DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

Date: May 27, 2008

From: Division of Food Contact Notifications (DFCN)
Michelle L. Twaroski, Ph.D. (HFS-275)
Yan Gu, Ph.D. (HFS-275)

Subject: Acceptance of "Bisphenol A – Effects on onset of puberty in female and prostate and urinary tract in male rodents" reviewed by Drs. K. Barry Delclos (HFT-110) and Deborah K. Hansen (HFT-130) at FDA's National Center for Toxicological Research (NCTR).

To: Food Master File (FMF) 580 – Administrative Record

As part of the FDA Task Force on Bisphenol A (BPA, CAS RN 80-05-7), the Office of Food Additive Safety (OFAS) requested a review of several publications in the peer reviewed literature specific to the endpoints concluded to be of some concern in the National Toxicology Program's draft Brief on BPA[1]. These included the developmental endpoints of onset of puberty in females and prostate and urinary tract development in males. These studies were reviewed by Drs. K. Barry Delclos (HFT-110) and Deborah K. Hansen (HFT-130) at FDA's National Center for Toxicological Research (NCTR).

A few of the studies cited in the review were summarized as based on the literature; however, it is noteworthy that OFAS/DFCN has previously reviewed them as full study reports. The literature references and the full reviews are as follows:

- Tyl RW, Myers CB, Marr MC, Sloan CS, Castillo NP, Veselica MM, Seely JC, Dimond SS, Van Miller JP, Shiotsuka RN, Beyer D, Hentges SG, Waechter JM Jr (2008) Toxicol Sci. Two-generation reproductive toxicity study of dietary bisphenol A (BPA) in CD-1 (Swiss) mice. *Tox Sci* Advance Access published April 29, 2008. Complete study reviewed in: Shackelford/Twaroski, 05/25/2007: Review of Two-Generation Reproductive Toxicity Evaluation of Bisphenol A Administered in the Feed to CD-1® Swiss Mice

- 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, Waechter JM (2002) *Toxicol Sci.* Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats. 68(1): 121-146. Complete study reviewed in: Gu/Twaroski, 06/01/2007: Review of study entitled "Three-Generation Reproductive Toxicity Evaluation of Bisphenol A in the Feed of CD® (Sprague-Dawley) Rats".

- Nagel SC, vom Saal FS, Thayer KA, Dhar MG, Boechler M, Welshons WV (1997) *Environmental Health Perspectives.* Relative Binding Affinity-Serum Modified Access (RBA-SMA) Assay Predicts the Relative In Vivo Bioactivity of the Xenoestrogens Bisphenol A and Octylphenol. 105(1): 70-76. Previously reviewed in Sprando/Biddle, 02/04/1999.

The assessment of the literature has been reviewed by OFAS/DFCN toxicology and is

[1] Dated April 14, 2008; accessible at http://cerhr.niehs.nih.gov/chemicals/bisphenol/BPADraftBriefVF_04_14_08.pdf.

acceptable for the record. The secondary reviewers (M. Twaroski and Y. Gu) agree with the conclusions as stated by the NCTR reviewers.

M. Twaroski, Ph.D. Y. Gu, Ph.D.

[redacted]

Bisphenol A – Effects on onset of puberty in female and prostate and urinary tract in male rodents

Prepared for FDA/CFSAN

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Bisphenol A – Effects on onset of puberty in female and prostate and urinary tract in male rodents

Executive Summary

There are shortcomings in nearly all of the studies reviewed that limit their usefulness in a risk assessment. Some studies had only small numbers of animals tested, some used only 1 or 2 doses of BPA so a dose-response could not be determined, some used a non-oral route of administration which would have affected blood levels and embryonic exposures, and several lacked experimental details which would allow complete analysis of their reported results. Results from these studies may be more useful for hazard identification than for risk assessment purposes.

One of the most common weaknesses among these studies is a lack of a measure of internal exposure. A very small number of studies evaluated blood levels of BPA and/or its metabolites. This is particularly important for studies using other than oral administration. BPA is reported to be glucuronidated more quickly by the oral route than by other routes which bypass the liver and first pass metabolism. Plasma levels of free BPA would be different under these dosing scenarios, so embryonic exposure would be expected to be higher in studies using non-oral administration. Coupled with a decreased ability of fetuses and neonates to metabolize BPA, this would result in prolonged exposure to higher levels of BPA than would be present in studies using oral dosing. Such studies might be expected to demonstrate more adverse effects or to demonstrate effects at doses lower than adverse effects observed in oral dosing studies. An example of this may be a comparison of the age of vaginal opening in the studies of Honma *et al*. (2002) and Ashby *et al*. (1999) in which the same doses of BPA were administered during the same gestation period. Honma *et al*. observed an acceleration of vaginal opening at 20 μg/kg bw/day whereas Ashby *et al*. observed no effect at the same dose; Honma *et al*. used the subcutaneous route while Ashby *et al*. used the oral route. There were other differences in experimental design that may have contributed to the different observations (differences in mouse strains used, in environmental exposure, and in numbers of animals examined), but certainly the different routes of administration may have played a major role in the differing results.

In addition, effects have been reported after direct subcutaneous injections of neonates with low doses of BPA. Because of the relatively low glucuronidation capacity of neonates, it is not entirely clear that exposure resulting from the subcutaneous route is not similar to that resulting from direct oral ingestion at this specific stage of development. For example, persistent effects of exposures to doses as low as 10 to 100 μg /kg/day administered by subcutaneous injection to neonatal rodents in the first five days of life in both males (Ho *et al*., 2006) and females (Newbold *et al*., Reproductive Toxicol., **24**: 253-258, 2007) have been demonstrated. Normally in the first five days of life, rodents would be ingesting test compound only via the mother's milk in experiments that involve dosing of dams. The question of the level of exposure to the pups during the lactation period under the conditions of the rat and mouse multigeneration studies seems critical. There is evidence cited in the reports on these studies for transfer to milk, but no measurements of levels in pups under the conditions of the studies are available.

However, smaller studies that have often used non-traditional (for toxicology studies) dose routes and endpoints raise issues that may need to be addressed in a more rigorous fashion. These issues persist since several of the endpoints reported to be affected in these studies would

not be readily detectable in standard toxicology studies utilizing organ weights and histopathology on hematoxylin and eosin sections. This is particularly true for effects on male endpoints, since a variety of non-traditional endpoints have been reported to be adversely impacted by BPA. The brief reviews of the Ho *et al*., 2006 and Ogura *et al*., 2007 studies in the present document touch on the issue of effects in the prostate that would not be detected in standard assays. A major concern from the prostate studies seems to be sensitization to later hormonal stimulation rather than overt toxicity to the prostate, with only subtle treatment-related changes in gene expression control evident prior to hormonal challenge. This would not be picked up in a standard reproductive toxicity study. Additionally, collecting data for some of the endpoints is very labor-intensive and technically demanding; it would be extremely costly, if even possible, to collect such data under the conditions of a traditional guideline GLP study. This is true for the studies on female puberty. It has been argued that a better endpoint for the determination of puberty in the female rodent is the presence of cornified epithelial cells in the vaginal lavage rather than examining only the day of vaginal opening (Safranski *et al.*, Biol. Reprod., **48**:669-673, 1993). It is far less labor-intensive to examine the day of vaginal opening compared to looking at large numbers of slides for a determination of the day of first estrus.

Studies on the acceleration of puberty in female rodents

Three studies were judged to be useful in a risk assessment; these are the multigeneration studies by Tyl *et al.* (2002, 2008) and Ema *et al.*(2001). All three studies were conducted under GLP conditions and examined only the day of vaginal opening as the endpoint for determination of the onset of puberty in the female. Tyl *et al.* (2008) used mice; the other studies used rats.

The study by Ema *et al.* (2001) reported no effects on the day of vaginal opening at oral doses up to 200 μg/kg bw/day. Although the authors did not identify a NOAEL, it would appear that 200 μg/kg bw/day would be a NOAEL for the timing of female puberty; this was the maximum dose used in this study. The study by Tyl *et al.* (2002) reported a delay in vaginal opening at 7500 ppm (approximate intake of 500 mg/kg bw/day); this appeared to be due to a decrease in body weight. The authors identified a NOAEL for developmental and reproductive toxicity of 750 ppm (approximate intake of 50 mg/kg bw/day). The study by Tyl *et al.* (2008) used CD-1 mice and reported no effect on the day of vaginal opening at any dose, including the maximum dose of 3500 ppm (approximate intake of 600 mg/kg bw/day). The authors identified a NOAEL for reproductive and developmental toxicity of 300 ppm (approximate intake of 50 mg/kg bw/day) due to some adverse effects on male development at 3500 ppm. Due to the very thorough nature of these studies, one can have a high level of confidence in the results.

All of these doses are well above the normal range of human exposure (up to approximately 15 μg/kg bw/day – NTP Brief, 2008) and are also above the human occupational exposure range of up to 100 μg/kg bw/day (NTP Brief, 2008). Therefore, perinatal BPA exposure does not appear to accelerate vaginal opening in rodents at doses of 0.2 – 50 mg/kg bw/day.

