National Toxicology Program
U.S. Department of Health and Human Services

Center For The Evaluation of Risks To Human Reproduction

NTP-CERHR MONOGRAPH ON THE POTENTIAL HUMAN REPRODUCTIVE AND DEVELOPMENTAL EFFECTS OF BISPHENOL A

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NTP BRIEF ON BISPHENOL A

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Table 3. Blood and Breast Milk Biomonitoring of bisphenol A in People (United States)

Biological Medium	Population (sample size)	Free bisphenol A (µg/L) Mean or Median [range]	Total bisphenol A (µg/L) Mean or Median [range]	Reference
Blood	Pregnant women (40)	*Mean:* 5.9 [0.5–22.4]		*(12)*
Breast milk	Lactating women (20)	*Mean:* 1.3; *Median:* 0.4 [<0.3 (LOD)–6.3]	*Mean:* 1.3; *Median:* 1.1 [<0.3 (LOD)–7.3]	*(5)*

LOD = limit of detection

It is helpful in interpreting the biomonitoring data for bisphenol A to understand how the body processes and excretes it once exposure occurs. Following ingestion, the majority of bisphenol A is quickly bound to glucuronic acid to produce bisphenol A-glucuronide, a metabolic process called glucuronidation that is carried out by enzymes primarily in the liver [reviewed in *(2)]*. Glucuronidation makes bisphenol A more soluble in water and, therefore, easier to eliminate in the urine and also minimizes its ability to interact with biological processes in the body. To a lesser extent, unconjugated parent (commonly referred to as "free")[5] bisphenol A is converted to other metabolites, primarily bisphenol A sulfate. Understanding the degree to which bisphenol A is metabolized is very important in determining whether bisphenol A poses a potential risk to human reproduction and development. While free bisphenol A and its major metabolites (bisphenol A-glucuronide and bisphenol A-sulfate) can all be measured in humans, only free bisphenol A is considered to be biologically active. Bisphenol A is metabolized more quickly following oral exposure compared to non-oral exposures such as inhalation because of "first pass effects" (see below).

[5] Unmetabolized bisphenol A is commonly referred to as "free"; however, the majority of "free" bisphenol A circulating in human blood is bound to plasma proteins.

There is evidence in laboratory rodents that very young animals metabolize bisphenol A less efficiently than adult animals *(18–20)*. Neonatal rats have higher circulating concentrations of free bisphenol A in their blood compared to older animals given an equal exposure, presumably due to an underdeveloped ability to glucuronidate early in life *(18)*. However, neonatal rats do have the capacity to metabolize and eliminate bisphenol A. The specific enzymes that glucuronidate bisphenol A have not been identified in people, but there is evidence of postnatal maturation for a number of glucuronidation enzymes in humans. For this reason, a reduced ability or efficiency to glucuronidate is generally predicted for human fetuses and infants [reviewed in *(2)]*. However, a number of the enzymes involved in metabolizing bisphenol A to bisphenol A sulfate in humans are known and have been shown to be active in fetal and neonatal life *(21, 22)*, suggesting that this metabolic pathway may be more important than glucuronidate early in life relative to adulthood.

CAN BISPHENOL A AFFECT HUMAN DEVELOPMENT OR REPRODUCTION?

Possibly. Although there is no direct evidence that exposure of people to bisphenol A adversely affects reproduction or development, studies with laboratory rodents show that exposure to high dose levels of bisphenol A during pregnancy and/or lactation can reduce survival, birth weight,

and growth of offspring early in life, and delay the onset of puberty in males and females. These effects were seen at the same dose levels that also produced some weight loss in pregnant animals ("dams"). These "high" dose effects of bisphenol A are not considered scientifically controversial and provide clear evidence of adverse effects on development in laboratory animals. However, the administered dose levels associated with delayed puberty (≥50 mg/kg bw/day), growth reductions (≥300 mg/kg bw/day), or survival (≥500 mg/kg bw/day) are far in excess of the highest estimated daily intake of bisphenol A in children (<0.0147 mg/kg bw/day), adults (<0.0015 mg/kg bw/day), or workers (0.100 mg/kg bw/day) (Table 1).

In addition to effects on survival and growth seen at high dose levels of bisphenol A, a variety of effects related to neural and behavior alterations, potentially precancerous lesions in the prostate and mammary glands, altered prostate gland and urinary tract development, and early onset of puberty in females have been reported in laboratory rodents exposed during development to much lower doses of bisphenol A (≥0.0024 mg/kg bw/day) that are more similar to human exposures. In contrast to the "high" dose developmental effects of bisphenol A, there is scientific controversy over the interpretation of the "low" dose findings. When considered together, the results of "low" dose studies of bisphenol A provide limited evidence for adverse effects on development in laboratory animals (see Figures 2a & 2b).

Recognizing the lack of data on the effects of bisphenol A in humans and despite the limitations in the evidence for "low" dose effects in laboratory animals discussed in more detail below, the possibility that bisphenol A may alter human development cannot be dismissed (see Figure 3).

SUPPORTING EVIDENCE

The NTP finds that there is clear evidence of adverse developmental effects at "high" doses of bisphenol A in the form of fetal death, decreased litter size, or decreased number of live pups per litter in rats (≥500 mg/kg bw/day) *(36, 37)* and mice (≥875 mg/kg bw/day) *(38–40)*, reduced growth in rats (≥300 mg/kg bw/day) *(36, 37)* and mice (≥600 mg/kg bw/day) *(38, 39, 41)*, and delayed puberty in male mice (600 mg/kg bw/day) *(41),* male rats (≥50 mg/kg bw/day) *(37, 42)* and female rats (≥50 mg/kg bw/day) *(37, 43)*.

In addition to these "high" dose effects on survival and growth, the NTP recognizes that there are studies that provide evidence for a variety of effects at much lower dose levels of bisphenol

Figure 2a. *The weight of evidence that bisphenol A causes adverse developmental or reproductive effects in humans*

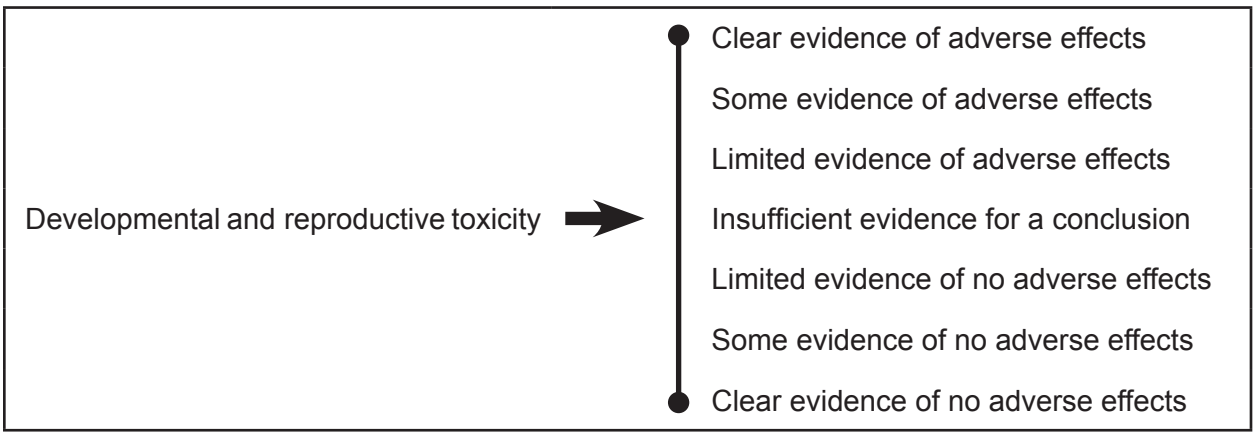
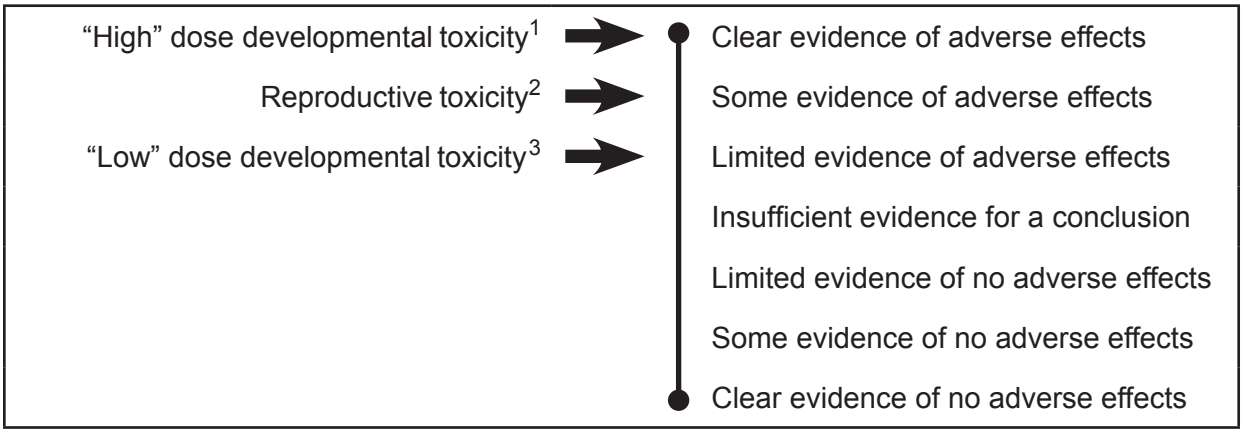


Figure 2b. *The weight of evidence that bisphenol A causes adverse developmental or reproductive effects in laboratory animals*

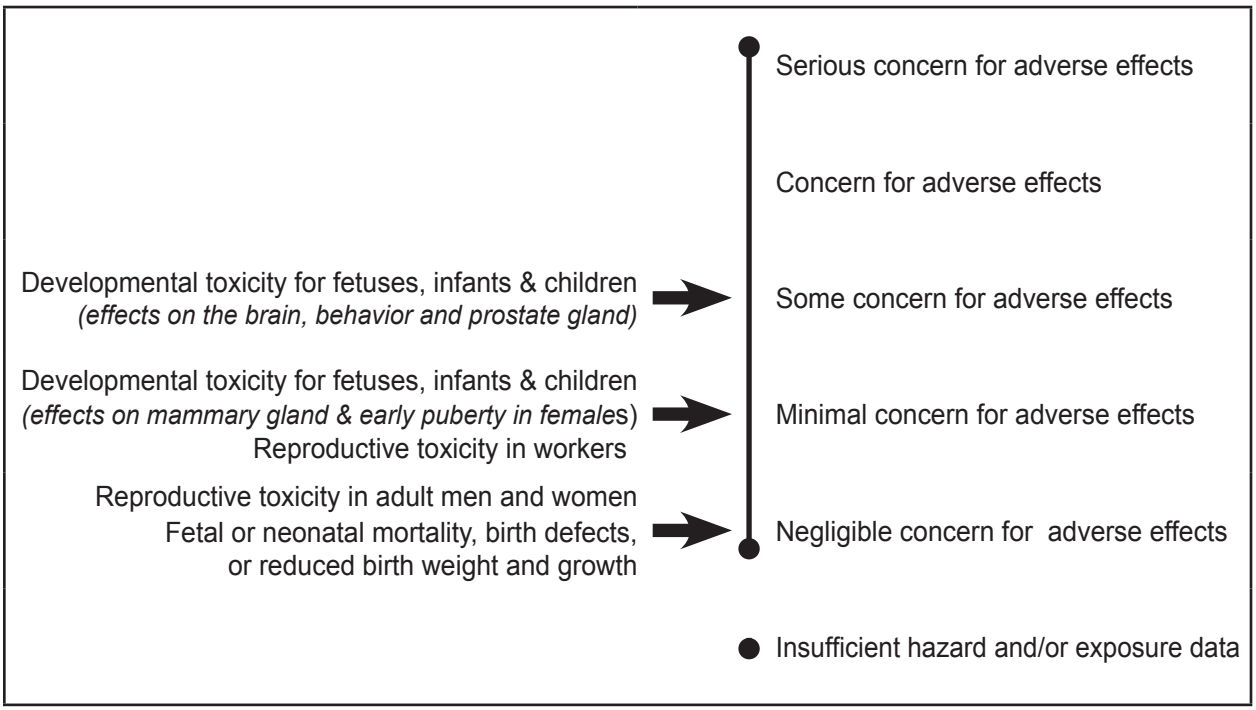


[1]Based on reduced survival in fetuses or newborns (≥500 mg/kg bw/day) *(36–40)*, reduced fetal or birth weight or growth of offspring early in life (≥300 mg/kg bw/day) *(36, 37, 41)*, and delayed puberty in female rats (≥50 mg/kg bw/day) and male rats and mice (≥50 mg/kg bw/day) (*37, 41–43*).

[2]Based on possible decreased fertility in mice (≥875 mg/kg bw/day) (*40*); altered estrous cycling in female rats (≥600 mg/kg bw/day) *(110),* and cellular effects on the testis of male rats (235 mg/kg bw/day) *(111).*

[3]Based a variety of effects related to neural and behavior alterations (≥10 µg/kg bw/day) *(44–50)*, lesions in the prostate (10 µg/kg bw/day) (*51*) and mammary glands (0.0025–1 mg/kg bw/day) *(52, 53)*; altered prostate gland and urinary tract development (10 µg/kg bw/day) *(54),* and early onset of puberty (2.4 and 200 µg/kg bw/day) *(48, 55)*.

Figure 3. *NTP conclusions regarding the possibilities that human development or reproduction might be effected by exposure to bisphenol A*



A related to neural and behavioral alterations in rats and mice (≥ 0.010 mg/kg bw/day) *(44 – 50)*, preneoplastic lesions in the prostate and mammary gland in rats (0.010 mg/kg bw/day and 0.0025 mg/kg bw/day, respectively) *(51 – 53)*, altered prostate and urinary tract development in mice (0.010 mg/kg bw/day) *(54),* and early onset of puberty in female mice (0.0024 and 0.200 mg/kg bw/day) *(48, 55)*.

These "low" dose findings in laboratory animals have proven to be controversial for a variety of reasons including concern for insufficient replication by independent investigators, questions on the suitability of various experimental approaches, relevance of the specific animal model used for evaluating potential human risks, and incomplete understanding or agreement on the potential adverse nature of reported effects. These issues have been extensively addressed elsewhere *(2, 56 – 60)* and were considered by the NTP when evaluating the bisphenol A literature.

HOW WAS THIS CONCLUSION REACHED?

Scientific decisions concerning health risks are generally based on what is known as the "weight-of-evidence." In the case of bisphenol A, evidence from the limited number of studies in humans exposed to bisphenol A is not sufficient to reach conclusions regarding possible developmental or reproductive hazard. In contrast, there is a large literature of laboratory animal studies. These include studies of traditional designs carried out to assess the toxicity of bisphenol A, as well as a wide variety of studies examining the possibility that exposure to "low" doses of bisphenol A, defined in the NTP Brief on Bisphenol A as ≤ 5 mg/kg bw/day *(61),* during critical periods of development might result in adverse health outcomes later in life due to its estrogenic or other biological properties. Many of these latter studies were designed not as toxicology studies but rather to probe very specific experimental questions, and their results are not always easily interpreted with regard to how they contribute to the weight-of-evidence for human health risks.

Many of the laboratory animal studies of bisphenol A have technical or design shortcomings or their reports do not provide sufficient experimental details to permit an assessment of technical adequacy *(2)*. As discussed in more detail below, the NTP did not establish strict criteria for determining which studies from the bisphenol A literature to consider for the evaluation. Rather, in an effort to glean information that might contribute to understanding the numerous reported effects of bisphenol A, NTP evaluated many individual study reports. Attention was paid to issues of sample size, control for litter effects, and various other aspects of experimental design; however, experimental findings were initially evaluated in relation to their biological plausibility and consistency across studies by multiple investigators. Studies were then evaluated as to their adequacy of experimental design and the likelihood that any inconsistent outcomes resulted from differences or shortcomings in experimental design. The NTP considered several overarching issues when evaluating the bisphenol A literature:

Are the *in vivo* effects biologically plausible?

Historically, bisphenol A has been characterized as being weakly estrogenic. For this reason the most common type of positive control compounds used in bisphenol A studies are potent estrogens. There is wide variability in *in vitro* estrogenic potency estimates for bisphenol A, although the mean estimate is ~1,000 to 10,000 times less potent than positive control compounds *(2)*. However, a number of the "low" dose studies suggest that bisphenol A has a higher *in vivo* potency than would be predicted based on binding to estrogen receptor alpha. The lack of concordance in potency estimates based on estrogen receptor binding and *in vivo* biological activity has been a point of debate

use of a positive control to be a required study design component particularly in animal model systems that are well characterized regarding the background incidence of "effects" and their variability. For bisphenol A studies, potent estrogens, such as diethylstilbestrol, ethinyl estradiol, 17β-estradiol, and estradiol benzoate, are the most commonly used positive control chemicals given bisphenol A's historical classification as a weak estrogen. Failure to obtain predicted responses with these chemicals is generally interpreted as a "failed" experiment, perhaps reflecting the selection of a relatively insensitive animal or experimental model or insufficient chemical challenge. Studies where no responses are observed in the positive control group have generally contributed less weight to evaluations of bisphenol A *(2, 60)*. The significance of a "failed" positive control for bisphenol A varies from endpoint to endpoint and reflected more negatively on a study in the NTP evaluation when the predicted effect on reproductive tissue or function was not observed at dose levels that should be sufficiently high to produce an effect. In addition, although potent estrogens are used as positive controls for bisphenol A, as discussed earlier an increasing number of molecular or cell-based studies suggest that interpreting the toxicological effects of bisphenol A solely within the context of their consistency with a classic estrogenic mechanism of action is overly simplistic.

HUMAN STUDIES

Only a very small number of studies have looked at associations between bisphenol A exposure and disorders of reproduction or developmental effects in humans [*(12, 99, 100)*, studies prior to mid-2007 reviewed in *(2, 3)*]. The human studies have looked at the relationship between urine or blood concentrations of total or free bisphenol A and a variety of health measures including levels of certain hormones that help regulate reproduction *(32, 101)*, markers of DNA damage *(102),* miscarriage *(103),* chromosomal defects in fetuses *(104),* fertility and obesity in women *(16, 99, 105)*, effects on the tissue that lines the uterus ("endometrium") *(99, 106)*, polycystic ovary syndrome *(101, 105)*, and birth outcomes and length of gestation *(12, 100)*.

In these studies, there are reports of associations between higher urine or blood concentrations of bisphenol A and lower levels of follicle–stimulating hormone in occupationally exposed men *(32),* higher levels of testosterone in men and women *(101, 105)*, polycystic ovary syndrome *(101, 105)*, recurrent miscarriage *(103),* and chromosomal defects in fetuses *(104).* In addition, one study reported that patients with endometrial cancer and complex endometrial hyperplasia had lower blood levels of bisphenol A than healthy women and women with simple endometrial hyperplasia *(106).* Bisphenol A was not associated with decreased birth weight or several other measures of birth outcome in two recent studies *(12, 100)*. Drawing firm conclusions about potential reproductive or developmental effects of bisphenol A in humans from these studies is difficult because of factors such as small sample size, cross-sectional design, lack of large variations in exposure, or lack of adjustment for potential confounders. However, the CERHR Expert Panel on Bisphenol A *(2)* concluded that several studies collectively suggest hormonal effects of bisphenol A exposure *(32, 101, 105)* including one in occupationally exposed male workers likely exposed through multiple routes including inhalation *(32).*

The NTP concurs with findings of the recent evaluations *(2, 3)* that while these studies may suggest directions for future research, there is currently insufficient evidence to determine if bisphenol A causes or does not cause reproductive toxicity in exposed adults. There is also insufficient evidence from studies in humans to determine if bisphenol A does or does not cause developmental toxicity when exposure occurs prenatally or during infancy and childhood.

LABORATORY ANIMAL STUDIES

In contrast to the limited literature evaluating possible effects of bisphenol A in humans, the scientific literature on the toxic effects of bisphenol A in laboratory animals is extensive and expanding. For example, between February 2007 (the cut-off date for literature included in the CERHR Expert Panel Report on Bisphenol A) and April 11, 2008, more than 400 new articles related to bisphenol A were identified by PubMed search. All new studies related to the potential reproductive and developmental effects of bisphenol A were considered during preparation of the NTP Brief on Bisphenol A. However, only those studies that were considered the most informative for developing NTP conclusions are cited in the Brief. In addition to the new literature cited, many key studies reviewed in the expert panel report are cited herein.

Reproductive Toxicity Studies

The reproductive toxicity studies of bisphenol A include assessment of fertility, sperm counts, estrous cycling, and growth or cellular damage in reproductive tissues. Reproductive toxicity can be studied in animals exposed during adulthood, during development, or both. Conclusions on reproductive toxicity presented in this section of the NTP Brief on Bisphenol A are limited to the assessment of fertility in laboratory animals, regardless of when exposure occurred, and other indicators of reproductive effects in animals exposed only during adulthood. Assessments of aspects of the reproductive system other than fertility in animals exposed during development are discussed under the headings of "High" Dose and "Low" Dose Developmental Toxicity Studies below.

Studies show that bisphenol A does not reduce fertility in laboratory animals exposed in adulthood and/or during development at dose levels up to 500 mg/kg bw/day in rats *(37, 107)*. Fertility may be negatively impacted at higher dietary doses (≥875 mg/kg bw/day) in mice exposed as adults as indicated by a decreased number of litters per breeding pair *(40),* although two multigenerational reproductive toxicity studies did not report effects on fertility in mice at doses up to 1669–1988 mg/kg bw/day *(39, 41)*. There are occasional reports of decreased fertility in smaller sample size studies of rodents exposed to much lower dose levels of bisphenol A during adulthood, such as oral treatment with 0.025 and 0.100 mg/kg bw/day in male mice *(108).* In the Al-Hiyasat *et al.* study, decreased pregnancy rates and increased incidence of resorptions in untreated female mice were attributed to effects in treated adult males, i.e., reductions in the number of testicular or epididymal sperm and hypothesized impaired sperm quality. However, the magnitude of the impact on weight–corrected testicular or epididymal sperm number, ~16 to 37%, is not generally considered severe enough to account for the observed pregnancy rate decrease of ~33 to 40%.[9]

At high oral dose levels, adult exposure to bisphenol A caused reproductive toxicity in the form of altered estrous cycling in female rats (≥600 mg/kg bw/day)[10] *(110)* and cellular effects on the testis of male rats (235 mg/kg bw/day) *(111).* In addition, more subtle effects on maternal behavior, i.e., decreased duration of licking and grooming of pups, are reported at a lower oral dose in treated adult female rats (0.04 mg/kg bw/day) *(112).*

"High" Dose Developmental Toxicity Studies (> 5 mg/kg bw/day)

Results from developmental toxicity studies in mice and rats show adverse effects on pup

[9]Sperm counts in laboratory rodents and rabbits generally have to be severely impacted to cause infertility. Rats may still be fertile with a 90% reduction in sperm count *(109).*

[10]Animals were treated with 1000 mg/kg bw/day for 1-week and then the dose was reduced to 600 mg/kg for 22–25 additional days.

survival and growth following maternal exposure to dose levels of bisphenol A defined by the NTP as "high" (>5 mg/kg bw/day). In rats, a ~20–36% decrease in the number of pups per litter is reported following maternal dosing with ≥500 mg/kg bw/day *(36, 37)*. Increases in fetal death and post-implantation loss are seen in rats treated with 1000 mg/kg bw/day during pregnancy *(36)*. Reductions in fetal weight or growth during postnatal life occur at oral dose levels of ≥300 mg/kg bw/day in rats *(36, 37)*. In mice, developmental toxicity is generally reported at higher oral doses in the form of fetal death, decreased number of live pups, reduced fetal or pup body weight at ≥875 mg/kg bw/day *(38–40)*, and reductions in body weight during postnatal life in the F1 generation (but not the F2 generation) at 600 mg/kg bw/day *(41)*. Fetal death in mice has also been observed in a recent study that reported embryo lethality following subcutaneous dosing with 10 mg/kg bw/day bisphenol A to pregnant mice *(113)*. Occasionally, decreases in pup survival have been reported at much lower oral dose levels, such as 0.0024 mg/kg bw/day in mice *(114)*. However, this effect is not typically reported at oral doses in this range even in studies from the same laboratory using a similar dosing regimen and the same source of mice *(115)*.

Delayed onset of puberty (assessed by day of vaginal opening) has been reported in the female offspring of rats orally treated with bisphenol A at 50 mg/kg bw/day during gestation *(43)* or 500 mg/kg bw/day during gestation and lactation *(37)*. In the study by Tyl *et al. (37),* this effect has been attributed to a decrease in body weight also observed at that dose and has not necessarily been considered a direct developmental effect *(27)*. However, decreased body weight was not observed in females at the dose where delayed vaginal opening was reported by Tinwell *et al. (43)*. This high dose effect of delayed vaginal opening is not the predicted effect of exposure to an estrogenic compound. It is worth noting that Tinwell *et al. (43)* did not detect any difference in onset of puberty in female rats when age at first estrus assessed by vaginal smear was used as the marker of puberty. Other "high" dose studies report no effect on onset of puberty in female rats exposed during gestation and lactation at maternal oral doses ranging from 3.2 to ~1000 mg/kg bw/day *(116–119)*. One "high" dose study reported an accelerated onset of puberty in female rats following subcutaneous injection of bisphenol A during early postnatal life at 105 and 427 mg/kg bw/day *(120)*. Delayed puberty in male rats treated during development has also been reported at oral doses of ≥50 mg/kg bw/day *(37, 42)*. This effect was associated with decreased body weight in the study by Tyl *et al. (37),* but not in the study by Tan *et al. (42)*. A delay in puberty of 1.8 days has also been reported in male mice at 600 mg/kg bw/day in a 2-generation reproductive toxicity study *(41)*.

With the exception of a possible morphological alteration of the urethra (discussed below) *(54),* bisphenol A has not been shown to cause malformations, such as skeletal birth defects or abnormally shaped or absent organs, in rats or mice at oral doses up to 1000 and 1250 mg/kg bw/day, respectively *(36, 38)*. An indication of a possible developmental delay, apparent delayed bone formation ("ossification"), was reported at an oral dose level of 1000 mg/kg bw/day *(36)*. A more subtle effect, cellular changes in the liver, in developmentally exposed animals has been reported at ≥50 mg/kg bw/day *(41)*.

"Low" Dose Developmental Effects (≤5 mg/kg bw/day)

Neural and Behavioral Alterations

The NTP concurs with the CERHR Expert Panel on Bisphenol A that there is a sufficiently consistent body of literature to suggest that perinatal or pubertal exposure to "low" doses of bisphenol A causes neural and behavioral alterations in rats and mice, especially related to the development

(96). In brief, estradiol has a clearer role in regulating male-typical brain and behavioral sexual differentiation in rodents compared to primates and humans. The sexual dimorphism of a number of the neural and behavioral endpoints affected by bisphenol A exposure, i.e., AVPV and LC volume/cell number, locomotor activity, exploration in the plus maze, have been shown to depend on estradiol formed perinatally in the male rodent brain via aromatization of testosterone secreted from the fetal and neonatal testes. To the extent that these effects of bisphenol A are due to its interactions with the estrogen receptor the translation of these findings to humans is not clear because there is currently no evidence that estrogen receptor signaling plays an essential role in male-typical brain and behavioral sexual differentiation in primates including humans *(96)*.

However, as discussed previously a number of studies suggest that bisphenol A may also exert biological effects by mechanisms that do not involve estrogen receptor binding. For this reason, future neural and behavioral studies of bisphenol A should not focus exclusively on estrogen receptor-mediated endpoints. For example, a number of male-typical brain and behavioral sexual dimorphisms in rodents depend on androgen receptor signaling, i.e., spinal nucleus of the bulbocavernosus, the posterodorsal medial amygdalar nucleus, the ventromedial hypothalamic nucleus, the suprachiasmatic nucleus, forebrain response to pheromones, play fighting and the preference of males to seek out female vs same-sex (male) urinary odors. These androgen receptor-mediated behaviors are considered to have more direct relevance to humans and should be more thoroughly assessed following bisphenol A exposure given suggestions that bisphenol A can modulate androgen receptor activity or expression levels.

In addition, several studies suggest that bisphenol A may interfere with the dopaminergic system. The CERHR Expert Panel on Bisphenol A did not consider most of these studies useful in the evaluation by due to experimental design limitations and/or use of a non-oral route of administration. Additional research that includes assessment of dopaminergic-related endpoints to address the limitations in the current literature would be helpful.

Future studies should also take precautions to distinguish between "organizational" and "activational" effects of hormones. Organizational effects are permanent and induced by hormones during perinatal life whereas activational effects are acute, generally reversible, and occur throughout life *(174)*. Many sexual and other behaviors reflect both organizational and activational influences of hormones *(175)*. Observed behavioral effects of perinatal bisphenol A could reflect organizational changes in the brain accomplished during the normal perinatal period of brain sexual differentiation or, alternatively, they could simply reflect group differences in plasma levels of circulating sex hormones at the time of adult testing. Only a small number of the studies in the bisphenol A literature controlled for the influence of differences in plasma levels of circulating sex hormones at the time of testing, for example by testing ovarectomized females, using females at the same stage of the estrous-cycle, or conducting assessments on pre-pubertal animals *(46, 48, 125)*.

Mammary Gland

There is evidence from rodent studies suggesting that perinatal exposure to bisphenol A via subcutaneous mini-pump at administered doses of 0.0025 to 1 mg/kg bw/day causes tissue changes ("lesions") in the mammary gland that may signal an increased susceptibility to develop mammary gland tumors later in life *(52, 53)*. Although these lesions have been described as preneoplastic, currently no data are available that assess whether the reported lesions progress to invasive carcinoma. For this reason, the evidence is not sufficient to conclude that bisphenol A is a rodent mammary

gland carcinogen or that bisphenol A presents a breast cancer hazard to humans.

While bisphenol A has not been shown to cause cellular changes or cancer of the mammary gland in female rats and mice exposed as adults *(176),* two recent studies suggest that exposure of rats to bisphenol A during gestation may lead to the development of "preneoplastic" lesions in adulthood, ductal hyperplasia and carcinoma *in situ*, that may potentially progress to tumors *(52, 53)*.

[*Technical comment:* During preparation and peer review of the draft NTP Brief on Bisphenol A a number of pathologists questioned the classification of the lesions with cribriform-like structures described as carcinoma *in situ* in the studies by Murray *et al.* and Durando *et al.* In addition, the degree of hyperplasia reported in these studies was described during the peer review as being of a relatively mild form and not necessarily of the type that presents the most concern for development of invasive breast cancer in women, i.e., focal areas of atypical hyperplasia (see the Peer Review Report for Bisphenol A *(96)* for additional discussion)].

In the study by Murray *et al. (53),* rats were treated with 0.0025 – 1 mg/kg bw/day bisphenol A during pregnancy by subcutaneous mini-pump. Significant increases in the incidence of hyperplastic ducts were reported in all dose groups of female offspring on postnatal day 50 and only in the lowest dose group of 0.0025 mg/kg bw/day on postnatal day 95 (sample sizes range from 4 – 6). Lesions described as carcinoma *in situ* were reported in female offspring in the 0.25 and 1 mg/kg bw/day groups on postnatal day 50 (25% incidence for both treatment groups) and postnatal day 95 (33% incidence for both treatment groups). These findings are supported by a study by Durando *et al. (52)*[13] where pregnant rats were treated with 0.025 mg/kg bw/day, again using a subcutaneous mini-pump. In this study, the percent of hyperplastic ducts was significantly increased in the female offspring at both postnatal days 110 and 180 (~2 – 5-fold). A non-significant increase in the incidence of ductal carcinoma *in situ* was noted following adult treatment with a subcarcinogenic dose of N-nitroso-N-methylurea, a chemical used in cancer research to assess susceptibility to carcinogens (2/15 compared to 0/10 in control animals).

These findings are generally consistent with other reports of changes in mammary gland growth and development following perinatal exposure to bisphenol A that are related to an altered rate of maturation, e.g., advanced fat pad maturation, delayed lumen formation, enhanced duct growth, adoption of a pregnancy-like state, enhanced responsiveness to secondary estrogenic exposures, and potentially increased susceptibility to carcinogenesis, e.g., increased number or density of terminal end buds and ducts *(52, 53, 177 – 183)*. These findings have been interpreted by some authors as indicating that developmental exposure to bisphenol A causes effects on breast tissue maturation that may lead to a predisposition to disease onset later in life *(52, 53, 181 – 183, 191)*.

[13]The study by Durando *et al. (52)* implied that 99.9% DMSO was used in the mini-pump ["Pumps are designed to deliver 25 bisphenol A (Sigma-Aldrich de Argentina S.A., Buenos Aires, Argentina) or only DMSO (99.9% molecular biology grade, Sigma-Aldrich de Argentina S.A.)"]. The manufacturer of the mini-pump does not recommend use of DMSO concentrations greater than 50% because it can degrade the pump reservoir material and potentially result in tissue inflammation and edema. For this reason, the CERHR Expert Panel on Bisphenol A considered this study critically flawed *(2).* The NTP concurs that use of a high concentration of DMSO is a technical short-coming, but is not convinced that this factor could account for the observed results. The NTP also considered the possibility that potential pump degradation could result in variations in administered dose, but concluded that the study was still useful to consider in the context of other findings.

With the exception of an oral dosing study conducted by Moral *et al. (183)* that reported an increased number of mammary gland terminal ducts in the female offspring of rats treated during gestation with 0.250 mg/kg/day, the cellular and tissue-level effects on the mammary gland occurred in studies where bisphenol A was administered by subcutaneous treatment via mini-pump at doses of 0.000025 to 10 mg/kg/day *(52, 53, 177, 179–182)*.

Certain aspects of mammary gland cancer differ between rats and humans, e.g., metastases are uncommon in rodents, but ductal hyperplasia and carcinoma *in situ*, are generally recognized as intermediary steps in chemical-induced mammary gland cancer in the rat and as preneoplastic lesions in the human *(184–187)*. The appearance of ductal hyperplasia and carcinoma *in situ* are similar enough between rats and humans that these findings in the rat are considered relevant to humans *(185)*. In humans, a greater than mild degree of ductal hyperplasia and ductal carcinoma *in situ* are associated with increased relative risk of developing invasive breast carcinoma. It is important to note that the development of these lesions does not guarantee the formation of tumors or cancer in rats or humans and they are most appropriately interpreted as risk factors. If similar changes occur in women, the increased relative risks for developing invasive breast cancer range from 1.5 to 5-fold for moderate and atypical ductal hyperplasia and 8.0 to 10.0-fold for ductal carcinoma *in situ (188)*. The relative risk is based on a comparison to women of the same age in the general population. For example, a 50-year old woman has a 1 in 39 chance of developing invasive breast cancer in the next 10 years. If a 50-year woman has atypical ductal hyperplasia, a form of ductal hyperplasia associated with a moderate level of increased relative risk (4 to 5-fold), then her chance of developing invasive breast cancer in the next 10 years increases to approximately 1 in 10 to 1 in 8.

The current literature is not sufficient to establish the reproducibility of the ductal lesion findings by multiple independent investigators. Bisphenol A was not shown to induce neoplastic or non-neoplastic lesions in the mammary gland of female rats (~74 and 135 mg/kg bw/day) or mice (650 and 1300 mg/kg bw/day) in two-year dietary cancer bioassays where exposure was initiated in young adult animals (5-weeks of age) *(176)*. However, these studies did not include perinatal exposure and the NTP recognizes that adult-only exposure may not be sufficient to detect chemical carcinogens in hormonally-responsive tissues such as the mammary gland *(187)*. Most of the toxicology studies of bisphenol A that included assessment of females following developmental exposure either (1) did not report examination of the mammary gland *(37, 43, 120, 189, 190)*, or (2) collected mammary gland tissue but did not prepare the tissue in a manner that would readily reveal these changes, i.e., whole mounts *(41, 107)*. The limited assessment of the mammary gland in these studies is critical because it is not clear that, if present, intraductal epithelial proliferations would have been detected during the routine histopathologic examinations. More severe mammary lesions were not reported in these studies. Although severe lesions or tumors could be detected during routine necropsy, the studies by Ema *et al. (107)* and Tyl *et al. (41)* were primarily designed to detect effects on reproduction and development and not tumor incidence. Animals were not followed up for a sufficiently long period of time to necessarily expect to observe tumors in control animals or differences in tumor incidence between treatment groups. In both of these studies, mammary gland tissues in the parental (F0) and F1 generations of females were only examined after weaning of their pups and the animals would have been well under one year of age at the time of tissue collection.

The NTP concurs with recent reviews *(2, 191)* that additional data are needed to more completely

understand the possible long-term consequences of disrupting mammary gland development in animals by bisphenol A exposure and its significance for human health. Namely, long-term follow-up studies with sufficient statistical power should be conducted to evaluate if the ductal hyperplasia and carcinoma *in situ* progress to mammary gland tumors, preferably without the use of a secondary chemical challenge in adulthood. In addition, conducting the appropriate pharmacokinetic studies to better understand the distribution of bisphenol A to target tissues with the subcutaneous mini-pump would aid in interpreting the results. While researchers predict that circulating levels of total and free bisphenol A in the subcutaneous mini-pump studies would be quite low based on the administered dose (≤ 1 mg/kg bw/day), the lack of supporting pharmacokinetic information limits the ability to make comparisons to human exposures.

Prostate Gland and Urinary Tract

There is some evidence that perinatal exposure to bisphenol A in rodents may alter prostate gland and urinary tract development and predispose the prostate to develop hormonally-induced pre-neoplastic lesions later in life. The evidence is not sufficient to conclude that bisphenol A is a rodent prostate gland carcinogen or that bisphenol A presents a prostate cancer hazard to humans.

In mice, exposure of pregnant dams to bisphenol A at an oral dose of 0.010 mg/kg bw/day has been shown in one study to alter prostate development in offspring by increasing the number of prostatic ducts, ductal volume, and the proliferation of a cell population implicated in the development of prostate cancer (basal epithelial cells) in one or more regions of the prostate *(54, 192)*. This study also reported a urinary tract deformation where the urethra narrows near the neck of the bladder, an effect that, if permanent, could contribute to urine flow disorders. These effects were observed in fetal mice and it is unclear if they persist into adulthood or relate to a clear adverse health outcome. It is important to note that other studies have not reported severe consequences of urinary tract constriction in adult animals exposed during development that might be predicted based on the finding by Timms *et al.* including bladder stones, hydronephrosis, hydroureter, or other indications of kidney toxicity.

In Sprague-Dawley rats, subcutaneous injection of neonates with 0.010 mg/kg bisphenol A followed by adult hormone treatment[14] was reported to cause 100% of the animals to develop "low" grade (3/10 animals) or "high" grade (7/10 animals) prostate intraepithelial neoplasia *(51)*.[15, 16] The incidence of prostate intraepithelial neoplastic (PIN) lesions in animals that did not receive the adult hormone

[14]Animals were given Silastic capsule implants packed with estradiol and testosterone that result in serum concentrations of ~75 pg/ml estradiol and 3 ng/ml testosterone. This hormone treatment is intended to mimic the ratio of estradiol to testosterone in the aging male.

[15]The classification scheme of "low" and "high" grade PIN lesions used by Ho *et al.* (2006) appears to be their own *(96)*.

[16]One other study assessed bisphenol A's ability to predispose the prostate to develop prostate intraepithelial neoplasia lesions and tumors *(193)*. In this study, female F344 rats were orally dosed with 0.05, 7.5, 30, or 120 mg/kg bw/day of bisphenol A during pregnancy and lactation. In order to induce prostate lesions and tumors, male offspring were treated with a chemical carcinogen, 3,2'-dimethyl-4-aminobiphenyl (DMAB). No statistically significant changes in prostate intraepithelial neoplasia lesions or carcinomas were observed. Differences between this study and the report of Ho *et al.* may be related to age at exposure (fetal versus neonatal and fetal), rat strain (F344 versus Sprague–Dawley), carcinogenic insult (DMAB versus estradiol+testosterone), route of administration (subcutaneous versus oral to dams), or other factor such as animal husbandry and housing.

strategies to improve the sensitivity of rodent models for detecting prostate cancer included using alternative models, e.g., genetically modified, and/or initiating exposure in perinatal life. In addition, NTP workshop participants suggested a more detailed histopathologic evaluation of the prostate because the assessment of human carcinogenic potential may be better determined based on chemical-induced preneoplastic changes rather than tumor incidence.

During its evaluation of bisphenol A exposure and prostate development, the NTP also considered a number of studies in rats or mice that have detected increased prostate weight at low doses *(115, 196)* or failed to detect this effect *(37, 41, 43, 107, 116, 122, 193, 197–201)*. Prostate weight effects have taken on a special significance in the controversy surrounding bisphenol A because elevated prostate weight was the first "low" dose finding reported in laboratory animals *(115)* and prompted numerous follow-up studies. Attempts to understand the basis for discordant findings has generated considerable scientific discussion and debate including their review at the NTP Low-Dose Peer Review workshop mentioned earlier *(97)*. In brief, the NTP believes that the overall conclusions of the Bisphenol A Subpanel of the NTP Low-Dose Peer Review remain valid with respect to "low" dose effects on prostate weight, i.e., increased prostate weight cannot be considered a general or reproducible finding.

More importantly, it is not clear that prostate weight should continue to be considered a critical endpoint in risk evaluations of bisphenol A given the relative crudeness of this measure. Changes in organ weight may be useful to identify potential target tissues, but become less important when additional data relating to structural, cellular, or functional integrity are available. Prostate enlargement does not correlate with the development of prostate histopathology or cancer in rodents, and the evaluation of prostate weight without corresponding assessment of histopathologic changes is not considered useful for determining carcinogenic potential *(202)*.

In addition, changes in prostate weight are not necessarily observed in the same bisphenol A studies that report prostatic cellular or tissue-level changes. For example, no effects on prostatic lobe weight were observed in studies that reported (1) increased incidence and susceptibility to develop prostate intraepithelial neoplastic lesions *(51)*, (2) changes in the prostatic periductal stroma and decreases in androgen-receptor positive stromal cells and epithelial cells positive for prostatic acid phosphatase (PAS), an enzyme produced by the prostate that can be found in higher amounts in men with prostate cancer *(203)*, and (3) increased expression of CK10 in adult mice exposed as fetuses to 0.020 mg/kg bw/day via treatment of the dam or during adulthood to high doses of bisphenol A (2–200 mg pellets implanted under the skin for 3-weeks) *(195)*.

Puberty and Sexual Maturation

NTP concurs with the CERHR Expert Panel on Bisphenol A that limited data are available at low doses to suggest an effect of accelerating the onset of puberty in female mice. In humans, early onset of puberty in girls is associated with elevated risk of developing breast cancer, early bone age maturation, and psychosocial impacts that include influencing age at first sexual intercourse and increasing risk for certain adolescent risk behaviors *(204–206)*. Depending on the magnitude of the effect, early onset of puberty in laboratory animals can be considered an "adverse" effect in reproductive toxicology *(204)*.

The consistency of the literature on "low" dose effects of bisphenol A related to puberty in female rodents is different in rats and mice. Of the eight studies in rats evaluated by the NTP, seven were interpreted as being "negative" *(37, 43, 53, 107, 122, 173, 207)* and one study was

considered "positive" *(52)*. Overall, the NTP considered the rat data to indicate that "low" doses of bisphenol A do not affect the onset of puberty in female rats. A total of six "low" dose mouse studies were identified and evaluated by the NTP. Of these, two reported effects consistent with accelerated puberty and one showed an alteration in events related to sexual maturation in females and were interpreted as "positive" and the other three were considered "negative."

The NTP strategy for evaluating the entire "low" dose literature on puberty in female rodents was to first conduct a detailed assessment of the mouse studies to determine whether any aspect of study design could account for the apparently contradictory results in this species. The most consistent difference between the "positive" and "negative" studies in mice is the approach used to measure a puberty-related event. Age at first estrus is the most accurate indicator of puberty in rodents. This occurs at the same time as vaginal opening in rats. However, in mice, vaginal opening does not correlate well with puberty and the first day of detecting cornified cells in a vaginal smear, a sign of first estrus, is the preferred measure used to indicate the onset of puberty *(208)*. Although accelerated vaginal opening is an expected response to estrogens in mice, the lack of simultaneous occurrence of vaginal opening and first estrus suggests that these events may be differentially regulated even though they are both estrogen responsive. Thus, the NTP considers vaginal opening to be a marker of sexual maturation, but not a surrogate measure of puberty, i.e., first estrus. The three mouse studies that reported effects consistent with an acceleration of a puberty-related event used first estrus as the marker of puberty *(48, 55, 189)*. In contrast, the "negative" studies used vaginal opening *(41, 178, 197)*. Each study also has its own limitation that complicates a straight-forward interpretation of the results, e.g., small sample size, positive control response, or use of subcutaneous injection to pregnant dams. Based on the analysis outlined below, the NTP concluded that the "positive" mouse studies provided limited evidence of an acceleration of puberty.

In contrast, the NTP concluded that the rat studies do not indicate an effect on puberty at "low" doses. The differences in outcomes cannot be attributed to the use of an insensitive strain or stock because a variety of rat models were used in the "negative" studies: Sprague-Dawley, Wistar, Wistar-Furth rats, Wistar-derived Alderley Park, CD, and Donryu. Moreover, three of the "negative" rat puberty studies reported other "low" dose effects *(53, 122, 173)*. The effects of bisphenol A on puberty in rats at "high" doses are more inconsistent than the "low" dose studies. Only one study has reported an effect on puberty in the predicted direction, i.e., acceleration following subcutaneous treatment on postnatal days 0 to 9 *(120)*. Other studies reported no effect *(116–119)* or a delay in puberty at ≥ 50 mg/kg bw/day *(37, 43)*. Four of these studies used a positive control group *(43, 116, 118, 120)*. In these studies, responses to potent estrogens based on age at vaginal opening ranged from no effect *(116)*, to a statistically significant small or moderate acceleration [1.7 days *(43)*; 2.4 days *(120)*; 3.6 days *(118, 119)*].

Mouse Studies

"Positive" Mouse Studies

- The largest magnitude of effect on puberty in female mice was reported by Ryan *et al.* *(48)*. In this study, age at first estrus was accelerated by 4.5 days in C57BL/6 mice whose dams were orally dosed with 0.2 mg/kg/day bisphenol A during gestation and lactation (GD3–PND21) *(48)*. Acceleration in puberty of approximately 6 days was reported in the ethinyl estradiol positive control group. The major limitation of this study was the relatively small sample sizes used for this endpoint (4–5 dams per treatment group).

- Howdeshell et *al. (55)* reported that female CF-1 offspring of dams orally-treated with 0.0024 mg/kg/day of bisphenol A during gestation (GD11–17) had a 2.5 day shorter interval between age at vaginal opening and first estrus. This study also evaluated whether fetal response to prenatal bisphenol A treatment differed based on intrauterine position (IUP). In some rodent species, females surrounded by 2 females *in utero* (0 male or 0M) have higher serum concentrations of estradiol and lower serum concentrations of testosterone compared to females surrounded by 2 males (2M). Intrauterine position effects have been reported for some behaviors or physiological characteristics, mostly in mice but also other rodents and swine *(209)*. The shorter interval of ~2.5 days between vaginal opening and first estrus in control and bisphenol A-treated animals was primarily attributed to a 5-day shortening of this interval in the 0M females leading the authors to hypothesize that animals with higher background exposures to estradiol may be more sensitive to bisphenol A exposure. The most significant limitation of this study is the interpretation of the interval between vaginal opening and first estrus. No effect was observed on age at first estrus in bisphenol A-treated mice[17] or age at vaginal opening. A shortening of the interval between these two events should not be interpreted as an acceleration in the onset of puberty and is more appropriately characterized as a alteration in the timing of events related to sexual maturation.

- The findings of Ryan *et al. (48)* and Howdeshell *et al. (55)* are supported by a subcutaneous injection study that noted a statistically significant 1-day earlier onset of first estrus and vaginal opening in female ICR/Jcl mice whose mothers were treated with 0.02 mg/kg bw/day bisphenol A during gestation (GD11–17) *(189)*. Although the reported magnitude of the effect in the bisphenol A-treated animals was small, ~1-day, the authors also reported that females in both the 0.002 and 0.02 treatment groups had significantly longer estrous cycles (a "classic" estrogenic response) compared to control animals. Accelerations in vaginal opening and puberty and lengthened estrous cycles were also observed in the diethylstilbestrol positive control groups. The interpretation of this study is limited by the small magnitude of an effect on age at first estrus and use of subcutaneous injection as the route of administration to pregnant dams.

"Negative" Mouse Studies

- Ashby *et al. (197)* did not detect an effect of bisphenol A on vaginal opening in the female offspring of CF-1 mice dosed orally with 0.002 or 0.02 mg/kg bw/day. The interpretation of this study is complicated by the response in the positive control group where diethylstilbestrol (0.0002 mg/kg bw/day) caused a significant 3.6-day delay in the age of vaginal opening compared to the vehicle control group. A delay in puberty is inconsistent with the predicted estrogenic effect of accelerated puberty for the diethylstilbestrol group.

- Markey *et al. (178)* did not report an effect of bisphenol A on age at vaginal opening in female CD-1 mice whose dams were dosed with 0.000025 or 0.00025 mg/kg bw/day via subcutaneous mini-pump. The authors noted that a portion of the mice showed partial vaginal opening approximately 4 days earlier than control animals. In addition, both bisphenol A groups had longer estrous cycles than control animals.

[17] Age at first estrus was significantly accelerated for 0M females versus controls in post hoc analyses (personal communication Frederick vom Saal, August 13, 2008)

- Tyl *et al.* (41) reported no effect of bisphenol A on age at vaginal opening in CD-1 mice using a multigenerational study design at dietary doses that ranged from 0.003 to 600 mg/kg bw/day. The positive control group used in the bisphenol A study, 17β-estradiol (0.08 mg/kg bw/day) caused the expected effect of accelerated vaginal opening. However, the experimental model used in this study did not appear to be sensitive in detecting estrogenic effects at low doses. In a separate multigenerational study designed to characterize the response in CD-1 mice to 17β-estradiol (0.0002 – 0.1 mg/kg bw/day), the authors did not report any effect on vaginal opening at doses below ~0.03 mg/kg/day *(210)*. In addition, estrous cycle length was unaffected at all doses. Thus, detecting an estrogenic effect of a weaker estrogen such as bisphenol A at very low doses (≤ ~0.03 mg/kg bw/day) would not be expected in this animal model.

Species Differences

There are other indications of species differences between rats and mice that may contribute to the inconsistent literature for bisphenol A. Research on the effects of pheromones in regulating puberty suggests that puberty may be more easily perturbed in mice compared to rats. Puberty in female mice can be accelerated when the mice are exposed to urine from a male. This effect has been reported more often and more consistently for mice than for rats *(211, 212)*. In addition, the IUP effect, which was an important factor in the Howdeshell *et al.* study, is better documented in mice compared to rats suggesting that this effect may be more robust in mice *(209)*. IUP was not considered in other studies in the bisphenol A literature, but it is worth noting that the studies by Markey *et al.* *(178)* and Ryan *et al.* *(48)* also show indications of a subpopulation of mice that may be more responsive to bisphenol A. As discussed earlier, Markey *et al.* commented that a portion of mice exhibited partial vaginal opening ~4 days earlier than control animals. In the Ryan *et al.* study, two of the five animals in the 0.200 mg/kg bw/day group that had a ~4.5 day statistically significant acceleration in puberty showed a much greater acceleration than the other animals in that group, i.e., ~10 days early onset of first estrus compared to the mean of the control group (public comment on the draft NTP Brief from Dr. Earl Gray, received May 23, 2008).

Other Effects Considered

A variety of other effects in laboratory animals have been linked to "low" dose bisphenol A exposure during development, including decreased sperm quantity or quality, obesity, disruption of meiosis, changes in reproductive hormone levels, or cellular effects in reproductive tissues. These effects had less impact in shaping NTP's conclusions on potential risks to humans from bisphenol A exposure than the developmental effects observed at "high" doses on survival and growth and the "low" dose effects on brain and behavior, mammary gland, prostate gland, and onset of puberty in females described above.

In some cases, the relationship between a specific cellular- or tissue-level finding and a potential health effect in the whole organism is unclear. This is because there is often uncertainty about the functional impact of a cellular or mechanistic finding, such as the altered level of a receptor protein or change in enzyme activity. For example, the potential health impact that may result from uterine changes characterized by altered ERα and ERβ expression and from an increase in the number and appearance of uterine epithelial cells is unclear *(213)*.

In other cases, the literature is not sufficiently developed. Newbold *et al.* *(214)* recently described a number of morphological changes in the ovaries and uteri of 18-month old mice that had received subcutaneous injections of bisphenol A at doses of 10, 100, or 1000 μg/kg on days 1 – 5 of life. Increases in cystic ovaries

and cystic endometrial hyperplasia were statistically significant in the 100 µg/kg dose group but not at 1000 µg/kg. Non-statistically significant increases in the incidence of a variety of other ovarian and uterine proliferative lesions and cysts were also reported. Replication of these findings and further study of the linkage of early and late occurring events will be important in establishing a better understanding of any long-term consequences of exposures of the developing organism to bisphenol A.

As mentioned earlier, NTP Briefs are not meant to serve as comprehensive reviews of the scientific literature. Only key study findings and issues that relate to NTP conclusions on concerns for potential reproductive and developmental health effects in humans are typically presented. However, three reported "low" dose health effects (obesity, decreased sperm count or quality, and abnormalities of meiosis) that ultimately had less impact in determining the NTP's conclusions are briefly discussed below in order to illustrate the interpretive challenges associated with this literature. Two examples of such effects, obesity and impacts on sperm, demonstrate findings that are not reported consistently enough to be considered reproducible. The third example relates to abnormalities of meiosis and is presented to demonstrate that effects predicted from *in vitro* studies are not necessarily observed in the *in vivo* studies.

Obesity

There is currently insufficient evidence to conclude that bisphenol A exposure during development predisposes laboratory animals to develop obesity or metabolic diseases such as diabetes, later in life. Obesity and metabolic disruption have become a research focus for bisphenol A based on several reports of increased postnatal growth following "low" dose exposure during development and several *in vitro* and *in vivo* studies that report effects related to altered carbohydrate and lipid regulation.

The NTP concurs with the CERHR Expert Panel on Bisphenol A that the effects of bisphenol A on body weight at "low" doses are inconsistent *(2)*. A number of studies in rats and mice report increases in postnatal growth following developmental exposure to bisphenol A at oral doses of 0.0024 – 1.2 mg/kg bw/day *(55, 155, 173, 215)* or a subcutaneous dose of 0.5 mg/kg bw/day *(179)*. Other "low" dose (≤ 5 mg/kg bw/day) studies in rats and mice have either not detected any significant effect on body weight *(43, 48, 50, 52, 107, 116, 122, 200, 214, 216)* or reported growth reductions *(37, 115, 146, 189, 217)*. Differences in study outcomes cannot easily be attributed to the use of a potentially insensitive rodent model or experimental protocol because several studies that did not detect any significant difference in body weight reported other effects at "low" dose levels *(48, 50, 52, 122, 217)*. The bases for the inconsistent findings are unclear, but may relate to factors such as diet and differences in experimental design or analysis.

The data are currently too limited to conclude that developmental exposure to bisphenol A causes diabetes or other metabolic disorders later in life. Two studies in laboratory animals have assessed endpoints related to carbohydrate or lipid regulation. In adult male mice, a single subcutaneous dose of 0.010 or 0.100 mg/kg bw/day bisphenol A caused decreased blood glucose and increased plasma insulin *(218)*. Additionally, increased pancreatic insulin content and insulin resistance were reported at 0.100 mg/kg bw/day (administered orally or by subcutaneous injection) after a slightly longer period of dosing (4-days) *(218)*. A recent study by Miyawaki *et al. (215)* assessed a variety of endpoints related to carbohydrate and lipid regulation in 1-month old mice that were exposed through maternal treatment during gestation and lactation with 0.001 or 0.010 µg/ml bisphenol A in drinking water (~0.26 and 2.42 mg/kg bw/during gestation). Endpoints included body weight, adipose tissue weight, and blood concentrations of leptin,

REFERENCES

1. Wilson NK, Chuang JC, Morgan MK, Lordo RA, Sheldon LS (2007) *Environ Res.* An observational study of the potential exposures of preschool children to pentachlorophenol, bisphenol-A, and nonylphenol at home and daycare. 103:9 – 20.

2. Chapin RE, Adams J, Boekelheide K, Gray LE, Jr., Hayward SW, Lees PS, McIntyre BS, Portier KM, Schnorr TM, Selevan SG, Vandenbergh JG, Woskie SR (2008) *Birth Defects Res B Dev Reprod Toxicol.* NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Bisphenol A. 83:157 – 395.

3. Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV (2007) *Reprod Toxicol.* Human exposure to bisphenol A (Bisphenol A). 24:139 – 177.

4. Le HH, Carlson EM, Chua JP, Belcher SM (2008) *Toxicol Lett.* Bisphenol A is released from polycarbonate drinking bottles and mimics the neurotoxic actions of estrogen in developing cerebellar neurons. 176:149 – 156.

5. Ye X, Kuklenyik Z, Needham LL, Calafat AM (2006) *J Chromatogr B Analyt Technol Biomed Life Sci.* Measuring environmental phenols and chlorinated organic chemicals in breast milk using automated on-line column-switching-highperformanceliquid chromatography-isotope dilution tandem mass spectrometry. 831: 110 – 115.

6. European-Union (2008) Updated European Risk Assessment Report 4,4'-Isopropylidenediphenol (bisphenol-A). Environment Addendum of February 2008 (to be read in conjunction with published EU RAR of Bisphenol A, 2003) *http://ecb.jrc.it/documents/Existing-Chemicals/RISK_ASSESSMENT/ADDENDUM/bisphenola_add_325.pdf.*

7. Lopez-Espinosa MJ, Granada A, Araque P, Molina-Molina JM, Puertollano MC, Rivas A, Fernandez M, Cerrillo I, Olea-Serrano MF, Lopez C, Olea N (2007) *Food Addit Contam.* Oestrogenicity of paper and cardboard extracts used as food containers. 24:95 – 102.

8. Calafat AM, Ye X, Wong LY, Reidy JA, Needham LL (2008) *Environ Health Perspect.* Exposure of the U.S. Population to bisphenol A and 4-tertiary-Octylphenol: 2003 – 2004. 116:39 – 44.

9. Moors S, Blaszkewicz M, Bolt HM, Degen GH (2007) *Mol Nutr Food Res.* Simultaneous determination of daidzein, equol, genistein and bisphenol A in human urine by a fast and simple method using SPE and GC-MS. 51:787 – 798.

10. Murakami K, Ohashi A, Hori H, Hibiya M, Shoji Y, Kunisaki M, Akita M, Yagi A, Sugiyama K, Shimozato S, Ito K, Takahashi H, Takahashi K, Yamamoto K, Kasugai M, Kawamura N, Nakai S, Hasegawa M, Tomita M, Nabeshima K, Hiki Y, Sugiyama S (2007) *Blood Purif.* Accumulation of bisphenol A in hemodialysis patients. 25:290 – 294.

11. Fernandez MF, Arrebola JP, Taoufiki J, Navalon A, Ballesteros O, Pulgar R, Vilchez JL, Olea N (2007) *Reprod Toxicol.* Bisphenol-A and chlorinated derivatives in adipose tissue of women. 24:259 – 264.

12. Padmanabhan V, Siefert K, Ransom S, Johnson T, Pinkerton J, Anderson L, Tao L, Kannan K (2008) *J Perinatol.* Maternal bisphenol-A levels at delivery: a looming problem? 28:258–263.

13. Dekant W, Volkel W (2008) *Toxicol Appl Pharmacol.* Human exposure to bisphenol A by biomonitoring: Methods, results and assessment of environmental exposures. 228:114–134.

14. Engel SM, Levy B, Liu Z, Kaplan D, Wolff MS (2006) *Reprod Toxicol.* Xenobiotic phenols in early pregnancy amniotic fluid. 21:110–112.

15. Schönfelder G, Wittfoht W, Hopp H, Talsness CE, Paul M, Chahoud I (2002) *Environ Health Perspect.* Parent bisphenol A accumulation in the human maternal-fetal-placental unit. 110:A703–707.

16. Kuroda N, Kinoshita Y, Sun Y, Wada M, Kishikawa N, Nakashima K, Makino T, Nakazawa H (2003) *J Pharm Biomed Anal.* Measurement of bisphenol A levels in human blood serum and ascitic fluid by HPLC using a fluorescent labeling reagent. 30:1743–1749.

17. Tan BLL, Mohd MA (2003) *Talanta.* Analysis of selected pesticides and alkylphenols in human cord blood by gas chromatograph-mass spectrometer. 61:385–391.

18. Domoradzki JY, Thornton CM, Pottenger LH, Hansen SC, Card TL, Markham DA, Dryzga MD, Shiotsuka RN, Waechter JM, Jr. (2004) *Toxicol Sci.* Age and dose dependency of the pharmacokinetics and metabolism of bisphenol A in neonatal Sprague-Dawley rats following oral administration. 77:230–242.

19. Miyakoda H, Tabata M, Onodera S, Takeda K (2000) *J Health Sci.* Comparison of conjugative activity, conversion of bisphenol A to bisphenol A glucuronide, in fetal and mature male rat. 46:269–274.

20. Matsumoto J, Yokota H, Yuasa A (2002) *Environ Health Perspect.* Developmental increases in rat hepatic microsomal UDP-glucuronosyltransferase activities toward xenoestrogens and decreases during pregnancy. 110:193–196.

21. Suiko M, Sakakibara Y, Liu MC (2000) *Biochem Biophys Res Commun.* Sulfation of environmental estrogen-like chemicals by human cytosolic sulfotransferases. 267: 80–84.

22. Hines RN (2008) *Pharmacol Ther.* The ontogeny of drug metabolism enzymes and implications for adverse drug events. 118:250–267.

23. Biles JE, McNeal TP, Begley TH (1997) *J Agric Food Chem.* Determination of bisphenol A migrating from epoxy can coatings to infant formula liquid concentrates. 45:4697–4700.

24. FDA (1996) Cumulative Exposure Estimated for bisphenol A (Bisphenol A), Individually for Adults and Infants from Its Use in Epoxy-Based Can Coatings and Polycarbonate (PC) Articles, verbal request of 10-23-95, memorandum to G. Diachenki, Ph.D, Division of Product Manufacture and Use, HGS-245, from Allan B. Bailey, Ph.D., Chemistry Review Branch, HFS-245. Department of Health and Human Services, Food and Drug Administration.

25. EFSA (2006) European Food Safety Authority: Opinion of the Scientific Panel on Food Additives, Flavourings, Pro-

cessing Aids and Materials in Contact with Food on a request from the Commission related to 2,2-bis(4-hydroxyphenyl) propane (bisphenol A). *http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620772817.htm*

26. European-Commission (2002) Opinion of the Scientific Committee on Food on Bisphenol A. *http://europa.eu.int/comm/food/fs/sc/scf/out128_en.pdf.*

27. European-Union (2003) Risk Assessment Report–4,4'-isopropylidenediphenol (Bisphenol A). *http://ecb.jrc.it/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/bisphenolareport325.pdf.*

28. Sun Y, Irie M, Kishikawa N, Wada M, Kuroda N, Nakashima K (2004) *Biomed Chromatogr.* Determination of bisphenol A in human breast milk by HPLC with column-switching and fluorescence detection. 18:501–507.

29. Wilson NK, Chuang JC, Lyu C, Menton R, Morgan MK (2003) *J Expo Anal Environ Epidemiol.* Aggregate exposures of nine preschool children to persistent organic pollutants at day care and at home. 13: 187–202.

30. Miyamoto K, Kotake M (2006) *Environ Sci.* Estimation of daily bisphenol A intake of Japanese individuals with emphasis on uncertainty and variability. 13:15–29.

31. Thomson BM, Grounds PR (2005) *Food Addit Contam.* Bisphenol A in canned foods in New Zealand: an exposure assessment. 22:65–72.

32. Hanaoka T, Kawamura N, Hara K, Tsugane S (2002) *Occup Environ Med.* Urinary bisphenol A and plasma hormone concentrations in male workers exposed to bisphenol A diglycidyl ether and mixed organic solvents. 59:625–628.

33. US EPA (1988) Recommendations and documentation of biological values for use in risk assessment. *http://www.epa.gov/iris/backgr-d.htm.*

34. Brenn-Struckhofova Z, Cichna-Markl M (2006) *Food Addit Contam.* Determination of bisphenol A in wine by sol-gel immunoaffinity chromatography, HPLC and fluorescence detection. 23:1227–1235.

35. LaKind JS, Naiman DQ (2008) bisphenol A (Bisphenol A) daily intakes in the United States: Estimates from the 2003–2004 NHANES urinary bisphenol A data. *J Expos Sci Environ Epidemiol*. In Press.

