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CONTRIBUTED ARTICLES

Weight of the Evidence Evaluation of Low-Dose Reproductive and Developmental Effects of Bisphenol A

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ABSTRACT

A panel convened by the Harvard Center for Risk Analysis (HCRA) evaluated the weight of evidence for potential developmental and reproductive toxicity of bisphenol A (BPA, CASRN 80-05-7) in animals at doses well below the Lowest Observed Adverse Effect Level (LOAEL) of 50 mg/kg-day previously identified by the U.S. Environmental Protection Agency (US EPA) and even US EPA's reference dose (RfD) of 0.05 mg/kg-day. The effects are hypothesized to occur through an endocrine-modulating mode of action, specifically through estrogen receptors. The panel focused on potential male reproductive effects but also examined other endpoints possibly associated with hormone-like effects. The review considered studies published through April 2002. A formal deliberation framework focused on consistency, generalizability, and biological plausibility. The panel found no consistent affirmative evidence of low-dose BPA effects for any endpoint. Inconsistent responses across rodent species and strains made generalizability of low-dose BPA effects questionable. Lack of adverse effects in two multiple-generation reproductive and developmental studies casts doubt on suggestions of significant physiological or functional impairment. The panel was concerned about generalization of non-oral administration results to oral exposures. Differences in the pattern of BPA responses compared to estradiol or diethylstilbestrol (DES) cast doubt on estrogenicity as a low-dose mechanism of action for BPA. Finally, there is indirect evidence that humans may be less

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sensitive to possible estrogenic effects from BPA exposure due to pharmacodynamic factors. The panel recommended replication of existing studies under carefully controlled conditions and further study of BPA's pharmacokinetics and pharmacodynamics. The study was funded by a grant from the American Plastics Council.

Key Words: bisphenol A, endocrine disruption, reproductive toxicity, developmental toxicity, weight of evidence.

1. INTRODUCTION

1.1. Charge to Panel

Experiments have reported potential developmental and reproductive toxicity of bisphenol A (BPA, CASRN 80-05-7) in animals at doses well below those previously identified to be Lowest Observed Adverse Effect Levels (LOAELs) (*e.g.*, USEPA's LOAEL of 50 mg/kg-day). The effects are hypothesized to occur through an endocrine-modulating mode of action. Efforts to replicate these findings have generally been unsuccessful. In an effort to characterize what is known about the effects of BPA at low levels of exposure, the American Plastics Council awarded a grant to the Harvard Center for Risk Analysis (HCRA) in 2000. HCRA convened an expert panel of scientists to help assess the evidence regarding the existence of low dose BPA effects in laboratory animals and in humans. The panel was chaired by Donald Mattison, former medical director of the March of Dimes, and currently Senior Advisor to the Directors of the Center for Research for Mothers and Children and the National Institute of Child Health and Human Development. Our goal was to investigate the possibility of "low dose effects," that is, effects at levels at least an order of magnitude below the current LOAEL of 50 mg/kg-day. Such effects would call into question the USEPA's reference dose (RfD) of 0.05 mg/kg-day, which serves as a regulatory benchmark and was derived by dividing the LOAEL by a safety factor of 1,000.

The panel focused on potential male reproductive effects but also examined other endpoints possibly associated with hormone-like effects. The panel and HCRA investigators reviewed relevant scientific literature published as of April 2002. Some studies published after this date are used for general information or background. We did not consider studies available only in abstract form. To evaluate the hypothesis that BPA may cause effects in humans at low levels of exposure, the panel made use of criteria developed in Gray *et al.* (2001) for the use of toxicology results in risk assessment. We have grouped those criteria (slightly modified) to address three questions.

The first question (Section 2 of this report) asks if there is evidence for low-dose reproductive and developmental effects in experimental animals. Addressing this question requires an assessment of the extent to which results from different studies are consistent. Where results differ, study design differences and potential weaknesses must be evaluated. The Gray *et al.* criteria used to address these issues are the following:

- Corroboration—The replication of findings among similar studies and the observation of similar effects under relevant conditions increases the confidence that the findings represent a real effect in experimental animals. Conversely,

lack of corroboration is grounds to doubt the validity of single experimental results. In a multi-generational study, an exposure-related effect should appear across generations.
- Rigor—Studies are evaluated for their proper conduct and analysis. Greater weight is given to better-conducted studies (*e.g.*, those that use "good laboratory practices"), and some studies may have been performed poorly enough that their results are substantially discounted.
- Power—The statistical power of an experimental design should be examined for its ability to detect effects of a certain magnitude, expecially for "negative" studies, where a low level of response could be mistaken for lack of response.

The second question (Section 3 of this report) asks if a positive finding in test animals can be generalized to humans subjected to environmentally relevant exposures. This generalization to humans involves an extrapolation across species, and sometimes an extrapolation from high dose to low dose. Accordingly, the criteria to address this question are as follows:

- Universality—The degree to which an effect is consistently reproduced in valid test systems increases the confidence that it applies to humans. In contrast, if an effect is restricted to a certain species, strain, or route of administration, the ability to generalize the response to other species or routes becomes more questionable.
- Proximity—When effects have been shown in a species similar to humans or at a dose level similar to that expected in humans, such results weigh more heavily than those in dissimilar species, by inappropriate routes, or at markedly different dose levels.

The final question (Section 4 of this report) asks if the evidence is consistent with a biologically plausible mode of action. The first criterion (cohesion) addresses the extent to which the available literature is consistent with a coherent mode of action, while the second (relevance) addresses the extent to which the mode of action is applicable to humans.

- Cohesion—The extent to which all of the data are consistent and are subject to a single, biologically plausible explanation increases weight compared to a situation where inconsistencies require ad hoc explanations and exceptions to general patterns.
- Relevance—From what is known about the underlying biological basis for a toxic response in animals, it may be possible to judge (based on knowledge of animal and human physiology) whether similar metabolism, mechanisms of damage and their repair, and molecular targets of toxic action should be expected to operate in humans. Confidence in applicability to humans can increase or decrease, accordingly.

Section 5 of this article discusses our findings.

1.2. Background

1.2.1. Evidence of BPA effects

Efforts in the 1930s to synthesize and characterize non-steroidal chemicals with estrogen-like activity for potential therapeutic use led to the discovery of Diethylstibestrol (DES) and BPA. Structure activity studies were undertaken using *in vivo* pharmacological responses determined with the rodent uterotrophic assay (Dodds and Lawson 1936). The structure of these two synthetic estrogens differs from the structure of the natural estrogen 17β-estradiol.

In vitro experiments using isolated receptors from a number of species, including human ERs, have uniformly indicated that BPA, like estradiol, is a full ER_α agonist, but considerably less potent (Gould *et al.* 1998; Kim *et al.* 2001; Matthews *et al.* 2001). The measured potency depends on the assayed system used (*e.g.*, rat uterine cytosol *vs.* recombinant human ER). For example, in an assay in MCF-7 cells BPA (10–25 nM) induced progesterone receptors at a potency of approximately 1:5000 compared to that of estradiol. The activity of BPA in the assay was blocked by the antiestrogen tamoxifen (Krishnan *et al.* 1993). A reporter gene assay system (Gould *et al.* 1998) indicated that BPA was only a partial ER_β agonist. These authors also indicated that combinations of 0.5 μg/day estradiol and 5.0 mg/kg-day BPA would attenuate the responses of estradiol for a number of uterine estrogenic activities (*e.g.*, peroxidase activity). Taken together, these data indicate the potential for BPA to have differential *in vivo* (anti) estrogenic activity that depends on the dose and the tissue undergoing response.

Most of the studies that evaluated the estrogenicity of BPA examined the potential presence of a uterotrophic response, typically measured as an increase in the uterine weight in immature or ovariectomized rats dosed daily for three to four days. The uterotrophic assay is based on the ability of estrogenic subtances to increase uterine weight. The capacity for a chemical to induce a uterotrophic effects depends on several factors, including the route of administration, the animal species and strain used, and the sexual maturity of the test animals in the experiment. BPA has been shown to have a very weak estrogenic effect in most of the studies conducted in rats (Dodds and Lawson 1936; Ashby and Tinwell 1998; Gould *et al.* 1998; Laws*et al.* 2000).

Cook *et al.* (1997) observed a 67% increase in uterine weight among female Crl:CD BR rats administered 500 mg/kg-day BPA for four days *via* intraperitoneal (ip) injection at age seven weeks. Other investigators (Dodge *et al.* 1996) reported an increase of 37% more than controls after oral administration of 30 mg/kg-day BPA in 1% carboxymethylcellulose to ovariectomized Sprague-Dawley rats. High BPA doses administered orally or *via* subcutaneous injection (400 mg/kg-day) to immature rats have also increased uterine weight (Ashby and Tinwell 1998). Gould *et al.* (1998) observed uterotrophic effects in immature Sprague-Dawley rats following three days of oral BPA administration at 150 mg/kg-day. Coldham *et al.* (1997) observed no uterotrophic effects in CFLP mice following oral BPA administration at doses ranging from 0.05 to 5 mg/day.

The National Toxicology Program (NTP) (1982) conducted a chronic (103 week) study in which 50 F344 rats of each gender were fed diets containing, 0, 1,000, or 2,000 ppm BPA. In the same study, male B6C3F1 mice (N = 50) were fed diets containing 0, 1,000, or 5,000 ppm BPA, and female B6C3F1 mice (N = 50) received

0, 5,000, or 10,000 ppm BPA. USEPA established its Reference Dose (RfD) (Barnes and Dourson 1988) based on the effect occuring at the lowest dose (reduced body weight gain in male and female rats at 1,000 ppm BPA, corresponding to 50 mg/kg-day). In mice, USEPA identified a LOAEL of 130 mg/kg-day (the health effect in this case was also reduced body weight gain). The RfD reflects application of an uncertainty factor of 1,000 to the rat LOAEL of 50 mg/kg-day, yielding a value of 0.05 mg/kg-day (USEPA 2003).

1.2.2. Human exposure to BPA

BPA has a number of uses that can result in exposure to the general public. The greatest potential exposures come from food contact uses. BPA monomer is the starting material for manufacture of polycarbonate plastics used for plastic bottles, jugs, baby bottles, and many other products. It is also a starting material for resins used to coat the inside of food and beverage cans. In addition, similar resins are used as a coating for the interior surfaces of wine storage vats.

Using measured and modeled estimates of migration potential, exposures have been recently evaluated by European Union scientific bodies using data from Europe, Japan, the United States, and around the world. The most extensive evaluation can be found in the Risk Assessment for BPA (European Union 2003). Recently, the Scientific Committee on Food of the European Commission Health and Consumer Protection Directorate-General reviewed and updated exposure estimates in the process of evaluating the safety of food uses of BPA (Scientific Committee on Food 2002). Both assessments reported estimated adult exposures associated with consumption of food and wine. The European Union assessment (2003) estimated exposures can amount to 0.009 mg/kg-day, whereas the Scientific Committee on Food (2002) estimated this exposure can amount to 4.8×10^{-4} mg/kg-day. These estimates differ by almost 20-fold.[1]

BPA is also a component in some dental sealants and papers, although these products have been found to make little or no contribution to exposure (European Union 2003). In addition, there may be local environmental exposure to BPA in the vicinity of polyvinylchloride (PVC) manufacturing facilities or other industrial sites that use BPA in their production processes.

2. EVIDENCE FOR LOW-DOSE REPRODUCTIVE AND DEVELOPMENTAL EFFECTS IN EXPERIMENTAL ANIMALS

We identified 19 studies satisfying our inclusion criteria that have investigated BPA's potential for endocrine modulating activity at low doses (*i.e.*, with tested doses extending at least as low as 5 mg/kg-day, which is an order of magnitude

[1]The main source of difference was related to exposure in wine. Both assessments assumed consumption of 750 ml (one standard bottle) of wine per day but with different estimates of BPA levels in wine. Wine accounted for 23% of the total BPA exposure estimated by the Scientific Committee on Food, and 83% of the total BPA exposure estimated in the European Union Risk Assessment.

below EPA's LOAEL of 50 mg/kg-day). Evaluating the extent to which these studies produced consistent results is complicated by methodological differences, including differences in the type of animal studied, the health effects evaluated, the route of exposure, and the age at which the animals were exposed. Tables 1–4, which summarize the studies, are organized by species because of potential cross species differences in sensitivity, and by route of exposure because this factor may have a substantial impact on the delivered dose (see Section 3.2.1). Section 2.1 addresses the corroboration criterion by looking at the qualitative impact of BPA exposure on various endpoints across studies in an effort to identify patterns (*e.g.*, a monotonic or U-shaped dose response relationship). Section 2.2 addresses the rigor and power criteria by investigating the factors that may have contributed to some of the discrepancies across studies. Unless otherwise noted, the following discussion ignores results reported at exposures exceeding 5 mg/kg-day.

2.1. Dose-Response Patterns and Potential Corroboration of Effects Across Studies

To investigate the potential corroboration of effects across studies, we grouped the studies by endpoint using the same four categories specified in the National Toxicology Program's 2001 review of BPA (2001). The endpoints identified in the studies we reviewed include the following:

- Organ weight—Cervix, epididymis, ovaries, preputial gland, prostate, seminal vesicles, testes, uterus, vagina;
- Perinatal characteristics—Anogenital distance (AGD);
- Pubertal characteristics—Estrus cycle characteristics, time until first estrus, mammary gland maturation rate, mammary gland ductal migration rate, preputial separation date, testes descent date, vaginal opening date;
- Other endpoints—Avoidance behavior, brain anatomy, daily sperm production, litter gender ratio, hormone levels, mammary gland percent ducts, ovary histopathology, prostatic acid phosphatase activity, sperm characteristics, sperm production efficiency, testes histopathology, testosterone levels.

Tables 5–8 group the results of the studies we reviewed by species and route of exposure. The tables group study results for each endpoint by order of magnitude dose. Each study result is represented by a single character, with "0" indicating no statistical difference from controls, "−" indicating a decrease relative to controls, and "+" indicating an increase. A "Δ" represents a difference that does not have an obvious directionality (*e.g.*, changes in avoidance behavior). Statistically significant differences were generally characterized as either "+" or "−". However, in the case of multigenerational studies, we designated results as a change relative to controls only if the difference was evident in more than one generation. Section A of the online supplemental information for this article (Gray *et al.* 2004) provides a more detailed listing of the results.