Of the studies that were reviewed, only 3 mouse studies and 1 rat study used the day of first estrus as their endpoint for the onset of puberty. The rat study (Tinwell *et al.*, 2002) reported no effect at doses up to 50 mg/kg bw/day which is the same dose at which Tyl *et al.* (2002) reported no adverse effects using the day of vaginal opening as the endpoint. Tinwell *et*

al. (2002) did observe a delay in vaginal opening at 50 mg/kg bw/day in the Alderley Park strain of rats, but there was no effect on the day of first estrus at this dose. This suggests that the slight delay observed in the day of vaginal opening had no consequence on the subsequent attainment of estrus. The three mouse studies (Howdeshell *et al.*, 1999; Ryan and Vandenbergh, 2006 and Honma *et al.*, 2002) all observed acceleration of the day of first estrus at doses of 2.4, 20 or 200 µg/kg bw/day. All of these studies have weaknesses in their experimental designs which decrease the confidence in the results. Only Honma *et al.* (2002) evaluated the fertility of the animals demonstrating an acceleration in first estrus and found no effect on fertility. Although the multigeneration study by Tyl *et al.* (2008) did not evaluate the time of first estrus, they observed no adverse effects on fertility. Taken together, these results suggest that even if puberty were accelerated, it did not adversely affect the ability of the mice to reproduce. Other possible adverse effects of accelerated puberty have not been examined in these rodent studies.

Studies on altered prostate and urinary tract development in males

"Guideline" GLP studies using oral exposure (Tyl *et al*., 2002; Tyl *et al*., 2008) throughout the life span, including gestation and weaning, show no evidence of selective reproductive toxicity or effects on male development or prostate at doses below 750 ppm (approximate intake of 50 mg/kg bw/day) in the rat or 3500 ppm (approximate intake of 500 mg/kg bw/day) in the mouse. Although there were no effects on the prostate at this dose, there was evidence of adverse effects on other male reproductive tissues, including decreased testis weight and delays in preputial separation and testicular descent. Therefore, the authors identified the NOAEL for reproductive and developmental toxicity as 300 ppm (approximate intake of 50 mg/kg bw/day). A third such study (rat two generation reproductive study with Sprague-Dawley rats, Ema, *et al*., 2001 that was summarized in the section considering effects on puberty), likewise found no effect on prostate weight or histology at doses up to 200 µg /kg/day. These studies clearly contain datasets that are most useful in a risk assessment because of their size, comprehensive endpoint evaluation, rigorous attention to the certification of doses, and control of experimental conditions. The study of Tyl *et al.* (2008) is particularly important because it utilizes a strain of mouse that has been reported by others to be sensitive to BPA under different treatment conditions. These studies indicate that perinatal BPA exposure does not adversely affect prostate weight or histology at doses of 0.2 – 50 mg/kg bw/day. As discussed above, prostate weight and histology are somewhat crude endpoints, and more functional endpoints as examined in some of the smaller studies might uncover more subtle effects of BPA exposure.

There are conflicting results on the effects of BPA on the mouse prostate after oral dosing of dams during gestation only. Some studies report effects at doses between 2 and 50 µg /kg/day, while others show no effects at these dose levels using reportedly similar conditions or even at much higher dose levels. Levels of BPA reaching the fetus in this situation must be extremely low due to glucuronidation in the dam. As has been concluded by other review groups, there is no clear reason why the results of these studies differ, although the effects of environmental factors, including background diet estrogenic activity, animal strain and/or genetic background remain as possible contributory factors.

Studies on the acceleration of puberty in female rodents

Howdeshell KL, Hotchkiss AK, Thayer KA, Vandenbergh JG, vom Saal FS (1999) *Nature.* Exposure to bisphenol A advances puberty. 401(6755): 763-764.

The effect of prenatal bisphenol A (BPA) exposure on the age of puberty in female CF-1 mice was examined in a brief communication. Pregnant mice were given either oil vehicle or BPA at 2.4 μg/kg bw/day on GD 11 – 17 (day of vaginal plug not defined). On GD 19, pups were removed by cesarean section, and intrauterine position was determined (position near male/female pups). Pups were fostered to untreated mothers and were weaned on PND 22. Female pups were monitored for the day of vaginal opening and the day of first estrus. Data were analyzed on a litter basis.

Observations: Results were presented for all pups from each dose group (for body weight) or in relation to intrauterine position (for time between vaginal opening and first estrus). BPA significantly increased body weight at weaning. This effect was greater if the fetus was positioned next to other female fetuses *in utero*. There was no effect of BPA on the day of vaginal opening, but the period between vaginal opening and first estrus was accelerated by BPA only in female fetuses positioned next to other female fetuses *in utero*. The authors concluded that prenatal exposure to BPA altered postnatal growth and reproductive function in female mice, but that natural variation in endogenous hormone levels may influence the response to BPA.

Comments: These authors used the oral route of exposure and a large N of 21 per group. They observed both the time of vaginal opening and the day of first estrus. However, only a single dose of BPA was used so no dose-response could be determined. There was also a lack of experimental details including no description of animal care so it is unclear if there might have been exposure to environmental estrogens in either food, water or bedding. They were concerned about endogenous hormones affecting the endpoints but apparently were less concerned about uncontrolled environmental factors that might have affected these endpoints. Overall this report is of limited usefulness in determining a risk assessment.

Ryan BC, Vandenbergh JG (2006) *Horm Behav.* Developmental exposure to environmental estrogens alters anxiety and spatial memory in female mice. 50(1): 85-93.

The effects of perinatal exposure to BPA were evaluated using several behaviors and onset of puberty as endpoints in C57BL/6 mice. Mice were maintained in polycarbonate cages (that were checked frequently for condition) with chip bedding; however, estrogenicity of the food, water and bedding were not determined. Females were mated, and the day that a vaginal plug was identified was considered GD 1. Beginning on GD 3, dams were gavaged with BPA at 0 (tocopherol-stripped corn oil), 2 or 200 μg/kg bw/day, or ethinyl estradiol at 5 μg/kg bw/day. Animals were dosed daily from GD 3 to PND 21, when pups were weaned. Female mice were checked for vaginal opening, and vaginal smears were taken daily subsequently. Puberty was defined as the first day on which cornified cells were detected in the vaginal lavage.

Observations: Puberty was advanced by exposure to ethinyl estradiol and the high dose of BPA. The authors concluded that BPA and ethinyl estradiol accelerated puberty in female mice.

Comments: A positive control group, ethinyl estradiol, was included in this study. Weaknesses include the failure to determine estrogenicity of the animal environment; the chow used in this study (Purina rodent chow 5001) is known to have a high soy content. A particular weakness of the study is the small number of females examined in each group for the determination of the time of puberty (N = 4 - 7), and it was not described how these females were selected. There were apparently 16 litters in each treatment group, so far less than one female from each litter was examined for this endpoint. It also appears that the individual was used as the experimental unit for statistical analysis of this endpoint. Primarily due to the small numbers of animals examined for detection of puberty and the failure to describe how these animals were chosen, this report is of limited usefulness in determining a risk assessment.

Honma S, Suzuki A, Buchanan DL, Katsu Y, Watanabe H, Iguchi T (2002) *Reprod Toxicol.* Low dose effect of in utero exposure to bisphenol A and diethylstilbestrol on female mouse reproduction. 16(2): 117-122.

The effect of prenatal BPA exposure on the reproductive system of female ICR/Jcl mice was examined. Groups of 10 pregnant mice were injected subcutaneously with BPA in sesame oil at 0, 2 or 20 μg/kg bw/day on GD 11–17. Additional groups of mice were injected with diethylstilbestrol at 0.02, 0.2 or 2 μg/kg bw/day. At birth, pups were sexed, counted, and weighed, and litter size was adjusted to 8 pups. After weaning, females were monitored daily for vaginal opening and subsequently for the time of first estrus (defined as only cornified cells in the vaginal lavage). Female offspring were mated with untreated males from 90 to 120 days of age, and F_2 pups were counted and sexed at birth. The litter was considered the experimental unit in statistical analyses.

Observations: There were no adverse effects on pregnancy outcome. The age of vaginal opening (by about 1 day) and time of first estrus (by less than 1 day) were accelerated in the high dose group females, and body weight at vaginal opening was lower in both BPA dose groups. Among F_1 females that were mated, there were no significant effects on number of pups/litter or the sex ratio of F_2 pups. Females exposed to any of the 3 DES doses also demonstrated acceleration in the age at vaginal opening and age at first estrus with no effects on fertility. The authors concluded that prenatal exposure to low doses of BPA results in early vaginal opening in mice but did not affect female reproductive function.

Comments: This study included 3 doses of DES as a positive control also used low doses of BPA. Ten litters per group is adequate, and the litter was used as the experimental unit in the statistical analysis. However, there was no description of possible estrogen exposure in the food, water or bedding materials. Additionally, the subcutaneous route of exposure was used; this could lead to higher plasma levels of biologically active BPA. Blood levels of BPA and/or its metabolites were not determined in this paper. This paper is of limited utility in determining a risk assessment for BPA.

Ashby J, Tinwell H, Haseman J (1999) *Regul Toxicol Pharmacol.* Lack of effects for low dose levels of bisphenol A and diethylstilbestrol on the prostate gland of CF1 mice exposed in utero. 30(2 Pt 1): 156-166.

The effects of prenatal BPA exposure on the reproductive system of CF-1 mice was evaluated. Although the chow was not tested for estrogenicity, it was noted that the chow used during pregnancy and lactation contained 18.5% soy, and the chow used at all other times contained 6.5% soy. On GD 11–17, groups of 8 mice were dosed with BPA at 0 (tocopherol-stripped corn oil), 2 or 20 μg/kg bw/day (N=6); a positive control group (N=5) was included in the study design and was dosed with DES at 0.2 μg/kg bw/day. A naïve group of 5 dams was not weighed or dosed. The dosing solution was administered orally by being slowly expelled from a pipette placed in the animals' mouths and allowing them to lick the solution. All female offspring were checked daily for vaginal opening after weaning and were weighed at various intervals. Care was taken to reduce any stress to the animals and included administering test agents by drip feeding, minimal handling of pups, and minimal environmental noise. Data were analyzed using the litter as the experimental unit.

