Environmental exposure to hormonally active chemicals has coincided with an increase in the incidence in breast cancer (Davis et al. 1993), testicular cancer (Skakkebaek et al. 1998), and other endocrine-related diseases (Sharpe and Skakkebaek 1993). These outcomes are thought to result from endogenous exposure to synthetic estrogens during fetal development (Sharpe and Skakkebaek 1993) and have motivated the worldwide formation of government-sponsored committees to evaluate evidence for this hypothesis. For instance, in the United States, the U.S. Environmental Protection Agency (EPA) developed a screening program to test chemicals that may contaminate water and food to assess potential endocrine disruptor activity (Endocrine Disruptor Screening and Testing Advisory Committee 1998).

At the request of the U.S. EPA, the National Toxicology Program (NTP) convened a meeting to consider whether environmentally relevant doses of endocrine disruptors caused biological effects. In 2001, the NTP Low-Dose Peer Review Panel published a final report (NTP 2001), which stated that there was “credible evidence for low-dose effects” and suggested that different experimental animal strains may account for reports of both positive and negative effects for the same parameters. In this regard, Spearow et al. (1999) observed that rodent strains selected for high fecundity and rapid growth rates, such as CD-1 mice, are more estrogen resistant than the less fecund C57Bl6.

An additional controversy identified by the NTP was the shape of the dose–response curve, which was reported as nonmonotonic for some effects of prenatal xenoestrogen exposure. For example, prenatal methoxychlor exposure altered the response of the adult uterus to 17β-estradiol (E2); low doses increased uterine weight, and higher doses reduced it (Alworth et al. 2002). This type of nonmonotonic response was also observed for other endpoints with other estrogenic chemicals (Rubin et al. 2001; Vandenberg et al. 2006; vom Saal et al. 1997), arguing that low-dose effects cannot be deduced from effects observed at high doses.

Our research focuses on the effects of prenatal exposure to environmentally relevant levels of the xenoestrogen bisphenol A (BPA). In the present study we examined strain sensitivity and the shape of the estrogen dose–response curve in the context of our ongoing work in the mouse mammary gland. In addition, we explored the effects of prenatal BPA exposure on subsequent estrogen sensitivity at puberty.

BPA, a compound used in the manufacture of polycarbonate plastics and epoxy resins, leaches from food and beverage containers (Biles et al. 1997; Brotons et al. 1994) and dental sealants and composites (Olea et al. 1996) under normal conditions of use (Markey et al. 2001b, 2003b). BPA levels have been measured in human urine (Calafat et al. 2005), serum (Takeuchi and Tatsuoka 2002), and maternal and fetal plasma, amniotic fluid, and placental tissue at birth (Ikekuzu et al. 2002; Schonfelder et al. 2002). We chose to administer perinatally 0 or 250 ng BPA/kg body weight (bw)/day to mice. Based on data reported by Arakawa et al. (2004), we estimated that this level of BPA should fall within the range of reported human exposures.

In the mammary gland, perinatal exposure to BPA alters ductal invasion of the stroma at puberty and increases lateral branching and the number of terminal ends during adulthood (Markey et al. 2001a; Munoz de Toro et al. 2005). Although the mechanisms by which BPA induces developmental abnormalities in the mammary gland are unknown, it is plausible that estrogen receptors (ER), which are expressed in the fetal mammary gland, may mediate BPA-induced effects.

In the present study, we compared the response of the mammary glands to E2 in outbred CD-1 mice (the strain we have used previously) with inbred C57Bl6 mice, which have been used in numerous studies involving development of the mammary gland. Different levels of biological complexity within the mammary gland (i.e., tissue organization and gene expression) were examined. We also examined the effects of perinatal BPA exposure on the response to E2 at puberty in both strains.