The remainder of this section addresses rat studies with BPA exposure *via* oral administration (Section 2.1.1), rat studies with exposure *via* other routes (Section 2.1.2), mouse studies with BPA exposure *via* oral administration (Section 2.1.3), and mouse studies with BPA exposure *via* other routes (Section 2.1.4).

Table 1. Rat studies—Oral BPA administration.

Study	Strain	BPA treatment groups	Doses (mg/kg-day)[a]	Other design features	Endpoints		Reported positive findings
Cagen *et al.* (1999a)	Han Wistar	28 dams/group—drinking water containing 0, 0.01, 0.1, 1, and 10 ppm BPA	0 0.001–0.004[b] 0.008–0.038 0.100–0.391 0.775–4.022	Animals culled at pnd 4 so that animals/litter ≤ 8. At pnd 22, 4 males selected from each litter and housed in groups of 4. Animals terminated at age 90 days.	Other Weight	Fertility, pup survival, gender ratio, DSP, SE Body, prostate, SV, epididymis, Testes, preputial gland	None
Ema *et al.* (2001)	IGS	25 M and 25 F adults/group—gastric intubation	2×10^{-4} 0.002 0.02 0.2	Two generation study—M exposed before mating, F during mating, gestation, and lactation. Necropsies at pnd 22 and in adulthood.	Other Perinatal Puberty Weight	DSP, SE AGD Vaginal opening, preputial separation, testes descent, estrus cycle Body, prostate, testes, epididymis, SV, ovaries, uterus	None[c]
Kubo (2001)	Wistar	5 dams/group	0 1.5	Dams exposed during gestation. Litters reduced to 5 M and 5 F.	Other Puberty Weight	Avoidance behavior, brain anatomy, hormone levels, sperm characteristics, testes, and ovary histopathology Estrus cycle Testes, epididymis, prostate, uterus, ovaries	Avoidance behavior, Brain anatomy, both at 1.5 mg/kg-day
Sakaue *et al.* (2001)	S-D	5/group	0 0.02 0.2 2 20 200	BPA by gavage for 6 days at 13 wks of age. Sacrifice at wk 14 or wk 18	Other Weight	DSP, SE Body, testes	DSP, SE decreased at 0.02 mg/kg-day and at higher doses.[d]
Sakue *et al.* (2001)	S-D	8/group	0 2×10^{-6} 2×10^{-5} 2×10^{-4} 0.002 0.02 0.2 2	BPA by gavage for 6 days at 13 wks of age. Sacrifice at wk 14 or wk 18.	Other Weight	DSP, SE Body, testes	DSP, SE decreased at 0.02, 0.2, and 2 mg/kg-day

(Continue on next page)

Table 1. Rat studies—Oral BPA administration.(Continued)

Study	Strain	BPA treatment groups	Doses (mg/kg-day)[a]	Other design features	Endpoints		Reported positive findings
Tyl *et al.* (2002)	CD S-D	30/gender/group	0 0.001 0.02 0.3 5	BPA in diet *ad libitium* for 3 generations. F0 exposed for 10 wks pre-breeding, 2 wks during mating, 3 wks during gestation, 3 wks during lactation. F1, F2, F3 exposed after weaning until lactation. Litters culled to ≤ 10 pups. Sacrifice of 3 pups/litter at pnd 21.	Other	DSP, SE, mating, fertility, gestational indices, ovarian primordial follicle counts, estrous cyclicity, precoital interval, gestational length, offspring sex ratios, postnatal survival, nipple retention in pre-weanling males, epididymal sperm number, motility, morphology	None at ≤ 5 mg/kg-day[c]
					Perinatal	AGD	
					Puberty	Vaginal opening	
					Weight	Body, testes, epididymis, prostate, SV, preputial gland, ovaries, uterus	
Tinwell *et al.* (2002)	S-D and Wistar	7 dams/strain/group	0 0.02 0.1	BPA exposure *via* oral gavage from gd 6–21. Positive controls exposed to 0.2 mg/kg-day EE by gavage. Litter culled to ≤ 8 pups on pnd 5 to get equal number of M and F pups. Sacrifice at pnd 90–91 (M) and pnd 98 (F).	Other	Fertility, DSP	No positive findings among BPA exposed animals. EE exposed animals experienced high resorption rate *in utero*.
					Perinatal	AGD	
					Puberty	Vaginal opening, 1st estrus, preputial separation	
					Weight	Liver, SV, epididymis, testes, prostate, uterus, vagina, cervix, ovaries	

Abbreviations: AGD: Anogenital distance; DSP: Daily sperm production; EE: Ethinylestradiol; gd: Gestation day; i.p.: Intraperitoneal; pnd: Post natal day; s.c.: Subcutaneous; S-D: Sprague-Dawley; SE: Sperm efficiency; SV: Seminal vesicles. *Notes*: [a]Reflects results from dose groups below USEPA's LOAEL of 50 mg/kg-day. [b]Cagen *et al.* (1999a) had two negative control groups, each with 28 dams: vehicle controls and unhandled females. [c]In multigeneration studies, we considered a change to be significant only if it appeared in at least two generations. Ema *et al.* (2001) discounted various statistically significant findings because they did not appear consistently. For example, the authors did not consider AGD changes to be treatment-related because they were consistently small (within 5% of control group values), and were not consistent across genders or generations. Testicular descent was statistically delayed in F2 males exposed to either 0.02 and 0.2 mg//kg-day BPA. However, the delay was small in absolute terms (mean of 0.7 days). Moreover, the authors noted that the time until descent for these F2 males showing the statistically significant delays (mean of 19.0 days) was nearly identical to the control value for the F1 males (mean of 19.1 days). [d]A recent paper, not reviewed by the panel, was unable to replicate the findings of Sakaue *et al.*—J. Ashby, H. Tinwell, P. A. Lefevre, R. Joiner, and J. Haseman, *The Effect on Sperm Production in Adult Sprague-Dawley Rats Exposed by Gavage to Bisphenol A between Postnatal Days 91–97* Toxicol. Sci. 2003 74: 129–138.

Table 2. Rat studies—BPA administration *via* non-oral routes.

Study	Strain	BPA treatment groups	Doses (mg/kg-day)[a]	Other design features		Endpoints	Reported positive findings
Colerangle and Roy (1997)	Noble	6 animals/group	0 0.1	Exposure *via* s.c. for 11 days at age 5–6 wks.	Puberty	Mammary gland maturation rate	Mammary gland maturation rate
Steinmetz *et al.* (1998)	F-344 SD	Not reported	0 0.3	Silastic implant capsules—admin. for 3 days. Admin. of estradial in silastic capsules (0.006 mg/kg-day) served as positive controls.	Other Weight	Uterine cell height Uterus	Uterine height and uterus weight in F-344 but not SD
Long *et al.* (2000)	F-344 SD	3–5 animals/ group	0 0.2	BPA administered *via* i.p. at 9–11 weeks of age.	Other	Vaginal epithelium DNA synthesis	None
Ramos *et al.* (2001)	Wistar	4 dams/group	0 0.025 0.25	s.c. pump inserted in dams on g.d. 8 and left implanted through pregnancy. Male offspring sacrificed at pnd 30.	Other Perinatal	Ventral prostate androgen receptor activity, prostatic acid phospatase activity, gender ratio AGD	Prostate androgen receptor activity decreased. Prostatic acid phosphatase expression decreased.

Abbreviations and notes: see Table 1.

Table 3. Mouse studies—Oral BPA administration.

Study	Strain	BPA treatment groups	Doses (mg/kg-day)	Other design features		Endpoints	Reported positive findings
vom Saal *et al.* (1998) and Nagel *et al.* (1997)	CF1	11 control dams 7 dams/treatment group	0 0.002 0.02	BPA administered to dams on gd 11 to 17. One male selected per litter and housed individually for 1 month.	Other Weight	SE, DSP Body, preputial gland, epididymis, testes, SV	Epididymis wt down at 0.002 μg/kg-day, DSP down at 0.02 μg/kg-day, prostate weight up at 0.002 and 0.02 μg/kg-day.
Ashby *et al.* (1999)	CF1	16 control dams 8 dams/treatment group	0 0.002 0.02	BPA administered to dams on gd 11 to 17. Three males from each litter selected at pnd 71 and housed individually. Males sacrificed at pnd 183–187. Female offspring sacrificed at pnd 310.	Other Puberty Weight	DSP, gender ratio, SE Vaginal opening date Body, testes, epididymis, SV, prostate, cervix, uterus, vagina, ovaries	Increased body wt (0.002 μg/kg-day), testes wt at both doses, epididymis (0.02 μg/kg-day), DSP at both doses.[a]
Cagen *et al.* (1999b)	CF1	56 control dams 28 dams/treatment group	0 2×10^{-4} 0.002 0.02 0.2	BPA administered to dams on gd 11 to 17. Four pups per litter selected at pnd 22 and housed individually. Animals sacrificed at pnd 90.	Other Weight	Fertility, pup survival, gender ratio Body, prostate, SV, epididymis, testes	None
Gupta (2000)	CD-1	15 dams/group	0 0.05	BPA administered to dams on gd 16–18. Litters adjusted to contain 8 pups with $\geq$ 3 males. Measurements made at pnd 3, pnd 21, pnd 60.	Perinatal Weight	AGD Body, epididymis (only at pnd 60), prostate	AGD and prostate weight increased, epididymis weight decreased
Howdeshell *et al.* (1999); Howdeshell and vom Saal (2000)	not spec.	21 dams/group	0 0.0024	BPA administered to dams on gd 11–17.	Other Puberty Weight	Survival Time to 1st estrus, vaginal opening Body	Time to 1st estrus and survival decreased, body weight increased.
Tinwell *et al.* (2000)	AP	12 animals/group	0 0.5 1 5	BPA administered at pnd ~ 20–22.	Other Weight	Uterine hyperplasia and uterine hypertrophy Uterus	None

Nagao *et al.* (2002)	C57 BL/6N	50 dams 10 dams/treatment group	00.002 0.02 0.2	BPA administered to dams at gd 11–17. Pups removed from dam *via* cesarean on gd 18. Three pups per litter selected and housed individually after weaning from foster dam.	Other Weight	SE Body, testes, epididymis, SV	Decrease in SV wt at 0.002 μg/kg-day.
Nagao *et al.* (2002)	C57 BL/6N	30 males/group	0 0.002 0.02	BPA administered at pnd 21 through pnd 43.	Other Weight	SE Body, testes, epididymis, SV	None
Nagao *et al.* (2002)	C57 BL/6N	20 males/group	002 0.002 0.02 0.2	BPA administered at age 10 weeks for a total of 6 days.	Other Weight	SE Body, testes, epididymis, SV	None

Notes: [(a)]Ashby *et al.* (1999) considered the statistically significant findings to be "equivocal." In the case of the organ weights, they noted that use of body weight corrected organ weight values (*i.e.*, organ weight divided by body weight) were *not* significantly associated with BPA treatment. They suggested that the statistically significant associations identified in their original design (*i.e.*, using analysis of covariance analysis) may have resulted because "*the underlying variability of the observed data may result in an underestimate of the true association between organ and body weight*" (p. 163). They also stated that sperm production efficiency, which was not associated with exposure, is of greater biological importance than daily sperm production, which was associated with exposure. **Abbreviations**: see Table 1.

Table 4. Mouse studies—BPA administration *via* non-oral routes.

Study	Strain	BPA treatment groups	Doses (mg/kg-day) [a]	Other design features	Endpoints		Reported positive findings
Tinwell *et al.* (2000)	AP	6–25 animals/ group	0 2×10^{-5} 2×10^{-4} 0.002 0.02 0.2 0.5 1 2 5	Paper describes 8 experiments in which BPA was administered *via* s.c. injection for 3 days starting at pnd 19–20. Animals exposed to 10 μg/kg-day DES served as positive controls	Other Weight	Uterine hyperplasia (two experiments) and uterine hypertrophy Uterus	Hyperplasia significant at 5 mg/kg-day but no dose response.
Markey *et al.* (2001)	CD-1	6–10/group	0 0.025 0.25	BPA administered *via* osmotic pump from gd 9 to end of pregnancy. Mammary glands of female offspring examined at pnd 10, age 1 month, and 6 months.	Other	Mammary gland ductal migration rate, percent of ducts, percent terminal ducts, percent terminal end buds, percent alveolar buds	Ductal migration up at low dose, down at high dose. Percent terminal end buds up in low dose group only. Other endpoints up for both exposed groups.

Abbreviations and notes: see Table 1.

Table 5. Results for rat studies—Oral administration.

	Dose (mg/kg-day) order of magnitude						
Endpoint	$\leq 10^{-5}$	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1	10
Other							
Androgen levels	00	0	0	0			
Avoidance behavior						Δ	
Brain anatomy						0	
Fertility	00	0	00	0000	000	0	
Gender ratio			0	0	0	0	
Hormone levels	00	0	0	0		0	
Ovary histopathology						0	
Pup survival			0	0	0	0	
Sperm characteristics						0	
Sperm—daily production	0000	000	00000	000000	00000	0	0
				−	−	−	
Sperm efficiency	00	00	0000	000	000	0	0
				−	−	−	
Testes histopathology						0	
Ventral prostate androgen activity	00	0	0	0			
Perinatal							
AGD	00	00	000	00000	0000		0
Puberty							
Estrus cycle		0	0	0	0	0	
Time until first estrus				00	00		
Preputial separation date	00	00	00	0000	000		
Testes descent date		0	0	0	0		
Vaginal opening date		0	00	0000	0000		0
Weight							
Body	00	00	0000	000000	000000	00	0
Cervix				00	00		
Epididymis	00	00	0000	000000	00000	00	0
Liver				00	00		
Ovaries		0	00	0000	0000	0	0
Preputial gland			00	00	00	0	0
Prostate and ventral prostate	00	00	0000	000000	00000	00	0
Seminal vesicles	00	00	0000	000000	00000	0	0
Testes	0000	000	00000	0000000	000000	000	0
				−			0
Uterus		0	00	0000	0000	0	0
Vagina				00	00		

Key: Each study result is represented by a single character, "0" indicating no statistical difference from controls, "−" indicating a decrease relative to controls, and "+" indicating an increase. A "Δ" represents a difference that does not have an obvious directionality (*e.g.*, changes in avoidance behavior). Statistically significant differences were generally characterized as either "+" or "−". See text for further explanation.