Observations: There were no adverse effects on pregnancy outcome including litter size or the sex ratio. In female offspring from the BPA groups, there were no significant effects on body weight or organ weights when compared to the vehicle control group. Age and weight at vaginal opening were also unaffected in groups exposed to BPA. Vaginal opening was delayed in the diethylstilbestrol-treated group and in the naïve control group compared to the vehicle control group.

Comments: This study used low doses of BPA, and the litter was used as the experimental unit for most of the statistical analyses. The group sizes are fairly small, but the study was designed to replicate findings in published work. Somewhat bothersome is the unexpected effect on vaginal opening in the positive control group and the naïve control group. Vaginal opening was delayed in the naïve control group by about 3 days, and DES delayed vaginal opening by over 3.5 days, rather than causing an acceleration. The dose of DES used (0.2 μg/kg bw/day) is apparently borderline for producing effects on the reproductive tract and was probably not a good choice. The small sample size and the unexpected effects in the two control groups limit the usefulness of this study in determining a risk assessment.

Markey CM, Luque EH, Munoz De Toro M, Sonnenschein C, Soto AM (2001) *Biol Reprod.* In utero exposure to bisphenol A alters the development and tissue organization of the mouse mammary gland. 65(4): 1215-1223 [Erratum: Biol Reprod 2004; 71: 1753].
Muñoz-de-Toro M, Markey CM, Wadia PR, Luque EH, Rubin BS, Sonnenschein C, Soto AM (2005) *Endocrinology.* Perinatal exposure to bisphenol-A alters peripubertal mammary gland development in mice. 146(9): 4138-4147.

The primary endpoint examined in these studies was the effect of prenatal BPA exposure on mammary gland development. Markey et al. (2001) did not determine the onset of puberty; Munoz-de-Toro et al. (2005) did determine this endpoint (time of first proestrus), but these data

were not reported. Additionally, a non-oral route of administration was utilized, and it appears that pure DMSO may have been used as the vehicle in an osmotic mini-pump which may have caused failure of the pump. Since blood levels were not determined, actual exposure to BPA cannot be determined.

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

These authors examined the effects of perinatal BPA exposure on reproductive development in CD-1 mice. Care was taken to decrease exposure from environmental estrogens by testing the cages, bedding, and chow and using only glass water bottles. From GD 9 through PND 4, groups of 6–10 mice were exposed to BPA at 0 (DMSO), 25 or 250 μg/kg bw/day via a subcutaneously implanted osmotic mini-pump. Age at vaginal opening was determined and classified as either partial or complete. It appears that the individual pup was evaluated as the experimental unit.

Observations: Although there were trends toward a younger age for partial vaginal opening as well as for the time between partial and complete vaginal opening, these differences were not statistically significant. It is not clear how many animals were evaluated for this endpoint or how those animals were selected.

Comments: The authors used environmentally relevant doses of BPA and were careful to decrease exposure to environmental estrogens. However, in addition to using a non-oral route of administration, it appears that pure DMSO was used as the vehicle in the osmotic mini-pump. As stated earlier, this vehicle is not recommended by the manufacturer and could have caused pump failure leading to inaccurate BPA dosing. Blood levels were not determined in this work. Overall, this report is of limited usefulness in determining a risk assessment.

Tyl RW, Myers CB, Marr MC, Sloan CS, Castillo NP, Veselica MM, Seely JC, Dimond SS, Van Miller JP, Shiotsuka RN, Beyer D, Hentges SG, Waechter JM Jr (2008) *Toxicol Sci.* Two-generation reproductive toxicity study of dietary bisphenol A (BPA) in CD-1® (Swiss) mice. Tox Sci Advance Access published April 29, 2008.

Developmental and reproductive toxicity of BPA was examined in a two generation study in CD-1 mice. Dietary doses were 0.018, 0.18, 1.8, 30, 300 and 3500 ppm; these doses resulted in estimated intakes of approximately 0.003, 0.03, 0.3, 5, 50 or 600 mg/kg bw/day. A positive control group was fed 17β-estradiol at 0.5 ppm with an estimated intake of about 0.08 mg/kg bw/day. Groups of 28 mice were fed the diets for 8 weeks before breeding and throughout breeding, gestation and lactation. Concentration, stability, and homogeneity of BPA and E_2 in feed were verified, and animal body weights and food intake were monitored throughout the study. Animals were housed in cages with chip bedding with glass water bottles. The chow was analyzed by the manufacturer and contained isoflavones at 394.2 ppm. F_1 litters were culled to 10 pups on PND 4, with equal numbers of each sex when possible. At weaning on PND 21, 28

F_1 offspring/sex/group were randomly selected and exposed to BPA in the diet according to the same protocol as F_0 mice. Those selected offspring were monitored for vaginal opening and preputial separation and later mated. At weaning, an additional 1 male/litter was randomly retained with BPA exposure continuing for an additional 3 months; preputial separation was also determined in those males. Pregnant F_1 females were followed through gestation, birth and lactation. At weaning, all F_2 animals and F_1 parents were sacrificed and necropsied.

Observations: There were no adverse effects among F_0 animals regarding mating, fertility, number of live pups/litter or birth weight of F_1 pups. Preputial separation was significantly delayed at 3500 ppm BPA whether considering the absolute time or the time adjusted for body weight at the time of acquisition. If the time of preputial separation was adjusted for body weight on PND 30, this difference was not statistically different. Estradiol delayed preputial separation (absolute time as well as time adjusted for body weight at time of acquisition or PND 30). BPA had no effect on the time of vaginal opening. Females exposed to 3500 ppm BPA weighed less than control animals

Comments: This is a well-conducted, thorough study done under GLP conditions. Concentration and stability of dosing solutions were verified, exposure to environmental estrogens was controlled, two vehicle control groups were used to help define the instrinsic variability in the endpoints evaluated in the study, six doses of BPA were used covering a wide dose range, oral administration was used, group sizes were large, a large number of endpoints was evaluated, the litter was used as the experimental unit, and fertility of exposed animals was evaluated. However, blood levels of BPA were not determined, and the time of first estrus was not evaluated. Additionally, markers of puberty were not determined among F_2 offpsring. Overall this study is highly useful for determination of a risk assessment.

Durando M, L. K, Piva J, Sonnenschein C, Soto AM, Luque E, Muñoz-de-Toro M (2007) *Environ Health Perspect.* Prenatal bisphenol A exposure induces preneoplastic lesions in the mammary gland in Wistar rats. 115 (1): 80-86.

The main endpoint in this study was the effect of prenatal BPA exposure on susceptibility to mammary tumors in Wistar rats. These rats were housed in stainless steel cages, provided water from glass bottles and fed commercially available chow. On GD 8–23, groups of 11–14 dams were dosed subcutaneously using an osmotic mini-pump with a DMSO vehicle or 25 μg/kg bw/day BPA. During the study, body weights and day of vaginal opening were monitored.

Observations: BPA exposure had no significant adverse effects on pregnancy outcome or the sex ratio, and there were no effects on AGD on PND 1 or 5. Vaginal opening was accelerated by 5 days by BPA. Body weight was not determined at the time of vaginal opening, but it was not affected by BPA treatment at any of the postnatal time points examined. The authors concluded that rats exposed prenatally to a low dose of BPA may demonstrate accelerated puberty.

Comments: Consideration of estrogen exposure in the environment is a strength of this study. Although the estrogenic potential of the chow was not determined, food consumption was apparently monitored, and the groups ate approximately the same amount of chow so their

exposure via this route would be expected to be similar. Also the use of stainless steel cages and glass water bottles would minimize environmental estrogen exposure. However, this study examined only a single dose of BPA so no dose response could be determined. A major weakness is the lack of an indication of the concentration of DMSO used in the mini-pump; pure DMSO is not recommended by the pump manufacturer and could cause its failure. Additionally, a non-oral exposure route was utilized, and blood levels were not determined. Overall, this report is of limited usefulness in determining a risk assessment.

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, Waechter JM (2002) *Toxicol Sci.* Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats. 68(1): 121-146.

Developmental and reproductive toxicity of BPA was examined in a three generation study in Sprague-Dawley rats. Dietary doses were 0, 0.015, 0.3, 4.5, 75, 750, or 7500 ppm, and actual intakes were 0.0007–0.003, 0.015–0.062, 0.22–0.73, 4.1–15.4, 37.6–167.2, and 434–1823 mg/kg bw/day. Concentration, stability, and homogeneity of BPA in feed were verified, and animal body weights and food intake were monitored throughout the study. Animals were housed in stainless steel cages, and water was supplied by an automatic watering system. During mating, pregnancy and lactation, animals were housed in cages with chip bedding with glass water bottles; the authors stated that none of these items were made from materials containing BPA. The chow was analyzed by the manufacturer and contained isoflavones at 309.2 µg/g feed. F_1 litters were culled to 10 pups on PND 4, with equal numbers of each sex when possible. At weaning on PND 21, 30 F_1 offspring/sex/group were randomly selected and exposed to BPA in the diet according to the same protocol as F_0 rats. Those selected offspring were monitored for vaginal opening and preputial separation and later mated. The same procedures were repeated in F_2 rats and F_3 litters during the lactation period. Thirty rats/sex/dose were selected for evaluation of vaginal patency, preputial separation, and estrous cyclicity. Dietary BPA exposure continued in the F_3 offspring until they were killed approximately 10 weeks after weaning.