Materials and Methods

Animals. CD-1 (Crl: CD-1; Charles River Laboratories, Wilmington, MA) and C57Bl6 Animals were purchased by the Tufts University Laboratory Animal Resources. CD-1 (n = 10) and C57Bl6 (n = 10) female mice were ovariectomized and treated for 10 days with one of eight doses of E2. Morphological mammary gland parameters were examined to identify doses producing half-maximal effects. Mice were exposed perinatally to 0 or 250 ng BPA/kg body weight (bw)/day from gestational day 8 until postnatal day (PND) 2. On PND25, female offspring were ovariectomized and given an estrogen challenge of 0, 0.5, or 1 μg E2/kg bw/day for 10 days. Morphometric parameters of the mammary gland were compared between strains.

Results: Both strains exhibited similar responses to E2. Perinatal BPA exposure altered responses to E2 at puberty for several parameters in both strains, although the effect in CD-1 was slightly more pronounced.

Conclusion: Both mouse strains provide adequate models for the study of perinatal exposure to xenoestrogens.

Key words: BPA, C57Bl6, CD-1, estradiol, estrogen bioassay, mammary gland bioassay, mouse strains, nonmonotonic response, uterotopic assay.

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pumps were filled with solutions delivering 0, 0.25, 0.5, 1, 2.5, 5, 10, or 50 μg E2/kg bw/day (Steraloids Inc., Newport, RI) dissolved in 50% dimethylsulfoxide (DMSO; vehicle control; Sigma-Aldrich, St. Louis, MO) for 20 days. The pumps were implanted subcutaneously as previously described (Markey et al. 2001a). Uteri were dissected, blotted on filter paper, and weighed.

Morphometric analysis of mammary glands. Slides were coded and analysis was performed in a blind fashion. Mammary gland images were captured with a Zeiss dissection scope and AxioCam digital camera (Carl Zeiss Inc., Germany). Morphometric analysis was performed as described previously (Vandenberg et al. 2006). The parameters examined included number and area of terminal end buds (TEBs), area subtended by the ductal tree, and ductal extension measured as the distance from the midpoint of the lymph node to the leading edge of the ductal tree. In cases where the leading edge of the ductal tree did not grow beyond the center of the lymph node, ductal extension was assigned a negative value.

RNA isolation and real-time quantitative RT-PCR. Mammary gland RNA was isolated and quantitative real time reverse transcription-polymerase chain reaction (RT-PCR) was performed as described previously (Munoz de Toro et al. 2005). The primers used were Msx2, forward: ggaagaccagatggaccaga, and reverse: tcgtataagggccgtctgtcag; amphiregulin, forward: aaggagcttgccagaagg, and reverse: gtcgaagctctctctctctcc; and ribosomal protein L19 (a housekeeping gene), forward: atcggccagcatacccgag, and reverse: tctacgccctctctcatcaggca.

Analysis of dose–response curves. For each parameter, the following were calculated: a) the lowest observable effect level (LOEL) dose (the lowest dose causing a statistically significant effect); b) the peak response (the dose(s) at which the response reaches a plateau or the highest response is achieved); c) the half-maximal dose (the amount of E2 inducing half the maximal response); and d) the fold change (the peak response divided by the response of the ovariectomized control).

Curve-fitting analysis was performed for each parameter to determine whether the response to estradiol best conformed to a sigmoidal (monotonic) curve, or to a polynomial (inverted-U–shaped) curve. To determine which responses could be considered statistically nonmonotonic, we assessed whether the peak response of a given parameter occurred at one of the intermediate doses and whether the peak response could be statistically distinguished from the response at the highest dose. When both criteria were met, the parameter of interest was defined as having a nonmonotonic response to E2.

Statistical analysis. Statistical significance was determined using SPSS software (SPSS, Chicago, IL). To determine statistical differences between the responses at each dose or the effects of BPA, we used nonparametric Mann-Whitney U-tests as well as parametric t-tests when data were normally distributed. To compare the responses of CD-1 and C57Bl6 mice, we used two-way analysis of variance (ANOVA). Post hoc tests (Bonferroni or planned t-tests) were used to make comparisons between groups. For all statistical tests, results were considered significant at p < 0.05. Values in figures are mean ± SE.