Table 6. Results for rat studies—Administration route other than oral.

	Dose (mg/kg-day) order of magnitude						
Endpoint	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1	10
Other							
Prostatic acid phosphatase activity				−	−		
Uterine cell height					0		
					+		
Vaginal epithelium DNA synthesis					00		
Ventral prostate androgen receptor activity				−	−		
Perinatal							
AGD				0			
Puberty							
Mammary gland maturation rate	+						
Weight							
Body				0	00		
Uterus					0 +		

Key: See Table 5.

2.1.1. Rat studies—oral administration

Table 5 summarizes the studies in this category.

Organ Weight. Investigators who studied the impact of oral exposure to BPA on organ weights in rats included Cagen *et al.* (1999a), Ema *et al.* (2001), Kubo *et al.* (2001), and Tyl *et al.* (2002). Organs studied included the epididymis, ovaries, preputial gland, prostate, seminal vesicles, testes, and uterus. The vast majority of the findings revealed no difference between treatment animals and controls (see Table 5). Because all organs may not be equally sensitive to a possible BPA effect, corroboration judgments are based primarily on those putative BPA responsive tissues and tissues that would be expected to be sensitive to estrogen effects.

Perinatal Characteristics. Ema *et al.* (2001) reported no association between BPA exposure and anogenital distance. Tyl *et al.* (2002), did report a statistically significant increase in AGD among females born to treated animals. However, we do not consider the effect to be treatment related because it was not consistent across generations. In addition, Tinwell *et al.* (2002) found no change in AGD with BPA exposure (Table 5).

Pubertal Characteristics. Investigators evaluating the impact of BPA exposure on pubertal characteristics included Ema *et al.* (2001), Kubo *et al.* (2001), and Tyl *et al.* (2002). Endpoints investigated included estrus cycle characteristics, preputial separation date, testicular descent date, and vaginal opening date. Neither Ema *et al.* nor Kubo *et al.* reported changes in estrus cycle characteristics. Tyl *et al.* identified a delay in vaginal patency among treated animals.

Other Endpoints. Sakaue *et al.* (2001) reported a decrease in daily sperm production and sperm production efficiency when adult rats were exposed to BPA at a wide

Table 7. Results for mouse studies—Oral administration

	Dose (mg/kg-day) order of magnitude						
Endpoint	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1	10
Other							
Fertility		0	0	0	0		
Gender ratio		0	00	00	0		
Pup survival		0	0	0	0		
			−				
Sperm—daily production		0	00	00	00	0	
			+	+			
Sperm efficiency		0	0000	000	00		
				−			
Uterine hyperplasia						00	0
Uterine hypertrophy						00	0
Perinatal							
AGD					+		
Puberty							
Time until first estrus			−				
Preputial separation date		0	0	0	0		
Vaginal opening date			00	0			
Weight							
Body		0	00	0000	000		
			−				
			++				
Cervix			0	0			
Epididymis		0	000	000	00		
			−	+	−		
Ovaries			0	0			
Preputial gland			+	0			
Prostate and ventral prostate		0	00	00	0		
			+	+	+		
Seminal vesicles		0	000	0000	00		
			−				
Testes		0	000	000	00		
			+	+			
Uterus			0	0		00	0
Vagina			0	0			

Key: See Table 5.

range of doses. However, a variety of other studies with fetal rats did not find any effects at the same or similar doses (Cagen *et al.* 1999a; Ema *et al.* 2001; Tyl *et al.* 2002).[2] It should be noted that Ema *et al.* (2001) and Tyl *et al.* (2002) performed multigenerational studies that by design included BPA exposures during the adult

[2]A recent paper, not reviewed by the panel, was unable to replicate the findings of Sakaue *et al.*—J. Ashby, H. Tinwell, P. A. Lefevre, R. Joiner, and J. Haseman, The Effect on Sperm Production in Adult Sprague-Dawley Rats Exposed by Gavage to Bisphenol A between Postnatal Days 91–97, Toxicol. Sci. 2003 74: 129–138

Table 8. Results for mouse studies—Administration route other than oral.

	Dose (mg/kg-day) order of magnitude						
Endpoint	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1	10
Other							
Mammary gland ductal migration rate				+	−		
Mammary gland percent alveolar buds				+	+		
Mammary gland percent ducts				+	+		
Mammary gland percent terminal ducts				+	+		
Mammary gland percent terminal end buds				+	0		
Uterine hyperplasia				0	0	00	+
Uterine hypertrophy	0	0	0	0	0	000	0
Weight							
Uterus	0	0	0	0	0	000	0

Key: See Table 5.

period. Analysis of the data of Tyl *et al.* showed no decrease in daily sperm production in the F0 generation and a 7% decline in sperm production at 0.02 mg/kg-day (which was not statistically significant). Ema *et al.* did not analyze daily sperm production but found no significant decreases in testicular weight or cauda epididymal sperm counts in the F0 or F1 generations.

Kubo *et al.* (2001) reported no association between exposure to 1.5 mg/kg-day BPA and testosterone or gonadotropin levels, ovarian histopathology, sperm characteristics, testicular or epididymal weights, or testicular histopathology. There were no other studies of these endpoints in rats orally exposed to BPA.

Conclusion. The panel concludes that there is no corroboration of positive findings for an association between BPA exposure *via* oral administration and any effects on rats.

2.1.2. Rat studies—administration *via* routes of exposure other than oral

Table 6 summarizes the results from studies of rats exposed to BPA *via* routes of exposure other than oral. Because there is no more than one study for each endpoint, there cannot be any corroborated findings.

2.1.3. Mouse studies—oral administration

As was the case with rats, the results of the majority of the studies for mice orally exposed to BPA at low doses (*i.e.*, 0.001 mg/kg range) (Table 7) do not indicate a difference between treatment animals and controls for many of the endpoints analyzed.

Body Weight and Organ Weight. vom Saal *et al.* (1998) reported that BPA exposure decreased body weight in CF1 mice exposed *in utero*, whereas Ashby *et al.* (1999) (CF1 mice) and Howdeshell *et al.* (2000) (strain unspecified) reported an increase in body

weight among mice exposed to similar doses of BPA (0.002 and 0.0024 mg/kg-day BPA, respectively).

Ashby *et al.* (1999) reported no change in cervix weight among animals exposed to 0.002 or 0.02 mg/kg-day BPA. However, no other studies investigated this endpoint.

vom Saal *et al.* (1998) observed a decrease in epididymis weight at a dose of 0.002 mg/kg-day but Cagen *et al.* (1999b) did not. At 0.02 mg/kg-day, Ashby observed an increase in epididymis weight, but neither vom Saal *et al.* nor Cagen *et al.* observed any change at that dose. However, in Ashby *et al.* the ratio of epididymis weight to body weight did not statistically differ from controls at 0.02 mg/kg-day. At a dose of 0.05 mg/kg-day, Gupta (2000) observed a decrease in epididymis weight. There were no other study observations at that dose. However, at 0.2 mg/kg-day, Cagen *et al.* (1999b) observed no change in epididymis weight. None of the changes observed for this endpoint were corroborated by multiple studies. Those changes that were observed are not consistent with either a monotonic or a u-shaped dose-response relationship. It is possible that the observed changes are simply due to a treatment-independent shift from control values.

Ashby *et al.* (1999) reported no change in ovarian weight among animals exposed to 0.002 mg/kg-day or 0.02 mg/kg-day BPA. However, no other studies investigated this endpoint.

vom Saal *et al.* (1998) reported an increase in preputial gland weight at a dose of 0.002 mg/kg-day, but no change at a dose of 0.02 mg/kg-day. No other study results are available to which these findings could be compared.

Nagel *et al.* (1997) reported an increase in prostate weight among mice receiving 0.002 mg/kg-day or 0.02 mg/kg-day BPA. However, neither Cagen *et al.* (1999b) nor Ashby *et al.* (1999) observed any changes in prostate weight at these doses. Gupta (2000) reported an increase in prostate weight at a dose of 0.05 mg/kg-day. No other studies reported results for this dose. Ashby *et al.* reported no change in prostate weight at a dose of 0.2 mg/kg-day. The soundness of the negative findings reported by Ashby *et al.* and Cagen *et al.* have been questioned because neither of these groups detected a change in prostate weight among DES-treated animals. To this point, Ashby (1999) has responded that at low doses, DES should not be considered a positive control because its effects are equivocal at that level. In particular, Ashby notes that only vom Saal has reported *in utero* DES effects following low dose oral exposure. A single additional study has reported *in utero* DES effects at low doses, but the DES was administered *via* ip injection, complicating direct comparison to oral administration studies because the orally administered DES is subjected to first-pass hepatic metabolism (Neumann 1976).

None of the studies that measured seminal vesicle weight (vom Saal *et al.* 1998; Ashby *et al.* 1999; Cagen *et al.* 1999b) reported changes compared to controls at doses ranging from 2×10^{-4} to 0.2 mg/kg-day.

Ashby *et al.* (1999) reported about a 13% increase in absolute testicular weight among animals exposed to either 0.002 mg/kg-day or 0.02 mg/kg-day but no increase when testis weight was expressed as a percentage of body weight. This increase in testicular weight appears to be an unusual finding, and there is no evidence that it can be explained by the increase in body weight. Neither vom Saal *et al.* (1998) nor Cagen *et al.* (1999b) reported any change in testicular weight at these doses.

Neither Ashby *et al.* (1999) nor Tinwell *et al.* (2000) observed any change in uterine weight among animals exposed to BPA. The doses tested in these two studies did not overlap. Ashby *et al.* tested doses of 0.002 and 0.02 mg/kg-day, and Tinwell *et al.* tested doses of 0.5, 1, and 5 mg/kg-day.

Perinatal Characteristics. Gupta (2000) observed an increase in anogenital distance among animals exposed to 0.05 mg/kg-day. However, Gupta did not report results for any other doses. Nor did any other investigators report results for this endpoint among orally exposed mice.

Pubertal Characteristics. Howdeshell *et al.* (1999) reported a decrease in time until first estrus among animals exposed to 0.0024 mg/kg-day BPA. However, no other investigators reported results for this endpoint for mice exposed orally to BPA.

Cagen *et al.* (1999b) reported no change in preputial separation date among animals exposed to between 2×10^{-4} and 0.2 mg/kg-day BPA, but no other investigators reported results for this endpoint.

Ashby *et al.* (1999) reported no change in vaginal opening date for animals exposed to either 0.002 mg/kg-day or 0.02 mg/kg-day BPA, but no other investigators reported results for this endpoint.

Other Endpoints. Ashby *et al.* (1999) reported that daily sperm production increased among mice exposed to 0.002 mg/kg-day or 0.02 mg/kg-day BPA. However, sperm production efficiency did not show any increase because of the increased testicular weight. Nevertheless, we find the increase in daily sperm production reported by Ashby to be a very unusual finding. Neither vom Saal *et al.* (1998) nor Cagen *et al.* (1999b) reported any significant change in sperm production at these doses, but at 0.02 mg/kg-day, vom Saal *et al.* (1998) observed a significant decrease in sperm production efficiency. Tinwell *et al.* (2000) reported a significant increase in uterine hyperplasia at 5 mg/kg-day.

Conclusion. Among the studies that reported positive findings on the male reproductive system of mice exposed to BPA *via* oral administration (Nagel *et al.* 1997; vom Saal *et al.* 1998; Ashby *et al.* 1999; Gupta 2000), there are two cases in which multiple studies produced positive findings for the same endpoint.

In the first case, although three studies reported that BPA exposure influenced epididymis weight (vom Saal *et al.* 1998; Ashby *et al.* 1999; Gupta 2000), the panel concludes that the results do not form a convincing pattern. First, Ashby *et al.* concluded that the positive association they observed was not directly due to BPA exposure because it vanished when epididymis weight was divided by body weight. Second, although vom Saal *et al.* reported that 0.002 mg/kg-day BPA exposure decreases epididymis weight, they reported no impact at 0.02 mg/kg-day exposure although the decreases were of a similar magnitude (-12% at 0.002 mg/kg-day and -8% at 0.02 mg/kg-day). Third, although Ashby *et al.* reported that 0.02 mg/kg-day BPA exposure increases epididymis weight, both vom Saal and Gupta reported that BPA exposure decreased epididymis weight (decreases of about 10% and 35%, respectively). Finally, Cagen *et al.* (1999b) reported no change in epididymis weight at 2×10^{-4}, 0.002, 0.02, and 0.2 mg/kg-day exposure.

The second case pertains to changes in prostate weight. Although Nagel *et al.* (1997) reported an increase in prostate weight among animals exposed to 0.002 and 0.02 mg/kg-day and Gupta reported an increase in prostate weight among animals exposed to 0.05 mg/kg-day, two other studies that replicated the Nagel *et al.* study (Ashby *et al.* 1999; Cagen *et al.* 1999b) did not find this same effect. Section 2.2.2 discusses the methodological quality of these competing studies. For the purpose of this section, it is sufficient to state that there was at best limited consistency across the studies that evaluated this endpoint.

2.1.4. Mouse studies—administration *via* routes of exposure other than oral

Table 8 summarizes the results for studies of mice exposed to BPA *via* routes other than oral administration. Markey *et al.* (2001) reported an increase in the rate of mammary gland ductal migration into the stroma in mice exposed to 0.025 mg/kg-day BPA *via* sc pump, but a decrease among animals exposed to 0.25 mg/kg-day BPA. However, no other studies investigated this endpoint in mice. Markey *et al.* also reported an increase in the percentage of ducts, terminal ducts, terminal end buds, and alveolar buds among six-month-old mice exposed to either 0.025 mg/kg-day or 0.25 mg/kg-day BPA. Again, there were no other studies of BPA's impact on these endpoints.