Observations: The day of vaginal opening was delayed in females in the 7500 ppm group in all 3 generations. The body weights of females in all 3 generations were also significantly decreased at the 7500 ppm dose; when corrected for body weight, there was still a significant delay in vaginal opening in all 3 generations. The absolute day of vaginal opening was also delayed in the 75 ppm females in only the F_2 generation; when adjusted for body weight, this difference was no longer statistically significant. The authors concluded that the delay in acquisition of puberty in the high dose animals was probably due to the decreased body weight. There were no effects of continual exposure to BPA on fertility. The authors identified a NOAEL of 750 ppm (approximately 50 mg/kg bw/day) for offspring and reproductive effects; this would also be the NOAEL for age at vaginal opening. The authors concluded that BPA should not be considered a reproductive toxicant.

Comments: This is a well-conducted, well controlled, thorough study done under GLP conditions. Exposure to environmental estrogens was controlled, concentration and stability of dosing solutions were verified, 6 doses were used covering a wide dose range, oral administration was used, a large number of appropriate endpoints was evaluated, large group

sizes were used, the litter was used as the experimental unit, and multiple generations were evaluated. Blood levels of BPA were not determined. Overall this study is highly useful for determination of a risk assessment.

Tinwell H, Haseman J, Lefevre PA, Wallis N, Ashby J (2002) *Toxicol Sci.* Normal sexual development of two strains of rat exposed in utero to low doses of bisphenol A. 68(2): 339-348.

Tinwell et al. (2002) examined the effects of *in utero* exposure to low doses of BPA on sexual development in both Sprague-Dawley and Alderley Park (derived from Wistar rats) rats. Pregnant females were housed in cages with plastic bottoms containing sawdust and shredded paper as bedding. Each treatment group contained 6 - 7 rats which were gavaged on GD 6 – 21 with BPA at 0 (arachis oil), 20 or 100 μg/kg bw/day or 50 mg/kg bw/day. A fifth group was a positive control group which was dosed with ethinyl estradiol at 200 μg/kg bw/day; however, maternal toxicity caused this dose to be reduced to 100 μg/kg bw/day on GD 11 in Alderley Park rats and on GD 14 in Sprague-Dawley rats. HPLC was used to verify dosing solution concentrations and stability. Soy content of chow was noted. Litters were standardized to 8 pups on PND 5 with equal numbers of males and females when possible. Body weights were monitored at regular intervals after birth, and the ages at which preputial separation, vaginal opening, and first estrus occurred were assessed. Data were analyzed using the litter as the experimental unit.

Observations: There were no adverse effects on pregnancy outcome, including the number of live pups and the sex ratio. There were also no effects on birth weights or AGD. There was a significant delay in vaginal opening in Alderley-Park rats of the high dose group, but no effects at any BPA dose in Sprague-Dawley rats. There was a significant correlation between body weight and time of vaginal opening in the Alderley Park rats. There were no effects of BPA on time of first estrus (defined as first day when only cornified epitheilial cells were found in the vaginal lavage) at any BPA dose in either strain of rats. There were also no effects on preputial separation at any BPA dose in either strain of rats. Treatment with ethinyl estradiol did result in an earlier day of vaginal opening in the Alderley Park rats, but there was no effect on the age at first estrus. No Sprague-Dawley rats survived past birth. The authors concluded that this study failed to confirm low-dose endocrine effects.

Comments: The authors used 2 strains of rats, a range of doses of BPA, oral exposure, included a positive control group, verified dosing solution concentrations by HPLC and used the litter as the experimental unit for statistical analysis. A drawback is the somewhat small number of litters used in each treatment group, and the lack of blood levels of BPA. Additionally, the ethinyl estradiol data are somewhat difficult to interpret due to the production of significant maternal toxicity. This study is useful in determining a risk assessment for effects of BPA on timing of puberty.

Ema M, Fujii S, Furukawa M, Kiguchi M, Ikka T, Harazono A (2001) *Reprod Toxicol.* Rat two-generation reproductive toxicity study of bisphenol A. 15(5): 505-523.

Ema et al. (2001) examined developmental toxicity endpoints in a 2-generation study conducted in Crj:CD (SD) rats. Two generations of rats were gavaged daily with 0, 0.2, 2, 20, or 200 μg/kg bw/day BPA. Bedding, chow and water were assayed for BPA, and levels were found to be below the detection limits of 0.03 - 0.003 μg/g. Animals were housed in suspended stainless steel cages except from GD 17 through lactation when they were housed in cages with wood chips as bedding. The purity and stability of BPA was assayed by HPLC, and dosing concentrations were also assayed. Each treatment group consisted of 25 rats. Preputial separation was observed daily beginning on PND 35 in F1 and F2 male pups; vaginal opening was observed daily beginning on PND 28. Body weight was recorded at the time of preputial separation or vaginal opening.

Observations: BPA exposure did not affect either preputial separation or vaginal opening at any BPA dose; additionally, there were no effects on prenatal or postnatal growth, survival, developmental landmarks, AGD or fertility.

Comments: This study was well-conducted, thorough and done under GLP conditions. Concentrations and stability of dosing solutions were determined throughout the study. Exposure to environmental estrogens was monitored. Four doses were used, and these covered a wide dose range. A large number of animals were used in each treatment group. The litter was used as the experimental unit for statistical analysis. However, blood levels of BPA were not determined. This study is highly useful in determining a risk assessment.

Yoshida M, Shimomoto T, Katashima S, Watanabe G, Taya K, Maekawa A (2004) *J Reprod Dev.* Maternal exposure to low doses of bisphenol A has no effects on development of female reproductive tract and uterine carcinogenesis in Donryu rats. 50(3): 349-360.

The effects of BPA exposure on development of the female reproductive tract in Crj:Donryu rats was examined. Groups of 12 – 19 pregnant females were gavaged with BPA at 0 (0.05% carboxymethylcellulose solution), 0.006, or 6 mg/kg bw/day from GD 2 (plug day not defined) to PND 20. Animals were housed in plastic cages with wood chip bedding; they were given tap water that had been stored in plastic containers and fed a commercial pelleted chow. HPLC was used to examine samples of tap water, drinking water, and chow for BPA content. Litter sizes were adjusted to 8-10 pups on PND 4 or 6. All female offspring were examined for vaginal opening. BPA levels were measured in maternal and pup tissues. It appears that the litter was the experimental unit selected for statistical analyses.

Observations: There were no effects on gestation length, on the number of live pups/litter or on pup body weights through weaning. There were also no effects on the time of vaginal opening. BPA was detected in the chow and drinking water stored in plastic containers. BPA was detected in all tissues examined at all time points among rats of all 3 groups. The presence of BPA in the serum of control animals appears to be due to environmental exposure. There were no differences between tissue BPA concentrations among control and low dose animals, but significantly more BPA was observed in the serum of rats dosed with 6 mg/kg bw/day. BPA was

present in milk (milk was collected from stomachs of pups on PNDs 10 and 14), but there were no differences in the concentrations between treatment groups. BPA was also observed in offspring serum and liver at PNDs 10, 14 and 21, and there were no differences in concentrations at any postnatal time among any of the treatment groups. These data suggest that transplacental and lactational transfer of BPA do occur. The authors concluded that perinatal exposure to BPA at levels comparable to human exposure did not affect the reproductive system of female rats.

Comments: This study determined BPA levels in the environment, used large group sizes, an oral route of exposure, and determined BPA levels in dam and offspring tissues. However, the authors reported that maternal body weights were determined weekly during pregnancy, but the animals were dosed daily. Due to the large amount of weight gain during pregnancy, this would result in lower daily doses and inconsistency in dosing across pregnancy. Blood levels in dams were determined only at the end of lactation, and small numbers of samples were examined in some groups. Although only 2 doses were used, these doses covered a wide range. This study is of limited usefulness in a risk assessment.

Kubo K, Arai O, Omura M, Watanabe R, Ogata R, Aou S (2003) *Neurosci Res.* Low dose effects of bisphenol A on sexual differentiation of the brain and behavior in rats. 45(3): 345-356.

The effect of prenatal BPA exposure on sexually dimorphic behavior and brain structure in groups of 5 - 6 Wistar rats was examined. The rats were dosed with 0.1% ethanol in distilled water or BPA. It was not stated, but it appears that the compound was administered in the drinking water. The authors estimated BPA intake at 30 and 300 μg/kg bw/day. It appears that exposure occurred from the day of finding sperm in the vaginal smear to PND 21. A positive control group was dosed with diethylstilbestrol at an estimated intake of 6.5 μg/kg bw/day. Body weight and AGD were measured in pups on PND 1 (the day following birth), and litters were adjusted to 10 pups at that time. The time of vaginal opening was determined after weaning. The litter was considered the statistical unit in analyses of data collected prior to weaning of animals. Individual animals were considered the statistical unit for data collected after weaning (time of vaginal opening).

Observations: The day of vaginal opening was not affected by BPA. Body weight at vaginal opening was significantly increased by the high dose of BPA. Vaginal opening was significantly accelerated by DES, and the body weights of those pups were significantly lighter than controls. A number of tests of sexual behavior were also examined, and the only significant finding was a decrease in the rate of intromission among low dose males. The authors concluded that BPA exposure had no effects on male or female sexual behavior.

Comments: It appears that oral dosing was used. The lack of details in experimental methods makes it unclear if this is actually how the animals were dosed or their actual BPA intake. Determination of water consumption can overestimate the actual exposure since there can be more loss of dose by this route. There was also no description of environmental conditions and exposure that might have occurred in this manner. Fairly small number of dams were used in each group. Additionally, although the litter was used as the experimental unit for analyses of

endpoints collected prior to weaning, the individual animal was the experimental unit for endpoints collected after weaning, including the age at vaginal opening. Since treatment occurred to the litter, the litter should have been the experimental unit for all analyses. Overall, this study is of limited utility in determining a risk assessment.

Murray TJ, Maffini MV, Ucci AA, Sonnenschein C, Soto AM (2007) *Reprod Toxicol.* Induction of mammary gland ductal hyperplasias and carcinoma in situ following fetal bisphenol A exposure. 23(3): 206-210.