36. Kim JC, Shin HC, Cha SW, Koh WS, Chung MK, Han SS (2001) *Life Sci.* Evaluation of developmental toxicity in rats exposed to the environmental estrogen bisphenol A during pregnancy. 69: 2611–2625.

37. 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. 68:121–146.

38. Morrissey RE, George JD, Price CJ, Tyl RW, Marr MC, Kimmel CA (1987) *Fundam Appl Toxico.* The Developmental Toxicity of bisphenol A in Rats and Mice. 8: 571–582.

39. Tyl R, Myers CB, Marr MC. Abbreviated one-generation study of dietary bisphenol A (Bisphenol A) in CD-1® (Swiss) mice.

In. Research Triangle Park, NC: RTI (sponsored by the Society of the Plastics Industry, Inc.); 2002.

40. NTP (1985) Bisphenol A: reproduction and fertility assessment in CD-1 mice when administered in the feed. NTP-85-192. Research Triangle Park, NC.

41. 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 (Bisphenol A) in CD-1(R) (Swiss) mice. 104:362–384.

42. Tan BL, Kassim NM, Mohd MA (2003) *Toxicol Lett.* Assessment of pubertal development in juvenile male rats after subacute exposure to bisphenol A and nonylphenol. 143:261–270.

43. 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. 68:339–348.

44. Palanza PL, Howdeshell KL, Parmigiani S, vom Saal FS (2002) *Environ Health Perspect.* Exposure to a low dose of bisphenol A during fetal life or in adulthood alters maternal behavior in mice. 110:415–422.

45. Laviola G, Gioiosa L, Adriani W, Palanza P (2005) *Brain Res Bull.* D-Amphetamine-related reinforcing effects are reduced in mice exposed prenatally to estrogenic endocrine disruptors. 65:235–240.

46. Gioiosa L, Fissore E, Ghirardelli G, Parmigiani S, Palanza P (2007) *Horm Behav.* Developmental exposure to low-dose estrogenic endocrine disruptors alters sex differences in exploration and emotional responses in mice. 52:307–316.

47. Ceccarelli I, Della Seta D, Fiorenzani P, Farabollini F, Aloisi AM (2007) *Neurotoxicol Teratol.* Estrogenic chemicals at puberty change ERalpha in the hypothalamus of male and female rats. 29:108–115.

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

49. Della Seta D, Minder I, Belloni V, Aloisi AM, Dessi-Fulgheri F, Farabollini F (2006) *Horm Behav.* Pubertal exposure to estrogenic chemicals affects behavior in juvenile and adult male rats. 50:301–307.

50. Negishi T, Kawasaki K, Suzaki S, Maeda H, Ishii Y, Kyuwa S, Kuroda Y, Yoshikawa Y (2004) *Environ Health Perspect.* Behavioral alterations in response to fear-provoking stimuli and tranylcypromine induced by perinatal exposure to bisphenol A and nonylphenol in male rats. 112: 1159–1164.

51. 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: 5624–5632.

52. 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. 115:80–86.

53. 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. 23:383–390.

54. 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: 7014–7019.

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

56. Willhite CC, Ball GL, McLellan CJ (2008) *J Toxicol Environ Health B Crit Rev.* Derivation of a bisphenol A oral reference dose (RfD) and drinking-water equivalent concentration. 11:69–146.

57. Goodman JE, McConnell EE, Sipes IG, Witorsch RJ, Slayton TM, Yu CJ, Lewis AS, Rhomberg LR (2006) *Crit Rev Toxicol.* An updated weight of the evidence evaluation of reproductive and developmental effects of low doses of bisphenol A. 36:387–457.

58. vom Saal FS, Akingbemi BT, Belcher SM, Birnbaum LS, Crain DA, Eriksen M, Farabollini F, Guillette LJ, Jr., Hauser R, Heindel JJ, Ho SM, Hunt PA, Iguchi T, Jobling S, Kanno J, Keri RA, Knudsen KE, Laufer H, LeBlanc GA, Marcus M, McLachlan JA, Myers JP, Nadal A, Newbold RR, Olea N, Prins GS, Richter CA, Rubin BS, Sonnenschein C, Soto AM, Talsness CE, Vandenbergh JG, Vandenberg LN, Walser-Kuntz DR, Watson CS, Welshons WV, Wetherill Y, Zoeller RT (2007) *Reprod Toxicol.* Chapel Hill bisphenol A expert panel consensus statement: integration of mechanisms, effects in animals and potential to impact human health at current levels of exposure. 24:131–138.

59. vom Saal FS, Hughes C (2005) *Environ Health Perspect.* An extensive new literature concerning low-dose effects of bisphenol A shows the need for a new risk assessment. 113:926–933.

60. Richter CA, Birnbaum LS, Farabollini F, Newbold RR, Rubin BS, Talsness CE, Vandenbergh JG, Walser-Kuntz DR, vom Saal FS (2007) *Reprod Toxicol. In vivo* effects of bisphenol A in laboratory rodent studies. 24:199–224.

61. Melnick R, Lucier G, Wolfe M, Hall R, Stancel G, Prins G, Gallo M, Reuhl K, Ho SM, Brown T, Moore J, Leakey J, Haseman J, Kohn M (2002) *Environ Health Perspect.* Summary of the National Toxicology Program's report of the endocrine disruptors low-dose peer review. 110:427–431.

62. Wetherill YB, Akingbemi BT, Kanno J, McLachlan JA, Nadal A, Sonnenschein C, Watson CS, Zoeller RT, Belcher SM (2007) *Reprod Toxicol. In vitro* molecular mechanisms of bisphenol A action. 24: 178–198.

63. Nadal A, Ropero AB, Laribi O, Maillet M, Fuentes E, Soria B (2000) *Proc Natl Acad Sci U S A.* Nongenomic actions of estrogens and xenoestrogens by binding at a plasma membrane receptor unrelated to estrogen receptor alpha and estrogen receptor beta. 97:11603–11608.

64. Nadal A, Ropero AB, Fuentes E, Soria B, Ripoll C (2004) *Steroids.* Estrogen and xenoestrogen actions on endocrine pancreas: from ion channel modulation to activation of nuclear function. 69:531–536.

91. Calabrese EJ, Baldwin LA (2003) *Toxicol Sci.* The hormetic dose-response model is more common than the threshold model in toxicology. 71:246–250.

92. Taylor JA, Welshons WV, Vom Saal FS (2008) *Reprod Toxicol.* No effect of route of exposure (oral; subcutaneous injection) on plasma bisphenol A throughout 24h after administration in neonatal female mice. 25:169–176.

93. Pottenger LH, Domoradzki JY, Markham DA, Hansen SC, Cagen SZ, Waechter JM, Jr. (2000) *Toxicol Sci.* The relative bioavailability and metabolism of bisphenol A in rats is dependent upon the route of administration. 54:3–18.

94. Yamasaki K, Sawaki M, Takatsuki M (2000) *Environ Health Perspect.* Immature rat uterotrophic assay of bisphenol A. 108:1147–1150.

95. Kurebayashi H, Harada R, Stewart RK, Numata H, Ohno Y (2002) *Toxicol Sci.* Disposition of a low dose of bisphenol A in male and female cynomolgus monkeys. 68:32–42.

96. NTP (2008) Peer Review Report for the NTP Brief on Bisphenol A (*http://cerhr.niehs.nih.gov/chemicals/bisphenol/bisphenol.html)*.

97. NTP (2001) National Toxicology Program's Report of the Endocrine Disruptors Peer Review. *(http://ntp.niehs.nih.gov/ntp/htdocs/liason/LowDosePeerFinalRpt.pdf)*.

98. Haseman JK, Bailer AJ, Kodell RL, Morris R, Portier K (2001) *Toxicol Sci.* Statistical issues in the analysis of low-dose endocrine disruptor data. 61:201–210.

99. Itoh H, Iwasaki M, Hanaoka T, Sasaki H, Tanaka T, Tsugane S (2007) *Environ Health Prev Med.* Urinary bisphenol-A concentration in infertile japanese women and its association with endometriosis: A cross-sectional study. 12:258–264.

100. Wolff M, Engel S, Berkowitz G, Ye X SM, Zhu C, Wetmur J, Calafat A (2008) *Environ Health Perspect.* Prenatal phenol and phthalate exposures and birth outcomes 116:1092–1097.

101. Takeuchi T, Tsutsumi O (2002) *Biochem Biophys Res Commun.* Serum bisphenol A concentrations showed gender differences, possibly linked to androgen levels. 291:76–78.

102. Yang M, Kim SY, Chang SS, Lee IS, Kawamoto T (2006) *Environ Mol Mutagen.* Urinary concentrations of bisphenol A in relation to biomarkers of sensitivity and effect and endocrine-related health effects. 47:571–578.

103. Sugiura–Ogasawara M, Ozaki Y, Sonta S, Makino T, Suzumori K (2005) *Hum Reprod.* Exposure to bisphenol A is associated with recurrent miscarriage. 20: 2325–2329.

104. Yamada H, Furuta I, Kato EH, Kataoka S, Usuki Y, Kobashi G, Sata F, Kishi R, Fujimoto S (2002) *Reprod Toxicol.* Maternal serum and amniotic fluid bishenol A concentrations in the early second trimester. 16:735–739.

105. Takeuchi T, Tsutsumi O, Ikezuki Y, Takai Y, Taketani Y (2004) *Endocr J.* Positive relationship between androgen and the endocrine disruptor, bisphenol A, in normal women and women with ovarian dysfunction. 51:165–169.

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

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

108. Al-Hiyasat AS, Darmani H, Elbetieha AM (2002) *Eur J Oral Sci.* Effects of bisphenol A on adult male mouse fertility. 110:163–167. [Erratum: *Eur J Oral Sci.* (2003) 2111:2547].

109. Mangelsdorf I, Buschmann J, Orthen B (2003) *Regul Toxicol Pharmacol.* Some aspects relating to the evaluation of the effects of chemicals on male fertility. 37: 356–369.

110. Yamasaki K, Sawaki M, Noda S, Imatanaka N, Takatsuki M (2002) *Arch Toxicol.* Subacute oral toxicity study of ethynylestradiol and bisphenol A, based on the draft protocol for the "Enhanced OECD Test Guideline no. 407". 76:65–74.

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

112. Della Seta D, Minder I, Dessi-Fulgheri F, Farabollini F (2005) *Brain Res Bull.* Bisphenol-A exposure during pregnancy and lactation affects maternal behavior in rats. 65:255–260.

113. Tachibana T, Wakimoto Y, Nakamuta N, Phichitraslip T, Wakitani S, Kusakabe K, Hondo E, Kiso Y (2007) *J Reprod Dev.* Effects of bisphenol A (Bisphenol A) on placentation and survival of the neonates in mice. 53:509–514.

114. Howdeshell KL, vom Saal FS (2000) *Amer Zool.* Developmental exposure to bisphenol A: Interaction with endogenous estradiol during pregnancy in mice. 40: 429–437.

115. Nagel SC, vom Saal FS, Thayer KA, Dhar MG, Boechler M, Welshons WV (1997) *Environ Health Perspect.* Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative *in vivo* bioactivity of the xenoestrogens bisphenol A and octylphenol. 105:70–76.

116. 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. 55:399–406.

117. Takashima Y, Tsutsumi M, Sasaki Y, Tsujiuchi T, Kusuoka O, Konishi Y (2001) *J Toxicol Pathol.* Lack of effects of bisphenol A in maternal rats or treatment on response of their offspring to N-nitrosobis(2-hydroxypropyl)amine. 14:87–98.

118. Takagi H, Shibutani M, Masutomi N, Uneyama C, Takahashi N, Mitsumori K, Hirose M (2004) *Arch Toxicol.* 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. 78: 97–105.

119. US EPA (2007) Integrated Summary Report for Validation of a Test Method for Assessment of Pubertal Development and

Thyroid Function in Juvenile Female Rats as a Potential Screen in the Endocrine Disruptor Screening Program Tier-1 Battery (U.S. EPA Office of Science Coordination and Policy and Office of Research and Development, Washinton D.C. October 2007; *http://www.epa.gov/endo/pubs/assay-validation/pubertal_female_pr.htm)*.

120. Kato H, Ota T, Furuhashi T, Ohta Y, Iguchi T (2003) *Reprod Toxicol.* Changes in reproductive organs of female rats treated with bisphenol A during the neonatal period. 17:283 – 288.

121. Kubo K, Arai O, Ogata R, Omura M, Hori T, Aou S (2001) *Neurosci Lett.* Exposure to bisphenol A during the fetal and suckling periods disrupts sexual differentiation of the locus coeruleus and of behavior in the rat. 304:73 – 76.

122. 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. 45:345 – 356.

123. Funabashi T, Kawaguchi M, Furuta M, Fukushima A, Kimura F (2004) *Psychoneuroendocrinology.* 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. 29:475 – 485.

124. Patisaul HB, Fortino AE, Polston EK (2006) *Neurotoxicol Teratol.* Neonatal genistein or bisphenol-A exposure alters sexual differentiation of the AVPV. 28: 111 – 118.

125. Rubin BS, Lenkowski JR, Schaeberle CM, Vandenberg LN, Ronsheim PM, Soto AM (2006) *Endocrinology.* Evidence of altered brain sexual differentiation in mice exposed perinatally to low, environmentally relevant levels of bisphenol A. 147: 3681 – 3691.

126. Patisaul HB, Fortino AE, Polston EK (2007) *Neurotoxicology.* Differential disruption of nuclear volume and neuronal phenotype in the preoptic area by neonatal exposure to genistein and bisphenol-A. 28:1 – 12.

127. Nagao T, Saito Y, Usumi K, Kuwagata M, Imai K (1999) *Reprod Toxicol.* Reproductive function in rats exposed neonatally to bisphenol A and estradiol benzoate. 13: 303 – 311.

128. Toufexis D (2007) *J Neuroendocrinol.* Region- and sex-specific modulation of anxiety behaviours in the rat. 19: 461 – 473.

129. Dessi-Fulgheri F, Porrini S, Farabollini F (2002) *Environ Health Perspect.* Effects of perinatal exposure to bisphenol A on play behavior of female and male juvenile rats. 110:403 – 407.

130. Farabollini F, Porrini S, Della Seta D, Bianchi F, Dessi-Fulgheri F (2002) *Environ Health Perspect.* Effects of perinatal exposure to bisphenol A on sociosexual behavior of female and male rats. 110:409 – 414.

131. Kawai K, Nozaki T, Nishikata H, Aou S, Takii M, Kubo C (2003) *Environ Health Perspect.* Aggressive behavior and serum testosterone concentration during the maturation process of male mice: the effects of fetal exposure to bisphenol A. 111: 175 – 178.

132. Carr R, Bertasi F, Betancourt A, Bowers S, Gandy BS, Ryan P, Willard S (2003) *J Toxicol Environ Health A.* Effect of neo-

stanceschimiques.gc.ca/challenge-defi/batch-lot_2_e.html#release.

173. 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. 109:675–680.

174. Arnold AP, Breedlove SM (1985) *Horm Behav.* Organizational and activational effects of sex steroids on brain and behavior: a reanalysis. 19:469–498.

175. Li AA, Baum MJ, McIntosh LJ, Day M, Liu F, Gray LE, Jr. (2008) *Neurotoxicology.* Building a scientific framework for studying hormonal effects on behavior and on the development of the sexually dimorphic nervous system. 29:503–518.

176. NTP (1982) Carcinogenesis Bioassay of bisphenol A (CAS No. 80–05–7) in F344 Rats and B6C3F1 Mice (Feed Study). TR-215. *http://ntp.niehs.nih.gov/go/14366.*

177. 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: 1215–1223 [Erratum: *Biol Reprod. (*2004) 1271:1753].

178. 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. 5:67–75.

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

180. 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. 146:4138–4147.

181. Vandenberg LN, Maffini MV, Wadia PR, Sonnenschein C, Rubin BS, Soto AM (2007) *Endocrinology.* Exposure to environmentally relevant doses of the xenoestrogen bisphenol-A alters development of the fetal mouse mammary gland. 148: 116–127.

182. Wadia PR, Vandenberg LN, Schaeberle CM, Rubin BS, Sonnenschein C, Soto AM (2007) *Environ Health Perspect.* Perinatal bisphenol A exposure increases estrogen sensitivity of the mammary gland in diverse mouse strains. 115:592–598.

183. Moral R, Wang R, Russo IH, Lamartiniere CA, Pereira J, Russo J (2008) *J Endocrinol.* Effect of prenatal exposure to the endocrine disruptor bisphenol A on mammary gland morphology and gene expression signature. 196:101–112.

184. Thompson HJ, Singh M (2000) *J Mammary Gland Biol. Neoplasia.* Rat models of premalignant breast disease. 5:409–420.

185. Singh M, McGinley JN, Thompson HJ (2000) *Lab Invest.* A comparison of the histopathology of premalignant and malignant mammary gland lesions induced in sexually immature rats with those occurring in the human. 80:221–231.

186. Russo J, Gusterson BA, Rogers AE, Russo IH, Wellings SR, van Zwieten MJ (1990) *Lab Invest.* Comparative study of human and rat mammary tumorigenesis. 62: 244–278.

187. Thayer KA, Foster PM (2007) *Environ Health Perspect.* Workgroup Report: National Toxicology Program workshop on hormonally induced reproductive tumors-relevance of rodent bioassays. 115: 1351–1356 [see also *http://ntp.niehs.nih.gov/go/18592].*

188. Fitzgibbons PL, Henson DE, Hutter RV (1998) *Arch Pathol Lab Med.* Benign breast changes and the risk for subsequent breast cancer: an update of the 1985 consensus statement. Cancer Committee of the College of American Pathologists. 122: 1053–1055.

189. 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: 117–122.

190. Suzuki A, Sugihara A, Uchida K, Sato T, Ohta Y, Katsu Y, Watanabe H, Iguchi T (2002) *Reprod Toxicol.* Developmental effects of perinatal exposure to bisphenol-A and diethylstilbestrol on reproductive organs in female mice. 16:107–116.

191. Keri RA, Ho SM, Hunt PA, Knudsen KE, Soto AM, Prins GS (2007) *Reprod Toxicol.* An evaluation of evidence for the carcinogenic activity of bisphenol A. 24: 240–252.

192. Wang S, Garcia AJ, Wu M, Lawson DA, Witte ON, Wu H (2006) *Proc Natl Acad Sci USA.* Pten deletion leads to the expansion of a prostatic stem/progenitor cell subpopulation and tumor initiation. 103: 1480–1485.

193. 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. 28:165–171.

194. 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: 902–908.

195. 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. 75:745–756.

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

197. 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*. 30:156–166.

198. Cagen SZ, Waechter JM, Jr., Dimond SS, Breslin WJ, Butala JH, Jekat FW, Joiner RL, Shiotsuka RN, Veenstra GE, Harris LR (1999) *Regul Toxicol Pharmacol.* Normal reproductive organ development in Wistar rats exposed to bisphenol A in the drinking water. 30:130–139.

199. Kato H, Furuhashi T, Tanaka M, Katsu Y,

Watanabe H, Ohta Y, Iguchi T (2006) *Reprod Toxicol.* Effects of bisphenol A given neonatally on reproductive functions of male rats. 22:20 – 19.

200. Howdeshell KL, Furr J, Lambright CR, Wilson VS, Ryan BC, Gray LE, Jr. (2008) *Toxicol Sci.* Gestational and lactational exposure to ethinyl estradiol, but not bisphenol A, decreases androgen-dependent reproductive organ weights and epididymal sperm abundance in the male Long Evans Hooded rat. 102:371 – 382.

201. Nagao T, Saito Y, Usumi K, Yoshimura S, Ono H (2002) *Reprod Toxicol.* Low-dose bisphenol A does not affect reproductive organs in estrogen-sensitive C57BL/6N mice exposed at the sexually mature, juvenile, or embryonic stage. 16:123 – 130.

202. Milman HA, Bosland MC, Walden PD, Heinze JE (2002) *Regul Toxicol Pharmacol.* Evaluation of the adequacy of published studies of low-dose effects of bisphenol A on the rodent prostate for use in human risk assessment. 35:338 – 346.

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

204. Golub MS, Collman GW, Foster PM, Kimmel CA, Rajpert-De Meyts E, Reiter EO, Sharpe RM, Skakkebaek NE, Toppari J (2008) *Pediatrics.* Public health implications of altered puberty timing. 121 Suppl 3:S218 – 230.

205. Trichopoulos D, Adami HO, Ekbom A, Hsieh CC, Lagiou P (2008) *Int J Cancer.* Early life events and conditions and breast cancer risk: from epidemiology to etiology. 122:481 – 485.

206. Partsch CJ, Sippell WG (2001) *Hum Reprod Update.* Pathogenesis and epidemiology of precocious puberty. Effects of exogenous oestrogens. 7:292 – 302.

207. 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. 50:349 – 360.

208. Cooper RL, Goldman, J.M., Vandenbergh, J.G. (1993) Monitoring of the estrous cycle in the laboratory rodent by vaginal lavage. In: Heindel, J.J., Chapin, R.E. (Eds.), Female Reproductive Toxicology. *Methods Toxicol.*, Vol. 3B. Academic Press, pp. 45–56.

209. Ryan BC, Vandenbergh JG (2002) *Neurosci Biobehav Rev.* Intrauterine position effects. 26:665 – 678.

210. Tyl RW, Myers CB, Marr MC, Sloan CS, Castillo NP, Veselica MM, Seely JC, Dimond SS, Van Miller JP, Shiotsuka RS, Stropp GD, Waechter JM, Jr., Hentges SG (2008) *Toxicol Sci.* Two-generation reproductive toxicity evaluation of dietary 17beta-estradiol (E2; CAS No. 50 – 28 – 2) in CD-1 (Swiss) mice. 102:392 – 412.

211. Vandenbergh JG (1989) *J Anim Sci.* Coordination of social signals and ovarian function during sexual development. 67: 1841 – 1847.

212. Schank JC, Alberts JR (2000) *Horm Behav.* Effects of male rat urine on reproductive

and developmental parameters in the dam and her female offspring. 38:130–136.

213. Schönfelder G, Friedrich K, Paul M, Chahoud I (2004) *Neoplasia.* Developmental effects of prenatal exposure to bisphenol A on the uterus of rat offspring. 6:584–594.

214. Newbold RR, Jefferson WN, Padilla-Banks E (2007) *Reprod Toxicol.* Long-term adverse effects of neonatal exposure to bisphenol A on the murine female reproductive tract. 24:253–258.

215. Miyawaki J, Sakayama K, Kato H, Yamamoto H, Masuno H (2007) *J Atheroscler Thromb.* Perinatal and postnatal exposure to bisphenol A increases adipose tissue mass and serum cholesterol level in mice. 14:245–252.

216. Kobayashi K, Miyagawa M, Wang RS, Sekiguchi S, Suda M, Honma T (2002) *Ind Health.* Effects of *in utero* and lactational exposure to bisphenol A on somatic growth and anogenital distance in F1 rat offspring. 40:375–381.

217. Talsness CE, Fialkowski O, Gericke C, Merker HJ, Chahoud I (2000) *Congen Anom.* The effects of low and high doses of bisphenol A on the reproductive system of female and male rat offspring. 40:94–107.

218. Alonso-Magdalena P, Morimoto S, Ripoll C, Fuentes E, Nadal A (2006) *Environ Health Perspect.* The estrogenic effect of bisphenol A disrupts pancreatic beta-cell function *in vivo* and induces insulin resistance. 114:106–112.

219. Masuno H, Iwanami J, Kidani T, Sakayama K, Honda K (2005) *Toxicol Sci.* Bisphenol A accelerates terminal differentiation of 3T3-L1 cells into adipocytes through the phosphatidylinositol 3-kinase pathway. 84: 319–327.

220. Masuno H, Kidani T, Sekiya K, Sakayama K, Shiosaka T, Yamamoto H, Honda K (2002) *J Lipid Res.* Bisphenol A in combination with insulin can accelerate the conversion of 3T3-L1 fibroblasts to adipocytes. 43:676–684.

221. Phrakonkham P, Viengchareun S, Belloir C, Lombes M, Artur Y, Canivenc-Lavier MC (2008) *J Steroid Biochem Mol Biol.* Dietary xenoestrogens differentially impair 3T3-L1 preadipocyte differentiation and persistently affect leptin synthesis. 110:95–103.

222. Sakurai K, M. Kawazuma, T. Adachi, T. Harigaya, Y. Saito, N. Hashimoto and C. Mori (2004) *Br. J. Pharmacol.* Bisphenol A affects glucose transport in mouse 3T3-F442A adipocytes. 141:209–214.

223. Adachi T, Yasuda, K., Mori, C., Yoshinaga, M., Aoki, N., Tsujimoto, G. and Tsuda, K. (2005) *Food Chem Toxicol.* Promoting insulin secretion in pancreatic islets by means of bisphenol A and nonylphenol via intracellular estrogen receptors. 43:713–719.

224. Ropero AB, Alonso-Magdalena P, Garcia-Garcia E, Ripoll C, Fuentes E, Nadal A (2008) *Int J Androl.* Bisphenol-A disruption of the endocrine pancreas and blood glucose homeostasis. 31:194–200.

225. Aikawa H, Koyama S, Matsuda M, Nakahashi K, Akazome Y, Mori T (2004) *Cell Tissue Res.* 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. 315:119–124.

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Expert Panel Report

NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Bisphenol A

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Preface

The National Toxicology Program (NTP)[1] established the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) in June 1998. The purpose of the CERHR is to provide timely, unbiased, scientifically sound evaluations of the potential for adverse effects on reproduction or development resulting from human exposures to substances in the environment. The NTP-CERHR is headquartered at NIEHS, Research Triangle Park, NC, and is staffed and administered by scientists and support personnel at NIEHS.

Bisphenol A is a high-production volume chemical used in the production of epoxy resins, polyester resins, polysulfone resins, polyacrylate resins, polycarbonate plastics, and flame retardants. Polycarbonate plastics are used in food and drink packaging; resins are used as lacquers to coat metal products such as food cans, bottle tops, and water supply pipes. Some polymers used in dental sealants and tooth coatings contain bisphenol A. Exposure to the general population can occur through direct contact with bisphenol A or by exposure to food or drink that has been in contact with a material containing bisphenol A. CERHR selected bisphenol A for evaluation because of (1) high production volume; (2) widespread human exposure; (3) evidence of reproductive toxicity in laboratory animal studies; and (4) public concern for possible health effects from human exposures.

Relevant literature on bisphenol A was identified from searches of the PubMed (Medline) and Toxline databases through February 2007 using the term ''bisphenol'' and the bisphenol A CAS RN (80-05-7). References were also identified from databases such as REPROTOX, HSDB, IRIS, and DART, from the bibliographies of the literature reviewed, by members of the expert panel, and in public comments.

CERHR convened a 12-member, independent panel of government and non-government scientists to evaluate the scientific studies on the potential reproductive and developmental hazards of bisphenol A. The expert panel met publicly on March 5–7, 2007 and August 6–8, 2007. The Expert Panel Report on Bisphenol A is intended to (1) interpret the strength of scientific evidence that bisphenol A is a reproductive or developmental toxicant based on data from in vitro, animal, or human studies; (2) assess the extent of human exposures to include the general public, occupational groups, and other sub-populations; (3) provide objective and scientifically thorough assessments of the scientific evidence that adverse reproductive and developmental health effects may be associated with such exposures; and (4) identify knowledge gaps to help establish research and testing priorities to reduce uncertainties and increase confidence in future evaluations. This report has been reviewed by members of the expert panel and by CERHR staff scientists. Copies of this report have been provided to

[1]NTP is an interagency program headquartered in Research Triangle Park, NC, at the National Institute of Environmental Health Sciences, a component of the National Institutes of Health.

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litter as the unit. It is unclear when the dams were killed and analyzed. The absence of understandable dose-related effects complicates interpretation at these low doses; although the possibility of unusual low dose effects cannot be discounted.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for inclusion into the evaluation process, due to small sample size and poor documentation and communication about what was done.

3.2.1.2 Evaluation of reproductive organ development: Talsness et al. (2000), supported by the German Federal Ministry for Environmental Protection and Radiation Security, examined the effect of prenatal bisphenol A exposure on the reproductive systems of male and female rats. **[No information was provided about feed, caging, and bedding materials used.]** On GD 6–21, Sprague–Dawley rats ($n = 18–20$/group) were gavaged with 2% corn starch vehicle or bisphenol A [purity not indicated] at 0.1 or 50 mg/kg bw/day. A group of 11 dams was gavaged with 0.2 mg/kg bw/day ethinyl estradiol. Litters were weighed during the lactation period. Pups were weaned on PND 22 (according to Table 1 of the study, PND 1 was apparently the day of birth) and males and females were separated around PND 30. Vaginal opening was examined in 42–91 female offspring/group, and estrous cyclicity was monitored over a 3-week period in 42–53 females/group. At 4 months of age, 5–10 females/group were killed during diestrus and 20 females/group were killed while in estrus. A histopathological evaluation of vaginal tissue was conducted in 5 animals **[assumed 5/group]**. In 44–112 male offspring/group, anogenital distance was measured on PND 3, 15, and 21 and days of testicular descent and preputial separation were recorded. Males were killed on PND 70 ($n = 20$/group) or 170 ($n = 17–20$/group). Blood LH and testosterone concentrations were measured in 14–20 animals/group/time period. Sperm and spermatid numbers and sperm production and transit rates were determined in all offspring. Histopathological evaluation of the testis was conducted in 2 animals **[assumed/group]**. Body, reproductive organ, and liver weights were measured in all male and female offspring killed. Data from female rats were analyzed by ANOVA with post-hoc Dunnett test or Fisher test. Data from male rats were analyzed by ANOVA and Dunnett test. **[It appears that offspring were considered the statistical unit.]**

Pup body weights at birth were unaffected in the bisphenol A group, but on PND 22, pup body weights were lower **[by 28%]** in the low-dose group than in the control group. Study authors noted that the mean litter size in the low-dose group was larger by 2.6 pups than in the control group. Vaginal opening was delayed in the low-dose group and accelerated in the high-dose group. When estrous cyclicity data were evaluated according to total number of cycles, there was an increase in estrous phases lasting more than 1 day and prolongation of the cycle length in the high-dose group. Evaluation of estrous cycles by individual rat indicated a decrease in the percentage of low-dose females with 3 consecutive 1-day estrus phases. The only terminal body and organ weight effects occurred in the low-dose group and included decreased absolute liver weight in females killed in estrus and decreased body and uterus weights in females killed in diestrus or in estrus. There were no effects on relative organ weights. Histological observations in vaginal tissue of bisphenol A-exposed rats included less pronounced cornification during estrus and more pronounced mucification during diestrus, with magnitude of effect greater in the low- than the high-dose group. Observations in the animals exposed to ethinyl estradiol included decreased pup birth weight, delayed vaginal opening, near-persistent estrus, decreased absolute and relative uterus weights, and changes in vaginal histology similar to those described for the low-dose bisphenol A group.

Decreased anogenital distances was observed in the bisphenol A groups during all three time periods for male offspring, but the effect remained statistically significant only in the high-dose group when normalized for body weight. Testicular descent and preputial separation were delayed in the low-dose group. Organ weight effects that remained significant following adjustment for body weight included increased prostate weight in the high-dose group on PND 70 and increased testicular and epididymal weights in the low-dose group on PND 170. There was no effect on sperm morphology. Blood testosterone concentration was decreased in the high-dose group on PND 70, and blood LH concentration was increased in the high-dose group on PND 170. Testicular histopathology observations in the low-dose group on PND 70 included cellular debris in lumens, pyknotic nuclei in spermatids, and apoptotic debris in the region of the spermatogonia and primary spermatocyte. In testes of 70-day-old animals of the high-dose group, there were central necrotic masses, low numbers of meiotic figures in spermatocytes, and low spermatozoa numbers. On PND 170, observations in testes from the low-dose group included low spermatozoa numbers, a thin layer of spermatocyte meiotic figures, and apoptotic debris in region of spermatids. Low spermatocyte meiotic figures were the only testicular observation in the high-group on PND 170. Effects observed in the ethinyl estradiol group included increased anogenital distance, delayed testicular descent, accelerated preputial separation, decreased testis and prostate weights, decreased sperm counts and production, increased LH concentrations, increased testosterone concentrations on PND 170, apoptotic debris, and/or low sperm numbers in testes.

Study authors concluded that prenatal exposure to bisphenol A disrupts the reproductive systems of both male and female rats and that the effects do not occur according to a classic dose–response curve, which is generally observed in toxicology studies.

Strengths/Weaknesses: Strengths are the postnatal evaluation of various endpoints to "pup" adulthood and that the concentration of the dosing solutions was verified. Based on the description of numbers of pups contributing to various endpoints, however, the authors do not appear to have used the litter as the unit of analysis. These inflated numbers subjected to analysis complicate the interpretation of findings, especially for PND 1–21 measures. A weakness also is that only 2 dose levels were examined. The vaginal opening data for the controls were outside the normal range for Sprague–Dawley rats. It is unclear how the estrous cycle data were analyzed. The F_1 data were not analyzed correctly. Data may be suggestive of developmental disruptions at both doses, but the magnitudes are likely unreliable, and the

group, as well as 19 offspring from 11 dams in the 0.2 mg/kg/d 17α-ethinyl estradiol group (G. Schönfelder, personal communication, July 20, 2007)]. [Exact litter representation for animals collected during diestrus was not provided.]Vaginas were fixed in Bouin solution and a histopathological evaluation was conducted. Western blot analyses were conducted to measure expression of ERα and ERβ. **[It does not appear that statistical evaluations were conducted.]**

Qualitative descriptions of vaginal histopathology changes and ER expression were provided by the study authors. Low-dose animals killed during the estrous stage lacked keratinization of the surface epithelium and demonstrated reduced thickness of the total epithelium. Similar but less pronounced effects were observed in rats of the high-dose bisphenol A group. Vaginal findings were similar in the positive control group, and slight desquamation of the superficial layers was also observed. There were no differences in vaginal histopathology findings in rats killed during the diestrous stage. No ERβ was observed in vaginas of rats from any treatment group. Full-length ERα expression was not observed in either bisphenol A group during estrus, but ERα in the bisphenol A-exposed groups did not differ from the control group during the diestrous stage. ERα in vaginas obtained from the positive control group was either reduced or was not detected. The study authors concluded that altered vaginal morphology following bisphenol A treatment appears to be due to down-regulation of ERα.

Strengths/Weaknesses: Vaginal histopathology of female offspring is of interest but the quality of the study cannot be judged due to unclear methodology. Uncertainty about the numbers of animals, the number of offspring examined and the lack of statistical accounting for litter effects are significant weaknesses.

Utility (Adequacy) of the CERHR Evaluation Process: This study is inadequate for the evaluation process for the reasons detailed above.

Schönfelder et al. (2004), supported by the German Federal Ministry for Environmental Protection and Radiation Security, examined the effects of prenatal bisphenol A exposure on the rat uterus. **[No information was provided about composition of feed, caging, or bedding.]** Sprague–Dawley rats **[number treated not specified]** were gavaged with bisphenol A **[purity not reported]** at 0 (2% corn starch vehicle), 0.1, or 50 mg/kg bw/day on GD 6–21. **[Author clarified that the purity of bisphenol A was ≥98%; Altromin 1324 rodent chow was used (obtained from Altromin GmbH); bedding was wood shavings obtained from Altromin GmbH; caging was Type III macrolon cages (G. Schönfelder, personal communication, July 20, 2007).]** The high bisphenol A dose was selected because it was reported to be the no observed effect level (NOEL) recommended by the Society of the Plastics Industry. A positive control group was gavaged with 0.2 mg/kg bw/day ethinyl estradiol on GD 6–21. Estrous cyclicity was examined for 3 weeks in 6 female offspring/group beginning at 3 months of age. Six female offspring/group were killed at 4 months of age on the day of estrus. Body and reproductive organ weights were measured. Uteri were fixed in methacarn solution and sectioned. Examinations of uterine morphology were conducted. Immunohistochemistry techniques were used to detect ERα and ERβ in the uterus, and results were verified by Western blot. Data were analyzed by Mann–Whitney test. **[It was not clear if data were analyzed on a per litter or per offspring basis.] [Author states that each female came from a different litter so the data were analyzed on a per litter basis (G. Schönfelder, personal communication, July 20, 2007).]** Statistically significant findings are summarized in Table 72. Effects observed at both dose levels were increased epithelial cell nuclei, epithelial nuclei with condensed chromatin, and epithelial cells with cavities and reduced ERβ-positive cells in uterine tissue. Additional effects observed only at the high-dose included decreased thickness of luminal epithelium and increased ERα-positive cells in the epithelium. Similar findings were observed following treatment with ethinyl estradiol. The study authors concluded that prenatal bisphenol A exposure causes uterine effects in rat offspring.

Table 72
Uterine Effects in Rats Exposed to Bisphenol A During Prenatal Development

	Dose, mg/kg bw/day	
Endpoint	0.1	50
Thickness of luminal epithelium	↔	↓38%
Epithelial nuclei[a]	↑69%	↑89%
Epithelial nuclei with condensed chromatin	↑2.7-fold	↑3.1-fold
Epithelial cells with cavities	↑2.1-fold	↑ 1.9-fold
ERα positive cells in epithelium	↔	↑67%
ERβ-positive cells in uterine tissue	↓88%	↓88%

[a]It is unclear if authors were referring to numbers of nuclei.

Strengths/Weaknesses: A strength is the examination of effects on uterine indices in female offspring. A slight weakness is the use of only 6 females per group; however, the panel noted that the results appeared to be consistent across animals and across endpoints, especially in the 50 mg/kg bw/day treatment group.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation process.

Wistuba et al. (2003), supported by the German Federal Ministry of Education and Science, examined the effects of prenatal exposure on testicular histology and sperm endpoints in rats. **[No information was provided about chow, bedding, or caging.]** Sprague–Dawley rats were gavaged with 0 (2% corn starch suspension vehicle), 0.1, or 50 mg/kg bw/day bisphenol A **[purity not reported]** on GD 6–21 (GD 0 = day of sperm detection). A third group was treated with 0.02 mg/kg bw/day ethinyl estradiol. The high-dose was said to correspond to the current accepted no observed adverse effect level (NOAEL) and the lower dose was selected to determine if effects occurred at lower doses. It appears that the number of dams treated was 2 in the control group, 4 in the low-dose group, 1 in the high-dose group, and 4 in the ethinyl estradiol group. Litters were weighed during the lactation period. Pups were weaned on PND 22 **[day of birth not defined]**. Male offspring were killed between the ages of ~9–12 months. The number of males killed was 5 from 2 litters in the control group, 15 from 4 litters in the low-dose

significance of the changes. Weaknesses include use of the s.c. pump as a route of administration and use of DMSO as a vehicle.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for inclusion due to the use of 99.9% DMSO as a vehicle to administer BPA via s.c. pump. As discussed in earlier, the use of >50% DMSO as a vehicle for ALZET mini-pump studies is a clear contravention of the directions for mini-pump use, as it accelerates the breakdown of the mini-pumps

Naciff et al. (2002), from the Procter and Gamble Company, examined the effects of prenatal bisphenol A exposure on gene expression and, to a limited extent, development in female rat reproductive organs. Pregnant Sprague–Dawley rats were fed Purina 5K96, a casein-based soy- and alfalfa-free diet. **[Composition of caging and bedding materials was not reported.]** The rats were assigned to groups (≥7 rats/group) s.c. injected with bisphenol A (~99% purity) in DMSO vehicle at 0, 5, 50, or 400 mg/kg bw/day on GD 11–20 (day of sperm detection = GD 0). Dams were killed on GD 20, and ovaries and uteri were removed from fetuses. In 4 litters/group, 1 female fetus/litter was examined for ovarian and uterine histopathology. In 5 litters/group, ovaries and uteri from at least 5 littermates were pooled for a microarray analysis of gene expression. Changes in gene expression were further quantified using RT-PCR. Data were analyzed by *t*-test, ANOVA, and Jonkheere-Terpstra test. Comparisons of gene expression among estrogenic compounds were made by Wilcoxon-Mann–Whitney and Jonkheere-Terpstra tests. Results of gene expression assays are discussed in Section 2. Vaginal bleeding and early parturition occurred in 1 of 8 dams in the high-dose group. Bisphenol A treatment had no effect on maternal body weight or number of live fetuses/litter, and there were no gross or histopathological effects on ovary or uterus. Prominent nipples and areolas were observed in males and females in the high-dose bisphenol A group **[number of fetuses and litters affected were not reported]**.

Strengths/Weaknesses: Strengths are that these endpoints appear appropriate; weaknesses are the limited nature of the endpoints and the use of neat DMSO as vehicle. The sample sizes are 4–5/endpoint/group and judged to be inadequate. Of additional concern is the route of administration.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process.

Naciff et al. (2005), from The Procter and Gamble Company, examined the effect of prenatal bisphenol A exposure on male rat reproductive organ histology and gene expression. Pregnant Sprague–Dawley rats were fed Purina 5K96, a casein-based soy- and alfalfa-free diet. Rats were housed in stainless steel cages before mating. Rats were randomly assigned to groups (≥8 rats/group) and s.c. injected with bisphenol A (~99% purity) in DMSO at 0, 0.002, 0.02, 0.5, 50, or 400 mg/kg bw/day on GD 11–20 (day of sperm detection = GD 0). Dams were killed on GD 20, and testes and epididymides were removed from fetuses. In 4 litters/dose group, 1 male fetus/litter was examined for testicular histopathology. In 5 litters/group, testes and epididymides from 5 littermates were pooled for a microarray analysis of gene expression. Changes in gene expression were further quantified using RT-PCR. Data were analyzed by *t*-test, ANOVA, and Jonkheere-Terpstra test. Comparisons of gene expression among estrogenic compounds were analyzed by Wilcoxon-Mann–Whitney and Jonkheere-Terpstra tests.

Bisphenol A treatment had no effect on maternal body weight or number of live fetuses/litter, and there were no gross or histopathological effects on the testis or epididymis. Prominent nipples/areolas were observed in male and female fetuses from the high-dose group **[numbers of fetuses and litters affected were not reported]**. In pooled testis and epididymis samples from the high-dose bisphenol A group, expression of 15 genes was significantly altered in a dose-related manner. When bisphenol A data were pooled with data obtained from ethinyl estradiol and genistein and globally analyzed, there were 50 genes that were significantly altered in the same direction by all three compounds. The study authors concluded that transplacental exposure to high-doses of bisphenol A alters the expression of certain genes in the testis and epididymis of fetal rats without causing malformations in those organs. The study authors noted that the dose response to bisphenol A was monotonic with no evidence of robust quantifiable responses at low doses.

Strengths/Weaknesses: Strengths are that these endpoints appear appropriate; weaknesses are the limited nature of the endpoints and the use of neat DMSO as vehicle. The sample sizes are 4–5/endpoint/group and judged to be inadequate. Of additional concern is the route of administration.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process.

Saito et al. (2003b), support not indicated, examined the effect of prenatal bisphenol A exposure on testosterone production during adulthood in rats. On GD 12–19 (day of vaginal plug not reported), 2 Wistar rats were s.c. injected with the corn oil vehicle, 4 rats were s.c. injected with 0.005 mg/day bisphenol A **[purity not indicated]**, and 2 rats were injected with 5 μg/day 17β-estradiol. **[Assuming a pregnant Wistar rat weights ~0.33 kg, 0.005 mg/day would be equivalent to 0.015 mg/kg bw/day bisphenol A.]** Other materials found in dental composites were also evaluated but will not be discussed. During the lactation period, rats were housed in polypropylene cages with synthetic bedding. **[No information was provided on feed.]** Offspring were housed separately at 3 weeks of age and killed at 13 weeks of age. Body and testis weights were measured in all male offspring (22 in the bisphenol A group, 11 in the vehicle control group, and 5 in the 17β-estradiol group). Plasma testosterone level was measured by RIA, and plasma cholesterol level was measured using a kit. Activities of testicular enzymes involved in the production of testosterone from progesterone were also examined in an in vitro assay in which testicular microsomes were incubated with ^{14}C-progesterone and ^{14}C-δ^4-androstenedione for 20 min. Data were analyzed using unspecified post-hoc tests. **[Although not clear, it appears that offspring were considered the statistical unit for some analyses.]**

Bisphenol A exposure had no effect on pup sex ratio. No effects on body weight or absolute testicular weight were observed in the bisphenol A group at 13 weeks of age. However, relative (to body weight) testicular weight was lower **[by 6%]** in rats of the bisphenol A compared to the control group. Also observed in the bisphenol A

group was a reduction in plasma testosterone level **[by ~28%]**. No effect was observed on cholesterol level. In the ex vivo study, prenatal bisphenol A exposure increased activities of 17α -hydroxysteroid dehydrogenase **[by ~140%]** and 17β-hydroxysteroid dehydrogenase **[by ~70%]**. Observations in the 17β-estradiol compared to the control group included decreased numbers of offspring delivered, higher body weight of male offspring at 13 weeks of age, reduced plasma testosterone level, and increased testicular 17α-hydroxysteroid dehydrogenase activity. The study authors concluded that bisphenol A had an estrogenic effect on the testis but did not decrease activities of enzymes involved in testosterone production.

Strengths/Weaknesses: A strength of this study is the examination of testosterone levels at 13 weeks of age. This strength is negated by the sample size ($n = 2$–4), which is too small to draw any firm conclusions.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate based on insufficient sample size.

Murray et al. (2007), supported by NIH, examined the effect of prenatal bisphenol A exposure on in situ induction of mammary tumors. Wistar-Furth rats were fed Harlan Teklad 2018, which was reported to contain 20 fmol/g estrogen equivalents. Water was supplied in glass bottles. Caging and bedding materials were not reported, but they were stated that to test negative in the E-SCREEN. From GD 9 (GD 1 = day of vaginal sperm) through PND 1 **[The day of birth was PND 0 (A. Soto, personal communication, March 2, 2007)]**, rats received the 50% DMSO vehicle or bisphenol A **[purity not reported]** at 0.0025, 0.025, 0.250, or 1 mg/kg bw/day. Dosing solutions were delivered by implanted **[assumed s.c.]** osmotic pumps. **[Number of dams treated was not reported. Based on a limited amount of information provided on the number of offspring examined, it appears that ≤6 dams/group were treated.]** Pup viability was assessed on PND 1. On PND 2 pups were sexed and litters were culled to 8 pups. Anogenital distance was measured on PND 4. Litters were weighed during the lactation period. Female offspring were monitored for body weight and vaginal opening in the post weaning period. Female offspring were killed on PND 50 or 95. Mammary glands were collected and whole-mounted or sectioned for histopathological examination. Morphometric analyses were conducted to examine possible presence of preneoplastic lesions. Mammary glands were examined for ERα and Ki-67 protein by an immunohistochemistry technique. Maximal numbers of "maternal units" were represented in each dose group. One female/litter was included in histological examinations. **[Apparently ≤6 offspring/group were examined in histopathological examinations. Number of offspring examined for other endpoints was not reported in the manuscript. According to an author, $n = 7$–21 for the other endpoints (A. Soto, personal communication, March 2, 2007).]** Statistical analyses included ANOVA followed by post-hoc tests (Bonferroni or *t*-test) when significant effects were observed by ANOVA. **[It was not clear if dams or offspring were considered the statistical unit.]**

Bisphenol A exposure did not affect offspring viability, sex ratio, age at vaginal opening, or female anogenital distance. Anogenital distance was reduced on PND 4 in males from the 0.250 mg/kg bw/day group. Percent hyperplastic ducts was increased in all dose groups on PND 50 and in the 0.0025 mg/kg bw/day group on PND 95; the study authors noted that the effect on PND 50 was quantitatively similar in all dose groups (i.e. 3–4-fold increase). Cribriform structures were observed in the 0.25 and 1 mg/kg bw/day groups. **[Incidence was not reported for the control and lower dose groups.]** The structures were classified as carcinomas-in-situ and were characterized by increased ductal size resulting from luminal epithelium proliferation, enlarged luminal epithelial cells, presence of a nucleolus, variable chromatin pattern, and rounded luminal spaces consisting of trabecular rods of cells perpendicularly aligned to the longer duct axis. Numbers of Ki-67- and ER-α positive cells were increased in aberrant compared to normal tissues, regardless of dose. **[Results in treated compared to control groups were not reported.]** The study authors concluded that fetal bisphenol A exposure is rats is sufficient to induce development of preneoplastic and neoplastic mammary lesions.

Strengths/Weaknesses: Relevance of endpoints is a strength, as is the use of multiple dose levels. Weaknesses include an unstated number of dams (and by inference, a small number of these, and thus, because of dam-related effects, a small overall n), the uncertainty of the response rate of histopathology in the controls, and the use of 50% DMSO as vehicle.

Utility/Adequacy for CERHR Evaluation: This study was inadequate due to small sample size, route of administration, and lack of clarity on statistical analysis.

Durando et al. (2007), supported by Universidad National del Litoral, Argentine National Agency for the Promotion of Science and technology, and NIH, examined the effects of prenatal bisphenol A exposure on susceptibility to mammary tumors in rats. Wistar rats were fed Cooperación (Buenos Aires, Argentina) and housed in stainless steel cages. **[It was not clear if bedding was used.]** On GD 8–23 (GD 1 = day of vaginal sperm), 11–14 dams/group were s.c. dosed by osmotic pump with the DMSO vehicle or 0.025 mg/kg bw/day bisphenol A **[purity not indicated]**. Pups were delivered on GD 23 and weaned on PND 21. It was not indicated if day of birth was considered PND 0 or 1. During the study, body weights and day of vaginal opening were monitored. Offspring were killed before puberty (PND 30), after puberty (PND 50), or in adulthood (PND 110 and 180). In mammary gland stroma and epithelium, proliferation was assessed by BrdU incorporation and apoptotic cells were identified by TUNEL method. Morphometric analyses were conducted in sectioned mammary glands. Mast cells were identified by immunostaining for proteinase. At least 6 offspring/group/time point were evaluated. **[No littermates were used in the evaluation at any given time point (A. Soto, personal communication, March 2, 2007).]** Additional offspring were examined for responsiveness to chemically-induced mammary preneoplastic or neoplastic lesions. On PND 50, *N*-nitroso-*N*-methylurea was administered to 10–16 offspring from the vehicle control group at 25 or 50 mg/kg bw and 21 offspring from the bisphenol A group at 25 mg/kg bw. Based on findings from a pilot study, 25 mg/kg bw was considered a subcarcinogenic dose and 50 mg/kg bw was considered a positive control. During the study, rats were palpated for tumors. Rats that received 50 mg/kg bw *N*-nitroso-

N-methylurea were killed on PND 180 and rats that received 25 mg/kg bw *N*-nitroso-*N*-methylurea were killed on PND 110 or 180. Whole-mounted mammary glands were examined for tumors. Immunostaining was conducted to identify cytokeratin 8 (an epithelial marker) and p63 (a myoepithelial marker). Data were statistically analyzed using the Mann–Whitney *U*-test.

Bisphenol A exposure did not affect successful pregnancies, dam weight gain, pregnancy duration, number of pups/litter, or percent females/litter. Anogenital distance on PND 1 or 5 and postnatal body weights were unaffected in pups exposed to bisphenol A. Vaginal opening was accelerated in pups from the bisphenol A group (mean 34 days of age compared to 39 days of age in controls). On PND 50, the BrdU/apoptosis ratio was significantly increased and apoptosis was significantly decreased in mammary parenchyma and stroma of bisphenol A-exposed animals; the effects were not observed on PND 30 or 110. Significantly increased percentages of hyperplastic ducts, density of stromal nuclei, and numbers of mast cells were observed in the bisphenol A group on PND 110 and 180. Exposure to bisphenol A resulted in formation of a dense stroma layer around mammary epithelial structures and replacement of normal adipose tissue with a fibroblastic stroma. In rats exposed to 25 mg/kg bw *N*-nitroso-*N*-methylurea on PND 50, incidence of hyperplastic lesions on PND 180 was significantly higher in the group with prenatal bisphenol A compared to DMSO exposure (mean incidence of 35.5% compared to 15.7% in controls). Although statistical significance was not achieved, exposure to 25 mg/kg bw *N*-nitroso-*N*-methylurea resulted in tumors in 2 of 15 rats in the prenatal bisphenol A group and 0 of 10 rats in the prenatal vehicle control group on PND 180. Cytokeratin 8 immunostaining revealed no invasion by stromal epithelial cells. The study authors concluded that rats prenatally exposed to environmentally relevant doses of bisphenol A may have an increased risk of developing mammary tumors.

Strengths/Weaknesses: Weaknesses include route of administration and the high single dose is a weakness as is the use of pure DMSO.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for inclusion due to the use of 99.9% DMSO as a vehicle to administer bisphenol A via s.c. osmotic pump.

Hong et al. (2005), sponsored by the Korea Research Foundation, investigated the effects of acute exposures to bisphenol A during late pregnancy on expression and protein level of calbindin-D_{9k}, a putative biomarker of estrogen activity, in the uteri of offspring and lactating rats on PND 5. Pregnant Sprague–Dawley rats were given free access to water and a diet of soy-free pellets in polycarbonate caging. **[Housing conditions (individual or group) and bedding material were not indicated.]** On GD 17–19, pregnant rats were s.c. injected daily with 200, 400, or 600 mg/kg bw/day bisphenol A **[purity not provided]** in corn oil ($n = 5$/group). Negative and positive control groups ($n = 10$/group) were administered corn oil or 17β-estradiol 40 μg/kg bw/day. On PND 5, lactating dams and female pups were killed and their uteri harvested. Dose response changes in calbindin-D_{9k}, expression levels in uteri of lactating dams and female offspring (3/group) were analyzed by Northern blot and RT-PCR, with appropriate housekeeping gene controls. Protein levels and localization of calbindin-D_{9k} were performed by Western blot and immunohistochemistry for lactating dams only. Statistical analyses were performed using the Kruskall–Wallis and Dunnett tests. **[It was not clear if dams or offspring were considered the statistical unit.]**

Northern blot analysis revealed a significant increase **[~6.4-fold]** in the level of calbindin-D_{9k} expression in the uteri of lactating dams exposed to 600 mg/kg bw/day bisphenol A compared to oil controls. 17β-Estradiol treatment produced a significant **[~3.9-fold]** increase in calbindin-D_{9k} mRNA expression in the dam uterus that was not statistically distinct from the effect of the high bisphenol A dose. Uteri of offspring exposed to the highest dose level of bisphenol A also showed a significant upregulation **[~4.4-fold]** in calbindin-D_{9k} expression. Expression levels of *ER*α were unaffected in maternal uteri exposed to bisphenol A. However, *ER*α expression was increased significantly in uteri of pups exposed to 400 and 600 mg/kg bw bisphenol A **[↑33% and 66%, estimated from a graph]**. Protein levels of calbindin-D_{9k} in lactating dam uteri were elevated significantly at all dose points **[50, 40, and 50%, for 200, 400, and 600 mg/kg bw/day, respectively]**. 17β-Estradiol-treatment was not associated with a significant increase in calbindin-D_{9k} protein. The density of calbindin-D_{9k}-immunopositive cells was increased in uterine sections from lactating dams exposed to all doses of bisphenol A relative to oil controls, correlating with Western blot results. Authors note insufficient material or low detectability of calbindin-D_{9k} protein in offspring tissue, and protein analyses were not performed.

The authors suggest that calbindin-D_{9k} can serve as a reliable biomarker of acute estrogenic exposure, particularly for insight into maternal-fetal metabolic exchange, given that calbindin-D_{9k} is tightly regulated and rapidly induced by 17β-estradiol, diethylstilbestrol, alkylphenols, and now, bisphenol A. They further point out that calbindin-D_{9k} expression is absent in immature rat and ovariectomized rat uteri.

Strengths/Weaknesses: This study supports the use of calbindin-D_{9k} as a uterine biomarker of estrogenic effect in the perinatal period in the rat, and provides some dose–response information for bisphenol A induction of an estrogenic response. Limitations are the subcutaneous route of exposure, small sample size, high-doses and uncertain statistical analyses of the F1 data.

Utility (Adequacy) for CERHR Evaluation Process: While providing some dose–response information regarding bisphenol A-induced estrogenic effects following exposure of rats in the perinatal period, the lack of clarity regarding whether the dam or offspring was considered the statistical unit, route of exposure, and use of high doses render this study inadequate for consideration in the evaluation process.

3.2.3 Rat—oral exposure postnatally with or without prenatal exposure.

3.2.3.1 Reproductive studies: The International Research and Development Corporation (General Electric, 1976), sponsored by General Electric, examined the effects of bisphenol A exposure on CD rats and their offspring. Male and female F_0 rats were housed in wire mesh cages and fed Purina Laboratory Chow. Ten rats/sex/group (body weights of 110–170 g for males and

100–151 g for females) were given feed containing bisphenol A **[purity not specified]** at 0, 1000, 3000, or 9000 ppm for 17 weeks. **[It was not clear how long before mating that the dosing was started or if dosing was continued through the gestation and lactation periods.]** The European Union (2003) estimated bisphenol A intake at 0, 70, 200, or 650 mg/kg bw/day in males and 0, 100, 300, or 950 mg/kg bw/day in females. F0 rats were mated at ~100 days of age and assessed for fertility. F_1 pups were counted and weighed at birth and on PND 21 (day of birth not defined). Fifteen male and female F_1 rats/group/sex that were exposed in utero were selected for a 13-week feeding study and were fed diets containing the same concentration of bisphenol A as their parents. F_1 rats were weighed and observed for clinical signs. Hematological, clinical chemistry, and urinalysis parameters were examined in 5 rats/sex/group in the control and 2 highest dose groups at 1, 2, and 3 months of F_1 exposure. Ophthalmoscopic examinations were conducted at 3 months of F_1 exposure. After 13 weeks of dosing, the F_1 rats were killed and necropsied. Organs were weighed and fixed in 10% neutral buffered formalin. Included among organs weighed were testis and ovary. Histopathological examinations were conducted in tissues from 10 rats/sex/group in the control and high-dose group. Included among organs histologically examined were prostate, uterus, testis, and ovary. Statistical analyses included χ^2 test with Yates correction, Fisher exact probability test, Mann–Whitney *U*-test, ANOVA, *t*-test, and Dunnett multiple comparison test.

Fertility was unaffected in F_0 rats. Body weight gain was lower in F_0 rats from the 3000 and 9000 ppm groups. Body weight at Week 17 followed the same patterns as body weight gain **[6–7% decrease in the 3000** ppm **group and 12–18% decrease in the 9000** ppm **group compared to controls]**. There were no differences in food intake. **[Statistical significance for body weight effects was not reported. It was not clear if statistical analyses were not conducted or if the effects did not attain statistical significance.]**

There were no effects on number of F_1 pups/litter or survival of pups. Pup birth weights in the 9000 ppm group were slighter decreased but were said to be within normal range. Body weight gains on PND 21 were slightly decreased in pups from the 3000 and 9000 ppm dose groups. Body weights on PND 21 were significantly lower in pups from the 3000 and 9000 ppm groups **[7 and 12% lower compared to controls; benchmark dose analysis not conducted because variances not reported]**. One male F_1 rat in the control group and 2 female F_1 rats in each of the 3000 and 9000 ppm group died during the study. Post-weaning body weight gain was lower in females from all dose group and in males from the 3000 and 9000 ppm dose groups. Body weight at week 13 followed the same patterns as body weight gain **[13% decrease in the 1000** ppm **group, 11–17% in the 3000** ppm **group, and 22–24% decrease in the 9000** ppm **group compared to controls]**. Food intake was decreased in females from all dose groups and in males from the 9000 ppm group. Examination by ophthalmoscopy revealed no treatment-related effects. No treatment-related effects were observed for hematology, biochemistry, or urinalysis. No changes in organ weights or gross or histopathological lesions were considered treatment-related. The study authors noted increases in mean weights of spleen, brain, thyroid, and adrenals in the treated groups but concluded that the effects resulted from decreased body weight. **[With the exception of PND 21 pup weights, there was no discussion of statistical significance for effects observed in F_1 rats. It was not clear if statistical analyses were not conducted or if statistical significance was not attained.]**

Strengths/Weaknesses: This study is a conventional, state-of-the-art-at-the-time two-generation toxicity study. The inclusion of a breeding period and a second generation are strengths. Weaknesses are magnified in hindsight: these include the limited number of animals examined, the lack of close examination of the reproductive processes in the F_1 animals, and uncertainty about the statistical significances. The study has not been peer-reviewed.

Utility (Adequacy) for CERHR Evaluation Process: Although this study was not designed to find non-linear dose–responses, it represents a conventional-for-the-time 2-generation toxicity study, and is adequate for the evaluation process but of limited utility because the high doses preclude evaluation of low dose effects and limit its utility in showing a lack of marked organ toxicity or gross reproductive toxicity in a limited number of animals at very high-doses.

The International Research and Development Corporation (General Electric, 1978), sponsored by General Electric, examined the effects of bisphenol A exposure on male and female CD rats and their offspring. In the first part of the experiment, male and female rats were housed in wire mesh cages and were fed Purina Laboratory Chow containing bisphenol A **[purity not specified]** for 18 weeks. Ten rats/group (body weights of 135–179 g for males and 114–158 g for females) were assigned to each treatment group based on even distribution of body weight and litter mates. **[Based on information provided in study tables, it appears that the rats were ~30 days old at the start of dosing.]** Bisphenol A was added to feed at concentrations of 0, 100, 250, 500, 750, or 1000 ppm. The European Union (2003) estimated bisphenol A intake at 0, 5, 15, 30, 50, and 60 mg/kg bw/day in males and 0, 10, 25, 50, 75, and 100 mg/kg bw/day in females. Rats were examined for clinical signs, body weight gain, and food intake throughout the study. Estrous cyclicity was examined in females for 3 weeks before breeding and during breeding. At 100 days of age (Week 10 of the study), rats were moved to plastic cages with corncob bedding and mated for 3 weeks. GD 0 was defined as the day that vaginal sperm or plug was observed. Rats were assessed for fertility and gestation length. Day of delivery was designated lactation day 0 (PND 0). Pups were counted, sexed, and weighed, assessed for viability at birth and through the lactation period. After weaning, 15 male and female F_1 rats/group that were exposed in utero were selected for a 90-day feeding study. Parental rats and unselected F_1 rats were killed and discarded.

During a 90-day period, F_1 rats were fed diets containing the same concentration of bisphenol A as their parents. **[Ages at the start of dosing were not reported, but based on body weight ranges reported (64–138 g for males and 57–118 g for females) it appears that rats were different ages at the start of dosing.]** F_1 rats were weighed and observed for clinical signs. Hematological, clinical chemistry, and urinalysis

parameters were examined at Day 30, 60, and 90 of the study. Ophthalmoscopic examinations were conducted before initiation of and following 90 days of dosing. The rats were killed and organs weighed. Adrenals, pituitary, ovaries, and thyroid were weighed following fixation in 10% neutral buffered formalin. Histopathological examinations were conducted in tissues from 10 rats/sex/group in the control and high-dose groups. Organs histologically examined included prostate, uterus, testis, and ovary. Statistical analyses included χ^2 test with Yates correction, Fisher exact probability test, Mann–Whitney *U*-test, ANOVA, *t*-test, and Dunnett multiple comparison test.

In parental rats, bisphenol A exposure did not affect general behavior, appearance, or survival. Mean body weight of males in the 1000 ppm group was 6% lower than control males. Food intake was increased **[by ~7–11%, no dose–response]** in females of all dose groups. Bisphenol A exposure had no effect on estrous cyclicity or gestation length **[data were not shown]**, male or female fertility, number of pups/litter, or pup survival Body weights of pups in the 750 ppm group were significantly higher **[by ~10%]** compared to controls on PND 21, but the study authors did not consider the effect to be treatment-related.

In the F_1 offspring, a slight decrease in body weight gain was observed for males in the 750 ppm group. **[At the end of the study, body weights of males in the 750 ppm group were ~7% less than controls]**. Food intake was similar in treated and control groups. Ophthalmoscope examinations did not reveal any treatment-related effects. Although mean blood urea nitrogen levels were slightly lower and mean serum glutamic-oxaloacetic transaminase values were sporadically increased in treated rats, the study authors noted that the values were within physiological ranges. There were no effects on hematological or urinalysis parameters. Some significant organ weight changes were noted by the study authors, but they stated that the biological significance of the effects was not known. **[There did not appear to be dose–response relationships for any organ weight change.]** The study authors stated that no compound-related lesions were observed in organs, including reproductive organs.

Strengths/Weaknesses: The use of multiple dose levels (going down to fairly low exposure levels) is a plus, as is a breeding phase. Weaknesses include the limited number of animals per group, discarding of the parental animals without examination, the fact that not all F_1 animals were examined at least for structural effects, the lack of close examination of F_1 animals for reproductive effects (cyclicity and sperm measures), and the use of the conventional "top-down" pathology evaluation, wherein the lower dose groups were examined only if effects were noted in the high-dose. The study has not been peer-reviewed.