Results

Effect of E2 on uterine weight. Uterine wet weight represents the classical end point for assessing estrogenicity. As expected, the uterus demonstrated a monotonic dose–response curve to E2 in both mouse strains at the doses tested (Table 1, Figure 1A). We observed

Table 1. Dose–response curves for uterine wet weight and mammary gland morphological parameters in both mouse strains.

| Parameter                  | CD-1 Mice | C57Bl6 mice |
|----------------------------|-----------|-------------|
|                            | LOEL      | Half-max dose | Dose of max effect | Fold change | Curve shape | LOEL      | Half-max dose | Dose of max effect | Fold change | Curve shape |
| Ductal extension           | 0.5       | 0.42         | 2.5–10       | 3.2         | Inverted-U | 2.5       | 0.6          | 1–50          | –3.34       | Inverted-U    |
| No. of TEBs                | 2.5       | 1.6          | 5            | 9.8         | Inverted-U | 1.0       | 0.5          | 2.5–50       | –0.25       | Inverted-U    |
| TEB area                   | 0.5       | 0.4          | 1–50         | 1.4         | Inverted-U | 2.5       | 0.78         | 1–50         | 3.3          | Inverted-U    |
| Ductal area                | 2.5       | 2            | 9–10         | 7.0         | —          | 0.5       | 0.85         | 5–50         | 62.25        | —            |
| TEB area/ductal area       | 0.5       | 1.6          | 5            | 8.6         | —          | 0.5       | 0.8          | 5–50         | 75.96        | —            |
| Uterine weight             | 0.25      | 4.25         | 10–50        | 4.7         | Monotonic  | 1.0       | 3.55         | 5–50         | 5.95         | Monotonic     |
| Uterine weight/bw          | 0.5       | 4.1          | 10–50        | 7.8         | Monotonic  | 1.0       | 3.4          | 5–50         | 6.09         | Monotonic     |
| Msx2                       | 2.5       | 2.5          | 5–50         | 4.7         | Monotonic  | 0.5       | 1.5          | 5–50         | 10.6         | Monotonic     |
| Amphiregulin               | 3.6       | 5–50         | 64.8         | Monotonic   |            | 2.5       | 4            | 5–50         | 81.81        | Monotonic     |

max, maximal. The LOEL, half-maximal dose, dose of maximal effect, fold change, and curve shape were calculated.

The curve shape for these parameters is “derived” data, from a quotient between two direct measurements, each one of them being affected by E2, therefore, the shape of the derived curve is irrelevant.
significant differences between strains at E2 doses ≥ 0.5 µg/kg bw/day. However, because CD-1 females are significantly larger than their C57Bl6 counterparts (25.5 ± 0.2 g and 16.3 ± 0.2 g, respectively; p < 0.001), significant differences between strains were not apparent when uterine weight was normalized to body weight (not shown). When normalized to the ovariectomized controls, the fold change in uterine weight was also comparable in both strains (Figure 1E).

Morphometric parameters of the mammary gland: response to E2. In CD-1 females, E2 treatment increased the number and area of TEBs (Table 1, Figure 1B) and increased ductal extension (Figure 1C). Each of these parameters revealed an inverted-U–shaped, nonmonotonic response (Table 1). Graphic representation of the ductal area also showed an obvious inverted-U–shaped response to E2 (Figure 1D).

In C57Bl6 females, we found that the effect of E2 on the number of TEBs, area of TEBs, ductal area, and ductal extension generated an inverted-U–shaped dose response (Figure 1C, Table 1). For every parameter, the doses showing peak responses were similar to those obtained in CD-1 females (Table 1). The LOEL for the number and area of TEBs occurred at a lower dose than in CD-1 females. However, for ductal area and ductal extension, the LOEL was found at a higher dose.

When CD-1 and C57Bl6 responses were compared, we detected no significant differences at any E2 dose for number or area of TEBs. However, significant differences were detected in parameters related to the overall growth of the epithelium (i.e., ductal extension and ductal area; Figure 1C–D). Because they were detected even in ovariectomized females (without E2 treatment), these differences appeared to be largely due to the divergent body size of these two strains. In fact, when normalized to body weight, strain differences only remained for ductal area; disparities between strains disappeared for the other parameters (not shown). Finally, 2-way ANOVAs did not indicate a significant interaction variable between E2 dose and mouse strain for any mammary gland parameter, indicating that the shape of the dose–response curves cannot be statistically distinguished for CD-1 and C57Bl6 strains.