The effect of prenatal BPA exposure on in situ induction of mammary tumors in Wistar-Furth rats was examined. Cages and bedding tested negative for estrogenicity, and water was supplied in glass bottles. The chow was reported to contain 20 fmol/g estrogen equivalents. From GD 9 through PND 1, rats received BPA at 0 (50% DMSO), 2.5, 25, 250, or 1000 µg/kg bw/day. Group sizes were not indicated. Dosing solutions were delivered by implanted osmotic mini-pumps. Litters were adjusted to 8 pups on PND 2. AGD was measured on PND 4, and female offspring were monitored for body weight and vaginal opening after weaning. It is unclear if the litter or the individual pup was considered as the experimental unit, but the authors attempted to "maximize the number of maternal units represented in each group."

Observations: Exposure to BPA had no adverse effects on the number of live pups or the sex ratio at PND 1. Age at vaginal opening was not affected by any dose of BPA.

Comments: Care was taken to decrease exposure to environmental estrogens, and the authors used four doses covering a wide range. However, there was no indication of the number of dams treated in each group; a comment in the CERHR report indicated that an N of 7 – 21 was used for all endpoints other than histopathology, but it was not indicated whether the litter or the individual pup was considered the experimental unit. According the manufacturer, 50% DMSO can be used in the Alzet mini-pumps, but this is a non-oral exposure. Blood levels of BPA were not determined. This paper is not useful in determining a risk assessment.

Rubin BS, Murray MK, Damassa DA, King JC, Soto AM (2001) *Environ Health Perspect.* Perinatal exposure to low doses of bisphenol A affects body weight, patterns of estrous cyclicity, and plasma LH levels. 109(7): 675-680.

This study was designed to assess the effects of perinatal BPA exposure on estrus cyclicity and LH levels in Sprague-Dawley rats. The animals were housed in plastic cages, but these cages tested negative for estrogenicity; water was provided in glass bottles. Groups of 6 rats were randomly assigned to treatment with BPA at 0 (1% ethanol vehicle), 1, or 10 mg/L from GD 6 (plug day not indicated) through PND 21; administration was via the drinking water. Based on water consumption, mean BPA doses were estimated by the authors to be 0.1 and 1.2 mg/kg bw/day. Offspring gender was determined on PND 2, and body weights were monitored throughout the study. The day of vaginal opening was monitored beginning on PND 28. It is not clear if the litter was used as the experimental unit for statistical analysis.

Observations: There were no adverse effects on litter size or sex ratio. Measurement of the AGD during the neonatal period (exact times not specified) was also not affected by BPA, nor was the time of vaginal opening.

Comments: Oral dosing was used, and the authors took care to minimize some exposure to environmental estrogens. However, the assessment of BPA exposure by determining the amount of water consumed (and not lost through other means) can lead to overestimates of dose [as noted by the authors]. Also the lack of experimental details concerning the experimental unit used for statistical analysis as well as the rather small number of animals in each group are limitations. Overall, this paper is of limited utility in determining a risk assessment.

Table 1. Summary of reviewed studies – onset of puberty in females.

Authors	Species/Strain/Source	BPA Doses	Route	N	Exposure period	Results on Day of Vaginal Opening	Results on Day of First Estrus
Howdeshell et al., 1999	Mouse?CF-1/Charles Rive	2.4 µg/kg bw/day	gavage	21	GD 11-17	↔	↓ 2.4
Ryan & Vandenbergh, 2006	Mouse/ C57/Bl-6/Charles River	2 and 200 µg/kg bw/day	gavage	4-7	GD 3-PND 21	Not Evaluated	↓ 200
Honma et al., 2002	Mouse/ ICR/Jcl/Not indicated	2 and 20 µg/kg bw/day	sc injection	10	GD 11-17	↓ 20	↓ 20
Ashby et al., 1999	Mouse/CF-1/Charles River	2 and 20 µg/kg bw/day	Oral	7-8	GD 11-17	↔	Not Evaluated
Markey et al., 2003	Mouse/CD-1/Charles River	25 and 250 µg/kg bw/day	sc pump	6-10	GD 9-PND 4	↔	Not Evaluated
Tyl et al., 2008	Mouse/CD-1/Charles River	0.018, 0.18, 1.8, 30, 300 and 3500 ppm	in chow	28	lifetime	↔	Not Evaluated
Durando et al., 2007	Rat/Wistar-derived/ University colony (Santa Fe, Argentina)	25 µg/kg bw/day	sc pump	11-14	GD 8-23	↓	Not Evaluated
Tyl et al., 2002	Rat/Sprague-Dawley/Charles River	0.15, .3, 4.5, 75, 750 and 7500 ppm	in chow	30	lifetime	↑ 7500	Not Evaluated
Tinwell et al., 2002	Rat/Sprague-Dawley/Harlan and Rat/Alderley Park (Wistar- derived) /AstraZeneca	20 and 100 µg/kg bw/day, 50 mg/kg bw/day	gavage	6-7	GD 6-21	↑ 50 (AP rats)	↔
Ema et al., 2001	Rat/Sprague-Dawley/Charles River (Japan)	0.2, 2, 20 and 200 µg/kg bw/day	gavage	25	lifetime	↔	Not Evaluated
Yoshida et al., 2004	Rat/Crj:Donryu/Charles River (Japan)	6 and 6000 µg/kg bw/day	gavage	12-19	GD 2-PND 20	↔	Not Evaluated

Kubo et al., 2003	Rat/Wistar/Kyudo	30 and 300 µg/kg bw/day	drinking water	5-6	GD 0-PND 21	↔	Not Evaluated
Murray et al., 2007	Rat/Wistar-Furth/Harlan	2.5, 25, 250 and 1000 µg/kg bw/day	sc pump	?	GD 9-PND 1	↔	Not Evaluated
Rubin et al., 2001	Rat/Sprague-Dawley/Taconic Farms	100 and 1200 µg/kg bw/day	drinking water	6	GD 6-PND 21	↔	Not Evaluated

↓ Indicates a significant acceleration in the age at vaginal opening or first estrus.
↑ Indicates a significant delay in the age at vaginal opening or first estrus.
↔ Indicates no change in the age at vaginal opening or first estrus.

Studies on altered prostate and urinary tract development in males

Timms BG, Howdeshell KL, Barton L, Bradley S, Richter CA, vom Saal FS (2005) *Proc Natl Acad Sci U S A*. Estrogenic chemicals in plastic and oral contraceptives disrupt development of the fetal mouse prostate and urethra. 102(19): 7014-7019.

This study used CD-1 mice (Charles River) fed Purina 5008 diet, a soy containing diet. Drinking water was administered from glass bottles and polypropylene cages were used to minimize background BPA. A total of 21 pregnant mice were divided into 4 groups : tocopherol-stripped corn oil control; ethinyl estradiol, 0.1 microgram/kg; DES 0.1 μg/kg; BPA 10 μg/kg (n=6 for BPA, n = 5 for the other groups). There was also a separate experiment with a dose of 200 μg /kg reported. Test compounds were administered **orally** by pipettor (to avoid the stress of gavage) from GD 14-19. Animals were sacrificed on GD 19 (before parturition) and only male fetuses (one per litter) developing between a male and a female were used to minimize variations in in utero endogenous hormone exposure.

Observations: All three compounds (BPA, ethinyl estradiol, and low dose DES) increased dorsolateral prostate duct volume and number of ducts (about 40% increase) as determined from 3D reconstruction from serial sections. PCNA staining (500-1,000 cells counted in dorsal, lateral and ventral ducts, and urethra) increased about 100%. The urethra near the neck of the bladder was also narrowed in the BPA and ethinyl estradiol groups. The high dose of DES (200 μg /kg) inhibited prostate duct formation.

Comments: There are no measures of internal dose available, but it would be expected that after oral administration to the dam the circulating BPA would be almost entirely glucuronidated. The 3D reconstruction of the fetal prostate used in this study is technically demanding and not something that would be attempted in large scale toxicology studies. From this paper alone, it is not clear if the effects would produce adverse effects later in life, although the results of this study suggest the potential for BPA to alter prostate development, either by direct action on the fetus or a maternally-mediated effect. Since only a single effective dose of BPA (10 μg /kg) was used, no dose response information is available and no NOAEL can be determined. The diet used in this study was a soy-containing diet that contains phytoestrogens, although later work from this laboratory has suggested that the absence of phytoestrogens in the diet of these mice can cause significant physiological changes consistent with *in utero* estrogenization (Ruhlen *et al*., EHP 116: 322-328, 2008). A potentially important finding from this study is that intrauterine position had a significant effect on the response, suggesting that the response to BPA could differ depending upon the level of endogenous hormone exposure *in utero*. This is an extemely difficult variable to control in standard toxiciology studies and could add to observed variability.

Ho SM, Tang WY, Belmonte de Frausto J, Prins GS (2006) *Cancer Res.* Developmental exposure to estradiol and bisphenol A increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4. 66(11): 5624-5632.

Sprague-Dawley rats (Zivic-Miller Labs) were shipped on GD12. Polysulfone cages with steel tops and water from glass bottles were used to minimize background BPA, although polysulfone, like polycarbonate, contains BPA-derived polymers. A soy-free, low phytoestrogen diet (Ziegler) containing 12 ppm phytoestrogens (presumably isoflavones) was used. Litters were culled to 10 pups, and males within a litter were randomly assigned to dose groups to avoid litter effects. A single dose of BPA (10 μg /kg) was used, along with two doses of estradiol benzoate (EB) (0.1 μg /kg and 2500 μg /kg) and a vehicle control. Tocopherol stripped corn oil was the vehicle. Animals were dosed by subcutaneous injection on PND 1, 3, and 5. At PND 90, half of rats in each group were implanted with silastic tubes containing testosterone and estradiol (T/E2), the other half were implanted with empty tubes (n=10 for each treatment group). Animals were necropsied 16 weeks after the implants were place. Other animals (n=5-7) were used for gene methylation analyses on PND 10 and 90.