Utility (Adequacy) for CERHR Evaluation Process: For what it is, this study is adequate and of limited utility for the evaluative process, showing no gross changes in the structure of a limited number of tissues in a limited number of F_1 animals, exposed from preconception. This study was not designed to find unusual effects or non-linear dose–response relationships or to address the issue of low-dose functional responses or non-linear responses.

Ema et al. (2001), supported by the Japanese Ministry of Health and Welfare, examined developmental toxicity endpoints, in a 2 generation rats study described in detail in Section 4.2.3.1. Two generations of rats were gavaged with 0, 0.0002, 0.002, 0.020, or 0.200 mg/kg bw/day bisphenol A (99.9% purity) before and during mating and throughout the gestation and lactation period. These doses were based on previous studies that found effects at 0.002 and 0.020 mg/kg bw/day. There were some non-dose-related and sporadic effects, but the study authors concluded that none of the effects were related to bisphenol A treatment. Bisphenol A exposure did not adversely affect prenatal or postnatal growth or survival, developmental landmarks, anogenital distance, or age of puberty. In adult animals exposed to bisphenol A during development, there was no evidence of adverse effects on reproductive endpoints such as fertility, estrous cyclicity, or sperm counts. Prostate and other male reproductive organ weights were unaffected.

Strengths/Weaknesses: Strengths of this study were the thoroughness of the evaluation, the size of the dose range, the large number of animals, the litter-based analysis, and the verification of the dosing solution. A minor weakness is the lack of a positive control group, which leaves a question about the ability of this group of rats to respond.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation process.

Tyl et al. (2002b), supported by The Society of the Plastics Industry, Inc., reported some developmental toxicity effects in a multigeneration bisphenol A study in Sprague–Dawley rats that is reported in detail in Section 4.2.3.1. In that study, F_1, F_2, and F_3 rats were exposed to bisphenol A [99.70%-99.76% pure] indirectly during gestation and lactation and directly through feed after weaning. Dietary doses were 0, 0.015, 0.3, 4.5, 75, 750, or 7500 ppm, and target intakes were ~0.001, 0.02, 0.30, 5, 50, and 500 mg/kg bw/day. At the 7500 ppm dose there were fewer pups and live pups/litter and body weight gain of pups was lower during the lactation period. Delayed puberty in both males and females of the 7500 ppm group was most likely related to reduced body weights according to the study authors. Bisphenol A exposure during development did not increase the weight of the prostate in adult rats. Although some decreases in epididymal sperm concentration and daily sperm endpoints were each observed in 1 generation of males from the high-dose group, the study authors concluded there were no treatment-related effects on sperm endpoints or reproductive function. The study authors identified an offspring and reproductive NOAEL of 750 ppm (~50 mg/kg bw/day). A systemic NOAEL for adult rats was identified at 75 ppm (~5 mg/kg bw/day) by the study authors; therefore, bisphenol A was not considered a selective developmental toxicant.

Strengths/Weaknesses: This study has numerous strengths, including the quality and number of the endpoints evaluated, the number of dose groups and generations examined, and the confirmation of dosing solutions. This study incorporated screening-level endpoints within the context of a multigeneration study. As such, it addresses gross issues but does provide helpful data regarding the NOAEL.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation process.

3.2.3.2 Development of the reproductive or endocrine systems: Cagen et al. (1999b), support not indicated (but all authors affiliated with industry), conducted a study to examine the effects of prenatal and lactational bisphenol A exposure on reproductive development of rats. The study attempted to replicate findings by Sharpe et al. that appeared in an unpublished meeting abstract. The protocol used by Cagen et al. (1999b) was the same as that used by Sharpe et al. with the exception that more dose levels were included, group sizes were larger, and a greater number of reproductive endpoints were examined. Animals were fed Certified Rodent Chow 5002. Music was played at a low volume to provide background noise. Female Han-Wistar rats were randomly assigned to groups. For 2 weeks before mating, during a 2-week mating period, and during the gestation and lactation periods, 28 rats/group were given drinking water containing bisphenol A (>99% purity) at 0.01, 0.1, 1.0, or 10 ppm (0.001–0.004, 0.008–0.038, 0.100–0.391, or 0.775–4.022 mg/kg bw/day). Two negative control groups of 28 rats each were given undosed drinking water. Because the two control groups were determined to be statistically equivalent, data from the two groups were pooled. A positive control group of 28 rats was given drinking water with diethylstilbestrol at 0.1 ppm (0.006–0.036 mg/kg bw/day). Dosing solutions were prepared weekly, and concentrations were verified. Dams were evaluated for food and water intake, weight gain, and fertility endpoints. Pups were sexed, weighed, and counted at birth. During the postnatal period, pups were evaluated for growth and survival. On PND 4, litters were culled to 8 pups with as many male pups retained as possible. At weaning on PND 22, up to 4 males/litter (86–109 pups/group) were randomly selected to continue in the study until 90 days of age and were individually housed. At necropsy, brain, liver, kidneys, and reproductive organs were weighed, daily sperm production was determined, and testes were examined histologically. Technicians were blinded to treatment group. The litter was considered the experimental unit in statistical analyses. Data were analyzed by Levene test, ANOVA, Dunnett test, rank transformation, and Wilcoxon rank sum test with Bonferroni correction.

In the bisphenol A groups, there were no significant effects on dam body weight gain or food or water intake. **[Food and water intake data were not shown.]** There were also no effects on dam fertility, mating, gestation index and duration, live litter size, or pup survival and body weight gain during the postnatal period. Male sex ratio was increased in the 0.1 ppm bisphenol A group (56.7% males vs. 48.4% in control), but the study authors did not consider the effect to be treatment-related due to the lack of a dose response relationship. Dams in the diethylstilbestrol group experienced decreased body weight gain and food intake, increased duration of gestation, smaller litter size at birth, and decreased pup survival in the postnatal period.

In adult offspring from the bisphenol A groups, there were no significant effects on terminal body weight or organ weights including prostate, epididymis, preputial gland, seminal vesicle, or testis. There were also no significant effects on epididymal sperm concentration, efficiency of sperm production, or daily sperm production. No histopathological alterations were observed in the testis. Reproductive development in male offspring was also unaffected by prenatal exposure to diethylstilbestrol. The study authors noted that the reduced testis weight and sperm production reported by Sharpe et al. was not confirmed in this study and that bisphenol A should not be considered a selective reproductive or developmental toxicant.

[The NTP Statistics Subpanel (NTP, 2001) concluded that the statistical methods used by Cagen et al. (1999b) were appropriate. Although the Subpanel agreed with the study author conclusions, 2 matters were noted. The first was that a significant ANOVA is not a requirement for Dunnett test. The second was that a Bonferroni correction of Wilcoxon-rank sum test was not needed because the authors already required significance by ANOVA, which was sufficient.]

Strengths/Weaknesses: Significant strengths of this study include the large number of dose levels and animals per dose level and the technical care with which the study was performed, as well as the inclusion of a positive control group and two negative controls. The lack of much effect with diethylstilbestrol treatment is a weakness.

Utility (Adequacy) for CERHR Evaluation Process: Although only weak effects were observed for the diethylstilbestrol positive control the panel considered this study adequate and of high utility.

Elswick et al. (2000), from the Chemical Industry Institute of Toxicology [CIIT], examined the effects of sampling design on conclusions made about bisphenol A effects on prostate weight. Two of the 3 studies discussed in the study relate to bisphenol A research in Sprague–Dawley rats performed at CIIT between 1997–1999. One study is Kwon et al. (2000) which is discussed in detail in Section 3.2.3.3. The other study was unreferenced at the time and remains so. This section discussed the analysis of the unpublished study. In that study, the litter was considered the experimental unit in statistical analyses. Organ weights were analyzed using a nested ANOVA with litter within dose as the random effect. Post-hoc tests were conducted when appropriate.

Dams were given drinking water containing 0, 0.005, 0.05, 0.5, 5, or 50 mg/L bisphenol A **[purity not indicated]** from GD 2 to PND 21. The study authors estimated bisphenol A intakes at ~0.001–10 mg/kg bw/day. The lowest doses were reported to be similar to human exposure levels. The study was conducted in 2 blocks separated by 4 months. A total of 16 dams/group were exposed, and the overall sample size was ultimately 13–16/group. In the first block, 2 males/litter were most often retained and in the second block, 1 male/litter was retained until 6 months of age. Fresh ventral prostate weights were recorded. Analysis of data from the first study block revealed no treatment-related effects on ventral prostate weight. Within litters, ventral prostate weights were observed to be very variable, with weights sometimes differing by values of 2-fold or more. In the second study block, mean weights in the 0.05, 5, and 50 mg/kg bw/day groups were significantly higher than those of the control group. It was noted that mean prostate weight in the control group from Block 2 (0.387 g) was much lower than the mean weight observed in Block 1 (0.517 g) and that the standard error in Block 2 (0.174 g) was almost two times higher than the standard error in Block 1 (0.092 g). When data from the 2 blocks were combined, statistical significance remained. The

study authors noted that no historical control database was available at CIIT at the time of the analysis.

[The NTP Statistics Subpanel (NTP, 2001) reanalyzed these data agreed with its results and conclusions showed a consistent increase in ventral prostate weight in the 2 replicates. Note that the NTP Statistics Subpanel rejected the conclusions in Elswick et al. (2000) that use of multiple pups per litter can decrease false positive rates in these studies.]

Strengths/Weaknesses: This study demonstrated an increase in ventral prostate weight. These data argue for multiple pup/litter sampling, a characteristics that has been uncommon in this literature. The fact that significant effects were noted in only in 1 block raise the question of a lack of experience or training among the technicians. The study referred to in Elswick et al. (2000) is unpublished and not peer-reviewed.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate because it is primarily a discussion of results published previously and the new data presented have inconsistencies in block replicates.

Rubin et al. (2001), supported by the Tufts Institute of the Environment and NIH, examined the effects of perinatal bisphenol A exposure on estrous cyclicity and LH levels in rats. Uterotropic responses were examined in a second group of rats, and those results are listed in Table 53. Sprague–Dawley rats were fed Purina Rodent Chow and provided drinking water in glass bottles. The rats were housed in plastic cages; estrogenicity testing of ethanol extracts indicated that estrogenic compounds did not leach from cages at detectable levels. **[No information was provided about bedding.]** Dams were weighed and randomly assigned to treatment groups of 6 animals given drinking water containing bisphenol A **[purity not reported]** at 0 (1% ethanol vehicle), 1, or 10 mg/L from GD 6 (plug day not indicated) through the lactation period. Mean bisphenol A doses were estimated by study authors at 0.1 and 1.2 mg/kg bw/day. At weaning, pups were given untreated water. Dams were examined and weighed during the studies. Offspring were sexed on PND 2 and weighed beginning in the postnatal period and continuing through adulthood ($n = 40$–53/group during the neonatal period and 19–27/sex/group during adulthood). Anogenital distance was examined during the neonatal period. **[It was not clear how many time points and animals were examined. According to 1 study author, anogenital distance was measured on PND 2 (A. Soto, personal communication, March 2, 2007).]** Genital tracts were examined for gross abnormalities in males killed during the neonatal period, at 3 months, and at 5 months of age and in females killed during the neonatal period, at 8 months, and at 12–16 months of age. **[The total number of animals examined at each time period was reported as 12–34, but it is not known how many/dose group were examined.]** Animals were selected from as many different litters as possible at each time point. Day of vaginal opening was monitored. Estrous cyclicity was evaluated daily for 18 days at 4 and 6 months of age in 18–28 rats/group. Eight female offspring/group were killed 3 months later following ovariectomy to measure serum LH levels using an LH assay kit; a total of 6–8 values/group were obtained. Body and uterine weights and LH levels were analyzed by ANOVA followed by *t*-test, Tukey test, or least significant difference test. Mammary tumors were analyzed by χ^2 test, and estrous cyclicity data were analyzed by Kruskall–Wallis test and Mann–Whitney *U*-test. **[It appears that offspring were considered the statistical unit.]**

On PND 4, 7, and 11, body weights were significantly higher in pups from the bisphenol A groups than in the control group; body weights were higher in animals of the low compared to the high-dose group. Body weights of low-dose females were higher than body weights of control and high-dose females at PND 28 and beyond. Although the percentage of control females with regular estrous cycles was 83% at 4 months of age and 60% at 6 months of age, the values were reduced significantly in the high-dose group to 21% at 4 months of age and 23% at 6 months of age. There were no clear patterns of estrous cycle changes. Periods of diestrus were extended in some animals and other animals had extended periods of proestrus and/or estrus. The mean number of 4–5-day estrous cycles was reduced significantly in rats of the high-dose group at 6 months of age. Serum LH levels in the high-dose group were reduced significantly by ~19% compared to the control group **[$BMD_{10} = 0.94$, $BMDL_{10} = 0.48$, $BMD_{1\ SD} = 1.6$, and $BMDL_{1\ SD} = 0.78$ mg/kg bw/day]**. The treatment group incidences of females with mammary tumors (10% in controls, 20% in the low-dose group, and 28% in the high-dose group) were not statistically different. The study authors noted that the study was not designed to detect mammary tumors and that the tumors were detected during routine handling. No effects were reported for mean number of pups/litter, sex ratio, day of vaginal opening, or anogenital distance in the neonatal period. **[Data were not shown for anogenital distance.]** In comparing the effects on estrous cycles and LH levels in animals exposed in the perinatal period to the lack of uterotropic effects in animals exposed in the post-pubertal period, the study authors concluded that there was evidence of increased sensitivity to bisphenol A during the perinatal period.

Strengths/Weaknesses: This study incorporates a range of basic developmental and gross functional reproductive endpoints, but the sample sizes are small (6 dams/group) and the statistical approach does not appear to use litter as the unit. Actual exposures are poorly defined, particularly postnatally. The plausibility of the estrous cycle changes is a strength.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process, based on a lack of adequate control for litter effects

Takashima et al. (2001), supported by a Grant-in-Aid for Health Sciences Research **[sponsor not indicated]**, examined the effect of bisphenol A exposure during development on carcinogenicity induced by *N*-nitrosobis (2-hydroxypropyl)amine. **[No information was provided about caging and bedding materials used in this study.]** Female Wistar rats were fed either MF diet or soybean-devoid powder diet (Oriental Yeast Co.). In each dietary group, 10–11 rats/group received bisphenol A **[purity not indicated]** at 0 or 1.0% diet. Bisphenol A exposure commenced 10 weeks before mating and was continued through the mating, gestation, and lactation periods. Total intakes of bisphenol A were reported at 21–22 g/rat. **[Assuming an exposure period of ~16 weeks, mean bisphenol A intake over the course of the study was estimated at ~200 mg/day. Based on reported body**

weights, bisphenol A intake was ~1600 mg/kg bw/day during the prebreeding stage and 1000 mg/kg bw/day during gestation and at weaning.] The rats were mated to males fed CE-2 basal pellet diet (Clea, Inc.), and GD 0 was defined as the day of the vaginal plug. Endpoints associated with pregnancy, delivery, and nursing were evaluated. Dam body weight and food intake were measured. Offspring were not culled and were weaned at 3 weeks of age. Dams were killed following weaning of offspring. Serum levels of thyroid hormones were measured in 2–4 dams/group. Implantation sites were evaluated. Weights of several organs, including ovary, were measured. The organs were fixed in 10% buffered formalin and processed for histopathological evaluation. Offspring ($n = 32$–50/group) were evaluated for body weight gain, preputial separation, and vaginal opening. Beginning at 5 weeks of age and continuing for 12 weeks, offspring in each group were subdivided into 2 groups ($n = 17$–21/group/sex) that received either undosed tap water or tap water containing 2000 ppm *N*-nitrosobis (2-hydroxypropyl)amine. Offspring were killed at 25 weeks of age. Serum thyroid hormone levels were measured. Organs, including testis, ovary, and uterus were weighed. In 5–19 offspring/sex/group, histopathological examinations were conducted in organs targeted by *N*-nitrosobis (2-hydroxypropyl)amine (lungs, thyroid, esophagus, liver, and thymus). Data were analyzed by Dunnett and χ^2 tests. **[Data for pre-and postnatal survival were presented and apparently analyzed on a litter basis. The offspring were apparently used as the statistical unit in body weight analyses. It was not clear if the dam or offspring were considered the statistical unit in other analyses.]**

Dam body weight was lower in the 1.0% bisphenol A group fed MF diet compared to the MF diet control during the gestation period and at weaning. Food intake and maternal serum levels of triiodothyronine, thyroxine, and thyroid-stimulating hormone were unaffected by bisphenol A exposure. Changes in weights or histopathological alterations of maternal organs, including uterus and ovary, were not observed in the bisphenol A groups. **[Data were not shown by the study authors.]** Bisphenol A had no significant effect on mating, fertility, duration of gestation, live-born pups, implantation loss, or offspring viability through PND 21. In pups from dams exposed to 1.0% bisphenol A fed MF diet compared to pups from MF controls, body weights were higher **[by 11%]** in females at 3 days of age and lower in males and females at 10 days and 2 weeks of age **[16–22% decreases in males and 12–19% decreases in females]**. In pups from dams exposed to 1.0% bisphenol A and fed soybean-free diet compared to pups from the soybean-free controls, body weights of pups were increased in males at 3 weeks of age **[13% increase]** and in females at 10 days and 3 weeks of age **[13–19% increase]**. Prenatal exposure to bisphenol A did not affect preputial separation or vaginal opening. In 25-week-old rats that were not exposed to N-nitrosobis (2-hydroxypropyl)amine, prenatal bisphenol A exposure was associated with some thyroid-stimulating hormone elevations in males and females from the MF and soybean-free diet groups. According to a statement in the study abstract, the study authors did not consider the effect on thyroid-stimulating hormone to be related to bisphenol A exposure. There were no effects of *N*-nitrosobis (2-hydroxypropyl)amine exposure on serum thyroid-stimulating hormone, triiodothyronine, or thyroxin levels or on thyroid histopathology. No effects were observed on offspring organ weights. **[With the exception of uterus and ovary, no organ weight data were shown.]** Prenatal bisphenol A exposure was not associated with significant differences in the development of *N*-nitrosobis (2-hydroxypropyl)amine-induced neoplasms in the offspring. The study authors concluded that bisphenol A exposure did not induce tissue injury in rat dams or their offspring or affect the development of tumors in offspring exposed to *N*-nitrosobis (2-hydroxypropyl)amine.

Strengths/Weaknesses: Weaknesses include high-doses and inadequate sample sizes. This study appears to discount the importance of certain effects on body weight and thyroid-stimulating hormone levels that might have received more attention in a study with a non-tumor focus. Sample size is inadequate to address neoplasm endpoints. Information is insufficient to judge the appropriateness of the statistical analyses and hence the reliability of findings.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process due to small sample size, high-dose levels, and inappropriate statistics.

Kobayashi et al. (2002), supported by the Japanese Ministry of the Environment, examined the effect of prenatal and lactational bisphenol A exposure on somatic growth and anogenital distance in Sprague–Dawley rats. The same rats were used to measure plasma hormone levels and testicular testosterone content in a study by Watanabe et al. (2003) and apparently thyroid function in a study by Koybayashi et al. (2005). Rats were fed standard laboratory feed (CE-2, CLEA Japan, Inc.). **[No information was provided about caging or bedding materials.]** Rats were randomly assigned to groups and 6 rats/group were gavaged with bisphenol A (99.8% purity) at 0 (corn oil vehicle), 4, 40, or 400 mg/kg bw/day from GD 6 through PND 20. GD 0 was defined as the day a vaginal plug was observed, but the day of birth was not defined. Doses were based on the study by Kwon et al. (2000) **[discussed in Section 3.2.3.3]**. On PND 7, litters were culled to 10 pups, with equal numbers of males and females when possible. Offspring were weaned on PND 21. Dams were weighed during the study. Body weight and anogenital distance were measured in offspring at 1, 3, and 9 weeks of age. Plasma and testicular testosterone levels were measured at 9 and 36 weeks of age, and plasma LH and FSH concentration were measured at 9 weeks of age Weights of liver, kidney, and testis were examined in offspring at 3 and 9 weeks of age. One to 10 (most often 6–10) offspring/group/sex were examined for body weight and anogenital distance at 1 week of age and 4–6/sex/group at 3 and 9 weeks of age. A pair of male and female offspring/litter **[assuming authors meant 1/sex/litter]** was examined for organ weights, and 4–6 males/group were used in hormone analyses at 3 and 9 weeks of age. Statistical analyses included ANOVA followed by Dunnett test. **[It was not clear if the dam or litter was considered the statistical unit.]**

In the 40 mg/kg bw/day group, all pups from 1 dam were found dead on PND 2. Four of 6 dams of the 400 mg/kg bw/day group died on GD 21, and all pups

120 mg/kg bw/day bisphenol A groups were injected with corn oil during the same time period. Surviving male offspring were killed and necropsied at 65 weeks of age. Blood was collected for analysis of serum testosterone levels in 5 rats/group. Reproductive organs were examined for gross abnormalities, weighed, and fixed in 10% buffered formalin. A histopathological examination of the prostate was conducted. Body and organ weight data were analyzed by Student *t*-test. The incidence of histopathological lesions was evaluated by Fisher exact probability test. **[It appears that the litter was considered the statistical unit in analyses for numbers and survival of pups at birth. Offspring were apparently considered the statistical unit for other analyses.]**

Body weights of dams in the 120 mg/kg bw/day group were significantly lower than control values from GD 14–20. There were no consistent or dose-related effects on dam body weights during lactation, although a significant increase in body weight was observed in dams of the 0.05 mg/kg bw/day group on PND 14. Exposure to bisphenol A had no effect on gestation period duration or number of implantation sites. In pups exposed to bisphenol A, there were no differences in number of live births, sex ratio, external anomalies, or body weights during the lactation period. **[Data for pup body weights were not shown by study authors.]** Terminal body weight of pups exposed to 0.05 mg/kg bw/day bisphenol A before treatment with 3,2-dimethyl-4-aminobiphenyl were significantly higher than controls **[by 12%]**. Exposure to bisphenol A had no effect on weights of prostate, testis, or epididymis. Incidences of prostatic intraepithelial neoplasia, carcinoma, and atypical hyperplasia were not increased by bisphenol A treatment, and there were no increases in tumors found in non-reproductive organs. No effect was observed on serum testosterone levels. The study authors concluded that exposure of rat dams to bisphenol A during the gestation and lactation periods does not predispose their offspring to prostate cancer development.

Strengths/Weaknesses: Strengths are the range of low dose levels, the use of an additional strain (Fischer 344 rat), and the endpoints evaluated. The design is reasonable for some of the endpoints measured, but sample sizes are inadequate for the prostate cancer endpoint and hormonal endpoints in particular. Statistical accounting for litter effects is unclear for neonatal measures, body weight, and fertility endpoints.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate based on insufficient sample size given the endpoints (i.e., tumors response) and lack of consistently accounting for litter effects.

Yoshida et al. (2004), supported by the Japanese Ministry of Health, Labor, and Welfare, examined the effects of bisphenol A exposure on development of the rat female reproductive tract. Donryu rats (12–19/group) were gavaged with bisphenol A **[purity not reported]** at 0 (carboxymethylcellulose solution), 0.006, or 6 mg/kg bw/day from GD 2 to the day before weaning of pups at 21 days post delivery. The low dose was said to represent average daily intake from canned foods and the high-dose was reported to represent the maximum dose level detected in plastic plates for children. **[It is assumed the authors meant estimated exposure levels for children eating off plastic plates.]** Bisphenol A levels were measured in maternal and pup tissues, and those values are reported in Section 2.1.2.2.1. After delivery, dams and litters were housed in plastic cages with wood chip bedding. Tap water was stored in plastic containers. The only information provided about feed was that it was a commercial pellet diet. Samples of tap water, drinking water from plastic containers, and feed were measured for bisphenol A content by HPLC. Offspring were sexed, weighed, and examined for external abnormalities on PND 1 and then weighed weekly through PND 21. Litters were adjusted to 8–10 pups at PND 4 or 6 (day of birth = PND 0). Dams were weighed, and observed during the study and killed following weaning of litters on PND 21. Implantation sites were examined and organs including uterus, vagina, and ovaries were fixed in 10% neutral buffered formalin and examined histologically. **[It does not appear that results of histopathological testing in dams were reported.]** All female offspring were examined for vaginal opening, and following vaginal opening, vaginal smears were taken for the remainder of the study. Three to 5 offspring/group from different litters were killed on PND 10, 14, 21, or 28 and at 8 weeks of age. At most time periods, uteri were weighed, preserved in 10% neutral buffered formalin, and examined histopathologically to determine development of uterine glands. Ovaries and vagina were also examined histologically. ERα was determined using an immunohistochemical method. Serum was collected for measurement of FSH and LH by RIA. Four offspring/group from different litters were killed at 8 weeks of age on the morning of estrus to examine ovulation by counting ova in oviducts. Initiation of carcinogenesis following injection of the uterine horn with *N*-ethyl-*N*′-nitro-nitrosoguanidine was examined at 11 weeks of age in 35 or 36 animals/group. At ~24 weeks following cancer initiation, the 24–30 surviving animals/group were killed and uteri were examined histologically to determine the presence of tumors and other lesions. Statistical analyses included ANOVA and Dunnett test. **[Most of the data for endpoints evaluated at birth appeared to be presented and apparently analyzed on a litter basis. For other data, it appears that offspring were considered the statistical unit.]**

Bisphenol A was not detected in fresh tap water but was detected at ~3 ng/mL following storage in plastic containers. The bisphenol A concentration in feed was ~40 ng/g. In dams exposed to bisphenol A, there were no clinical signs of toxicity or effects on body weight, implantation sites, or gestation length. Bisphenol A exposure had no effect on litter size, pup body weight at birth and through PND 21, external abnormalities in pups or age of vaginal opening. In uteri of bisphenol A-exposed offspring, there were no effects on weight, gland development, ERα, or cell proliferation. No increase in lesions was reported in organs of the alimentary, urinary, respiratory, or nervous system. **[Data were not shown by study authors.]** Bisphenol A exposure had no effect on ovulation, estrous cyclicity, or serum FSH or LH levels. There were no effects on uterine preneoplastic or neoplastic lesions or ovarian histopathology following bisphenol A treatment. The study authors concluded that perinatal exposure to bisphenol A at levels comparable to human exposure did not affect the reproductive system of female rats.

Strengths/Weaknesses: Strengths of this study were the bisphenol A determinations that were made and the anchoring of animal exposure levels to human exposures. The design appears sound with a good range of endpoints measured. Small numbers of animals were sacrificed at several time points and cellular analyses were performed; these numbers were too small for a definitive cancer evaluation and were, in fact, too small for definitive conclusions to be reached for most of the adult reproductive endpoints. Statistics are not described in enough detail to determine how data from multiple sampling points were evaluated. This experiment represents a thorough screening study.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate based on insufficient sample size (3–5/group).

Takagi et al. (2004), supported by the Japanese Ministry of Health, Labor, and Welfare, examined the effect of perinatal bisphenol A exposure on the reproductive and endocrine systems of rats. Nonylphenol was also examined but will not be discussed. Sprague–Dawley rat dams were fed a soy-free diet (Oriental Yeast Co.) prepared according to the formula for NIH-07. At weaning, the offspring were fed CRF-1 diet (Oriental Yeast Co.), which contains soybean and alfalfa-derived proteins. Rats were housed in polycarbonate cages containing wood chip bedding. Dams were randomly assigned to groups, and 5–6 dams/group were fed diets containing bisphenol A (96.5% purity) at 0, 60, 600, or 3000 ppm from GD 15 (GD 0 = day of vaginal plug) to PND10 (PND 1 = day of birth). The study authors estimated bisphenol A intake at ~5, 49, and 232 mg/kg bw/day during the gestation period and ~9, 80, and 384 mg/kg bw/day during the lactation period. Dose levels were based on results of preliminary studies, and selected with a goal of achieving weak to moderate toxicity in dams at the highest dose. In a separate study, rats were fed diets containing ethinyl estradiol at 0 or 0.5 ppm from GD 15 to PND 10. On PND 2, offspring were counted, sexed, and weighed and anogenital distance was measured. Litters were culled to 6 pups on PND 10, and pups were weaned on PND 21. Five pups/sex/group (1/sex/litter) were selected for necropsy on PND 21 and brain, adrenals, testis, ovary, and uterus were weighed. Eight offspring/sex/group (at least 1/sex/litter) were selected for evaluation in adulthood, and these rats were observed for age and body weight at puberty. Estrous cyclicity was observed from 8–11 weeks of age. Offspring were killed at 11 weeks of age, on the day of diestrus for cycling female rats. Brain, pituitary, thyroid, adrenal mammary gland, epididymis, prostate, seminal vesicles, ovary, uterus, and vagina were weighed and examined histologically. The testis was fixed in Bouin solution, and other organs were fixed in 10% neutral buffered formalin. The volume of the sexually dimorphic nucleus of the preoptic area (SDN-POA) was measured. It appears that endpoints were assessed in 8 adult rats/sex/group, with the exception of histopathological evaluations, which were conducted in 5 rats/sex/group. The litter was considered the experimental unit in statistical analyses of data from PND 21 offspring, and the individual animal was considered the statistical unit for data obtained from adult offspring. Homogenous numerical data were analyzed by ANOVA and Dunnett test, and heterogeneous numerical data were analyzed by Kruskall–Wallis *H*-test and Dunnett-type rank sum test. Data for histopathological lesions and vaginal cyclicity were analyzed by Fisher exact probability test or Mann–Whitney *U*-test.

Maternal body weight gain was significantly decreased the high-dose bisphenol A group during gestation, but there were no effects on body weight gain during lactation or food intake. In offspring evaluated on PND 2, there were significant decreases in body weight in low- and high-dose males **[13 and 22%]** and in high-dose females **[20%]**, but there were no effects on number of live offspring or anogenital distance. Body weight gain was lower in high-dose males **[21%]** and females **[29%]** from PND 2–10. Increased relative brain weight as a result of growth retardation was reported in high-dose offspring evaluated on PND 21. **[Data were not shown by study authors.]** Exposure to bisphenol A did not affect onset of vaginal opening, preputial separation, or estrous cyclicity. Body weight of males was significantly lower **[by 9.3%]** at adult necropsy. Weights and histopathology of brain, pituitary, thyroid, adrenal mammary gland, epididymis, prostate, seminal vesicles, ovary uterus, and vagina in adulthood were unaffected in rats from the bisphenol A group. **[Organ weight data were not shown by study authors.]** Bisphenol A did not affect SDN-POA volume. Effects observed in offspring from the ethinyl estradiol study included reduced numbers of live offspring, increased male:female ratio, decreased body weight and body weight gain, accelerated vaginal opening, delayed preputial separation, increased estrous cycle irregularities, and histopathological alterations in pituitary, ovary, uterus, vagina, and mammary gland. The study authors concluded that bisphenol A did not affect endocrine or reproductive system development of rats at doses that induced maternal toxicity.

Strengths/Weaknesses: Strengths include the range of doses and endpoints measured and the use of the ethinyl estradiol comparator group. The study used small sample sizes of dams (n = 5–6/group) and inadequate statistical procedures to control for litter effects.

Utility (Adequacy) for CERHR Evaluation Process: This study is considered inadequate for the evaluative process, based on sample size and statistical procedures.

Akingbemi et al. (2004), supported by NIEHS, USEPA, NICHHD, and NIH, conducted a series of studies in Long–Evans rats to determine the effects of postweaning and perinatal exposure to bisphenol A on testicular steroidogenesis. In vitro studies were also conducted and are described in Section 4 because cells used in the studies were obtained from adult animals. Rats were fed Purina chow, which contains soybean meal, and given drinking water in polycarbonate bottles. Pregnant and nursing dams were housed in polycarbonate cages lined with wood bedding, but no information was provided on caging used at the other life stages. To reduce leaching of bisphenol A, the cages were washed, rinsed, and dried at least twice/week and were discarded once they began getting cloudy; water bottles were cleaned daily. Corn oil vehicle was used for bisphenol A and was administered to control animals. Rats were stratified according to body weight and randomly assigned to treatment groups. RIA methods were used to measure steroid hormone concentrations in serum or testicular fluid. RT/PCR methods were used to examine changes in mRNA expression. Statistical analyses included ANOVA with multiple

the findings of this study, the study authors concluded that prenatal and lactational exposure of rats to bisphenol A does not appear to affect thyroid function.

Strengths/Weaknesses: Strengths of this study include the use of a range of dose levels of bisphenol A. Weaknesses include the limited endpoints addressed (thyroid function), concern that the number of animals used (6 dams per treatment group) may not provide adequate statistical power to assess changes in hormone levels and response given the variability inherent in these measures, and failure to account for litter in the analyses.

Utility (Adequacy) for CERHR Evaluation Process: As presented, this study is inadequate because it is unclear whether there were adequate controls for litter effects.

Zoeller et al. (2005), supported in part by NIH, examined the effect of bisphenol A exposure on the thyroid of developing rats. Sprague–Dawley rats were housed in plastic cages. **[No information was provided about composition of feed or bedding materials.]** Before initiation of dosing, rats were trained to eat an untreated wafer each day. On GD 6 (day of vaginal plug not defined) through the remainder of the experiment (the remainder of the gestation and lactation periods), 9 rats/group were given a wafer dosed with bisphenol A **[purity not reported]** at levels resulting in exposure to 0 (methanol vehicle), 1, 10, or 50 mg/kg bw/day. Doses were based on those used in the study by Tyl et al. (2002b). Pups ($n = 7$–9/group/sex/time period) were weighed and killed on PND 4, 8, 15, or 35 (day of birth not defined). During each of those time periods, serum thyroxin was measured by RIA. On PND 15, brains of male pups were sectioned and examined for presence of RC3/neurogranin mRNA, a thyroid hormone-responsive gene, using an in situ hybridization and autoradiography technique. Serum thyroid-stimulating hormone was measured using an unspecified method in 6–8 male pups/group (1/litter) on PND 15. Statistical analyses included ANOVA and Bonferroni *t*-test.

The text of the study indicated a significant reduction in maternal body weight gain during pregnancy in the high-dose group, while Figure 1 of the study indicated a significant reduction in maternal body weight gain during pregnancy at all dose levels. Maternal body weight gain during the lactation period was unaffected by bisphenol A treatment. Bisphenol A exposure had no effect on litter size at birth. **[Data were not shown by study authors.]** Bisphenol A had no effect on pup body weights on PND 4, 8, or 15. On PND 15, but at no other time period, there was a significant increase in serum thyroxin levels in all dose groups of male and female pups **[percent increases compared to controls were ~11, 35, and 37% in each respective dose group.]** Significant increases in expression of RC3/neurogranin mRNA were observed in the upper and lower dentate gyrus in males from each treatment group **[with no apparent dose–response relationship]**. Expression of RC3/neurogranin mRNA in cortex was unaffected by bisphenol A treatment. No significant effects were observed for thyroid-stimulating hormone levels in males on PND 15. The study authors concluded that bisphenol A acts as a thyroid antagonist at these concentrations.

Strengths/Weaknesses: Strengths of the study include use of a range of doses and examination of a role of bisphenol A as a thyroid hormone antagonist. Weaknesses include the lack of litter-based analysis.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate based on inappropriate statistics (i.e., not accounting for repeated measures over time or the use of more than one pup per litter for a given endpoint).

3.2.3.3 Studies with neurobehavioral endpoints: **Kwon et al. (2000)**, from CIIT, examined the effects of bisphenol A exposure during pre- and postnatal development on reproductive endpoints and the SDN-POA of rats. Sprague–Dawley rats were fed NIH-07 feed and housed in polycarbonate cages with cellulose fiber-chip bedding. Water was provided in glass bottles with Teflon-lined caps. Pregnant rats were randomly assigned to groups according to body weight. Rats ($n = 8$/group) were gavaged with bisphenol A (~99% purity) at 0 (corn oil vehicle), 3.2, 32, or 320 mg/kg bw/day from GD 11 (GD 0 = day of sperm detection) through PND 20, excluding the day of parturition. It was not stated if the day of parturition was considered PND 0 or 1. A positive control group was treated with 15 μg/kg bw/day diethylstilbestrol. Rats were examined for clinical signs of toxicity and weighed during the study. Pups were weighed on PND 1 and 7. Pups were weaned on PND 21. After pups were weaned, dams were killed for assessment of body and organ weights. On PND 10, brains were collected from 1–3 female offspring/litter from 6–8 litters/group for measurement of SDN-POA volume. All remaining female pups were examined for age of vaginal opening and day of first estrus, and estrous cyclicity was monitored for 22 days, beginning at ~4 months of age. Lordosis behavior was examined at 6 months of age in 1–2 female offspring/litter from 7–9 litters/group that had been ovariectomized 2 weeks before reproductive behavior testing and primed with estradiol benzoate and progesterone. Male offspring were killed on PND 180 for measurement of body and reproductive organ weights and histopathological evaluation of ventral prostates fixed in 10% neutral buffered formalin. **[Based on information presented in the Results section, it also appears that ovaries and uteri were examined in an unspecified number of female offspring at 6 months of age.]** Statistical analyses included ANOVA, Dunnett test, ANCOVA, and pair-wise comparison of least square means. The litter was considered the experimental unit.

Bisphenol A treatment had no significant effect on maternal body weight during pregnancy or lactation or on maternal liver, kidney, adrenal, ovary, or uterus weights. There was no effect on number of live pups/litter at birth or pup weight on PND 1 or 7. In female offspring, bisphenol A exposure had no significant effect on volume of SDN-POA, age or weight at vaginal opening or first estrus, estrous cyclicity, or mean lordosis intensity. In male offspring, there were no significant effects on body weight or weights of testis, epididymis, seminal vesicle, or prostate. The study authors noted that a 23% increase in ventral prostate weight in the high-dose group did not reach statistical significance. No treatment-related histopathological alterations were reported for ventral prostate, ovary, or uterus. Effects observed in the diethylstilbestrol group included increased maternal liver weight, increased SDN-POA volume in female offspring, and disrupted estrous

cycles. The study authors concluded that indirect exposure of offspring to bisphenol A at these levels during gestation and lactation did not affect estrogen-mediated reproductive endpoints. A similar study with comparable findings in females was reported in abstract form (Kwon et al, 1999).

Strengths/Weaknesses: This study was well performed and presented. The wide coverage of the dose range (across a three-log range) is a major strength. The use of diethylstilbestrol as a positive control is a strength, as is the number of reproductive organs and endpoints evaluated. A weakness was the limited analysis of those reproductive organs (wet weight only; histopathology was only performed on the prostate) and a lack of determination of pup exposure during lactation.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation process.

Kubo et al. (2001), supported by the Japanese Ministry of Education, Science, and Culture, examined the effect of prenatal bisphenol A exposure on sexually dimorphic behavior and brain development in rats. **[No information was provided about the type of chow, bedding, or caging materials used.]** Throughout the gestation (from the day that sperm were detected in the vagina) and lactation periods, 5 Wistar rats/group were administered bisphenol A through drinking water at 0 or 5 mg/L. **[No information was provided on bisphenol A purity or use of a vehicle.]** The study authors estimated the bisphenol A dose at 1.5 mg/kg bw/day. **[It is not clear whether this is an estimate based on water consumption or dosing by a separate, unspecified route. If based on drinking, this estimate is suspect because it implies a daily consumption of approximately 70 mL water (because the weight of the rats is not supplied this must of necessity be a guess), which is well in excess of published intakes for post-partum rats (generally noted as around 20 mL/day). It is also noted that water consumption varies widely in non-lactating rats and throughout the period of lactation in rats, reflecting milk production, so any such estimate would of necessity be suspect, and doses will vary with time post-partum.]** Litters were adjusted to 5 pups/sex on the day following birth. Pups were weaned on PND 21 **[day of birth not defined]** and housed according to sex and litter. Behavior was tested for 10 min in an open-field apparatus at 6 weeks of age ($n = 11–14$) **[It was not clear if the number of animals examined included total animals, total/group, or total/sex/group. Litter distribution was not indicated.]** A passive avoidance test was conducted at 7 weeks of age ($n = 11–14$); the test included a habituation period and testing of retention 24 hr later. An unspecified number of rats were killed and necropsied at 12 weeks of age, with females killed in diestrus. Reproductive organs were weighed ($n = 12–14$) and sperm endpoints were evaluated in an unspecified number of rats. Serum hormone levels were measured by RIA ($n = 5–10$/group). At 20 weeks of age, 6 rats/sex from the control group and 7 rats/sex from the treated group were killed to measure the volume of the SDN-POA and the locus ceruleus. Behavioral data were analyzed by Student *t*-test or Mann–Whitney *U*-test, and brain morphology data were analyzed by Student *t*-test. **[It was not clear if the litter or offspring was considered the statistical unit.] No information was provided on data analyses for reproductive organ weight, serum hormone levels, or sperm endpoints.]**

In open-field testing of controls, females moved significantly greater distances, reared more times, and stayed in the center of the apparatus for a longer period of time than males. In passive avoidance testing of controls, latency to enter the dark chamber following electric shock was significantly longer in male than female rats. In rats exposed to bisphenol A, there were no significant differences in the behaviors of males compared to females. Study authors attributed the loss of sexually dimorphic behaviors to demasculinization of male behavior and defeminization of female behavior. Bisphenol A treatment did not affect brain weight, which was higher in male than female controls. The larger size of SDN-POA in male compared to female controls was retained following bisphenol A treatment. The volume of the locus ceruleus was significantly larger in females than males of the control group. In the bisphenol A group, the volume of the locus ceruleus was described as larger in males than females, but the stated increase was not statistically significant ($P = 0.12$). **[Graphically, there is an estimated 14% difference between male and female locus ceruleus volume in controls and in bisphenol A-exposed animals, with the direction of the difference apparently reversed by treatment.]** Bisphenol A treatment had no effect on absolute weight of the testis or epididymis or relative weights of the ventral prostate, ovaries, or uterus. There were no significant effects on serum levels of LH, FSH, testosterone, or 17β-estradiol. Sperm count and motility were also unaffected by bisphenol A exposure. The study authors concluded that current methods for establishing NOAELs may not be sufficient to detect disrupted sexual dimorphism in the brain.

Strengths/Weaknesses: A strength is the variety of biological and behavioral endpoints assessed. The major weakness of the study is the lack of experimental detail, which makes it difficult to determine whether litter effects were adequately controlled for and how much bisphenol A was received by the animals.

Utility (Adequacy) for CERHR Evaluation Process: Given the lack of methodological data provided in the study, this communication is inadequate for the evaluation process.

Kubo et al. (2003) examined the effect of prenatal bisphenol A exposure on sexually dimorphic behavior and brain structure of rats. **[No information was provided on the type of feed or materials used in bedding or caging.]** Wistar rats were dosed with the 0.1% ethanol in distilled water vehicle ($n = 5$ dams/group) or bisphenol A **[purity not reported]** at 0.1 or 1 mg/L ($n = 6$ dams/group). The study authors estimated bisphenol A intake at 0.030 and 0.3 mg/kg bw/day and noted that the levels were below the tolerated daily intake. **[Although not clearly stated, it appears that as in the previous study by Kubo et al. (2001), exposures occurred through drinking water during the entire gestation and lactation period.]** Five dams/group were exposed to *trans*-resveratrol, an estrogenic compound found in grapes, at 5 mg/L or diethylstilbestrol at 50 μg/L. Body weight and anogenital distance were measured in pups on PND 1 (the day following birth). **[All litters were examined and although the number of pups examined in each litter was not clearly stated, it was implied that all pups were**

analyzed.] Anogenital distance was adjusted by the cube root of body weight. Following the evaluations on PND 1, litters were standardized to 5 pups/sex. Pups were weaned on PND 21 and housed according to sex and litter. Day of testicular descent or vaginal opening was monitored in all remaining offspring ($n = 25$/sex in the control group and 30–31/sex in the treated group). Open-field testing was conducted in 20–24 animals/group at 6 weeks of age. **[It is not clear if the authors meant 20–24 animals/group or animals/group/sex]**. Sexual behavior of 7–13 male and female rats/sex/group was tested at 11–12 weeks of age. Males and females ($n = 11$–15/sex/group) were killed at 12 weeks of age, females during proestrus. Reproductive organs were weighed. Serum hormone levels were measured by RIA. Sperm from one testis and cauda epididymis were counted. Histological examinations were conducted on testis fixed in Bouin solution and ovary fixed in 10% neutral buffered formalin. Rats were killed at 14 weeks of age for measurement of SDN-POA and locus ceruleus volume in 7–8 males and females/group.

Because of the large number of animals used, the experiment was conducted in 3 blocks representing identical experiments. All data were collected and analyzed following completion of the third block of the study. The litter was considered the statistical unit in analyses of data collected before weaning of animals. Individual animals were considered the statistical unit in analyses of data collected subsequent to weaning.

Behavior and brain structure data were analyzed by ANOVA and differences between sexes were analyzed by Student *t*-test. Reproductive data were analyzed by ANOVA followed by Fisher protected least significant difference test for each sex.

Bisphenol A exposure had no significant effect on body weight on PND 1, anogenital distance in males and females, day of testicular descent, or day of vaginal opening. Body weight at vaginal opening was significantly higher **[by 7%]** in the high-dose bisphenol A group. In sexual behavior testing of males, a non-dose-related decrease in the intromission rate observed in the low-dose group was the only significant effect reported following bisphenol A exposure. There were no effects on mounting or ejaculation. Bisphenol A exposure had no significant effects on female sexual behavior as measured by ear wiggle, lordosis quotient, and rejection of males. The study authors concluded that bisphenol A exposure had no remarkable effects on male or female sexual behavior. The only significant effect on organ weights was an **[9%]** increase in testis weight in the high-dose bisphenol group. There were no significant effects on absolute weight or relative (to body weight) weights of ventral prostate, seminal vesicle, uterus, or ovary. Bisphenol A treatment did not affect sperm count or motility or estrous cycles. Serum levels of LH, FSH, prolactin, testosterone, and 17β-estradiol were also unaffected by treatment. No histopathological findings were observed in testis or ovary. **[Data were not shown.]**

In open-field testing of control rats, females moved greater distances, reared more often, and spent more time in the center of the testing apparatus. Following treatment with the low or high-dose of bisphenol A, there were no longer significant differences between males and females in frequency of rearing and or duration of time spent in the center of the apparatus. Differences in distances moved by males versus females were no longer significant following exposure to the high bisphenol A dose. Males in the low bisphenol A group reared significantly more times than males in the control group. Bisphenol A treatment had no significant effect on the sex-related difference in size of the SDN-POA, which was significantly larger in males than females in the control and treatment groups. Although the volume of the locus ceruleus was significantly greater in females than males of the control group, locus ceruleus volume was significantly larger in males than females of both bisphenol A groups. The change was due to a significant increase in volume in males at the low dose and significant decrease in volume in females at both dose levels of bisphenol A. **[Magnitude of locus ceruleus volume changes in males and females was ~12–17% compared to controls, as estimated from a graph.]** The numbers of neurons in the locus ceruleus was affected in the same manner as volume by bisphenol A treatment, except that increases in neuron numbers following bisphenol A treatment were also significant in males of the high-dose group.

Diethylstilbestrol mainly affected open-field behavior, locus ceruleus volume, and the reproductive system. *Trans*-resveratrol mainly affected locus ceruleus volume and the reproductive system. The study authors concluded that the brain is highly sensitive to bisphenol A at levels below the tolerable daily intake and disruptions in sexual differentiation may differ from effects observed with diethylstilbestrol and *trans*-resveratrol.

Strengths/Weaknesses: As with the previous study by this group (Kubo et al., 2001) the main weakness of the study lies in the failure to accurately describe the methods to allow a reader to determine how much bisphenol A the dams received during the experiment. Despite well-selected endpoints, the sample size of 5 dams/group and lack of clarity on the number of pups analyzed per litter are weaknesses.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process due to insufficient sample size and lack of experimental detail.

Facciolo et al. (2002), supported in part by the Italian Ministry of University Education and Research, examined the effects of developmental exposure to bisphenol A on the somatostatin receptor subtype sst_2 in the limbic circuit of rats. Sprague–Dawley dams were exposed orally to bisphenol A at 0 (arachis oil vehicle), 0.040, or 0.400 mg/kg bw/day. **[No information was provided on the specific method of oral dosing, the purity of bisphenol A, or the number of dams treated/group. There was no information on the type of chow used or composition of cage and bedding materials.] [Author states that 32 dams were subdivided into three treatment subgroups: controls ($n = 8$;), low bisphenol A and high bisphenol A ($n = 12$/group) (R. Facciolo, personal communication, July 17, 2007).]** The authors stated that the doses selected were relevant to human exposures from can linings and dental sealants and had been reported to induce morphometric changes in offspring. The rats were mated for 5 days during the treatment period, and treatment was continued through gestation and lactation. Litters (minimum 8/group) were culled to 8 pups at birth and 1 pup/litter was randomly assigned to a dam in the same treatment group for

slightly higher than environmental exposures irreversibly affected perception of fear-provoking stimuli and monoaminergic neural pathways in male offspring.

Strengths/Weaknesses: The use of a single dose level is a weakness. Strengths include the variety of endpoints used to provide data, which point to effects that are not gross structural changes but relatively subtle behavioral effects.

Utility (Adequacy) for CERHR Evaluation Process: These data are adequate and of high utility for the evaluation process.

Farabollini et al. (1999), supported by the University of Siena, University of Firenze, MURST, and the Italian National Research Council, examined the effects of perinatal bisphenol A exposure on behavior in male and female rats. **[No information was provided in the manuscript about chow or composition of cage and bedding materials. The Expert Panel has been informed that Morini MIL chow, lignocel bedding, and polysulfone cages were used (F. Farabolli et al., personal communication, March 1, 2007).]** Three groups of Sprague–Dawley rats were orally dosed with the arachis oil vehicle or bisphenol A **[purity not reported in the manuscript; ≥95% according to the authors (F. Farabolli et al., personal communication, March 1, 2007)]** by micropipette. One group of 11 rats was administered 0.040 mg/kg bw/day bisphenol A from 10 days before conception until weaning of pups at 21 days of age. A second group of 11 rats was given arachis oil from 10 days before conception through GD 13, 0.400 mg/kg bw/day bisphenol A from GD 14 **[day of vaginal plug not stated]** through 6 days following delivery of pups, and arachis oil until weaning of pups. A control group of 9 rats was given arachis oil from 10 days before conception until weaning of pups. Beginning at 85 days of age and continuing for 3 days, behavioral testing was conducted using a hole board and elevated-plus maze in 15 offspring/sex from the low-dose group, 11–12 offspring/sex from the high-dose group, and 14 pups/sex from the control group. **[Litter distribution was not reported.]** Separate sessions were conducted for each sex and treatment group. Data were analyzed by ANOVA and Fisher least significant difference test. A factor analysis was conducted using the principal components method with an orthogonal rotation of the factor matrix. **[It appears that offspring were considered the statistical unit.]**

In general, head dipping (extending head over edge of apparatus) and arm entries were reduced and self-grooming was increased in exposed females. Head dipping and stretched-attend posture (moving body forward without moving paws and then returning to original position) were inhibited and arm entries were increased in exposed males. A factor analysis indicated reduced anxiety and motivation to explore in treated males and reduced activity and motivation to explore in treated females. The study authors concluded that although sex-related differences in behavior were noted following bisphenol A treatment, there was no clear masculinization of behavior in females. The authors also noted the lack of substantial differences in results between the two exposure protocols.

Strengths/Weaknesses: The unusual exposure scenario in this study is both a strength and a weakness; however, the use of 11–15 pups from 9–11 litters raises concern for possible litter effects that were unaccounted for in the statistical analysis.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate due to insufficient control for possible litter effects.

Farabollini et al., (2002), supported by the University of Siena, University of Firenze, and MURST, examined the effects of perinatal bisphenol A exposure on sociosexual behavior in rats. Sprague–Dawley rats were housed in polysulfone cages. **[No information was provided in the manuscript on type of feed or composition of bedding materials. The Expert Panel has been informed that Harlan Teklad 2018 chow and Lignocel bedding were used (F. Farabolli et al., personal communication, March 1, 2007).]** Dams received arachis oil vehicle ($n = 13$) or 0.040 mg/kg bw/day bisphenol A **[purity not indicated]** ($n = 7$) through a micropipette from mating through weaning of pups. On Day 2 following delivery, litters were culled to 4 pups/sex and cross-fostered to obtain the following exposure groups of 12 animals/sex:

- Prenatal exposure group: born to bisphenol A-treated dams and nursed by vehicle-treated dams;
- Postnatal group: born to vehicle-treated dams and nursed by bisphenol A-treated dams; and
- Control group: born to and nursed by vehicle-treated dams.

Litters were weaned on PND 21 (day of birth not defined). On day 45 **[assumed to be PND 45]**, animals of the same sex were randomly chosen and housed 4/cage, with no siblings in any cage. At 100 days of age, behavior in the presence of an intruder rat was observed. In female rats, vaginal smears were taken at the end of intruder testing and only females in diestrus were considered ($n = 8$–9/group). One week later, sexual orientation was tested in 12 rats/sex/group by placing a rat between cages containing a sexually receptive female and sexually mature male and recording the number of visits to each rat. Sexual performance was tested next in males; evaluation was restricted to only males that ejaculated ($n = 10$–12 group). One week later, sexual behavior was tested in females during the diestrous or proestrous phase. **[It is not clear how many females were evaluated for sexual behavior.]** Behavior testing sessions were video recorded and later evaluated by a blinded observer. Data were analyzed by ANOVA followed by post-hoc Fisher least significant difference test. Litter effects were purposely confounded through cross-fostering.

In intruder testing, statistically significant effects observed in males exposed prenatally to bisphenol A included an increased number showing defensive behavior (9 of 10 vs. 4 of 10 in the control group), a decreased number showing ambivalent behavior (3/10 vs. 8/10 in the control group), and increased ratio of defensive/agonistic behaviors **[by 280% compared to controls]**. No significant effects were observed in intruder testing of female rats. There was no effect on sexual preference of males or females. For sexual behavior testing of females, data from the pre- and postnatal exposure groups were pooled because there were no significant differences between groups. Bisphenol A exposure significantly decreased exit latency in females in diestrus **[by ~66%]** and proestrus **[by ~83%]** and significantly ($P < 0.05$)

increased lordosis frequency in females in proestrus **[∼11.75 vs. 3.75 times in controls]**. Statistically significant effects on sexual performance of treated males included an increased number of intromissions **[∼15 compared to 11 in controls]** in the postnatal exposure group and increased duration of intromission latency **[∼115 vs. 40 sec in controls]** and genital sniffing **[∼40 vs. 16 sec in controls]** in the prenatal exposure group. The study authors stated that the results suggested a slight intensification of sexual behavior in females, slightly reduced performance in a limited number of endpoints in males, but no effect on other important sexual endpoints in males (e.g., latency of ejaculation and refractory period). It was concluded that pre- or postnatal exposure to bisphenol A potentiated female behavior and depotentiated male behavior.

Strengths/Weaknesses: The work was carefully performed. The use of a single dose level of bisphenol A is a weakness; however, this dosing paradigm is consistent with many other studies by this group making comparisons between the studies relevant. Addressing aggressive/defensive behavior as well as sexual performance and interest in both male and female offspring is a strength. The failure to address underlying biological mechanisms is a weakness. Further weaknesses include the inability to account for litter effects as the use of multiple pups from some litters without appropriate statistical control raises concern for possible litter effects due to unequal litter representation

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for evaluation purposes due to the inability to fully account for possible litter effects.

Dessi-Fulgheri et al. (2002), supported by the University of Firenze, University of Siena, and MURST, examined the effect of perinatal bisphenol A exposure on play behavior in rats. Sprague–Dawley rats were housed in polysulfone cages. **[No information was provided in the manuscript on chow or bedding material. The Expert Panel has been informed that Morini MIL chow and Lignocel bedding were used (F. Farabolli et al., personal communication, March 1, 2007).]** Using a pipette, rats were fed solutions containing the arachis oil vehicle and/or bisphenol A according to 1 of 3 exposure scenarios. A control group of 9 rats was given arachis oil from 10 days before mating until weaning of pups on PND 21 **[day of birth not defined]**. Eleven rats in the low-dose group were given 0.040 mg/kg bw/day bisphenol A **[purity not provided]** from 10 days before mating until weaning of pups. Eleven rats in the high-dose group received arachis oil vehicle from 10 days before mating until GD 13 **[day of vaginal plug not defined]**, 0.400 mg/kg bw/day bisphenol A from GD 14–PND 6, and arachis oil from PND 7 until weaning. Both doses were considered to be within the range of human exposure. The low dose was said to represent exposures through food occurring over a long period of time. The high-dose was said to represent exposures occurring through dental procedures occurring over a short period of time. Litters were culled to 8 pups at birth. **[No information was provided in the study on the sex distribution of the retained pups; the Expert Panel was advised that there were 4 males and 4 females/litter (F. Farabolli et al., personal communication, March 1, 2007).]** After pups were weaned, 3 male and 3 female pups were randomly caged together, with no siblings co-housed in any cage. Behavioral testing was conducted on PND 35, 45, and 55. For the behavioral testing, rats from the same cage were individually identified by marking them with dye. On each day of testing, the 6 cage mates were transferred to a neutral arena that was covered in clean sawdust and video recorded for 6 min. Behaviors recorded during the second and third minute of each testing session were evaluated. There were 12–15rats/sex/group. **[The methods section indicates that 15 rats/sex were tested at the high-dose, 12 rats/sex at the low dose, and 15 rats/sex in the control group. According to Table 4 of the study, which gives the pooled number of rats tested for 3 age periods, it appears that 12/sex were tested in the high-dose group, 15/sex in the low-dose group, and 15/sex in the control group. The Expert Panel has been informed that Table 4 is correct (F. Farabolli et al., personal communication, March 1, 2007).] For statistical analyses, individual factor scores were used as independent variables in a 3-way ANOVA that considered treatment, sex, and age. Fisher least significant difference test was used when appropriate. At weaning, housing conditions confounded litter of origin that was not then accounted for in statistical analyses.**

Behavioral elements were categorized under 8 general factors. The authors first presented results that were pooled for the 3 different age groups. In females of the low-dose group, bisphenol A treatment was found to significantly increase factors addressing play directed toward females. Factors affecting low-intensity mating elements (e.g., crawling-under behavior) were reduced significantly in high-dose males and females. Factors of sociosexual exploration (e.g., genital and body sniffing) were reduced significantly in high-dose females and in males from both dose groups. Factors of social interest (e.g., approaching) were reduced significantly in both sexes at the high-dose but increased in low-dose males. The authors next discussed results for PND 35, because it is the approximate time period of vaginal opening in females. Factors that were affected significantly at PND 35 included increased social interest by males and females of the low-dose group, decreased low-intensity mating elements by females of both dose groups, and decreased sociosexual exploration by males of both dose groups. The study authors concluded that 2 factors of female behavior were masculinized by treatment: play with females and sociosexual exploration.

Strengths/Weaknesses: A strength of this work is that it evaluated the socio-sexual consequences of exposure, and specifically at a young age. Weaknesses include absence of accounting for litter influences and inadequate statistical procedures (i.e., failure to consider the repeated measures design). In addition, the hypothesis is not biologically plausible (i.e., consistent with expected effects of a chemical with an estrogenic mode of action) and the factor analysis does not necessarily cluster the play behaviors that are known to be sexually dimorphic.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for evaluation process due to faulty statistical procedures.

Porrini et al. (2005), supported by MURST, the University of Firenze, and the University of Siena, examined the effects of perinatal bisphenol A exposure on play behavior of female rats. **[No information was provided in the manuscript about the type of feed or**

duration of adverse effects induced by estrogenic compounds were broadly similar to the estrogenic potencies of the compounds.

Strengths/Weaknesses: This is a carefully performed study, although the inclusion of many methodological details (*vide supra*) would have improved it. Strengths include the use of a wide range of estrogenic compounds to alter testicular development. A limitation for the present purpose is that only a single dose level of bisphenol A was administered subcutaneously. A weakness is that tissues other than the testis were not examined. Other weaknesses include sample sizes ranging from 3–20 examined pups across groups and s.c. administration.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for evaluation due to lack of clarity about experimental or statistical control for litter effects.

Nagao et al. (1999), supported by the Japanese Ministry of Health and Welfare, examined the effects of neonatal bisphenol A exposure on reproductive function of male and female Sprague–Dawley given CE-2 feed (Clea Japan). **[No information was provided about caging or bedding materials.]** From PND 1–5 (birth by 16:00 considered PND 0), 28–31 pups/sex/group were s.c. injected with corn oil vehicle, 300 mg/kg bw/day bisphenol A **[purity not reported]**, or 2 mg/kg bw/day estradiol benzoate. Pups within litters were treated with the same dose. Doses were based on results of preliminary studies that demonstrated no effect on growth or viability at bisphenol A doses up to 300 mg/kg bw/day administered by s.c. injection in the neonatal period. Pups were examined for viability from PND 6–21. On PND 21, 5 pups/sex/group were randomly selected and killed. Pups were transcardially perfused, and reproductive organs were collected for histopathological evaluation. At 12 weeks of age, 22–25 rats/sex were mated with untreated rats. Females were killed on GD 13 for an evaluation of implant number and viability of embryos. After fertility evaluation, sexual behavior with a sexually receptive female was assessed in 10 males/group. Following evaluation of sexual behavior, 15 male rats/group were killed for measurement of reproductive organ and brain weight. Histopathology of reproductive organs and SDN-POA volume were measured in 5 males/group. Copulation and fertility indices were analyzed by χ^2 and Fisher exact one-tailed test. Data for other endpoints were analyzed by Student *t*-test.

In rats treated with bisphenol A, there were no clinical signs of toxicity or effects on pup viability or body weight gain during or following the lactation period **[data for pup viability not shown by study authors]**. There were no effects on age of vaginal opening or preputial separation. Copulation and fertility indices and numbers of live embryos/litter were not affected in male or female rats treated with bisphenol A. Bisphenol A treatment did not affect sexual behaviors of males, as determined by number of mounts, intromissions, and ejaculations. No histopathological alterations were observed in the ovaries of treated females at 21 days of age or in the epididymis, prostate, or seminal vesicles of treated male rats at 21 days or 14 weeks of age. **[The prostatic lobe not specified; based on the figure provided, the lobe appears to have been ventral prostate. The Expert Panel notes that the number of apically located nuclei may be elevated by 14 weeks of age over what would normally be expected; however, this observation cannot be determined definitively based on a single high power field and in the absence of a matched control.]** No effect of treatment was observed on the SDN-POA of males. In contrast to the bisphenol A groups, rats treated with estradiol benzoate experienced decreased body weight gain, compromised male sexual behavior, infertility, lesions in reproductive organs, and reduced volume of the SDN-POA. The study authors concluded that neonatal exposure to a relatively high-dose of bisphenol A had no effect on morphological development or function of the reproductive system.

Strengths/Weaknesses: Strengths include a well performed and documented study that compared effects of bisphenol A and estradiol benzoate. Additional strengths include documentation of both behavioral (mating behavior) and biological (genital tract development) endpoints in both male and female rats. Weaknesses include the use of only a single dose level of bisphenol A via s.c. injection, and no accounting for litter effects within the context of individual animal treatments within litters.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for evaluation, however, utility is limited by subcutaneous administration.

Stoker et al. (1999), support not indicated, examined the effects of prepubertal bisphenol A exposure on prolactin secretion and prostate size in rats. **[No information was provided about feed, bedding, or caging materials.]** On PND 22–32 (day of birth = PND 0), 15–17 male Wistar rats from different litters/group were s.c. injected with bisphenol A **[purity not reported]** at 0 (sesame oil vehicle) or 50 mg/kg bw **[assumed to be 50 mg/kg bw/day]**. Another group of rats was administered 17β-estradiol through a s.c. Silastic tube implant **[dose administered not clear]**. On PND 29, 6 animals/dose were killed and blood was collected for measurement of serum prolactin concentration. The remaining rats ($n = 9$–11/group) were killed at 120 days of age. Prolactin levels were measured in serum and anterior pituitary by RIA. Inflammation was visually examined in the ventral and lateral prostate. Left lateral and ventral prostates were weighed and lateral prostate was analyzed for myeloperoxidase (an indicator of neutrophil numbers) and DNA. The right lateral prostate was subjected to histological examination. Statistical analyses included ANOVA, Dunnett *t*-test for multiple comparison, and Fisher exact probability test.