The number of TEBs per ductal area (TEBs/area) and the area of all TEBs per ductal area (TEB area/area) were calculated to determine the TEB density. We found significant differences between these strains at 1, 10, and 50 µg E2/kg bw/day regarding both TEB density parameters (data not shown). At all three doses, the response observed in C57Bl6 females was more pronounced than that in CD-1 females. Because these parameters are ratios of the number or area of TEBs (no significant differences shown between strains) and ductal area (significant differences shown between strains, which correlate with animal size), the overall response to E2 may be more striking in the C57Bl6 strain because of their smaller size and/or slower development.

To explore this concept further, we normalized the number and area of TEBs, ductal area, and ductal extension to ovariectomized controls. The mammary glands of ovariectomized C57Bl6 mice displayed almost complete developmental arrest, whereas those of their CD-1 counterparts maintained a few TEB structures. Accordingly, E2 treatment increased the number of TEBs by 80-fold in C57Bl6 and only 10-fold in CD-1 mice (Figure 1F, Table 1), while the maximal TEB number was similar (Figure 1B). The increase in ductal extension was comparable in both strains (Figure 1G, Table 1), whereas the increase in ductal area was 1.4-fold in CD-1 and 3.3-fold in C57Bl6 mice (Figure 1H).

Mammary gland gene expression: response to E2. The expression of Mx2 and amphiregulin mRNAs, two estrogen-regulated genes (Mallepell et al. 2006; Phippard et al. 1996), increased monotonically with increasing doses of E2 in both CD-1 and C57Bl6 mice (Figure 2 and Table 1).

Selection of doses for pubertal E2 challenge. To investigate the effects of perinatal BPA exposure on the response to E2 at
growth of the ductal epithelium, measured as increased ductal area. However, perinatal exposure to BPA had no effect on this parameter (Table 2).

In C57Bl6 mice, the number of TEBs increased with higher doses of E2, as expected from the dose–response data. In 250BPA females, the mean number of TEBs induced by 0.5 µg E2/kg bw/day was increased compared with 0BPA animals (Figure 3D), similar to the response seen in the CD-1 strain; however, these differences were not statistically significant, likely due to variability in the data. Additionally, treatment with 1 µg E2/kg bw/day induced significantly fewer TEBs in 250BPA compared with 0BPA females (Figure 3D). The total TEB area induced by administration of 1 µg E2/kg bw/day was lower in the 250BPA mice compared with 0BPA animals (Table 2), although this decrease was not statistically significant. As in CD-1 mice, TEB parameters such as TEBs/area (Table 2) and TEB area/area (Figure 3E) were significantly lower in the 250BPA mice treated with 1 µg E2/kg bw/day compared with 0BPA in C57Bl6 mice.

Parameters associated with overall ductal growth (ductal area and extension) were also measured in the C57Bl6 mammary gland. As expected, higher doses of E2 induced larger areas (Table 2) and greater ductal extensions (Figure 3F) than in ovariectomized controls. As observed in CD-1 mice, perinatal exposure to BPA did not alter ductal area; BPA also did not alter ductal extension in C57Bl6, contrasting with growth patterns observed in CD-1 mice.

Uterine weight: response to BPA. As expected, treatment with increasing doses of E2 resulted in increased uterine wet weight. In

Figure 3. The effects of perinatal BPA exposure on an E2 challenge at puberty shown as the (A, D) number of TEBs, (B, E) TEB area/ductal area, and (C, F) ductal extension. There were no TEBs in the mammary glands of C57Bl6 E2 controls. (A–C) represent CD-1 mice and (D–F) represent C57Bl6 mice. Values shown are mean ± SE. Letters that are not in common indicate significant differences (p < 0.05).
both strains, perinatal treatment with 250 ng BPA/kg bw/day did not alter this parameter (Table 2).