Observations: The endpoints reported included prostatic intraepithelial neoplasia (PIN, precancerous lesions), Ki-67 as a marker of cell proliferation, TUNEL as a measure of apoptosis (counted 1000 cells per slide), and analysis of gene methylation patterns. It was indicated that histopathological evaluations were done in blinded fashion.

There was no effect of neonatal BPA on prostate weight or on the prostate weight increase that was induced by the T/E2 implant. There was an increased high grade PIN in BPA animals treated with T/E2, but not in animals that received control implants. This increase in high grade PIN was also observed with the high dose of EB, and high dose EB also increased PIN scores in animals with control implants. There were also increased proliferative and apoptotic indices in BPA-treated animals with T/E2 implants, and similar effects were seen with high EB. BPA and both doses of EB caused gene methylation changes, including hypomethylation of PDE4D4 (phosphdiesterase 4 variant 4). The methylation changes were detectable before any microscopic lesions could be detected and were present in both hormone-treated and control BPA-treated animals. The authors conclude that these data indicate that animals exposed to BPA as neonates are sensitized to prostatic hormonal stimulation later in life.

Comments: Subcutaneous exposure was used in this study. However, as indicated in the Draft NTP Brief on BPA (April 14, 2008), several studies have indicated considerably lower glucuronidation of BPA in neonates than adults and a recent study (Taylor *et al*., Reprod. Toxicol. **25**: 169-176, 2008) has reported similar levels of unconjugated BPA in neonatal mice following oral and subcutaneous administration. It may thus be reasonable, for this particular life stage, to consider results from subcutaneoous treatment similar after oral and subcutaneous dosing, although there were no direct measurements of internal dose in this experiment.

Strengths of the study include blinded histopathology, the use of two doses of an estrogen for comparison, attention to minimizing background BPA and phytoestrogens in the feed (although the importance of this is not clear given that there is no clear link between the use of phytoestrogen-containing diets and effects or lack of effects of BPA). A weakness is that only a single dose of BPA (10 μg /kg) was used, so no dose response or NOAEL can be determined.

This is a key paper in suggesting some concern for developmental exposure to BPA as far as effects on the prostate.

Richter CA, Taylor JA, Ruhlen RL, Welshons WV, Vom Saal FS (2007) *Environ Health Perspect.* Estradiol and Bisphenol A stimulate androgen receptor and estrogen receptor gene expression in fetal mouse prostate mesenchyme cells. 115(6): 902-908.

This study used CD-1 mice (Charles River) fed Purina 5008 (pregnancy/lactation) and 5001 (maintenance). Mesenchymal cells were isolated from the urogenital sinus (UGS) of male fetuses at GD 17 and cultured. Responses to a range of doses of estradiol and BPA were studied. Cells were incubated with test compounds for 4 days, with daily media change.

Observations: BPA was a weak stimulator of growth, about 20% at 1 and 10 uM. There was a significant induction of AR and ER-alpha at 1 nM and above, less than 2-fold at max stimulation. The authors argue that induction of the receptors occurs in the range of concentrations found in human serum. High dose estradiol also increased AR, although it would be predicted to decrease AR based on in vivo studies.

Comments: This is an *in vitro* study that is useful primarily because it provides information on possible mechanisms of BPA action in the prostate. It is not useful for risk assessment purposes. It is not clear if the mesenchymal cell cultures can metabolize BPA over the culture period or how the aglycone BPA concentrations achieved would compare to those in vivo.

Ogura Y, Ishii K, Kanda H, Kanai M, Arima K, Wang Y, Sugimura Y (2007) *Differentiation.* Bisphenol A induces permanent squamous change in mouse prostatic epithelium. 75(8): 745-756.

This study utilized Balb/c mice (CLEA, Japan) fed a low phytoestrogen diet (NIH-07 PLD, phytoestrogen level not specified) and housed in polyolefin cages with chip bedding. The containers used to deliver tap water were not specified. The experiments described included both in vivo and in vitro studies.

Observations: The main endpoint evaluated was the expression of cytokeratin 10 (CK10) as a marker of squamous metaplasia of basal epithelial cells of the prostate. This lesion is established to be related to estrogen exposure. Histological evaluation of H&E sections of the prostate and analysis of estrogen receptor alpha expression levels by real time PCR are also reported. Three experiments are reported:

1) 9-week-old male mice received subcutaneous implants containing 0,0.2, 2, 20, 200 mg BPA or 2 mg DES (n=7-9) per group. Prostates were evaluated after 3 weeks of exposure. The anterior prostate (AP) and dorsolateral prostate (DLP) expressed CK10 in animals receiving BPA pellets (2 mg and above) or DES pellets. The ventral prostate (VP) expressed CK10 in the DES-treated animals and the high dose (200 mg) BPA-treated animals. The data are reported in terms of intensity of staining and are not quantitative.

2) Explants of adult (8-9 weeks) prostate were incubated for 6 days with 1 nM DES or 1nM or 1 uM BPA. Squamous metaplasia was evident in the DES-treated cultures and in the 1 uM BPA-treated cultures. The 1 nM BPA-treated cultures were histologically normal, but showed CK10 staining.
3) Pregnant mice (n=3) were treated with 20 μg /kg/day BPA or 0.2 μg /kg/day DES by gavage from GD 13-18. Tocopherol-stripped corn oil was the vehicle. Males (2-5 pups per litter) were evaluated at 12 weeks of age. The prostates were morphologically normal when assessed by H&E. However, BPA- and DES-treated animals "appeared" to express CK10, with the most intense staining in the AP, intermediate staining in the DLP, and lowest staining in the VP . This order of intensity corresponds to the relative levels of ER alpha expression as measured in the adult prostate.

Comments: This study used a small number of animals and did not report the rate of release of BPA from the implanted pellets or the internal dose achieved by the subcutaneous or oral routes of exposure. The degree of metabolism of BPA over the in vitro culture period is also not known. A strength of the study is the use of the combination of *in vivo* and *in vitro* studies. The results are of interest because they provide evidence for effects of BPA on the prostate, particularly after developmental exposure, that have the potential to lead to adverse effects and are not evident by standard evaluation (H&E histology, organ weight).

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

This study used CF-1 mice (Charles River) fed Purina 5001 diet and housed in polypropylene cages with corn cob bedding. Animals were dosed orally via a micropipet to avoid gavage stress daily from GD 11- GD 17 with 0, 2, and 20 μg /kg BPA. The vehicle was tocopherol-stripped corn oil. There were 11 controls (5 unhandled, 6 dosed with vehicle) and 7 animals per dose group. One male per litter was used to avoid litter effects.

Observations: Prostate weight at 6 months was significantly increased in both dose groups. There was no dose-response evident as both treated groups showed approximately the same degree of increase (54-55 mg in treatment groups versus 41 from controls.

Comments: The issue of the diet used in this study was mentioned in the summary of the Timms *et al*., 2005 study above. Later studies have also shown that corn cob bedding can contain potent phytoestrogens (reviewed in Dixon, Ann. Rev. Plant Biol., 55: 225-261). This study does indicate that prostate effects resulting from neonatal oral treatment with BPA persist to adulthood, although the toxicological significance of a prostate weight increase of this degree is not known. There was no histological examination of the prostate in this study. There was no NOAEL determined, and the LOAEL was 2 μg /kg. No measures of internal dose are available, and the amount of non-glucuronidated BPA resulting from oral dosing of the dam would be expected to be extremely low.

Ashby J, Tinwell H, Haseman J (1999) *Regul Toxicol Pharmacol.* Lack of effects for low dose levels of bisphenol A and diethylstilbestrol on the prostate gland of CF1 mice exposed in utero. 30(2 Pt 1): 156-166.

This study represents an attempt to repeat results of Nagel *et al*. (1997). CF-1 mice (Charles River) were used, but RM1 diet (6.5% soy) was used instead of Purina 5001. All other experimental parameters were similar, except that additional animals were used from each litter (n=5-7 litters) to examine possible litter effects. Potential effects of single versus group housing were also examined. Doses of BPA were 0.2 and 2 μg /kg and DES was used as an estrogenic control compound at 0.2 μg /kg.

Observations: The DES treatment had no statistically significant effecs on any of the endpoints measured in males (terminal body weight, testis weight, epididymal weight, seminal vesicle weight, daily sperm production, and sperm efficiency). BPA did not affect prostate weight; there were no additional evaluations of the prostate. Body weight was significantly higher in the low dose BPA group, and testes weights and daily sperm production were significantly elevated in both BPA dose groups. Epididymal weight was increased in the high dose BPA group. The authors noted that the body weight difference clouded interpretation of the organ weight changes and considered all of the observed BPA effects equivocal and not of biological significance. In any case, increased prostate weight reported in the Nagel et al. (1997) study, as well as the indication of decreased sperm production by BPA reported in the separate study of vom Saal *et al*. (Toxicol. Indus. Health 14: 239-260, 1997) were not confirmed in this study.

Comments: A strength of the study is the attempt to replicate the conditions of the earlier study and extend the analysis to the possible effects of housing and litter effects. The study authors raise several possible issues that could account for the different results between the two laboratories and for the lack of activity of the DES dose used, but there is no clear explanation for the differences. The studies use relatively small numbers of litters and relatively crude measures of effect. No adverse effects of BPA were found in this study for oral doses up to 2 μg /kg.