On PND 29, serum prolactin levels were significantly increased by ~210% in rats of the bisphenol A group compared to the control group. On PND 120, there was no effect on prolactin levels in serum or pituitary in the bisphenol A group. Ventral prostate weight was unaffected but lateral prostate weight was increased **[by ~25%]** in the bisphenol A group. Exposure to bisphenol A had no effect on body or testis weight. **[Data were not shown by study authors.]** The myeloperoxidase assay was reported to show a "trend" for lateral prostate inflammation in the bisphenol A group. **[Trend was not defined; there was no statistical difference between the bisphenol A group and the control in the myeloperoxidase assay.]** No histological evidence of inflammation was observed in prostates from the control group. In the bisphenol A group, histopathological analyses revealed

that 44.4% of prostates contained increased a focal luminal polymorphonuclear cellular infiltrate that was milder in severity compared to prostates from the 17β-estradiol group. The study authors noted the discrepancy between the results obtained by myeloperoxidase assay and histological observation in the bisphenol A group and stated that the discrepancy may have been due to evaluation of the whole tissue by myeloperoxidase assay versus only one section of the tissue by histological evaluation. Bisphenol A had no effect on prostate DNA content. In addition to prostate inflammation, effects observed in the 17β-estradiol group were increased serum prolactin levels on PND 29 and elevated myeloperoxidase and DNA content in lateral prostate on PND 120. Based on these findings, the study authors concluded that chemically induced, transient increases in prolactin secretion in the prepubertal period can lead to increased incidence of lateral prostate inflammation in 120-day-old rats.

Strengths/Weaknesses: Comparison with other agents is a strength. Weaknesses include low to moderate sample sizes and the use of a single high-dose level of bisphenol A through subcutaneous administration.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for the evaluation process but has limited utility due to concerns about sample sizes and route of administration of treatment.

Atanassova et al. (2000), supported by the European Center for the Ecotoxicology of Chemicals and AstraZeneca, examined the effects of neonatal bisphenol A exposure on the reproductive system of male rats. Wistar rats were fed rat and mouse breeding diet No. 3, which contains 15.5% soy meal flour. **[No information was provided about caging and bedding materials.]** Litters of 8–12 male rats from randomized litter origin were assembled by cross-fostering pups on PND 1 (day of birth). On PND 2–12, rats were s.c. injected with corn oil vehicle or bisphenol A **[purity not given]** 0.5 mg/day. **[Assuming a 5–25 g body weight during this interval, this dose would be ~100 mg/kg bw/day at the beginning of the interval and ~20 mg/kg bw/day at the end of the interval.]** Other groups of rats were s.c. injected with 0.01–10 μg diethylstilbestrol every other day between PND 2–12 or 2 mg 4-*tert*-octylphenol/day during PND 2–12. Rats were killed on PND 18, 25, and 90–100. At PND 18 and 25, testes were weighed and fixed in Bouin solution. Testicular cell numbers and seminiferous tubule lumen formation were determined by standard point counting of cell nuclei. Apoptosis was assessed by DNA fragmentation detected by in situ DNA 3′-end labeling. Spermatocyte nuclear volume as a fraction of Sertoli cell nuclear volume was calculated as ''an index of spermatogenic efficiency.'' Plasma FSH and inhibin B were measured by RIA and ELISA methods, respectively. Fertility was assessed at 80–90 days of age; rats were mated for 7 days and number of pups was counted at birth. The number of rats/group examined was 7–14 at 18 days of age, 4–12 at 25 days of age, and 6 in fertility testing. Data were analyzed by ANOVA.

Significant effects observed on PND 18 were advanced testicular lumen formation and increases in testis weight, Sertoli cell volume/testis, and spermatocyte nuclear volume/unit Sertoli cell. A decrease in germ cell apoptosis was also described on PND 18 but was not statistically significant. Plasma FSH levels were increased significantly on PND 18, but there was no effect on plasma inhibin B concentration. The only significant effect observed on PND 25 was increased plasma FSH levels. Testis weight was increased in adulthood, but there were no effects on fertility or litter size. Effects observed with octylphenol were similar to those observed with bisphenol A. In contrast, exposure to one or more doses of diethylstilbestrol resulted in increased apoptosis, decreased plasma inhibin levels, decreased Sertoli cell nuclear volume, and changes in spermatocyte/Sertoli cell ratios. The study authors concluded that the effect of bisphenol A on spermatogenic processes is benign.

Strengths/Weaknesses: Comparison with other agents is a strength. Weaknesses include low to moderate sample sizes, the use of a single high-dose level of bisphenol A through subcutaneous administration, and no accounting for litter effects within the context of individual animal treatments within litters.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for the evaluation process but has limited utility due to concerns about sample sizes and route of administration of treatment.

Williams et al. (2001), supported by the European Centre for Ecotoxicology, examined the effect of neonatal bisphenol A exposure on seminal vesicle structure and expression of sex steroid receptors in rats. On PND 2 (day of birth = PND 1), litters consisting of 8–14 male Wistar rat pups were derived through cross-fostering. Rats were s.c. injected with corn oil vehicle or 0.5 mg/day bisphenol A on PND 2–12. **[Assuming a 5–25 g body weight during this interval, the dose would be ~100 mg/kg/day at the beginning of the interval and ~20 mg/kg bw/day at the end of the interval.]** The dose was based on the highest amount that could remain in solution. A positive control group was injected with diethylstilbestrol at 0.1, 1, or 10 μg/day on PND 2, 4, 6, 8, 10, and 12. Ethinyl estradiol was administered at 10 μg/day, according to the protocol for diethylstilbestrol. Control animals for each compound were dosed with vehicle on the appropriate days, and because no differences were noted for controls, data were pooled. The effects of 4-*tert*-octylphenol, genistein, Antarelix, flutamide, and tamoxifen were also examined but will not be discussed. **[No information was provided about feed, caging or bedding materials, or purity of compounds.]** Animals were killed on PND 18, and seminal vesicles from 11–15 animals/group were collected and stored in Bouin solution. Seminal vesicles were examined for gross abnormalities in stroma and epithelium. Immunolocalization studies were conducted to assess ERβ, ERα androgen receptor, and progesterone receptor proteins in the seminal vesicle. Studies were replicated 3–5 times using samples from at least 6 animals/group. Results were scored subjectively.

The gross structure of the seminal vesicles from bisphenol A-treated rats appeared normal, and there were no changes in ERβ, ERα, androgen receptor, or progesterone receptor proteins in the seminal vesicle. In contrast, diethylstilbestrol induced changes in seminal vesicle morphology, increased ERα and progesterone receptor, and decreased androgen receptor. Effects of ethinyl estradiol were similar to those observed with diethylstilbestrol. The study authors concluded that the lack of bisphenol A effects suggested that only high-

injected with the tocopherol-stripped corn oil vehicle, bisphenol A **[purity not indicated]** at 0.1 or 0.5 mg/day, diethylstilbestrol at 5 μg/day, or octylphenol at 0.1 or 0.5 mg/day. **[Assuming a pup body weight of 5 g, bisphenol A intakes were estimated at 20 and 100 mg/kg bw/day.]** On PND 15, 20, and 25, blood was collected for measurement of serum prolactin level by RIA. A final sample for prolactin analysis was obtained when animals were killed on PND 30. Medial basal hypothalamus, anterior pituitary, uterus, and prostate were collected for measurement of *ER*α and *ER*β mRNA expression by RT-PCR in animals of the low-dose group. Statistical analyses included ANOVA followed by Student-Newman–Keuls test.

In male and female rats, hyperprolactemia was observed on PND 25 and 30. **[On PND 30, prolactin levels in the low- and high-dose groups compared to the control group were ~150 and 95% higher in females and 120 and 80% higher in males]**. In females exposed to the low dose, *ER*α mRNA in the medial basal hypothalamus was higher **[by 25%]** than control levels. In anterior pituitary of low-dose males, *ER*α mRNA was higher **[by ~80%]** and *ER*β mRNA was higher by 35–40% compared to control levels. There were no effects on *ER*β mRNA in female tissues. Most effects observed with octylphenol exposure were similar to those observed with bisphenol A exposure. Diethylstilbestrol induced transient increases in prolactin levels, decreased expression of *ER*α in medial basal hypothalamus of males, upregulated *ER*α and *ER*β expression in the pituitary of males, decreased expression of *ER*α in the uterus, and upregulated *ER*β expression in prostate. The study authors concluded that exposure of neonatal rats to bisphenol A resulted in delayed and sustained hyperprolactemia and changes in *ER* mRNA expression.

Strengths/Weaknesses: A strength is that both male and female animals were assessed following administration of two dose levels. Weaknesses include small treatment groups consisting of unclear numbers of litters and composition and limited experimental details regarding design.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process due to lack of design clarity.

Fukumori et al. (2003), support not indicated, examined the effect of postnatal bisphenol A exposure on ultrastructure of the prostate in rats. **[The study was published in Japanese; a translation was provided by the American Plastics Council.]** On Day 1–21 following birth, F344 rats were s.c. injected with bisphenol A 5 days/week at doses of 0 (DMSO vehicle), 0.0008, 0.004, 0.020, and 0.500 mg/kg bw/day. A positive control group received 100 μg/kg bw 17β-estradiol by s.c. injection during the same time period. Rats were killed at 22 days of age. Ventral prostates were fixed in glutaraldehyde, sectioned, and examined by electron microscopy. **[The number of rats treated and examined/group and the number of litters represented were not reported. No information was provided on purity of bisphenol A, type of feed, or composition of bedding and caging. The translated version of the report did not include figures from the original report.]**

In ventral prostates obtained from rats exposed to 17β-estradiol, there was an increase in secretory granules accompanied by reductions in microvilli on the surface of the glandular epithelium. Proliferation of fibroblasts was observed in the fibromuscular layer of the stroma in rats from the 17β-estradiol group. In the 0.020 and 0.500 mg/kg bw/day bisphenol A groups, a slight increase in secretory granules and slight decrease in microvilli was observed in glandular epithelium. Effects in stroma were described as unremarkable for the bisphenol A groups. The study authors concluded that bisphenol A may have ultrastructural effects on the ventral prostates of suckling rats.

Strengths/Weaknesses: This is a translation of an apparently carefully performed study to assess the effects of low doses of perinatal bisphenol A on prostatic structure. A major weakness is that the original figures were not provided in the translated version of the report, and the route is s.c. injection in DMSO. The young age at which the animals were sacrificed is also a concern because prostatic development is not complete at 22 days of age making comparisons with the bulk of established data problematic. The lack of data specifics raise the level of uncertainty about this study.

Utility (Adequacy) for CERHR Evaluation Process: This study is considered inadequate for inclusion in the evaluative process because of the lack of detail on study design (i.e., litter representation, number of animals per group).

Kato et al. (2003), supported by the Japanese Ministry of Education, Culture, Sports, Science, and Technology and the Ministry of Health, Labor, and Welfare, examined the effects of neonatal bisphenol A exposure on the reproductive organs of rats. Sprague–Dawley rats were fed CRF-1 diet. **[No information was provided on caging or bedding materials.]** Female offspring from 8 dams were grouped to achieve equal distribution of body weight. At least 8 female offspring/group were s.c. injected with 0 (ethanol/corn oil vehicle), 0.25, 1, or 4 mg/day bisphenol A **[purity not reported]** from PND 0 to 9 (day of delivery = PND 0). **[Based on body weights reported on PND 0 and 9, CERHR calculated mean bisphenol A intakes of ~26, 105, and 427 mg/kg bw/day.]** A positive control group was given 10 μg/day 17β-estradiol **[~3 mg/kg bw/day]** during the same time period. Rats were weighed during and following the lactation period and examined for day of vaginal opening. External reproductive organs were examined on PND 60, and estrous cycles were assessed from PND 61–94. One group of rats was ovariectomized on PND 80; ovaries were weighed, and fixed in 10% neutral buffered formalin for evaluation of corpora lutea and polyovular follicles. Another group of bisphenol A-exposed and the vehicle-treated control females were given 1 μg/kg 17β-estradiol from PND 94–96 and killed the day following final injection; uterus and vagina were weighed, and fixed in 10% formalin. For all endpoints, 5–8 rats/group were examined. Statistical analyses included Student *t*-test and Fisher exact probability test.

Treatment–related results are summarized in Table 74. Two rats of the high-dose group died. Body weights of rats in the high-dose group were lower than controls on PND 9–30 but higher than controls on PND 61–97. Effects observed at the mid- and high-dose included accelerated vaginal opening, increased incidence of polycystic ovaries, decreased area of corpora lutea, and decreased uterine fluid weight. All rats of the mid-dose group had partial clefts in the clitoris, and all rats of the high-dose

Table 74
Effects in Female Rats Exposed to Bisphenol A During the Neonatal Period[a]

Endpoint	Dose, mg/kg bw/day **[CERHR estimate]**						
	26	105	427	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$
Body weight gain							
PND 9	↔	↔	↓16%	286	200	233	156
PND 97	↔	↔	↑10%	432	261	430	253
Day of vaginal opening	↔	↓2.9 days	↓4.1 days	345	267	159	116
No. with normal estrous cycles[b]	↔ (8/8)	↔ (2/8)	↓ (0/6)	81	28		
No. with cleft clitoris[c]	↔ (0/8)	↑ (0/8)	↑ (6/6)	299	failed		
Relative organ weight							
Ovary	↔	↔	↓59%	85	59	140	93
Uterus, wet	↔	↔	↓60%	66	55	128	96
Uterus, blotted	↔	↔	↓21%	273	128	318	168
Uterine fluid weight	↔	↓42%	↓97%	42	34	139	104
No. with polycystic ovaries[c]	No data	↑ (4/8)	↑ (5/5)	81	24		
No. with corpora lutea[b]	No data	↔ (8/8)	↓ (0/5)	238	90		
No. of corpora lutea	No data	↔	↓ (none)	65	38	137	83
Corpora lutea area	No data	↓ 30%	↓ (none)	42	37	84	66

[a]Kato et al. (2003).
[b]Control rate 8/8.
[c]Control rate 0/8.
↑,↓ Statistically significant increase or decrease compared to controls; ↔ no statistically significant effect.

group had deep clefts in the clitoris. Additional effects observed in rats of the high-dose group included disrupted estrous cycles (e.g., irregular cycles or persistent estrous) and decreased relative (to body weight) ovary and wet or blotted uterus weights. Absolute weights of wet uterus and ovary were also reduced in the high-dose group. No corpora lutea were observed in rats of the high-dose group. Qualitatively similar effects were observed in the group treated with 17β-estradiol. The study authors concluded that exposure of rats to bisphenol A during the neonatal period resulted in changes in female reproductive organs.

Strengths/Weaknesses: The strengths are the carefully performed and documented experiments. The major limitation is that the s.c. route of administration and the doses of bisphenol A were relatively high. The changes in the female reproductive organs seen are well documented, but given the extremely high-dose of agent used, broadly unsurprising.

Utility (Adequacy) for CERHR Evaluation Process: The results of this study reflect a careful documentation of the experiments performed. The study is adequate for the evaluation process but has limited utility due to concerns about the route of administration.

Toyama and Yuasa (2004), supported in part by the Japanese Ministry of Environment and Ministry of Education, Science, Sports and Culture, examined the effects of neonatal bisphenol A **[purity not reported]** exposure on spermatogenesis during puberty and adulthood in rats and mice. **[No information was provided about chow or bedding and caging materials. The mouse data are reported in Section 3.2.8.]** Wistar rats were s.c. injected on a μg/pup basis with bisphenol A in a DMSO and olive oil vehicle on PND 1, 3, 5, 7, 9, and 11 (PND 0 = day of birth). Bisphenol A doses were 1.0, 10.0, 100.0, and 600.0 μg/pup. Additional animals were treated with 17β-estradiol and estradiol benzoate. Animals were killed weekly at 2–10 weeks of age, and other pups were killed at 24 and 31 days of age. There were 5 animals/dose/time point in bisphenol A groups and apparently 5 vehicle control rats/time period. Testes were examined by light and electron microscopy. Males from each experimental group (a total of 11 rats) were mated with 2 females **[number tested in each dose group not reported]**. A total of 11 rat dams were allowed to complete pregnancy. **[It does not appear that statistical analyses were conducted.]**

All rats given 0.600 μg/pup bisphenol A died before 20 days of age and were excluded from analysis. In mature spermatids of 8-week-old rats in the vehicle control group, the incidences of deformed acrosomes, deformed nuclei, and abnormal ectoplasmic specialization were <0.3%. In 8-week-old rats treated with ≥0.010 μg/pup bisphenol A, the incidence of deformed acrosomes was >50–60%, the incidence of deformed nuclei was >40%, and the incidence of abnormal ectoplasmic specialization was >60–70%. **[Data were not shown for individual dose levels.]** Similar effects were observed in the groups treated with 17β-estradiol and estradiol benzoate. No effects were reported at other ages. **[Data were not shown by study authors.]** The blood–testis barrier remained intact based on histologic observations. All tested males from the bisphenol A group were fertile, and sex ratio, litter sizes, and pup weights were reported to be normal. **[No results were shown for individual dose levels. Fertility data presented in Table 4 and 5 of the study, were not clearly identified by dose level.] The study authors concluded that bisphenol A acts as an estrogen and induces transient changes in the male reproductive system of rodents that resolve in adulthood.**

Strengths/Weaknesses: The strengths include the use of multiple doses of bisphenol A and the use of both rats and mice, allowing interspecies comparisons. Weaknesses include selective and unclear data presentation, absence of statistical analyses, subcutaneous injection on a per pup basis, and failure to examine sperm morphology in the fertile 15-week-old animals to

determine whether the changes in sperm maturation seen at earlier time points had resolved or whether the animals were fertile in the face of such abnormalities.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate and not useful for the evaluation process due to lack of clarity of design and analyses, route of administration, and dosing procedures.

Kato et al. (2006), supported by the Japanese Ministry of Education, Culture, Sports, Science and Technology and Ministry of Health, Labor and Welfare, examined the effects of neonatal exposure to bisphenol A on reproductive function of male rats. Sprague–Dawley rats were fed CRF-1 diet, which was described as having relatively low estrogenic activity compared to other Japanese rodent feeds. **[No information was provided on caging or bedding materials.]** Male rats used in this study were born to 12 dams, assigned to 8 foster dams in groups of seven based on body weights, and distributed to dose groups. From PND 0–9 (PND 0 = day of birth), 24 male pups/group were s.c. injected with bisphenol A **[purity not indicated]** at 0 (ethanol/corn oil vehicle), 0.000024, 0.000120, 0.000600, 0.003, or 1 mg/pup/day bisphenol A. Study authors calculated average exposures of 0.002, 0.011, 0.056, 0.277, or 97 mg/kg bw/day. An additional group was treated with 10 μg/day 17β-estradiol (0.9 mg/kg bw/day) during the same time period. Eight rats/group were killed and necropsied at PND 10, 35, and 150. At the PND 10 necropsy, serum testosterone levels were measured by RIA, the testis was weighed and examined histologically, and expression changes in genes for hormone receptors and steroidogenic enzymes were determined by RT-PCR. The same endpoints were examined at the PND 35 necropsy in addition to measuring seminal vesicle, ventral prostate, and epididymis weights. The remaining rats were assessed for day of preputial separation. From PND 105–130, they were mated for 1 day a maximum of 4 times with an untreated female in proestrus. Females were killed on GD 13 (day of sperm = GD 0) and examined for corpora lutea, embryonic mortality, and implantation sites. Male rats were killed on PND 150. In addition to endpoints examined at earlier time periods, sperm endpoints and histopathology of ventral prostate were assessed. Statistical analyses included Bartlett method for homogeneity of variance followed by Dunnett method for homogeneous variances or Dunnett-type method with rank order for heterogeneous variances. Reproductive data were analyzed by Fisher exact probability test. Data obtained from the 17β-estradiol group were analyzed by Student *t*-test.

There were no deaths or decreases in body weight in animals of the bisphenol A group. There were no effects on age of preputial separation, copulation rate, or fertility. In dams impregnated by bisphenol A-treated males, there were no effects on numbers of implantation sites, implantation losses, or live fetuses. Bisphenol A treatment had no adverse effects on sperm count, motility, or morphology. There were no effects on serum testosterone levels, histopathology of testis or prostate, or weights of testis, epididymis, seminal vesicle, ventral prostate, or penis. No significant changes were observed in mRNA for estrogen, androgen, or progesterone receptor or steroidogenic enzymes. In contrast to the bisphenol A groups, rats treated with 17β-estradiol experienced decreases in reproductive organ weights, altered gene expression, delayed and incomplete preputial separation, decreased copulatory rate, and decreased sperm numbers. The study authors concluded that neonatal bisphenol A exposure caused no adverse effects on reproductive function or gene expression of steroidogenic enzymes in the rat testis.

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Strengths/Weaknesses: This study has a number of major strengths, notably the wide range of doses, appropriate use of statistics, inclusion of a positive control, and use of relevant endpoints. Weaknesses include route of administration and dosing on a per pup basis, thus not adjusting for body weight.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate but of limited utility due to route of administration and dosing on a per pup basis.

Noda et al. (2005), support not indicated, examined the effect of neonatal bisphenol A exposure on reproductive organs of Sprague–Dawley rats. For 5 days beginning on PND 1 (day of birth = PND 0), 6–10 pups/sex/group (drawn from 2 litters) were s.c. injected with olive oil vehicle or bisphenol A **[purity not reported]** at 0.0001, 0.001, or 0.010 mg/rat/day. According to the study authors, the doses were equivalent to ~0.010, 0.100, or 1 μg/kg bw/day. A positive control group received diethylstilbestrol at the same doses as bisphenol A. Nonylphenol and genistein were also examined but will not be discussed here. Dose selection was based on diethylstilbestrol doses reported to have an effect. Stability, homogeneity, and concentration of dosing solutions were verified. Pups in each group were obtained from 2 dams. On PND 7, litters were adjusted to 4 males and 4 females/dam when possible. Dams and pups were housed in polycarbonate cages until weaning at PND 21. At that time, pups were housed in wire mesh cages. Animals were fed MF feed (Oriental Yeast Co.). **[No information was provided on bedding used in polycarbonate cages.]** During the study, animals were examined for clinical signs, body weight, anogenital distance on PND 7, and day of vaginal opening or preputial separation. Estrous cycles were assessed from the time of vaginal opening until animals were killed on PND 47–50 (females in diestrus). Rats in persistent estrus were killed on PND 70. Reproductive organs were weighed. Testis was fixed in Bouin solution and all other

reproductive organs were fixed in 10% neutral buffered formalin for histopathological examination. **[It was not indicated, but it is assumed that all pups were examined in each analysis.]** Data were analyzed by Bartlett test for homogeneity of variance, ANOVA, Dunnett test, or Kruskal–Wallis test.

In the bisphenol A groups, there were no abnormal clinical signs or effects on body weight. Absolute anogenital distance was not affected, but anogenital distance adjusted by the square root of body weight cubed was decreased in females treated with the mid- and high-bisphenol A dose. There were no effects on day of vaginal opening or preputial separation or on estrous cycles. **[Data were not shown.]** No gross or histopathological abnormalities were reported in male or female reproductive organs. The study authors only reported organ weight effects relative to body weight, because the rats were killed at different ages. The only dose-related effect on reproductive organ weight was increased relative ventral prostate weight in the high-dose group. Relative pituitary weight was increased in males of the low-dose group and females of the high-dose group. There were no effects on weights of testis, epididymis, seminal vesicle, uterus, or ovary in bisphenol A-treated animals. Effects observed in animals treated with 1 or more dose of diethylstilbestrol included delayed or incomplete preputial separation, estrous cycle disruption, underdeveloped reproductive organs (including ventral prostate), malformations in male and female reproductive organs, ovarian cysts, and uterine squamous metaplasia in glandular epithelium. The study authors noted that the shortened anogenital distance in females appeared to be biologically significant. However, it was stated that the effect is of unknown relevance in female rats and was not observed in the rats treated with diethylstilbestrol. The study authors concluded that findings observed with bisphenol A were not toxicologically relevant.

Strengths/Weaknesses: Strengths of this report include the use of 3 dose levels, the use of a positive control (diethylstilbestrol), and the use of multiple endpoints to evaluate estrogenic effects. Weaknesses include the use of only 2 litters to constitute exposure groups, exposure by the subcutaneous route to bisphenol A (not the anticipated route of exposure in humans), and dosing on a per pup basis.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate due to the combination of small sample size (i.e., 2 litters) and s.c. route of administration.

Ho et al. (2006b), supported by NIH and Department of Defense, examined the effect of developmental exposure to bisphenol A on susceptibility of Sprague–Dawley rats to prostate cancer. The dams and offspring used in this study were fed a soybean-free phytoestrogen-reduced diet (Zeigler Reduced Rodent Diet 2, Ziegler Brothers, Inc.), housed in polysulfone cages **[with unspecified bedding]**, and provided drinking water in glass bottles. On PND 1, 3, and 5 (day of birth = PND 0), 20–30 male pups/group were s.c. injected with tocopherol-stripped corn oil vehicle, bisphenol A **[purity not indicated]** at 0.1 μg/pup (0.010 mg/kg bw), or estradiol benzoate at 0.001 μg/pup (0.1 μg/kg bw) or 25 μg/pup (2500 μg/kg bw). Male rats from each litter were randomly assigned to treatment groups, but the total number of litters from which the pups were selected was not reported. Likewise, it is unclear, but assumed, that all doses were represented within litter rearing units. Pups were weaned on PND 21. At PND 90, half the rats from each treatment group were implanted with Silastic capsules containing 17β- estradiol and testosterone and the other half were implanted with empty capsules; the capsules were left in place for 16 weeks. The treatment was designed to result in a serum 17β-estradiol level of ~75 ng/L and testosterone level of ~3 μg/L, levels reported to induce prostatic intraepithelial neoplasia in 33% of Sprague–Dawley rats. Rats were killed at 28 weeks of age. Prostates were removed, and histopathological evaluations were conducted on each lobe. Immunohistochemistry techniques were used to measure proliferation. Apoptosis was measured using the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) technique. PCR techniques were used to study methylation pattern and expression changes in prostate cell signaling proteins on PND 10, 90, and 200. Statistical analyses included χ^2 test, ANOVA, Fisher exact test, and Bonferroni test. The study authors stated that similar responses were observed in each of the 3 prostate lobes; and thus results were presented only for dorsal prostate. In bisphenol A-exposed compared to vehicle controls rats that did not receive 17β-estradiol/testosterone exposure in adulthood, there were no effects on dorsal prostate weight, histopathology alterations, proliferation index, or apoptotic index. In bisphenol A-treated compared to vehicle control rats that received 17β-estradiol/testosterone exposure in adulthood, there was increased incidence and severity of prostatic intraepithelial neoplasia (100 vs. 40% incidence). In the bisphenol A/17β-estradiol/testosterone group, proliferation and apoptosis indices were increased in regions where prostatic intraepithelial neoplasia (PIN) was observed. In humans PIN is an accepted precursor lesion to prostate cancer. In rodents the significance of PIN is less clear. Some transgenic mouse models will form PIN lesions that progress to adenocarcinoma in a manner broadly similar to that seen in humans. However, there are many examples in which mice form PIN lesions that do not progress to invasive disease. In rats, testosterone plus estradiol classically induces PIN lesions that progress to adenocarcinoma. The increase in incidence of PIN lesions seen following testosterone and estradiol treatment in BPA exposed rats in this study are certainly a cause for concern. The data presented do not address whether these lesions progress to cancer in a manner similar to PIN lesions seen in the classic testosterone plus estradiol model, or whether such progression occurs at a higher or lower rate. Changes observed in rats exposed to the high estradiol benzoate dose in the neonatal period but not 17β-estradiol/testosterone during adulthood included increased incidence and severity of prostatic intraepithelial neoplasia and elevated apoptosis and proliferation indices. The same effects, in addition to decreased prostate weight, were observed in rats receiving neonatal exposure to the high estradiol benzoate dose and adult exposure to 17β-estradiol/testosterone.

In the investigation of a molecular basis for increased susceptibility to PIN, exposure to estrogenic compounds altered methylation pattern in several cell signaling genes. Phosphodiesterase type 4 variant, an enzyme

include small sample size (5 treated dams) and lack of adequate experimental and statistical control for litter effects.

Utility (Adequacy) for CERHR Evaluation Process: Despite certain strengths, this study is inadequate for the evaluation process for the reasons cited above.

Shikimi et al. (2004), supported by the Japan Society for the Promotion of Science for Young Scientists, examined the effects of bisphenol A exposure on Purkinje cell development in rats. **[No information was provided about feed or composition of caging and bedding materials.]** At 6–9 days of age, 4 male or female Fisher rats/group received bisphenol A **[purity not provided]** at 0 (sesame oil vehicle), 0.050, or 0.500 mg/day by injection into the cerebrospinal fluid near the region of the cerebellum. During the same time period, additional groups of 4 rats received 0.500 mg/day tamoxifen, 0.500 mg/day bisphenol A + 0.500 mg/day tamoxifen, or 5 μg/day estradiol benzoate through the same exposure route. **[Both male and female rats were treated, but it was not indicated if there were equal numbers in each group; both sexes were apparently evaluated together.]** At 10 days of age, pups were killed and vermal cerebella were removed and sectioned. Purkinje cells were examined morphologically following identification by calbindin-D28 K immunostaining. Data were analyzed by ANOVA, followed by Duncan multiple range test.

Treatment with the high-dose of bisphenol A increased Purkinje fiber length. There was no effect on cross-sectional soma area or Purkinje cell number as a result of bisphenol A treatment. Co-treatment with tamoxifen inhibited the increase in dendritic length that was observed following treatment with bisphenol A alone. Estradiol benzoate also induced an increase in dendritic length of Purkinje fibers that was blocked by tamoxifen. Treatment with tamoxifen alone also reduced dendritic fiber length. The effects of octylphenol were also examined and an increase in dendrite length was observed. The study authors concluded that bisphenol A induced Purkinje dendritic growth, possibly through the ER.

Strengths/Weaknesses: The use of estradiol benzoate as a positive control is a strength of this study. Weaknesses are the injection into cerebrospinal fluid.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process due to uncertainties surrounding the route of administration (i.e., difficulty of relating a cerebrospinal injection to human exposures).

Zsarnovszky et al. (2005), supported by NIH, NIEHS, and the American Heart Association, evaluated the effect of intracerebellar injection of bisphenol A on the development of activated extracellular signal-regulated kinase (ERK)-positive cells in cerebellar sections in Sprague–Dawley rats. Neonatal rats on PND 4–19 underwent a single direct injection under anesthesia of bisphenol A or 17β-estradiol under stereotactic guidance into cerebellar folia 6 and 7. **[For bisphenol A, only PND 10 results were given. The number of animals at each age was not specified, but a figure legend indicated at least 6/dose group. The purity of the chemicals was not specified. The day of birth was not defined.]** Concentrations of the chemicals were 10^{-12}–10^{-6} M **[bisphenol A concentrations of 0.23 ng/L to 0.23 mg/L]**. Uninjected, mock-injected, and vehicle-injected controls were used. Brains were removed and fixed 6 min after the onset of the injection. Sections were processed for immunohistochemistry using an antibody that recognized activated ERK. Quantitative analysis was performed on images of folium 9. Statistical analysis was performed using ANOVA with post-hoc Tukey–Kramer multiple comparison test. Response to different chemicals and different concentrations on PND 10 were compared using 2-factor ANOVA with post-hoc Bonferroni test. Adult rats were also treated but were not included in the quantitative analysis.

The qualitative appearance of the immunostained sections was similar after bisphenol A and 17β-estradiol. In the 10^{-12}–10^{-9} M dose range, the quantitative responses to the two chemicals were similar. Activated ERK-positive cells increased with a median effect concentration of 7.46 pM for 17β-estradiol and 3.25 pM **[0.74 ng/L]** for bisphenol A. Both chemicals were described as having an inhibitory effect at higher doses. **[The data graph shows drop-offs to control densities at 10^{-9} and 10^{-10} M, with a second increase in density at 10^{-7} and 10^{-5} M.]** Co-administration of 10^{-10} M 17β-estradiol with bisphenol A 10^{-12}–10^{-10} M **[0.23–23 ng/L]** resulted in a concentration-dependent decrease in activated ERK-positive cells compared to the administration of 17β-estradiol alone. The authors concluded that 17β-estradiol regulates ERK signaling in the developing cerebellum and that bisphenol A can mimic and also inhibit this estrogenic effect, with potentially adverse affects on brain development and function.

Strengths/Weaknesses: The use of 17β-estradiol as a positive control is a strength of this study. Weaknesses are the intracerebellar injection and the administration on a per pup basis.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process due uncertainties surrounding the route of administration (i.e., difficulty of relating a cerebrospinal injection to human exposures).

3.2.5 Mouse—oral exposure only during pregnancy.

3.2.5.1 Studies without neurobehavioral endpoints: Morrissey et al. (1987), supported by NTP/NCTR, examined the effects of prenatal bisphenol A exposure in rats and mice in studies conducted according to GLP. The studies are also available as NTP publications for rats (NTP, 1985c) and mice (NTP, 1985b). The study was conducted in two sets of rats and mice and data were pooled for each species. **[The data for rats were discussed in Section 3.2.1.]** Animals were fed Purina 5002 diet, housed in polypropylene or polycarbonate cages with stainless steel wire lids with Ab-Sorb-Dri cage bedding. Pregnant CD-1 mice were randomly assigned to groups of ≥10 animals in each set of the study, for a total of ≥20 animals/dose. On GD 6–15 (GD 0 = sperm or plug), mice were gavaged with bisphenol A at 0 (food-grade corn oil), 500, 750, 1000, or 1250 mg/kg bw/day. Doses were based on results of preliminary studies and were expected to result in 10% maternal mortality at the high-dose and no toxicity at the low dose. The purity of bisphenol A was >95%, and 2,4′-bisphenol A was reported as an impurity. Concentrations of dosing solutions were verified. Pregnant animals were

Table 75
Maternal and Developmental Toxicity in Mice Gavaged With Bisphenol A[a]

	Dose in mg/kg bw/day							
Endpoint	500	750	1000	1250	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$
Dam weight in treatment period	↔	↔	↔	↓43%	881	661	1159	1039
Gravid uterine weight	↔	↔	↔	↓32%	983	690	1243	1123
Relative dam liver weight	↑9%	↑13%	↑17%	↑26%	618	411	755	541
Resorptions/litter	↔	↔	↔	↑2.8-fold	817	377	1245	1162
Fetal body weight/litter	↔	↔	↔	↓15%	1079	785	1249	1024

[a]Morrissey et al. (1987).
↑,↓ Statistically significant increase, decrease; ↔ no statistically significant change.

Table 76
Sperm Production and Male Reproductive Organ Weights in Mice Exposed to Bisphenol A During Gestation[a]

	Dose in mg/kg bw/day[b]					
Endpoint	0.002	0.020	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$
Sperm production efficiency	↔	↓ 19%	0.011	0.007	0.010	0.007
Body weight	↓ 9%	↔				
Preputial weight	↑ 36%	↔				
Seminal vesicle weight	↓ 12%	↔				
Epididymal weight	↓ 12%	↓ 8%				

[a]vom Saal et al. (1998).
[b]Benchmark doses were not estimated for values obtained from graphs and non-dose-related effects; errors were assumed to be SEM, as reported earlier in the study.

weighed during the study. Mice were killed on GD 17. Liver and uteri were weighed, and corpora lutea and implantation sites were examined. Fetuses were sexed, weighed, and examined for viability and external, visceral, and skeletal malformations. Data were analyzed by Bartlett test for homogeneity of variance, ANOVA, and/or William multiple comparison, Dunnett, and/or Fisher exact probability tests. **[Data were presented and analyzed on a per litter basis.]**

Clinical signs reported in mice treated with bisphenol A included arched back, lethargy, piloerection, rough coat, vaginal bleeding, vocalization, alopecia, weight loss, and wheezing. One or 2 of 29–34 dams died in each of the 3 lowest dose groups and 6 of 33 dams died in the 1250 mg/kg bw/day group. Statistically significant effects are summarized in Table 75. Absolute liver weight was increased in the 500, 750, and 1000 mg/kg bw/day dose groups, and relative liver weights were increased in all bisphenol A dose groups. Decreased gravid uterine weight and dam body weight gain during the gestation and treatment periods attained statistical significance at the 1250 mg/kg bw/day dose. The number of litters available for evaluation in the control and each dose group was 26, 23, 21, 23, and 21. Increased resorptions/litter and decreased fetal body weights/litter attained statistical significance in the high-dose group. There was no effect on the number of live fetuses/litter at birth or on fetal malformations/litter. The study authors concluded that bisphenol A is not teratogenic in mice at doses that result in maternal toxicity.

Strengths/Weaknesses: Strengths include the oral route of exposure as well as the design and sample sizes used. The use of very high-doses is a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility in the evaluation in providing information on conventional teratogenic endpoints.

vom Saal et al. (1998), supported by NIH, examined the effects of bisphenol A exposure on male reproductive organs and sperm production in mice. The CF-1 mice used in this study were purchased in 1979 and maintained as an outbred stock in a closed colony. Dams were fed Purina breeder chow (5008) during pregnancy and lactation, and male offspring were fed Purina 5001 standard lab chow after weaning. Housing consisted of polypropylene cages with corn cob bedding. Bisphenol A **[purity not reported]** in tocopherol-stripped corn oil vehicle was fed to 7 mice/group by electronic micropipette at 0.002 or 0.020 mg/kg bw/day on GD 11–17 (day of vaginal plug = GD 0). One group of 6 mice was given the vehicle control, and a group of 5 mice was not handled. Based on results of in vitro assays conducted by the study authors, the 0.02 mg/kg bw/day bisphenol A dose was predicted to be bioactive in mice. Additional mice were treated with the same doses of octylphenol. Females delivered pups naturally on GD 19, and pups were weaned on PND 23 (day of birth not defined). Male siblings were housed 3/cage until 5 months of age. Randomly selected males were housed individually at 5 months of age and killed 1 month later. Body, testes, epididymides, preputial glands, and seminal vesicles were weighed in 11 control mice and 7 treated mice/group. Data from the two control groups did not differ significantly and were combined for analyses of organ and body weight. Data for prostate weight were reported by Nagel et al. (1997). Daily sperm production was determined in 8 control males/group and 5 treated

with 28 dams each were given the tocopherol-stripped corn oil vehicle. Because results from the two vehicle control groups were statistically equivalent, data from the two groups were pooled. A positive control group of 28 mice was given 0.2 μg/kg bw/day diethylstilbestrol. Dosing solutions were dripped into the animals' mouths using a micropipette. Concentrations of dosing solutions were verified before dosing. Animals were fed certified rodent chow 5002. Water was provided in glass bottles with Teflon seals. Cages were made of polypropylene with steel lids. Corn cob bedding was used. Music was played at low volume to provide background noise. Dams were monitored for clinical signs, food intake, body weight gain, and fertility endpoints. Pups were counted and sexed at birth (PND 0) and monitored for survival and weight gain until weaning on PND 22. Litters were culled to 8 pups on PND 4, leaving as many males as possible. At weaning, no more than 4 males/litter (65–95 males/group) were randomly selected to continue in the study and housed individually. The males were monitored for body weight gain and feed intake until they were killed on PND 90. Brain, liver, kidneys, and reproductive organs were weighed. Daily sperm production and epididymal sperm counts were determined and a histopathological examination of testes was conducted. The litter was considered the experimental unit in statistical analyses. Data were analyzed by Levene test, ANOVA, Dunnett test, rank transformation, Wilcoxon rank sum test with Bonferroni correction, Fisher exact probability test, and binomial distribution test.

There were no clinical signs or significant differences in body weight gain or feed intake in dams. The numbers of dams that died of unknown causes during the study were: 2 receiving vehicle controls; 1 dosed with diethylstilbestrol; 3 dosed with 0.0002 mg/kg bw/day bisphenol A; and 1 each in the 0.002 and 0.020 mg/kg bw/day bisphenol A groups. The number of total pups/litter was significantly lower than controls in the 0.2 mg/kg bw/day bisphenol group (mean ± SD = 9.60 ± 3.85 compared to 12.37 ± 3.02 in the control group). In communications with the animal vendor, it was determined that litter size in the control group exceeded typical litter sizes (9–10 pups), and the study authors therefore concluded that the effect was not treatment-related. Bisphenol A had no significant effects on gestation index or duration, percentage of male pups at birth, or pup survival and body weight during the lactation period. The same endpoints were unaffected in the diethylstilbestrol group.

Terminal body weights were increased **[by 7%]** in the 0.020 mg/kg bw/day group and **[by 5%]** in the 2 mg/kg bw/day group. Bisphenol A did not affect absolute or relative (to body or brain) weights of reproductive organs including prostate, preputial gland, seminal vesicle, or epididymis. Non-dose-related effects were observed for brain and kidney weights, and the study authors concluded that the effects were not treatment-related. There were no significant effects on cauda epididymal sperm concentration, daily sperm production, or efficiency of sperm production. Testicular histopathology was not affected by bisphenol A treatment. **[Data were not shown by authors.]** Reproductive development of male offspring was also unaffected by diethylstilbestrol. The study authors noted that the diethylstilbestrol dose was considered the "maximum effect" oral dose by vom Saal but was lower than doses affecting male offspring in other studies. The study authors also noted that the effects of bisphenol A on prostate weight and sperm production reported by vom Saal et al. (1998) and Nagel et al. (1997) were not repeated in this study. They concluded that bisphenol A should not be considered a selective reproductive or developmental toxicant.

[The NTP Statistics Subpanel (NTP, 2001) concluded that the statistical methods used by Cagen et al. (1999a) were appropriate. Although the Subpanel agreed with the study author conclusions, they noted that (1) a significant ANOVA is not a requirement for Dunnett test; and (2) a Bonferroni correction of the Wilcoxon-rank sum test was not needed because the study authors already required significance by ANOVA, which was sufficient.]

Strengths/Weaknesses: The attempt to replicate the studies of vom Saal et al. (1998) and Nagel et al. (1997), the use of litter analysis, the large sample sizes, and the agreement of the NTP Subpanel with the author conclusions are strengths. With respect to this study as a replication, weaknesses include design differences relating to strain, dietary differences, age at evaluation, and the use of solo housing rather than small group housing. The lack of response of the positive control DES group is problematic.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process due to absence of response of the positive control group.

Ashby et al. (1999), support not indicated [2 authors from industry], examined the effects of prenatal bisphenol A exposure on the mouse reproductive system. The study attempted to duplicate the findings reported by vom Saal et al. (1998) and Nagel et al. (1997). Both generations of CF-1 mice were fed RM1 diet containing 6.5% soy during periods when they were not pregnant or lactating, and dams were fed RM3 diet containing 18.5% soy during pregnancy and lactation. On post-conception days 11–17, 8 dams/group were dosed with bisphenol A (99% pure) at 0, 0.002, or 0.020 mg/kg bw/day. The negative control group was administered the tocopherol stripped corn oil vehicle. A positive control group of 7 dams received diethylstilbestrol at 0.2 μg/kg bw/day. A naïve group of 7 dams was not weighed or dosed. The dosing solution was slowly expelled from a pipette placed in the animals' mouths. Day of vaginal plug detection was designated post-conception day 1, however. females that had no vaginal plugs but gained >3.5 g were arbitrarily considered to be 10 days pregnant. Females with vaginal plugs and those that gained >3.5 g were distributed evenly among treatment and control groups. Females that gained >1 but <3.5 g were considered to be pregnant, but because the day of pregnancy could not be determined, they were assigned to the naïve control group. Dams were allowed to litter. All female offspring were weighed and monitored for vaginal opening. Females were killed at ~44 weeks of age, and liver, kidney, and reproductive organs were weighed. Male pups were housed as littermates until PND 112 (day of birth designated as PND 1). To determine the effects of housing, ~3 males from 4–7 litters/group (11–21 males/group) were randomly selected and housed separately from PND 112 until study

termination, which occurred ~71 days later. The remaining male pups from 4–5 litters/group from each litter (11–17/group) were housed together. Singly housed males were weighed and killed on PND 183–185, and group-housed males were weighed and killed on PND 186–187. Equal numbers of males from each group were killed each day. Liver, kidney, and reproductive organs were weighed, and testicular sperm count and efficiency were determined. Technicians were blinded to experimental conditions. Measures taken to reduce stress to animals included administering test agents by drip feeding, minimal handling of pups, and minimal environmental noise. Selection of 3 males from each litter increased statistical power compared to previous studies (Nagel et al., 1997; vom Saal et al., 1997). Statistical analyses were dually conducted using the individual offspring and the litter as the statistical unit. Data were evaluated by ANOVA and Dunnett test. Results from vehicle-treated and naïve controls were pooled when there was no evidence of a vehicle effect. Data from individually housed and group housed-males were pooled when they did not differ significantly.

There were no significant differences in litter sizes or percentage of males/litter. In female offspring from the bisphenol A groups, there were no significant effects on body weight or organ weights, including cervix, uterus, vagina, and ovary. Age and weight at vaginal opening were also unaffected in groups exposed to bisphenol A. Vaginal opening was delayed in the diethylstilbestrol-treated group and in the naïve control group.

Significant effects included increased terminal body weights in the low-dose group, increased testis weight in both dose groups, and increased epididymis weight in the high-dose group. Because testis and epididymis weights relative to body weights were nearly identical to controls **[data not shown by study authors]**, the authors considered the finding equivocal. Although prostate weights were slightly higher in the bisphenol A groups, there were no statistically significant effects on prostate weight when adjusted for body weight and litter effects. Daily sperm production was increased in both dose groups, but the study authors considered the finding equivocal due to low biological significance. The study authors noted that the study failed to confirm the increase in prostate weight and decrease in sperm production reported in the studies by vom Saal et al. (1997) and Nagel et al. (1997), but results were consistent with those reported by Cagen et al. (1999a). Possible reasons for variability between studies were stated as differences in background sound level, diet, and animal body weights. The study authors also mentioned the possibility of genetic drift occurring in mice bred in-house in the vom Saal laboratory.

[The NTP Statistics Subpanel (NTP, 2001) essentially reproduced the findings reported by Ashby et al. (1999).]

Strengths/Weaknesses: Strengths are the rather close replication of the designs of the studies by vom Saal et al. (1998) and Nagel et al. (1997) with diet as the only major difference, the use of both solo and group housed mice, and the support of the conclusions by the NTP Statistics Subpanel. The use of small samples is an understandable weakness given that this study was designed to be a replicate study. The lack of response of the positive control DES group is problematic.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process due to absence of response of the positive control group and small sample sizes.

Howdeshell et al. (1999), support not indicated, examined the effect of prenatal bisphenol A exposure on age of puberty in female mice. **[No information was provided about chow or composition of bedding and cage materials.]** CF-1 mice ($n = 21$/group) were fed oil vehicle **[type of oil not specified]** or bisphenol A **[purity not reported]** at 0.0024 mg/kg bw/day on GD 11–17 **[day of vaginal plug not defined]**. On GD 19, pups were obtained by cesarean section. Intrauterine position of pups (i.e., located next to male or female pups) was noted at that time. Pups were fostered by untreated mothers and weaned on PND 22. Body weights were measured, and pups were monitored for vaginal opening and time to estrus. Results were analyzed according to all pups from each dose group or in relation to intrauterine position. The study authors stated that fetuses positioned between 2 male mice were exposed to the lowest levels of 17β-estradiol, while exposures to 17β-estradiol were highest in fetuses positioned next to female fetuses. Data were analyzed on a litter basis to control for maternal effects. Age of vaginal opening was covaried with weight at weaning. Numbers of female offspring evaluated were 75–111/group for body weight and 51–58/group for vaginal opening. The study authors attempted to evaluate females from each intrauterine position in each litter. **[No additional information was provided for statistical analysis in this brief communication.]**

Body weight at weaning was significantly increased in females in the bisphenol A group. When analyzed according to intrauterine position, body weights were 22% higher than controls in females who were not positioned next to a male fetus and 9% higher in females who had been positioned next to 1 male in utero. There were no significant effects on age of vaginal opening. **[It was not clear if the data presented were covaried with body weight.]** Bisphenol A treatment significantly reduced the period between vaginal opening and first estrus by ~2.5 days. When evaluated according to intrauterine position, a significant decrease in time to first estrous was observed in females who were not positioned next to a male pup (accelerated by ~5 days) and in females positioned next to 1 male **[~2 days]**. No statistically significant findings were observed in females who had been positioned next to 2 males in utero. The study authors concluded that prenatal exposure to bisphenol A at environmentally relevant levels altered postnatal growth and reproductive function in female mice but that natural variations in individual endogenous 17β-estradiol levels influenced the response to bisphenol A.

The results of this study were also discussed in a publication by Howdeshell and vom Saal (2000), which indicated that the work was supported by NIH and reported additional findings. There was a bisphenol A-associated reduction in pup survival between birth and weaning. Complete litter death occurred in 6 of 21 litters in the bisphenol A group compared to 1 of 21 litters in the control group. Significantly increased body weight of male pups at weaning was also reported for the bisphenol A group. Body weights were highest in males who were positioned next to 2 female pups in

utero and were 10% higher than body weights of control males positioned next to 2 female fetuses in utero. No increase in body weight occurred in males that were positioned between two male fetuses in utero. Although the authors identified a litter-based analysis, it was not always clear that this applied to all analyses (in Study Figure 1, the n-values exceed the number of dams, suggesting that some of the data were analyzed on a per pup basis.

[The NTP Statistics Subpanel (NTP, 2001) requested the Howdeshell et al. (1999) data set for reanalysis, but it was not provided by study authors.]

Strengths/Weaknesses: Strengths are the oral route of exposure and the use of a low dose level of bisphenol A. The omission of a description of husbandry conditions and lack of clarity of statistical procedures are weaknesses. Use of only a single dose is a weakness. Further, the use of time from vaginal opening to first estrus is not a standard endpoint for assessing puberty in mice and is of questionable biological significance.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for the evaluation process but utility is limited due to uncertainties in data analyses.

Gupta (2000), supported by NIH, examined the effects of bisphenol A exposure on the reproductive system of male mice. CD-1 mice were received on GD 12 (GD 0 = day of breeding). The mice were fed Purina Chow-5 L9 at the Charles Rivers Laboratory and Purina Chow 5012 at the study author's laboratory. **[No information was provided on bedding or caging materials.]** On GD 16–18, 15 mice/group were fed the corn oil/12% ethanol vehicle or 0.050 mg/kg bw/day bisphenol A **[purity not reported]**. Additional groups of mice were administered diethylstilbestrol at 0.1 and 200 μg/kg bw/day and Aroclor at 0.050 mg/kg bw/day during the same time period. The bisphenol A dose level was based on a level reportedly considered safe by the FDA. Following delivery, litters were culled to 8 pups, with at least 3 males. Body weight and anogenital distance were examined in 3 pups/litter (45 pups) on PND 3, 2 pups/litter (30 pups) on PND 21, and 1 offspring/litter (15 offspring) on PND 60. **[Although Table 1 of the study lists the n-value as 15–45/group, a statement in the methods section indicated that an equal number of pups (n = 1–3) were pooled from each litter.] Prostate and epididymis were weighed in 15 offspring/group on PND 3, 21, and 60. Whole-tissue mounts of prostate were examined for growth in 15-day-old offspring (n = 4/group). Androgen binding was measured in prostates isolated at 3, 21, and 60 days of age, with 2–6 prostates pooled, depending on age; an n of 5 was reported in Figure 2 of the study. Data were analyzed by ANOVA. [It was not clear if the offspring or litter was considered the statistical unit.]**

Body weights of male offspring were not affected by bisphenol A treatment. In male pups of the bisphenol A group compared to the control group, anogenital distance adjusted for body weight was significantly increased **[by 22%]** on PND 3, **[by 25%]** on PND 21, and **[by 33%]** on PND 60. Prostate weights in males of the bisphenol A group were significantly increased **[by 56%]** on Day 3, **[by 39%]** on Day 21, and **[by 101%]** on Day 60. Relative (to body weight) epididymis weight in the bisphenol A group was significantly reduced **[by 35%]** on PND 60. Prostate growth was reported to be qualitatively increased by bisphenol A exposure. Androgen receptor binding was increased on PND 21 and 60 **[by ~344% on PND 21 and 358% on PND 60, estimated from a graph]**. Similar effects were reported following treatment with the low dose of diethylstilbestrol and Aroclor. In contrast, the high-dose of diethylstilbestrol reduced body weights, anogenital distance, prostate weight, and androgen receptor binding. Presentation of pathology data are superficial, thus questioning interpretation.

The report also included an in vitro study to examine the effects of bisphenol A on prostate growth. The urogenital sinus was dissected from GD 17 fetuses and cultured for 7 days in media containing 0, 5, or 50 ng/L bisphenol A with and without the addition of testosterone. The urogenital sinus was also incubated in 0.1 or 0.5 ng/L diethylstilbestrol and 5 or 30 ng/L Aroclor. Prostates obtained from cultures were then fixed in Bouin solution and examined histologically. A similar protocol was used to examine androgen binding in cultured prostates, except that only the high-doses of each compound were examined, and cells were cultured for 6 days. Bisphenol A at 50 ng/L increased prostate size **[by 140%]** in the absence of testosterone and **[by 150%]** in the presence of testosterone. Androgen binding in prostate was increased **[by 200%]** following treatment with bisphenol A. Similar effects were reported with diethylstilbestrol and the high Aroclor dose. The study authors concluded that the effects of in vivo studies were reproduced in in vitro studies, which suggests a direct effect on reproductive organs of fetal mice.

In a subsequent commentary, Elswick et al. (2000) noted several concerns and requested clarification of the data analysis performed by Gupta. It was noted that statistical analyses were insufficiently described to determine if analyses in addition to ANOVA were conducted. It was not indicated if post-hoc tests were used or if corrections were made for multiple comparisons. Table 1 of the study was noted to contain a footnote indicating $P < 0.05$ (larger) or $P < 0.05$ (smaller). It was stated that determining a mean and conducting a one-tailed post-hoc test based on whether the mean is larger or smaller is a source of potential bias in the statistical analyses. Analyses conducted by Elswick et al. (2000) indicated that the assumption of homogeneity of variance, a requirement for ANOVA, was not met for some data such as anogenital distance on PND 3 (Table 1 of the study) and prostate size (Table 3 of the study). Therefore, questions were raised about whether homogeneity testing was done or if data were transformed to account for lack of homogenous variances before ANOVA. Failure to consider the litter as the experimental unit was noted in cases where the sample size was listed as 30 and 45, while only 15 dams/group were treated. It was noted that if anogenital distance was measured in the same animal at different time points, a repeated-measures ANOVA would have been the appropriate statistical test. It was stated that correction of anogenital distance by the cube root of body weight instead of body weight would have been preferred to avoid overcorrection; ANCOVA with body weight as a covariate would have been a better method for correcting anogenital distance, and the best method would have been a nested ANCOVA (dam within treatment). Questions were raised about whether sampling 1 pup/litter on PND 60

Table 77
Effects on Prostate Development in Mice After Prenatal Exposure to 0.010 mg/kg bw/day Bisphenol A[a]

	Prostate region		
Endpoint[b]	Dorsolateral	Ventral	Dorsolateral and ventral
No. of prostate ducts	↑ 41%	↔	↑ 40%
Prostate duct volume	↑ 99%	↑ 78%	↑ 91%
Proliferating cell nuclear antigen staining	↑ 44%	↔	No data

[a]Timms et al. (2005).
[b]Percent changes calculated by CERHR differed slightly from values presented by authors; it was not clear which part of the prostate the authors' values represented.
↑,↓ Statistically significant increase, decrease; ↔ no statistically significant effect.

Table 78
Maternal Behavior Effects in Mice Exposed to Bisphenol A During Gestation or Adulthood[a]

	Bisphenol A exposure during gestation/adulthood		
Percent time[b]	Bisphenol A/vehicle	Vehicle/ bisphenol A	Bisphenol A/bisphenol A
Nursing	↓ 15%	↓ 14%	↔
Nest building	↑ 73%	↑ 146%	↔
Resting alone	↑ 67%	↑ 29%	↑ 46%
Grooming	↑ 25%	↑ 18%	↔
Active	↔	↑ 18%	↔
In nest	↓ 12%	↓ 10%	↔
Out of nest	↑ 17%	↑ 12%	↔

[a]Palanza et al. (2002).
[b]Data were presented graphically. Values were provided by the study author (personal communication, P. Palanza, February 26, 2007).
↑,↓ Statistically significant increase/decrease compared to vehicle-vehicle group, ↔ no statistically significant effect.

between a male and female fetus was examined. Prostate morphology was determined by a 3D computer reconstruction technique. Immunohistochemistry techniques were used to measure levels of proliferating cell nuclear antigen and mouse keratin 5. Statistical analyses included ANOVA, followed by Fisher least-squares mean test when statistical significance was obtained. In a separate study, prostate morphology was examined in 4 pregnant mice/group that were dosed with vehicle or 200 μg/kg bw/day diethylstilbestrol according to the procedures described above.

Bisphenol A increased numbers of ducts, volume, and proliferation in one or more prostate regions, as outlined in Table 77. The pattern of proliferating cell nuclear antigen staining was similar to that observed with mouse keratin 5, a basal epithelial cell maker. The study authors also reported a 56% increase in the volume of the coagulating glands. **[Data were not shown by study authors.]** An abnormal narrowing was observed in the portion of the urethra near the neck of the bladder. **[The volume of the cranial urethra was reduced by 35% compared to controls. Malformation of prostatic sulci was reported, but no information was provided on incidence or severity.]** Similar effects on the prostate were reported in mice exposed to ethinyl estradiol and the low dose of diethylstilbestrol. Narrowing of the cranial urethra was observed in mice exposed to ethinyl estradiol. In contrast, exposure to the high diethylstilbestrol dose resulted in inhibited morphogenesis of the prostate. The study authors concluded that the differentiating urogenital system of male mice is very sensitive to a low dose of bisphenol A.

Strengths/Weaknesses: Strengths are the oral route of administration, the low dose level of bisphenol A, the use of diethylstilbestrol and ethinyl estradiol as positive controls, and the sophisticated measures applied to the prostate. Weaknesses are the use of a single dose level and small sample size, although the Panel judged it to be adequate for the methodology.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation.

Palanza et al. (2002), supported by NIEHS, NIH, MURST, the University of Parma, and the National Council for Research, examined the effects of bisphenol A treatment on maternal behavior following exposure of mice during prenatal development and/or adulthood. The CD-1 mice used in this study were maintained as an outbred colony. Mice were housed in polypropylene cages with corn cob bedding. During pregnancy and lactation, mice were fed Purina 5008 (soy-based) chow. After weaning, mice were fed Purina 5001 (soy-based) chow. Water was provided in glass bottles. On GD 14–18 (GD 0 = day of vaginal plug), 14 mice were fed the tocopherol-stripped corn oil vehicle and 9 mice were fed 0.010 mg/kg bw/day bisphenol A **[purity not reported]** using an electronic micropipette. Dams were housed 3/cage after mating and individually housed on GD 17. Body weights of dams were measured during gestation. The day of birth was considered PND 1, and offspring were weaned on PND 20. At 2–2.5 months of age, F_1 female offspring from vehicle- and bisphenol A-treated dams were mated and exposed to vehicle or 0.010 mg/kg bw/day bisphenol A on GD 14–18. There were 4 groups of F_1 females that were exposed during gestation–adulthood to vehicle–vehicle ($n = 20$), vehicle–bisphenol A ($n = 15$), bisphenol A–vehicle ($n = 15$), and bisphenol A–bisphenol A ($n = 15$). Maternal behavior was observed in F_1 dams every 4 min during a 120-min period on PND 2–15. On PND 1, F_2 pups were weighed, sexed, and counted. Litters were then culled to 10 pups, with equal numbers of male and female pups when possible. Pups were weighed during the lactation period and cliff-drop aversion and righting reflex were evaluated in all pups of a subset of 8 litters/group on PND 3, 5, 7, and 9. For statistical analyses, all pup data were adjusted for litter. Data were analyzed by ANOVA, Holms *t*-test, and/or Fisher protected least-squared difference test.

Bisphenol A treatment did not affect gestational body weight gain in F_0 or F_1 dams. Statistically significant effects for F_1 maternal behavior collapsed across 14 observation days are presented in Table 78. Exposure to bisphenol A either in gestation or in adulthood resulted in decreases in the percentage of time the dams spent nursing and in the nest and increases in the percentage of time the dams spent nest building, resting alone,

grooming, and out of the nest. Increased activity was also observed in the group exposed to bisphenol A in adulthood. The only significant effect observed in mice exposed to bisphenol A during gestation and adulthood was increased time resting. When data were presented for individual evaluation days, time resting was significantly increased on PND 9, 10, 11, 12, and 14 in the group exposed to bisphenol A during gestation. Time spent resting was significantly increased on PND 9 and 14 in the group exposed to bisphenol A during gestation and adulthood. No other significant effects were observed on specific evaluation days. There were no significant differences in the number of live F_2 pups/litter, sex ratio, or body weight at birth or in weight gain during the lactation period. **[Data were not shown]**. No significant effects were observed for cliff aversion or righting reflexes. The study authors concluded that reduced levels of nursing behavior were observed in mice exposed to bisphenol A only as fetuses or only as adults. **[Because this study involves effects of adult exposure on maternal behaviors, it is also discussed in Section 4.2.]**

Strengths/Weaknesses: Strengths are the oral route of administration, the low dose level of bisphenol A, and the exploration of effects on complex maternal behaviors. It is unusual that pre- and postnatal exposure had effects but not the combination of pre- and postnatal exposure, and failure to explain this finding is a weakness. The use of a diet high in soy isoflavones is an additional weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation process.

Nishizawa et al. (2003), supported by the Japanese Ministry of Education, Culture, Sports, Science, and Technology, examined the effects of prenatal bisphenol A exposure on expression of retinoic acid receptor α and retinoid X receptor α in mouse embryos. ICR mice were fed standard feed (CM; Oriental Yeast). **[No information was provided about caging and bedding materials.]** Mice were orally dosed with bisphenol A **[purity not indicated]** at 0 (olive oil vehicle) or 0.002 mg/kg bw/day on 6.5–11.5, 6.5–13.5, 6.5–15.5, and 6.5–17.5 days post-coitum. Day of vaginal plug was considered 0.5 days post-coitum. **[No information was provided about the specific method of oral dosing.]** Twelve dams/group were killed at 12.5, 14.5, 16.5, and 18.5 days post-coitum, 24 hr after receiving the last dose. Expression of mRNA for retinoic acid receptor α and retinoid X receptor α was measured by RT-PCR in fetal cerebrum, cerebellum, and gonads. Data were analyzed by ANOVA. **[It was not clear if the litter or offspring was considered the measurement unit.]** Numerous changes in mRNA expression were observed following in utero exposure to bisphenol A, and they varied according to sex, tissue, and dosing period. The study authors concluded that these findings suggest a novel mechanism of bisphenol A toxicity mediation by disruption of the expression of retinoic acid receptor α and retinoid X receptor α.

Strengths/Weaknesses: Strengths are the oral route of delivery, the use of a low dose level of bisphenol A, and the exposure at different time periods. The study has value for understanding mechanisms of action although these changes were not tied to any adverse findings that might be related to these changes. Weaknesses include the use of a single dose level and lack of clarity on number of embryos per litter sampled. This is not considered a critical weakness because it is known that standard procedures for these methods require pooling of embryos within litter.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate but of limited utility for the evaluation because of the mechanistic nature of the endpoints.

Nishizawa et al. (2005b), supported by the Japanese Ministry of Education, Culture, Sports, Science, and Technology and by the Japan Society for the Promotion of Science, examined the effects of bisphenol A exposure on expression of mRNA for arylhydrocarbon and retinoid receptors in mouse embryos. ICR mice were fed standard diet (CM; Oriental Yeast). **[No information was provided about caging or bedding materials.]** Pregnant mice were orally dosed with bisphenol A **[purity not indicated]** at 0 (olive oil vehicle), 0.00002, 0.002, 0.20, or 20 mg/kg bw/day from 6.5 to 13.5 days post-coitum or 6.5 to 17.5 days post-coitum. Day of vaginal plug detection was considered 0.5 days post-coitum. **[No information was provided about the specific method of oral dosing.]** Twelve pregnant mice/group were killed on 14 and 18.5 days post-coitum, 24 hr after the last bisphenol A dose was administered. RT-PCR analyses were conducted to determine expression of mRNA for retinoic acid, retinoid X, and arylhydrocarbon receptors in fetal cerebrum, cerebellum, ovary, and testis. Data were analyzed by ANOVA. **[It was not clear if the litter or offspring was considered the measurement unit.]** Numerous changes in mRNA expression were observed following bisphenol A exposure and they varied according to dose, sex, tissue, and exposure period. The study authors concluded the this study shows a novel mechanism by which bisphenol can induce endocrine disruption through upregulation of arylhydrocarbon receptor (a key factor in the metabolism of some xenobiotics compounds) and retinoid receptors (key factors in nuclear receptor signal transduction).

Strengths/Weaknesses: The wide dose range from 0.00002 to 20 mg/kg bw/day and the oral route are strengths. The study has value for understanding mechanisms of action although these changes were not tied to any adverse findings that might be related to these changes. Weaknesses include the lack of specification of the method of oral dosing and lack of clarity on sample origins and sizes for each assay. Again, this is not considered a critical weakness because it is known that standard procedures for these methods require pooling of embryos within litter.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate but of limited utility for the evaluation because of the mechanistic nature of the endpoints.