**Discussion**

In the present study we examined two important issues regarding estrogen action—strain sensitivity and dose–response curve shape—always in the context of the effects of perinatal BPA exposure. Previously, we reported that perinatal exposure to environmentally relevant levels of BPA results in altered postnatal development of the mammary gland (Markey et al. 2001a, 2003a; Munoz de Toro et al. 2005). One of the most striking observations was that the sensitivity of the mammary gland to E2 increased in perinatally BPA-exposed CD-1 females that were ovariectomized before puberty (Munoz de Toro et al. 2005). The present study revealed that both outbred CD-1 and inbred C57Bl6 strains respond quite similarly to E2 and BPA in many parameters. The C57Bl6 strain is of particular interest because it is widely used to study mammary gland development and in the generation of genetically modified mice.

There are concerns regarding the use of laboratory strains selected for large litter size (i.e., CD-1 mice) stemming from the possible correlation with resistance to endocrine disruptors including xenoestrogens. It has been suggested that testing chemicals in these strains may underestimate the endocrine disruptor potential of the agent being examined (Spearow et al. 1999). Specifically, CD-1 males were shown to be E2-resistant when compared with C57Bl6 mice regarding effects on testes weight and spermatogenesis (Spearow et al. 1999). Strain susceptibility was also observed in rats, often specific to the chemical being studied. For example, Sprague-Dawley rats were resistant to BPA compared with the Fischer 344 rats regarding proliferation of vaginal epithelium. However, both strains responded equally to E2. Thus, the differences between Sprague-Dawley and Fischer 344 rats regarding E2 sensitivity and dose–response curve shape should also be considered when assessing strain sensitivity. When a given parameter exhibits a monotonic dose–response curve, all effective doses should result in a qualitatively similar effect. To the contrary, if the dose–response curve is nonmonotonic or has an inverted-U shape, opposite effects might be observed at different doses. It has been proposed that nonmonotonic dose–response curves are generated by the integration of two or more monotonic dose–response curves that are occurring through different pathways and affecting a common end point with opposing effects (Conolly and Lutz 2004). For those end points, one cannot test a single high dose of a given chemical to assess whether or not it produces a biological effect (vom Saal and Hughes 2005; Welshons et al. 2003). In the present study, morphometric parameters of the mammary glands of both CD-1 and C57Bl6 mice displayed inverted-U–shaped dose–response curves to E2. Although most parameters in CD-1 mice met the criteria for statistical nonmonotonicity, variability in responses prevented the C57Bl6 from meeting this standard. One striking difference between these strains was the state of quiescence of the mammary gland of C57Bl6 ovariectomized mice compared with their CD-1 counterparts. There were practically no TEBs in the former, whereas a few TEBs were present in the CD-1 females 10 days after ovariectomy. It is possible that these results are indicative of different rates of development or onset of puberty in females of these two strains. Although there is no evidence in the literature to support this conclusion, no studies have examined age of puberty in these two strains under the same conditions (food supplied, light cycle, temperature, etc.) In the present study, females were ovariectomized before puberty, and thus this information could not be collected.

In contrast to the morphometric parameters, the induction of estrogen-target genes in the mammary gland was maintained in both strains. The magnitude of the response was comparable in the two strains, and the sensitivities of these responses were lower than those of several morphological end points.

**E2 sensitivity of the uterus.** Diel et al. (2004) found that the uterus and vagina of several rat strains responded similarly to three different doses of estrogen in a 3-day assay (Diel et al. 2004). In the present study, we arrived at a similar conclusion using a set of seven different E2 doses and a 10-day assay in mice. Both mouse strains displayed a monotonic dose–response curve regarding uterine weight at the doses tested, and their response was comparable when uterine weight was normalized to body weight. Similar dose–response curves were reported in a 3-day (Padilla-Banks et al. 2001) and a 10-day mouse assay (Skarda 2002).