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

This study utilized Sprague Dawley rats (Charles River) fed NIH-07 diet (phytoestrogen content not specified) and given deionized water from glass bottles. Animals were housed in polycarbonate cages with cellulose fiber chip bedding. Dams (n=8 per group) were dosed daily by gavage from GD 11 – PND 20, except for the day of parturition. BPA doses were 0, 3.2, 32, 320 mg/kg/day and DES at 15 μg /kg/day was included as a reference estrogen. Corn oil was the vehicle. Males were evaluated at PND 180, and litter means were used in the statistical analyses.

Observations: There were limited evaluations conducted on the males. Body and reproductive organ weights, including ventral and dorsolateral prostate weights, were reported. In addition, the ventral prostate underwent histopathological evaluation. There were no significant effects of

BPA at any dose. DES likewise did not have effects on these endpoints, although some female endpoints were affected by the dose of DES used.

Comments: As with nearly all of the studies, there are no measurements of internal dose available for the various stages of the experiment. This study gives a NOAEL of 320 mg/kg/day for orally administered BPA in the Sprague-Dawley rat for prostate weight and histopathology. In light of the results of other studies, particularly that of Ho *et al*. (2006), a determination of the dose received by the pups during lactation would have been particularly useful. Also, the standard analyses of the prostate performed in this or other standard toxicology tests may be insufficient to detect the effects indicated by Ho *et al*. It is not clear why only the ventral lobe of the prostate underwent evaluation. In studies conducted at later dates by others, other prostate lobes are reported to be affected.

Ichihara T, Yoshino H, Imai N, Tsutsumi T, Kawabe M, Tamano S, Inaguma S, Suzuki S, Shirai T (2003) *J Toxicol Sci.* Lack of carcinogenic risk in the prostate with transplacental and lactational exposure to bisphenol A in rats. 28(3): 165-171.

This study utilized Fisher 344 rats as the test animals. Minimal information on the experimental conditions is provided. Caging was not specified, but animals were housed on wood chip bedding, given free access to tap water, and fed NMF diet during pregnancy and lactation and MF diet after weaning. The compositon or phytoestrogen content of this diet is not provided.

Dosing was by gavage to the dams throughout pregnancy and lactation. There were 8-14 dams per group and dose levels were 0, 0.05, 7.5, 30, and 300 mg/kg. The vehicle was carboxymethylcellulose. At 5 weeks, 12 rats from each BPA dose group were given 10 sc vehicle injections at 2 week intervals. 21 rats from each group received 50 mg/kg of the carcinogen 3, 2'-dimethyl-4-aminobiphenyl (DMAB); rats were sacrificed at 65 weeks old. It was not clear how the pups were allocated to these treatment groups from the litters.

Observations: There were no treatment-related effects on male reproductive organ weights or on preneoplastic or neoplastic lesions in the prostate (atypical hyperplasia, prostatic intraepithelial neoplasia, carcinoma) in either control rats or carcinogen-treated rats. There were also no significant effects on testosterone levels, although the hormone measurements were highly variable.

Comments: These results indicate no effects of BPA on the prostate of F344 rats following oral exposure to dams at doses up to 300 mg/kg. In contrast to the results of Ho *et al*. (2006) where neonatal treatment sensitized the prostate of Sprague-Dawley rats to later hormonal stimulation, the BPA treatments in this study did not sensitize the prostate to later treatment with a carcinogen. As noted above, the authors provide limited information on the experimental conditions, and neither the phytoestrogen content of the diet nor the background BPA levels are reported. A critical question is what the level of exposure (i.e. internal dose) of the fetus and the nursing pup to BPA actually was in this study.

Gupta C (2000) *Proc Soc Exp Biol Med.* Reproductive malformation of the male offspring following maternal exposure to estrogenic chemicals. 224(2): 61-68.

This study utilized CD-1 mice (Charles River), which were received in the study laboratory on GD 12. Animals were maintained on Purina 5-L9 at the breeder and were switched to Purina 5012 at the study laboratory. No information on the phytoestrogen content of the diets or on other husbandry conditions is provided. Animals were dosed orally ("fed" by an unspecified method, the vehicle was corn oil with 10% ethanol) from GD 16-18, which is indicated by the author as a critical time for prostate development. There were 15 litters per treatment, and litter size was adjusted to 8, with at least 3 males per litter, at birth. A single dose of BPA, 50 μg /kg, was administered and two doses of DES, 0.1 and 200 μg /kg, were also tested.

In addition, organ culture studies were conducted. Fetal urogenital sinus was dissected from GD 17 male pups and exposed to 5 and 50 pg/ml BPA and 0.1 and 0.5 pg/ml DES for 7 days with media changed every other day.

Observations: Prostate weight was increased at PND 3, 21 and 60 and anogenital distance, adjusted for body weight, was also significantly increased at a all time points. Epididymal weight was decreased at PND60. Prostate size was increased by BPA in animals examined at PND 15. In organ cultures, BPA at 50 pg/ml increased prostate growth both in the absence and presence of testosterone. Androgen receptor (AR) levels were reported to be increased at PND 21 and 60, and *in vitro* studies also showed an increase in AR at 50 pg/ml. This persistent increase in AR is proposed as a mechanism whereby BPA alters prostate growth.

Comments: This study provides evidence in support of effects of orally administered BPA on the prostate that are persistent. A single dose of 50 μg /kg was used, so no dose response evaluation is possible. As with other studies using this dosing regimen, it is not clear what level of free BPA would be available to the fetus after such an oral exposure. The organ culture study suggests that the effect of BPA is a direct effect on the fetal prostate rather than an indirect effect mediated through the dam. The CERHR review of this study discussed concerns over the statistical treatment of the data in some instances where litter effects appeared not to be accounted for, but did not dispute the findings at the terminal time point.

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, Waechter JM (2002) *Toxicol Sci.* Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats. 68(1): 121-146.

This study used the Sprague-Dawley rat (source not indicated in paper, presumably Charles River) fed Purina 5002 diet (isoflavone content provided by the manufacturer) and housed in polypropylene cages with Sani-chip bedding. During acclimation and breeding, animals were held in stainless steel hanging cages. Water was administered from glass water bottles. BPA was mixed in the diet at 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, which translated to approximate doses of 0.001, 0.02, 0.3, 5, 50, and 500 mg/kg/day. The study was conducted under GLP, and dose stabilities and certifications were conducted. There were 30 pairs per dose group, with 10 week prebreed exposure for F_0, followed by continuous feeding through necropsy of F_3

generation at about 14 weeks. Multiple reproductive endpoints as specified in current regulatory guidelines were measured in both sexes.

Observations: There was a reduction in absolute prostate weight at the high dose in all generations, but body weight was also reduced and the relative organ weight was not significantly affected. There were multiple male reproductive organ weight depressions at the highest dose and delays in preputial separation at the high dose in all generations as well as sporadic effects on this endpoint at lower doses that were not consistent across generations. There were some sporadic lower dose reductions in testes weights (absolute), but there was no pattern to suggest biological significance. There were no significant treatment-related histopathology findings in male reproductive organs. The high dose effects were considered to be secondary to systemic toxicity and not to represent specific reproductive toxicity.

Comments: This was a large dietary administration study covering a broad dose range and conducted under GLP according to regulatory guidelines. The authors report a NOAEL for reproductive endpoints of 750 ppm, or 50 mg/kg/day and a LOAEL of 500 mg/kg/day based on the observation of reproductive effects only at the highest dose. There is presumed exposure *in utero* and during lactation, but this has not been directly measured. With regard to effects on prostate development for comparison to other studies (e.g. Ho *et al*., 2007) these measurements would be particularly useful. As mentioned in previous summaries, it would be difficult to detect some of the prostate effects of BPA that have been reported using standard evaluations of organ weight and H&E histopathology.

Tinwell H, Haseman J, Lefevre PA, Wallis N, Ashby J (2002) *Toxicol Sci.* Normal sexual development of two strains of rat exposed in utero to low doses of bisphenol A. 68(2): 339-348.

This study used Sprague Dawley rats (supplied by Harlan) and Alderly Park (Wistar-derived from AstraZeneca) rats fed RM-1 diet and housed in plastic cages with sawdust bedding. There was no mention of plastic type or type of water bottles used. Pregnant rats (6-7 per group per strain) were treated by gavage on GD6-GD 21 with 20 and 50 μg /kg BPA, 50 mg/kg BPA or ethinyl estradiol, 200 μg /kg using arachis oil as the vehicle. Males were terminated at PND 90, females at PND 98. Data was presented both as means of individual animals and as litter averages and the results of the analyses of the litter averages were used in data interpretation.

Observations: There was a decrease in daily sperm production in AP rats, but not in Sprague-Dawley rats, observed at 50 mg/kg. Histological evaluations of testes and (for SD) epididymides were conducted, and no lesions or abnormalities were noted.

Comments: This was a relatively small study, but was appropriately analyzed and utilized two strains of rat to attempt to address possible differences in strain sensitivity and included a potent reference estrogen for comparison. Effects on male reproductive endpoints were observed only at the 50 mg/kg dose in AP rats, and no treatment effects on prostate weight were seen. There was not histological evaluation of the prostate.

Tyl RW, Myers CB, Marr MC, Sloan CS, Castillo NP, Veselica MM, Seely JC, Dimond SS, Van Miller JP, Shiotsuka RN, Beyer D, Hentges SG, Waechter JM Jr (2008) *Toxicol Sci.* Two-generation reproductive toxicity study of dietary bisphenol A (BPA) in CD-1® (Swiss) mice. ToxSci Advance Access published April 29, 2008.

