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# Large Effects from Small Exposures. III. Endocrine Mechanisms Mediating Effects of Bisphenol A at Levels of Human Exposure

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**Over 6 billion pounds per year of the estrogenic monomer bisphenol A (BPA) are used to manufacture polycarbonate plastic products, in resins lining metal cans, in dental sealants, and in blends with other types of plastic products. The ester bond linking BPA molecules in polycarbonate and resins undergoes hydrolysis, resulting in the release of free BPA into food, beverages, and the environment, and numerous monitoring studies now show almost ubiquitous human exposure to biologically active levels of this chemical. BPA exerts estrogenic effects through the classical nuclear estrogen receptors, and BPA acts as a selective estrogen receptor modulator. However, BPA also initiates rapid responses via estrogen receptors presumably associated with the plasma membrane. Similar to estradiol, BPA causes changes in some cell functions at concentrations between 1 pM and 1 nM, and the mean and median range of unconjugated BPA measured by multiple techniques in human pregnant maternal, fetal, and adult blood and other tissues exceeds these levels. In contrast to these published findings, BPA manufacturers persist in describing BPA as a weak estrogen and insist there is little concern with human exposure levels. Our concern with human exposure to BPA derives from 1) identification of molecular mechanisms mediating effects in human and animal tissues at very low doses, 2) *in vivo* effects in experimental animals caused by low doses within the range of human exposure, and 3) widespread human exposure to levels of BPA that cause adverse effects in animals. (*Endocrinology* 147: S56–S69, 2006)**

BISPHENOL A (BPA) is a small (228 Da) estrogenic monomer that is polymerized to produce polycarbonate plastic and resins used to line metal cans. BPA is also used as an additive in other types of plastic, such as polyvinyl chloride (PVC), used in medical tubing, toys and water pipes, and polyethylene terephthalate (PET), used in soda and mineral water bottles. BPA is also used to make some dental sealants. With total worldwide production capacity exceeding 6 billion pounds in 2003, BPA is one of the highest volume chemicals in commerce (1). Brominated BPA is one of the major flame retardants and is also a known endocrine-disrupting chemical (EDC) (2). It has been known for decades that BPA has the efficacy of the hormone estradiol in some tissues (3), and BPA has recently been shown to also antagonize thyroid hormone action (4) and antagonize androgen action (5). However, BPA acts as an agonist for a mutant form of the androgen receptor found in some prostate cancer cells (6).

Until recently, BPA had been considered to be a very weak environmental estrogen, because in some bioassays (for example, in the uterus of some rats and mouse strains or for some responses in human breast cancer cells), BPA can be 10,000- to 100,000-fold less potent than estradiol (7). For example, a common statement about BPA is that it "elicits weak estrogenic activity in *in vitro* and *in vivo* test systems" (8). However, studies of molecular mechanisms have revealed a variety of pathways through which BPA can stimulate cellular responses at very low doses in addition to effects initiated by binding of BPA to the classical $\alpha$- or more recent $\beta$-form of the estrogen receptor (ER$\alpha$ and ER$\beta$). We discuss below, in *Molecular Mechanisms of BPA Action*, recent findings showing that in a variety of tissues, BPA not only has the efficacy of estradiol but is also equally potent, with changes in cell function being observed at a dose of 1 pM (0.23 pg/ml culture medium) (9).

We discuss that the high potency of BPA *in vivo*, particularly during fetal and neonatal development, is explained not only by limited binding of BPA to plasma estrogen-binding proteins that evolved to regulate uptake of endogenous estradiol into tissues but also by the limited capacity for the liver to conjugate (deactivate) BPA in fetuses and newborns. We review in *Sources and Levels of Exposure of Animals and Humans to BPA* evidence that bioaccumulation of BPA occurs during pregnancy, although this does not occur in the nonpregnant adult female or in adult males. There is evidence that endogenous steroids modulate BPA metabolism, which contributes to higher circulating levels of BPA in males relative to females (10, 11).

Measurement of parent (unconjugated) BPA in human blood, tissues, and urine in studies conducted in the United States, Europe, and Japan are remarkably consistent in showing much higher levels than would be expected based on the assumption that BPA is very rapidly metabolized and ingested only infrequently and that BPA thus does not pose a threat to the public health (12).

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Abbreviations: BPA, Bisphenol A; DES, diethylstilbestrol; EDC, endocrine-disrupting chemical; ER, estrogen receptor; GC-MS, gas chromatography mass spectrometry; RBA-SMA, relative binding affinity-serum modified access; SERM, selective ER modulator.