Overall, this study revealed little or no difference in the sensitivity to E2 between CD-1 and C57Bl6 mice regarding the uterotrophic response and a variety of morphometric and gene-expression end points in the mammary gland. This is in contrast to the marked differences observed between these strains in tests end points (Spearow et al. 1999). The mechanisms underlying the latter differences have yet to be determined. However, steroid metabolism by the liver is subject to hormonal imprinting (Gustafson et al. 1977), which may explain differences in response between males and females of the same strain.

**Effect of perinatal exposure to BPA on the pubertal response to E2.** Perinatal BPA exposure did not alter the uterine response to E2 in these two strains under the same conditions (food supplied, light cycle, temperature, etc.) In the present study, females were ovariectomized before puberty, and thus this information could not be collected.

**Table 2. Mammary gland morphological parameters of CD-1 and C57Bl6 mice exposed perinatally to BPA (OBPA and 250BPA) and postnatally to 0, 0.5, or 1 µg E2/kg bw/day.**

| Treatment                  | 0, OBPA | 0.5, OBPA | 0.5, 250BPA | 1, OBPA | 1, 250BPA |
|----------------------------|---------|-----------|-------------|---------|-----------|
| **CD-1 mice**              |         |           |             |         |           |
| Ductal extension           | 2.62 ± 0.53a | 2.80 ± 0.76a | 4.41 ± 0.74ab | 4.69 ± 0.58b | 4.54 ± 0.53a | 6.00 ± 0.46b |
| # TEBs                     | 1.4 ± 0.58a | 2.1 ± 0.78a | 5.6 ± 1.1b  | 10.0 ± 0.85b | 11.9 ± 1.3c  | 10.1 ± 0.50c  |
| TEB area                   | 0.048 ± 0.020a | 0.078 ± 0.029a | 0.219 ± 0.054b | 0.428 ± 0.040d | 0.546 ± 0.049c | 0.440 ± 0.034d |
| Ductal area                | 79.1 ± 8.7a | 82.26 ± 7.0a | 126.1 ± 8.2b | 118.6 ± 8.26a | 124.58 ± 8.65b | 139.8 ± 6.85b  |
| # TEBs/ductal area         | 0.015 ± 0.006a | 0.023 ± 0.008a | 0.042 ± 0.009a | 0.085 ± 0.006b | 0.096 ± 0.009b  | 0.075 ± 0.005b  |
| TEB area/ductal area       | 0.0005 ± 0.0002a | 0.0009 ± 0.0003a | 0.0011 ± 0.0004a | 0.0036 ± 0.0003c | 0.0045 ± 0.0004a | 0.0032 ± 0.0002c |
| Uterine weight             | 15.32 ± 1.58a | 14.97 ± 0.73a | 20.59 ± 1.02b | 19.46 ± 0.81a | 25.47 ± 1.46a | 29.32 ± 1.35a |
| **C57Bl6 mice**            |         |           |             |         |           |
| Ductal extension           | −3.00 ± 0.34a | −2.39 ± 0.93a | 0.99 ± 0.34b | 1.68 ± 0.59a | 2.13 ± 0.25a | 2.05 ± 0.36b |
| # TEBs                     | 0.0 ± 0.0a | 0.0 ± 0.0a  | 3.1 ± 0.6b  | 7.7 ± 0.5a | 10.1 ± 1.1c | 6.0 ± 0.8b |
| TEB area                   | 0.00 ± 0.000a | 0.00 ± 0.000a | 14.57 ± 2.72a | 36.83 ± 16.21a | 54.14 ± 8.23b | 30.50 ± 5.95b |
| Ductal area                | 6.40 ± 0.80a | 10.10 ± 2.64a | 23.13 ± 1.33b | 36.03 ± 4.94b | 40.58 ± 3.41c | 44.69 ± 2.6b |
| # TEBs/ductal area         | 0.00 ± 0.00a | 0.00 ± 0.00a  | 0.11 ± 0.019b | 0.18 ± 0.072abc | 0.25 ± 0.018c | 0.15 ± 0.035ac |
| TEB area/ductal area       | 0.00 ± 0.00a | 0.00 ± 0.00a  | 0.44 ± 0.080b | 0.91 ± 0.31abc | 1.34 ± 0.16c | 0.67 ± 0.12ab |
| Uterine weight             | 5.1 ± 0.26a | 8.05 ± 2.6ab | 9.31 ± 0.71b | 11.00 ± 0.51bc | 12.20 ± 0.76c | 13.0 ± 1.53c |