Nishizawa et al. (2005a), supported by the Japan Society for the Promotion of Science, examined the effects of bisphenol A exposure on expression of aryl hydrocarbon receptors, related factors, and metabolizing enzymes in mouse embryos. ICR mice were fed standard diet (CM; Oriental Yeast). **[No information was provided about caging and bedding materials.]** Mice were orally dosed with bisphenol A **[purity not indicated]** at 0 (olive oil vehicle), 0.00002, 0.002, 0.2, or 20 mg/kg bw/day from

method of oral dosing was not described.] At 8 weeks of age (Day 77) 5 mice/group/sex were randomly selected and immunized by i.p. injection with hen egg lysozyme. Representation of litter was not specified. Blood was collected and spleens were removed 3 weeks following immunization (Day 98). Serum levels of hen egg lysozyme-specific immunoglobulin G (IgG), IgG1, and IgG2A were measured by ELISA. Spleen cell suspensions were prepared, and proliferation was assessed by incorporation of ^{3}H-thymidine following a 72-hr incubation with hen egg lysozyme. Spleen cell suspensions were also prepared for measurement of interferon-γ and interleukin-4 secretion by ELISA. An additional 6 mice/group/sex were killed at 8 weeks of age (Day 77). Spleens were removed and expression of $CD3^+CD8^+$ and $CD3^+CD4^+$ molecules on splenic lymphocytes was examined using monoclonal antibodies and flow cytometry. Thymus and spleen were fixed in 4% formaldehyde and examined histologically. Data were analyzed by Mann–Whitney *U*-test. **It was not clear if the litter of origin was accounted for in statistical analyses.**

Bisphenol A treatment had no significant effect on pregnancy rate, sex ratio, or body weight of offspring. There were several significant immune responses for male mice. **[Results in female mice were said to be similar to those observed in male mice but the data were not show by study authors.]** At bisphenol A doses ≥0.03 mg/kg bw/day, production of anti-hen egg lysozyme IgG2a following immunization was increased. Effects observed at ≥0.3 mg/kg bw/day included increases in production of anti-hen egg lysozyme IgG and secretion of interferon-γ and interleukin-4. Additional findings at the high-dose (3 mg/kg bw/day) were increases in spleen cell proliferation and production of anti-hen egg lysozyme IgG1 following immunization. Augmentation of interferon-γ and interleukin-4 secretion following incubation of spleen cells with hen egg lysozyme was examined in the high-dose group only and found to be increased. **[Increases in $CD3^+CD8^+$ and $CD3^+CD4^+$ expression on lymphocytes were reported in males and females exposed to bisphenol A, but the doses at which the effects occurred were not specified.]** No histopathological alterations were reported for the spleen or thymus. The study authors explained that effects on IgG2a and interferon-γ were indicators of T helper 1 immune responses and effects on IgG1 and interleukin-4 were indicators of T helper 2 responses. They concluded that the findings suggest that prenatal exposure to bisphenol A may upregulate immune responses in mice.

Strengths/Weaknesses: The oral route of administration and the wide range of doses are strengths. Weaknesses include small sample size ($n = 5$), lack of clarity regarding statistical handling of factors such as litter and sex effects.

Utility (Adequacy) of CERHR Evaluation Process: This study is inadequate for the evaluation process due to the reasons stated above.

Berger et al. (2007), supported by The Natural Sciences and Engineering Research Council of Canada, examined the effect of bisphenol A exposure on ovum implantation and pup survival in mice. CF-1 mice were housed in polypropylene cages and were fed Harlan Teklad 22/5 rodent feed, which was stated to contain soy. **[No information was provided about bedding materials.]** On GD 1–4 or 5 **[described as GD 1–5 in Methods section and GD 1–4 in study figures and tables]** (GD 0 = day of vaginal plug), 31 mice in the control group were s.c. injected with peanut oil vehicle and 5–15 mice/group were s.c. injected with bisphenol A (97% purity) at 0.0005, 0.0015, 0.0046, 0.0143, 0.0416, 0.125, 0.375, 1.125, 3.375, or 10.125 mg/animal/day. In a second experimental group, BPA was administered through a diet containing 3% or 6% BPA added to peanut butter and chow. In a third experimental group maintained on chow, BPA was administered at 0.11, 1.0, 3.0, or 9.0% in separate offerings of peanut butter alone. Pregnancy disruptions in orally exposed mice are discussed in Section 3.2.5.1. **[In the first experimental group, if it is assumed that the mice weighed 0.02 kg at the start of gestation (USEPA, 1988), CERHR estimated bisphenol A intakes of 0.025, 0.075, 0.23, 0.72, 2.1, 6.3, 19, 56, 170, and 500 mg/kg bw/day.]** Mice were allowed to litter. Pups were counted on the day of parturition and observed for survival for 5 days. Pups were weaned at 28 days after birth and at that time, body weight and sex ratio were determined. Data were analyzed by ANOVA, χ^2 test, and Newman–Keuls multiple comparisons. **[It was not clear if all offspring data were analyzed on a pup or litter basis.]** A study examining implantations in s.c.-treated females is discussed in Section 4.2.1.1 Percent of females giving birth was significantly decreased in the 10.125 mg/day group (~28% vs. 97% in control group). Numbers of pups born were significantly decreased in the 3.375 and 10.125 mg/day group (~8 and 2 pups in each of the dose groups and 13 pups in the control group). There were no treatment-related effects on pup weight or sex ratio at weaning. **[As discussed in Section 3.2.5.1, it appears that with oral exposure, pregnancy disruption occurred at higher bisphenol A levels (68.8 mg/day, 3440 mg/kg bw/day) than with s.c. exposure (10.125 mg/day, ~500 mg/kg bw/day)]**. The study authors concluded that the amount of bisphenol A required for pregnancy disruption was higher than typical environmental levels but that it is not known if bisphenol A could have additive or synergistic effects with other environmental estrogens.

Strengths/Weaknesses: A strength of the subcutaneous study is that it examined a wide range of bisphenol A dose levels. The comparison of the differential effects of s.c. and oral routes of bisphenol A administration is also a strength. Weaknesses include the limited/unequal number of mated mice in each dose group, absence of maternal data to ascertain the potential impact of maternal toxicity on pregnancy, methodological deficiencies regarding fertility assessment, and the use of a diet that contains phytoestrogens.

Utility (Adequacy) for CERHR Evaluation Process: Due to the limited number of mated mice per dose level ($n = 5$–15), methodological concerns, absence of key statistical information as well as maternal information, this study is inadequate for the CERHR evaluation process.

3.2.5.2 Studies with neurobehavioral endpoints:

Narita et al. (2006), supported by the Japanese Ministry of Health, Labor, and Welfare, and Ministry of Education, Culture, Sports, Science, and Technology, conducted a series of studies to examine the effects of bisphenol A on the dopaminergic system of mice exposed during development. Only brief details were provided about

has been informed that there were 20–30/group (A. Soto, personal communication, March 2, 2007).] Fetal mammary glands were mounted whole or sectioned to examine mammary gland development in 36–40 offspring/group. Immunohistochemistry techniques were used to measure expression of *Ki67* and *Bax* in mammary structures from 4–8 offspring/group. Mammary collagen localization was assessed using Masson Trichrome stain in 6–17 mice/group. Expression of mRNA for *ER*α, *ER*β, adipocyte lipid binding protein, *Col-I*, and *PPAR*γ were measured by RT-PCR in mammary glands from 4–6 offspring/group. Litter was accounted for in design and analyses by assigning 1 individual/litter to each group or endpoint. Statistical analyses included *t*-tests, ANOVA, Mann–Whitney *U* non-parametric tests, and χ^2 tests.

Morphometric analysis revealed significantly higher ductal area and extension in the bisphenol A group than in controls. In the control group, females positioned next to two females in utero had significantly fewer branching points than females positioned next to 1 or 2 males; this difference was not observed in the bisphenol A group. In fetuses that were not positioned next to a male, significantly more branching points were observed in the bisphenol A than in the control group. Control females positioned next to 2 males had significantly larger epithelial duct area than control females not positioned next to a male; this difference was not observed in the bisphenol A group. In bisphenol A-treated females positioned next to 1 male, ductal extension was significantly greater than in control females positioned next to 1 male.

In the bisphenol A group, epithelial cells were less rounded, more evenly spaced, and more dense than in controls. Bisphenol A did not significantly affect Ki67 (a proliferation marker) expression in mammary epithelium. Lumen formation was observed in 6 of 16 control mice and 0 of 10 bisphenol A-exposed mice. Significantly decreased numbers of Bax-positive (apoptotic) cells were observed in the inner epithelial cord (not in contact with basement membrane) of bisphenol A-exposed than control mice. Optical density of histological staining was significantly lower in the fat pad of the bisphenol A-exposed than control group. Fat pads of the bisphenol A group compared to control group were found to be significantly less cellular, contain more Bax-positive cells, and have more vacuoles at a distance <1 mm from the epithelial compartment. Study authors interpreted the effect as increased epithelial penetration and advanced maturation of fat pads. No significant differences were observed for PPARγ or adipocyte lipid binding protein mRNA expression. Density of collagen deposits was lower in the entire mammary gland but higher in the periductal stroma (within 10 μM of the epithelium) of the bisphenol A than the control group. Bisphenol A exposure did not affect collagen type I, *ER*α, or *ER*β mRNA expression. ERα protein expression in the stroma was also unaffected by bisphenol A exposure. Study authors concluded that advanced maturation of fat pad and changes in extracellular matrix may be the cause of altered growth, cell size, and lumen formation in mammary epithelium of mouse fetuses exposed to bisphenol A.

Strengths/Weaknesses: Strengths of this study are the rigor with which the measurements were made, and the fact that the authors were trying to quantify endpoints that are difficult to measure (e.g., the relationship of the stroma to the epithelium). The relevance of the endpoints is a strength as is the low dose used. The single dose and subcutaneous route of administration are weaknesses. A critical weakness is inappropriate statistical analysis of a complex study design that may have produced too many positive findings and a lack of statistical accounting for litter effects (i.e.,. 36–40 pups presented in Table 1 of study and only 20–30 litters treated).

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process because of insufficient control for litter effects.

Honma et al. (2002), supported by the Japanese Ministry of Education, Culture, Sports, Sciences, and

Table 79
Effects in Mice Exposed to Bisphenol A During Prenatal Development[a]

	Dose (mg/kg bw/day)					
Endpoint	0.002	0.020	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$
Female body weight						
Weaning	↓ 10%	↓ 7%	0.065	0.017	0.088	0.021
PND 60	↔	↓ 4%	0.054	0.021	0.11	0.021
Male body weight						
Birth	↔	↓ 5%	0.054	0.020	0.031	0.015
PND 60	↔	↓ 6%	0.048	0.020	0.044	0.020
Anogenital distance						
Females at weaning	↑ 6%	↔				
Males on PND 60	↑ 6%	↑ 8%	0.035	0.020	0.035	0.020
Age at vaginal opening[b]	↔	↓ 1.3 days				
Body weight at vaginal opening[b]	↓ 10%	↓ 11%				
Age at 1st estrus[b]	↔	↓ 1 day				
Estrous cycle length	↑ 1.3 day	↑ 1 day	0.021	0.007	0.12	0.021
Cornified cells in vaginal smear	↑ 3.1 days	↔	0.17	0.020	0.44	0.021
Lymphocytes in vaginal smear	↓ 2.2 days	↔	0.26	0.020	0.26	0.020

[a]Honma et al. (2002).
[b]Value estimated from a graph by CERHR; data from graphs were not modeled.
↑,↓ Statistically significant increase, decrease; ↔ no significant effect.

Technology, examined the effect of prenatal bisphenol A exposure on the reproductive system of female mice. Mice were fed commercial diet (CE-2, CLEA, Tokyo, Japan). **[No information was provided about bedding or caging materials.]** Ten ICR/Jcl mice/group were s.c. injected with bisphenol A **[purity not reported]** in sesame oil at 0, 0.002, or 0.020 mg/kg bw/day on GD 11–17 (GD 0 = vaginal plug). Additional mice were injected with diethylstilbestrol at 0.02–2 μg/kg bw/day. Pups were sexed, counted, and weighed at birth. At 22 days of age, offspring were weaned and litter sizes were adjusted to 8 pups. Male and female offspring were weighed during the postnatal period. Anogenital distance was measured in males and females at 22 and 60 days of age. Females were monitored for vaginal opening. Vaginal smears were obtained for 30 days following vaginal opening. Female offspring were mated with untreated males from 90 to 120 days of age. F_2 pups were counted and sexed at birth. The litter was considered the experimental until in statistical analyses. Data were analyzed by ANOVA and Student or Welch *t*-test.

Statistically significant findings are summarized in Table 79. There were no effects on gestation duration, number of pups/litter, or sex ratio. Body weights were slightly lower in high-dose males at birth, both dose groups of females at weaning, and high-dose males and females at 60 days of age. Anogenital distance was increased in low-dose females at weaning and both dose groups of males at 60 days of age. Age of vaginal opening and first estrus was accelerated in the high-dose group, and body weight at vaginal opening was lower in both dose groups. Estrous cycle length was increased in both dose groups. Total days that cornified cells were present in vaginal smears was increased and total days that lymphocytes were detected was decreased in the low-dose group. In F_1 offspring there were no significant effects on mating, number of F_2 pups/litter, or sex ratio of F_2 pups. Results in mice dosed with diethylstilbestrol were similar to those observed in mice dosed with bisphenol A. The study authors concluded that prenatal exposure to low doses of bisphenol A results in early vaginal opening in mice but did not affect female reproductive function.

Strengths/Weaknesses: Strengths are that this study represents one of the few studies that appropriately examines the onset of puberty in the mouse as an endpoint, it uses low dose levels of bisphenol A, relatively large sample sizes, and effectively uses a positive control at 3 dose levels. The lack of AGD measurement at birth and difficulty of measurement at PND 60 are weaknesses. he Expert Panel was unable to confirm the statistical significance of the effects shown in Table 2 of the manuscript.

Utility (Adequacy) for CERHR Evaluation Process: The study is adequate for inclusion but of limited utility due to statistical questions about body weight and AGD and subcutaneous route of exposure.

Iwasaki and Totsukawa (2003), support not indicated, examined the effect of prenatal bisphenol A exposure on reproductive development of female mice. ICR mice were fed F1 diet (Funabashi, Chiba, Japan) and housed in polycarbonate cages containing an unspecified chip bedding. On GD 7–18 (GD 0 = day of copulatory plug), 6 dams/group received bisphenol A **[purity not reported]** at 0 (DMSO vehicle) 0.00025, 0.025, or 2.5 mg/kg bw/day by s.c. injection. A positive control group of mice received 100 μg/kg bw/day 17β-estradiol **[route not specified]**. Dams were weighed during the study. Pups were counted and sexed on PND 0, and pup viability was determined on PND 4. Pups were weaned on PND 21, and male pups were killed and discarded. Female pups (24–41/group) were observed for vaginal opening. On PND 21, 1 pup/litter(4/group) from the low- and mid-dose group was injected with 3 μg/kg bw/day 17β-estradiol for 2 days and then killed. Uterine weights were assessed and expression of the *ERα* gene in uterus was determined using a colorimetric method. Statistical analyses included ANOVA, ANOVA on ranks (Kruskall–Wallis test), and Dunnett test. **[It was not clear if the litter or offspring was considered the statistical unit.]**

Weight gain was described as increased in all treated dams compared to control dams, but there was no evidence of a dose–response relationship and statistical significance was not achieved. Pup birth weight was significantly lower **[6%]** in the low-dose group compared to the control group. There were no differences in litter size at birth. Pup viability on PND 4 was significantly reduced **[by 26%]** in the low-dose group. Age of vaginal opening was significantly delayed by 3 days in the low-dose group, but significantly accelerated by 2.2 days in the high-dose group. Following 17β-estradiol exposure, uterine weight was significantly decreased **[by ~85%]** in the low-dose bisphenol A group and significantly increased **[by ~29%]** in the mid-dose bisphenol A group. Although expression of *ERα* mRNA was observed at 132% of control levels in the mid-dose bisphenol A group following exposure to 17β-estradiol, the effect did not attain statistical significance. Expression of *ERα* gene was not detectable in the low-dose bisphenol A group following 17β-estradiol exposure. No significant effects were reported in mice treated with 17β-estradiol. The study authors concluded that "The levels tested in this study appear to be dangerous."

Strengths/Weaknesses: The use of 3 dose levels, including low doses, and the use of 17β-estradiol as a positive control are strengths of this study. Weaknesses include the use of DMSO as a vehicle, the subcutaneous route of administration, the small sample size, lack of significant effects detected in the 17β-estradiol positive control group, and the failure to account for litter in statistical analyses.

Utility (Adequacy) for CERHR Evaluation Process: The study is inadequate for the evaluation process.

Nakamura et al. (2006), supported by grants from the Japanese government, examined the effects of prenatal exposure to bisphenol A on the morphology and expression of certain genes related to brain development in the mouse neocortex. In the first experiment ICR/Jc1 mouse dams were injected subcutaneous with either 0 (sesame oil vehicle) or 20 μ/kg bw/day bisphenol A **[purity not indicated]** daily from GD 0 (defined as the day that a vaginal plug was detected) until GD 10.5, GD 12.5, GD 14.5 or GD 16.5. **[No information was provided on feed, caging materials, bedding.]** Dams were then given a single i.p. injection of 5-bromo-2′-deoxyuridine (BrdU). Fetuses were collected either 1 hr following BrdU treatment (to assess precursor cell proliferation) or 2 or 3 days following BrdU treatment (to assess neuronal migration and differentiation). Brains were fixed in 4%

buffered paraformaldehyde for morphometry and immunohistochemical evaluation. The sections of the neocortex were sectioned into three zones: ventricular zone, intermediate zone, and cortical plate (the neocortex at GD 12.5 was divided into the ventricular zone and the primordial plexiform layer). Ten fetuses from two or more dams were collected at each time point. In the second study, ICR/Jc1 dams were treated as described above and fetal telencephalons were collected on GD 12.5, GD 14.5, or GD 16.5 and frozen in liquid nitrogen and stored at −80°C for mRNA expression analyses ($n = 10$–15 fetuses in each group).

There were no significant differences in the pattern of immunoreactivity for K1-67 (a marker for cell proliferation), nestin (a marker for neural progenitors), Musashi (another marker for neural progenitors), and histone H_3. However, a marker for young neurons, Tuj1, was more prominent in the intermediate zone at GD 14.5 and GD 16.5 in the bisphenol A group. The authors also looked at the immunoreactivity pattern for PDI, a microsomal enzyme that contains binding sites for T3 and estradiol. PDI is believed to act as a buffer for these hormones in cells. PDI is of interest because bisphenol A has been reported to bind to the T_3 binding sites of PDI with 10 to 100-fold lower affinity than T_3 (Hiroi, 2006) and inhibit the binding of T_3 to PDI when bound. PDI immunoreactivity was increased in the neocortex of bisphenol A treated fetuses from GD 12.5–GD 16.5 and in subplate cells at GD 14.5.

There were no differences in BrdU labeled cells in any neocortical zone from brains collected 1 hr following BrdU treatment. However, the BrdU-labeled cells analyzed 2 days following BrdU injection were decreased in the ventricular zone of BPA-treated mice at GD 14.5 (labeled at GD 12.5) and GD 16.5 (labeled at 14.5) and increased in the cortical plate at GD 14.5 (labeled at GD 12.5). The authors used quantitative RT-PCR to examine the expression of several genes involved in brain development including those that help regulate the maintenance of neural stem cells and promote gliogenesis (*Hes1* and *Hes5*), promote neurogenesis (*Mash1*, *Math3*, and *Ngn2*), and relate to thyroid hormone action (*L1CAM*, *THR*-α, and *THR*-β). The gene expression of *Math3*, *Ngn2*, *Hes1*, *LICAM*, and *THR*-α were upregulated significantly in the bisphenol A-treated group at GD 14.5 (*Hes1* and *Hes5* were downregulated significantly at GD 12.5). Overall, the authors interpreted these findings as suggesting that bisphenol A might disrupt normal neocortical development by accelerating neuronal differentiation and migration.

Strengths/Weaknesses: The strengths of this study are that a reasonable sample size (10) for this type of study was used although the presumed dam effect was only partly controlled for by choosing 10 pups from two different dams. The study used a low dose (20 ug/kg) delivered s.c. to a pregnant mouse. The results revealed an effect on neocortical development in developing fetuses. Neurogenesis and gene expression were affected by BPA.

Utility (Adequacy) for CERHR Evaluation Process: This is an adequate study for evaluation purposes but of limited utility because dam effects were only partly controlled for and because of the subcutaneous route of administration.

Nikaido et al. (2004), supported by the Japanese Ministry of Health, Labor, and Welfare examined the effects of bisphenol A exposure on mammary glands and reproductive systems of mice. Outbred CD-1 (ICR) mice were fed NIH-07 (a low-phytoestrogen diet) and provided with water supplied in polycarbonate bottles with rubber stoppers. The mice were housed in polyisopentene cages with white pine chip bedding. Beginning on GD 15 (plug day not specified), mice were s.c. injected with 0 (DMSO vehicle), 0.5, or 10 mg/kg bw/day bisphenol A (≥99% purity) or 0.5 or10 μg/kg bw/day diethylstilbestrol for 4 days. **[The control group contained 6 dams/group, but the number of dams in treated groups was not clear.]** Additional groups of mice were treated with the same doses of genistein, resveratrol, or zearalenone. Female pups were weaned at 21 days of age. Onset of vaginal opening was monitored. Estrous cyclicity was monitored in 12 mice/group at 9–11 weeks of age. At 4, 8, 12, and 16 weeks of age, 6 randomly selected mice/group were weighed and killed. Ovaries, uterus, vagina, and mammary glands were preserved in 10% formalin for histopathological evaluation. Differentiation of mammary structures was evaluated in whole mounts. Statistical analyses included homogeneity of variance tests followed by ANOVA or Kruskal–Wallis test. When P-values were <0.05, Fisher protected least significant difference test was conducted. **[It appears that offspring were considered the statistical unit.]**

Body weight gain of offspring was increased by bisphenol A treatment, and at 16 weeks of age, body weight compared to controls was higher **[by ∼50%]** in the low-dose group and **[by ∼23%]** in the high-dose group. Vaginal opening was accelerated by 1.2 days at the high-dose group. Estrous cycle length was increased by 2.8 days in the low-dose group and 3 days in the high-dose group as a result of increased time spent in diestrus. Corpora lutea were observed in all control mice at each age. No corpora lutea were observed in 2 of 6 mice of the low-dose group and 3 of 6 mice of the high-dose group at 4 weeks of age, but all mice had corpora lutea at 4, 8, 12, and 16 weeks of age. With the exception of vaginal cornification observed in mice lacking corpora lutea, no histopathological abnormalities were observed in the uterus or vagina. Two of three mice with corpora lutea in the high-dose bisphenol group had greater mammary alveolar differentiation compared to control mice at 4 weeks of age. No differences in mammary differentiation were observed at later ages. The study authors concluded that both the high and low dose of bisphenol A produced transient changes in the mammary gland and reproductive tracts of mice. Transient effects on the reproductive tract and mammary gland were also observed with genistein and diethylstilbestrol, whereas prolonged effects were induced by zearalenone.

Strengths/Weaknesses: The lack of clarity regarding sample size and the weak description of the histopathology findings are weaknesses, as are the use of DMSO as a vehicle, the subcutaneous route of administration, and statistical concerns.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process.

Park et al. (2005b), support not indicated, treated ICR mice during pregnancy. Bisphenol A **[purity not indicated]** in corn oil was given i.p. at dose levels of 0, 0.05, 0.5, or 5 mg/kg bw on the day of mating and every 3 days for a total of six doses ($n = 12$/group). Dams were

killed on GD 18 (plug = GD 0) for determination of litter size, fetal weight, and sex ratio. The uterus and right ovary were removed from each dam, fixed in Bouin fluid, and sections were stained with hematoxylin and eosin for light microscopy. Results were analyzed with least significant difference test **[apparently on a per fetus basis]**.

Maternal weight was not altered by treatment. Fetal body weight was decreased in the high-dose group by 14% for males and 12% for females. There was no effect on litter size or sex ratio. There was no treatment effect on dam uterine or ovarian weight. Histopathology of the dam ovary was reportedly not affected by treatment. Histopathology of the dam uterus showed thickening of the endometrium in the 0.05 and 0.5 mg/kg bw groups and uterine muscle damage in the 5 mg/kg bw group. **[The damage is not otherwise described. The photomicrographs available in the report were not interpretable due to poor reproduction quality.]** The authors concluded that bisphenol A at low doses does not produce reproductive toxicity in mice. **[This study was written in Korean with an English abstract and tables. A translation was provided to CERHR by the American Plastics Council.]**

Strengths/Weaknesses: The use of 3 dose levels is a strength. The lack of information on husbandry conditions, the i.p. dose route, failure to account for litter effects in statistical analyses, and the poor presentation of histopathology results are weaknesses.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process.

Park et al. (2005a), support not indicated, treated ICR mice during pregnancy. Bisphenol A **[purity not indicated]** in corn oil was given i.p. at dose levels of 0, 0.05, 0.5, or 5 mg/kg bw on the day of mating, and every 3 days for a total of 6 doses ($n = 3$–6/group). Offspring were evaluated on PND 45 for body weight, reproductive organ weight and histopathology, semen analysis, complete blood count, and serum chemistry. **[There were 24 female and male offspring evaluated per dose group (not indicated whether 12 of each sex). Litter of origin appears not to have been considered. No information was provided on standardization of litters, diet, or cage/bedding materials.]** Statistical analysis was performed using the least significant difference test. **[It was not clear if the litter or offspring was considered the statistical unit.]**

There was a statistically significant 6% decrease in male body weight in the high-dose group; a comparable body weight decrement in female offspring was not statistically significant. There were no statistically significant treatment effects on the weights of the testis, epididymis, seminal vesicles, coagulating glands, uterus, or ovary. Sperm concentration, viability, motility, and morphology were not affected by treatment. Blood endpoints were not affected by treatment except for a statistically significant 6% increase in erythrocyte count in male offspring and a 2% decrease in serum albumin in female offspring. An 11% increase in blood urea nitrogen in mid-dose female offspring was not dose-related. Histopathology of the testis and ovaries was described as unaffected by treatment. Uterine intimal proliferation was described in the mid- and high-dose female offspring. **[The histological methods were not described. The photomicrographs available in the report were not interpretable due to poor reproduction quality.]** The authors concluded that bisphenol A at low doses does not produce reproductive toxicity in mice. **[This study was written in Korean with an English abstract and tables. A translation was provided to CERHR by the American Plastics Council.]**

Strengths/Weaknesses: The inadequate description of methods, unacceptable small sample size, the i.p. dosing, inappropriate statistical analyses, and the poor presentation of histology results are weaknesses of this study.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process due to the reasons stated above.

Sato et al. (2001), support not indicated, investigated the effects in mice of in utero exposure to bisphenol A on fetal growth, offspring reproductive and brain development, and behavior. Pregnant Jcl-ICR mice ($n = 20$) were given s.c. injections of bisphenol A **[purity not indicated]** 100 mg/kg bw/day, ethinyl estradiol 0.2 or 0.02 mg/kg bw/day, or olive oil vehicle on GD 11–19 **[Plug day was not defined. Information regarding caging material, animals per cage, feed, culling, and weaning was not provided.]** Pups were evaluated for onset of pivoting, righting, straight line walking, and grasp reflex. Open field testing was conducted at 40 days of age. Offspring were killed at 40 or 60 days of age and organs were weighed and processed for histology using hematoxylin and eosin **[fixation method not given]**. Brain myelin was evaluated using Klüver-Barrera staining. Statistical analyses were performed using the Student *t*-test. **[The pup appears to have been used as the statistical unit.]**

There were 11/93 stillborn fetuses after in utero exposure to bisphenol A, but no data were provided for the control group. There were no significant effects of bisphenol A treatment on litter size or offspring body weight at birth, 20, or 60 days of age. There were no significant effects of bisphenol A treatment on days at acquisition of pivoting, righting, straight-line walking, or grasp reflexes. In open field testing, mice in the bisphenol A-treated group showed significantly less defecation than controls **[39% less]**. There was no statistically significant difference between groups in grooming, rearing, line-crossing of inner and outer fields, or latency to first line crossing. At 60 days of age, seminiferous tubules from bisphenol A-exposed male offspring had a significant reduction in mean diameter **[↓16.6%]** and cell layer thickness **[↓25%]** compared to controls. There was no significant bisphenol A effect on brain myelination at 60 days of age or in mean diameter at 40 and 60 days of age of the tractus mamillothalamics. The authors suggest that in utero exposure to 100 mg/kg bw/day bisphenol A induces alterations in behavior similar to that seen at reduced plasma corticosterone levels and that bisphenol A exposure induces gross and cellular changes in seminiferous tubules, suggesting potential perturbation in hormone pathways involved in development.

Strengths/Weaknesses: The use of multiple doses of estrogen as a positive control is a strength. Weaknesses include the evaluation of a single dose of BPA, subcutaneous dosing, and lack of details regarding husbandry. Behavioral methods were chosen from less sophisticated screening approaches and data were not analyzed appropriately using the litter as the statistical

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process based on the limitations noted above.

Matsumoto et al. (2004), support not indicated, examined the effect of maternal bisphenol A exposure on growth of offspring in mice. Mice were fed standard rodent chow (CE-2, Japan Clea). **[No information was provided on caging and bedding materials.]** Mice of the ddY strain were exposed to bisphenol A ($\geq$97% purity) through feed at 0 or 1% from GD 14–PND 7. The study authors stated that the bisphenol A dose was equivalent to 1000 mg/kg bw/day. **[The number of dams treated was not indicated. Day of vaginal plug and day of birth were not defined]**. Mice delivered pups on PND 21. During the postnatal period, body weight was monitored in 31 pups from the control group and 61–89 pups from the bisphenol A group. Serum prolactin levels were measured by RIA in 3 dams/group 4 days following delivery. Pups were killed on PND 7, and stomach weight was measured. Data were analyzed by Student *t*-test. **[It was not clear if the litter or offspring was considered the statistical unit.]**

No differences were reported for live pups at birth. During the postnatal period, body weights of pups in the bisphenol A group were significantly lower **[by ~40%]** than control group pups. No deaths were reported for pups in the control group, but 30% of pups in the bisphenol A group died before PND 7. On PND 1, milk could be seen in stomachs of pups from the control group, but not the bisphenol A group. **[The number of pups evaluated for milk in stomach was not reported.]** On PND 7, stomach weight was significantly lower **[by 40%]** in pups from the bisphenol A than control group. Serum prolactin level was significantly reduced **[by 46%]** in dams from the bisphenol A group. The authors concluded that administration of a high bisphenol A dose to mice resulted in suppressed postnatal growth of offspring that probably resulted from an insufficient supply of milk, which might have been due to decreased prolactin secretion. **[Because of the implications of this study for lactation competence, this study will be discussed again in Section 4.2.]**

Strengths/Weaknesses: Weaknesses of the study are the difficulty in calculating bisphenol A intake, the likely high exposure level, the lack of information on dam number and husbandry, and the high level of pup body weight decrement and mortality.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process due to the reasons stated above.

Suzuki et al. (2003), supported by the Japanese Ministry of Health, Labor, and Welfare and the Ministry of Education, Culture, Sports, Science, and Technology conducted a study to determine the effect of prenatal bisphenol A exposure on dopamine-receptor mediated actions in mice. Female ddY mice were fed chow containing bisphenol A at 0.002, 0.5, or 2 mg/g feed from mating through weaning of offspring. **[No information was provided on the number of dams treated, purity of bisphenol A, or the type of chow, bedding, or caging materials. Assuming a female mouse eats ~0.2 kg feed/kg bw/day (USEPA, 1988), bisphenol A intake would have been 0.4, 100, or 400 mg/kg bw/day.]** Male offspring were subjected to a series of tests **[age at testing not stated]**. In a conditioned place-preference test, groups of 6–10 mice were injected with 0.5 mg/kg bw methamphetamine and placed in either the dark or light area of the test apparatus for 3 days. On the other 3 days, males were injected with saline and placed in the other compartment of the testing apparatus. On Day 7, the divider in the apparatus was raised and the time spent in each compartment was measured. Activity was measured in groups of 9–10 mice for 3 hr following injection with saline or 2 mg/kg bw methamphetamine. Dopamine-induced binding of ^{35}S-guanosine-5′[γ-thio]-triphosphate in the limbic system was measured ($n = 3$ samples/group). Protein levels of dopamine and vesicle monoamine transporters in brain were determined by Western blot ($n = 6$ samples), and mRNA levels of dopamine receptor in brain were determined by RT-PCR. Data were analyzed by ANOVA with Bonferroni/Dunnett test. **[It was not clear if the litter or offspring was considered the statistical unit.]**

In conditioned-preference testing, exposure to all 3 bisphenol A doses resulted in a significant and dose-related increase in preference for compartments associated with methamphetamine exposure. **[Control mice showed no compartment preferences while the times spent in the methamphetamine-associated compartment were ~150, 200, and 275 sec by animals in each respective dose group.]** Preference for the methamphetamine compartment was eliminated by injecting the animals with SCH23390, A dopamine D_1 receptor antagonist. In mice exposed to the high-dose of bisphenol A, activity was significantly increased **[by ~80% at peak]** compared to the control group following methamphetamine challenge, and sensitization to methamphetamine-induced activity was also enhanced. Dopamine-induced binding of ^{35}S-guanosine-5′[γ-thio]-triphosphate in the limbic system was potentiated **[increased by ~15%; not clear if statistically significant]** and G-protein activation was increased **[by ~75%]** in mice exposed to the high bisphenol A dose. The effects on G-protein activation were eliminated following injection with SCH23390 or sulpiride, a dopamine D_2 receptor antagonist. No changes were observed for expression of dopamine and vesicle monoamine transporter proteins. Expression of dopamine D_1 receptor mRNA was upregulated significantly to 130% of control levels in the high-dose bisphenol A group. **[For all endpoints except for conditioned preference, only the data from the high-dose bisphenol A group was shown. It was not clear if that was the only dose tested for those endpoints or if the high-dose data were shown because it was the only dose that resulted in a statistically significant effect.]** The study authors concluded that "prenatal and neonatal exposure to bisphenol A can potentiate central dopamine D_1 receptor-dependent neurotransmission, resulting in supersensitivity of methamphetamine-induced pharmacological actions related to psychological dependence on psychostimulants."

Strengths/Weaknesses: Strengths include a wide range of doses administered orally. Weaknesses include absence of adequate experimental details, inappropriate statistical procedures that did not account for litter or repeated measurement, inadequate presentation of body weight data, and use of high-doses.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process due to the reasons stated above.

3.2.8 Mouse—parenteral exposure postnatally with or without prenatal exposure.

3.2.8.1 Female reproductive endpoints: Suzuki et al. (2002), supported by Japanese Ministry of Education, Culture, Sports, Sciences, and Technology, the Special Coordination Funds of Science and Technology Agency of the Japanese Government, and the Japanese Ministry of Health, Labor, and Welfare, conducted a study to examine the effects of bisphenol A exposure on the reproductive system of the female mouse. Two sets of studies were conducted, one with prenatal exposure, and one with postnatal exposure. In both studies, ICR/Jcl strain mice were fed a commercial diet (CE-2, CLEA, Tokyo, Japan). **[No information was provided about bedding or caging materials.]** Bisphenol A **[purity not reported]** was administered by s.c. injection in sesame oil vehicle. For histological examinations, organs were fixed in Bouin solution. Parametric data were analyzed by ANOVA, with post-hoc Student *t*-test or Welch *t*-test. Data expressed as proportions were analyzed by Fisher exact probability test. For exposures occurring in the prenatal period, the litter was maintained as the statistical unit.

In the prenatal exposure study, mice were administered bisphenol A by s.c. injection at 0 (vehicle), 10, or 100 mg/kg bw/day on GD 10–18 (day of vaginal plug = GD 0). Other groups of mice were treated with diethylstilbestrol at 0.0067–67 μg/kg bw/day during the same period. **[Numbers of dams treated were not specified.]** On GD 19, fetuses were removed by cesarean section, weighed, adjusted to 7 pups/litter **[numbers for each sex not indicated]**, and fostered to untreated mothers. Pups were weaned at 22 days of age. Some pups were ovariectomized at 30 days of age, and some were killed at 30 or 40 days of age for histological examination of reproductive organs, polyovular follicle numbers, corpora lutea numbers, and mitotic index in uterine and vaginal cells. In the remaining pups, vaginal smears were examined from 41–70 days of age. Fertility was then assessed by mating the mice with untreated males (2 or 3 females/male). Offspring were counted and sexed. The authors stated that 2 or 3 pups/litter were used in each analysis. Data tables list the sample size as 8–11/group/time period for the bisphenol A and control groups.

Bisphenol A treatment did not affect the histology of the uterus or vagina in ovariectomized mice. The study authors stated there was no evidence of increased mitogenicity compared to controls in uterine cells of intact or ovariectomized mice exposed to bisphenol A. **[**Figure 3 **of the study indicated a higher mitotic index in epithelial cells of ovariectomized mice of the high-dose group.]** Mitotic indices were significantly lower in stromal cells of intact mice of both dose groups and in glandular cells of the low-dose group. There was no increase in mitogenicity of vaginal cells compared to the control group; in intact mice, the mitotic index was lower than control values in vaginal epithelial cells of the high-dose group and stromal cells of the low-dose group. Number of vaginal epithelial layers was increased in both bisphenol A dose groups of intact mice compared to control mice. No effect was reported for uterine or vaginal epithelial stratification. There were no effects on numbers of polyovular follicles. **[Data were not shown by study authors.]** The number of mice with corpora lutea at 30 days of age was significantly reduced in the low-dose group (4 of 9 mice in low dose group compared to 7 of 9 mice in control group). Estrous cyclicity was not affected by bisphenol A treatment. In mating studies, bisphenol A exposure did not affect the number of mice giving birth, number of fetuses/litter, or sex ratio. Several effects were observed in mice prenatally exposed to diethylstilbestrol, and most of the effects occurred at the high-dose of 67 μg/kg bw/day. In the high-dose diethylstilbestrol group, there were changes in vaginal and uterine histology, increases in mitotic indices in vaginal and uterine cells of ovariectomized animals, vaginal stratification and increased layers of epithelial cells in ovariectomized animals, disrupted estrous cycles, and complete infertility. The number of mice with corpora lutea at 30 days was decreased at the two highest diethylstilbestrol doses ($\geq$6.7 at μg/kg bw/day).

In the postnatal exposure experiment, female mice (1.5 g bw) were s.c. injected with bisphenol A at 0.015 or 0.150 mg/pup/day or diethylstilbestrol at 0.3 or 3 μg/pup/day for 5 days, beginning on the day of birth. **[The number of animals treated was not stated. Based on body weights provided by authors, bisphenol A doses were estimated at 10 and 100 mg/kg bw/day; diethylstilbestrol doses were estimated at 200 and 2000 μg/kg bw/day.]** Two-thirds of mice were ovariectomized at 30 days of age and then killed at 30, 40, or 90 days of age for histological examination of reproductive organs. Numbers of polyovular follicles were determined at 30 days of age, and number of corpora lutea were counted at 30 and 90 days of age. Estrous cyclicity was monitored in the remaining mice at 61–90 days of age. The 90-day-old mice were s.c. injected with 5 mg/kg bw colchicine and killed 5 hr later. Mitotic rates of uterine and vaginal cells were determined, and histological examinations of reproductive organs were conducted. Sample sizes were 6–17/group/time period in analyses conducted in mice exposed postnatally.

Vaginal stratification was observed at 40 days of age in 4 of 7 ovariectomized mice of the high-dose bisphenol A group, which was higher than in the control. The incidence of vaginal stratification in 90-day-old ovariectomized mice of the high-dose group (4 of 10) did not attain statistical significance compared to control. In ovariectomized mice, significant increases in the mitotic rate compared to controls were observed in uterine stromal cells and vaginal epithelial cells at the high-dose. The number of vaginal epithelial layers was also increased in the high-dose bisphenol A group ($\sim$4 layers in treated group compared to 3.5 layers in control group). There were no significant changes in estrous cycles or number of mice with corpora lutea. In 30-day-old mice of the high-dose group, significant increases were observed in the number of mice with polyovular follicles (15 of 17 in exposed group compared to 6 of 15 in control group) and the numbers of polyovular follicles/mouse (mean $\pm$ SE: 0.8 ± 0.2 in the exposed group and 0.2 ± 0.1 in control group); polyovular follicles contained 2 oocytes in the control and bisphenol A groups. Effects observed in mice treated with both doses of diethylstilbestrol included increased stratification of vaginal cells in ovariectomized mice at 40 and 90 days of age, increased mitotic rates of vaginal and uterine cells in ovariectomized mice, disrupted estrous cycles, and increased polyovular follicles. The study authors concluded that

high-doses of bisphenol A induce ovary-independent vaginal stratification and polyovular follicles when administered during postnatal but not prenatal development.

Strengths/Weaknesses: The use of diethylstilbestrol as a positive control is a strength as are an experimental design that appropriately examined litter effects. The use of relatively high-doses by s.c. injection and small sample sizes for ovarian histopathology are weaknesses.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate but of limited utility due to the route and dose level.

Nikaido et al. (2005), supported by the Japanese Ministry of Health, Labor, and Welfare, examined the effects of bisphenol A exposure on the development of the reproductive system in female mice. Mice used in this study were housed in polyisopentene cages with white pine chip bedding. The mice were fed a low-phytoestrogen diet (NIH-07 PLD; Oriental Yeast Co.) and provided water in polycarbonate bottles with rubber stoppers. At 15 days of age, 17–24 female CD-1 mice/group were s.c. injected with DMSO vehicle, 10 mg/kg bw/day bisphenol A ($\geq$99% purity), or 10 μg/kg bw/day diethylstilbestrol for 4 days. Additional groups were dosed with other compounds, but those results will not be discussed. **[No information was provided on the numbers of litters represented.]** Mice were weaned at 21 days of age. Body weights were measured weekly. Day of vaginal opening was determined and estrous cyclicity was assessed over 21-day periods beginning at 5, 9, and 21 weeks of age. Six mice/group/time period were killed and necropsied at 4, 8, 12, and 24 weeks of age. **[In contrast to the Materials and Methods section, there was no mention of animals killed at 12 weeks of age in the Abstract or Results section of the study.]** Ovaries, uteri, vaginas, and inguinal mammary glands were fixed in 10% neutral buffered formalin. Histopathological analyses were conducted of the ovary, uterus, and vagina. Mammary glands were examined as whole-mount preparations. It appears that all endpoints were assessed in every mouse. Statistical analyses included homogeneity of variance analysis and ANOVA or Kruskal–Wallis test. If statistical significance was obtained, data were further analyzed by Fisher protected least significant difference test.

Exposure to bisphenol A resulted in no effects on body weight gain, age of vaginal opening, estrous cyclicity, histopathological changes in the uterus or vagina, or growth or development of the mammary gland. At 4 weeks of age, 33% of mice in the control group, 83% of mice in the bisphenol A group, and 100% of mice in the diethylstilbestrol group lacked corpora lutea. **[It appears that the study authors considered the lack of corpora lutea to be normal based on the age of mice.]** No effects on corpora lutea numbers or numbers of polyovular follicles were observed at later ages. Mice treated with diethylstilbestrol experienced accelerated vaginal opening and increased time in estrus. In their conclusion, the study authors reiterated the lack of effects in the bisphenol A group.

Strengths/Weaknesses: The use of diethylstilbestrol as a positive control is a strength, but the lack of information on sample size of dams, small sample size for postnatal endpoints, subcutaneous route, and high-dose level are weaknesses.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate and not useful in the evaluation.

Markey et al. (2005), supported by NIH, examined the effects of perinatal bisphenol A exposure on reproductive development in mice. CD-1 mice were fed Purina rodent chow that tested ''negligible for estrogenicity in the E-SCREEN assay.'' Cages and bedding tested negative for estrogenicity in the E-SCREEN assay. Tap water was supplied in glass bottles. From GD 9 (GD 1 = day of vaginal plug) through PND 4, 6–10 mice/group were exposed to bisphenol A **[purity not reported in the manuscript; 97 ± 2% per A. Soto, personal communication, March 2, 2007]** at 0 (DMSO vehicle), 0.000025, or 0.000250 mg/kg bw/day through a s.c. pump. Offspring were culled to 10/litter on PND 7 and weaned on PND 20. One pup/litter from 6–10 litters/treatment group was killed on the day of proestrus at 3 months of age. The uterus and vagina were weighed and subjected to morphometric analysis. The uterus was also assessed for cell proliferation by bromodeoxyuridine (BrdU) incorporation, apoptosis by TUNEL method, and expression of ERα and progesterone receptor by an immunostaining procedure. Data that were normally distributed and showed homogeneity of variance were analyzed by ANOVA and least significant difference test. Other data were analyzed by Kruskall–Wallis and Mann–Whitney U test.

Significant effects observed in 3-month-old offspring exposed to the high-dose included decreased absolute and relative (to body weight) vaginal weight, decreased volume of uterine lamina propria, and increased percentage of proliferating uterine glandular epithelial cells. In mice of both dose groups, there were significant increases in expression of ERα and progesterone receptor in uterine luminal epithelial cells; levels of both receptors were also increased in the subepithelial stroma. No treatment effects were observed for apoptosis in uterine luminal and glandular epithelial cells. No treatment effects were observed for vaginal morphometry or cell proliferation. The study authors concluded that environmentally relevant doses of bisphenol A affect the development of the genital tract at the gross and cellular level in the female offspring of mice exposed during pregnancy.

Strengths/Weaknesses: The administration of very low doses is a strength. A critical weakness is the use of DMSO as a vehicle which is known to degrade the pump apparatus, and is inappropriate as a vehicle for in vivo studies. A critical weakness is the uncertainty of the DMSO concentration as a vehicle and therefore pump performance.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process given exposure uncertainties.

Muñoz-de-Toro et al. (2005), supported by NIH and National University of Litoral (Argentina), examined the effect of perinatal bisphenol exposure on mammary gland development in mice. Food, caging, and bedding material were reported to test negligible for estrogenicity in the E-SCREEN. Water was provided in glass bottles. CD-1 mice (n = 6–10/group) were implanted with osmotic pumps designed to deliver bisphenol A **[purity not indicated]** at 0 (DMSO vehicle), 0.000025, or 0.000250 mg/kg bw/day from GD 9 (GD 1 = day of vaginal plug) through PND 3 (not defined). Offspring

were culled to 10 pups/litter on PND 7. One female offspring/litter, from 6–10 litters/group, was killed on PND 20 and 30 and at 4 months of age. The 4-month-old mice were killed on proestrus. Another group of mice **[number not specified]** was killed on the first proestrus. Mammary glands were collected for evaluation of mammary structures at 20 and 30 days and 4 months of age and day of first proestrus. Mammary glands were also collected from 30-day-old mice for analysis of DNA synthesis by BrdU incorporation, expression of ERα and progesterone receptor using immunohistochemistry techniques, apoptosis by TUNEL method, and *Wnt4* mRNA by RT-PCR. Plasma 17β-estradiol levels were measured in mice killed at first proestrus. In an experiment to monitor response to 17β-estradiol, 1 pup/litter ($n = 10$/group) was ovariectomized at 25 days of age and implanted with a s.c. pump supplying vehicle or 0.5 μg 17β-estradiol/kg bw/day on PND 25–35. Mice were killed following 17β-estradiol treatment for examination of mammary structures. Statistical analyses included ANOVA and Dunn post-hoc test. If the data were not normally distributed, statistical analyses were done by Kruskall–Wallis and Mann–Whitney test.

In 30-day-old mice, bisphenol A exposure increased numbers of terminal end buds at both doses and area of terminal end buds at the high-dose. Percentages of apoptotic cells were decreased on PND 30 in mice from both bisphenol A dose groups. The percentage of stromal cells undergoing proliferation on PND 30 was reduced in the high-dose bisphenol A group. The number of epithelial cells expressing progesterone receptors was increased in both dose groups on PND 30, but there were no treatment-related changes in ERα receptor expression. Clusters of progesterone receptors were often observed in the ductal epithelium of bisphenol A-treated mice. Slopes of the correlation between age of first proestrus and mammary length were significantly reduced in the high-dose group, suggesting slower ductal invasion of stroma. There were no significant differences in plasma 17β-estradiol levels in mice killed at first proestrus. Trends for increasing expression of mRNA for *Wnt4*, a mediator of lateral branching downstream from progesterone receptors, did not attain statistical significance. The number of lateral branches in mammary gland at 4 months of age was significantly increased at the low but not the high-dose. In mice exposed to the high-dose of bisphenol A during perinatal development and 17β-estradiol during postnatal development compared to mice who were exposed to 17β-estradiol but not bisphenol A, there were increases in numbers, area, and size of terminal end buds, terminal end bud numbers/ductal area, and terminal end bud area/ductal area. The study authors concluded that "…perinatal exposure to environmentally relevant [bisphenol A] doses results in persistent alterations in mammary gland morphogenesis."

Strengths/Weaknesses: This study was a follow-up on the study of Markey et al. (2005) and tested the same doses using a similar schedule for effects on mammary tissue. The administration of very low doses is a strength. The statistics appear to be inappropriate in not accounting for the significant number of comparisons made. A critical weakness is the uncertainty of the DMSO concentration as a vehicle and therefore pump performance.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process given exposure uncertainties.

3.2.8.2 Male reproductive endpoints:: Nakahashi et al. (2001), supported by the Japanese Ministry of Education, Science, Sports, and Culture, examined the effect of neonatal bisphenol A exposure on adult sperm count in mice. On the first 5 days of life, 10–15 neonatal SHN mice/group were injected **[route not indicated]** with sesame oil/DMSO vehicle or with bisphenol A **[purity not reported]** in sesame oil at 0.0005 or 0.050 mg/day. **[Assuming a neonatal mouse weights 2 g, the mice received doses of 0.25 and 25 mg/kg bw/day.]** A group of 12 mice received 0.050 mg/day bisphenol A in sesame oil in combination with 100 IU retinol acetate in DMSO vehicle. In a second exposure protocol, pregnant mice were fed a vitamin A-deficient diet (Low Vitamin A diet; Clea Japan) from 3 days before gestation to PND 5. After PND 5, the dams were fed commercial diet (CE-7, Clea Japan). On the first 5 days of life, their pups ($n = 7$–9/group) were injected with bisphenol A at 0 (sesame oil) or 0.0005 mg/day. Male offspring from both studies were weaned at 20 days of age and fed the CE-7 diet. Mice were killed at 14 weeks of age and epididymal sperm counts were obtained. **[No information was provided about caging and bedding materials. Numbers of litter represented were not indicated. Procedures for statistical analyses were not discussed.]**

A 35% reduction in sperm counts was observed in mice from the 0.050 mg/day group compared to the control group. A significant reduction in sperm counts was not observed in the group co-treated with 0.050 mg/day bisphenol A and retinol acetate. Administration of a vitamin A-deficient diet to dams had no effect on sperm counts in their offspring, but sperm counts were reduced in mice born to mothers fed a vitamin A-deficient diet and injected with 0.0005 mg/day bisphenol A in the neonatal period. The study authors concluded that vitamin protects infants from the effects of environmental xenoestrogens.

Strengths/Weaknesses: The subcutaneous route of administration, lack of clarity on exposure issues, lack of husbandry and statistical information are weaknesses.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for inclusion and not useful.

Aikawa et al. (2004), supported by the Japanese Ministry of Education, Science, Sports, and Culture, examined the effects of neonatal bisphenol A exposure on sperm endpoints in adult mice. Unless otherwise specified, dams were fed CE-7 and CA-1 (Clea Japan Inc.). **[No information was provided about caging or bedding materials.]** In the first experiment, SHN mice were s.c. injected with bisphenol A, bisphenol A plus retinol acetate, or vehicle for 5 days beginning on the day of birth. Doses of each compound were 0.5 or 50 μg/day bisphenol A **[purity not reported]** ($n = 10$–14/group), 50 μg bisphenol A plus 100 IU retinol acetate/day ($n = 5$), and vehicle control (sesame oil for bisphenol A and or DMSO for retinol acetate; $n = 11$). **[Assuming a neonatal mouse weighs 2 g, these bisphenol A doses would be 0.25 and 25 mg/kg bw/day.]** In another group, pregnant mice were fed a low vitamin A diet from 3 days before gestation to PND 5 and were fed a normal vitamin A-containing diet (CE-7 and CA-1) beginning on Day 6 following parturition **[number/group not stated]**. Pups

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate and not useful due to critically small sample size, route of administration, lack of clarity of design, and inappropriate statistical procedures.

3.2.9 Sheep. **Evans et al. (2004)**, supported by the British Council, Irish Health Research Board, and the Royal Society, examined the effects of bisphenol A exposure on gonadotropin secretion on prepubertal female lambs. **[No information was provided about feed or composition of bedding or caging materials.]** Starting at 3 weeks of age, female Poll Dorset lambs were weighed weekly, and blood samples were collected 2 times/week for measurement of LH and FSH levels. At 4 weeks of age, lambs were assigned to treatment groups according to body weight. From 4–11 weeks of age, 6 lambs/group received biweekly i.m. injections with the 10:1 corn oil/alcohol vehicle, 3.5 mg/kg bw bisphenol A **[purity not reported]**, 0.175 mg/kg bw diethylstilbestrol **[listed as 0.0175 in the legend for Figure 1 of the study]**, or 3.5 mg/kg bw octylphenol. Lambs were ovariectomized at 9 weeks of age. **[The text of the methods sections reported ovariectomy at the beginning of treatment, but that statement appears to be an error because it is not indicated elsewhere in the study.]** On the last day of treatment, blood was collected every 15 min for 6 hr to assess pulsatile LH secretion. All lambs were then killed. Adrenal glands, kidneys, and ovaries were weighed. Uteri were examined as discussed in Morrison et al. (2003). Data were analyzed by ANOVA, Dunnett multiple comparison post-hoc test, regression analysis, Munro algorithm, and paired *t*-tests.

Compared to the control group, the bisphenol A group did not experience significant changes in body, kidney, adrenal, or ovarian weights. **[No data were shown for body, kidney, and ovarian weights in the control vs. bisphenol A group.]** Uteri from the bisphenol A group were reported to be visually larger, but no uterine weights were provided. Over the 7-week treatment period, bisphenol A did not significantly affect blood LH or FSH levels compared to controls. Compared to controls, the bisphenol A group experienced significant decreases **[% change compared to controls]** in concentration **[48%]**, amplitude **[77%]**, and frequency **[66%]** of pulsatile LH secretion. Octylphenol did not have any effect on the endpoints examined. Diethylstilbestrol treatment resulted in decreased blood levels of LH and FSH over the treatment period, including the period following ovariectomy. Concentration, amplitude, and frequency of pulsatile LH secretion were also lower in the diethylstilbestrol group, with a greater magnitude of effect compared to bisphenol A. The study authors concluded that the bisphenol A dose tested can inhibit LH secretion in lambs.

Strengths/Weaknesses: The unique animal model and the use of LH pulsatile response are uncommon but interesting. The high-dose level via i.m. injection is a weakness as are small sample sizes ($n = 6$). The statistical tests for LH trends did not appear to take into account the repeated nature of the sampling leading to over stating the significance of trend effects.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for inclusion but of limited utility for the evaluation process.

Morrison et al. (2003), supported by the Wellcome Trust, Dr. Ferranti, and the Irish Health Research Board, examined the effects of bisphenol A exposure on the lamb uterus. **[No information was provided on feed or composition of bedding or caging materials.]** At 4 weeks of age, female Poll Dorsett lambs were randomly assigned to treatment groups according to body weight. Beginning at 4 weeks of age and continuing for 7 weeks, 6 lambs/group received biweekly i.m. injections with the 10:1 corn oil:alcohol vehicle, 3.5 mg/kg bw bisphenol A **[purity not reported]**, 0.175 mg/kg bw diethylstilbestrol, or 3.5 mg/kg bw octylphenol. Lambs were ovariectomized during the fifth week of exposure. Throughout the study, blood was collected for measurement of gonadotropin levels and the results of those analyses were reported in the study by Evans et al. (2004). Lambs were killed following 7 weeks of exposure. Uteri and cervices were fixed in Bouin solution for histopathological examination, morphometric measurement, and immunohistochemical detection of ERα and ERβ. Statistical analyses included ANOVA with Fisher protected least significant difference.

Significant effects observed with bisphenol A treatment **[% change compared to controls]** were increased uterine/cervical tract weight **[87%]**, endometrial area **[154%]**, and endometrial/myometrial ratio **[65%]**. Qualitative histopathological observations in uteri from bisphenol A-treated lambs included endometrial edema, decreased endometrial gland density compared to controls, and crowding of cells in the uterine epithelium, which contained substantial amounts of eosinophilic, non-vacuolated cytoplasm. In contrast to uteri from control lambs, mononuclear cell exocytosis was not a common observation in uteri from the bisphenol A group. The cervical epithelium was keratinized in the bisphenol A group. Qualitative analyses revealed that diffuse intracellular staining for ERα and ERβ in the uterine subepithelium was most pronounced in the bisphenol A and diethylstilbestrol groups. Similar to animals treated with bisphenol A, the diethylstilbestrol group had increased uterine weight, keratinized cervical epithelium, changes in uterine histology, and keratinized cervical epithelium, but there was no change in endometrial/myometrial ratios. No changes were observed following exposure to octylphenol. The study authors concluded that bisphenol A exposure altered the uterocervical environment of lambs.

Strengths/Weaknesses: This is a companion to the study of Evans et al. (2004) with similar strengths. The single high-dose level via i.m. injection is a weakness as is the exclusion of data from 2 lambs based on responses for E/M ratio endpoints, thus reducing the n to 5 and potentially biasing the data. The statistical analyses do not appropriately account for the number of multiple comparison made which can increase the probability of detecting an effect by chance.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for inclusion but of limited utility in the evaluation process.

Savabieasfahani et al. (2006), supported by the U.S. Public Health Service, NIH, and the University of Michigan, used Suffolk ewes to investigate the effects of maternal exposure to bisphenol A or methoxyclor **[not discussed here]** during gestation. Pregnant Suffolk ewes used in this experiment were exposed to a natural photoperiod in the same pasture and fed a diet of 1.25 kg alfalfa/grass hay. Pregnant ewes ($n = 10$) of similar

average weight were injected s.c. on GD 30–90 with 5 mg/kg bw day bisphenol A (99+% purity) dissolved in cottonseed oil. Control pregnant ewes ($n = 16$) were administered vehicle injections. Lambs were born over about a 1-month interval in early spring. Birth outcome measurements included number and gender of offspring, weight, height, chest circumference, genital development, and measurement of blood insulin and insulin-like growth factor-1. Lambs were cross-fostered and group housed on PND 3. Lactating ewes were fed a diet of corn and alfalfa hay. Lambs had free access to standardized Shur Gain feed pellets. **[The authors note the presence of phytoestrogens in the feed but did not provide quantification.]** At weaning, female were separated from male offspring, and the females were housed in open air pens under natural photoperiod with free access to feed pellets, as described above.

Maternal blood samples were taken on GD 50, 70, and 90 for measurement of bisphenol A using HPLC. The number and sex of offspring in each treatment group, weight, height, chest circumference, and genital development were noted. Blood levels of insulin and insulin-like growth factor 1 were assayed by RIA on PND 1. In female offspring **[n not indicated]**, blood was drawn biweekly during the first 2 postnatal months for determination of LH by RIA. Timing of puberty onset was estimated through twice weekly blood draws for progesterone ($n = 11$/group). Estrus cycling patterns were determined by frequent measurement of FSH, LH, and progesterone by RIA in 3 female offspring/group after synchronization with prostaglandin F2α at 40 weeks of age. Statistical analyses were performed using ANOVA, repeated measures ANOVA, or a linear mixed model. A cluster algorithm was used to identify LH pulses, with Student *t*-test to determine LH nadirs.

Blood levels of bisphenol A were significantly higher in exposed pregnant ewes than controls at all sampling times. The levels reached (37.4 ± 3.3 μg/L) were compared to exposure levels reported in pregnant women [0.3–18.9 μg/L (Schönfelder et al., 2002b)]. No statistical difference was reported in gestation length, number of offspring, or sex. There were no significant differences in female lambs in anogenital distance, insulin, or insulin-like growth factor levels on PND 1. In female offspring, prenatal bisphenol treatment significantly decreased birth weight **[by ~11%]**, height **[by ~5%]**, and chest circumferences **[by ~7%, all comparisons estimated from a graph]**. In male offspring exposed to bisphenol A, there were no significant differences from control in birth weight, height, chest circumference, or anogenital distance, but anoscrotal:anonavel ratio was increased significantly **[by 21%]**. Bisphenol A treatment increased significantly levels of circulating LH **[by ~89%, estimated from a graph]** during the first 2 months of life in female offspring. Onset of puberty was not affected by treatment in bisphenol A-exposed female offspring, but these females had a significantly longer first breeding season **[by ~2 weeks]** and larger number of cycles during the first breeding season). Estrous cycle length and progesterone levels were not different from controls. The bisphenol A group had significantly lower peak and total LH, and the amplitude of LH pulses was increased significantly, whereas frequency showed no difference from control group. No differences in FSH were seen between groups. Progesterone secretion pattern showed no difference between groups, despite perturbations in LH patterns.

The authors concluded that prenatal exposure to bisphenol A impairs growth in female fetuses and is associated with dampening of the LH surge. Although there was no apparent effect on progesterone production, the authors suggested that the changes induced by prenatal exposure of females could interfere with fertility.

Strengths/Weaknesses: This study appears to have been well-conducted with the utilization of multiple endpoints in sheep. Weaknesses are the use of a single dose level and the relatively small sample size. The single time point for bisphenol A plasma determination at an unknown time relative to s.c. injection is a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate though of limited utility.

3.2.10 Non-mammalian species. Although these studies in non-mammalian species can be quite useful for understanding mechanisms and environmental impacts, the studies are not considered useful for the evaluation process, because of the uncertain relationship between human biology and that of the model species.

3.2.10.1 Invertebrates: Hill et al. (2002) supported by the Council on Undergraduate Research and the Association for Biological Laboratory Education, examined the effects of bisphenol A on the development of 2 freshwater sponge species. (*Heteromyenia* sp. and *Eunapius fragilis*). Sponge gemmules were incubated in tissue culture wells containing bisphenol A **[purity not indicated]** at 0, 0.16, 16, 80, or 160 ppm **[mg/L]**. The control group was incubated in the spring water vehicle. There were 5 replicates/treatment. Nonylphenol and ethylbenzene were also examined. Growth was measured on Days 3, 6, and 9. Because growth patterns were similar at all 3 evaluation periods, statistical analyses were conducted only for Day 6 data. Data were analyzed by ANOVA and Tukey multiple comparison test. In both species, abnormal development or malformation of the water vascular system was observed at a bisphenol A dose of 16 ppm and germination was completely inhibited at 80 and 160 ppm. Significantly reduced growth rates were observed in *Heteromyenia* sp. at 160 ppm. Similar effects were observed with nonylphenol and ethylbenzene. The study authors stated that sponges may prove useful for examining endocrine-disrupting compounds.

Strengths/Weaknesses: This study used a unique model with a focus on the aquatic system.

Utility (Adequacy) for CERHR Evaluation Process: This study may have utility for environmental assessment, but is not useful for human risk assessment.

Roepke et al. (2005), supported by the National Oceanic and Atmospheric Administration, examined the effects of bisphenol A exposure on development of two species of sea urchin, *Strongylocentrotus purpuratus* and *Lytechinus anamesus*. In dose–response studies, sea urchin embryos were incubated from 1–96 hr post-fertilization in media containing bisphenol A **[purity not indicated]** at 0, 250, 500, 750, or 1000 μg/L **[culture ware not discussed]**. Development toxicity was assessed at 96 hr by examining larvae at the pluteus stage. The larvae were categorized as normal, delayed, abnormal, elongated, or hatched. Data were obtained in 3 replicates.