Tinwell *et al.* (2000) did not find any association between uterine weight and BPA exposures *via* sc pump ranging from 2×10^{-5} to 5 mg/kg-day.

2.2. Experimental Design—Power and Rigor

Sections 2.2.1 and 2.2.2 evaluate the rat study results and mouse study results, respectively, and explore potential factors that may help to explain inconsistent findings among studies. Power refers to the number of analytical units (litters or pups). Factors influencing judgment of rigor include use of Good Laboratory Practice (GLP), appropriateness of control groups, and others. Because no two studies exposing animals *via* routes other than oral administration investigated the same endpoint, this section limits attention to studies exposing animals *via* oral administration.

2.2.1. Rat study results

Organ Weight. With the exception of Tyl *et al.* (2002), who reported a decline in testes weight at one dose in the F2 and F3 generations, none of the rat studies we have included in this analysis reported an association between BPA exposure and organ weights. The absence of any large changes reported by various combinations of these studies (Cagen *et al.* 1999a; Ema *et al.* 2001; Tyl *et al.* 2002) are unlikely to be the result of inadequate statistical power. All three studies were relatively large: Ema *et al.* conducted a multigenerational study with 25 dams per zero-generation treatment group; Tyl *et al.* conducted a multigenerational study with 30 dams per zero-generation treatment group; and Cagen *et al.* (single generation study) had 28 dams per treatment group.

Perinatal Characteristics. Both studies of perinatal characteristics in rats (Ema *et al.* 2001; Tyl *et al.* 2002) investigated the potential impact of BPA exposure on AGD.

Neither study reported an association at any dose level. As noted earlier, these studies had large sample sizes, decreasing the likelihood that the negative findings are the result of inadequate statistical power.

Pubertal Characteristics. Both studies of pubertal characteristics in rats reported no association between BPA exposure and any of the endpoints investigated, including preputial separation date, testes descent date (Ema *et al.* 2001), and vaginal opening date (Ema *et al.* 2001; Tyl *et al.* 2002). Once again, these studies had large sample sizes, decreasing the likelihood that the negative findings are the result of inadequate statistical power.

Other Endpoints. Sakaue *et al.* (2001) reported that BPA exposure of adult rats decreased daily sperm production by 30% at doses of 0.02 mg/kg-day and above. Ema *et al.* (2001), Cagen *et al.* (1999a), and Tyl *et al.* (2002) all reported no such association. The Cagen *et al.* study is not comparable to Sakaue because it involved treatment of the rats during gestation only, whereas the Ema *et al.* and Tyl *et al.* studies were multigenerational and included adult exposure. Although the numbers of animals in each treatment group in the Sakaue *et al.* study (five in one experiment and eight in the other) were low, the results at the doses tested in both experiments were consistent. However, the numbers were substantially fewer than the number of animals per treatment group in the Ema *et al.* and Tyl *et al.* studies. Ema *et al.* did not measure daily sperm production but did measure cauda epididymal sperm counts and showed no significant decline. There is no information available suggesting that the design difference, multigenerational as opposed to adult only exposure, could reduce the effect seen; in fact, the opposite would be expected.

Conclusion. For those endpoints for which large changes have been reported, there are studies indicating no change or at most small changes in the endpoints; and the latter studies are well conducted and larger than the positive studies. We conclude that the negative findings for reproductive endpoints for rats are more compelling than the positive findings.

2.2.2. Mice

Five publications contain the vast majority of the reported results from studies of mice exposed to BPA *via* oral administration. The vom Saal *et al.* (1998) and Nagel *et al.* (1997) analyses are based on the same set of animals. The studies of Ashby *et al.* (1999) and Cagen *et al.* (1999b) were conducted independently in an effort to replicate the vom Saal *et al.* and Nagel *et al.* experiment, although there were several differences among these studies, as described later. Gupta (2000) reported the impact of BPA exposure on AGD, prostate weight, and epididymis weight at one dose level (0.05 mg/kg-day).

Another study, described by Howdeshell *et al.* (1999) and Howdeshell and vom Saal (2000), reported that BPA exposure (0.0024 mg/kg-day) reduced time until first estrus, but only at one dose level. Because no other study measured that endpoint and only one dose was tested, it will not be considered further in this discussion of corroboration.

A final study (Tinwell *et al.* 2000) investigated BPA's uterotrophic activity at doses ranging from 2×10^{-5} to levels exceeding 5 mg/kg-day and within this range reported a statistical association only at 5 mg/kg-day. Because the findings of this study are negative and because no other studies investigated this endpoint in mice, further discussion of this finding is unnecessary in this section.

As noted by the NTP peer review group (p. A-8 in National Toxicology Program (NTP) 2001), the potential association between BPA and prostate weight has been regarded as particularly important. We therefore turn our attention to the four studies that have measured prostate weight in mice exposed to BPA (Nagel *et al.* 1997; Ashby *et al.* 1999; Cagen *et al.* 1999b; Gupta 2000). The following discussion first evaluates factors that may have masked the presence of an association between BPA exposure and prostate weight in the negative studies, and then evaluates factors that may have given rise to non-causal association in the positive studies. The comments made with respect to the Nagel *et al.* (1997) study also apply to the vom Saal *et al.* study (1998) because vom Saal *et al.* used pups taken from the same dams and a very similar methodology.

Factors Potentially Masking an Association Between BPA Exposure and Prostate Weight. Prostate weight measurement accuracy: Perhaps the most important confounding factor in all of the prostate studies is that prostatic wet weight is an extremely poor measure of prostatic growth, which substantially diminishes the strength of data advanced both for and against an effect of BPA on prostatic growth. Wet weight potentially reflects number of cells, amount of secretion, and amount of extracellular matrix. The mouse prostate is multi-lobar, and exact descriptions of the dissection process, in particular, what tissue was included or excluded, is not clear in the various papers on effects of low dose BPA. We note that none of the studies reporting that BPA exposure influences prostatic weight report any other prostatic size measures (*e.g.*, proliferation or apoptotic indices) that might provide confirmatory evidence.

In addition, dissection of the mouse prostate is subject to considerable variation between different operators. It has been suggested that expertise in the dissection of the mouse prostate may have been better in the case of studies conducted by vom Saal *et al.* and Nagel *et al.* compared to the studies conducted by Ashby *et al.* and Cagen *et al.* because the Ashby *et al.* and Cagen *et al.* investigators were less experienced in prostatic anatomy (vom Saal and Welshons 2000). However, in assays of other chemicals (*e.g.*, cyproterone acetate), Ashby and LeFevre (2000) reported a statistically significant impact on prostate weight in rats. Although rat prostates are larger than mouse prostates (and thus presumably easier to measure), the Ashby *et al.* study measured prepubertal rats (age 23 days) with prostate weights averaging from 43.0 mg (cyproterone acetate treatment group) to 76.5 mg (anastrozole treatment group), values that do not differ substantially from those of Nagel *et al.* (1997), who measured prostatic weights in adult mice of 41 mg (controls) to 55 mg (0.02 mg/kg-day BPA).

Weight of the control prostate in five studies (Nagel *et al.* 1997; vom Saal *et al.* 1998; Ashby *et al.* 1999; Cagen *et al.* 1999b; Thayer *et al.* 2001) ranged from 36 mg (Thayer *et al.* 2001) to 49 mg (Ashby *et al.* 1999). Average wet weight of the control prostate for these five studies was 41 mg. The Ashby *et al.* control group prostate weight was at the high end of the range. The question arises as to whether the control prostatic

weight reported by Ashby *et al.* is artifactually high. At 41 mg, the control prostatic weight reported by Nagel *et al.* is nearly equal to the average for all five studies. This discussion highlights the difficulties in using prostatic wet weight as the measure of low dose BPA or DES effects. Whereas all of the vom Saal *et al.* studies showed a significant increase in prostatic weight induced by DES, EE, or BPA, the studies by Cagen *et al.*, with a control group average prostatic weight of 39 mg, and Ashby *et al.*, with a control group average prostatic weight of 49 mg, showed no increase in prostatic weight in response to low dose DES or BPA.

Finally, intra-uterine position effects may have played a role in the Nagel *et al.* study (which included only a single animal from each litter) and the Gupta (2000) study (which used one animal from each litter at each of three ages). However, in contrast, the negative studies conducted by Cagen *et al.* and Ashby *et al.* used multiple animals from each litter. Therefore, any intra-uterine position effects should have been averaged out.

Statistical power: The Ashby *et al.* study included a substantially larger number of animals (11 control dams yielding 54 control animals, 7 dams producing 37 animals receiving 0.002 mg/kg-day BPA, and 6 dams producing 29 animals receiving 0.02 mg/kg-day BPA) than did Nagel *et al.* (11 control group dams yielding 11 males included in the experiment, and 7 dams in each exposed group, yielding 7 exposed males in each dose group). The Cagen *et al.* study was also substantially larger than the Nagel *et al.* study (56 dams in the control group and 28 in each exposed group, yielding 179 control pups surviving to at least age 22 days, and between 81 and 92 pups in each of the exposed treatment groups). As a result, it is unlikely that a lack of statistical power explains the negative results reported by Ashby *et al.* and Cagen *et al.*

Test animal strain: Ashby *et al.* and Cagen *et al.* used commercially available CF1 mice (Charles River Laboratories), whereas vom Saal *et al.*/Nagel *et al.* used mice bred in-house (University of Missouri) for over two decades. Unfortunately, this strain no longer exists, and it is therefore impossible to conduct a confirmatory study in this strain. It is possible that due to genetic drift, the mice used by vom Saal *et al.*/Nagel *et al.* had become more sensitive to estrogenic effects than the CF1 mice obtained by Ashby *et al.* directly from Charles River (Ashby *et al.* 1999; Ashby 2000). Spearow *et al.* have identified differences in sensitivity to inhibition of spermatogenesis during pubertal development by estradiol in different mouse strains (Spearow *et al.* 1999, 2001). Because differences in strain sensitivity to estrogenic effects have been well documented, it is plausible that this factor could explain the difference in results. Spearow *et al.* also showed that inbreeding of CD-1 mice resulted in an increase in sensitivity to the testicular effects of estradiol. It is more likely that the mice bred in-house in the vom Saal *et al.*/Nagel *et al.* studies might have been more inbred. Gupta, who reported that BPA did influence several factors, including prostate weight, used commercially available CD-1 mice, the strain reported by Spearow *et al.* (1999) to be relatively resistant to the testicular effects of estradiol.

Feed administered to test animals: Ashby *et al.* (1999) hypothesized that the prostate weights in their study were elevated relative to those reported by Nagel *et al.* due to phyto-estrogen activity in the soy Ashby *et al.* used as feed, and that this increase in prostate weight might make the Ashby *et al.* study less sensitive to detection of further prostate weight increases. They dismissed this possibility, however, pointing

out that the prostate weights in their study were only modestly elevated relative to those reported by Nagel *et al.*; that the feed Ashby *et al.* used (Rat and Mouse No. 1 maintenance, "RM1") has been used successfully in other estrogen studies conducted in their lab; and that the estrogen activity in the RM1 diet is markedly lower than the estrogen activity of the Purina 5001 feed used in the vom Saal *et al.* and Nagel *et al* studies (Ashby 2000). Thus, it seems unlikely that phytoestrogens in the feed used by Ashby *et al.* could explain their negative findings.

Cagen *et al.* used Purina 5002, whereas vom Saal *et al.*/Nagel *et al.* used Purina 5001. We are unaware of any studies published as of our review cutoff date that have compared the estrogenic activity of Purina 5001 and 5002. However, given the fact that the relatively elevated estrogenic activity of Purina 5001 did not mask the putative effects observed by vom Saal *et al.*/Nagel *et al.*, there is at the very least an absence of evidence that the use of the Purina 5002 feed could mask effects in the Cagen *et al.* study.

Age at sacrifice: Cagen *et al.* sacrificed and examined the animals at an earlier age (90 days) than did vom Saal *et al.*/Nagel *et al.* (180 days). Cagen *et al.* stated that they sacrificed the animals at an earlier age "*because effects on male sex accessory organs were reportedly driven by the in utero BPA exposure* [according to the vom Saal/Nagel group]*, and sacrifice time after puberty was not critical (vom Saal and Thayer, 1997, personal communication)*" (p. 37 in Cagen *et al.* 1999b). There is no evidence that sacrifice of the animals at an earlier age may have masked effects that would have otherwise been evident.

Factors Casting Doubt on Whether the Associations Reported Between BPA Exposure and Prostate Weight are Causal

Inadequate Confounder Control: Ashby *et al.* controlled for either of two factors (body weight and individual *vs.* group housing) whenever they were significant predictors of the endpoints of interest (prostate weight, seminal vesicle weight, testis weight, epididymal weights, and sperm production). They reported that body weight was a significant predictor for all organ weight measures, but was not a significant predictor for the sperm production measures. Conversely, housing was not a significant predictor for any of the organ weights, but was a significant predictor for daily sperm production.

vom Saal *et al.* and Nagel *et al.* evaluated only the associations between body weight and organ weight measures (prostate, testis, epididymis, seminal vesicle, and preputial gland). Body weight was correlated with testis weight and epididymal weight. Despite the fact that they did not detect a significant association between body weight and prostate weight, they verified that controlling for body weight (*i.e.*, conducting an analysis of covariance) did not eliminate the significant association between BPA treatment and increased prostate weight. Although NTP (2001) raised several technical issues with respect to the authors' adjustment for body weight, the association between BPA and prostate weight remained significant in the panel's reanalysis (although the P value increased from <0.01 to <0.05).

Although Ashby *et al.* considered a larger set of potential confounders, we conclude that these statistical issues do not *per se* disqualify the findings reported by vom Saal *et al.*/Nagel *et al.* In particular, effects on the prostate and testis remained significant even after control for body weight, and it does not seem likely that controlling

for body weight in the other cases (*i.e.*, in those cases where body weight was not significantly correlated with organ weight) would eliminate statistical significance. vom Saal *et al.*/Nagel *et al.* did not consider housing effects. Although they state that their approach of group housing (three male littermates/cage) followed by one month of individual housing for a randomly chosen individual "eliminates the prior effect of subordination" (Nagel *et al.* 1997), there is concern that the known effects of social hierarchies on accessory sex organs (Bartos and Brain 1993) may not have been completely eliminated leading to residual confounding.