Letters that are not in common indicate significant differences (p < 0.05).
administered from PND25 to PND35 in either mouse strain. In contrast, the response of the mammary gland to E2 was significantly altered by perinatal BPA exposure. A pattern emerged for TEB-related parameters, which increased in response to 0.5 μg E2/kg bw/day relative to controls, suggesting a shift to the left of the dose–response curve. Increased responses at 0.5 μg E2/kg bw/day were significantly reduced in the BPA-pretreated C57Bl6 mice. These results suggest a difference between the two strains in the level of the response to E2 after perinatal BPA exposure, such that increased sensitivity to E2 was manifested at a lower dose in CD-1 than in C57Bl6 mice. Alternatively, the sensitivity of the two strains may be similar, but the higher variability in the C57Bl6 TEB-related parameters may have precluded reaching statistical significance for end points that were significantly altered in the CD-1 strain. However, in both strains perinatal exposure to BPA significantly altered the response to E2 later in life.

Estrogen exposure represents a main risk factor for breast carcinogenesis. Increased sensitivity to E2 may have similar effects. Consistent with this concept, previous studies of CD-1 mice exposed perinatally to BPA revealed an increased number of TEBs at puberty and terminal ends in adulthood (Markey et al. 2001a; Munoz de Toro et al. 2005). These two structures are thought to be the sites where neoplasias arise. Also, there is an overexpression of progesterone receptors in BPA-exposed animals, which in turn induces excessive lateral branching of the mammary gland ducts (Munoz de Toro et al. 2005), resulting in an increased ductal density of the gland. In humans, increased mammmographic density is also a risk factor for breast cancer (McCormack and dos Santos Silva 2006). In the present study we extend these findings to C57Bl6 mice, suggesting that the enhanced sensitivity to E2 resulting from perinatal BPA exposure may represent a general phenomenon in mice rather than a strain idiosyncrasy.

Nonmonotonic dose–response curves. In vitro studies that used human breast epithelial cells and other estrogen-target cell lines showed nonmonotonic dose–response curves in response to increasing E2 doses (Amarra and Dannies 1983; Soto and Sonnenschein 1985). This type of curve suggests that estrogens can evoke different effects, such as induction (Soto and Sonnenschein 1987) or inhibition of cell proliferation (Sze et al. 2000), depending on the dose tested. The combined effect of these variable responses is reflected in the overall cell number (Soto and Sonnenschein 2001). In the mammary gland, estrogens promote proliferation—manifested as ductal growth (Nandi 1958)—and induce apoptosis—manifested as lumen formation (Munoz de Toro et al. 2005).

Conclusions

Nonmonotonic dose–response curves are observed in cultured cells and in animal models, and are oftentimes observed for estrogen end points. These patterns highlight the unreliability of assuming that the effect of exposure to low doses of a hormone, endocrine disruptor, or other toxicant can be extrapolated from the response to high doses of the compound (Conolly and Lutz 2004; vom Saal and Hughes 2005).

Contrary to the clear differences in the testicular response to postnatal administration of E2 between CD-1 and C57Bl6 mice (Spearow et al. 1999), the differences observed in the uterus and mammary gland of these different mouse strains are subtle. In addition, the mammary glands of both strains are sensitive to perinatal exposure to low doses of BPA, in that the postnatal response to E2 is significantly modified. This observation suggests that both strains provide adequate models for the study of perinatal exposure to xenoestrogens. Even though the outbred CD-1 strain has been selected for larger litter size and reproductive efficiency, these results show that this strain provides an excellent model for the study of estrogen action in the uterus and mammary gland. Additionally, mice of the C57Bl6 strain may be used advantageously in the study of endocrine disruption when an inbred strain is required. Confirmation of the suitability of the C57Bl6 strain for this work is important because many genetically modified animals have been developed on this background.

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