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The higher-than-predicted tissue levels of parent BPA in human populations in developed countries, including a study as part of the National Health Survey by the Centers for Disease Control and Prevention (CDC) that detected BPA in over 95% of samples (13), suggests that exposure to significant amounts of BPA is continuous for these populations. This is not surprising given the massive amount of BPA used to produce products each year. Exposure occurs because when BPA molecules are polymerized, they are linked by ester bonds that are subject to hydrolysis, which is accelerated as temperature increases and in response to contact with acidic or basic substances (Fig. 1). The consequence is that as polycarbonate products are repeatedly washed, or polycarbonate plastic or metal cans are exposed to heat and/or acidic or basic conditions, significant leaching of BPA due to hydrolysis of the ester bond occurs (14–18).

The focus of this review will be on mechanisms mediating responses that occur as a result of exposure to very low doses of BPA. As of November 2005, there were over 125 studies showing significant effects in experimental animals of administering doses of BPA that were once thought to be below the no-observed-effect level based on experiments in which only very high doses (50–1200 mg/kg·d) were administered to rats and mice (19). In contrast to the traditional approach used by toxicologists to predict the possibility of health effects at human exposure levels based on only testing doses thousands or even millions of times higher than doses that are environmentally relevant (based on human exposure studies), a new paradigm has emerged in the study of EDC research in which much lower, environmentally relevant doses are used to assess directly the hazards posed by EDCs. For example, in the United States, a daily exposure dose of 50 $\mu$g/kg·d is stated by the U.S. Food and Drug Administration (FDA) and the U.S. Environmental Protection Agency (EPA) to be safe for humans, but this is based on toxicological studies conducted in the 1980s in which the lowest dose tested was 1000-fold higher than this predicted safe dose (20). In 1997, we published the first study that directly tested whether this prediction of the safe dose of BPA was valid (21).

During the 1990s, endocrinologists began to challenge the assumptions used to design the high-dose toxicological studies used by regulatory agencies to assess the risk to humans posed by chemicals. Previously, no experiment had ever been conducted by toxicologists to determine experimentally whether the predicted safe dose of 50 $\mu$g/kg·d BPA actually was, in fact, safe (7). This prediction was simply accepted by toxicologists and government regulatory agencies as valid in the absence of any data. We will discuss in *Prediction of Biological Activity of BPA* that the application of basic approaches used by endocrinologists for decades to determine physiologically relevant doses for hormones has led to a paradigm inversion in toxicology with regard to dose selection in experiments with chemicals that cause effects by interacting with endogenous endocrine signaling systems. We will use BPA as our model hormone-mimicking chemical, because there are now over 40 published studies reporting significant effects in rats and mice at doses below the predicted safe dose of 50 $\mu$g/kg·d (22, 23). However, the approach we describe applies to other EDCs as well.

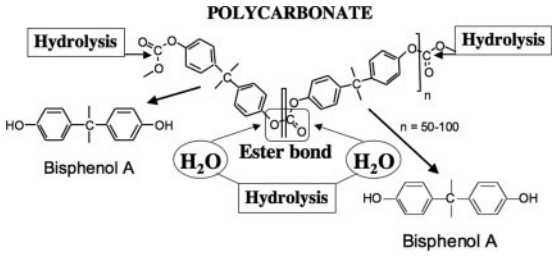


FIG. 1. Schematic diagram depicting hydrolysis of the ester bond linking BPA molecules to form polycarbonate plastic. BPA is a symmetrical aromatic molecule that reacts on both phenolic ends in polymerization reactions. For polycarbonate, BPA typically reacts with phosgene forming an ester linkage, which is subject to an increase in hydrolysis as temperature increases and in response to acidic or basic conditions.

## Prediction of Biological Activity of BPA by Reference to the Levels of Estradiol and Diethylstilbestrol (DES) that Act on Fetal Development

*Low doses of BPA selected based on estrogenic activity not toxicity*

To characterize the high-dose acute toxicity of a chemical with unknown activities, the effects must be determined first, and then the doses of the chemical are characterized that bring about those effects, which will be specific to each chemical. But for the low-dose endocrine-disruptor effects of an estrogenic chemical, it is a different matter (24). Many of the targets and effects that are subject to disruption by an estrogen are already known. The targets will be the estrogen-responsive tissues and cells of the organism, although the effects that may augment, inhibit, or modify the endogenous response, depending on the activity of the chemical, will potentially include all effects known to be under the direct regulation of endogenous estrogen.