Rather than using analysis of covariance to control for the impact of body weight on organ weight, Gupta used body weight normalized prostate weights (*i.e.*, prostate weight divided by body weight). However, because body weights of control animals and BPA-treated animals were very similar, it is unlikely that this aspect of the analysis introduced an apparent association between BPA treatment and prostate weight.

Failure to Account for Litter Effects: Ashby *et al.* controlled for litter effects when litter membership was significantly associated with the outcome of interest (all organ weights), and used the pup as the unit of analysis otherwise. Cagen *et al.* used the litter as the unit of analysis for all endpoints. Treating each pup as a statistically independent observation when measurements from pups coming from the same litter are correlated in effect overstates the amount of information available and thus overstates the statistical power available to distinguish among treatment groups.

vom Saal *et al.* and Nagel *et al.* selected a single (different) pup from each litter and treated each pup as statistically independent. Gupta also selected a single pup from each of 15 litters to measure the impact of BPA exposure on prostate weight. Elswick *et al.* (2000) claim that selecting a single pup from each litter can increase the probability of a false-positive finding. However, Haseman *et al.* (2001) point out that treating each pup as statistically independent does not increase the false positive probability when the pups each come from different litters, although failure to use information from all pups in the litter can increase the probability of a false negative finding because less information is available to distinguish among treatment groups. We conclude that this aspect of the vom Saal *et al.*/Nagel *et al.* study design did not increase the risk of a false positive finding beyond the nominal P value.

Prostate weight variance: Inconsistencies between the Nagel et al. *study and other studies*: During gestation, rodent fetuses are arrayed linearly in the two horns of the uterus. Because the gender of each fetus is random, individual fetuses may develop with two males as neighbors, or two females, or one of each sex, or (if the fetus is at the end of a horn's array) one neighbor, either male or female. There is evidence that the genders of neighboring fetuses can influence the hormonal milieu and can therefore affect the state of hormonally influenced characteristics of the developing organism (Nonneman *et al.* 1992). In particular, an effect of intrauterine position (IUP) has been shown on anogenital distance (Vandenbergh and Guggett 1995), and, importantly, on prostate weight (Nonneman *et al.* 1992). Accordingly, IUP constitutes a potentially important source of intralitter variability in hormonally influenced endpoints. Moreover, IUP may have a systematic effect dependent on the (usually unknown) sexes of the fetus' neighbors.

Nonneman *et al.* (1992) found that prostate weights in 100–115-day-old males having zero males (0M) as neighbors *in utero* were about 20% larger on average than those in males with two male neighbors (2M) (males with one neighbor of

each sex, or with only one neighbor, were not studied). Androgen receptor density (fmol/mg protein) in these prostates was over threefold higher in 0M males than in 2M males. Nonneman *et al.* measured free serum estradiol concentrations that were 30% higher in 0M than in 2M fetuses.

The magnitude of the IUP effect on prostate weight is nearly as large as that observed for low doses of BPA in the Nagel *et al.* (1997) study. The small sample size and the fact that Nagel *et al.* did not record IUP raises the concern that, by chance, the control group could have been enriched in 2M males and the exposed groups in 0M males, leading to an apparent effect of BPA that should in fact be attributed to the happenstance effect of IUP. Section B of the online supplement to this article (Gray *et al.* 2004) describes a simulation of the Nagel *et al.* study, taking into account the IUP variance contribution to prostate weight. We find that even if BPA exposure has no impact on prostate weight, the analysis conducted by Nagel *et al.* would have incorrectly identified a statistically significant effect 13% of the time, that is, with greater probability than the nominal statistical significance level of $P < 0.05$ would suggest.

Conclusion. We did not identify any factor or factors that clearly invalidate either the negative or the positive findings discussed earlier. With respect to the negative studies (Ashby *et al.* 1999; Cagen *et al.* 1999b), we are unconvinced that the difficulty of measuring prostate weights contributed substantially to their findings. First, more than one independent group failed to replicate the positive prostate weight findings. Second, at least one of these groups detected a statistically significant impact on prostate weight in juvenile rats exposed to a different chemical agent. The fact that both negative studies are substantially larger than the positive studies casts doubt on the possibility that inadequate statistical power is responsible for these findings. Nor is there compelling evidence that either the type of feed administered or the age at sacrifice can explain the negative results reported. The one factor that cannot be readily dismissed is the potential differences in sensitivity to estrogenic effects among the strain types used in different studies. In particular, it is possible that the in-house bred stock of CF1 mice used by vom Saal *et al.*/Nagel *et al.* is more sensitive than the commercially available stock used by Ashby *et al.* and Cagen *et al.* It is not clear what to make of Gupta's use of the CD-1 mouse that Spearow *et al.* (1999) found to be relatively resistant to testicular effects, compared to B6 mice, following exposure to estradiol. However, we do not have data comparing the sensitivity of CD-1 mice used by Gupta (and yielding a positive finding) to the sensitivity of commercially available CF1 mice used by Ashby *et al.* and Cagen *et al.* (and yielding a negative finding).

With respect to the positive studies, it does not appear that failure to control for either potential confounders or for litter effects are responsible for the reported associations. However, as suggested by the analysis in Section B of the online supplement (Gray *et al.* 2004), the false positive probability for the Nagel *et al.* study may have exceeded the nominal statistical significance threshold of 5%. This finding reflects the anomaly that the variability in the Nagel *et al.* control group is smaller than what would be expected (*i.e.*, due to the influence of intrauterine position). The Gupta data do not exhibit these anomalies. However, given that there are two relatively large, apparently well-conducted studies that found no association between BPA exposure and prostate weight, we conclude that the evidence does not support the presence of a causal association.

3. EVIDENCE THAT EFFECTS IN EXPERIMENTAL ANIMALS CAN BE EXTRAPOLATED TO HUMANS

This section evaluates the extent to which effects observed in experimental animals would be expected to occur in humans at levels of exposure and *via* routes of exposure that might occur in the actual population. Section 3.1 addresses the universality criterion, which characterizes the degree of confidence in the possibility that the effects of BPA observed in animals can occur in humans at some level of exposure. Section 3.2 addresses the proximity criterion, which characterizes the degree of confidence in the possibility that such an effect will occur at levels and routes of exposure experienced by members of the human population.

3.1. Universality—Extrapolation of Effects from Experimental Animals to Humans

In the absence of information comparing the pharmacodynamics and pharmacokinetics of an agent in humans and test animals, we can characterize confidence in the hypothesis that an effect observed in a test species will likewise occur in humans by examining the degree to which that effect occurs in multiple test species and test strains. This section surveys the results in Tables 1–4 to assess the degree of cross species and cross strain effect concordance. We review only those effects that were observed to occur in at least one test species/strain.

Daily sperm production: Sakaue *et al.* (2001) reported a decrease in daily sperm production in adult Sprague-Dawley rats exposed to 0.02, 0.2, or 2 mg/kg-day mg/kg-day BPA administered orally. In CF1 mice Ashby *et al.* (1999) reported that exposure to BPA (0.002 or 0.02 mg/kg-day administered orally) *increased* daily sperm production. Such an increase is contrary to the expected action of an estrogen and was not observed by any other investigators. No effect on sperm production was observed in any of the following rat studies, including Ema *et al.* (2001) (2×10^{-4}, 0.002, 0.02, 0.2 mg/kg-day in IGS rats), Cagen *et al.* (1999a) (0.001, 0.023, 0.25, 2.4 mg/kg-day in Wistar rats), and Tinwell *et al.* (2002) (0.02, 0.1 mg/kg-day in Sprague-Dawley and AP-derived Wistar rats), and only small effects appear to have been present in the study by Tyl *et al.* (2002) (0.001, 0.02, 0.3, 5 mg/kg-day in Sprague-Dawley rats). Although the two studies reporting an association between BPA exposure and daily sperm production evaluated different species and at different times, the reported effects were in opposite directions, lending weight to our conclusion that these findings do not constitute concordance across test species.

Sperm Efficiency: Sakaue *et al.* (2001) reported a decrease in sperm efficiency in Sprague-Dawley rats exposed to 0.02, 0.2, or 2 mg/kg-day BPA administered orally. This effect was not observed in any other rat strain. In CF1 mice, vom Saal *et al.* (1998) reported that exposure to BPA (0.02 mg/kg-day administered orally) decreased sperm efficiency in CF1 mice. These findings amount to weak evidence of concordance because other investigators observed no change in sperm efficiency in rats (Cagen *et al.* 1999a; Ema *et al.* 2001; Tyl *et al.* 2002) and in mice (Ashby *et al.* 1999; Cagen *et al.* 1999b; Nagao *et al.* 2002).[3]

[3]Also see footnote 2.

Pup Survival: Howdeshell *et al.* (1999) reported that exposing female mice (strain unspecified) to BPA (0.0024 mg/kg-day *via* oral administration) decreased the survival rate for their offspring. However, this result was not observed in rats by Cagen *et al.* (1999a). We therefore conclude there is no evidence of concordance for this endpoint.

Anogenital Distance: Gupta (2000) reported that *in utero* exposure of CD-1 mice to 0.05 mg/kg-day BPA increased anogenital distance. However, results across a range of oral exposures had no effect on anogenital distance in several different studies, including Ema *et al.* (2001) (2×10^{-4}, 0.002, 0.02, 0.2 mg/kg-day in IGS rats), Tyl *et al.* (2002) (0.001, 0.02, 0.3, 5 mg/kg-day in Sprague-Dawley rats), and Tinwell *et al.* (2002) (0.02, 0.1 mg/kg-day in both Sprague-Dawley and AP-derived Wistar rats). There were no other reported results in mice. We therefore conclude there is no evidence of concordance for this endpoint.

Time Until First Estrus: Howdeshell *et al.* (1999) reported that exposure *in utero* to 0.0024 mg/kg-day BPA by oral administration decreased the time until first estrus in mice (strain unspecified). However, Tinwell *et al.* (2002) reported that *in utero* exposure to BPA by oral administration had no effect on this measurement in both Sprague-Dawley and AP-derived Wistar rats. There were no other reported results in mice. We therefore conclude there is no evidence of concordance for this endpoint.

Body Weight: Several investigators have reported that oral administration of BPA to mice *in utero* influences body weight. In CF1 mice, vom Saal *et al.* reported a decrease in body weight in animals exposed to 0.002 mg/kg-day relative to controls. Ashby *et al.* (1999) reported an increase in body weight relative to controls in CF1 mice also exposed *in utero* to 0.002 mg/kg-day. Howdeshell *et al.* (1999) reported an increase in body weight relative to controls among mice exposed to 0.0024 mg/kg-day BPA (strain unspecified). In rats, no body weight effects were observed across a range of BPA exposure studies, including Sakaue *et al.* (2001) (2×10^{-6}, 2×10^{-5}, 2×10^{-4}, 0.002, 0.02, 0.2, 2 mg/kg-day in Sprague-Dawley rats), Ema *et al.* (2001) (2×10^{-4}, 0.002, 0.02, 0.2 mg/kg-day in IGS rats), Tyl *et al.* (2002) (0.001, 0.02, 0.3, 5 mg/kg-day in Sprague-Dawley rats), Cagen *et al.* (1999a) (0.0025, 0.023, 0.25, 2.4 mg/kg-day in Sprague-Dawley rats), and Tinwell *et al.* (2002) (0.02, 0.1 mg/kg-day in Sprague-Dawley and Wistar-derived AP rats). Because of the apparent absence of a body weight effect in rats, and because the limited observations in mice are not in a consistent direction, we conclude that there is no evidence of concordance for this endpoint.

Epididymis Weight: Two investigators have reported that *in utero* oral exposure to BPA affects epididymis weight. vom Saal *et al.* (1998) reported that CF1 mice exposed to 0.002 mg/kg-day BPA had lower epididymis weights than control mice. Likewise, Gupta (2000) reported lower epididymis weights in CD-1 mice exposed to 0.05 mg/kg-day BPA compared to controls. Ashby *et al.* (1999) observed higher epididymis weights in CF1 mice exposed to 0.02 mg/kg-day BPA compared to controls. Tyl *et al.* (2002) (0.001, 0.02, 0.3, 5 mg/kg-day in Sprague-Dawley rats) reported no statistically significant changes across all of the different doses. There was no evidence of an effect on epididymis weights among BPA exposed rats in several other studies, including Ema *et al.* (2001) (2×10^{-4}, 0.002, 0.02, 0.2 mg/kg-day in IGS rats), Cagen *et al.* (1999a) (0.0025, 0.023, 0.25, 2.4 mg/kg-day in Wistar rats), and Tinwell *et al.* (2002) (0.02, 0.1 mg/kg-day in Sprague-Dawley and AP-derived

Wistar rats). Because of the apparent absence of an effect on epididymis weight in most studies in rats, and because the limited positive observations in mice are not in a consistent direction, we conclude that there is no evidence of concordance for this endpoint.

Preputial Gland Weight: vom Saal *et al.* (1998) reported that BPA exposure increased preputial gland weight in CF1 mice at one of the two dose levels in their study (0.002 mg/kg-day). No other investigators measured this endpoint in mice. Neither of the studies that measured this endpoint in rats reported any association, including Tyl *et al.* (2002) (0.001, 0.02, 0.3, 5 mg/kg-day in Sprague-Dawley rats), and Cagen *et al.* (1999a) (0.001, 0.023, 0.25, 2.4 mg/kg-day in Wistar rats). Because only one investigator has measured this endpoint in mice, and because all measurements of this endpoint in rats have not indicated an association with BPA exposure, we conclude that there is no evidence of concordance for this endpoint.