Table 82
Summary of High Utility Developmental Toxicity Studies (Multiple Dose Levels)

		Bisphenol A dose level (mg/kg bw/day)						Reference
Model (treatment)	Endpoint	NOAEL	LOAEL	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$	
Rat								
Han–Wistar (drinking water from before mating through gestation and lactation)	Male reproductive organ weights, sperm production, testicular histopathology	≥0.775–4.022 (high dose)						Cagen et al. (1999b)
CD (gavage, 2-generations exposure including pre-and postnatal development periods)	Prenatal or postnatal growth or survival, developmental landmarks, anogenital distance, age of puberty, fertility, estrous cyclicity, or sperm counts	≥0.2 (high dose)						Ema et al. (2001)
Sprague–Dawley dam (gavage GD 1–20)	↓ Live fetuses/litter	300	1000	929	348	982	713	Kim et al. (2001b)
	↓ Male body weight	100	300	456	339	694	497	
	↓ Female body weight	300	1000	439	328	682	490	
	↓ Ossification	300	1000					
Sprague–Dawley dam (gavage GD 11–PND 20)	Volume of SDN-POA, age or weight at vaginal opening or first estrous, estrous cyclicity, mean lordosis intensity, prostate weight, or histopathology in ventral prostate, ovary, or uterus	≥320 (high dose)						Kwon et al. (2000)
CD dams (gavage GD 6–15)	Implantation sites, resorptions, body weight, viability, sex ratio, and malformations	≥640 (high dose)						Morrissey et al. (1987)
Sprague–Dawley dam (gavage GD6–PND 21)	↑ Uterine epithelial cell nuclei		0.1 (low dose)					Schönfelder et al. (2004)
	↑ Uterine epithelial nuclei with condensed chromatin		0.1 (low dose)					
	↑ Uterine epithelial cells with cavities		0.1 (low dose)					
	↓ ERβ-positive cells in uterine tissue		0.1 (low dose)					
	↓Thickness of uterine luminal epithelium	0.1	50					
	↑ ERα-positive cells in uterine epithelium	0.1	50					
Wistar-derived Alderley–Park dams (gavage GD 6–21)	Delayed vaginal opening	0.1	50	68	51	35	16	Tinwell et al. (2002)
	↓ Sperm count/testis	0.1	50	55	30	57	31	
	↓ Sperm count/g testis	0.1	50	81	41	68	34	
	↓ Daily sperm count/testis	0.1	50	56	31	59	31	
	↓ Daily sperm count/g testis	0.1	50	83	42	70	34	
Sprague–Dawley (dietary, multiple generations with exposure during pre-and post natal development)	Live F1 pups/litter	47.5	475	268	192	559	394	Tyl et al. (2000b, 2002b)
	Live F2 pups/litter	47.5	475	422	152	459	294	
	Live F3 pups/litter	47.5	475	236	174	376	286	
	F1 body weight, PND 4	47.5	475	406	283	561	400	
	F1, F2, or F2 body weight, PND 7	47.5	475	217–328	183–257	265–410	218–313	
	F1, F2, or F2 body weight, PND 14	47.5	475	183–243	163–209	177–227	153–191	
	F1, F2, or F2 body weight, PND 21	47.5	475	208–252	166–226	223–267	175–220	
	↑ Age at F1 vaginal opening	47.5	475	394	343	206	176	
	↑ Age at F2 vaginal opening	47.5	475	404	336	277	228	
	↑ Age at F3 vaginal opening	47.5	475	471	401	396	203	
	↑ Age at F1 preputial separation	4.75	47.5	466	411	188	163	
	↑ Age at F2 preputial separation	47.5	475	300	255	241	203	
	↑ Age at F3 preputial separation	47.5	475	547	473	222	189	
	Mating, fertility, pregnancy, or gestational indices; precoital interval, postimplantation loss, estrous cyclicity, and reproductive organ histopathology; sperm count, morphology or motility;	≥475 (high dose)						

Table 82
Continued

Model (treatment)	Endpoint	Bisphenol A dose level (mg/kg bw/day) NOAEL	LOAEL	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$	Reference
	anogenital distance in males or females; areolas/nipples in males							
Mouse								
CD-1 dam (gavage GD 6–15)	↑ Resorptions/litter	1000	1250	817	377	1245	1162	Morrissey et al. (1987)
	↓ Fetal body weight/litter	1000	1250	1079	785	1249	1024	
C57BL/6N males (gavage GD 11–17 or PND 21–43)	Sperm density or lesions in reproductive organs	≥0.200 (high dose)						Nagao et al. (2002)
	↓ Absolute seminal vesicle weight in group exposed during gestation		≤0.002 (low dose)[a,b]					
CF-1 (oral by pipette, GD 11–17)	↑ Prostate weight		≤0.002 (low dose)					Nagel et al. (1997)
C57BL/6 dam (gavage GD 3–PND 21)	no effect AGD or AGD corrected for body weight	≥0.2 (high dose)						Ryan and Vandenbergh (2006)
	No effect on errors in radial arm and Barnes mazes	≥0.2 (high dose)						
	↓ Time in open arms of plus maze	0.002	0.2					
	↓ Time in light part of light/dark preference box	0.002	0.2					
CD-1 (dietary, multiple generations with exposure during pre- and post-natal development)	↓ F1 body weight on PND 7, 14, and 21	50	600	548–560	267–313	580–617	370–506	Tyl et al. (2006)
	↓F1 male body weight at PND 21 necropsy	50	600	564	313	640	599	
	↓F1 female body weight at PND 21 necropsy	50	600	387	254	776	598	
	Hepatic cytoplasmic variation, F1 male	5	50	124	92.5			
	Hepatic cytoplasmic variation, F2 male	50	600	224	178			
	Hepatic cytoplasmic variation, F1 female	5	50	333	200			
	Seminiferous tubule hypoplasia, F1 male	50	600	591	406			
	Seminiferous tubule hypoplasia, F2 male	5	50	283	233			
	Age of preputial separation, F1 parental or non-mated males	50	600	727–754	572–576	491–551	364–414	
	Anogenital distance per body weight, F1 male on PND 21	5	50	1373	607	1769	616	
	Postnatal survival; daily sperm production; efficiency of daily sperm production; sperm motility or morphology; estrous cyclicity; numbers of ovarian primordial follicles; mating or fertility indices; or adult prostate weight	≥600 (high dose)						

[a]There was little to no evidence of a dose-response relationship.
[b]No effects were observed at one or more higher dose levels.

Table 83
Summary of Limited Utility Developmental Toxicity Studies (Single Dose Level)

Model (route)	Dose and dosing period (mg/kg bw/day)	Significant developmental findings	Reference
Rat			
Long–Evans male offspring (gavage) Experiment 3	0.0024, PND 21–90	↑ Serum LH level; ↓ weight of seminal vesicles; ↓ Testicular testosterone level; and ↓ basal and LH-induced ex vivo testosterone production	Akingbemi et al. (2004)
Wistar male pup (s.c. injection)	100, PND 2–12	Advanced testicular lumen formation, ↑testis weight, ↑ Sertoli cell volume/testis, ↑ spermatocyte nuclear volume/ unit Sertoli cell, and ↑ plasma FSH on PND 18; ↑ plasma FSH on PND 25; ↑ testicular weight in adulthood	Atanassova et al. (2000)
Wistar dam (drinking water)	~2.5, gestation[a]–PND 21	In rats 4–7 months of age: no effect on the number of corticotropin-releasing hormone neurons in the preoptic areas of males, a loss in sex difference in the anterior and posterior bed nuclei of the stria terminalis	Funabashi et al. (2004a)
Sprague–Dawley male pup (s.c. injection)	0.010, PND 1, 3, and 5; half the rats exposed to 17β-estradiol and testosterone in adulthood	In rats with no 17β-estradiol and testosterone exposure in adulthood: no effects on dorsal prostate weight, histopathology alterations, proliferation index, or apoptotic index In rats with 17β-estradiol and testosterone exposure in adulthood: ↑ incidence and severity of prostatic intraepithelial neoplasia; ↑ proliferation and apoptosis in regions of prostatic intraepithelial neoplasia	Ho et al. (2006b)
Sprague–Dawley pup (s.c injection)	300, PND 1–5	No effects on age of vaginal opening or preputial separation, copulation or fertility indices, sexual behavior of males, histopathologic alterations in males, or female reproductive organs, or effects on SDN-POA. **[Panel noted possible ↑ number of apically located nuclei in prostate, but a definitive conclusion could not be made based on 1 photograph]**	Nagao et al. (1999)
Wistar. male pup (s.c injection)	50, PND 22–32	↑ Serum prolactin levels on PND 29 but not PND 120; ↑ lateral but not ventral prostate weight; ↑ focal luminal polymorphonuclear cellular infiltrate in prostate No histological evidence of prostate inflammation	Stoker et al. (1999)
Mouse			
CF1 (oral)	0.0024, GD 11–17	↑ Body weight at weaning; ↓ postnatal pup survival; ↓ period between vaginal opening and first estrus No effect on age of vaginal opening	Howdeshell et al. (1999)
ICR/Jc1 mouse dams (s.c. injection)	0.02, GD 0 to GD 10.5, GD 12.5, GD 14.5, or GD 16.5	↑ Tuj1 in the intermediate zone at GD 14.5 and GD 16.5; ↑ PDI immunoreactivity in the neocortex from GD12.5 until GD16.5 and in subplate cells at GD 14.5; variable changes in BrdU labeling depending on when labeled and location; ↑ gene expression of *Math3*, *Ngn2*, *Hes1*, *LICAM*, and *THR-alpha*a at GD14.5; ↓ gene expression of *Hes1* and *Hes5* at GD 12.5 No effect on immunoreactivity pattern for KI-67, nestin, Musashi and histone H_3.	Nakamura et al. (2006)
ICR (oral)	0.002 mg/kg bw/day from 6.5–11.5, 6.5–13.5, 6.5–15.5, and 6.5–17.5 days post-coitum	Variable changes in retinoic acid retinoid X receptors á mRNA expression in brain, ovary, and testis, depending on brain region and day of exposure	Nishizawa et al. (2003)
Other			
Prepubertal Poll Dorset female lambs (i.m. injection)	3.5 biweekly, at 4–11 weeks of age (ovariectomy at 9 weeks of age)	↔ On blood levels during treatment; ↔ on body, kidney, adrenal, or ovarian weights; ↓ pulsatile LH secretion	Evans et al. (2004)
Prepubertal Poll Dorset female lambs (i.m. injection)	3.5 biweekly, at 4–11 weeks of age (ovariectomy at 9 weeks of age)	↑ Uterine/cervical tract weight, endometrial area, and endometrial/myometrial ratio Qualitative observations included endometrial edema, decreased endometrial gland density, crowding of cells in the uterine epithelium, keratinized cervical epithelium, ↑ intracellular staining for ERα and ERβ in the uterine subepithelium	Morrison et al. (2003)
Suffolk ewes (s.c. injection)	5 GD 30–GD 90	↓ Birth weight, height and chest circumference in female offspring at birth ↑ Anoscrotal:anonavel ratio in male offspring at birth ↑ LH and first breeding season in female offspring at PND 60	Savabieasfahani et al. (2006)

[a]Implied but not stated that exposure occurred during the entire gestation period.
↑,↓ Statistically significant increase, decrease compared to controls; ↔ no statistically significant effects compared to controls.

Panel members reviewed the literature on estrogen-sensitivity across rat strains and suppliers, the following is a summary of our findings.

Different strains of rats show clear, robust reproducible differences in response to potent estrogens and antiandrogens. Several traits have been shown to be

Table 84
Summary of Limited Utility Developmental Toxicity Studies (Multiple Dose Levels)

Model (treatment)	Endpoint	Bisphenol A dose level (mg/kg bw/day) NOAEL	LOAEL	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$	Reference
Rat								
Long–Evans males (gavage PND 21–35) Experiment 1	↓ Serum 17β-estradiol		0.0024 (low dose)[a,b]					Akingbemi et al. (2004)
	↓ Serum LH and testosterone		0.0024 (low dose)[a,b]					
Sprague–Dawley (dietary for 17 weeks)	↓ pup weight at weaning (PND 21)	70	200					General Electric, (1976)
Sprague–Dawley (dietary for 18 weeks)	No adverse effects reported	60 (high dose)						General Electric (1978)
Sprague–Dawley female pups (sc injection PND 0–9)	↓Body weight in lactation period	105	427	286	200	233	156	Kato et al. (2003)
	↑ Age of vaginal opening	26	105	345	267	159	116	
	↓ No. with normal estrous cycles	105	427	81	28			
	↑ No. with cleft clitoris	26	105	299	failed			
	↓ Ovary weight	105	427	85	59	140	93	
	↓ Uterus, wet weight	105	427	66	55	128	96	
	↓ Uterus, blotted weight	105	427	273	128	318	168	
	↓ Uterine fluid weight	26	105	42	34	139	104	
	↑ No. with polycystic ovaries		≤105 (lowest dose examined)	81	24			
	↓ No. corpora lutea	105	427	238	90			
	↓ No. with corpora lutea	105	427	65	38	137	83	
	↓Corpora lutea area		≤105 (lowest dose examined)	42	37	84	66	
Sprague–Dawley female pups (sc injection PND 0–9)	No adverse effects reported	≥97 (high dose)						Kato et al. (2006)
Sprague–Dawley (feed GD15–PND 10)	No adverse effects reported	3000 ppm [c]						Masutomi et al. (2004)
Mouse								
CF-1 (oral by pipette, GD 11–17)	Prostate weight and sperm production	≥0.020 (high dose)						Ashby et al. (1999)
ICR/Jcl (s.c. GD 11–17)	↓ Female body weight at weaning		≤0.002 (low dose)[a]	0.065	0.017	0.088	0.021	Honma et al. (2002)
	↓ Male body weight at birth	0.002	0.020	0.054	0.020	0.031	0.015	
	↑ Anogenital distance of females at weaning		≤0.002 (low dose)[a,b]					
	↑Anogenital distance of males on PND 60		≤0.002 (low dose)	0.035	0.020	0.035	0.020	

	↓ Age at vaginal opening	0.002	0.020				
	↓ Body weight at vaginal opening		≤0.002 (low dose)				
	↓ Age at 1st estrus	0.002	0.020				
	↑ Estrous cycle length		≤0.002 (low dose)[a]	0.021	0.007	0.12	0.021
	↑ Cornified cells		≤0.002 (low dose)[b]	0.17	0.020	0.44	0.021
	↓ Lymphocytes in vaginal smear		≤0.002 (low dose)[b]	0.26	0.020	0.26	0.020
ICR (oral GD 6.5–13.5 or 6.5–17.5)	↑ mRNA expression for arylhydrocarbon receptor in brain, testis, and ovary		≤0.00002 (low dose)[b]				Nishizawa et al. (2005b)
	↑ mRNA expression for retinoic acid α receptor in brain and ovary		≤0.00002 (low dose)[b]				
	↑ mRNA expression for retinoic acid α receptor in testis	0.20	20				
	↑ mRNA expression for retinoid X α receptors in brain		≤0.00002 (low dose)[b]				
	↑ mRNA expression for retinoid X α receptor in testis and ovary	0.002	0.020[b]				
ICR (oral GD 6.5–13.5 or 6.5–17.5)	↑ mRNA expression for arylhydrocarbon receptor, arylhydrocarbon receptor repressor, and arylhydrocarbon receptor nuclear translocator in brain, testis, and ovary		≤0.00002 (low dose)[b]				Nishizawa et al. (2005a)
ICR/Jcl (s.c. GD 10–18; female offspring ovariectomized)	↑ No. of vaginal epithelial layers		≤10 (low dose)				Suzuki et al. (2002)
	↓ No. with corpora lutea		≤10 (low dose)[b]				
ICR/Jcl (s.c. for 5 days beginning at birth; mice later ovariectomized except those used to monitor estrous cycles)	↑ Mitotic rate in uterine stromal cells and vaginal epithelial cells	10	100				Suzuki et al. (2002)
	↑ Vaginal epithelial layers	10	100				
	↑ No. with polyovular follicles and no. polyovular follicles/mouse	10	100				
	Estrous cyclicity	100 (high dose)					
CF-1 (oral by pipette, GD 11–17)	↓ Body weight		≤0.002 (low dose)[b]				vom Saal et al. (1998)

[a]There was little-to-no evidence of a dose-response relationship.
[b]No effects were observed at one or more higher dose levels.
[c]Feed consumption and dam weight not reported-dose not calculable.

600 mg/kg bw/day), but the effect was not observed in a second generation exposed according to the same protocol (Tyl et al., 2006). An increase in hepatic histopathologic findings (cytoplasmic variation) at weaning was also observed in offspring of mouse dams exposed during gestation and lactation (LOAEL 50–600 mg/kg bw/day) (Tyl et al., 2006). A single dose level study with gestational exposure in mice reported increased lactational body weight gain and decreased postnatal pup survival at 0.0024 mg/kg bw/day (Howdeshell et al., 1999).

Reproductive system development.

Rat studies: Delays in vaginal opening were observed in offspring of rat dams receiving high oral doses of bisphenol A on GD 6–15 or during the entire gestational and lactational period (Tyl et al., 2000a, 2002b; Tinwell et al., 2002). No delays in vaginal opening were observed with doses of bisphenol A $\leq$1.2 mg/kg bw/day administered to dams during gestation or lactation (Tyl et al., 2000a, 2002b; Ema et al., 2001).

Estrous cycle alterations were not reported in rat oral exposure studies covering a wide range of doses (<1–475 mg/kg bw/day) administered during all or part of the gestational or lactational periods (Kwon et al., 2000; Tyl et al., 2000a, 2002b; Ema et al., 2001).

Studies suggest that preputial separation is delayed following oral administration of high bisphenol A doses (LOAELs = 47.5–475) to male rat offspring in the post weaning period (Tyl et al., 2000a, 2002b; Tan et al., 2003). No effects on preputial separation were observed when treatment of rat dams with high-doses (50–384 mg/kg bw/day) ended during the gestation or lactation period (Tinwell et al., 2002) Oral doses of bisphenol A $\leq$1 mg/kg bw/day also had no effect on preputial separation (Ema et al., 2001; Tyl et al., 2000a, 2002b).

Effects on rat sperm parameters were inconsistent. Decreased sperm count and daily sperm production were reported in offspring of dams exposed during gestation (LOAEL 50 mg/kg bw/day for sperm count/g testis, LOAEL 50 mg/kg bw/day for daily sperm count/g testis) (Tinwell et al., 2002). A single dose level study reported decreased numbers of rats undergoing spermatogenesis following post-weaning exposure of males to 100 mg/kg bw/day (Tan et al., 2003). In contrast, no consistent effects on sperm parameters were observed in rats following exposures with up to 475 mg/kg bw/day during the prenatal, lactational, and post-weaning periods (Tyl et al., 2000a, 2002b). Other rat studies with gestational and lactational doses ranging from <1–4 mg/kg bw/day also reported no effects on sperm parameters (Cagen et al., 1999b; Ema et al., 2001). Testicular histopathology (multinucleated giant cells in seminiferous tubules and absent spermatogenesis) was only reported in a single dose level study at a bisphenol A dose of 100 mg/kg bw/day administered in the post-weaning period (Tan et al., 2003).

Although some sporadic effects were reported for anogenital distance in male and female rats, study authors concluded that the endpoint was not affected by prenatal, lactational, and/or post-weaning exposure to bisphenol A (Ema et al., 2001; Tinwell et al., 2002; Tyl et al., 2000b, 2002b).

No effects on rat prostate weight were observed with bisphenol A doses of <1–475 mg/kg bw/day administered during the gestational, lactational, and/or post-weaning periods (Cagen et al., 1999b; Kwon et al., 2000; Tyl et al., 2000b, 2002b; Tinwell et al., 2002). The study of Timms et al. (2005) in mice raise a level of concern.

Mouse studies: Exposure of mice to bisphenol A during pre- and post-natal development delayed preputial separation (LOAEL 600 mg/kg bw/day) (Tyl et al., 2006). Effects reported for anogenital distance were inconsistent. A single dose study reported an increase in anogenital distance in male mice at 0.050 mg/kg bw/day (Gupta, 2000). A second study with a wide dose range (0.003–600 mg/kg bw/day) reported no consistent or dose-related effects on anogenital distance (Tyl et al., 2006).

One group of investigators reported increased prostate weight at 0.002 and 0.020 mg/kg bw/day in offspring of mouse dams exposed during pregnancy (Nagel et al., 1997). These prostate effects were consistent with findings in single dose level studies with gestational exposure of mice, however, it is noted that the studies had differing periods of exposure and ages of evaluation. One of these studies demonstrated increased prostate weight at 0.050 mg/kg bw/day (Gupta, 2000). Another study demonstrated increased numbers of prostate ducts and proliferating cell nuclear antigen staining in dorsolateral prostate and increased prostate duct volume in dorsolateral and ventral prostate at 0.010 mg/kg bw/day (Timms et al., 2005). However, no effects on prostate or sperm production were observed in more robust studies with multiple dose levels and larger group sizes. A third mouse study with exposures occurring during gestation, lactation, and post-lactational periods also reported no effects on prostate weight, daily sperm production, or efficiency of daily sperm production at doses of 0.003–600 mg/kg bw/day (Tyl et al., 2006). A fourth mouse study demonstrated no effect on sperm density following low-dose exposure ($\leq$0.200 mg/kg bw/day) during gestation or the post-weaning period (Nagao et al., 2002).

Seminiferous tubule hypoplasia in association with undescended testes in mouse weanlings was reported following exposure during pre- and post-natal development (LOAEL 50–600 mg/kg bw/day; BMD_{10} 283–591 mg/kg bw/day) but the effect was not observed in mice examined in adulthood (Tyl et al., 2006). The findings were similar to those in studies reporting no testicular histopathology or lesions in reproductive organs following pre- and post-natal exposure to bisphenol A at $\leq$0.2 mg/kg bw/day (Nagao et al., 2002).

Following exposure of mice during pre- and postnatal development; no effect on age of vaginal opening, estrous cyclicity, or numbers of ovarian primordial follicles were observed at doses ranging from 0.003–600 mg/kg bw/day (Tyl et al., 2006). No effect on age of vaginal opening was reported but there was a shortened period between vaginal opening and first estrus following gestational exposure to 0.0024 mg/kg bw/day in a single dose level study (Howdeshell et al., 1999).

Body Weight: All rat and mouse multigenerational studies have measured body weight as an endpoint. No consistent differences have been detected in the weights of offspring of animals exposed to low to moderate doses of BPA (Table 86).

Hormone Levels: Several studies have measured testosterone and LH levels in rats, there have also been investigations of thyroid hormone (T4) levels. No

consistent effects on the levels of these hormones have been seen (Ema et al., 2001).

Fertility and ability to raise pups to weaning following developmental exposure: Multigenerational studies in both rats and mice have shown that BPA over a wide dose range does not compromise the ability of animals exposed during development to successfully produce offspring, raise them to weaning and for those offspring to successfully give rise to a subsequent generation of animals (Ema et al., 2001; Tyl et al., 2002b, 2006).

Neural and Behavioral Endpoints Following Oral Administration: Several studies addressing effects on neural and behavioral endpoints have been conducted following gestational and lactational exposure [rats: (Funabashi et al., 2004a; Negishi et al., 2004a; Della Seta et al., 2005)]; mice: [(Palanza et al., 2002; Nishizawa et al., 2003, 2005b; Laviola et al., 2005; Ryan and Vandenbergh, 2006)], pubertal exposure [rat: (Akingbemi et al., 2004; Della Seta et al., 2006; Ceccarelli et al., 2007)], and exposure during adulthood [gerbils: (Razzoli et al., 2005)].

Gestational and lactational exposures in rats have reported subtle effects on sexually-dimorphic brain nuclei (Funabashi et al., 2004a), hormonal receptors in brain (Nishizawa et al., 2003, 2005b), and certain sexually-dimorphic or reproductively relevant behaviors (Negishi et al., 2004a; Ryan and Vandenbergh, 2006). Most of this work has utilized single doses across the range of 2–40 μg/kg, and none has been confirmed or linked to other functional or clearly adverse effects. No effects on the volume of the SDN-POA of the hypothalamus were observed in offspring of rats orally exposed to bisphenol A doses ranging from 3.2–320 mg/kg bw/day during the gestation and lactation period (Kwon et al., 2000). Single dose level rat studies demonstrated reduced sexually dimorphic difference in corticotropin-releasing hormone neurons in anterior stria terminalis at 2.5 mg/kg bw/day (Funabashi et al., 2004a). No changes in sexual behavior were reported for female rats exposed to 0.3–320 mg/kg bw/day or males exposed to $\leq$0.3 mg/kg bw/day during the gestation and/or lactation period (Kwon et al., 2000).

Maternal behavior of dams has also been suggested to be altered in two studies of dams exposed during gestation and lactation (Palanza et al., 2002; Della Seta et al., 2005).

One study involving exposures during puberty (Della Seta et al., 2005) suggested alterations in exploratory and sexual behavior of males following 40 μg/kg on PND 23–30. Certain changes in hypothalamic estrogen receptors (Ceccarelli et al., 2007) following 40 μg/kg exposures on PND 23–30 have been reported. Akingbemi et al. (2004) reported effects on gonadal hormonal and receptor endpoints in the pituitary following 2.4 μg/kg/day on PND 21–35.

Other endpoints: Following oral exposure of mice to bisphenol A during gestation, changes were observed for mRNA expression of arylhydrocarbon receptors, receptor repressor, or nuclear translocator and retinoic acid and retinoid X receptors in brain, testes, and/or ovary at 0.00002–20 mg/kg bw/day (Nishizawa et al., 2003, 2005a,b). The strongest effects were found at the lowest doses following exposures during organogenesis (GD 6.5–13.5 or 6.5–17.5) (Nishizawa et al., 2005a,b). The study authors suggested those changes as possible mechanisms for bisphenol A-induced toxicity.

A summary of LH and testosterone effects observed in humans and in bisphenol A-exposed experimental animals is included in Section 4.4.

Summary and Conclusion of Developmental Hazards: There are sufficient data to conclude that bisphenol A does not cause malformations or birth defects in fetuses exposed during gestation at levels up to 640 mg/kg/day (rats) and 1000 mg/kg/day (mice) (Morrissey et al., 1987). This is consistent with the lack of malformations seen in offspring in multigenerational studies (Tyl et al., 2002b, 2006).

There are sufficient data to conclude that bisphenol A does not alter male or female fertility in rats or mice after gestational exposure up to doses of 450 mg/kg/day (Cagen et al., 1999b; Tyl et al., 2000a, 2002b; Ema et al., 2001).

There are sufficient data to conclude that bisphenol A does not change the age of puberty in male or female rats [NOAELs of 0.2 mg/kg/day (Ema et al., 2001) and 1823 mg/kg/day (Tyl et al., 2002b)]. While limited data available suggest an effect on the onset of female puberty in mice [LOAEL 0.2 mg/kg/day (Ryan and Vandenbergh, 2006), 0.002 mg/kg/day, (Howdeshell et al., 1999)], the data are insufficient to conclude that bisphenol A accelerates puberty in female mice. The limited data available suggest, but are insufficient to conclude, that bisphenol A slightly delays the age of puberty in male mice at a LOAEL of ca. 550–800 mg/kg/day (Tyl et al., 2006).

There are sufficient data to conclude that bisphenol A exposure during development does not permanently affect prostate weight in adult rats or mice [NOAELs of: 1823 mg/kg/day (Tyl et al., 2002b), 600 mg/kg/day (Tyl et al., 2006), 4 mg/kg/day (Cagen et al., 1999b), 0.2 mg/kg/day (Ema et al., 2001), 50 mg/kg/day (Tinwell et al., 2002), and 320 mg/kg/day (Kwon et al., 2000). There are sufficient data to conclude that bisphenol A does not cause prostate cancer in rats or mice after adult exposure [calculated dose ranges of 25–400 mg/kg/day for rats, 600–3000 mg/kg/day, mice (NTP, 1982)]. There are slight suggestions, but insufficient data to conclude, that bisphenol A might predispose toward prostate cancer in rats in later life following developmental exposure [at 10 μg/kg (Ho et al., 2006a)]. There are slight suggestions, but insufficient evidence to conclude, that fetal exposure to bisphenol A can contribute to urinary tract deformations in mice [10 μg/kg (Timms et al., 2005)].

There are sufficient data to suggest that developmental exposure to bisphenol A causes neural and behavioral alterations related to sexual dimorphism in rats and mice (ca. 2.5 mg/kg/day, gestation and lactation in rats, (Funabashi et al., 2004a); LOEL 0.00002 mg/kg/day, fetal mice, (Nishizawa et al., 2005a); 0.0002 mg/kg/day, fetal mice, (Nishizawa et al., 2003), 0.04 mg/kg/day, weaning to puberty, rats, (Ceccarelli et al., 2007); 0.1 mg/kg/day, GD 3–PND 20, rats, (Negishi et al., 2004a); 0.2 mg/kg/day, GD 3–PND 20, mice, (Ryan and Vandenbergh, 2006); 0.01 mg/kg/day, GD 11–18, mice, (Laviola et al., 2005), although other studies report no change in a related measure, the size of the sexually dimorphic nucleus of the pre-optic area (SDN-POA) [300 μg/kg/day, rats

(Nagao et al., 1999); NOEL of 320 mg/kg/day, rats, (Kwon et al., 2000)].

4.0 REPRODUCTIVE TOXICITY DATA

4.1 Human

4.1.1 Female. **Takeuchi and Tsutsumi (2002)**, supported by the Japanese Ministry of Education, Science, Sports, and Culture, the Ministry of Health and Welfare, and the Science and Technology Agency, measured bisphenol A in the blood serum of 14 healthy women, 11 healthy men, and 16 women with polycystic ovary syndrome **[diagnostic criteria not discussed]**. The healthy women were evaluated in the mid-follicular phase of the menstrual cycle. Bisphenol A was measured using a competitive ELISA. Serum was also evaluated for total and free testosterone, 17β-estradiol, androstenedione, dehydroepiandrosterone sulfate, LH, FSH, and prolactin. Statistical analysis was by ANOVA. Correlation coefficients were obtained from a linear regression analysis. Mean ± SEM bisphenol A serum concentrations (ng/mL) were 0.64 ± 0.10 in normal women, 1.49 ± 0.11 in normal men, and 1.04 ± 0.10 in women with polycystic ovary syndrome. Bisphenol A serum concentrations were correlated significantly with total testosterone ($r = 0.595$) and free testosterone ($r = 0.609$) in all subjects and in all female subjects ($r = 0.559$ for total testosterone and 0.598 for free testosterone). Bisphenol A serum concentrations were not significantly correlated with any other hormone measures. The authors concluded that either bisphenol A stimulates testosterone production or metabolism of bisphenol A is inhibited by testosterone. They further suggested that displacement of sex steroids from sex-hormone binding globulin by bisphenol A might disrupt the estrogen-androgen balance.

Strengths/Weaknesses: Quality assurance for the hormone radioimmunoassays appeared adequate; however, there was no standardization for time of day for the serum samples, which may result in variable testosterone levels. ELISA has not been standardized for human sera, and may overestimate bisphenol A due to nonspecific binding (see Section 1.1.5). Very little descriptive information was given on any of the groups beyond mean age and body-mass index. No information was given on recruitment methods and participation rates/exclusions. The lack of diagnostic criteria for polycystic ovary syndrome is a weakness. No potential confounders or effect modifiers were identified except mean age and body-mass index. Mean values appear to have been similar between groups. The positive correlations between bisphenol A level and total/free testosterone levels in all women and in entire study group were noted, but these analyses were not adjusted for potential confounders or effect modifiers. No information was given on whether the data were normally or lognormally distributed. The study was limited by small numbers in each group and the results should be regarded as descriptive epidemiology.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate but has limited utility given its small size, and limited design. The study provides some insight for potential mechanisms affecting the levels of bisphenol A in the body.

Takeuchi et al. (2004a), supported by the Japanese Ministry of Education, Science, Sports, and Culture, the Ministry of Health, Labor, and Welfare, the National Institute for Environmental Studies, and the Science and Technology Agency, examined relationships between serum sex hormone and bisphenol A concentrations in women with ovarian dysfunction and obesity. Fasting blood samples were collected during the midfollicular phase from 19 non-obese and 7 obese healthy women with normal menstrual cycles. Blood samples were also obtained from 7 women with hyperprolactinemia, 21 patients with hypothalamic amenorrhea, and 13 non-obese and 6 obese patients with polycystic ovary syndrome. **[It not known whether any of these subjects were the same as those reported earlier by this group (Takeuchi and Tsutsumi, 2002).]** Mean ages for the subgroups ranged from 25–29 years old. Blood serum was analyzed for bisphenol A levels using an ELISA technique, and total and free testosterone, 17β-estradiol, androstenedione, dehydroepiandrosterone sulfate, LH, FSH, prolactin, and insulin levels were measured using by RIA. Statistical analyses included ANOVA and linear regression analysis.

Compared to non-obese healthy women, concentrations of bisphenol A in serum were significantly higher in non-obese women with polycystic ovary syndrome **[48% higher]**, obese women with polycystic ovary syndrome **[65% higher]**, and obese healthy women **[46% higher]**. Statistically significant positive correlations were found between bisphenol A level in serum and body mass index ($r = 0.500$) and serum levels of total testosterone ($r = 0.391$), free testosterone ($r = 0.504$), androstenedione ($r = 0.684$), and dehydroepiandrosterone sulfate ($r = 0.514$). The study authors concluded that there is a strong relationship between serum levels of bisphenol A and androgens, possibly due to androgen effects on metabolism of bisphenol A.

Strengths/Weaknesses: Quality assurance for the hormone radioimmunoassays appears adequate. In contrast to the 2002 article by these authors (Takeuchi and Tsutsumi, 2002), blood draws were time-standardized to 9:00–10:00 AM after overnight fasting. As noted in Section 1.1.5, ELISA may overestimate bisphenol A. It was not clear whether any of the women in this study were also included in their 2002 publication. No potential confounders or effect-modifiers were identified except mean age and body-mass index, and neither of these was controlled in the analyses. Positive correlations were observed for bisphenol A level with body-mass index, total testosterone, free testosterone, androstenedione, and dehydroepiandrosterone sulfate for all study groups. These correlations are also found (with the exception of total testosterone) in the control (''normal women'') group as well. Normality of the distributions of the hormones were not reported, and not transformed before analysis. The study was limited by small numbers and results should be regarded as descriptive epidemiology.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate but has limited utility in assessing possible relationships of bisphenol A with androgens (testosterone, free testosterone, androstenedione, dehydroepiandrosterone sulfate) and conditions that may promote hyperandrogenism (obesity, polycystic ovarian syndrome).

Hiroi et al. (2004), supported by the Japanese Ministry of Health, Labor, and Welfare, the National Institute for Environmental studies, and the Japan Science and Technology Agency, compared blood bisphenol A levels in women with and without endometrial hyperplasia. Volunteers were recruited from an outpatient clinic in Japan. Women included in the study consisted of 11 controls with normal endometrium, 19 with endometrial hyperplasia, and 7 with endometrial carcinoma. The hyperplasia group was further divided according to severity: 10 with simple hyperplasia and 9 with complex hyperplasia. Mean ages were 48.4–48.9 years in groups without cancer, and the mean age was 63.1 years in the group with endometrial cancer. Blood samples were collected at the time of endometrial examination. Serum bisphenol A levels were measured by ELISA. Data were analyzed by Student *t*-test, with the exception of gravidity and parity, which were analyzed by χ^2 test. There were no significant differences in age, gravidity, parity, or body height, weight, or mass index between the groups without endometrial cancer. Women with endometrial cancer were significantly older and had significantly lower values for gravidity, parity, height, and weight. Mean $\pm$ SD serum bisphenol A levels were reported at 2.5 ± 1.5 ng/mL in controls, 2.2 ± 1.6 ng/mL in women with hyperplasia, and 1.4 ± 0.5 ng/mL in women with endometrial cancer. When the group with hyperplasia was divided according to severity, serum bisphenol A blood levels were reported at 2.9 ± 2.0 ng/mL in the group with simple hyperplasia and 1.4 ± 0.4 ng/mL in the group with complex hyperplasia. Serum bisphenol A levels were significantly lower in women with complex endometrial hyperplasia or endometrial cancer than in controls. The study authors concluded that their preliminary findings demonstrated a possible link between bisphenol A exposure and endometrial hyperplasia or cancer. It was noted that modes of action for bisphenol A may be more complex than expected and that these contradictory results might provide a clue about mechanisms of production of estrogen-dependent diseases.

Strengths/Weaknesses: Because this was a small, cross-sectional study, it is not possible to determine whether this association preceded disease, or could have been associated with the disease process. As noted in Section 1.1.5, ELISA may over estimate bisphenol A.

Utility (Adequacy) for CERHR Evaluation Process: The cross-sectional study design is adequate but of limited utility for this evaluation, but raises research questions regarding mechanisms of production of estrogen-dependent diseases.

Sugiura-Ogasawara et al. (2005), supported by the Japanese Ministry of Health, Labor, and Welfare, conducted a study to determine if there is an association between recurrent miscarriage and bisphenol A levels in blood. The cases in this study were 45 patients with a history of 3 or more (3–11) consecutive first trimester miscarriages. Mean $\pm$ SD age of the cases was 31.6 ± 4.4. None of the cases had a history of live birth. All were seen at a Japanese hospital between August, 2001–December, 2002. Half of the cases were housewives and half were employed in various occupations. A hysterosalpingography analyses was conducted in cases, and chromosome analyses were conducted for both cases and their partners. Women were excluded from the study if uterine anomalies were observed or chromosomal abnormalities were detected in either partner. Serum bisphenol A levels were determined by ELISA. Immunological endpoints examined included antinuclear antibodies, antiphospholipid antibodies, and natural killer cell activity. Blood testing for hypothyroidism, diabetes mellitus, and hyperprolactinemia was conducted. Blood samples were obtained 5–9 days following ovulation in at least 2 cycles. Blood samples to determine progesterone and prolactin levels were taken at 3 months following the last miscarriage and before the next conception. For subsequent pregnancies, ultrasounds were conducted, and spontaneously aborted embryos/fetuses were karyotyped. Serum levels of bisphenol A in cases were compared to those of 32 healthy non-pregnant hospital employees with no history of live birth, infertility, or miscarriage. Mean $\pm$ SD age of controls was 32.0 ± 4.8. None were taking oral contraceptives. Like the cases, the controls lived near Nagoya City. Statistical analyses included Welch test, Mann–Whitney test, and Pearson correlation coefficient.

Bisphenol A levels (mean $\pm$ SD) were reported to be significantly higher in women with recurrent miscarriages (2.59 ± 5.23 ng/mL) compared to healthy controls (0.77 ± 0.38 ng/mL). In the 45 cases, incidences of abnormal conditions were 15.6% for hypothyroidism, 13.3% for antiphospholipid antibodies, 22.2% for antinuclear antibodies, 11.1% for hyperprolactinemia, and 20.5% for luteal phase defect. Serum levels of bisphenol A were significantly higher in patients who tested positive versus negative for antinuclear antibodies (mean $\pm$ SD = 7.382 ± 9.761 vs. 1.222 ± 1.54 ng/mL). Thirty-five of the patients became pregnant and 48.6% had another miscarriage. Serum bisphenol A levels in patients who miscarried were 4.39 ± 8.08 ng/mL, and serum bisphenol A in patients with successful pregnancies were 1.22 ± 1.07 ng/mL (not statistically significant). The study authors concluded that exposure to bisphenol A is associated with recurrent miscarriage.

In a letter to the editor, Berkowitz (2006) stated that this study did not support an association between bisphenol A blood levels and recurrent miscarriage. Several limitations were noted for the study. Timing and numbers of blood samples collected were not defined clearly. It was noted that because bisphenol A has a short half-life, it would be critical to know if blood samples were obtained in a timeframe relevant to the occurrence of miscarriage. Although differences in serum bisphenol A levels in cases compared to controls achieved statistical significance, it was noted that median levels of bisphenol A in serum were nearly identical in patients with recurring miscarriages (0.71 ng/mL) and controls (0.705). The similarities in median values suggested there were no differences between the two groups, and it was suggested that apparent differences in mean serum levels of bisphenol A were due to a few individuals, as was demonstrated in Figure 1 of the Sugiura-Ogasawara et al. (2005) report. Berkowitz (2006) stated that the Welch test was inappropriate for statistical analyses and noted that the two evaluation groups could not be considered comparable because of differences in occupation (housewives compared to medical workers) and unknown fertility of controls. Because the controls were not evaluated for factors such as hypothyroidism and systemic lupus erythematosus (associated with

antinuclear antibodies), the conditions may have been over represented in cases and may have been the cause of the reported differences between the 2 groups. Although mean bisphenol A levels were (non-significantly) lower in women who subsequently became pregnant and had a successful pregnancy compared to those who miscarried, Berkowitz (2006) noted that the median level of bisphenol A was actually higher in women with the successful pregnancies. Last, the ELISA method for measuring bisphenol A levels has not been validated and is subject to inaccuracy due to extensive cross-reactivity.

In a response to the comments by Berkowitz (2006), Sugiura-Ogasawara (2006) stated that although measurement of bisphenol A levels at various time points would have been ideal, obtaining samples every day during pregnancy would have been difficult. Sugiura-Ogasawara (2006) clarified that bisphenol A values were based on a single sample in each individual, but that similar tendencies were observed for a second blood sample. With respect to the use of women with live births as controls, Sugiura-Ogasawara (2006) explained that the same blood samples were used for measurements of other environmental compounds, some of which are known to decrease after delivery. It was noted that none of the cases had systemic lupus erythematosus, and that use of controls with hypothyroidism or antinuclear antibodies was not considered important for the study. Superiority of the HPLC method compared to the ELISA method for measuring serum bisphenol A levels was acknowledged, but the authors stated that the ELISA method was used because of limited funding, reiterated that the study was preliminary and used a small number of volunteers, and that additional studies using a larger sample and more appropriate analytical methods were needed.

Strengths/Weaknesses: The letter from Berkowitz (2006) summarizes many of the weaknesses of this study. No quality assurance information was given for the biomarker/hormone measurements. As the Berkowitz letter points out, the ELISA method is not standardized for human sera (and may overestimate bisphenol A due to nonspecific binding), the distribution of exposure was not normal, and median values of the two groups were similar, with two women skewing the mean. Little information was provided on the characteristics of the two study groups or response rates. Age and body-mass index were controlled in the analyses, but other potential confounders and effect modifiers were not. The time between exposure and observation was not appropriate. Spontaneous abortions have been associated with many factors which have not been addressed here. The authors' conclusions require the assumption that bisphenol A measurement levels represent those present during the important time frame for the spontaneous abortion. The authors do not report the time frame for collection of the blood samples. Nonnormal data were not appropriately transformed for analysis. Welch's test was used "…to compare bisphenol A levels…because the distribution of the two groups might have differed." Welch's test is a *t*-test for groups with unequal variance, not different distributions (both should be normal, which was probably not the case).

Utility (Adequacy) for CERHR Evaluation Process: Because of limitations in the design and analysis of this work, this study is inadequate has no utility in this evaluation.

Yang et al. (2006), supported by the Korean FDA, measured urine bisphenol A in 172 Korean men and women and evaluated the relationship of these values with UDP-glucuronosyl- and sulfotransferase polymorphisms, with sister-chromatid exchange testing, and with self-reported symptoms of possible endocrine origin. First-morning urine samples were collected at the time of a routine physical examination, as was a blood sample, and a questionnaire was completed. Urine bisphenol A was measured using reverse phase HPLC. DNA was isolated from blood samples and polymorphisms were determined at *SULT1A1* and *UGT1A6*. Sister chromatid exchange in response to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) was evaluated in blood cells **[not otherwise specified]**. The relationship between urine bisphenol A and continuous variables was assessed with simple or multiple regression analysis and the relationship with categorical variables assessed with the Wilcoxon test.

None of the subjects reported occupational exposure to bisphenol A. The median urine bisphenol A concentration was 7.86 μg/L. Urine bisphenol A was not different in men and women. Urine bisphenol A was associated with body-mass index ($P = 0.06$) and self-reported frequency of alcohol consumption ($P = 0.08$). *SULT1A1* and *UGT1A6* polymorphisms were not associated significantly with urine bisphenol A concentrations. No significant associations were observed between urine bisphenol A and MNNG-induced sister-chromatid exchange, although they were associated when lower levels of MNNG were used. There were no significant associations between urine bisphenol A and self-reported symptoms of possible endocrine origin, including thirst/frequent urination, dizziness, neck mass, heat intolerance, sweating, hot flashes, swelling of lymph nodes, dysmenorrhea, menstrual irregularity, or menorrhagia. The authors concluded that even though they had been unable to associate an endocrine disorder with urine bisphenol A, continuous biologic monitoring of bisphenol A would be prudent.

Strengths/Weaknesses: Bisphenol A was measured in urine using HPLC. No information was given regarding any selection criteria or response rates and some outcome measures were self-reported.

Utility (Adequacy) for CERHR Evaluation Process: While small, this study is useful for providing descriptive exposure information on BPA urinary levels (see Section I). This study does not have utility for evaluation of reproductive endpoints.

4.1.2 Male. **Luconi et al. (2001)**, supported by the Italian Public Health Project, examined the effects of in vitro exposure of human spermatozoa to bisphenol A. Semen was collected from normozoospermic men, and spermatozoa were separated. Intracellular calcium was measured using a spectrofluorometric method in cells treated with 1 μM bisphenol A, 1 μM 17β-estradiol, 10 μM progesterone, **[17 β-estradiol is noted as 10μM in Figure 6, text states 1μM]** or the same concentrations of bisphenol A in combination with 17β-estradiol or progesterone. Effects on acrosome reaction were examined using a fluorescent staining method in cells exposed to 1 μM **[0.23 mg/mL]** bisphenol A for 2 hr, with and without exposure to 10 μM progesterone. **[In the study figures summarizing results, sample numbers in studies involving bisphenol A were listed at 5–11. It**

is not known if the sample numbers represented total numbers of sperm donors. Very few protocol details were provided in the Methods section and many of the limited details presented above were obtained from the Results section.] Data were analyzed by Student *t*-test and 1-way ANOVA. Treatment of spermatozoa with bisphenol A resulted in a modest influx of calcium, but bisphenol A had no effect on calcium responses induced by 17β-estradiol or progesterone. Bisphenol A exposure did not affect basal acrosome reaction or acrosome reaction induced by progesterone. Results were in contrast to those observed with 17β-estradiol, which inhibited the acrosome reaction induced by progesterone. The study authors concluded, BPA did not exert any direct effect on calcium fluxes and acrosomal reaction in human spermatozoa either in basal conditions or in response to progesterone challenge.

Strengths/Weaknesses: Strengths of this study include examining human spermatozoa and use of a concurrent control (E2) to demonstrate the responsiveness of the system. Weaknesses include limited information on the spermatozoa samples, the single concentration of BPA used, and lack of clarity of concentrations of E2 versus bisphenol A administered.

Utility (Adequacy) for CERHR Evaluation Process: This study did not demonstrate that BPA-altered progesterone-mediated acrosomal reaction and is not useful in the evaluation process.

Hanaoka et al. (2002), supported by the Japanese Ministry of Health and Welfare and Ministry of Education, Science, Sports, and Culture, examined possible relationships between bisphenol A exposure and hormone levels in male workers. Exposed workers included 42 men in 3 Japanese plants who sprayed an epoxy hardening agent consisting of a mixture of bisphenol A diglycidyl ether (10–30%), toluene (0–30%), xylene (0–20%), 2-ethoxyethanol (0–20%), 2-butoxyethanol (0–20%), and methyl isobutyl ketone (0–30%). The workers were said to wear "protection devices" during spraying. Controls consisted of 42 male assembly workers from the same plants who did not use bisphenol A diglycidyl ether, were within 3 years of age to exposed workers (37 years vs. 38 years), and smoked the same number of cigarettes/day as exposed workers (21/day). Percentages of smokers were 86% in both groups, but percentages of alcohol drinkers were significantly lower in the exposed workers (43%) than in controls (57%) ($P = 0.03$). Urine and blood samples were obtained during periodic health examinations performed in June and July, 1999. Urinary bisphenol A was measured by HPLC, and urinary organic solvent metabolites were measured by GC or HPLC. Plasma LH, FSH, and free testosterone levels were measured by immunosolvent assay in a commercial laboratory. Data were log transformed and compared by paired *t*-test, Pearson correlation coefficient, and χ^2 test. Adjustments were made by linear regression for age and drinking habits, which were considered possible confounders.

Urinary bisphenol A concentrations were significantly higher in exposed workers (median: 1.06 μmol/mol creatinine **[0.043 μg/kg bw]**; range: <0.05 pmol to 11.2 μmol/mol creatinine) than in controls (median: 0.52 μmol/mol creatinine **[0.021 μg/kg bw]**; range: <0.05 pmol to 11.0 μmol/mol creatinine). Average difference was reported as 2.5 (95% CI = 1.4–4.7; $P = 0.002$). Bisphenol A was not detected in 3 exposed workers and 1 control. Urinary solvent metabolites were detected more frequently in exposed workers than controls. No differences in plasma testosterone or LH concentrations were observed between exposed workers and controls. Plasma FSH concentrations were significantly lower in exposed workers (median = 5.3 mIU/mL; range = 4.0–8.3 mIU/mL) than in controls (median = 7.6 mIU/mL; range = 5.4–11.0 mIU/mL; average difference = 1.3; 95% CI = −1.5 to −1.0). A "mild correlation" was reported between urinary bisphenol A and FSH ($r = -0.20$, $P = 0.071$) but was not observed for urinary solvent levels. A statistically significant relationship was observed between FSH and bisphenol A following adjustment for alcohol intake ($r = -0.23$; $P = 0.045$). The study authors concluded that bisphenol A may be generated endogenously following exposure to bisphenol A diglycidyl ether, and bisphenol A may disrupt gonadotropic hormone secretion in men.

Strengths/Weaknesses: Quality assurance for the hormone radioimmunoassays appeared adequate. Blood draws and urine samples were time standardized between 10:00 AM and 12:00 PM. Reference values were given and population values were considered in the discussion. Use of HPLC for bisphenol A and standard methods for the other urinary metabolites with creatinine-adjustment are strengths. The epoxy sprayer workers were matched to coworkers from other parts of the process. All selected workers participated in the study. Analyses were adjusted for age and alcohol use, and workers were matched on age (±3 years) and cigarette use. A plausible ($P = 0.07$) correlation between bisphenol A and decreasing FSH was reported. The authors took care to note that all FSH levels were within the clinical normal range. Correlations between other workplace exposures and hormones were not observed. Blood and urine samples were collected concurrently, but not on the first day of the week. Statistical methods were appropriate to the study size and distribution of the data. Nonnormal distributions were transformed or treated as nonnormal. Biomarker data were handled appropriately in analysis.

Utility (Adequacy) for CERHR Evaluation Process: This survey was methodologically sound and mechanistically thoughtful. This study is adequate and of high utility for the evaluation.

4.2 Experimental Animal

Studies in this section examine reproductive endpoints after administration of bisphenol A to sexually mature animals. Reproductive endpoints after administration of bisphenol A during pregnancy, the neonatal period, or puberty are discussed in Section 3.2.

4.2.1 Female

4.2.1.1 Rat: Goloubkova et al. (2000), supported by the Brazilian National Council of Scientific and Technological Development and the National University of Rio Grande Do Sul, examined the effects of bisphenol A exposure on the uterus and pituitary of ovariectomized rats. Wistar rats (60–67 days old) were fed a standard certified rodent diet. **[No information was provided on housing or bedding materials.]** Rats were subjected to bilateral ovariectomy or sham surgery. At 14 days post-surgery, rats were randomly assigned to groups of at

least 6 animals. Rats were s.c. injected with bisphenol A in DMSO vehicle (>99% purity) at doses of 11, 78, 128, or 250 mg/kg bw/day for 7 days. An ovariectomized vehicle control group was exposed to the 50% DMSO vehicle. A sham-operated control group was not exposed to the vehicle. Rats were killed following the dosing period, and body and uterine weight were measured. Trunk blood was collected for measurement of serum prolactin level by RIA. The anterior pituitary was weighed and preserved in 10% formalin. An immunohistochemical technique was used to identify pituitary cells expressing prolactin. A total of 3 or 4 rats per group were evaluated for prolactin-positive cells in the pituitary and 6–8 rats were evaluated for the other endpoints. Data were analyzed by ANOVA followed by post-hoc Student-Neuman–Keuls test or Kruskal–Wallis ANOVA followed by post-hoc Dunn test.

In the 250 mg/kg bw/day group, final body weight was 7% lower than in the ovariectomized vehicle control group, and body weight gain was lower compared to the ovariectomized vehicle and sham controls. There was no effect of treatment on food intake. A dose-related increase in uterine weight occurred in all groups of rats exposed to bisphenol A compared to the ovariectomized vehicle controls, but uterine weight in the bisphenol A groups was lower than in the sham controls. Ovariectomy resulted in decreased pituitary weight in ovariectomized vehicle controls and in the bisphenol A 11 and 78 mg/kg bw/day dose groups compared to sham controls. Pituitary weight did not differ from sham controls after 128 mg/kg bw/day bisphenol A and was greater than in sham controls after 250 mg/kg bw/day bisphenol A. Basal prolactin levels did not differ between the sham and ovariectomized vehicle controls. Serum prolactin levels were increased in the 128 and 250 mg/kg bw/day bisphenol A groups compared to the ovariectomized vehicle controls. Ovariectomy reduced the numbers of prolactin-positive cells in the pituitary. The number of prolactin positive cells in the pituitary was increased by 64% in the 250 mg/kg bw/day group compared to the ovariectomized controls. The study authors concluded that the reproductive tract and neuroendocrine axis of Wistar rats can respond to bisphenol A.

Strengths/Weaknesses: This study represents a comprehensive neuroendocrine assessment across multiple doses. Weaknesses are the absence of a positive control to demonstrate maximal response in endpoints examined, high-dose levels required to induce response, and the s.c. route of administration.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate but of limited utility for the evaluation process.

Funabashi et al. (2001), supported by Yokoyama City University, examined the effects of bisphenol A exposure on expression of progesterone receptor mRNA in the brain of ovariectomized rats. The effects of butylbenzyl phthalate were also examined but will not be discussed. **[No information was provided on feed, caging, or bedding materials.]** Wistar rats were ovariectomized at 7–8 weeks of age. Ten days following ovariectomy, 6 rats/group were s.c. injected with sesame oil vehicle, 10 mg bisphenol A **[purity not reported]**, or 10 μg 17β-estradiol. Rats were killed 24 hr later and the preoptic area, medial basal hypothalamus, and anterior pituitary were removed. Expression of mRNA for progesterone receptor, preproenkephalin, and neurotensin were assessed by Northern blot. Data were analyzed by ANOVA followed by Fisher protected least significant difference test. Exposure to bisphenol A resulted in increased expression of progesterone receptor mRNA in the preoptic area and anterior pituitary. Bisphenol A did not affect expression of mRNA for neurotensin in the preoptic area or preproenkephalin in medial basal hypothalamus. 17β-Estradiol increased expression of mRNA for progesterone receptor in the preoptic area, medial basal hypothalamus, and anterior pituitary and increased preproenkephalin mRNA expression in medial basal hypothalamus. The study authors concluded that bisphenol A increases expression of progesterone receptor mRNA in the preoptic area of adult ovariectomized rats.

Strengths/Weaknesses: Strengths are the use of a positive control and the biological plausibility of the model. Weaknesses include subcutaneous administration of a single high-dose level.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for inclusion but of limited utility.

Yamasaki et al. (2002a) conducted a 28-day exposure study that provided some information on the reproductive organs of male and female rats. **[Complete details of this study are included in Section 2. Results for females are discussed in this section, and results for males are discussed in Section 4.2.2.1.]** CD rats were fed a commercial diet (MF Oriental Yeast Co.) and housed in stainless steel wire mesh cages. Ten 7-week-old rats/sex/group were gavaged with bisphenol A **[98% purity]** at 0 (olive oil vehicle), 40, 200, or 1000 mg/kg bw/day for 28 days. Due to the death of 1 animal exhibiting clinical signs in the 1000 mg/kg bw/day group, the high-dose was reduced to 600 mg/kg bw/day on Day 8 of the study. In an additional study, rats were exposed to ethinyl estradiol at 0, 10, 50, or 200 μg/kg bw/day for 28 days. There were no treatment-related alterations in blood levels of thyroid hormones, FSH, LH, 17β-estradiol, prolactin, or testosterone. The numbers of females with diestrus lasting 4 or more days was increased in the high-dose group. Relative weights of ovary and uterus were unaffected. No gross or histopathological alterations were reported for reproductive organs. The study authors concluded that change in estrous cyclicity was the only useful endpoint for evaluating the endocrine-mediated effects of bisphenol A. In comparison, females from the mid- and/or high-dose ethinyl estradiol group experienced alterations in estrous cyclicity, decreased ovarian weight, increased uterine weight, and histopathological changes in the ovary, uterus, and vagina.

Strengths/Weaknesses: This study was well-conducted, used an appropriate route of administration, a positive control group, adequate sample sizes, a range of doses, and evaluations of both sexes. Weaknesses include failure to define the criteria for an abnormal estrous cycle, female necropsy at a point unrelated to stage of estrous.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation process.

Spencer et al. (2002), supported by NIH, evaluated the uterine response to bisphenol A before and after

deciduoma formation in pseudopregnant Sprague–Dawley rats. **[Cage and bedding materials and feed were not indicated.]** Adult females underwent mechanical cervical stimulation to induce pseudopregnancy **[pseudopregnancy day not indicated]**. On pseudopregnancy day 4, deciduoma formation was induced under ether anesthesia by antimesometrial uterine epithelial trauma, applied through a laparotomy under ether anesthesia. Rats were treated with s.c. bisphenol A **[97% purity]** 0 or 200 mg/kg bw in alcohol/saline on pseudopregnancy days 1–4 and killed on pseudopregnancy day 5, or treated on pseudopregnancy days 5–8 and killed on pseudopregnancy day 9. Uteri and pseudopregnancy day 9 endometria were harvested. Uteri were weighed and homogenized for measurement of protein and DNA content. Inducible nitric oxide synthase activity, decidual prolactin-related protein mRNA, *ER* mRNA, and cytosolic ER binding sites were measured in uteri and/or endometria. Blood was obtained for determination of serum 17β-estradiol and progesterone. **[*n* = 5 was indicated for some of the data presentations.]** Results are summarized in Table 87. The authors called attention to the difference in bisphenol A effect depending on whether exposure was before or after deciduoma induction. They concluded that there was a decrease in proliferation when bisphenol A was given during deciduoma induction, with a decrease in decidual proteins, in spite of a lack of differential effect on *ER* mRNA or cytosolic ER binding sites. The authors also concluded that bisphenol A activity appeared to be antagonized by progesterone **[although they probably meant that bisphenol A antagonized the action of progesterone]**.

Strengths/Weaknesses: These data are intriguing, but the functional consequences of bisphenol A administration on decidual formation were not assessed and the s.c. route of administration and the use of a single high-dose are a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate but of limited utility to the evaluation process.

Table 87
Bisphenol A Effects on Pseudopregnant Rats[a]

Endpoint	Treatment period, pseudopregnancy day 1–4	Treatment period, pseudopregnancy day 5–8
Uterus		
Wet weight	↑ 1.4-fold	↓ 63%
Protein content	↑ 1.4-fold	↓ 64%
DNA content	↔	↓ 53%
Decidual prolactin-related protein mRNA[b]	↔	↓ 44%
ER mRNA[b]	↓ 29%	↓ 50%
Cystosolic ER-binding sites	↓ 57%	↓ 37%
Nitric oxide synthase activity[b]	↔	↓ 50%
Pseudopregnancy day 9 endometrium		
Decidual prolactin-related protein mRNA[b]	Not applicable	↓ 48%
ER mRNA[b]	Not applicable	↓ 43%
Nitric oxide synthase activity[b]	Not applicable	↓ 40%
Serum		
17β-Estradiol	↔	↔
Progesterone	↔	↓ 49%

[a]Spencer et al. (2002).
[b]Estimated from a study graph by CERHR.
↑, ↓, ↔ Statistically significant increase, decrease, or no change compared to vehicle control.

Funabashi et al. (2003), supported by Yokohama City University, examined the effects of bisphenol A exposure on sexual behavior and progesterone receptor expression in adult rats. Wistar rats were ovariectomized at 7–8 weeks of age. **[No information was provided on feed, caging, or bedding materials.]** In two sets of experiments, an immunohistochemistry technique was used to measure expression of progesterone receptor in the preoptic area and ventromedial hypothalamus following bisphenol A exposure. In the first experiment, 3–5 rats/group were s.c. injected with sesame oil vehicle, 10 mg bisphenol A (~40 mg/kg bw) **[purity not reported]**, or 10 μg 17β-estradiol (~40 μg/kg bw) 2 weeks following ovariectomy. In the second experiment, ovariectomized rats (3–4/group) were s.c. injected with bisphenol A at 0.001, 0.010, 0.1, or 1 mg (~0.004, 0.040, 0.4, or 4 mg/kg bw). Rats were killed the day following dosing, and brains were removed and fixed in 2% paraformaldehyde. Statistical analyses included ANOVA followed by Scheffé post-hoc test and Kruskall–Wallis test. Sexual behavior was examined in a third experiment. Ovariectomized rats were s.c. injected with sesame oil vehicle, 10 mg bisphenol A, or 10 μg 17β-estradiol. The next day, rats were injected with 1 mg progesterone or vehicle to generate 4 treatment groups: sesame oil+progesterone ($n = 5$), bisphenol A+sesame oil ($n = 5$), bisphenol A+progesterone ($n = 8$), or estradiol+progesterone ($n = 6$). Examination of behavior with a sexually receptive male was conducted 5–7 hr following progesterone or vehicle injection. Statistical analyses included ANOVA followed by Scheffé post-hoc test.

In the first experiment, injection of rats with 10 mg bisphenol A increased progesterone-positive cells in both the preoptic area and ventromedial hypothalamus. The dose–response experiment demonstrated that dose-related increases in progesterone-positive cells in both brain regions occurred following exposure to ≥0.1 mg bisphenol A. In sexual behavior testing, treatment with bisphenol A had no effect on lordosis quotient. Rejection quotient was significantly higher in rats exposed to 10 mg bisphenol A and primed with 1 mg progesterone than in the vehicle control rats primed with progesterone. Treatment with 17β-estradiol resulted in increased numbers of progesterone positive cells in the preoptic area and ventral medial hypothalamus and increased lordosis quotient. The study authors concluded that the findings suggest that bisphenol A influences sexual behavior by altering the progesterone receptor system in the hypothalamus.

Strengths/Weaknesses: This study appears to have been relatively well conducted with the incorporation of a positive control group and examination of anatomical and functional endpoints. The number of animals per group is sufficient given the nature of this study design. However, the route of administration was sc.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for the evaluation process but of limited utility due to the route of administration.

Funabashi et al. (2004b), supported by Yokohama City University and the Japanese Ministry of Education, Culture, Sports, Science, and Technology, examined the effects of bisphenol A exposure on expression of progesterone receptor mRNA in brain of adult ovariectomized rats. *p*-Nonylphenol and 4-*tert*-octyl phenol were also examined, but will not be discussed. **[No information was provided on feed, housing, or bedding materials.]** Wistar rats were ovariectomized at 7 weeks of age, and experiments were conducted 10 days following ovariectomy. In the first experiment, 6 rats/group were s.c. injected with sesame oil vehicle or 10 mg bisphenol A (~40 mg/kg bw) **[purity not reported]**. Rats were killed 24 hr following injection, and frontal, parietal, and temporal cortex were removed. In a second experiment, frontal, temporal, and occipital cortex were collected from rats at 0, 6, 12, or 24 hr following injection with 10 mg bisphenol A; 5–6 rats were killed and examined at each time point. In both experiments, progesterone receptor mRNA expression was determined by Northern blot in each area of the cortex. Data were analyzed by ANOVA followed by Fisher protected least significant difference post-hoc test. At 24 hr following bisphenol A exposure, expression of progesterone receptor mRNA was increased in the frontal cortex and decreased in the temporal cortex. In the time-course experiments, expression of progesterone receptor mRNA was increased in the frontal cortex and decreased in the temporal cortex from 6–24 hr following exposure. Bisphenol A had no effect on expression of progesterone receptor mRNA in the parietal or occipital cortex. The study authors concluded that bisphenol A can alter the neocortical function through the progesterone receptor in adult rats, but the physiological significance of the effect is not known.

Strengths/Weaknesses: This study links relatively high single-dose (10 mg) s.c. bisphenol A administration to the induction of progesterone receptor mRNA, an estrogenic response. Weaknesses is the absence of a positive control to demonstrate maximal response in estrogen-mediated increases in progesterone mRNA and the failure to examine any physiological or functional endpoints. It was also not determined if increases in mRNA were associated with increases in progesterone receptor protein. There was only one dose level administered at a single time point. The s.c. route of dose administration is a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for inclusion but of limited utility.