The first unknown to be determined in the evaluation of an estrogenic EDC is therefore not the effects but rather the dose range to the animal that will lead to internal concentrations that are physiologically relevant to endogenous estrogen concentrations, and at which a battery of well described estrogen-related endocrine effects will be disrupted by the estrogenic EDC. This disruption in the target tissues is predictable from, and in fact requires, a concentration of the EDC at the target cell (dose at target) that can occupy ERs. However, for a change in response to occur, there must be a change in occupancy of ERs, so the dose range used must be substantially below the range that saturates receptors. This information can be obtained by comparison with the concentration range of estradiol that brings about normal hormonal signaling, because response varies as a function of receptor occupancy. However, the exact spectrum of consequences of exposure to a given EDC at the concentrations that occupy estrogen receptors may not be known without experimental determination, because a pattern of tissue-specific responses to some manmade estrogenic chemicals, different from the pattern observed for endogenous estrogen, has been demonstrated (25, 26). Some of the best-studied examples are the drugs tamoxifen and raloxifene, which act as selective ER modulators (SERMs) (27, 28).

We approached our studies with BPA by asking whether the range of activity of this estrogenic EDC could be understood by reference to the experimental levels of estradiol that acted on the development of the fetus. We decided to focus on effects in the fetus, because the fetus is highly sensitive to hormonal changes, and developmental changes are typically permanent and thus, by definition, adverse. Before conducting experiments with BPA, we determined the level of the natural estrogen, estradiol, required in the circulation of the fetus to produce biological effects. We reasoned that a level of any xenoestrogen, including BPA, equivalent in estrogenic activity to this level of estradiol, would also result in biological effects on development (24).

We first described effects of naturally occurring differences in circulating estradiol on the development of the male reproductive tract (30, 31). In subsequent studies, we determined the levels of free circulating estradiol (unconjugated and unbound to plasma proteins) that were active during normal development (32). Finally, we determined experimental increases of free circulating estradiol that disrupted development of the male reproductive system (33). This dose-response information for estradiol was used to predict the doses of both DES and BPA that would cause the same type of developmental effects in this system (24). Specifically, we first observed that the average free (unbound, bioactive) estradiol concentration during development of the male murine reproductive tract was 0.21 pg/ml in fetal serum and that an increase in this level of only 0.1 pg/ml free estradiol, to 0.31 pg/ml [via maternal administration of estradiol in a SILASTIC brand silicon capsule (Dow Corning, Midland, MI)], resulted in developmental changes (urethral constriction and an increase in number and hyperplasia of prostate gland ducts) detected in the fetus as well as permanent enlargement of the prostate and up-regulation of prostatic androgen receptors in the resulting adult many months after the exposure (33). Therefore, we predicted that an estrogenic EDC would be biologically active in the fetus if supplemental estrogenic activity of the free (unbound, bioavailable) chemical in blood was equivalent to an increase in free estradiol of only 0.1 pg/ml.

We modeled bioactivity of several environmental estrogens by addressing key factors that influence EDC activity (7, 21, 24, 34): 1) the intrinsic estrogenic activity of the molecule in interaction with ERs in the nucleus of the cell, 2) how the compound is carried in blood and what fraction is delivered free (unbound) to cells, 3) how the compound partitions between the circulation and body lipid, and 4) the absorption and metabolism relative to the route of exposure.

### *DES as an orally active positive control for orally administered BPA*

We chose as our positive control estrogenic EDC the well characterized, orally active estrogenic drug DES, which is widely used in endocrine-disruptor research (35). A National Toxicology Program Peer Review Panel that considered the scientific evidence in 2000 stated that DES was an appropriate positive control estrogenic drug (due to the extensive published literature on DES effects in experimental animals and in humans) to use in studies of estrogenic chemicals such as BPA (found on page iii of Ref. 36; http://ntp.niehs.nih.gov/index.cfm?objectid=06F5CE98-E82F-8182-7FA81C02D3690D47). (Requests for hard copies of the NTP report and inquiries about the Endocrine Disruptors Low-Dose Peer Review can be made to the NTP Liaison and Scientific Review Office at NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709; E-mail: liaison@starbase.niehs.nih.gov.) Importantly, all of the key factors above could be evaluated based on studies with pregnant mice. Factor 1, binding of DES to ERs, and factor 2, free concentration of DES in serum, were evaluated with an *in vitro* assay system that included effects of plasma binding proteins on uptake of estrogenic chemicals into cells, referred to as the relative binding affinity-serum modified access (RBA-SMA) assay (21, 34). Factors 3 and 4, concerning absorption, metabolism, and distribution to the fetus after maternal dosing, were evaluated from findings with tritiated DES (37); in this report, the authors concluded that approximately 3% of a maternal dose was retained for an extended period of time in fetal serum as unconjugated DES. The calculations involved in the evaluation of estrogenic endocrine-disrupting activity of DES by the RBA-SMA assay, and incorporation of the fetal distribution information on metabolism and fetal distribution, are detailed elsewhere (24).