Prostate Weight: vom Saal *et al.* (1998) (0.002, 0.02 mg/kg-day BPA in CF1 mice) and Gupta (2000) both observed an increase in prostate weights among exposed mice. However, no such association was observed by Ema *et al.* (2001) (2×10^{-4}, 0.002, 0.02, 0.2 mg/kg-day in IGS rats), Tyl *et al.* (2002) (0.001, 0.02, 0.3, 5 mg/kg-day in Sprague-Dawley rats), Cagen *et al.* (1999a) (0.001, 0.023, 0.25, 2.4 mg/kg-day in Wistar rats), and Tinwell *et al.* (2002) (0.02, 0.1 mg/kg-day in Sprague-Dawley and AP-derived Wistar rats). Given this negative evidence in rats, we conclude that the vom Saal *et al.* and Gupta results offer at most modest evidence of concordance across strains, but there is no evidence of concordance across species.

Seminal Vesicles Weight: Nagao *et al.* (2002) reported a decrease in seminal vesicles weight among C57BL/6N mice exposed *in utero* to 0.002 mg/kg-day BPA. However, vom Saal *et al.* (1998), Cagen *et al.* (1999b), and Ashby *et al.* (1999) observed no such effect in CF1 mice subject to the same BPA exposure (as well as others). No association between BPA exposure and seminal vesicle weight has been reported in rats by Ema *et al.* (2001) (2×10^{-4}, 0.002, 0.02, 0.2 mg/kg-day in IGS rats), Tyl *et al.* (2002) (0.001, 0.02, 0.3, 5 mg/kg-day in Sprague-Dawley rats), Cagen *et al.* (1999a) (0.001, 0.023, 0.25, 2.4 mg/kg-day in Wistar rats), and Tinwell *et al.* (2002) (0.02, 0.1 mg/kg-day in Sprague-Dawley and AP-derived Wistar rats). We conclude there is no evidence of concordance for this endpoint.

Testes Weight: Ashby *et al.* (1999) reported an association between exposure to 0.002 or 0.02 mg/kg-day BPA in CF1 mice and an increase in testes weight. Data from Tyl *et al.* (2002) (0.001, 0.02, 0.3, 5 mg/kg-day in Sprague-Dawley rats) indicate a slight decrease in testes weights, with this decrease achieving statistical significance in the F2 and F3 generations at a single dose of 0.02 mg/kg-day (average decrease at this dose in these generations of 7% compared to controls). Cagen *et al.* (1999b), vom Saal *et al.* (1997), and Nagao *et al.* (2002) observed no association between BPA exposure and testes weight in CF1 mice or in C57BL/6N mice. Furthermore, none of the other studies of BPA exposure in rats reveal an association with testes weight, including Sakaue *et al.* (2001) (2×10^{-6}, 2×10^{-5}, 2×10^{-4}, 0.002, 0.02, 0.2, 2 mg/kg-day in Sprague-Dawley rats), Ema *et al.* (2001) (2×10^{-4}, 0.002, 0.02, 0.2 mg/kg-day in IGS rats), Cagen *et al.* (1999a) (0.001, 0.023, 0.25, 2.4 mg/kg-day in Wistar rats), and Tinwell *et al.* (2002) (0.02, 0.1 mg/kg-day in Sprague-Dawley and AP-derived Wistar rats). We conclude there is no evidence of concordance for this endpoint.

Other Endpoints: As detailed in Tables 2 and 4, several other endpoints have been associated with BPA exposure in mice and rats. However, none of the endpoints associated with BPA exposure have been investigated in more than one study.

Conclusion. Evidence of effect concordance across strains or species is limited by the weakness or inconsistencies of the effects in the two test species, the mouse and the rat. These comparisons therefore offer no evidence for consistent BPA effects and do not suggest that BPA effects that may occur in test animals would be likely to occur in humans as well. This conclusion is similar to that of Milman *et al.* (2002) for prostate effects alone.

3.2. Proximity—Extrapolation of Effects to Levels and Routes of Exposure Experienced by Members of the Population

As noted in Section 1.2.2 of this report, conservative estimates of human exposure to BPA due to food and beverage consumption amount to between approximately 5×10^{-4} and 0.01 mg/kg-day. This section discusses the extent to which the studies of experimental animals described in Section 2 of this report are representative of the biologically relevant (*i.e.*, internal) dose associated with these levels of oral exposure. Section 3.2.1 compares internal BPA exposure levels associated with oral administration to internal BPA exposures associated with sc administration, a route of exposure used by several investigators (Markey *et al.* 2001; Ramos *et al.* 2001). Section 3.2.2 compares internal BPA exposures in rodents exposed orally to BPA to the corresponding exposure levels estimated in humans.

3.2.1. Oral vs. subcutaneous exposure

The potential estrogenic activity of BPA has been investigated following several routes of administration. Subcutaneous administration is often used to avoid loss of the test compound because of poor absorption and extensive first-pass effects caused by the intestine and/or liver. This route can be used to identify what effects can be produced if a chemical gets into systemic circulation.

Oral administration more closely mimics exposure from environmental sources and may produce results that differ dramatically from results following administration *via* other routes of exposure. One explanation for these route-dependent differences in response is differences in bioavailability. For BPA, studies by Pottenger *et al.* (2000) demonstrated in F-344 rats that the relative bioavailability values for BPA (area under the curve (AUC) administered subcutaneously (sc) divided by AUC administered orally) ranged from seven-fold in females to 245-fold in males. The most likely explanation for the reduced bioavailability of BPA following oral exposure is extensive first-pass metabolism of BPA by intestinal and hepatic enzymes. Such metabolism will reduce circulatory levels of the parent compound. In the case of BPA, the parent compound undergoes extensive conjugation by hepatic (and intestinal) glucuronsyl transferases. Because the resulting BPA-glucuronide has been shown to be devoid of estrogenic activity (Matthews *et al.* 2001), the reduced BPA-uterotropic response following oral administration can be explained by these first pass metabolic events.

Evidence for glucuronidation of BPA has been obtained in a number of studies. These include studies with isolated hepatocytes, in which BPA-glucuronide is the major, and in many cases, the only metabolite. In addition, BPA-glucuronide is the predominant circulating plasma and urinary metabolite of BPA following any route of administration. There is some evidence that glucuronidation rates are lower during the perinatal period than in adulthood (Lucier 1976; Lucier *et al.* 1979; Rane and Tomson 1980; de Wildt *et al.* 1999; Ring *et al.* 1999) and in males compared to females (Pottenger *et al.* 2000).

Because internal exposure levels associated with sc administration substantially exceed levels associated with oral administration, the panel concludes that inferences regarding health effects among humans exposed to BPA should be limited to those animal studies that also expose animals to BPA orally.

3.2.2. Internal BPA exposures in rodents vs. internal BPA exposures in humans

Pritchett *et al.* (2002) compared glucuronidation rates in mouse, rat, and human hepatocytes *in vitro*. Human hepatocytes showed the lowest glucuronidation rates, with V_{max} approximately 30 to 50% less than the corresponding rates for rats and mice. Although these *in vitro* rates of glucuronidation suggest compromised metabolism of BPA in humans compared to rats and mice, humans have been shown to glucuronidate BPA efficiently following oral administration of 5 mg per subject (Völkel *et al.* 2002). In fact, free BPA could not be measured in human blood or urine following this dose (*i.e.*, it was below the level of detection of the analytic procedure). The only circulating metabolite was the monoglucuronide of BPA. Urinary excretion of this glucuronide accounted for the dose of BPA. Available evidence indicates that as in rodents, oral exposure to BPA in humans yields very low internal doses because of efficient first pass metabolism (*e.g.*, extensive glucuronidation).

4. EVIDENCE OF BIOLOGICAL PLAUSIBILITY

Evidence of biological plausibility rests on two criteria. Cohesion refers to the extent that effects attributable to an agent can all be explained by a single, biologically plausible explanation. In the case of BPA the only proposed mechanism for low-dose effects is through modulation of estrogen receptors (see Section 1.2.1). Relevance refers to the level of confidence in the possibility that the applicable mechanism operates in humans.

4.1. Cohesion of the Biological Mechanism Hypothesized

4.1.1. Plausibility of *in utero* exposure

Because BPA has been reported to produce effects in pups as a result of *in utero* exposure, it is important to determine if BPA, BPA-glucuronide, or both BPA and BPA glucuronide are present in fetal tissue. Oral administration of a single large dose of BPA (1 g/kg) to pregnant F-344 rats (gd 18) resulted in distribution of BPA to the fetus (Takahashi and Oishi 2000). The maximum concentration in the fetus was 9 μg/g, which was 20- to 40-fold less than that found in maternal tissue (liver and kidney). The maximal fetal tissue to maternal blood ratio was 0.6, which

suggests passive distribution of BPA to the fetus. Following oral administration of a much lower dose of BPA to pregnant Wistar rats (10 mg/kg on GD 19), Miyakoda *et al.* (1999) reported that BPA was present in very low concentrations in fetal tissue. The concentrations were 11, 4.4, and 7.5 ng/g at 1, 3, and 24 hours after dosing the pregnant dams. These data show that BPA can diffuse to the fetus, albeit at low concentrations.

4.1.2. Similarity of BPA behavior to the behavior of other estrogens

If BPA behaves as an estrogen, it should qualitatively exhibit properties that are similar to those of other estrogens in general. Non-estrogen-like activity casts doubt on several statistically significant results detailed in Section 2.1.1 of this article. In particular, the increase in AGD among females born to BPA-treated animals reported by Tyl *et al.* (2002) is consistent with *in utero* exposure to an androgen, rather than to an estrogen. Likewise, the delay in vaginal patency among treated animals reported by Tyl *et al.* is the opposite of what would be expected following estrogen exposure.

The remainder of this discussion compares the carcinogenic properties of BPA to those of a known estrogen, DES. To pursue this issue, this section compares the chronic and subchronic effects of exposure to BPA and DES in experimental animals. Although this report focuses primarily on low-level exposures, we compare studies that addressed the carcinogenic potential of BPA or DES at high exposure levels. No low exposure studies addressing carcinogenicity were available. If BPA behaves like an estrogen such as DES, we would expect that it would cause the same effects (*e.g.*, similar lesions in the same organs) as DES at toxicologically equivalent doses. A finding that the two substances behave similarly at high doses would lend support to the hypothesis that they behave similarly at low doses, while differences in their behavior at high doses would cast doubt on this hypothesis. Similarly, a finding that both chemicals cause comparable lesions in one sex/species would lend support that they behave the same in other sex/species.

To compare the carcinogenicity of DES and BPA, we reviewed the results of the Highman *et al.* study (1980a) of chronic DES and 17β estradiol exposure in female mice and the National Toxicology Program study of chronic BPA exposure in female mice. These studies were selected for the following reasons. First, female mice appear to be highly susceptible to the estrogenic effects of DES. Second, both studies evaluate the same endpoints. Third, the exposures were similar in all studies in terms of dose level (*i.e.*, they would be considered to be at the maximum tolerated dose, or MTD), duration (they were administered over the animals' normal lifespan), and route of exposure (the chemicals were incorporated into the diet). Finally, in all of these studies, the animals were subjected to a complete necropsy, including a comprehensive pathological examination of the reproductive tract. The following discussion details each study's methodology and results.

Highman *et al.* (1980a) exposed female C3H/Hel mice to DES (concentrations in diet of 0, 10, 100, 500, or 1000 ppb) or to E2 (concentrations in diet of 100, 1,000, or 5,000 ppb) from the age of six to 110 weeks. They exposed C3Heb/Fej mice to DES (concentrations in diet of 0, 10, 100, and 500 ppb) from the age of 6 to 136 weeks. The investigators used two strains of mice because one (C3H/Hel) has a higher murine mammary tumor virus (MMTV) titer than the other (C3Heb/Fej), a

characteristic that predisposes the mice to the development of mammary neoplasms. The results of this study showed that both DES and E2 induced adenosis in the uterine cervix, cervical adenocarcinomas and granular cell myoblastomas, squamous cell carcinomas of the vagina, endometrial carcinomas, and possibly mesotheliomas. Interestingly, the incidence of neoplasms of the ovary was lower in the animals treated with DES than in the controls. There was also a significant decrease in ovarian tubular adenomas in both the MMTV+ and MMTV- DES exposed mice, but not in the E2 exposed mice. Mammary adenocarcinomas were not significantly increased by either DES or E2 exposure. However, the time to development of the tumors was decreased in the MMTV+ mice, but not in the MMTV- mice.

NTP (1982) used their standard protocol in a study of male and female F344 rats (dietary BPA exposure concentrations of 0, 1,000, or 2,000 ppm), B6C3F1 male mice (dietary BPA exposure concentrations of 1,000 or 5,000 ppm), and B6C3F1 female mice (dietary BPA exposure concentrations of 5,000 or 10,000 ppm). The animals were exposed for 104 weeks starting at approximately 6 weeks of age. The study concluded that there was no convincing evidence of carcinogenic activity in either sex of either species. However, subsequent to the report's publication, Huff (2001) proposed in a letter to the editor that BPA in this study caused tumors of the hematopoietic system in rats and mice, and both interstitial cell testicular tumors and mammary tumors in male rats. In a subsequent letter to the editor, Munro *et al.* (2002) refuted Huff's position. We note that although chemicals exhibiting carcinogenic behavior also typically cause preneoplastic changes in the same organ(s), there was no such evidence of preneoplastic lesions in the NTP study in the organs identified by Huff. A weight of the evidence review of BPA carcinogenicity (Haighton *et al.* 2002) concluded that BPA is unlikely to be carcinogenic to humans.

It is apparent that these studies produced different outcomes. Highman *et al.* (1980a) clearly showed that both DES and E2 are potent carcinogens in female mice, whereas the NTP (1982) study found no evidence of carcinogenic activity or other changes in the female reproductive tract caused by BPA that would suggest an estrogenic-like response. It should be noted that the BPA doses were approximately three orders of magnitude greater than the doses of DES or E2. It may be that based on *in vitro* measures of potency (*e.g*, Krishnan *et al.* 1993) exposures at the MTD are not sufficiently large to trigger estrogenic responses. But this possibility would cast doubt on the relevance of human exposures at doses well below the MTD.