CD-1 mice from (Charles River) were fed Purina 5002 with approx 400 ppm isoflavones, administered water from glass bottles, and housed in polypropylene cages with Sani-Chip bedding. BPA was mixed in the diet at 0, 0.018, 0.18, 1.8, 30, 300, 3500 ppm (corresponding ot approximate doses of 0, 0.003, 0.03, 0.3, 5, 50, 500 mg/kg). Estradiol was included as an estrogenic reference compound with the dose based on prior one and two generation studies in CD-1 mice. This was a two generation study, with 28 animals per sex per group.

Observations: For males, decreased testis weight, delayed PPS and delayed testicular descent were observed in the high dose group. The authors suggest that this is secondary to systemic toxicity. There were no effects on prostate weight and no treatment-related histological effects.

Comments: This was a large study conducted under GLP and according to regulatory guidelines. Strengths include the number of animals used, the use of a strain of animals used by others to show low dose effects, careful monitoring of conditions, and the use of an estrogen reference compound. There were no adverse effects on prostate observed at doses up to 500 mg/kg, and male reproductive effects were observed only at that dose. The same comments made for other studies hold here as far as lack of information on internal dose at the various dosing stages.

Ramos JG, Varayoud J, Sonnenschein C, Soto AM, Munoz de Toro, M, Luque EH (2001) Prenatal exposure to low doses of bisphenol A alters the periductal stroma and glandular cell function in the rat ventral prostate. Biology of Reproduction 65: 1271-1277.

This study used Wistar-derived rats (Dept. Human Physiol., Santa Fe, Argentina) housed in stainless steel cages and fed an unspecified pelleted chow. Tap water was provided from glass bottles. An osmotic minipump was implanted on GD 8 to release 25 or 250 ugBPA/kg/day (0.25 ul per hour) until parturition at GD 23. There were 4 dams per group. On PND 30, animals were injected with BrdU and killed 2 hours later. Sections were prepared and the tissue evaluated for proliferation and various immunohistochemical markers for stroma and epithelium. Androgen receptor and prostatic acid phosphatase were also evaluated.

Observations: There was no difference in BrdU labeling in stromal or epithelial regions of the prostates; prostatic acid phosphatase diminished in prostate ductal secretory cells after BPA. There was an increase in fibroblast to smooth muscle cell ratio and decreased stromal, but not epithelia, AR. There was no apparent dose response as both dose levels appeared to have similar effects. The authors conclude that gestational exposure to BPA altered differentiation in periductal stromal cells in ventral prostate.

Comments: This is a small study that is of interest primarily as an indication of potential effects of prenatal effects on the prostate and the suggestion of compartment-specific effects on the androgen receptor. The direction of change of androgen receptor levels (decrease) differs from that reported from the oral dosing of mice during gestation in those studies that reported effects.

The use of DMSO as the vehicle in an osmotic pump could be a problem for dose delivery. There is no information on the blood levels of BPA during the dosing period. Because this is a prenatal experiment, the levels of active nonconjugated BPA achieved are expected to be considerably higher than those that would be achieved by oral dosing of the dam.

Table 2. Summary of reviewed BPA studies – male endpoints

Authors	Species/Strain/ Source	BPA Doses	Route	N	Exposure period	Observations (Prostate, urinary tract)*
Timms *et al*., 2005	Mouse/CD-1/Charles River	0, 10 μg/kg/day	Oral (fed by micropipette tip)	6 (5 for controls)	GD 14-19	↑ dorsolateral prostate duct volume and number ↓ diameter of urethra near bladder neck
Ho *et al*., 2006	Rat/Sprague-Dawley/Zivic-Miller Labs	0, 10 μg/kg/day; Testosterone/estradiol (T/E) challenge at PND 90 in half of animals	s.c. injection	10	PND 1, 3, 5	↔ prostate weight ↑ high grade prostatic intraepithelial neoplasia, proliferative and apoptotic indices (only after T/E challenge) Altered gene methylation pattern

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Authors	Species/Strain/ Source	BPA Doses	Route	N	Exposure period	Observations (Prostate, urinary tract)*
Richter *et al*., 2007	Mouse/CD-1/Charles River	0,0.0001, 0.001, 0.1, 1, 10, 100, 1,000, 10,000, 100,000 nM	*In vitro* organ culture, fetal urogenital sinus	Number of animals used as source of cultures not specified	4 day exposure	↑ growth at 1 and 10 μM ↑ androgen and estrogen receptor α expression at 1 nM and above
Ogura *et al*., 2007 Experiment 1	Mouse/Balb/c/ CLEA(Japan)	0, 0.2, 2, 20, 200 mg pellets	s.c. implant	7-9	Adult males (9 weeks old) for 3 weeks	↑ squamous metaplasia as indicated by CK10 staining at 2 mg and above
Ogura *et al*., 2007 Experiment 2	Mouse/Balb/c/ CLEA(Japan)	1nM and 1 μM	*In vitro*, adult prostate explants	Number of animals used as source of explants not specified	6 days, *in vitro*	↑ squamous metaplasia and CK10 staining at 1 μM ↑ CK10 staining at 1 μM

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Authors	Species/Strain/ Source	BPA Doses	Route	N	Exposure period	Observations (Prostate, urinary tract)*
Ogura *et al*., 2007 Experiment 3	Mouse/Balb/c/ CLEA(Japan)	0, 20 μg/kg/day	Oral (gavage)	3	GD 13-18	↑ CK10 staining in absence of morphological difference by H&E
Nagel *et al*., 1997	Mouse/CF-1/Charles River	0, 2, and 20 μg/kg/day	Oral (fed by micropipette tip)	7 (11 controls: 5 unhandled, 6 vehicle-dosed)	GD 11- 17	↑ prostate weight at 6 months, 2 and 20 μg/kg/day
Ashby *et al*., 1999	Mouse/CF-1/Charles River	0, 2, and 20 μg/kg/day	Oral (fed by micropipette tip)	5-7	GD 11- 17	No effect on prostate weight
Kwon *et al*., 2000	Rat/Sprague-Dawley/Charles River	0, 3.2, 32, 320 mg/kg/day	Oral (gavage)	8	GD 11 – PND 20	At PND 180, no effect on prostate weight of histology of the ventral prostate (only lobe examined microscopically)

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Authors	Species/Strain/ Source	BPA Doses	Route	N	Exposure period	Observations (Prostate, urinary tract)*
Ichihara *et al*., 2003	Rat/Fisher 344/Charles River (Japan)	0, 0.05, 7.5, 30, and 300 mg/kg/day Challenge animals from each group with carcinogen (DMAB) at 5 weeks	Oral (gavage)	8 – 14 dams; 21 for DMAB challenge, 12 vehicle challenge	Daily throughout pregnancy and lactation	No effects on prostate in control or carcinogen-treated rats
Gupta, 2000 Experiment 1	Mouse/CD-1/Charles River	0, 50 μg/kg/day	Oral (administered in corn oil with 10% ethanol)	15	GD 16-18	↑ prostate weight at PND 3, 21, and 60 ↑ prostate size at PND 15 ↑ androgen receptor at PND 21 and 60
Gupta, 2000 Experiment 2	Mouse/CD-1/Charles River	0, 5, and 50 pg/ml *in vitro*	In vitro organ culture, GD 17 urogenital sinus	Number of animals used as source of cultures not specified	7 days	↑ prostate growthand androgen receptor, 50 pg/ml

Table 2. Summary of reviewed BPA studies – male endpoints

Authors	Species/Strain/ Source	BPA Doses	Route	N	Exposure period	Observations (Prostate, urinary tract)*
Tyl *et al*., 2002	Rat/Sprague-Dawley/Charles River	0, 0.015, 0.3, 4.5, 75, 750, 7500 ppm (Approximately 0, 0.001, 0.02, 0.3, 5, 50, and 500 mg/kg/day)	Mixed in diet; 3 generation exposure	30 males, 30 females per dose group per generation	10 weeks prior to mating of F_0 through postnatal week 14 of the F_3 generation	↓ absolute prostate weight, all generations at 7500 ppm (500 mg/kg/day) ↔ prostate histology
Tinwell *et al*., 2002	Rat/Sprague-Dawley/Harlan and Rat/Alderly Park (Wistar-derived)/AstraZeneca)	0, 20 and 50 μg/kg/day and 50 mg/kg BPA	Oral (gavage)	6-7	GD 6- 21	No effects on prostate weight (Reduction in daily sperm production in AP rats only at 50 mg/kg)
Tyl *et al.*, 2008	Mouse/CD-1/Charles River	0, 0.018, 0.18, 1.8, 30, 300, 3500 ppm (Approximately 0, 0.003, 0.03, 0.3, 5, 50, 500 mg/kg/day)	Mixed in diet; 2 generation exposure	28 males, 28 females per dose group per generation	8 weeks prior to mating of F_0 through postnatal week 14 of the F_2 generation	No effects on prostate weight or histopathology (decreased testis weight, delayed preputial separation and testicular descent at 3500 ppm (500 mg/kg/day)

Table 2. Summary of reviewed BPA studies – male endpoints

Authors	Species/Strain/ Source	BPA Doses	Route	N	Exposure period	Observations (Prostate, urinary tract)*
Ramos *et al*., 2001	Rat/Wistar-derived/University colony (Santa Fe, Argentina)	0, 25, and 250 μg/kg/day	s.c. (osmotic pump)	4	GD 8 to 23	All effects at 25 and 250 μg/kg/day ↔ proliferation as measured by BrdU ↑ fibroblast to smooth muscle cell ratio ↓ stromal androgen receptor and prostatic acid phosphatase

* ↑, increase; ↓, decrease; ↔, no change. Significantly affected male reproductive endpoints outside the prostate and urinary tract are listed in parentheses.