Della Seta et al. (2005), supported by a grant from MURST, Italy, examined the effects of bisphenol A exposure on maternal behavior in rats. **[No information was provided in the manuscript on the type of chow, bedding, and caging used. The Expert Panel has been informed that Harlan Teklad 2018 chow, Lignocel bedding, and polysulfone cages were used (F. Farabolli et al., personal communication, March 1, 2007).]** Female Sprague–Dawley rats were trained to ingest peanut oil from a micropipette. At 14 weeks of age, female rats were mated for 48 hr. On the day following mating, females were randomly assigned to groups administered peanut oil ($n = 23$) or 0.040 mg/kg bw/day bisphenol A **[purity not indicated in the manuscript; ≥95% according to the authors (F. Farabollini et al., personal communication, March 1, 2007)]** ($n = 17$) through a micropipette. Dosing was continued through the gestation and lactation periods. Two days following delivery, litters were culled to 4 male and 4female pups and were cross-fostered within treatment groups. Pups were weighed on Days 2, 7, and 21 following birth. Maternal behavior was tested at 3 and 4 days and at 8 and 9 days following delivery. In 30-min test sessions, frequency, duration, and latency of behaviors such as retrieving pups, licking pups, postures, and nest building were evaluated with pups of the same sex. Behavior with pups of the opposite sex was evaluated on the second day of the test period, and the order of testing with male and female pups was reversed during each testing period (Days 3–4 and 8–9). Data were analyzed by general linear model, Duncan multiple range test, and/or Mann–Whitney *U* test. The numbers of females giving birth were 9 of 17 in the bisphenol A group and 18 of 23 in the control group. Nine dams in the control group and 7 in the bisphenol A group were evaluated for maternal behavior. The only significant effect reported for bisphenol A was reduced duration of licking-grooming pups, which occurred with both sexes of pups during both observation periods **[~25–50 % decrease as estimated from a graph]**. Effects reported to be marginally significant were decreased frequencies of licking-grooming of pups ($P < 0.09$), anogenital licking of pups ($P < 0.08$), and arched back posture ($P < 0.07$). The study authors concluded that maternal behavior in rats is influenced by prolonged exposure to low bisphenol A doses during pregnancy and lactation.

This behavioral study suggested that a low, oral dose of bisphenol A (0.040 mg/kg bw/day) affects pregnancy and maternal behavior.

Strengths/Weaknesses: Weaknesses include the use of a single dose level and an unusually low pregnancy rate in the controls (18/23) as well as the authors emphasis on marginally significant bisphenol A effects.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation process.

4.2.1.2 Mouse: Park et al. (2004), support not indicated, examined the effects of bisphenol A exposure on the reproductive and hematological systems of male and female mice. **[Results for females are discussed here, and results for males are discussed in Section 4.2.2.2.]** Adult ICR mice were fed mouse formulation feed (Cheil Feed). **[No information was provided about caging or bedding materials.]** Fifteen mice/sex/group were i.p. injected with bisphenol A **[purity unknown]** in an ethanol/corn oil vehicle at 0.05, 0.5, or 5.0 mg/kg bw on 5 occasions (every 3 days over a 14-day period). One control group received no treatment and a second control group was i.p. injected with corn oil. Females were examined 7 days following administration. Reproductive organs were weighed and fixed in Bouin solution, and histopathological examination was conducted. Hematological and clinical chemistry endpoints were also assessed. Data were analyzed by least significant difference test.

Exposure to bisphenol A had no effect on body weight. Significant decreases were observed for right ovary weight in the mid- and high-dose group

and left ovary weight in the mid-dose group **[25–27% lower]**. No treatment effects were observed for uterine or ovarian histology. There were no effects of bisphenol A treatment on hematological endpoints in females. Blood urea nitrogen levels were decreased significantly **[by 28–32%]** in females of all dose groups. The study authors did not report conclusions regarding study findings.

Strengths/Weaknesses: The study design regarding frequency and route of administration and the lack of an appropriate positive control are weaknesses.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate though of limited utility for the evaluation process.

Berger et al. (2007), supported by the Natural Sciences and Engineering Research Council of Canada, examined the effect of bisphenol A exposure on blastocyst implantation in mice. CF-1 mice were housed in polypropylene cages and were fed Harlan Teklad 22/5 rodent chow, which was stated to contain soy. **[No information was provided about bedding materials.]** On GD 1–4 or 5 **[described as GD 1–5 in Methods section and GD 1–4 in study figures and tables]** (GD 0 = day of vaginal plug), 8–9 mice/group were s.c. injected with peanut oil vehicle or bisphenol A (97% purity) at 10.125 mg/animal/day. **[Assuming that the mice weighed 0.02 kg at the start of gestation (USEPA, 1988), CERHR estimated bisphenol A intake at 500 mg/kg bw/day.]** Mice were killed on GD 6 for an examination of implantation sites. Data were analyzed by χ^2 test or 2-sample *t*-test. The number of implantation sites was reduced significantly in the treated animals (mean of ~2.5 compared to ~15 in controls). Implantation sites were observed in 8 of 8 control females at a range of 12–17/female. Six of 9 females in the bisphenol group had no implantation sites. The study authors concluded that pregnancy disruption occurred during the period of implantation.

Strengths/Weaknesses: Weaknesses include lack of experimental details for examining the uteri, use of a single high-dose, number of corpora lutea were not recorded.

Utility (Adequacy) for CERHR Evaluation Process: Due to the absence of key information and faulty methodology, this study is inadequate for evaluation process.

Al-Hiyasat et al. (2004), supported by Jordan University of Science and Technology, examined the effect of bisphenol A and dental composite leachate on fertility of female mice. In this study, Swiss mice were fed a standard laboratory feed containing soy protein. **[No information was provided on caging and bedding materials.]** At 60 days of age, 11 mice/group were gavaged with distilled water or composite leachate for 28 days. Components of the composite leachate were identified by HPLC and included tri-(ethylene glycol)-dimethacrylate (5945 mg/L), bisphenol A glycerolate dimethacrylate (2097 mg/L), and bisphenol A (78 mg/L). **[Based on the reported volume of administration of 0.2 mL and a body weight of 34.4 g, CERHR estimated bisphenol A intake from leachate at 0.45 mg/kg bw/day.]** Additional 60-day-old mice ($n = 15$/group) were gavaged with bisphenol A (97% purity), at doses of 0 (ethanol/distilled water vehicle), 0.005, 0.025, or 0.1 mg/kg bw/day for 28 days. Five mice/group in the bisphenol A study were killed at the end of the dosing period for measurement of body, uterus, and ovary weights. All mice in the leachate study and 10 mice/group in the bisphenol A study were mated to untreated males (2 females to 1 male) for 10 days. One week following the end of the mating period, the mice were killed and examined for pregnancy, implantations, viable fetuses, and resorptions. Body, ovary, and uterus weights were measured in mice from the leachate study. Data were analyzed by Student *t*-test or Fisher exact test.

Effects in the leachate group included increased relative (to body weight) ovarian weight and decreased percentages of pregnant mice. In mice exposed to bisphenol A, body weights were decreased at all dose levels. Effects observed in mice exposed to the mid- and high-dose of bisphenol A included increased uterine weight, increased percentages of resorptions/implantations, and increased percentages of mice with resorptions. Ovarian weight was increased in mice of the high-dose bisphenol A group. **[Although the effects were not statistically significant, the percentages of pregnant females were 90, 77.7, 80, and 60% pregnant mice in the control and each respective dose group.]** In both the composite leachate and bisphenol A groups, there were no statistically significant effects on implantations or viable fetuses. The study authors concluded that bisphenol A and components leached from dental composite have adverse effect on fertility and the reproductive system of mice.

Strengths/Weaknesses: With only 5–10/group, this study was underpowered for determination of potential bisphenol A-related effects on fertility and other endpoints. Confirmation of mating was not performed (cohabitation was for 10 days; if the mice mated on day 10, the necropsy would have been performed on GD 7. Mean body weight and reproductive organ weights of bisphenol A-treated animals were only collected from 5 mice/dose level. Moreover, the normal body weight range for 10-week-old female Swiss mice is 28–35 g. Given that there are only 5 mice/group, it is hard to draw any meaningful conclusions from these data.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation based on small sample size.

Matsumoto et al. (2004), support not indicated, examined the effect of maternal bisphenol A exposure on growth of offspring in mice; this study was discussed in Section 3.2.7. Because the results of this study bear on lactation competence in treated dams, the study will also be considered here. Mice were fed standard rodent chow (CE-2, Japan Clea). **[No information was provided on caging and bedding materials.]** Mice of the ddY strain were exposed to bisphenol A (≥97% purity) through feed at 0 or 1% from GD 14–PND 7. The study authors stated that the bisphenol A dose was equivalent to 1000 mg/kg bw/day. **[The numbers of dams treated was not indicated. Day of vaginal plug and day of birth were not defined.]** Mice delivered pups on PND 21. Body weight of pups were monitored during the postnatal period in 31 pups from the control group and 61–89 pups from the bisphenol A group. Serum prolactin levels were measured by RIA in 3 dams/group 4 days following delivery. Pups were killed on PND 7, and

stomach weight was measured. Data were analyzed by Student *t*-test.

No differences were reported for live pups at birth. During the postnatal period, body weights of pups in the bisphenol A group were significantly lower **[by ~40%]** than control group pups. No deaths were reported for pups in the control group, but 30% of pups in the bisphenol A group died before PND 7. On PND 1, milk could be seen in stomachs of pups from the control group, but not the bisphenol A group. **[The number of pups evaluated for milk in stomach was not reported]**. On PND 7, stomach weight was significantly lower **[by 40%]** in pups from the bisphenol A compared to control group. Serum prolactin level was significantly reduced **[by 46%]** in dams from the bisphenol A group. The authors concluded that administration of a high bisphenol A dose to mice resulted in suppressed postnatal growth of offspring which probably resulted from an insufficient supply of milk, which might have been due to decreased prolactin secretion.

Strengths/Weaknesses: This study was conducted at a single high-dose that likely induced maternal toxicity (which was not assessed); therefore, it is difficult to delineate if the findings in the mouse pups are the result of potential bisphenol A-related effects of maternal toxicity or an effect on the pup.

Utility (Adequacy) for CERHR Evaluation Process: Given the likely confounding effects of maternal toxicity, this study is considered inadequate and of no utility.

4.2.1.3 Other mammals: Nieminen et al. (2002a), support not indicated, examined the effects of bisphenol A exposure on hormone levels in the European polecat (*Mustela putorius*). Five animals/group/sex **[age not reported]** were administered bisphenol A **[purity not reported]** in feed at concentrations providing doses of 0, 10, 50, or 250 mg/kg bw/day for 2 weeks. Body weight and length were measured during the study. Animals were killed at the end of the exposure period, with sampling conducted in random double-blinded order. Liver and kidney were weighed. Blood samples were obtained for measurement of hormone levels by RIA. Microsomal enzyme activities were determined. Statistical analyses included ANOVA, post-hoc Duncan test, Student *t*-test, Spearman correlation coefficient, Kolmogorov–Smirnov test, and/or Levene test.

There were no clinical signs of toxicity and no effects on body weight or body mass index following bisphenol A exposure. Absolute and relative liver weight were significantly increased in females of the high-dose group. Plasma cortisol levels were significantly reduced in females of the mid-dose group. Bisphenol A exposure had no significant effects on plasma levels of testosterone, estradiol, FSH, or thyroid hormones. Glutathione-*S*-transferase (GST) activity was significantly increased in females of the high-dose group. UDPGT activity was significantly higher in females of the mid- and high-dose group and males of the high-dose group. There was no effect on 7-ethoxyresorufin O-deethylase (EROD) activity. The study authors concluded that the endocrine effects in this study were not as remarkable as the effects on liver enzymes.

Strengths/Weaknesses: A strength of this study is the use of a non-rodent species and multiple doses. Weaknesses include small sample size and absence of reproductive endpoints.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate and not useful due to small sample size and absence of reproductive endpoints.

Nieminen et al. (2002b), support not indicated, examined the effects of bisphenol A exposure on endocrine endpoints in field voles (*Microtus agrestis*). Animals were housed in plastic cages with wood shavings and fed R36 diet (Lactamin, Sweden). Sexually mature field voles were randomly assigned to groups that received bisphenol A **[purity not reported]** in propylene glycol by s.c. injection for 4 days. Doses of bisphenol A (numbers of females in each group) were 0 ($n = 5$), 10 ($n = 7$), 50 ($n = 5$), and 250 ($n = 8$) mg/kg bw/day. Animals were killed the day following the last dose. Body and liver weights were measured. Blood was drawn for measurement of sex steroids, thyroxine, and weight-regulating hormone levels in plasma using RIA or immunoradiometry methods. The activities of EROD, UDPGT, and GST were measured in hepatic and renal microsomes using appropriate substrates. Statistical analyses included ANOVA, post-hoc Duncan test, Student *t*-test, Kolmogorov–Smirnov test, Levene test, Mann–Whitney *U* test, χ^2 test, and Spearman correlation. **[Results for males are discussed in Section 4.2.2.3.]**

Mortality was significantly increased by bisphenol A treatment, with incidences of 18, 36, and 20% in the low- to high-dose groups. No mortality was observed in the control group. Bisphenol A treatment did not significantly affect body or liver weight. Plasma testosterone levels increased with dose, and statistical significance was attained in high-dose females compared to control females. 17β-Estradiol levels decreased with dose in females. Pooled (male+female) LH levels were not significantly altered by treatment. Liver EROD activity **[apparently combined for males and females]** was significantly decreased at the mid- and high-dose, and liver GST activities **[not clear if for males or females or both]** was significantly decreased at the highest dose level. There were no other significant effects on microsomal enzymes examined. The study authors concluded that wild mammals such as field voles could be more susceptible to bisphenol A-induced toxicity than laboratory rodents.

Strengths/Weaknesses: A strength is the use of another species**.** The small number of voles/dose level, the subcutaneous route of administration, and questionable statistical procedures are weaknesses.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process.

Razzoli et al. (2005), supported by the Ministry of University Education and Research and the University of Parma, examined the effects of bisphenol A on sociosexual and exploratory behavior in female Mongolian gerbils, a monogamous species. Animals were fed Mil Morini Rodent Chow (Reggio Emilia, Italy) and housed in Plexiglas cages with wood shaving/cotton nesting material. At 11–12 weeks of age, female gerbils were trained to drink corn oil from a syringe, and 1 week later, they were paired with a male. From Days 1–21 of cohabitation, 12 females/group were fed 0 (corn oil vehicle), 0.002, or 0.020 mg/kg bw/day bisphenol A **[purity not indicated]** from a syringe. A group of 12 females received ethinyl estradiol, the positive control, 0.04 μg/kg bw/day during the same time period. During the cohabitation period, social behavior (e.g., agonism,

social investigation, huddling, and nest sharing) was observed and body weights of females were measured. A free exploratory test, which measured the amount of time females spent in an area of a cage with home nesting material compared to the time spent in an unfamiliar area of a cage, was conducted following the 21-day cohabitation period. Exploratory behavior was evaluated by an observer blinded to treatment groups. Statistical analyses included ANOVA and Duncan test for multiple comparisons.

Bisphenol A treatment did not affect body weight. Social sniffing was increased significantly **[by 60%]** in the low-dose bisphenol A group. Significant effects **[percent changes compared to control]** observed in the exploratory test were decreased time in the unfamiliar area at the low **[60%]** and high **[44%]** dose, fewer transitions to the unfamiliar area at the low **[60%]** and high **[50%]** dose, fewer transitions to the home cage at the high-dose **[29%]**, and less time in the unfamiliar area at the low dose **[46%]**. Similar results for both social sniffing and exploratory behavior were observed in the positive control group. According to the study authors, this study demonstrated that chronic exposure of adult female gerbils to environmentally relevant doses of bisphenol A during the hormonally sensitive period of cohabitation resulted in subtly altered social and exploratory behavior.

Strengths/Weaknesses: This study examined behavioral endpoints in gerbils, and included a positive control (ethinyl estradiol) and 2 doses of bisphenol A. It appears to be well conducted using oral dosing, respectable sample size (given study complexity), and use of a positive control. Weaknesses include failure to account for temporally repeated measures in statistical analyses.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for inclusion but of limited utility for the evaluation process.

4.2.1.4 Invertebrates: Although studies in invertebrates may be important for understanding mechanisms of action and environmental impact, the Panel views these studies as not useful for the evaluation process.

Oehlmann et al. (2000), supported by the Berlin Federal Environmental Agency, reported the effects of bisphenol A on reproductive organs in the freshwater ramshorn snail (*Marisa cornuarietis*) and the marine dog whelk (*Nucella lapillus*). In the first experiment, adult ramshorn snails were exposed for 5 months to bisphenol A in ethanol at 0, 1, 5, 25, or 100 μg/L. Thirty snails/group were removed every month for evaluation of reproductive organs. **[Culture ware type not indicated. The purity of bisphenol A and its stability during the exposure period were not reported. The snails removed for evaluation were adults; this species requires 8 months to attain sexual maturity. Octylphenol was also evaluated, but is not discussed here.]** In the second experiment, ramshorn snails were exposed to bisphenol A in ethanol at 0, 1, or 100 μg/L for 1 year. Thirty F_1 snails per time point were removed for evaluation at 6, 8, and 12 months. In the third experiment, dog whelk were exposed to bisphenol A in glacial acetic acid at 0, 1, 25, or 100 μg/L for 3 months. Thirty specimens were removed for evaluation each month. Evaluations included measurements of sex organs and the identification of sperm or oocytes in the genital tract. Statistical analyses included ANCOVA followed by Tukey or Student-Newman–Keuls test, Kruskal–Wallis test, χ^2 test, and Weir test.

Adult ramshorn snails were reported to show increases in volume of the capsule and albumen glands (portions of the oviduct). **[Apparently, the increase in volume was based on appearance rather than measurements. The measured lengths of the sex organs were not affected by treatment.]** Occasional specimens that had been exposed to bisphenol A showed rupture of the oviduct with protrusion of the egg mass. Enumeration of spawning masses and eggs showed statistically significant time-dependent increases in all bisphenol A groups. Histologic examination of the gonads did not suggest abnormalities of spermatogenesis or oogenesis. The F_1 snails also demonstrated a statistically significant increase in spawning mass and oocyte production at the 100 μg/L bisphenol A concentration, and some specimens showed rupture of the oviduct at 12 months of age in both bisphenol A groups. An increase in imposex **[the presence of vas deferens tissue]** was noted significantly more often in snails exposed to bisphenol A 100 μg/L than controls. Adult dog whelk demonstrated a significant increase in the length and weight of the sex glands and an increase in number of females with oocytes in the oviduct. The authors concluded that invertebrates are sensitive to bisphenol A toxicity at environmentally relevant concentrations.

Strengths/Weaknesses: The study appears to be well conducted and suggests that bisphenol A has stimulatory (17β-estradiol-like) effects on the spawning masses and eggs of snails. These changes occurred in the absence of a histological correlate. The potential stability/biotransformation was discussed in the introduction but not determined during the exposure period.

Utility (Adequacy) for CERHR Evaluation Process: This study is not considered useful for the evaluation process.

Forbes et al. (2007), supported by the Bisphenol A Global Industry Group, evaluated the effects of bisphenol A on reproduction in the freshwater ramshorn snail (*Marisa cornuarietis*). Bisphenol A **[purity not indicated]** concentrations in test water were 0, 0.10, 1.0, 16, 160, and 640 μg/L. Concentrations were periodically checked. Thirty breeding pairs per treatment level were observed for a 12-week period. The number of egg masses and number of eggs/egg mass were recorded. Hatchability was evaluated using 5 consecutive egg masses collected from 5females/replicate (75 egg masses/treatment). Juvenile growth rates were calculated for a subset of the offspring. Nested ANOVAs were used for data analysis. All snails survived. There were no significant treatment-related differences in adult egg production, hatchability, or juvenile growth rate. Interindividual variability in these parameters was prominent, and the authors concluded that a large number of replicates would be necessary using this animal model to detect reproductive effects.

Strengths/Weaknesses: This study examined dose response over a 12-week exposure of freshwater snails to bisphenol A with egg masses and number of eggs/egg mass as endpoints. Although no treatment-related effects were observed, interindividual variability was high.

Utility (Adequacy) for CERHR Evaluation Process: This study is not considered useful for the evaluation process.

are a primary (or secondary) effect or are the result of cytotoxicity. Calcium levels were also affected and collectively these changes may be the result of apoptosis initiated by some other mechanism.

Utility (Adequacy) for CERHR Evaluation Process: This study was not considered useful for the evaluation process.

Tabuchi et al. (2006), supported in part by the Japanese Ministry of Education, Culture, Sports, Science, and Technology, examined the effects of bisphenol A on gene expression in mouse Sertoli cell cultures. TTE3 cells were incubated in media containing bisphenol A **[purity not reported]** at 0 (DMSO vehicle) or 200 μM **[46 mg/L]** for up to 12 hr **[culture ware type not discussed]**. Cells were examined for viability using dye exclusion assays and for apoptosis by formation of DNA ladders. RNA was extracted from cells, and gene expression was determined by PCR and microarray analyses. Data were analyzed by Student *t*-test. Cell viability was decreased in a time-related manner between 3–12 hr of bisphenol A exposure, but there was no evidence of apoptosis. PCR analysis indicated that bisphenol A exposure significantly and time-dependently increased mRNA transcripts for 2 endoplasmic reticulum stress markers, *hspa5* and *ddit3*. Microarray analysis demonstrated that 661 sets of genes were downregulated and 604 sets of genes were upregulated >2-fold following bisphenol A exposure. Pathway analysis of decreased gene clusters revealed 2 significant genetic networks associated with the cell cycle or cell growth and proliferation. In increased gene clusters, two genetic networks were associated with cell death, DNA replication, recombination and repair, or injuries and abnormalities. The study authors concluded that the genes, genetic clusters, and genetic networks identified in this study are likely involved in Sertoli cell injury following bisphenol A exposure.

Strengths/Weaknesses: State-of-the-art technology was used in this study to examine gene expression changes after in vitro bisphenol A exposure of a Sertoli cell line. Only one dose level was examined. The use of hormone rich fetal bovine serum in the media may be a confounder. The absence of DNA laddering is not conclusive evidence of the absence of apoptosis (e.g., adherent cells undergoing apoptosis often are released into the culture media). Moreover, it is not surprising that given this ''high'' bisphenol A concentration, ''novel'' and likely non-specific gene changes were noted.

Utility (Adequacy) for CERHR Evaluation Process: This study was not considered useful for the evaluation process.

4.2.3 Male and female.

4.2.3.1 Rat: Two unpublished studies performed by the International Research and Development Corporation for General Electric (General Electric, 1976, 1978) provided some information on reproductive toxicity in rats orally exposed to bisphenol A. The studies are described in detail in Section 3.2.3.1. There was no effect on fertility in male and female rats given feed containing up to 9000 ppm bisphenol A (~650 mg/kg bw/day in males and 950 mg/kg bw/day in females) for an unspecified period before mating (General Electric, 1976). A second study reported no effects on estrus cyclicity or gestation length **[data not shown by study authors]** or male or female fertility in rats given feed containing bisphenol A at up to 1000 ppm (~60 mg/kg bw/day in males and 100 mg/kg bw/day in females) for ~70 days before mating (General Electric, 1978).

Ema et al. (2001), supported by the Japanese Ministry of Health and Welfare, conducted a multigeneration reproductive toxicity study of bisphenol A in CD rats. Animals were housed in suspended stainless steel cages at the beginning of the study. From GD 17, wood chips were used as bedding. Rats were fed CRF-1 chow (Oriental Yeast Co). In the study that was conducted according to GLP, F_0 male rats and female rats with 4–5-day estrous cycles were randomly assigned to groups of 25/sex. Five-week-old males and 10-week-old females were gavaged with 0 (distilled water vehicle), 0.0002, 0.002, 0.020, or 0.200 mg/kg bw/day bisphenol A (99.9% purity). Males were dosed for 10 weeks before mating and during the mating period, which lasted up to 2 weeks. Females were dosed from 2 weeks before mating, and during the mating, gestation, and lactation periods. Doses were based on results of studies by Nagel et al. (1997) and vom Saal et al. (1998). Stability and concentration of dosing solutions were verified. Dams delivered and nursed their pups. At weaning on PND 22 (day of birth defined as PND 0), 1 or 2 F_1 weanlings/litter/sex (25/sex/group) were selected to continue in the study. Dosing of F_1 animals began on PND 23 and continued for 10 weeks before mating and through the mating period, which lasted up to 3 weeks. Dosing was continued through the gestation and lactation periods. Twenty-five F_2 weanlings/sex/group were selected on PND 22. Beginning on PND 22, male F_2 rats were dosed for 4 weeks and females were dosed for 11 weeks before being killed.

Endpoints examined in adult rats included clinical signs, body weight, and food intake. Fertility, copulation, and gestational indices were examined in mating rats. Vaginal smears were evaluated for 2 weeks before mating in F_0 and F_1 females and at 9–11 weeks of age in F_2 females. Dams were killed and necropsied following weaning of their pups, and uterine implantation sites were examined. Males were killed following mating. Organs were weighed and histopathology examinations were conducted in control and high-dose animals. Sperm endpoints were measured in F_0 and F_1 adult males. Serum hormone levels were measured in 6 adult F_0 and F_1 males and proestrous females. At birth, pups were counted, sexed, and examined for viability and external malformations. On PND 4, litters were culled to 4 male and 4 female pups. At weaning, 1 male and female F_1 and F_2 weanling was killed for organ weight measurement; histopathology exams were conducted in seminal vesicles and coagulating glands of F_2 weanlings. Survival and growth were monitored during the postnatal period. Pups were examined for developmental landmarks and attainment of vaginal opening or preputial separation. Anogenital distance in pups was examined at numerous time points during the lactation period and through adulthood. Behavioral testing was conducted at 5–7 weeks of age. The litter was considered the experimental unit in data obtained before weaning. Statistical analyses included Bartlett test for homogeneity of variance, ANOVA, and/or Dunnett multiple comparison, Kruskal–Wallis, Mann–Whitney U, χ^2, or Fisher exact tests.

In F_0 and F_1 adult animals, there were no treatment-related effects on clinical signs, body weight gain, or death. The only significant reproductive effects reported in adult

animals were non-dose-related decreases in percentages of females with normal estrous cycles (76 vs. 96% in controls) and reduced gestation duration (by 0.5 days) in the F_1 group treated with 0.020 mg/kg bw/day. Bisphenol A did not significantly affect the precoital interval, copulation index, fertility index, gestation index, number of implantations, or delivery index. There were no adverse effects on sperm endpoints such as count, motility, or morphology in F_0 or F_1 males. A significant decrease in abnormal and tailless sperm was observed in F_1 males of the 0.020 mg/kg bw/day group. There was no evidence of histopathological effects in reproductive organs of F_0 animals that did not copulate or had totally resorbed litters or in F_1 animals of the high-dose group. **[Data were not shown by study authors.]** In F_0 females, there were significant decreases in serum LH concentrations at 0.0002, 0.002, and 0.020 mg/kg bw/day and in serum triiodothyronine levels at 0.200 mg/kg bw/day. **[Data were not shown by study authors.]** Organ weight changes in F_1 adult males included decreased absolute weights of lung at 0.0002 and 0.200 mg/kg bw/day, kidney at 0.2 mg/kg bw/day, and testis at 0.020 mg/kg bw/day. Absolute ovarian weight was decreased in females of the 0.0002 mg/kg bw/day group. Seminal vesicle weight was decreased in F_2 males of the 0.200 mg/kg bw/day group. **[Data were not shown by study authors]**.

There were no significant effects on number of F_1 or F_2 pups delivered, sex ratio, or pup survival during the lactation period. Body weights of F_1 pups in the 0.020 mg/kg bw/day group were significantly lower **[by 6–7%]** on PND 14 and 21. Testicular descent was delayed by 0.7 days in F_2 offspring from the 0.020 and 0.200 mg/kg bw/day groups. There were no significant effects on age of pinna detachment, incisor eruption, or eye opening. Some significant but non-dose-related effects on reflex development were observed. Day of mid-air righting reflex was accelerated by 1.2 days in F_1 males and 1.5 days in F_1 females of the 0.020 mg/kg bw/day group. In F_2 males, negative geotaxis was delayed by 0.8 days at 0.0002 mg/kg bw/day, 0.5 days at 0.002 mg/kg bw/day, and 0.8 days at 0.020 mg/kg bw/day. Bisphenol A treatment did not significantly affect age of vaginal opening or preputial separation in F_1 or F_2 offspring. Some sporadic and small (within 5% of control values) changes in anogenital distance were observed in F_1 and F_2 offspring. In F_1 males, decreased anogenital distance was observed in the 0.0002 mg/kg bw/day group on PND 57 and in the 0.020 mg/kg bw/day group on PND 106, 113, and on the day of sacrifice. In F_1 females, anogenital distance was decreased in the 0.200 mg/kg bw/day group on PND 4 and increased in the 0.002 and 0.020 mg/kg bw/day group on PND 7. Decreases in anogenital distance of F_2 females were observed in the 0.020 mg/kg bw/day group on PND 64, 71, 85, 92, and on the day of sacrifice and in the 0.200 mg/kg bw/day group on PND 57, 64, and on the day of sacrifice. In F_1 offspring, there were no significant effects on behavior, as determined by open-field testing and performance in a T-maze. **[Data were not shown by study authors.]** There was no evidence of histopathological effects in seminal vesicle or coagulating gland of F_2 pups from the high-dose group. **[Data were not shown by study authors.]** Organ weight changes in F_1 male weanlings included decreased absolute lung weight at 0.020 and 0.200 mg/kg bw/day group and decreased kidney weight at 0.020 mg/kg bw/day. In male F_2 weanlings, significant decreases were observed in absolute and relative seminal vesicle weight and absolute thyroid weight at 0.002 mg/kg bw/day, absolute lung weight at 0.020 mg/kg bw/day, and relative heart weight at 0.200 mg/kg bw/day; relative liver weight was significantly increased in F_2 males of the 0.002 mg/kg bw/day group. The study authors concluded that oral administration of bisphenol A at 0.0002 to 0.200 mg/kg bw/day to 2 generations of rats did not cause changes in reproduction or development.

[The NTP Statistics Subpanel (NTP, 2001) reviewed an unpublished study that appeared to be the same study later published as Ema et al. (2001). The subpanel noted that in general they agreed with the statistical methodology used in the study but stated that the Dunnett test does not require significance of ANOVA. It was noted that the anogenital distance findings were the most difficult to interpret. The Subpanel noted that many of the anogenital distance effects remained statistically significant when analyzed by ANCOVA, a method they considered superior to adjustment by body weight. The NTP Subpanel agreed with the author's conclusion that effects on anogenital distance were not biologically significant. They noted an error in the unpublished study abstract that described increases in anogenital distance in F_1 and F_2 females in the 0.020 and 0.2 mg/kg bw/day groups when actually the effect should have been decreased anogenital distance. [It was not clear to CERHR if this error was carried forward to the published report.]

Strengths Weaknesses: This well-designed comprehensive low-dose assessment of potential bisphenol A-related effects on multiple generations of rats examined a wide variety of hormonally sensitive endpoints. The study had appropriate power with an appropriate number of rats per group. Route of administration (oral) was appropriate. The concentrations of the dosing solutions were verified (both prior and after). It would have been helpful if a dose level that caused maternal toxicity was also used; however, given the objective of this study it is a minor point. This thorough multiple generation rat study is highly valuable for human risk assessment of low dose oral exposure to bisphenol A. This study indicates that the NOAEL for bisphenol A exceeds 0.2 mg/kg bw/day under the conditions of this study.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation process.

Tyl et al. (2000a, 2002b), sponsored by The Society of the Plastics Industry, Inc., conducted a multigeneration study of bisphenol A in rats. In the study that was conducted according to GLP, Sprague–Dawley rats were fed Purina Certified Rodent Chow 5002. F_0 rats (30/sex/group) were exposed to bisphenol A (99.5% purity) in feed for 10 weeks before mating. **[Age at start of exposure was not reported, but based on information provided in the discussion, it appears that the animals were adults at the start of exposure.]** Vaginal smears were evaluated during the last 3 weeks of the prebreeding period. Exposure continued through a 2-week mating period. Males were exposed an additional 3 weeks following mating, and females were exposed through gestation and lactation. Concentrations of bisphenol A added to feed were 0, 0.015, 0.3, 4.5, 75, 750, or 7500 ppm.

Target intakes were ~0, 0.0009, 0.018, 0.27, 4.5, 45, and 450 mg/kg bw/day in males and 0.001, 0.02, 0.30, 5, 50, and 500 mg/kg bw/day in females. 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. The study was designed to include low-dose exposures reported to increase prostate weights (Nagel et al., 1997; vom Saal and Sheehan, 1998) and maximally tolerated doses expected to result in toxicity. Concentration, stability, and homogeneity of bisphenol A in feed were verified. During the study, body weight and food intake were measured and animals were examined for clinical signs. F_0 males were killed and necropsied following delivery of the F_1 litter. Histopathological evaluation of organs was conducted in all control animals and 10 animals/bisphenol A dose group. Reproductive organs were weighed and sperm endpoints were evaluated. F_0 females were killed and necropsied following weaning of their litters. Selected organs were weighed and ovarian primordial follicles were counted.

On PND 4, F_1 litters were culled to 10 pups, with equal numbers of each sex when possible. Endpoints examined in pups included growth and survival in the prenatal period and retained areolae or nipples on PND 11–13. At weaning on PND 21, 30 F_1 offspring/sex/group were randomly selected and exposed to bisphenol A 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. Up to 3 F_1 weanlings/sex/litter were killed for organ weight measurement. Mating and evaluation of F_1 offspring were conducted according to the same procedures described for F_0 rats. The same procedures were repeated in F_2 rats and F_3 litters during the lactation period. Anogenital distance was measured in F_2 and F_3 rats at birth. Following weaning of F_3 offspring, up to 3/sex/litter were randomly selected for necropsy. Thirty/sex/dose were selected for evaluation of vaginal patency, preputial separation, and estrous cyclicity. Bisphenol A exposure was continued in those offspring until they were killed ~10 weeks following weaning. F_3 offspring were not mated, but necropsy evaluations were conducted as described above for previous generations.

Statistical analyses for quantitative continuous data included Bartlett test for homogeneity of variances, ANOVA, Dunnett, linear trend, Kruskal–Wallis, or Mann–Whitney *U* tests. Frequency data were analyzed by χ^2, Fisher exact, and Cochran–Armitage tests. Covariance and correlations analyses were also conducted.

Treatment-related systemic findings with available quantitative information in adult rats are summarized in Table 93. Body weights and body weight gain were consistently lower in F_0, F_1, F_2, and F_3 adult rats of the 750 and 7500 ppm dose groups, including during gestation and lactation periods. Terminal body weight effects are summarized in Table 93. Terminal body weight was reduced in all generations at 7500 ppm and in F_1 females and F_1 and F_2 males at 750 ppm. There were no consistent or clearly treatment-related effects on feed intake. No treatment-related clinical signs were reported. In the 7500 ppm group, absolute weights of the liver in males and the kidney in both sexes were decreased across generations. Relative weights were either increased or did not attain statistical significance. **[According to Table 2 of the study, absolute liver weights were also decreased in males of the 750 ppm group. The study authors also mentioned reductions in weights of adrenal glands, spleen, pituitary, and brain at the high-dose, but there were no data shown in the report for those endpoints.]** Other changes in non-reproductive organ weight occurred sporadically at lower dose and were not dose-related or consistent across generations. Relative organ weight changes that consistently attained statistical significance at the highest dose are summarized in Table 93. Histopathological analyses revealed a higher incidence of mild renal tubular degeneration and chronic hepatic inflammation in F_0, F_1, and F_2 but not F_3 females of the 7500 ppm group.

Treatment-related effects on reproductive endpoints in adult animals are summarized in Table 93. In evaluating organ weights, the study authors only considered organ weight effects to be biologically significant if statistically significant results were obtained in the same direction for absolute and relative weights. Therefore, the study authors concluded that the only treatment-related organ weight effects were reduced absolute and relative ovary weights. **[Numerous statistically significant effects on reproductive organ weights were reported in Table 2 of the study. Reductions in testes, epididymides, prostate, and seminal vesicle weights were observed in most generations of the 7500 ppm group. When adjusted for body weight, organ weights were either increased or did not differ significantly from controls.]** Relative reproductive organ weight changes that consistently attained statistical significance at the highest dose are summarized in Table 93. The authors reported no effect on mating, fertility, pregnancy, or gestational indices. **[With the exception of gestational length, data were not shown by study authors.]** Precoital interval, post-implantation loss, estrous cyclicity, and reproductive organ histopathology were also unaffected by bisphenol A treatment. In the high-dose group, there was no adverse effect on paired ovarian primordial follicle counts but counts were significantly increased by 43% in the F_0 generation. Implantation sites were decreased in F_0, F_1, and F_2 dams of the 7500 ppm group. The only significant effects on sperm endpoints were decreased epididymal sperm concentration in F_1 males and decreased daily sperm production in F_3 males of the 7500 ppm dose group. There were no effects on sperm morphology or motility. The study authors considered sperm to be unaffected by treatment.

Treatment-related effects observed in developing rats are summarized in Table 94. The number of live pups/litter was reduced in F_1, F_2, and F_3 litters of the 7500 ppm group. Body weights of F_1, F_2, and F_3 pups of the 7500 mg/kg bw/day groups were lower during the lactation period. Some small (~5%) decreases in pup body weight during the lactation period at lower doses were apparently not considered treatment-related by study authors. Postnatal survival was unaffected by bisphenol A treatment. In male rats, there were no effects on anogenital distance or the presence of areolas or nipples. Anogenital distance was significantly increased in F_2 females at all doses except 75 and 7500 ppm; there was no affect on anogenital distance in F_3 females. The study authors did not consider anogenital distance effects to be biologically or toxicologically significant. Vaginal patency was delayed in F_1, F_2, and F_3 females, and the effect remained significant

following adjustment for body weight. Preputial separation was delayed in F_1 males of the 750 and 7500 ppm groups, F_2 males in the 0.3, 75, 750, and 7500 ppm groups, and F_3 males of the 7500 ppm group. When adjusted for body weight, the effect remained significant in F_1 males of the 750 and 7500 ppm groups and F_2 and F_3 males of the 7500 ppm group. The study authors stated that reduced body weights were the most likely cause of puberty delay in males and females. **[In rats killed at weanling, absolute organ weights were said to be decreased at the high-dose but increased when adjusted for body weight. The specific organs affected were not reported and no data were presented. The exception was ovarian weights, which were reported to parallel effects observed in adult females with decreases in both absolute and relative weight at 7500 ppm.]**

The study authors concluded that there was no evidence of low-dose bisphenol effects (1 μg to 5 mg/kg bw/day) at any stage of the life cycle. They identified NOAELs of 75 ppm (~5 mg/kg bw/day) for adult systemic toxicity and 750 ppm (~50 mg/kg bw/day) for offspring and reproductive effects. The study authors concluded that bisphenol A should not be considered a selective reproductive toxicant.

[The NTP Statistics Subpanel (NTP, 2001) stated that the study by Tyl et al. (2000a) apparently lacked a check for outliers, but noted that the study was in draft form at the time of review. The NTP subpanel agreed with most author conclusions but disagreed with a conclusion that relative uterine weights were equivalent across all groups. The unnecessary use of ANOVA with Dunnett test was noted. Some possible outliers and 10-fold errors in data points that could have affected conclusions were observed. Overall, the NTP Subpanel concluded that Tyl et al. (2000a) study was the most comprehensive of the studies reviewed. They stated that the statistical methods were well thought out and appropriate.]

Strengths/Weaknesses: This assessment of potential bisphenol A-related effects on multiple generations of rats was well-designed and comprehensive. The large number of rats/group (30), the multiple endpoints examined, and the oral route of administration (diet) are strengths. The concentration of bisphenol A in the test diet was verified, and maternal and paternal toxicity was identified. This study explored a wide dose range and demonstrates an absence of adverse effects on reproductive function at very low bisphenol A dose levels. This study is highly valuable for human risk assessment for oral exposure to bisphenol A.

This study identified a NOAEL of 75 ppm (for general toxicity) and 750 ppm (for reproductive toxicity).

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation process.

4.2.3.2 Mouse: NTP (1984) and **Morrissey et al. (1989)** sponsored a continuous breeding study in CD-1 mice exposed to bisphenol A through s.c. implants. Mice were fed Purina certified ground rodent chow (#5002) and housed in polypropylene or polycarbonate cages containing Ab-Sorb-Dri bedding. Silastic implants were used for s.c. dosing of mice with bisphenol A (~95% purity) in corn oil vehicle. Stability and weight of bisphenol A in pumps was verified. In the dose-range finding portion of the study (Task 1), 8 mice/sex/group (8 weeks old) received implants containing vehicle or bisphenol A. Dosages were estimated by determining the difference in bisphenol A weight at the start and end of the 14-day dosing period. It was estimated that mice received 0, 6.25, 12.5, 25, 50, or 100 mg bisphenol A. Endpoints examined included body weight changes, survival, and uterine weight. Blood was collected to determine plasma bisphenol A levels. Data were analyzed by ANOVA, Duncan Multiple Range Test, χ^2 test, and Fisher exact test. The goal of Task 2 was to determine a maximum tolerated dose that produced signs of toxicity but did not reduce body weight or increase lethality by >10% and to identify a low dose that did not result in toxicity. Concentrations of bisphenol A in plasma were below the detection limit (3 ng/mL) in the 6.25 mg group but were reported at 7.0–7.7 μg/L in the 12.5 mg group, 8.4 μg/L in the 25 mg group, 13.1–18.5 μg/L in the 50 mg group, and 31.5–56.2 μg/L in the 100 mg group. In mice treated with bisphenol A, there were no increases in death or effects on body weight gain. The study authors noted that reproductive tract weight in the high-dose group was greater **[by 52%]** than in the control group but statistical significance was not achieved because of high variability.

In the continuous breeding portion of the study (Task 2), mice were 11 weeks old at the start of dosing. Forty mice/sex/group received implants containing the vehicle and 20/sex/dose received implants containing bisphenol A at 25, 50, or 100 mg. Over a dosing period of 18 weeks, it was estimated that animals in each treatment group received 11.65, 20.05, and 38.60 mg bisphenol A. **[Assuming body weights of ~38 g, as indicated in the study report, doses would have been ~306, 527, and 1015 mg/kg bw over 18 weeks or 2.4, 4.2, and 8.1 mg/kg bw/day.]** Mice were 11 weeks old at the start of dosing, which began during a 7-day premating period. The mice were then randomly paired with animals from the same dose group and housed together during a 98-day breeding period. Litters born during the breeding period were examined for viability, weighed, sexed, and discarded. Following the 98-day mating period, mice were separated for 21 days to allow for the birth of the last litter. Dosing was continued throughout the breeding and separation periods. However, implants were often expelled through cutaneous lesions or the incision site. When animals expelled their implant, a new one was inserted but pregnant mice were allowed to complete their pregnancy before insertion of the new implant. Therefore dosing was not uniform. Endpoints examined in adult mice included body weight, number of litters/pair, and fertility. Following delivery of the final litter, parental animals were killed and animals in the 0 and 100 mg group were necropsied. Liver, brain, and reproductive organs were weighed. Data were analyzed by χ^2 test, Fisher exact test, Kruskal–Wallis test, Jonckheere test, and Mann–Whitney *U* test.

With the exception of cutaneous lesions at the implantation site, there were no clinical signs of toxicity. In parental mice, there were no effects on body weight, mortality, fertility, or number of litters born. There were no changes in weights of organs including, liver brain, pituitary, the female reproductive tract, testis, epididymis, prostate, or seminal vesicles. Statistically

Table 93
Treatment-Related Effects in Adult Rats Fed Bisphenol A Through Diet in a Multigeneration Reproductive Toxicity Study[a]

	Dose, ppm diet **[mg/kg bw/day[b]]**													
Endpoint	0.015 **[0.0095]**	0.3 **[0.019]**	4.5 **[0.285]**	75 **[4.75]**	750 **[47.5]**	7500 **[475]**	BMD_{10}		$BMDL_{10}$		BMD_{1SD}		$BMDL_{1SD}$	
Terminal body weight														
F_0 males	↔	↔	↔	↔	↔	↓ 22%	3554	**[225]**	3137	**[199]**	3133	**[198]**	2701	**[171]**
F_1 males	↔	↔	↔	↔	↓ 6%	↓ 26%	2811	**[178]**	2548	**[161]**	2443	**[155]**	2153	**[136]**
F_2 males[c]	↔	↔	↔	↔	↓ 12%	↓ 29%	733	**[46]**	554	**[35]**	648	**[41]**	484	**[31]**
F_3 males[c]	↔	↔	↔	↔	↔	↓ 26%	1456	**[92]**	913	**[58]**	1260	**[80]**	786	**[50]**
F_0 females	↔	↔	↔	↔	↔	↓ 13%	5722	**[362]**	4753	**[301]**	4741	**[300]**	3876	**[245]**
F_1 females	↔	↔	↔	↔	↓ 6%	↓ 16%	4600	**[291]**	3950	**[250]**	3730	**[236]**	3142	**[199]**
F_2 females[c]	↔	↔	↔	↔	↔	↓ 14%	3863	**[245]**	1576	**[100]**	3115	**[197]**	1291	**[82]**
F_3 females	↔	↔	↔	↔	↔	↓ 20%	3664	**[232]**	3194	**[202]**	3456	**[219]**	2949	**[187]**
Relative paired kidney weight														
F_0 males	↔	↔	↔	↔	↔	↑ 14%	5903	**[374]**	4555	**[288]**	6536	**[414]**	5035	**[319]**
F_1 males	↔	↔	↓ 5%	↔	↔	↑ 10%	5729	**[363]**	4662	**[295]**	5053	**[320]**	4088	**[259]**
F_2 males	↔	↔	↔	↔	↑ 5%	↑ 18%	4524	**[287]**	3893	**[247]**	3471	**[220]**	2950	**[187]**
F_3 males	↔	↔	↔	↔	↔	↑ 16%	6986	**[442]**	4319	**[274]**	6720	**[426]**	3403	**[216]**
F_0 females	↔	↔	↔	↔	↔	↑ 7%	8008	**[507]**	7521	**[476]**	7712	**[488]**	6578	**[417]**
F_2 females	↔	↔	↔	↔	↔	↑ 6%	7930	**[502]**	7515	**[476]**	7621	**[483]**	6247	**[396]**
Relative paired testis weight														
F_0 males	↔	↔	↔	↔	↔	↑ 27%	2924	**[185]**	2567	**[163]**	2998	**[190]**	2596	**[164]**
F_1 males	↔	↔	↔	↔	↔	↑ 18%	3287	**[208]**	2763	**[175]**	4106	**[260]**	3428	**[217]**
F_2 males	↔	↔	↔	↔	↔	↑ 24%	3086	**[195]**	2874	**[182]**	3245	**[206]**	2779	**[176]**
F_3 males	↔	↔	↔	↔	↔	↑ 19%	4329	**[274]**	2593	**[164]**	5010	**[317]**	3298	**[209]**
Relative paired epididymis weight														
F_0 males	↔	↔	↔	↔	↔	↑ 19%	3804	**[241]**	3072	**[195]**	5044	**[319]**	4068	**[258]**
F_1 males	↔	↔	↔	↔	↔	↑ 19%	2963	**[188]**	2566	**[163]**	3255	**[206]**	2786	**[17]**
F_2 males[c]	↔	↔	↔	↔	↑ 8%	↑ 24%	884	**[56]**	596	**[38]**	951	**[60]**	641	**[41]**
F_3 males	↔	↔	↔	↔	↔	↑ 22%	3449	**[218]**	2516	**[159]**	4117	**[261]**	3095	**[196]**
Relative liver weight														
F_0 females	↔	↔	↔	↔	↔	↑ 11%	7663	**[485]**	5848	**[370]**	7965	**[504]**	7439	**[471]**
F_2 females	↑	↔	↔	↔	↔	↑ 19%	6912	**[438]**	3650	**[231]**	7454	**[472]**	5533	**[350]**
Relative paired ovary weight]														
F_0 females	↔	↔	↔	↔	↔	↓ 19%	4103	**[260]**	3149	**[199]**	7126	**[451]**	5387	**[341]**
F_1 females	↔	↔	↔	↔	↔	↓ 15%	5754	**[364]**	3964	**[251]**	10,237	**[648]**	6966	**[441]**
F_2 females	↓ 15%	↔	↓ 15%	↓ 11%	↔	↓ 24%	7053	**[447]**	3520	**[223]**	7646	**[484]**	6360	**[403]**
No. with renal tubule degeneration														
F_0 females	0/12	0/12	0/12	0/14	0/12	4/13	6491	**[411]**	3848	**[244]**				
F_1 females	0/10	0/10	0/10	0/10	0/10	8/11	5498	**[348]**	2470	**[156]**				
F_2 females	0/11	0/10	0/12	0/11	0/12	7/13	5884	**[373]**	3018	**[191]**				
No. females with chronic liver inflammation														
F_0 females	0/12	1/12	0/12	0/14	1/12	3/13	4867	**[308]**	3214	**[204]**				
F_1 females	0/10	0/10	3/10	1/10	1/10	3/11								
F_2 females	1/11	0/10	2/12	2/11	2/12	5/13	3029	**[192]**	1856	**[118]**				
No. of implantation sites														
F_0 dams	↔	↔	↔	↔	↔	↓16%	4088	**[259]**	3021	**[191]**	8020	**[508]**	5832	**[369]**
F_1 dams[c]	↔	↔	↔	↔	↔	↓26%	6120	**[388]**	2383	**[151]**	7000	**[443]**	4713	**[298]**
F_2 dams	↔	↓8%	↔	↔	↔	↓18%	4917	**[311]**	3597	**[228]**	7679	**[486]**	5631	**[357]**
Epididymal sperm concentration, F_1	↔	↔	↔	↔	↔	↓18%	5012	**[317]**	3407	**[216]**	11,050	**[700]**	7407	**[469]**
Daily sperm production, F_3	↔	↔	↔	↔	↔	↓19%	7399	**[469]**	4025	**[255]**	8279	**[524]**	7596	**[481]**

[a]Tyl et al. (2002b).
[b]Based on target doses provided by the study authors and expressed as an average of the dose for males and females.
[c]Benchmark dose values were estimated using a polynomial model.
↑,↓ Statistically significant increase, decrease, ↔ no statistically significant effect.

significant effects observed in pups included increased numbers of live male and total pups and increased adjusted (for litter size) pup weight in the mid-dose group. Unadjusted and adjusted male and female pup weights were significantly increased at the high-dose. The study authors noted that the effects observed in this study were random and most likely due to chance. They concluded that bisphenol A did not induce adverse effects on fertility in male or female mice. It was noted that further studies using a better route of exposure are needed for bisphenol A.

Strengths/Weaknesses: This study appears to have been well conducted. When compared to studies that used the oral route of exposure, this study provides evidence that the manifestation of maternal toxicity is dependent on the route of administration and that route-dependent metabolism may be important for toxicity. However, the administration of bisphenol A via silastic

Table 94
Treatment-Related Effects in Developing Rats in a Multigeneration Reproductive Toxicity Study of Bisphenol A[a]

	Dose, ppm diet **[mg/kg bw/day[b]]**											
Endpoint	0.015 **[0.0095]**	0.3 **[0.019]**	4.5 **[0.285]**	75 **[4.75]**	750 **[47.5]**	7500 **[475]**	BMD_{10}		$BMDL_{10}$		BMD_{1SD}		$BMDL_{1SD}$	
Live pups/litter														
F_1	↔	↔	↔	↔	↔	↓ 20%	4232	**[268]**	3033	**[192]**	8823	**[559]**	6225	**[394]**
F_2	↔	↔	↔	↔	↔	↓ 26%	6661	**[422]**	2405	**[152]**	7241	**[459]**	4645	**[294]**
F_3	↔	↓ 11%	↔	↔	↔	↓ 26%	3733	**[236]**	2742	**[174]**	5943	**[376]**	4518	**[286]**
Pup body weight														
F_1, PND 4	↔	↔	↔	↔	↔	↓ 11%	6412	**[406]**	4473	**[283]**	8860	**[561]**	6317	**[400]**
F_1, PND 7	↔	↔	↔	↔	↔	↓ 23%	3432	**[217]**	2891	**[183]**	4179	**[265]**	3448	**[218]**
F_2, PND 7	↔	↔	↔	↔	↔	↓ 15%	5179	**[328]**	4059	**[257]**	6023	**[381]**	4653	**[295]**
F_3, PND 7	↔	↔	↔	↔	↔	↓ 13%	4976	**[315]**	3854	**[244]**	6474	**[410]**	4940	**[313]**
F_1, PND 14	↔	↔	↔	↔	↔	↓ 27%	2890	**[183]**	2570	**[163]**	2789	**[177]**	2415	**[153]**
F_2, PND 14	↔	↔	↔	↔	↔	↓ 20%	3840	**[243]**	3302	**[209]**	3579	**[227]**	3013	**[191]**
F_3, PND 14	↔	↔	↔	↔	↔	↓ 20%	3704	**[235]**	3224	**[204]**	3323	**[210]**	2827	**[179]**
F_1. PND 21	↔[c]	↔	↔	↔[c]	↔[c]	↓ 27%	3284	**[208]**	2621	**[166]**	3523	**[223]**	2763	**[175]**
F_2, PND 21	↔[c]	↔	↔	↔[c]	↔[c]	↓ 20%	4253	**[269]**	3566	**[226]**	4219	**[267]**	3473	**[220]**
F_3, PND 21	↔[c]	↔	↔	↔[c]	↔[c]	↓ 19%	3972	**[252]**	3423	**[217]**	3575	**[226]**	3016	**[191]**
Anogenital distance, F_2 females	↑ 3%	↑ 3%	↑ 3%	↔	↑4%	↔								
Age of vaginal opening adjusted for body weight														
F_1	↔	↔	↔	↔	↔	↑ 3.6 days	6225	**[394]**	5422	**[343]**	3248	**[206]**	2786	**[176]**
F_2	↔	↔	↔	↔	↔	↑ 4 days	6381	**[404]**	5307	**[336]**	4367	**[277]**	3600	**[228]**
F_3	↔	↔	↔	↔	↔	↑ 3.2 days	7444	**[471]**	6325	**[401]**	6249	**[396]**	3198	**[203]**
Age of preputial separation adjusted for body weight														
F_1	↔	↔	↔	↔	↑ 1.7 days	↑ 4.9 days	7350	**[466]**	6485	**[411]**	2974	**[188]**	2580	**[163]**
F_2	↔	↔	↔	↔	↔	↑ 7.4 days	4740	**[300]**	4025	**[255]**	3809	**[241]**	3201	**[203]**
F_3	↔	↔	↔	↔	↔	↑ 4 days	8637	**[547]**	7466	**[473]**	3503	**[222]**	2984	**[189]**

[a]Tyl et al. (2002b).
[b]Based on target doses provided by the study authors and expressed as an average of the dose for males and females.
[c]A significant (~5%) decrease in pup body weights observed only in F_1 and/or F_2 litters was apparently not considered treatment-related by study authors.
↑,↓ Statistically significant increase, decrease, ↔ no statistically significant effect.

implants makes the extrapolation for human risk assessment difficult in the absence of an improved pharmacokinetic understanding.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate but of limited utility for the evaluation process.

NTP (1985a) and **Morrissey et al. (1989)** sponsored a continuous breeding study in CD-1 mice exposed to bisphenol A (98% purity). Additional information on ovarian follicle counts in F_0 and F_1 females was published in a report by Bolon et al. (1997). In this study, mice were fed NIH-07 open formula rodent chow and housed in polypropylene or polycarbonate cages containing Ab-Sorb-Dri litter. The laboratory at which the study was conducted was stated to be in full compliance with GLP regulations. In the preliminary study (Task 1), 8 mice/sex/group (8 weeks old) were fed diet containing bisphenol A at 0, 0.3125, 0.625, 1.25, 2.5, or 5.0% for 14 days. By assuming that a 40-g mouse ingests 7 g feed/day, the study authors estimated bisphenol intake at 0, 437.5, 875.0, 1750.0, 4375.0, 8750.0 mg/kg bw/day. The aim of the preliminary study was to determine a maximum tolerated dose that induced significant toxicity but resulted in ≥90% survival and ≤10% decrease in weight gain. Statistical analyses included ANOVA, and χ^2 test. Lethality was significantly increased in the high-dose group. Body weight gain was depressed in groups exposed to ≥1.25% bisphenol A. Clinical signs of toxicity were observed in the 2.5 and 5.0% dose groups and included dehydration, dyspnea, lethargy, tremors, ptosis, piloerection, and diarrhea.

In the reproduction and fertility study (Task 2), 11-week-old mice were randomly assigned to treatment groups according to body weight. The mice were fed diets containing 0, 0.25, 0.5, or 1.0% bisphenol A. The NTP stated that a 40-g mouse consuming 7 g of feed/day would be exposed to bisphenol A at 437.5, 875, and 1750 mg/kg bw/day. **[Based on body weight and feed intake values reported for males at ~3 week intervals, CERHR estimated mean bisphenol A intake at ~365, 740, and 1630 mg/kg bw/day. Feed intakes were reported only at Week 1 and 18 for females, and Week 18 most likely represented the lactation period. For Week 1, bisphenol A intake by females was estimated at 410, 890, and 1750 mg/kg bw/day. At Week 18, bisphenol A intake by females was estimated at 1090, 1785, and 3660 mg/kg bw/day.]** There were 40 mice/sex in the vehicle control group and 20/sex in each bisphenol A group. Exposure to bisphenol A began during a 7-day premating period. Following the premating period, males and females from the same treatment group were randomly paired and housed together for 98 days and following the mating period, each male and female was housed separately for 21 days. Bisphenol A dosing was continued throughout the mating and separation period. Concentration and stability of bisphenol A in feed were verified. During the 98-day cohabitation period, pups born were counted, sexed, and weighed. All litters

Table 95
Effects Observed in Adult Mice Dosed With Bisphenol A in a Continuous Breeding Study[a]

Endpoint	Dose, % in diet **[mg/kg bw/day]**						
	0.25 **[437.5]**	0.5 **[875]**	1.0 **[1750]**	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$
F_0 males and females							
Litters/pair	↔	↓ 5%	↓ 9%	1.0 **[1750]**	0.74 **[1295]**	0.96 **[1680]**	0.66 **[1155]**
Postpartum dam weight[b]	↔	↔	↓ 6–9%	1.0 **[1750]**	0.83 **[1452]**	0.87 **[1522]**	0.66 **[1155]**
Necropsy dam weight	No data	No data	↓ 4%				
Percent motile sperm	No data	No data	↓39%				
Relative organ weight, males[c]							
Liver	No data	No data	↑ 29%				
Kidney/adrenal	No data	No data	↑ 16%				
Seminal vesicle	No data	No data	↓ 19%				
Relative organ weight, females[c]							
Liver	No data	No data	↑ 27%				
Kidney/adrenal	No data	No data	↑ 10%				
Liver lesions, males and females[d]	No data	No data	↑[e]				
Kidney lesions, males and females[d]	No data	No data	↑[e]				
F_1 males and females							
Relative organ weight, males[c]							
Liver	↑ 7%	↑ 7%	↑ 29%	0.62 **[1085]**	0.42 **[735]**	0.59 **[1032]**	0.39 **[682]**
Kidney/adrenal[f]	↑ 16%	↑ 20%	↑ 20%	0.18 **[315]**	0.14 **[245]**	0.15 **[262]**	0.12 **[210]**
Left testis/epididymis[f]	↔	↓ 10%	↓ 9%	0.64 **[1120]**	0.32 **[560]**	0.53 **[928]**	0.27 **[472]**
Right testis[g]	↔	↓ 13%	↔				
Right epididymis[f]	↓ 11%	↓ 16%	↓ 18%	0.24 **[420]**	0.15 **[262]**	0.46 **[805]**	0.25 **[438]**
Seminal vesicle	↓ 11%	↔	↓ 28%	0.40 **[700]**	0.29 **[508]**	0.66 **[1155]**	0.47 **[822]**
Relative organ weight, females[c]							
Liver	↑ 6%	↑ 13%	↑ 20%	0.49 **[858]**	0.38 **[665]**	0.45 **[788]**	0.35 **[612]**
Kidney/adrenal[g]	↑ 13%	↑ 15%	↑ 13%				
Percent motile sperm[g]	↔	↓ 31%	↔				
Liver lesions, males[d]	↑[e]	↑[e]	↑[e]				
Liver lesions, females[d]	↔	↑[e]	↑[e]				
Kidney lesions, males and females	↑[e]	↑[e]	↑[e]				

[a]NTP (1985a).
[b]Values were reported following the birth of 5 litters, the benchmark doses are for values reported following the birth of the fifth litter because the greatest magnitude of effect was observed at that time point.
[c]Relative organ weights were adjusted for body weight; when absolute and relative organ weights changed in the same direction, only the relative organ weights were listed in this table.
[d]See text for a description of the types of lesions observed.
[e]It does not appear that statistical analyses were conducted for histopathology data, but incidence was increased compared to controls.
[f]Benchmark doses were estimated using a polynomial model.
[g]Benchmark doses were not estimated for endpoints without dose-response relationships.
↑,↓ Statistically significant increase, decrease compared to controls; ↔ no statistically significant effects compared to controls.

Table 96
Effects in Immature F1 Mice in a Continuous Breeding Study With Bisphenol A

Endpoint	Dose, % in diet **[mg/kg bw/day]**						
	0.25 **[437.5]**	0.5 **[875]**	1.0 **[1750]**	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$
Live pups/litter	↔	↓ 20%	↓ 48%	0.30 **[525]**	0.20 **[350]**	0.43 **[752]**	0.30 **[525]**
Proportion pups born alive	↔	↔	↓ 4%	3.0 **[5250]**	0.79 **[1382]**		
Live birth weight[b]	↔	↑5%	↑ 6%	0.43 **[752]**		0.34 **[595]**	
Mortality by PND 21[c]	↔	↔	↑ to 37.5%	0.48 **[840]**	0.40 **[700]**		

[a]NTP (1985a).
[b]Hill model used for benchmark dose calculations.
[c]Control mortality was 6.3%. Mortality was reported on a per pup basis, which limits the utility of the benchmark dose model.
↑,↓ Statistically significant increase, decrease compared to controls; ↔ no statistically significant effects compared to controls.

excluding the last one born were killed on the day of birth so that animals could continue mating. The last litter was raised by the dam and weaned on PND 21 (day of birth not defined). Birth weight and weight gain were recorded in the last litter. Reproductive endpoints in parental rats included the number of litters born and fertility. Statistical analyses included Kruskal–Wallis ANOVA on ranks, Mann–Whitney *U* test, χ^2 test, one-way ANOVA, arc sine square-root transformation, and Duncan multiple range test.

Table 97
Effects Observed in Mice Fed Bisphenol A-Containing Feed for One Generation[a]

Endpoint	Dose, % in diet (mg/kg bw/day)					
	0.5 (840–1055)[b]	1 (1669–1988)[b]	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$
F_0 females body weights and feed intake						
GD 17 body weight[c,d]	↓ 8%	↓ 11%	1292	646	742	404
PND 0 body weight[d]	↔	↓ 7%	2130	1675	1813	1193
GD 0–17 body weight change[c,d]	↓ 16%	↓ 19%	472	283	701	387
Study day 0–7 feed intake	↑11%	↔				
GD 14–17 feed intake (g/day)	↔	↓ 13%	1454	898	1840	1172
GD 0–17% food efficiency	↓ 16%	↓ 16%				
Relative (to body weights) organ weights in F_0[e]						
Liver, male	↑ 22%	↑ 24%	706	561	705	555
Liver, female	↑ 27%	↑ 29%	615	484	746	586
Kidney, female	↑ 8%	↑ 24%	973	529	1309	863
Clinical chemistry effects in F_0 females, not examined in males						
Blood urea nitrogen	↔	↑ 43%	628	266		
Sodium	↓ 9%	↔				
Potassium	↓ 18%	↔				
Chloride	↓ 8%	↔				
Histopathology in F_0 females (not examined in males)[f]						
Renal tubule epithelium degeneration (control: 0/20)	9/20	9/20				
Renal tubule epithelium necrosis (control: 0/20)	6/20	8/20	663	480		
Renal tubule regeneration (control: 2/20)	12/20	20/ 20	223	151		
Centrilobular hepatocyte hypertrophy (control 0/20)	2/20	11/20	902	612		
Diffuse hepatocyte hypertrophy (control 0/20)	6/20	6/20				
Reproductive/developmental effects						
Gestational length	↑ 2%	↑ 2%				
No. of live pups	↔	↓ 15%	1116	727	1925	1189
Total no. of pups	↔	↓ 15%	1116	727	1925	1189
Female pup body weight	↓0.6%[g]	↓4%[g]	2281	1728	2332	1733

[a]Tyl et al. (2002a).
[b]Bisphenol A intakes included values estimated for males and females during prebreeding or gestation; intake values for the appropriate sex were used in benchmark dose analyses; intakes during gestation were used for females.
[c]The effect was reported at earlier time period but is shown here only for the latest or longest time period evaluated.
[d]Benchmark doses were estimated using the polynomial model.
[e]Only effects on relative organ weights were shown.
[f]Histopathology data were not statistically analyzed.
[g]By trend test.
↑,↓ Statistically significant increase, decrease compared to controls; ↔ no statistically significant effect.