Differences between the results of shorter-term DES and BPA studies cast further doubt on the possibility that BPA exhibits carcinogenic activity similar to that of DES. If BPA exhibited carcinogenic activity one would expect preneoplastic changes, hyperplastic changes, or both types of changes in organs similar to those that have been found following subchronic DES exposure. We investigated the extent to which DES- and BPA-induced changes are similar by comparing the lesions from the 52-week sacrifice of female mice exposed to DES or E2 (Highman *et al.* 1980b) to the lesions in BPA-exposed mice from the NTP study (National Toxicology Program 1982). The slides of the genital tract from male and female rats and mice from the NTP BPA 13-week sacrifice were reviewed by a member of the panel (EEMc) because the histopathology from this time point was not described in the NTP Technical Report (see Appendix for details of the review). Highman *et al.* (1980b) found profound dose-related changes to both estrogenic compounds in the cervix characterized by

adenosis and mucoid stroma. In the uterus they observed glandular hyperplasia, adenomyosis and hyaline changes, and marked ovarian atrophy with ceroid pigment. In contrast, EEMc noted the opposite effect, that is, uterine gland atrophy and no changes in the cervix. He did, however, find evidence of a possible decrease in the number of corpora lutea in the ovary, although there were numerous germinal follicles, and the ovary appeared of normal size. In addition, there was no evidence of an increase in the amount of ceroid pigment in the ovary of BPA-exposed female mice. It should be noted that there was no evidence of pathology in the uterus, cervix, or ovary of female rats, or in the male genital tract of either species treated with BPA.

Some discussion of the male reproductive tract is also warranted. For decades it has been known that DES and other potent estrogens induce squamous metaplasia in the prostate when given chronically at pharmacologic doses (Tunn *et al.* 1979; Deschamps *et al.* 1987; Weijman *et al.* 1992; Risbridger *et al.* 2001). However, no such lesions were found on careful examination of the 13-week NTP bioassay of BPA in either mice or rats.

In summary, the panel concludes that there is no credible evidence that BPA exhibits carcinogenic activity at high doses in animals. Because other substances known to exhibit estrogenic effects also exhibit carcinogenicity at high doses, BPA's lack of carcinogenicity casts doubt on the hypothesis that it acts as an estrogen at low doses.

4.2. Relevance of the Biological Mechanism to Humans

An important difference between mice, the species in which most low dose effects of prenatal BPA exposure have been reported, and humans is the source and levels of estrogens produced during pregnancy. Witorsch (2002) reviewed these differences. The very low estrogen levels in mice during pregnancy may make them more sensitive to slight perturbations in the level of estrogens or estrogen-active compounds. These low levels could, for example, explain the reported impact of intrauterine position (IUP) on development in mice (*e.g.*, Nonneman *et al.* 1992; Timms *et al.* 1999), although other species show detectable but less robust signs of IUP effects (Faber and Hughes 1992). Witorsch (2002) suggests that the much higher levels of circulating hormones in human pregnancy greatly reduces the likelihood that exposure to small amounts of weakly estrogenic compounds could cause adverse effects.

Studies of the effect of the potent xenoestrogen DES in humans and rodents serve to illustrate potential differences in their sensitivity. Although no studies of BPA's reproductive consequences in humans have been conducted, a number of studies of prenatal exposure to DES have been carried out in both rodents and humans (McLachlan *et al.* 1975; Bibbo *et al.* 1977; Gill *et al.* 1977; Leary *et al.* 1984). Mice are exquisitely sensitive to prenatal DES exposure. Females at relatively low doses are rendered virtually sterile, and males at high doses show reproductive effects, principally on the excurrent duct structures and maldescent of the testes. During the period between the late 1940s and the early 1970s, DES was prescribed to between two- and three-million pregnant women in the United States because it was thought to prevent pregnancy complications, especially miscarriage. In 1971, women who had been exposed *in utero* to high levels of DES early in pregnancy were reported to have anatomical changes affecting both the uterus and cervix, and an increase

in the incidence of vaginal clear-cell adenocarcinoma, a rare tumor (Herbst *et al.* 1971). Subsequently, studies in male mice showed that prenatal DES exposure also produced malformations of the male genital tract's excurrent duct structures and prevented descent of the testes (McLachlan *et al.* 1975). In the human male, however, prenatal exposure to high levels of DES produced only a low incidence of cryptorchidism, and epididymal cysts, and appears to have had no subsequent effect on male fertility (Bibbo *et al.* 1977; Gill *et al.* 1977; Leary *et al.* 1984; Wilcox *et al.* 1995).

During the period from 1950 to 1952, a prospective, double blind, placebo controlled clinical trial of the efficacy of DES in preventing complications of pregnancy was carried out at the Chicago Lying-In Hospital. A total of 840 women received DES and 806 women received placebo. DES was administered orally starting in gestational week 7 at 5 mg/day, increasing every second week by 5 mg/day to a maximal dose of 150 mg/day. The study group ultimately consisted of 289 *in utero* DES-exposed males and 290 case-controlled unexposed males (Gill *et al.* 1979). A significant increase in epididymal cysts, cryptorchidism, and testicular atrophy was reported. No hormonal differences between exposed and unexposed males were found. No testicular cancer was reported to have occurred. Testicular atrophy was poorly characterized, and the semen studies were poorly controlled.

Four decades after their birth, from among 1,646 participants in the Chicago Lying-In Hospital study, 253 DES exposed males and 241 non-exposed males were reassessed for their fertility status (Wilcox *et al.* 1995). Including men who had genital malformations, DES-exposed men were as fertile as their unexposed controls. These data included measures of whether the men had ever established a pregnancy, age at the birth of their first child, average number of children, length of time to conception, cumulative probability of conception per menstrual cycle and sexual functioning (libido and erectile function). There was also no increase in the reported incidence of altered sexual orientation.

A small but provocative study from the Kinsey Institute for Sex, Gender and Reproduction among ten boys who were prenatally exposed to DES (at least 1,000 mg) suggested the presence of altered CNS differentiation toward the female phenotype (Reinisch and Sanders 1992). Compared to siblings who were not exposed to DES, study subjects demonstrated a decrease in hemispheric lateralization with respect to processing of non-linguistic spatial information, a decrease in cognitive factors related to spatial ability, but no change in I.Q. (Wechsler I.Q. test), and a decreased incidence in left handedness.

The Mayo Clinic reported a less well-controlled study of 828 DES-exposed males (Leary *et al.* 1984). From a retrospective chart review, no increase in the incidence of epididymal cysts, cryptorchidism, or testicular atrophy was found. There were also no differences noted in the spectrum of semen values or reports of infertility. Of note, DES dosing and timing of exposure were highly variable and poorly quantified.

This experience, with high doses of a very potent estrogenic compound, provides some insights into the relevance of potential BPA findings in rodents for humans. First, rodents are apparently much more sensitive (on a mg/kg basis) to DES than are humans. Second, in human males exposed *in utero* to high doses of DES, there is little evidence of developmental effects and no evidence of altered reproductive function. It could be argued that a U-shaped dose-response function, if it does exist, could cause effects at much lower doses. However, the human experience with high

dose exposures to DES suggests that rodent experiments over-predict both the range of effects and their magnitude in humans for this particular estrogenic compound.

Finally, another issue to be addressed in considering the relevance of animal findings to humans is the generalization of the animal effects to health outcomes in humans. Milman *et al.* (2002), for example, suggested that there is no consistent correlation between changes in prostate size and either prostate pathology or prostate cancer in model systems. It should be noted that rodents have low background rates of prostate cancer. None of the studies showing low dose effects of estradiol, DES, or BPA reported any prostatic histopathology, making it impossible to establish a link between changes in organ weight and adverse outcomes. Few, if any, of the potential low-dose effects of prenatal BPA administration have been linked to organ system or functional pathologies, even in multigeneration rodent studies (Ema *et al.* 2001; Tyl *et al.* 2002).

Conclusion. The panel considers possible findings of altered development in rodents exposed to low doses of BPA *in utero* to be of limited relevance to humans. Although metabolism of BPA is similar across species, evidence from BPA and other agents suggests that rodents are more sensitive to estrogenic agents than are humans, perhaps because of differences in levels of circulating hormones during pregnancy. If rodents are far more sensitive than humans to estrogenic BPA effects, satisfaction of the relevance criterion would depend on identifying an alternative mechanism of action or substantial differences in pharmacokinetics that favor an effect in humans.

5. DISCUSSION

5.1. Main Findings

The potential low dose effects following oral exposure to BPA have been studied extensively in rodents. A number of isolated biological effects have been observed in animals exposed to low (less than 5 mg/kg orally) doses of BPA including alterations in daily sperm production, prostate weight, epididymal weight, and testes weight, among others. However, the results of various long-term and multigenerational reproductive/developmental studies in rodents do not suggest that these small positive or negative changes in biological endpoints result in neoplastic or negative reproductive outcomes. In general, the positive findings have been in much smaller studies not conducted under GLP (good laboratory practice) guidelines.

Studies in rats have been predominantly negative or limited to small effects, with the positive findings generally refuted by negative results in larger, well-conducted studies. In mice, the results are not as clear. Much attention has focused on the potential relationship between BPA exposure and prostate weight because of efforts to replicate a reported association. We find that it is difficult to dismiss outright either the positive findings or the negative findings and believe that replication of this work addressing the research issues discussed later will be necessary before agreement can be reached.

If very low dose effects are established in rodents, the importance of such a finding to humans will remain an open question. At this time, effects at very low oral doses (*i.e.*, below 5 mg/kg-day) have not been reliably established in multiple strains or

species. As a result, we have little confidence that there will be effects of such low doses in other species, including humans. We note that further work in this area should focus on oral administration study designs because other routes of exposure are not common among humans. If other routes of exposure are used, the studies must account for the substantially higher internal dose than the corresponding oral exposure.

The panel has reservations regarding the applicability of the proposed low dose BPA effects to humans even if they are established in multiple rodent strains and species. Although BPA has been measured in rodent fetuses and can thus plausibly cross the placenta to reach human fetuses, there are differences between humans and rodents that suggest humans may be less sensitive to exposure based on pharmacodynamic considerations (in particular, the far higher estrogen levels in humans during pregnancy, compared to rodents). The robustness of humans against effects resulting from *in utero* exposure to estrogens is consistent with the relative magnitude and extent of response among rodents and humans to *in utero* DES exposure. Finally, the fact that DES and BPA do not have the same high dose carcinogenic effects casts further doubt on the role of BPA as a low-dose estrogen active agent.

5.2. Research Needs

As noted in Section 2.2.2 of this report, the most controversial evidence of a low-dose BPA effect relates to studies that have investigated the impact of BPA exposure on prostate weight in mice. Evaluating this evidence is complicated by several factors. First, one of the studies that reported an association (Nagel *et al.* 1997) used an inbred line of CF1 mice that are not available to other researchers. No attempt has been made to replicate the other positive study (Gupta 2000), which used CD-1 mice.

Second, prostatic wet weight is a difficult endpoint to measure accurately. If low level exposure to BPA during development does affect prostatic size, then more easily replicated measures of prostatic cell proliferation (BrdU labeling index) or cell death (apoptotic index) should reflect that influence. In particular, changes in epithelial differentiation should be reflected by well-known epithelial differentiation markers. The literature evaluating the effect of estrogen exposure on the prostate suggests that effects on epithelial differentiation might influence the normal conversion of the solid epithelial prostatic buds in the embryo into luminized ducts containing definitive luminal and basal cells expressing their respective markers in the maturing prostate (luminal cell = keratins 8 and 18, and secretory proteins; basal cells = keratins 5 and 14 and p63). ER_α is an estrogen-induced differentiation marker expressed in prostatic epithelial cells. Thus, a putative or actual estrogenic substance should induce expression of ER_α. The ratio of luminal to basal cells is another parameter that may be affected. Morphometric analysis on serial sections or on ductal microdissections can provide an accurate objective measure of the number of main ducts, ductal branch points, and ductal tips, which are excellent measures of prostatic growth. If effects of low dose DES or BPA were corroborated by changes in other objective endpoints, the interpretation would be strengthened. Separate analysis of the ventral, dorsal-lateral, and anterior prostates is critical because the expression of ER_α is lowest in the ventral prostate, highest in the anterior prostate and intermediate in the dorsal-lateral prostate. Use of αERKO and βERKO

mice could help resolve the issue of whether the putative effects of BPA on the mouse prostate are mediated by ER_α or ER_β.

Third, to the extent that other endpoints and species are studied, groups of investigators should communicate beforehand for the purpose of agreeing on a test species, dose range, route of exposure, and on other design issues. Doing so will facilitate evaluation of the extent to which results can be replicated, a task that is difficult at the present time.

Additional work must be done to determine the extent to which possible findings in test animals are applicable to humans. A widely available, peer-reviewed physiologically based pharmacokinetic (PBPK) model would be helpful in understanding potential similarities and differences in response in different species. As discussed in Section 4.2, the available evidence suggests that any differences that do exist will most likely reflect pharmacodynamic factors. At this time, there is only indirect evidence of these factors, including evidence that circulating estrogen levels are lower during pregnancy in rodents than they are in humans, and evidence that the effects of other estrogens (DES in particular) have a stronger effect on rodents than on humans.

Many important questions of public health risk confront conflicting evidence and interpretation. The progress of science will eventually provide resolution but often decisions must be made before the answers are clear. We believe that the organized and systematic approach demonstrated here can provide a characterization of the current "best estimate" of the scientific evidence. Because decisions to act, and not to act, may have public health implications (Graham and Wiener 1995), this is valuable information for risk management. In the case of BPA, the evidence considered by the panel suggests that the weight of the evidence for low-dose effects is very weak. Studies are conflicting, the effects are subtle with questionable functional importance if real, and there are data that conflict with the proposed mechanism of action. As more information is developed it can be incorporated into our framework to further refine the state of the science around low-dose effects of BPA.

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Appendix: Review of NTP Data—High Dose BPA and Lesions

The prostate is a known estrogen target organ expressing both ER_α and ER_β and has been shown to respond to estrogen in a variety of ways depending primarily on the timing of exposure and the dosage. A particularly reliable response of the developing and adult prostate to high chronic levels of estrogen is squamous metaplasia of the epithelium. In relation to other classical estrogen target organs such as the uterus, which shows a profound proliferative effect 18 hours after a single injection of estradiol or DES, the sensitivity of the prostate to exogenous estrogens is such that prostatic squamous metaplasia is not fully expressed in the mouse until about three weeks of continuous treatment with pharmacological levels of estrogen. Prostatic squamous metaplasia is seen normally in male human fetuses at the end of gestation. This spontaneous prostatic squamous metaplasia subsequently disappears postnatally after high levels of maternal estrogens have declined (Brody and Goldman 1940; Andrews 1951).

