spread were measured. At 24 h, more embryos exposed to 3 nM bisphenol A than to controls had reached the eight-cell stage. At 48 h, more embryos exposed to 1 nM and 3 nM bisphenol A than to controls had become blastocysts. At 100 microM, bisphenol A decreased frequency of development to blastocysts. Tamoxifen counteracted both stimulatory and inhibitory effects of bisphenol A on blastocyst formation. Although bisphenol A did not alter blastocyst morphology or cell number, early exposure to 100 microM bisphenol A increased subsequent trophoblast areas. These findings suggest that bisphenol A may not only effect early embryonic development via estrogen receptors even at low, environmentally relevant doses, but also exert some late effects on subsequent development of these embryos.

Effect of neonatal exposure to estrogenic compounds on development of the excurrent ducts of the rat testis through puberty to adulthood.
Fisher JS, Turner KJ, Brown D, and Sharpe RM.
Environ Health Perspect 1999;107(5):397-405.

Neonatal exposure to diethylstilbestrol (DES) can alter the structure of the testicular excurrent ducts in rats. We characterized these changes according to dose and time posttreatment and established whether potent estrogens (ethinyl estradiol), environmental estrogens (genistein, octylphenol, bisphenol A, parabens), and tamoxifen induce such changes. Rats were administered these compounds neonatally and assessed at several time points during (day 10, or day 18 for some treatments) and after (days 18, 25, 35, and 75) the treatment period to detect any changes in testis weight, distension of the rete testis and efferent ducts, epithelial cell height in the efferent ducts, and immunoexpression of the water channel aquaporin-1 (AQP-1). Treatment with DES (10, 1, or 0.1 microg/injection; equivalent to 0.37, 0.037, or 0.0037 mg/kg/day, respectively) induced dose-dependent changes in testis weight and all parameters. These effects were most pronounced at days 18 and 25 and appeared to lessen with time, although some persisted into adulthood. Neonatal treatment with ethinyl estradiol (10 microg/injection; equivalent to 0.37 mg/kg/day) caused changes broadly similar to those induced by 10 mg DES. Administration of tamoxifen (2 mg/kg/day) caused changes at 18 days that were similar to those induced by 1 microg DES. Treatment with genistein (4 mg/kg/day), octylphenol (2 mg/injection; equivalent to 150 mg/kg/day), or bisphenol A (0.5 mg/injection; equivalent to 37 mg/kg/day) caused minor but significant ($p<0.05$) decreases in epithelial cell height of the efferent ducts at days 18 and/or 25. In animals that were followed through to 35 days and/or adulthood, these changes were no longer obvious; other parameters were either unaffected or were affected only marginally and transiently. Administration of parabens (2 mg/kg/day) had no detectable effect on any parameter at day 18. To establish whether these effects of estrogens were direct or indirect (i.e., resulting from reduced follicle-stimulating hormone/luteinizing hormone secretion), the above end points were assessed in animals in which gonadotropin secretion was suppressed neonatally by administration of a gonadotropin-releasing hormone antagonist. This treatment permanently reduced testis weight, but did not affect any of the other end points, apart from a minor transient reduction in efferent duct epithelial cell height at 18 days. This study suggests that structural and functional (expression of AQP-1) development of the excurrent ducts is susceptible to impairment by neonatal estrogen exposure, probably as a consequence of direct effects. The magnitude and duration of adverse changes induced by treatment with a range of estrogenic compounds was broadly comparable to their estrogenic potencies reported from in vitro assays.

Environmental toxins: Exposure to bisphenol A advances puberty.
Howdeshell KL, Hotchkiss AK, Thayer KA, Vandenbergh JG, and Vom Saal FS.
Nature (London) 1999;401(6755):763-764.

Pregnant CF-1 mice were fed ether oil (vehicle) or bisphenol A dissolved in at a dose equiv. to that typically found in the environment (2.4 mg/kg), on days 11 to 17 of gestation. Prenatal

treatment with bisphenol A significantly reduced the no. of days between vaginal opening and first vaginal estrus, which is highly correlated with first postpubertal ovulation in 0M females but not in 2M females, based on anal. of covariance adjusted for body wt. at weaning.

Passage of bisphenol A into the fetus of the pregnant rat.
Miyakoda HA, Tabata MA, Onodera SA, and Takeda KRa.
Journal of Health Science 1999;45(6):318-323.

We examined whether orally administered bis-phenol A transfers from the maternal rat to the fetus. After oral dose of 10 mg/kg bisphenol A, it immediately appeared in maternal blood, and transferred into the fetuses. The concentration of bis-phenol A in both maternal blood plasma and fetuses peaked within 1 h after administration. The values were approximately 34 ppb and 11 ppb, respectively. At 3 h, the concentration of bisphenol A in maternal blood plasma had decreased to approximately 10% of the peak value. The 3-h decrease in fetuses was only about 40% of the peak, and by 24 h, the fetal concentration had increased again to the nearly 70% of the peak value. The results suggest that bisphenol A might easily pass through the placental barrier, unlike sex hormones such as estrogen.

Low-dose bioactivity of xenoestrogens in animals: fetal exposure to low doses of methoxychlor and other xenoestrogens increases adult prostate size in mice.
Welshons WV, Nagel SC, Thayer KA, Judy BM, and Vom Saal FS.
Toxicol Ind Health 1999;15(1-2):12-25.