In the cross-over trial (Task 3), ~20 males and females from the high-dose group were randomly paired with control mice for 7 days in order to determine the affected sex. Twenty control males and females were also paired. The animals were not exposed to bisphenol A during the 1-week mating period, but in animals from the high-dose group, dosing with bisphenol A was continued for 21 days on separation of the mating pairs. Vaginal smears were obtained from females that did not mate or did not appear to be pregnant. Fertility and offspring survival were determined. Parental mice from the control ($n = 38$/sex) and high-dose groups ($n = 19$/sex) were necropsied within a week following completion of the cross-over trial. Body, liver, kidney, and reproductive organ weights were obtained, and sperm count, morphology, and motility were determined. Testes, ovaries, and oviducts were fixed in Bouin solution and prostate, seminal vesicles/coagulating glands, uterus, liver, and kidney were fixed in 10% neutral buffered formalin for histopathological evaluation.

In Task 4 of the study, 20 F_1 mice/sex/group (at least 2/sex from 10 randomly selected litters/group) were mated within dose groups for 7 days and examined for reproductive function. Because fewer F_1 mice in high-dose group were available as a result of increased mortality, only 11 mice/sex were mated. The animals continued to receive the same diet given to their parents. Vaginal smears were obtained from females that did not mate or did not appear to become pregnant. One litter/pair was examined for sex, body weight, and viability. The parental F_1 animals from all dose group were killed and examined as described for Task 3 of the study.

Treatment-related effects observed in adult rats are summarized in Table 95, and effects occurring in immature rats are summarized in Table 96. Bisphenol A treatment had no effect on mating or fertility index in F_0 or F_1 mice. Postpartum body weights were reduced in F_0 dams of the high-dose group. In F_0 mice, the number of litters produced/pair and numbers of live F_1 pups/litter were reduced at the mid- and high-dose level. A decrease in the proportion of pups born alive occurred in F_0 mice of the high-dose group. No effects were observed on sex ratios of F_1 or F_2 pups. Weights of live F_1 pups were increased at the mid and high-dose. There were no significant effects when pup weights were adjusted for total numbers of live and dead pups in the litter.

Table 98
Treatment-Related Effects in Adult Mice Fed Bisphenol A Through Diet in a Multigeneration Reproductive Toxicity Study

	Dose, ppm diet **[mg/kg bw/day based on target intakes provided by study authors]**													
Endpoint	0.018 [0.003]	0.18 [0.03]	1.8 [0.3]	30 [5]	300 [50]	3500 [600]	BMD_{10}		$BMDL_{10}$		BMD_{1SD}		$BMDL_{1SD}$	
Body weight gain during lactation, F_0	↔	↔	↔	↔	↔	↑2.2–fold	249	[42.2]	150	[25.4]	4258	[722]	2941	[498]
Terminal body weight F_1 retained males	↔	↔	↔	↔	↔	↓10%	3455	[586]	2388	[405]	3503	[594]	2608	[442]
Relative liver to body weight														
F_0 males	↔	↔	↔	↔	↔	↑17%	2189	[371]	1820	[308]	2021	[343]	1668	[283]
F_1 parental males	↔	↔	↔	↔	↑5%	↑22%	1662	[282]	1425	[242]	1637	[277]	1389	[235]
F_1 retained males	↔	↔	↔	↔	↔	↑23%	1584	[268]	1383	[234]	1685	[286]	1405	[238]
F_0 females	↔	↔	↔	↔	↔	↑17%	2524	[428]	1595	[270]	3014	[511]	2155	[365]
F_1 females	↔	↔	↔	↔	↔	↑10%	3424	[580]	2438	[413]	3551	[602]	3024	[513]
Relative right kidney to body weight														
F_0 males	↔	↔	↔	↔	↑8%	↑20%	1861	[315]	1536	[260]	2100	[356]	1723	[292]
F_1 parental males	↔	↔	↔	↑11%	↑10%	↑21%	2079	[352]	1913	[324]	862	[146]	773	[131]
F_1 retained males	↔	↔	↔	↔	↔	↑27%	1501	[254]	1229	[208]	1978	[335]	1610	[273]
F_0 females	↔	↔	↔	↔	↔	↑13%	3568	[605]	2504	[424]	4326	[733]	3041	[515]
F_1 females	↔	↔	↔	↔	↔	↑8%	3629	[615]	2976	[504]	3702	[627]	3393	[575]
Relative left kidney to body weight														
F_0 males	↔	↔	↔	↔	↑9%	↑19%	1899	[322]	1548	[262]	2249	[381]	1825	[309]
F_1 parental males	↔	↔	↔	↑13%	↑10%	↑22%	2074	[352]	1650	[280]	2547	[432]	2020	[342]
F_1 retained males	↔	↔	↔	↔	↑11%	↑28%	1466	[248]	1205	[204]	1937	[328]	1582	[268]
F_0 females	↔	↔	↔	↔	↔	↑11%	3746	[635]	2550	[432]	4773	[809]	3258	[552]
Relative pituitary to body weight														
F_1 parental males[a]	↔	↔	↔	↔	↔	↑10%	3413	[578]	2087	[554]	3627	[615]	3182	[539]
F_1 retained males[a]	↔	↔	↔	↔	↔	↑16%	2678	[454]	1934	[328]	3476	[589]	2512	[426]
Relative brain to body weight F_1 retained males[a]	↔	↔	↔	↔	↔	↑9%	2678	[454]	1934	[328]	3476	[589]	2512	[426]
Hepatocyte centrilobular hypertrophy incidence (control incidence in parentheses)														
F_0 males (6/56)	1/10	2/10	2/10	0/10	4/10	10/10	122	[20.7]	70	[11.8]				
F_1 parental males (7/55)	0/10	0/10	4/10	2/10	1/10	6/10	879	[149]	578	[98.0]				
F_1 retained males (4/50)	1/10	3/10	2/10	2/10	5/10	7/10	656	[111]	442	[74.9]				
F_0 females (1/56)	0/10	0/10	0/10	0/10	1/10	6/10	1348	[228]	947	[161]				
F_1 females (2/55)	0/10	0/10	0/10	0/10	3/11	7/10	962	[163]	679	[115]				
Renal nephropathy incidence (control incidence in parentheses)														
F_0 males (12/56)	0/10	3/10	2/10	2/10	1/10	4/10	1556	[264]	750	[127]				
F_1 parental males (6/55)	2/10	0/10	1/10	2/10	0/10	4/10	1418	[240]	838	[142]				
F_1 retained males (8/50)	1/10	0/10	0/10	2/10	0/10	3/10	1991	[337]	992	[168]				
F_1 females (10/55)	1/10	3/10	0/10	1/10	1/11	4/11	1646	[279]	847	[144]				
Paraovarian cysts (control incidence in parentheses)														
F_0 (9/56)	1/11	2/12	1/11	1/12	3/14	7/17	1328	[225]	833	[141]				
F_1 (14/55)	1/11	1/11	1/10	2/10	2/11	7/15	1193	[202]	708	[120]				
Epididymal sperm concentration, F_0[a]	↔	↔	↔	↔	↔	↓15%	3343	[567]	1884	[319]	3581	[607]	3241	[549]
Gestational length														
F_0	↔	↔	↔	↔	↔	↑2%	21,351	[3619]	3770	[639]	6749	[1144]	3536	[599]
F_1	↔	↔	↔	↔	↔	↑2%	17,820	[3020]	3784	[641]	4552	[772]	3134	[531]

[a]Not considered a treatment-related effect by study authors.
↑,↓ Statistically significant increase, decrease; ↔ no statistically significant effect compared to controls.

Table 99
Treatment- or Dose-Related Effects in Developing Mice in a Multigeneration Reproductive Toxicity Study With Bisphenol A

	Dose, ppm diet **[mg/kg bw/day based on target intakes provided by study authors]**									
End point[a]	0.018 **[0.003]**	0.18 **[0.03]**	1.8 **[0.3]**	30 **[5]**	300 **[50]**	3500 **[600]**	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$
Body weight										
F_1, PND 7	↔	↔	↔	↔	↔	↓13%	3304 **[560]**	1849 **[313]**	3433 **[582]**	2403 **[407]**
F_1, PND 14	↔	↔	↔	↔	↔	↓11%	3453 **[585]**	2256 **[382]**	3639 **[617]**	2988 **[506]**
F_1, PND 21	↔	↔	↔	↔	↔	↓17%	3236 **[548]**	1577 **[267]**	3421 **[580]**	2342 **[370]**
F_1 male, PND 21 necropsy	↔	↔	↔	↔	↔	↓12%	3325 **[564]**	1845 **[313]**	3776 **[640]**	3536 **[599]**
F_1 female, PND 21 necropsy	↔	↔	↔	↔	↔	↓18%	2284 **[387]**	1501 **[254]**	4577 **[776]**	3529 **[598]**
Lactational survival indices (control index, %, in parentheses)										
F_2 PND 21 survival (100%)[c]	↔	↔	↔	↔	↓ to 86.6%	↔				
F_2 Lactational index (97.2%)[c]	↔	↔	↔	↔	↓ to 86.6%	↔				
Relative thymus to body weight, F_1 male, PND 21[b]	↔	↔	↔	↔	↑ 13%[b]	↑ 10%[b]				
Relative spleen to body weight										
F_1 male, PND 21	↔	↓ 12%	↔	↔	↔	↓ 30%	3123 **[529]**	1074 **[182]**	3538 **[600]**	3148 **[534]**
F_2 male, PND 21	↔	↔	↔	↔	↔	↓ 20%	2148 **[364]**	1425 **[242]**	7013 **[1189]**	3560 **[603]**
F_1 female, PND 21	↔	↔	↔	↔	↔	↓ 23%	3168 **[537]**	647 **[110]**	4571 **[775]**	3677 **[623]**
F_2 female, PND 21	↔	↔	↔	↔	↔	↓ 21%	1787 **[303]**	1311 **[222]**	5022 **[851]**	3517 **[596]**
Relative paired testes weight to body or brain weight										
F_1, PND 21 (body weight)	↔	↔	↔	↔	↔	↓ 8%	3578 **[606]**	2720 **[461]**	3861 **[654]**	3550 **[602]**
F_2, PND 21 (brain weight)	↔	↔	↔	↔	↔	↓ 11%	3316 **[562]**	2003 **[339]**	5342 **[905]**	3571 **[605]**
Relative paired epididymides to body weight, F_1[b]	↔	↑ 18%	↔	↔	↔	↔				
Relative brain to body weight F_1 female, PND 21[b]	↔	↔	↔	↔	↔	↑ 17%[b]	2219 **[376]**	1415 **[240]**	3576 **[606]**	2825 **[479]**
Relative left kidney to body weight, F_2 male, PND 21	↔	↔	↔	↔	↔	↑ 6%	6664 **[1129]**	3540 **[600]**	8501 **[1441]**	3589 **[608]**
Relative seminal vesicles with coagulating gland to brain weight, F_2 [b]	↔	↔	↔	↔	↓ 15%	↓ 16%	2389 **[405]**	1315 **[223]**	11,294 **[1914]**	3631 **[615]**
Uterus with cervix and vagina weight relative to bodyweight, F_2 PND 21[b]	↔	↔	↔	↔	↓16%	↔				
Relative paired ovary weights, F_1[b]	↔	↔	↑	↔	↔	↔				

Hepatic cytoplasm alteration (control incidence in parentheses)										
F_1 males (6/44)	1/26	0/17	1/22	6/24	10/20	13/20	732 **[124]**	546 **[92.5]**		
F_2 males (6/54)	1/25	1/25	1/25	1/24	2/20	9/23	1442 **[244]**	1050 **[178]**		
F_1 females (2/46)	1/27	2/21	3/24	4/26	8/16	6/22	1966 **[333]**	1182 **[200]**		
Unilateral hydronephrosis, F_1 males (0/44)[b]	0/26	0/17	0/21	0/24	0/21	3/21b				
Seminiferous tubule hypoplasia (control incidence in parentheses)										
F_1 (1/96)	0/54	0/37	1/45	3/51	2/45	5/43	3485 **[591]**	2398 **[406]**		
F_2 (5/114)	1/53	2/61	2/55	0/51	5/49	20/57	1670 **[283]**	1377 **[233]**		
Undescended testis, F_1 PND 21 (control 11/135)	5/79	5/54	10/70	5/78	7/50	12/600	2694 **[462]**	1755 **[301]**		
Anogenital distance adjusted for body weight F_1 male, PND 21[b]	↔	↔	↔	↔	↓4%	↓5%	8099 **[1373]**	3582 **[607]**	10,436 **[1769]**	3632 **[616]**
Age of preputial separation (adjusted per body weight)										
F_1 parental males	↔	↔	↔	↔	↔	↑2 days	4450 **[754]**	3397 **[576]**	3252 **[551]**	2445 **[414]**
F_1 retained males	↔	↔	↓0.6 days	↔	↔	↑1.8 days	4288 **[727]**	3375 **[572]**	2897 **[491]**	2145 **[364]**
Body weight on day of vaginal opening in F_1	↔	↔	↔	↔	↔	↓22%	3076 **[521]**	1281 **[217]**	3294 **[558]**	1972 **[334]**
Age of vaginal opening adjusted for PND 21 body weight[b]	↔	↔	↔	↔	↔	↓2.4	3501 **[593]**	2953 **[501]**	3404 **[577]**	2419 **[410]**

[a]Based on numbers of animals listed in data tables, it appears that statistical analyses in live animals before or on PND 21 considered the litter as the statistical unit, but statistical analyses conducted at PND 21 necropsy considered the individual pup as the statistical unit.
[b]Not considered treatment related by study authors.
[c]Effect was not discussed by study authors but it is unlikely to be related to treatment.

Therefore the NTP concluded that the increased pup weights resulted from the smaller litter size. Body weights were evaluated through PND 21 in F_1 pups, and no effects were found on pup body weight gain during the lactation period. Mortality in F_1 offspring during the postnatal period was increased in the high-dose group.

The cross-over test revealed no effect on mating or fertility in either males or females exposed to bisphenol A. Postpartum body weight was not affected in the treated females. The number of live pups/litter was significantly reduced **[by 26%]** in the group containing treated males and **[by 51%]** in the group containing treated females. Live pup weight was increased in the group containing treated females, but there was no significant effect following adjustment for litter size. There were no effects on the proportion of pups born alive or on sex ratio.

In sperm analyses conducted in high-dose F_0 males and all dose groups of F_1 males, sperm motility was reduced in high-dose F_0 males and mid-dose F_1 males. There were no effects on sperm count or morphology in either generation. Effects were observed on organ weights, which were examined in F_0 adults of the high-dose group and F_1 animals from each treatment group. Effects on absolute reproductive organ weights of F_1 mice included decreased right epididymis weight at all doses, decreased left testis/epididymis weight at the mid and high-dose, and decreased seminal vesicle weight at the high-dose. Significant effects on relative organ weights adjusted for body weight in F_1 rats included decreased right epididymis weight at all doses, decreased seminal vesicle weight at the low and high-dose, and decreased relative left testis and epididymis weight at the mid and high-dose. Reproductive organ weight effects observed in high-dose F_0 males included decreased absolute and relative seminal vesicle weight. There were no effects on prostate weight. No effects were reported for estrous cyclicity of F_0 females. There were no gross or histopathological alterations in F_0 or F_1 reproductive organs including testis, epididymis, prostate, seminal vesicles, ovary, vagina, and uterus. Effects observed in high-dose F_0 animals were also summarized in a report by Morrissey et al. (1988).

Effects were observed on non-reproductive organ weights, which were examined in F_0 adults of the high-dose group and F_1 animals from each treatment group. In the F_1 mice, dose-related effects on absolute organ weights included increased kidney/adrenal weight at all doses in both sexes and increased liver weight in mid- and high-dose females and high-dose males. Significant effects on relative organ weight adjusted for body weight in F_1 rats included increased liver and kidney/adrenal weights at all doses in both sexes. Organ weight effects observed in high-dose F_0 males included increased absolute and relative liver and kidney/adrenal weight. In F_0 female rats of the high-dose group, absolute and relative liver weight and relative kidney weights were increased. Body weights of high-dose F_0 females were reduced at necropsy. Histopathology was examined in F_0 rats of the high-dose group and F_1 rats from all dose groups. Treatment-related hepatic lesions observed in both generations included multifocal necrosis, multinucleated giant hepatocytes in males and females, and centrilobular hepatocytomegaly in males. Multifocal mineralization of liver cells was also observed in F_1 females of the high-dose group. Hepatic lesions were observed at all dose levels for F_1 males and in F_1 females of the mid- and high-dose group. Treatment-related renal lesions were observed in both generations and described as tubular cell nuclear variability, increased severity of spontaneous tubular interstitial lesions, cortical tubular dilatation, mineralization of renal cells, and micro-calculi in tubular epithelium that sometimes occurred with effaced tubular epithelium, tubular regeneration, and/or dilated tubules containing casts. **[It appears that the incidence of renal lesions was increased at all doses in F_1 rats.]** Renal lesions were stated to generally be more prominent in females than males. The study authors concluded that exposure of mice to bisphenol A resulted in toxicity to the reproductive system, kidney, and liver. The possibility was noted that some or all effects on reproductive performance may have been secondary to the generalized toxicity of bisphenol A.

This study demonstrates changes in F_1 male absolute reproductive weights (seminal vesicle with coagulating gland as well as epididymis; the testis and prostate appear not to have been appreciably affected). This study also suggested that reproductive toxicity and general toxicity occurred at similar dose levels. Bisphenol A-mediated general toxicity may have contributed to the observed female fertility effect, because this effect was noted with dosed females cohabiting with non-dosed males. In the male, however, the effect on motility is likely bisphenol A-related, resulting in the observed fertility deficits.

In Task 2, a clear effect on fertility was found with a NOAEL of 0.25% bisphenol A in the diet.

Strengths/Weaknesses: This comprehensive toxicology study was well-conducted. General toxicity was clearly demonstrated at all F_1 dose levels, and histopathological findings appear to be a sensitive indicator of effect. As a limitation of this design, because bisphenol A was in the diet, exposure to bisphenol A did not occur during cohabitation; therefore, direct exposure to bisphenol A was minimal or nonexistent during sperm maturation, capacitation and ovulation.

Utility (Adequacy) for CERHR Evaluation Process: These data are adequate and of high utility for the evaluation process.

Tyl et al. (2002a), sponsored by the Society of the Plastics Industry, conducted a one-generation reproductive toxicity study in mice. The study was conducted to verify the findings of reduced pup numbers at birth in a continuous breeding study conducted by the NTP (NTP, 1985a). GLP guidelines were applied in the conduct of the study. CD-1 mice were fed Purina Certified Rodent Diet Meal and housed in polycarbonate cages containing Sani-chip bedding. Mice were stratified according to body weight and randomly assigned to treatment groups. Starting at 9 weeks of age, 20 mice/sex/group were given feed containing bisphenol A (99.36% purity) 0, 5000, or 10,000 ppm. Males and females were fed the bisphenol A-containing diets during a 2-week prebreeding period and a 1-week mating period. The day of vaginal plug detection was defined as GD 0. Exposures in females continued through the gestation period of ~19 days. The study authors reported bisphenol A intakes of 0, 840, and 1669 mg/kg bw/day in males during the prebreeding period; 0, 1055, and

1988 mg/kg bw/day in females during the prebreeding period, and 0, 870, and 1716 mg/kg bw/day in females during the gestation period. **[Intake values were obtained from the Results section and study summary tables. They differed from values reported in text Table C, which were assumed to be in error.]** Homogeneity and stability of bisphenol A in feed were verified. Parameters evaluated during the study included clinical signs, body weight, and feed intake. Reproductive endpoints evaluated included implantation loss and indices of mating, fertility, pregnancy, and gestation. F_0 Males were killed at the end of the breeding period; liver and kidney were weighed. At birth, pups were counted, sexed, weighed, and evaluated for viability and external alterations. F_0 females and F_1 pups were killed on the day of parturition (PND 0). Dams were assessed for clinical chemistry parameters of liver and kidney function; corpora lutea and implantation sites; uterus, ovary, kidney, and liver weight; and liver and kidney histopathology. The male, female, pregnant female, or the litter were considered statistical units. Statistical analyses included ANOVA, Levene test, GLM procedure, Dunnett test, χ^2 test, Cochran–Armitage test, and Fisher exact probability test.

Treatment-related effects in F_0 animals are summarized in Table 97. There were no treatment-related changes in clinical signs, body weight gain, feed intake, or food efficiency in males or in females during the prebreeding period. A transient increase in food intake occurring in females of the low-dose group on study stays 0–7 did not appear to be treatment-related. Gestational body weight gain was decreased in the high-dose group, beginning on GD 7 and in the low dose group beginning on GD 10. Body weights of live F_0 females were significantly lower in the high-dose group on PND 0, but no significant differences were observed during necropsy conducted later in the day. A significant decrease in feed intake was reported for the high-dose group on GD 14–17, only when the values were expressed as g/day. **[The results section indicated that food efficiency during gestation was not significantly affected, but a downward trend was observed. Table 10 of the study reported a significant decrease in food efficiency.]** Significant necropsy findings observed in males included increased absolute and relative liver weight at both doses and increased absolute paired kidney weight at the low dose. Absolute and relative liver and paired kidney weight were increased significantly in females from both dose groups. Histopathological observations in females included dose-related increases in incidence and severity of hepatocyte hypertrophy and increased kidney lesions (renal tubular epithelial necrosis, degeneration, and regeneration) in both dose groups. Significant clinical chemistry findings in females included increased blood urea nitrogen in the high-dose group and decreased sodium, potassium, and chloride levels in the low-dose group.

Treatment-related reproductive or developmental effects are summarized in Table 97. No significant effects were observed for mating, fertility, or pregnancy indices; time to insemination; numbers of ovarian lutea or implantation sites; or implantation loss. Gestation duration was extended by ~10 hr in both dose groups; the study authors stated that the biological significance of the finding is not known. Total and live pup numbers were decreased in the high-dose group. No significant effects on pup weight were observed but a downward trend was statistically identified for female pup weight.

The study authors concluded that their study confirmed the NTP (1985a) finding of reduced litter size in mice fed 10,000 ppm bisphenol A in feed. The NTP finding of decreased litter size at 5000 ppm bisphenol A was not confirmed in this study, likely due, according to the authors, to the shorter exposure duration in the current study than in the NTP study. The study authors concluded that the litter size decreases in their study were likely caused by the compromised status of dams.

Strengths/Weaknesses: Strengths of this report include the comprehensive design with the assessment of multiple relevant endpoints. There were adequate numbers of animals, the doses and stability of the compound were verified, and the oral route of exposure was used. Weaknesses include the limited number of doses examined and the relatively high-doses studied.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and useful for the evaluation process.

Tyl et al. (2006), sponsored by the American Plastics Council, conducted a two-generation study of bisphenol A in mice. The study was conducted accorded to GLP. CD-1 mice were received in two cohorts approximately 2 weeks apart and data from the two cohorts were combined. Mice were fed Purina Certified Ground Rodent Diet No. 5002. The supplier provided information about phytoestrogen content of feed (177–213 ppm genistein, 173–181 ppm daidzein, and 39–55 ppm glycitein). Mice were housed in polypropylene cages with Sani-Chip bedding. Assignment of F_0 animals to groups involved randomization stratified by weight. F_0 and F_1 mice (28 sex/group/generation) were fed diets containing bisphenol A (99.70–99.76% purity) at 0.018, 0.18, 1.8, 30, 300, or 3500 ppm. Target intakes were 0.003, 0.03, 0.3, 5, 50, or 600 mg/kg bw/day, respectively. Based on measured feed intake, the study authors estimated bisphenol A intake in males at 0.0024–0.0038, 0.024–0.037, 0.24–0.37, 3.98–6.13, 39.1–60.8, or 529–782 mg/kg bw/day. Bisphenol A intakes (in mg/kg bw/day) by females were estimated at 0.0030–0.0041, 0.030–0.042, 0.32–0.43, 5.12–7.12, 54.2–67.8, 653–910 during the premating period; 0.0027–0.0029, 0.027–0.028, 0.28–0.29, 4.65–4.80, 47.0–48.6, 552–598 during the gestation period; and 0.0063–0.0087, 0.062–0.091, 0.61–0.89, 10.4–15.1, 103.2–146.4, 1264–1667 during the lactation period. In each generation, there were 2 vehicle control groups with 28 mice/sex/group. A positive control group was given feed containing 17β-estradiol at 0.5 ppm (target intake of 0.08 mg/kg bw/day). Estimated intakes for 17β-estradiol (in mg/kg bw/day) were 0.074–0.104 in males, 0.093–0.12 in females during the pre-mating period, 0.08–0.081 in females during the gestation period, and 0.160–0.25 in females during the lactation period. Dose selections were based on observations from several studies. **[The Expert Panel notes that a separate 2-generation study was used to characterize the dose–response relationship for 17β-estradiol.]** Homogeneity, stability, and concentration of bisphenol A in feed were verified. Exposure of F_0 mice began at ~6 weeks of age. Exposure of F_1 animals began at weaning, although it was noted that pups began eating the dosed feed in the late lactation period. F_0 and F_1 mice were fed the bisphenol A-containing diets for a minimum of 8

weeks before mating and during a 2-week mating period. Exposures of males continued through the gestation period of the litters they sired. Exposures of females continued through the gestation and lactation period. During the study, adult animals were monitored for clinical signs of toxicity, body weight, and food intake.

Estrous cycles were evaluated in F_0 and F_1 females during the last 3 weeks of the pre-breeding exposure period. Day of vaginal plug was defined as GD 0 and day of birth was considered PND 0. F_1 and F_2 pups were counted, sexed, weighed, and assessed for viability and physical abnormalities at birth and throughout the lactation period. Anogenital distance was measured in F_1 and F_2 pups at birth and on PND 21. On PND 4, F_1 and F_2 litters were standardized to 10 pups, with equal numbers per sex when possible. Pups removed on PND 4 were killed and examined for visceral alterations, with a focus on the reproductive system. The remaining pups were maintained and weaned on PND 21. At weaning, 28 F_1 pups/sex/group (1 per sex per litter) were randomly selected for mating and those animals were referred to as parental mice. An additional F_1 male/litter was selected for a 3-month exposure (referred to as retained males). Two F_1 pups/sex/litter were selected for gross necropsy and organ weight measurement at weaning. Histopathological examination of reproductive organs was conducted in one PND 21 pup/sex/litter. Histopathological evaluation of reproductive and systemic organs were conducted in the second F_1 pup from each group at weaning. All F_2 pups were killed at weaning and organ weights were measured. Vaginal opening and preputial separation were monitored in parental and retained F_1 mice. Parental F_0 and F_1 males were killed following delivery of the litters they sired. Retained F_1 males were killed at the same time as the parental F_1 males. Parental F_0 and F_1 females were killed after their pups were weaned. Organs, including those of the reproductive system, were weighed in adult F_0 and F_1 animals. Histopathological evaluations were conducted in all animals from the vehicle control group, in 10 F_0 and F_1 parental animals from each treatment group, in all F_1 retained males, and 10 animals from the 17β-estradiol positive control group. Histopathological evaluation of reproductive organs was also conducted in animals with suspected reduced fertility. Testes were preserved in Bouin fixative. Daily sperm production, efficiency of daily sperm production, and epididymal sperm count, motility, and morphology, were evaluated in F_0 and F_1 males. Data from the 2 control groups were analyzed separately and then pooled for statistical analysis of treatment groups. Statistical analyses included ANOVA, Levene test, robust regression methods, Wald χ^2 test, *t*-test, Dunnett test, Fisher exact probability test, and ANCOVA.

Treatment- or dose-related results and observations in reproductive organs of adult animals are summarized in Table 98. There were no consistent effects on body weight or body weight gain in F_0 males. Body weight gain during lactation was increased in F_0 females from the 3500 ppm group. During the premating period, body weights were decreased by $\leq 10\%$ in F_1 parental animals from the 3500 ppm group (study days 0, 7, 49, and 56 in males and study 0 in females). In retained F_1 males from the 3500 ppm group, body weights were decreased at most time periods between study days 7 and 84 and at necropsy. No consistent or dose-related changes in feed intake or efficiency were observed throughout the study in F_0 or F_1 animals. There were no clinical signs of toxicity or treatment-related deaths in F_0 or F_1 males or females. Increases in absolute and relative to body or brain weights of kidney and liver were consistently observed in F_0 and F_1 adults. Significant and dose-related organ weight changes relative to body weight are summarized in Table 98. Other effects on organ weight (e.g., seminal vesicles, epididymides, coagulating glands, and pituitary) were not considered to be treatment-related by study authors due to factors such as lack of a dose–response relationship, no consistency between absolute and relative weights, no histopathology, or no consistency across generations. Absolute and relative prostate weights were unaffected by bisphenol A exposure. There were no treatment-related gross systemic findings in F_0 or F_1 adults. Incidence of minimal to mild hepatocyte centrilobular hypertrophy was increased in both generations at 300 and/or 3500 ppm (Table 98). Renal nephropathy incidence was increased in F_0 males and in F_1 males and females of the 3500 ppm group. **[It did not appear that histopathological data were statistically analyzed.]**

Treatment- or dose-related reproductive effects in adult animals are summarized in Table 98. Bisphenol A exposure had no effect on numbers of implantation sites or resorptions or on mating, fertility, or gestational indices in F_0 or F_1 mice. Gestational length was increased in F_0 and F_1 females from the 3500 ppm group; the study authors stated the effect was of unknown biological significance. Epididymal sperm concentration was decreased in F_0 males of the 3500 ppm group but no effect was observed in F_1 parental or retained males. There was no effect on daily sperm production, efficiency of daily sperm production, or sperm motility or morphology in either generation. The study authors did not consider the decrease in sperm concentration in F_0 animals to be treatment-related based on lack of consistency between generations, no effect on any other andrological endpoint, and no effect on fertility. Estrous cyclicity and numbers of ovarian primordial follicle counts were not affected by bisphenol A exposure in F_0 or F_1 females. The only gross observation in reproductive organs was a slightly increased incidence of gross ovarian cysts in F_0 females from the 3500 ppm group. The incidence of paraovarian cysts was increased in F_0 and F_1 females from the 3500 ppm group. **[It did not appear that histopathological data were statistically analyzed.]**

Significant findings in developing mice are summarized in Table 99. Live F_1 and F_2 pups and litters at birth, sex ratio, and survival during the lactation period were not affected and there were no clinical or gross signs of toxicity in F_1 or F_2 offspring. A non-dose-related decrease in PND 21 survival index and lactational index (pups surviving on PND 21/PND 4) was described in F_2 pups of the 300 ppm group. **[The biological significance of the effect was not discussed by the study authors, but because the effect was not dose-related it is unlikely to be of biological significance.]** In F_1 pups from the 3500 ppm group, body weights were reduced during PND 7, 14, and 21 in F_1 females and both sexes combined and on PND 7 and 21 in F_1 males. Body weight results for both sexes combined are summarized in Table 99. An increase in male pup body weight observed on PND 7 in

the 1.8 ppm group was not considered to be treatment-related by the study authors because no dose–response relationship was observed. There was no effect on anogenital distance in F_1 or F_2 males or females on PND 0. Anogenital distance was also unaffected in F_2 males and F_1 and F_2 females on PND 21. Anogenital distance adjusted for body weight was reduced in F_1 males from the 300 and 3500 ppm groups on PND 21. Based on the lack of effect on anogenital distance at birth and inconsistencies between generations, the study authors did not consider the decreases in anogenital distance in F_1 males to be treatment-related. An increase in anogenital distance in F_2 females from the 0.018 ppm group on PND 0 was not considered to be treatment-related by the study authors. Preputial separation (absolute age and adjusted for body weight on day of acquisition) was delayed in parental and retained F_1 males of the 3500 ppm group. When adjusted for PND 30 body weight, preputial separation was delayed in retained but not parental F_1 males from the 3500 ppm group. Data for preputial separation adjusted for body weight on day of acquisition are shown in Table 99. Body weights on day of vaginal opening were lower in F_1 females from the 3500 ppm group. Day of vaginal opening was accelerated in the 3500 ppm group if adjusted for PND 21 body weight, but not body weight on the day of acquisition. Due to the lack of effect when adjusted for body weight on day of acquisition, the study authors did not consider effects on vaginal opening to be treatment-related.

Shown in Table 99 are significant organ weight effects relative to body weight. Dose-related organ weight changes in F_1 weanlings that were considered to be treatment-related by study authors included decreased absolute and relative (to body or brain weight) spleen and paired testes weights at 3500 ppm. Treatment-related absolute organ weight changes in F_2 weanlings included decreased weights of spleen, paired testes, and seminal vesicles with coagulating glands in the 3500 ppm group. Changes in organ weights relative to body weight in F_2 weanlings included decreased spleen weight in males and females and increased relative left kidney weight in 3500 ppm males. Treatment-related changes in organ weight relative to brain weight in F_2 weanlings were decreased spleen weight in both sexes and decreased paired testes weight at 3500 ppm and seminal vesicles with coagulating glands at 300 and 3500 ppm. Other organ weight effects (e.g., affecting epididymides, thymus, brain, ovaries, and/or uterus with cervix and vagina weights) were not considered to be dose-related due to lack of dose–response relationships or no consistent effects across generations. Included in Table 99 are significant organ weight effects relative to body weight. Significant organ weight effects relative to brain weight were included in Table 99 when the organ weight effect was significant only when normalized for brain weight. The study authors reported no gross findings in F_1 or F_2 weanlings. The incidence of undescended bilateral testes was increased in F_1 and F_2 weanling males of the 3500 ppm group. The incidence of hepatic cytoplasm alteration (clear hepatocellular cytoplasm, slightly more basophilic cytoplasm, and/or minute vacuoles) was apparently increased in F_1 males from the 300 and 3500 ppm groups and F_1 females and F_2 males from the 3500 ppm group. The incidence of seminiferous tubule hypoplasia was increased in F_1 and F_2 weanlings from the 3500 ppm group. **[Another histopathological finding that appeared to be possibly increased in weanlings from the 3500 ppm group was unilateral hydronephrosis in F_1 males. It did not appear that histopathological data were statistically analyzed.]**

Effects of 17β-estradiol in males were delayed preputial separation, reduced anogenital distance at weaning but not at birth, decreased weights of testes, epididymides, and seminal vesicles with coagulating gland, and increased incidence of seminiferous tubule hypoplasia and undescended testis. Effects of 17β-estradiol in female mice were accelerated vaginal patency, increased uterus with cervix and vagina weight, fluid filled/enlarged uterus, enlarged/thickened vagina, increased vaginal epithelial keratinization, and prolonged gestation. Reproductive effects in the 17β-estradiol group included decreased fertility, increased stillbirth, reduced live pups per litter, and increased dead pups.

The study authors identified bisphenol A NOELs of 30 ppm (~5 mg/kg bw/day) for systemic effects, 300 ppm (~50 mg/kg bw/day) for developmental toxicity, and 300 ppm (~50 mg/kg bw/day) for reproductive toxicity.

Strengths/Weaknesses: Strengths include the large number and range of doses examined, the rigor with which the study was performed, the large sample size in each group, the number of additional animals per litter that were retained and examined, the use of a concurrent estrogenic positive control group, and the thoroughness of the histologic evaluation.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation process.

4.2.3.3 Fish and invertebrates: Although studies in fish and invertebrates may be important for understanding mechanisms of action and environmental impact, the Panel views these studies as not useful for the evaluation process.

Kwak et al. (2001), supported by the Korean Ministry of the Environment, exposed adult male swordtail fish (*Xiphophorus helleri*) to bisphenol A 0, 0.4, 2, or 10 ppm **[mg/L]** for 72 hr ($n = 20$ fish/group). **[No information on purity or culture ware was provided.] [Nonylphenol was also studied but will not be discussed here.]** At the end of the exposure period, the fish were killed and livers were removed for measurement of vitellogenin. Testes of 10 fish/group were processed for flow cytometry by preparation of single cell suspensions stained with annexin V-fluorescein isothiocyanate and propidium iodide to detect necrosis and apoptosis. TUNEL staining was used to confirm apoptosis in testis sections. In a second experiment, juvenile male fish (30 days old) were exposed to bisphenol A in water at 0, 0.2, 2 and 20 ppb **[μg/L]** for 60 days, after which body length and sword length were measured. **[The sword is a portion of the caudal fin that elongates as a secondary sex characteristic.]** Statistical analysis used ANOVA followed by least significant difference test. Hepatic vitellogenin was increased by bisphenol A **[data were not shown]**. Apoptosis was increased in testes from fish exposed to bisphenol A at 10 ppm **[mg/L]** by TUNEL assay. **[Flow cytometry was said to be more sensitive, but data did not appear to have been statistically analyzed.]** Sword growth was decreased by bisphenol A

Table 101
Summary of High Utility Reproductive Toxicity Studies (Single and Multiple Dose Levels)

Model and treatment (doses in mg/kg bw/day)	Endpoint	Bisphenol A dose level (mg/kg bw/day)						Reference
		NOAEL	LOAEL	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$	
Female								
Sprague–Dawley rat (fed during pregnancy and lactation)	↓Duration of licking/grooming pups		0.04 (single dose)	Single dose study	Della Seta et al. (2005)			
CD rat (gavage, 40, 200, or 600/1000 × 28 days)	Altered estrous cycle	Unclear	≤1000/600	Data presentation does not permit modeling	Yamasaki et al. (2002a)			
Male								
Sprague–Dawley rat (gavaged, 0.020, 0.200, 2, 20, or 200 × 6 days)	No effect on daily sperm production, sperm count or reproductive organ weight	200	>200					Ashby et al. (2003)
F344 rats (drinking water with 0.011, 0.116, 1.094 or 11.846 × 13 weeks)	No adverse effects reported	11.846	-					Kim et al. (2002b)
C57BL/6N mouse (gavaged with 0.002, 0.020, or 0.200 × 6 days)	No effect on reproductive organ weight or epididymal sperm count	≥0.200	-					Nagao et al. (2002)
F344 rat (diet 235, 466, 950)	Histologic alterations in testis	<235	235	No dose response	Takahashi and Oishi (2001)			
CD rat gavaged with 40, 200, or 600/1000 × 28 days	↓ Relative ventral prostate weight	200	600/1000	Data presentation does not permit modeling	Yamasaki et al. (2002a)			
	↑ Relative testis weight	200	600/1000	Data presentation does not permit modeling				

Multigeneration								
CD rat (gavaged with 0.0002, 0.002, 0.020, or 0.200 before mating and × 2 generations)	No significant or dose-related reproductive effects	≥0.200	-					Ema et al. (2001)
Sprague–Dawley rat (dietary with ~0.0009, 0.018, 0.27, 4.5, 45, or 450 (male) and ~0.001, 0.02, 0.3, 5, 50, or 500 (female) × 3 filial generations)	↓ F1 epididymal sperm concentration	47.5a	475	317	216	700	469	Tyl et al. (2000b, 2002b)
	↓ F_3 daily sperm production	47.5	475	469	255	524	481	
	↓ Live pups/litter[b]	47.5	475	236	174	376	286	
	↓ Pup body weight[b]	47.5	475	183	163	177	153	
	Advanced vaginal opening[b]	47.5	475	394	343	206	176	
	Advanced F_1 preputial separation	4.75	47.5	466	411	188	163	
CD-1 mouse [F0 diet with ~840 or 1669 (male) and ~1055 or 1988 (female)]	↓ No. of live pups	840/1055	1669/1988	1116	727	1925	1189	Tyl et al. (2002a)
	↓ Female pup body weight (trend test)			2281	1728	2332	1733	
CD-1 mouse (diet with ~0.003, 0.03, 0.3, 5, 50, or 600 from 6 weeks of age × 2 filial generations)	↓ F_0 epididymal sperm concentration	50	600	567	319	607	549	Tyl et al. (2006)
	↑ Gestation length[b]	50	600	3619	639	1144	599	
	↓ Relative testis weight[b]	50	600	562	339	905	605	
	↓ Seminal vesicle weight relative to brain weight, F_2	30	50	405	223	1914	615	
Reproductive assessment by continuous breeding								
CD-1 mouse (diet with ~437.5, 875, or 1750 over 14-week continuous breeding period)	↓ Litters/breeding pair	437.5	875	1750	1295	1680	1155	NTP (1985a)

[a]Dose levels expressed as a mean of the estimated male-female target dose levels.
[b]Benchmark doses are shown for the generation with the lowest values.
↑,↓ Statistically significant increase, decrease compared to controls; ↔ no statistically significant effects compared to controls.

Table 102
Summary of Limited Utility Reproductive Toxicity Studies (Single and Multiple Dose Levels)

Model & treatment (doses in mg/kg bw/day)	Endpoint	Bisphenol A dose level (mg/kg bw/day) NOAEL	LOAEL	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$	Reference
Female								
Wistar rat (ovariectomized; s.c. dosed with ~40 × 1 day)	Altered progesterone receptor mRNA in different brain regions		~40 (single dose)	Single dose study				Funabashi et al. (2001, 2004b)
Wistar rat (ovariectomized; s.c. dosed with ~0.004, 0.04, 0.4, or 4 s.c., single dose)	↑ Progesterone receptor in brain regions	0.04	0.4	Data				presentation does not permit modeling
			Funabashi et al. (2003)					
Wistar rat (ovariectomized; s.c. dosed with 11, 78, 128, or 250 × 7 days)	↓ Body weight and body weight gain		250					Goloubkova et al. (2000)
	↑ Blotted uterine weight (compared to ovariectomized controls)		11 (low dose)					
	↑ Pituitary weight (compared to ovariectomized controls)		128					
	↑ Serum prolactin (compared to ovariectomized controls)		128					
	↑ Prolactin immunopositive cells in anterior pituitary		250					
ICR mice, i.p. every 3 days over 2 wks	No effect on body weight, uterine or ovarian histology, or hematological endpoints	5	> 5					Park et al. (2004)
	↓ Blood urea nitrogen		0.05 (low dose)					
	↓ Ovarian weight (right)		0.5					
	↓ Ovarian weight (left)		0.5 only					
Mongolian gerbil, fed 0.002 or 0.02 from Days 1–21 of cohabitation	↑Social sniffing	< 0.002	0.002	No dose response				Razzoli et al. (2005)
Sprague–Dawley (pseudopregnant; s.c.)	↑ Uterine wet weight and protein content on Days 1–4 with ~60% ↓ on Days 5–8		20 (single dose)	Single dose study				Spencer et al. (2002)
Male								
Long–Evans rat Leydig cells (cell culture)	↓ Testosterone production; various effects on mechanistic endpoints (e.g., LH-stimulated and basal testosterone production, mRNA expression)		0.0023 μg/L	No dose response				Akingbemi et al. (2004)
Swiss mouse, gavaged with 0.005, 0.025, and 0.1 × 30 days	↓ Body weight		0.005					Al-Hiyasat et al. (2002)
	↑ Relative testis weight[b]	0.005	0.025					
	↓ Seminal vesicle weight[b]	0.005	0.025					
Wistar rat, gavage for 45 days with 0.0002, 0.002, or 0.02	↓ Relative testis weight		0.0002	0.056	0.021	0.014	0.0087	Chitra et al. (2003a)

	↓ Relative epididymis weight		0.0002	0.011	0.0082	0.0069	0.0050	
	↓ Relative ventral prostate weight		0.0002	0.014	0.0083	0.015	0.0089	
ICR mice, i.p. every 3 days over 2 weeks	↓ Sperm concentration	0.5	5.0					Park et al. (2004)
	↑ Sperm abnormalities	0.5	5.0					
Sprague–Dawley rat, gavaged with 0.020, 0.200, 2, 20, or 200 × 6 days	↓ Daily sperm production (absolute and per g testis)	<0.020	0.020	No dose response				Sakaue et al. (2001)
Sprague–Dawley rat, gavaged with 0.000002, 0.00002, 0.0002, 0.002, 0.020, 0.200, or 2 × 6 days	↓ Daily sperm production (absolute and per g testis)	0.002	0.020	Data				presentation does not permit modeling.
			Sakaue et al. (2001)					
Wistar or Holtzman SD rat (diet)	No effect on reproductive organ histopathology, daily sperm production, epididymal sperm reserves, or serum testosterone			Single dose study				Takahashi and Oishi (2003)
Wistar rat (s.c.)	↓ Terminal body weight, absolute and relative reproductive organ weight; altered testicular histopathology		~200 (single dose)	Single dose study				Takahashi and Oishi (2003)
CD-1 (ICR) mouse (diet)	↑ Absolute testis weight, ↓absolute epididymis weight; no effect on testis histopathology, epididymal sperm reserves, daily sperm production, or serum testosterone		~400 (single dose)	Single dose study				Takahashi and Oishi (2003)
C57BL/6CrSlc mouse (diet)	No effect on reproductive organ weights; no effect on testis histopathology, epididymal sperm reserves, daily sperm production, or serum testosterone	~400 (single dose)		Single dose study				Takahashi and Oishi (2003)
Wistar rat, 2 or 20 i.p. 4 days/week × 1 month	↓ ventral prostate weight	2	20	7	5	9	6	Takahashi and Oishi (2003)
	↓ Serum testosterone	2	20	3	2	16	9	
	↓ Preputial gland relative weight	<235	235	124	86	171	114	
Reproductive assessment by continuous breeding								
CD-1 mouse, ~2.4, 4.2, or 8.1 over 18-week continuous breeding period, s.c. implant	No adverse effects on fertility	≤8.1						NTP (1984); Morrissey et al., (1989)

[a]Dose levels expressed as a mean of the estimated male-female target dose levels.
[b]Benchmark doses are shown for the generation with the lowest values.
↑,↓ Statistically significant increase, decrease compared to controls; ↔ no statistically significant effects compared to controls.

Table 103
Summary of Blood LH and Testosterone Changes in Experimental Animal Studies

Endpoints/protocol	LH effects[a]	Testosterone effects[a]	Reference
High Utility			
Experimental animal studies with oral exposure			
Adult male and female rats gavaged for 28 days	↔ at 40–1000 mg/kg bw/day	↔ at 40–1000 mg/kg bw/day	Yamasaki et al. (2002a)
4-week-old male rats fed bisphenol A in diet for 44 or 60 days	Not examined	↔ at 235–950 mg/kg bw/day or 200 mg/kg bw/day	Takahashi and Oishi (2001, 2003)
Multiple generation gavage dosing study in rats	↓ in F_0 adult females at 0.0002, 0.002, and 0.020 mg/kg bw/day but not at high dose (0.2 mg/kg bw/day); not considered treatment-related.	↔	Ema et al. (2001)
Experimental animal studies with parenteral exposure			
Female lambs i.m. injected at 4–11 weeks of age; ovariectomy at 9 weeks of age	↔ on blood levels during treatment; ↓ pulsatile secretion after treatment with 3.5 mg/kg bw biweekly	Not examined	Evans et al. (2004)
Limited utility			
Experimental animal studies with oral exposure			
Male rats gavaged from PND 21–35	↓ at 0.0024 mg/kg bw/day but ↔ at higher doses (0.010–200 mg/kg bw/day)	↓ at 0.0024 mg/kg bw/day but ↔ at higher doses (0.010–200 mg/kg bw/day)	Akingbemi et al. (2004)
Male rats gavaged from PND 21–90	↑ at 0.0024 mg/kg bw/day	↔ at 0.0024 mg/kg bw/day	Akingbemi et al. (2004)
4-week-old mice fed bisphenol A through diet for 2 months	Not examined	↔ at 400 mg/kg bw/day	Takahashi and Oishi (2003)
Experimental animal studies with parenteral exposure			
4-week-old male rats s.c. dosed on 4 days/week for 1 month	Not examined	↔ at 200 mg/kg bw	Takahashi and Oishi (2003)
4-week-old male rats i.p. injected for 1 month	Not examined	↓ at 20 mg/kg bw	Takahashi and Oishi (2003)

[a]Unless otherwise stated, animals were examined immediately after the treatment period.
↑,↓ Statistically significant increase/decrease compared to controls; ↔ no statistically significant effects compared to controls.

Utility (Adequacy) for CERHR Evaluation Process: This study suggests that fish are sensitive to bisphenol A-induced abnormalities in reproductive endpoints. Because this study was conducted in fish, it is not useful in the evaluation.

Ortiz-Zarragoitia and Cajaraville (2006), supported by the European Commission, examined the effects of bisphenol A exposure on the reproductive and digestive systems of adult blue mussels. For a period of 3 weeks, mussels were exposed to bisphenol A in acetone vehicle at 0 or 50 ppb **[μg/L] [no information on purity or culture ware was provided]**. Additional compounds were also tested but will not be discussed. Ten mussels/sex/group were examined at the end of the exposure period. The digestive gland was examined for volume of peroxisomes and peroxisomal proliferation. Gonads were histologically evaluated and assessed for alkali-labile phosphate level, a vitellogenin-like protein that is a possible biomarker of endocrine disruption. Statistical analyses included ANOVA followed by Duncan post-hoc test, Kruskall–Wallis, and Mann–Whitney *U* test. Bisphenol A had no effect on gonadal development, gonadal alkali-labile phosphate levels, or digestive gland peroxisomal proliferation or peroxisomal volume. However, observations of follicular brown cell aggregates and gonadal hemocyte infiltration in 35% of male and female mussels indicated severe gamete resorption.

Strengths/Weaknesses: This study evaluated bisphenol A-induced alterations in several reproductive endpoints in adult mussels. Severe gamete resorption was observed. Weaknesses include the failure to confirm bisphenol A concentrations in the test water and the use of only 1 concentration.

Utility (Adequacy) for CERHR Evaluation Process: Because this study was conducted in the mussel, it is not useful in the evaluation.

4.3 Utility of Reproductive Toxicity Data

4.3.1 Human. One high utility study of 42 men occupationally exposed to bisphenol A diglycidyl ether and 42 unexposed men evaluated the relationship

Table 104
Estimates of U.S. General Population Intake of Bisphenol A

Exposure source	Population	BPA mg/kg bw/day	Notes	Source
Formula	Infant	0.001	Assumes 4.5 kg bw, 700 ml formula at 6 μg/L BPA (U.S. canned formula max)	Expert Panel
Breast milk	Infant	0.001	Assumes 4.5 kg bw, 700 ml at 6.3 μg/L (U.S. breast milk max)	Expert Panel
Food	Infant (0–4 months old)	0.0016	European Commission	Table 11 Table 14
	Infant (6–12 months old)	0.0008–0.00165		
	Child (4–6 years old)	0.0012	European Commission	Table 11 Table 14
	Adult	0.00037 (canned food) 0.00048 (canned food + wine)	European Commission	Table 11 Table 14
Aggregate	Child (1.5–5 years old)	0.00004-0.00007	Max = 0.00007–0.00157 Assumes 50% absorption	Wilson et al. (2003, 2006)
Estimates based on urinary metabolites				
Aggregate	Child	0.00007	U.S. 6–8-year-old girls (max = 0.00217)	Table 15
	Adult	0.000026	U.S. population 95th percentile 0.0.00159	Table 15

between urinary levels of bisphenol A and plasma LH, FSH, and free testosterone, found reduced FSH levels among the exposed men. No fertility endpoints were evaluated. Three studies were considered to have low utility in the evaluation process due to limitations in design and analysis but suggest directions for future research. Two of these studies measured serum bisphenol A in healthy women, women with polycystic ovary syndrome and healthy men and evaluated correlations with serum gonadotropins, prolactin, testosterone, and other androgens. No fertility endpoints were included in these studies. The third study of 37 women found significantly lower bisphenol A concentrations among women with endometrial cancer and complex endometrial hyperplasia compared to healthy women and women with simple hyperplasia.

4.3.2 Experimental animal. Female reproductive toxicity testing using multiple dose levels has been evaluated in 2 rat, 1 mouse, and 1 gerbil study. Endpoints affected in these studies included brain progesterone receptor, estrous cyclicity, resorptions, and social sniffing. Male reproductive toxicity testing using multiple dose levels has been evaluated in 7 rat and 2 mouse studies. Affected endpoints in males included reproductive organ weight and histology, serum testosterone, daily sperm production, sperm motility, sperm concentration, percent pregnant females after mating, and females with resorptions after mating. There are 4 multigeneration tests, 2 in rats and 2 in mice, involving gavage or dietary treatments with bisphenol A with dose levels as low as 0.0009 mg/kg bw/day. There are also 2 reproductive assessments by continuous breeding, 1 of which involved subcutaneous implants for bisphenol A delivery and 1 of which used dietary administration in which the lowest dose level was ~437.5 mg/kg bw/day.

4.4 Summary of Reproductive Toxicity Data

The hypothesis has been advanced that the Charles River Sprague-Dawley (SD) rat is insensitive to estrogens and other EDCs and therefore it should not be used for developmental EDC studies and the studies of the effects of BPA that used this strain should be discounted. In order to address this important issue Expert Panel members reviewed the literature on estrogen-sensitivity among rat strains and the following is a summary of our findings.

Different strains of rats show clear, robust reproducible differences in responses to potent estrogens and antiandrogens. Several traits have been shown to be estrogen sensitive in rats including prolactin regulation in the pituitary, thymic involution, uterine pyometra, and liver carcinogenesis to name a few. It is evident that the SD rat and other rat strains are less sensitive to the effects of estrogens than the F344 rat. However, for some traits, the reverse is true. In addition, while the SD was less sensitive than the F344 to estrogen, the reverse was true for sensitivity to tamoxifen.

The sensitivity to estrogens has been mapped to specific chromosomes for several traits. In no case has it been demonstrated that the SD strain is completely insensitive to any known estrogen. It is evident that different traits map to different chromosomes and the degree of estrogen sensitivity varies from tissue to tissue, likely depending on the tissue-specific gene regulated by ER on the chromosome.

Therefore, one cannot conclude that the SD is insensitive to estrogens and the results of BPA studies with BPA should be ignored. In fact, there are several studies reporting low dose effects that used the SD rat. A comparison of the uterotrophic data from the OECD study with EE, BPA, and other estrogens does not indicate that the SD rat is less sensitive to any estrogen versus the Wistar. In this study, oral EE at 1 μg/kg/day for 3 days stimulated uterine weight whereas 0.3 μg/kg/day was uterotrophic when administered s.c. In addition, in the pubertal female rat assay, EE, the antiestrogen tamoxifen and the estrogenic pesticide methoxychlor produced equivalent responses in the Long–Evans and SD female rats.

Although some have hypothesized that the Crl: CD (SD) rat is more insensitive to estrogens than SD rats

from other suppliers, there are no data supporting this assertion.

4.4.1 Human. Human reproductive studies are summarized in Table 100. A study of 42 men occupationally exposed to an epoxy hardening agent containing bisphenol A diglycidyl ether found higher urinary bisphenol A concentrations, corrected for creatinine, than were found in 42 men who worked in the same factory but did not have known exposure to the hardening agent (Hanaoka et al., 2002). Differences were not detected between the worker groups in plasma testosterone or LH, but plasma FSH was significantly lower in exposed workers **[BPA: 0.043 μg/kg bw]** than in workers not exposed to the hardening agent **[BPA: 0.021 μg/kg bw]**. A significant correlation was noted between total urinary bisphenol A concentration and decreased FSH when adjusted for age and alcohol intake ($r = 0.23$, $P = 0.045$).

Two studies from Takeuchi and Tsutsumi (2002) and Takeuchi et al. (2004a) suggested a relationship between serum bisphenol A concentration and serum testosterone (total and free). The first study (Takeuchi and Tsutsumi, 2002) included women with and without polycystic ovary syndrome (POS), and healthy men. Statistically significant positive correlations were observed for women with and without POS (0.559 for total testosterone and 0.598 for free testosterone, $P < 0.01$), and with all participants (0.595 and 0.609, respectively, $P < 0.001$). The second study (Takeuchi et al., 2004a) reported only cycling women with and without obesity and women with POC, with and without obesity, hyperprolactinemia and hypothalamic amenorrhea. Statistically significant positive correlations were found for bisphenol A 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 dehydroepiandrosterone sulfate (DHEAS, $r = 0.514$, $P < 0.001$). Although these studies used ELISA, which may overestimate bisphenol A compared to HPLC, significant correlations between bisphenol A levels and higher serum testosterone levels were found. The authors speculated that androgens either may affect bisphenol A metabolism or the reverse.

A study of 37 women found differences in bisphenol A concentrations by health status. Significantly lower mean bisphenol A concentrations were found among women with endometrial cancer (1.4 ng/ml, $n = 7$) and complex endometrial hyperplasia (1.4 ng/ml, $n = 9$) compared to healthy women (2.5 ng/ml, $n = 11$) and women with simple hyperplasia (2.9 ng/ml, $n = 10$) (Hiroi, 2004).

4.4.2 Experimental Animal.

Reproductive toxicity studies of high and limited utility are summarized in Table 101 and Table 102 respectively.(single and multiple dose level studies in the same utility category are combined within a table). Based on reproductive studies using a single dose level, the lowest dose level at which an effect was seen in these studies was 0.04 mg/kg/day fed to female rats during pregnancy and lactation and resulting in a decreased duration of licking/grooming pups (Della Seta et al., 2005). This study of neural and behavioral effects is shown here for convenience but has been included with other studies focused on these endpoints for further discussion in Section 3.

For high utility female reproductive studies using multiple doses, the lowest effect level, for altered estrous cycle, was ≥600 mg/kg bw/day by gavage in rat for 28 days (Yamasaki et al., 2002a). For high utility male reproductive studies, the lowest effect level, for histologic alterations in the testis, was 235 mg/kg bw/day by gavage in rat for 28 days (Takahashi and Oishi, 2001). The value of the histologic observations may be limited due to the fixation and embedding techniques employed, raising some concern over the validity of this endpoint.

The reproductive assessments by continuous breeding included a study using very high-dose levels (NTP, 1985a), and this study is not the most informative for reproductive risk assessment. In a multigeneration study, CD rats did not show statistically significant or dose-related reproductive effects over 2 generations with bisphenol A gavage doses of 0.0002, 0.002, 0.020, or 0.200 mg/kg bw/day (Ema et al., 2001). In Sprague–Dawley rats treated for 3 generations, adverse reproductive effects consisted of decreased F_1 epididymal sperm concentration, decreased F_3 daily sperm production, decreased live pups/litter, decreased pup body weight, and delayed vaginal opening at an average dose level of 475 mg/kg bw/day. Delayed preputial separation was seen in F_1 and F_2 males at an average dose level of 47.5 mg/kg bw/day (Tyl et al., 2000a, 2002b). In CD-1 mice given bisphenol A for 2 generations in the diet at dose levels as low as ~0.003 mg/kg bw/day, the most sensitive effect was a reduction in F_2 seminal vesicle weight relative to brain weight at 50 mg/kg bw/day. Effects on F_0 epididymal sperm concentration, gestation length, and relative testis weight occurred at 600 mg/kg/day, the next highest dose level (Tyl et al., 2006).

A summary of LH and testosterone effects observed in bisphenol A-exposed experimental animals and in humans are included in Table 103.

Data sufficiency statement for human data: In summary, there are insufficient data to evaluate whether bisphenol A causes male or female reproductive toxicity in humans. However, several studies collectively suggest hormonal effects, including one study of exposed male workers likely to have multiple routes of exposure including inhalation (Hanaoka et al., 2002).

Data sufficiency statement for animal data: In summary, the experimental animal literature was assessed for its utility (high utility, limited utility, or no utility) based on the criteria established by this Expert Panel, including an evaluation of experimental design and statistical procedures. Studies with high and limited utility were further grouped according to female and male reproductive toxicity, their use of single or multiple dose levels, a multigenerational exposure paradigm, and the measurement of various hormonal endpoints. Greater weight was given to studies using the oral route of exposure, because of evidence that oral exposure predominates in humans and that target tissue exposure to parent compound (bisphenol A) is very low after oral exposure and first-pass metabolism as compared to subcutaneous or other routes of exposure.

There is sufficient evidence in rats and mice that bisphenol A causes female reproductive toxicity, characterized as delayed vaginal opening with subchronic or chronic oral exposure NOAELs of 47.5 mg/kg bw/day and a LOAEL of 475 mg/kg bw/day (Tyl et al., 2002b).

There is sufficient evidence in rats and mice that bisphenol A causes male reproductive toxicity, characterized as delayed preputial separation, with subchronic or chronic oral NOAEL of 4.75 mg/kg bw/day and a LOAEL of 47.5 mg/kg bw/day (Tyl et al., 2002b).

There is inconsistent evidence in rats and mice that bisphenol A alters testosterone and gonadotropin levels in males after oral postnatal exposure.

There is inconsistent evidence in male and female mice that bisphenol A produces aneugenic effects in germ cells after exposure.

5.0 SUMMARIES, CONCLUSIONS, AND CRITICAL DATA NEEDS

5.1 Developmental Toxicity

No data on the effects of human developmental exposure to bisphenol A are available. There is a large literature describing studies in rodents and some work in other species. A large experimental animal literature was reviewed, assessed for its utility, and weighed based on the criteria established by this Panel.

From the rodent studies we can conclude that bisphenol A:

- Does not cause malformations or birth defects in rats or mice at levels up to the highest doses evaluated: 640 mg/kg/day (rats) and 1250 mg/kg/day (mice).
- Does not alter male or female fertility after gestational exposure up to doses of 450 mg/kg bw/day in the rat and 600 mg/kg bw/day in the mouse (highest dose levels evaluated).
- Does not permanently affect prostate weight at doses up to 475 mg/kg/day in adult rats or 600 mg/kg/day in mice.
- Does not cause prostate cancer in rats or mice after adult exposure at up to 148 or 600 mg/kg/day, respectively.
- Does change the age of puberty in male or female rats at high doses (ca. 475 mg/kg/day).

Rodent studies suggest that bisphenol A:

- Causes neural and behavioral alterations related to disruptions in normal sex differences in rats and mice. (0.01–0.2 mg/kg/day).

The data on bisphenol A are insufficient to reach a firm conclusion about:

- A change in the onset of puberty in male rats or mice at doses up to 475–600 mg/kg/day.
- An acceleration in the age of onset of puberty at a low dose in female mice at 0.0024 mg/kg/day, the only dose tested.
- Whether Bisphenol A predisposes rats toward prostate cancer or mice toward urinary tract deformations.

5.2 Reproductive Toxicity

There are insufficient data to evaluate whether bisphenol A causes male or female reproductive toxicity in humans. A large experimental animal literature was reviewed, assessed for its utility, and weighted based on the criteria established by this expert panel, including an evaluation of experimental design and statistical procedures. These animal data are assumed relevant for the assessment of human hazard.

Female effects: There is sufficient evidence in rats and mice that bisphenol A causes female reproductive toxicity with subchronic or chronic oral exposures with a NOAEL of 47.5 mg/kg bw/day and a LOAEL of $\geq$475 mg/kg bw/day.

Male effects: There is sufficient evidence in rats and mice that bisphenol A causes male reproductive toxicity with subchronic or chronic oral exposures with a NOAEL of 4.75 mg/kg bw/day and a LOAEL of $\geq$47.5 mg/kg bw/day.

5.3 Human Exposures

Bisphenol A is FDA-approved for use in polycarbonate and epoxy resins that are used in consumer products such as food containers (e.g., milk, water, and infant bottles) food can linings (Staples et al., 1998; SRI, 2004) and in dental materials (FDA, 2006). Resins, polycarbonate plastics, and other products manufactured from bisphenol A can contain trace amounts of residual monomer and additional monomer may be generated during breakdown of the polymer (European-Union, 2003).

Environmental Exposures: Bisphenol A emitted from manufacturing operations is unlikely to be present in the atmosphere in high concentrations. However, it was found in 31–44% of outdoor air samples with concentrations of <LOD (0.9) to 51.5 ng/m^3 (Wilson et al., 2006). Indoor air samples found concentrations $\leq$29 ng/m^3 (Rudel et al., 2001, 2003; Wilson et al., 2003). Limited U.S. surface water sampling found bisphenol A in 0–41% of samples ranging from <0.1 to 12 ug/L (Kolpin et al., 2002; Boyd et al., 2003). Twenty-five to 100% of indoor dust samples contained bisphenol A with concentrations of <detectable to 17.6 μg/g (Rudel et al., 2001, 2003; Wilson et al., 2003, 2006).

Exposures Through Food: The highest potential for human exposure to bisphenol A is through products that directly contact food such as food and beverage containers with internal epoxy resin coatings and through the use of polycarbonate tableware and bottles, such as those used to feed infants (European-Union, 2003). Studies examining the extraction of bisphenol A from polycarbonate infant bottles in the U.S. found concentrations <5 μg/L. Canned infant formulas in the U.S. had a maximum levels of 13 μg/L in the concentrate that produced a maximum of 6.6 μg/L when mixed with water (FDA, 1996; Biles et al., 1997a). Breast milk studies in the U.S. have found up to 6.3 μg/L free bisphenol A in samples (Ye et al., 2006). Measured bisphenol A concentrations in canned foods in the U.S are <39 μg/kg (FDA, 1996; Wilson et al., 2006). Limited drinking water sampling in the U.S. indicates that bisphenol A concentrations were all below the limit of detection (<0.1 ng/L) (Boyd et al., 2003).

Biological Measures of Bisphenol A in Humans: The panel finds the greatest utility in studies of biological samples that use sensitive and specific analytical methods (LC-MS or GC-MS) and report quality control measures for sample handling and analysis. The panel further focused on biological monitoring done in