Because BPA has been reported to be a weak estrogen, examination of the effects of BPA on the prostate is well justified. In this regard chronic continuous exposure to BPA of young adult rats (4,000 ppm in the diet) and mice (25,000 ppm in the diet) for 13 weeks did not elicit squamous metaplasia of the prostate (NTP study), as would be expected following high doses of other classical estrogens such as estradiol or DES (Tunn *et al.* 1979; Weijman *et al.* 1992).

Brief exposure of developing rats and mice to high dose estrogen has elicited permanent alterations in the prostate. Changes induced by pre- and/or neonatal exposure to high dose estrogen include the following: dose-dependent reduction of adult prostatic size, impaired secretory activity in adulthood, alterations in androgen and estrogen receptors, perturbation in the TGFβ signaling system, and alteration in the cellular composition of the stroma. In a few cases "preneoplastic" and neoplastic changes in the prostates of mice and rats have been induced by high doses of estrogens given during development (Santti *et al.* 1990; Prins 1997).

To determine if short-term, high dose exposure to BPA causes lesions like those caused by short-term high dose exposures to substances known to act as estrogens, a member of the panel (EEMc) examined the genital tract histopathologic slides from the National Toxicology Program's (NTP) 90-day study of BPA in mice and rats. The study was conducted in male and female F344 rats and B6C3F1 mice at Litton Bionetics, Inc., Kensington, MD and is reported in NTP Technical Report

#215 (1982). It is not clear when this study was started or completed, but the long-term (two-year) study was completed in February 1979, which means it was started in February 1977. Therefore, the 90-day study had to be started sometime before 1977 because the results of it were used to identify the dose levels used in the two-year study. It is therefore reasonable to assume that the 90-day study was conducted in 1976.

Ten animals of each gender per dose were continuously exposed to BPA in their diet for 90 days: rats were exposed to BPA at concentrations of 0, 250, 500, 1,000, 2,000, and 4,000 ppm, and mice were exposed to BPA at concentrations of 0, 5,000, 10,000, 15,000, 20,000, and 25,000 ppm. These doses were selected based on the results of a 14-day feed study. A greater than 10% decrease in body weight gain was reported in male and female rats at exposures exceeding 1,000 ppm. A greater than 10% decrease in body weight gain in mice was reported at doses of 15,000 ppm and above in males and at all doses in females. However, the panel believes this finding may be misleading because differences were calculated relative to the change in body weight, rather than being calculated relative to total body weight. For example, the animals in the 90-day study all had a starting weight of 18 g. Although the final weights of the control group (25 g) and high dose group (23 g) would not be regarded as differing substantially by most toxicologists, the NTP's presentation of the results (a 29% decrease in the weight gain among high dose animals, *i.e.*, 7 − 5 g divided by 7 g) made the difference appear to be important. NTP no longer reports weight change results in this way.

The only treatment-related change noted in rats was cecal enlargement in males at exposures of 500 ppm and greater. However, no histopathologic changes were found in this organ. The only treatment-related lesion reported in mice was the presence of "multinucleated giant hepatocytes" in males exposed to 25,000 ppm BPA. No such lesions were noted in females. At the time of this study, the NTP practice was to first examine the histopathologic slides to determine if there were any lesions in animals from the highest dose group that did not appear in animals from the control group. If no difference was observed between these two groups, then the lower doses were not examined (at least for the tissues showing no lesions). Apparently, liver lesions observed in high dose animals were not considered significant, at least with respect to identifying dose levels for the chronic study (which was the primary function of the 90-day studies at that time). Therefore, the only slides available from the subchronic study were those from the control rats and mice, rats exposed to 4,000 ppm BPA, and mice exposed to 25,000 ppm BPA. This study did not record organ weights.

It appears that the male reproductive tract (except for the testicles and epididymis) was removed *in toto*, embedded in a single block, sectioned and stained with hematoxylin and eosin. As a result, some tissues were missing on a given animal. The protocol did not require sampling the epididymis.

Results for Males

Tables A-1 and A-2 list the male animals examined and identifies those tissues of the male reproductive tract that were present (P) and those not present (NP).

Table A-1. Histopathology of Bisphenol A—90-day NTP rat study; Date examined—24 September 2001.

					Prostate			
Animal	Dose	Testes	Epididymis	Seminal vesicles	Coagulation gland	Dorsal ventricle	Ampl. gland	Other
121	Control	P	NP	P[1]	P	NP	P	P
122	Control	P	P	P	P	NP	P	P
123	Control	P	NP	P	P	NP	P	P
124	Control	P	NP	NP	NP	NP	P	NP
125	Control	P	NP	P	P	P	P	P
126	Control	P	NP	P	P	NP	P	P
127	Control	P	P	P	P	P	P	NP
128	Control	P	NP	P	P[2]	NP	P	P
129	Control	P	P	P	P	NP	P	NP
130	Control	P	NP	P	P	NP	P	NP
1	4,000	P	NP	P	P	P	P	NP
2	4,000	P	NP	NP	NP	NP	P	NP
3	4,000	P	NP	P	P	NP	P	P
4	4,000	P	NP	P	P	NP	P	P
5	4,000	P	NP	P	P	P[1]	P	NP
6	4,000	P	NP	P	P	NP	P	P
7	4,000	P	NP	P	P	P	P	NP
8	4,000	P	NP	P	P	NP	NP	P
9	4,000	P	NP	P	P	NP	P	P
10	4,000	P	NP	P	P	NP	P	P

Abbreviations: P: Present; NP: Not present; P[1]: Inflammation; P[2]: Hyperplasia.

Superscripts denote tissues in which EEMc noted a lesion. Unless noted, no lesions were observed. The panel concludes that there is no evidence that BPA caused any lesions in male rats or mice in any of the tissues examined at these high exposures.

Results for Females

It appears that the female reproductive tract (except for the vagina) was removed *in toto*, was separated into various components, embedded in a single block, and sectioned and stained with hematoxylin and eosin. As a result, some tissues were missing for some of the animals. This was particularly true for mice, which have smaller tissues. The protocol did not require sampling the vagina. Tables A-3 and A-4 list the animals examined and identify those tissues that were present (P) and those that were not present (NP). Superscripts denote tissues in which EEMc noted a lesion. Unless noted, no lesions were observed.

The panel concludes that there is no evidence that BPA caused any lesions in any of the rat tissues examined at these high exposures. However, there were distinct changes in the uteri and possibly the ovary of female mice exposed to 25,000 ppm BPA. The uterine changes were characterized by moderate to marked atrophy of the uterine glands in seven of nine mice where this tissue was available. These changes were especially apparent because the uterine glands in mice are very prominent

Table A-2. Histopathology of Bisphenol A—90-day NTP mouse study; Date examined—25 September 2001.

					Prostate			
Animal	Dose	Testes	Epididymis	Seminal vesicles	Coagulation gland	Dorsal ventricle	Ampl. gland	Other
101	C	P	NP	P	P[2]	NP	P	NP
102	C	P	P	P	NP	NP	P	P
103	C	P	NP	P	P	P	P	NP
104	C	P	NP	P	P	P	P	P
105	C	P	P	P	P[2]	P	NP	P
106	C	P	P	P	P	NP	NP	P
107	C	P	NP	P	P[2]	NP	P	P
108	C	P	P	P	P	NP	NP	P
109	C	P	P	P	P[2]	NP	NP	P
110	C	P	P	P	P	NP	P	P
1	25,000	P	P	P	P	NP	P	P
2	25,000	P	P	P	P	P[1]	NP	NP
3	25,000	P	P	P	P	NP	NP	NP
4	25,000	P	P	P	NP	NP	P	NP
5	25,000	NP	P	P	P	NP	P	P
6	25,000	P	P	P	P	P	P	P
7	25,000	P	P	P	P[2]	P	NP	P
8	25,000	P	P	P	P	NP	NP	P
9	25,000	P	P	P	P[2]	NP	NP	NP
10	25,000	P	P	P	P[2]	P	P	NP

Abbreviations: P: Present; NP: Not present; P[1]: Inflammation; P[2]: Hyperplasia.

compared to those in rats, which are less numerous. There was also suppurative inflammation in the uterus in several of the same animals. It should be noted that NTP did not report this lesion. The epithelium lining the corpus uteri and cervix was within normal limits. No lesions were noted in the oviducts. There may also have been ovarian atrophy as evidenced by a lack of corpora lutea in most of the animals. However, corpus lutea in mice are more difficult to discern than corpus lutea in rats.

The atrophic changes in the uterine glands are probably related to exposure to BPA. At first glance, there are at least two possibilities. First, the atrophy may be related to non-specific intoxication or second, it may be directly related to BPA.

With regard to the first possibility, there is no indication of toxicity in females in the NTP report (1982). Although the report indicates that there was liver toxicity in males, the NTP specifically notes that none was observed in females. Review of the data from the two-year study does, however, suggest that female mice were intoxicated by BPA. Their body weights throughout the study were significantly lower (around 25%) than the controls at both doses (5,000 and 10,000 ppm), in contrast to the exposed males, which had body weights that were about the same as the those of the controls (Figure 3, p. 34 in National Toxicology Program 1982). It could not be ascertained if the decrease in body weight was related to a decrease in food consumption because these data were not reported for mice, although they were

Table A-3. Histopathology of Bisphenol A—90-day NTP female rat study; Date examined—29 November 2001.

Animal	Dose	Ovary	Fallopian tube	Uterus	Cervix
133	C	CL, GF	P	Hydrometra	P
134	C	CL, GF	P	P	P
135	C	CL, GF	P	P	P
136	C	CL, GF	P	Hydrometra	P
137	C	CL, GF	P	Hydrometra	P
138	C	CL, GF	P	P	NP
139	C	CL, GF	P	P	NP
140	C	CL, GF	P	P	P
141	C	CL, GF	P	Hydrometra	NP
142	C	CL, GF	P	P	NP
13	4,000	CL, GF	P	P	NP
14	4,000	CL, GF	P	Hydrometra	P
15	4,000	CL, GF	P	P	NP
16	4,000	CL, GF	P	P	P
17	4,000	CL, GF	P	P	P
18	4,000	CL, GF	P	Hydrometra	NP
19	4,000	CL, GF	P	P	P
20	4,000	CL, GF	P	P	P
21	4,000	CL, GF	P	P	P
22	4,000	CL, GF	P	P	P

Abbreviations: P: Present; NP: Not present; CL: Corpus lutea; GF Graffian follicles.

reported for rats. Importantly, this decrease in body weight was not associated with a decrease in survival. No treatment-related lesions (toxic or neoplastic) are reported in female mice. What is particularly striking is that while it appears that the uteri were examined in detail in the chronic study (Table D2, p. 94 in National Toxicology Program 1982), the only change that was noted was a decrease in the incidence of cystic hyperplasia of the endometrium (70% for controls, 49% in the low dose group, and 45% in the high dose group).

The second possibility is that the glandular atrophy was caused by BPA. Although this is possible, it is difficult to determine if the mechanism by which BPA acts is similar to that of DES. The only DES study of which we are aware that is comparable to the NTP study is that by Highman *et al.* (1977). This study administered relatively high doses (10 to 500 ppb of DES or 100 to 5,000 ppb E2 in feed) to mice from age 6 to 136 weeks. Although this study did not sacrifice animals at the age of 13 weeks (like the 90-day NTP study of BPA), we believe that the data collected following the 52-week sacrifice can serve as a reasonable surrogate. At 52 weeks, Highman *et al.* describe profound hyperplasia of the endometrial glands with growth into the subjacent muscle (adenomyosis) and into the wall of the cervix (adenosis) (Table 2, p. 5 in (Highman *et al.* 1977)). In addition, the authors report that the epithelium lining the cervix was covered by a layer of columnar epithelium (a normal lining is stratified squamous). Highman *et al.* also noted a reduced number of corpora lutea in the ovary, but also stressed that there were "*. . . a moderate to large number of ceroid cells. . .*" in the ovary (p. 92). Ceroid pigment was not seen with BPA. We

Table A-4. Histopathology of Bisphenol A—90-Day NTP female mouse study; Date examined—29 November 2001.

Animal	Dose	Ovary	Fallopian tube	Uterus	Cervix
111	C	CL, GF	P	P	P
112	C	CL, GF	P	P	NP
113	C	GF	P	P	NP
114	C	NP	NP	P	NP
115	C	CL, GF	P	P	P
116	C	CL, GF	P	P	P
117	C	CL, GF	P	P	P
118	C	CL, GF	P	P	P
119	C	CL, GF	P	P	NP
120	C	CL, GF	P	P	P
11	25,000	GF	P	P	P
12	25,000	NP	P	P	P
13	25,000	NP	P	Atrophy	P
14	25,000		Inadequate section to evaluate		
15	25,000	GF	P	Atrophy[1]	P
16	25,000	P, GF	P	Atrophy[1]	P
17	25,000	P, GF	P	Atrophy	P
18	25,000	P, GF	P	Atrophy[1]	P
19	25,000	P, CL, GF	P	Atrophy	NP
20	25,000	P, NP	NP	Atrophy	NP

Abbreviations: P: Present; NP: Not present; CL: Corpus lutea; GF: Graffian follicles; Atrophy [1]: Inflammation.

therefore conclude that the effect observed in the BPA-exposed mice appeared to be the opposite of that which was noted in animals exposed to DES and E2, that is, atrophy versus hyperplasia. We note that it is difficult to envision a situation in which there would be atrophy at 26 weeks, but hyperplasia at 52 weeks.

We conclude that continuous exposure of rats and mice to high levels of BPA in feed for 13 weeks failed to show any evidence of pathologic change in the reproductive organs in males of either species, or in female rats. However, profound uterine gland and possibly ovarian atrophy was observed in female mice.