The hormonal activity of natural estrogens is influenced by the degree to which they bind to serum proteins. In the pregnant female and in the fetus, greater than 99% of estradiol may be bound by serum binding proteins. Therefore, even though total serum levels of estradiol appear very high in fetuses, we have found that in rodent fetuses, there is a very low free concentration of estradiol (0.2 pg/ml). Naturally occurring variation in fetal serum estradiol predicts differences in numerous postnatal traits, including prostate size. In addition, when this low level of free estradiol was experimentally increased from 0.2 to 0.3 pg/ml during the last third of fetal life, treated male mice showed an increase in adult prostate weight. Fetal exposure to low doses of xenobiotic estrogens by feeding to pregnant females, including the compounds methoxychlor (20 and 2000 micrograms/kg body weight), DES (0.02 to 2 micrograms/kg body weight) and bisphenol A (2 and 20 micrograms/kg body weight), also led to increased prostate weight in adulthood. In contrast, fetal doses of natural estradiol and DES above the physiological range of estrogenic activity, and within a toxicological dose range, led to the opposite outcome, a reduction in subsequent adult prostate weight. This indicates that it may be impossible to assess endocrine-disrupting activities in response to low doses within a physiological range of activity by using high, toxic doses of xenoestrogens in testing procedures. We have developed approaches in vitro to predict the potential estrogenic bioactivity of compounds in the physiologically relevant range in animals and humans. We address the following factors in predicting the final observed endocrine-disrupting effect in the animal: (1) the intrinsic

estrogenic activity of a given molecule, (2) the effective free concentration determined by how the molecule is carried in serum, (3) partitioning between aqueous and lipid compartments in body and cell lipids, and (4) absorption and metabolism relative to the route of exposure. The studies and strategies we describe are important in developing criteria for a tiered testing system for the detection of estrogenic chemicals as well as endocrine-disrupting chemicals with different modes of action.

Differential follicle counts as a screen for chemically induced ovarian toxicity in mice: results from continuous breeding bioassays.
Bolon B, Bucci TJ, Warbritton AR, Chen JJ, Mattison DR, and Heindel JJ.
Fundam Appl Toxicol 1997 ;39(1):1-10.

Ovaries from National Toxicology Program Reproductive Assessment by Continuous Breeding (RACB) bioassays were used to directly compare differential ovarian follicle counts and reproductive performance for 15 chemicals. Ovaries of 10 animals per group from 16 studies in CD-1 mice and 1 study each in C3H and C57BL/6 mice were sectioned serially at 6 microm. Counts of small, growing, and antral follicles were obtained in every 10th section. For all follicle types, younger mice had more follicles than older mice, and CD-1 mice had more follicles than age-matched animals from either inbred strain. The in-life portion of the RACB protocols demonstrated that 9 of 15 chemicals altered reproductive outcome in one or both sexes of mice, with six agents affecting females (R. E. Morrissey et al., 1989, Fundam. Appl. Toxicol. 13, 747-777). Three of six female toxicants [2,2-bis(bromoethyl)-1,3-propanediol, BPD; ethylene glycol monomethyl ether, EGME; methoxyacetic acid, MAA] significantly decreased counts of small and/or growing follicles by 33 to 92% in CD-1 mice; EGME also reduced follicle counts in the other strains. Follicle counts were decreased in progeny of animals treated with EGME or its active metabolite, MAA. For BPD, reductions in follicle numbers were proportional to dose. In CD-1 mice, female toxicants di-N-hexyl phthalate, propantheline bromide, and tricresyl phosphate reduced reproductive performance but not follicle numbers. Counts were not affected by toxicants for which the susceptible sex could not be determined (bisphenol A, ethylene glycol, oxalic acid). Altered follicle counts without apparent reproductive impairment occurred in CD-1 mice at lower doses of BPD but were not observed for nontoxic chemicals. These data suggest that differential follicle counts (1) are a quantifiable endpoint of ovarian injury in conventional bioassays, and (2) in some instances, may provide a more sensitive indicator of female reproductive toxicity than fertility.

Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative in vivo bioactivity of the xenoestrogens bisphenol A and octylphenol.
Nagel SC, vom Saal FS, Thayer KA, Dhar MG, Boechler M, and Welshons WV.
Environ Health Perspect 1997;105(1):70-6.

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. 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. 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.

F. Titles only (abstracts not available)

Commercial animal feed: variability in estrogenic activity and effects on body weight in mice.
vom Saal, F. S., Richter, C. A., Mao, J., and Welshons, W. V.
Birth Defects Res A Clin Mol Teratol. 2005 Jul; 73(7):474-5.

Effects of bisphenol A on adult male mouse fertility. [Erratum to document cited in CA136:381603].
Al-Hiyasat AS, Darmani H, and Elbetieha AM.
European Journal of Oral Sciences 2003;111(6):547.

Support: final report, 3-generation reproductive toxicity evaluation of bisphenol A administered in the feed to CD (Sprague-Dawley) rats, w/ cover letter dated 102600.
Research Triangle Instititute..
2000; EPA/OTS Doc #89010000024; NTIS/OTS0558455-5; TSCATS/454162

Initial submission: letter from SPI to USEPA re: 3-generation reproductive toxicity evaluation of bisphenol A administered in the feed to CD (Sprague-Dawley) rats, dated 063099.
Research Triangle Instititute.
1999; EPA/OTS Doc #88990000192; NTIS/OTS0573933; TSCATS/453978

Support: evaluation of reproductive organ development in CF-1 mice following prenatal exposure to bisphenol A, with cover letter dated 10/14/1998.
MPI Research.
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George JD, Price CJ, Wolkowski-Tyl R, and Kimmel CA.
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