These calculations yielded an oral dose of DES of 0.077 $\mu$g DES/kg maternal body weight per day that was predicted to be active in the fetal prostate endocrine disruption model, equivalent to an approximately 50% increase in average free estradiol during development as shown in our study described above. The lowest dose of DES that was active in stimulating ad increase in prostate size during development due to feeding DES to pregnant mice was 0.02 $\mu$g/kg·d, and the maximum stimulating effect occurred at a maternal dose of 0.2 $\mu$g/kg·d; these doses were directly within the low-dose range predicted by our approach (24, 33). The lowest dose of DES tested in that study, 0.002 $\mu$g/kg·d, was not active in endocrine disruption of prostate development and was well below the predicted active dose of 0.077 $\mu$g/kg·d for that end point. Therefore, accurate prediction of active dose was possible by modeling the key factors listed above. These initial findings have been confirmed (38–41).

Of great importance, before conducting studies with a chemical of unknown estrogenic activity in various target tissues, we had established through extensive studies with positive control estrogens (initially estradiol and DES and subsequently also ethinyl estradiol) (41, 42) that the developing reproductive system in fetuses was responsive to very small changes in estrogenic activity. We describe in detail elsewhere that for toxicological studies conducted without appropriate positive controls and that report only negative findings for a test chemical, interpretation of the negative results is not possible and violates basic rules governing experimental research design and analysis, specifically the need for a valid positive control when test results for a drug or chemical with a known mode of action are uniformly negative (35).

### *Potency of BPA relative to DES*

At the time that we conducted our initial study with BPA, we could evaluate only the first two key factors influencing

dose at target receptor: binding affinity for ERs and free concentration in plasma. For BPA and a second EDC, octylphenol, we applied the RBA-SMA analysis to predict relative estrogenic activities (21, 34) and estimated actual active doses with less information than was available for DES (as described below in *Sources and Levels of Exposure of Animals and Humans to BPA*, all of this information is now available for BPA). We found that unconjugated BPA in blood showed very limited binding to SHBG and a free fraction in serum of around 8% (34); most BPA would thus only be bound weakly to albumin in blood and therefore delivered to cells with a physiological advantage compared with estradiol. The free fraction of octylphenol in serum was 0.3%, substantially lower than for BPA (34).

The effects of delivery of these EDCs to target cells by serum and its binding proteins changed the relative estrogenic activities of these two compounds and predicted that BPA would be more than 500-fold more active than octylphenol in endocrine disruption of the fetal mouse (21). This contrasted sharply with previously published results from *in vitro* experiments in which serum binding was not taken into account that octylphenol was approximately 10-fold more estrogenically active than BPA (21, 24). Specifically, the results of the analysis predicted not only 1) that BPA would be far more active than octylphenol but also 2) that BPA could be active in endocrine disruption at a dose of approximately 20 $\mu$g/kg·d, a dose lower than the presumed safe daily human exposure level of 50 $\mu$g/kg·d, which is assumed to be at least 100-fold lower than the no-observed-effect level (20).

When BPA and octylphenol were fed to pregnant mice at both 2 and 20 $\mu$g/kg body weight per day from gestation day 11–17, the prediction was confirmed that the 20 $\mu$g/kg dose of BPA was bioactive, whereas octylphenol was not bioactive in terms of an effect on prostate development. Importantly, even without full information on absorption, metabolism, and fetal distribution of these two compounds, our approach to estimating a low-dose active exposure range from the RBA-SMA assay yielded information that permitted a prediction of the low-dose exposure range for BPA that was orders of magnitude more accurate than had been predicted from previous studies that used only high doses (>50 mg/kg·d) to examine acute toxicity (21, 43).

In animal experiments, BPA proved even more biologically active than the 20 $\mu$g/kg·d dose that we had initially predicted based on our *in vitro* RBS-SMA assay (21), which has been confirmed in a large number of independently conducted studies reviewed elsewhere (22, 35). Therefore, we hypothesized that there were additional factors in 1) metabolism and delivery of BPA from the mother to the fetus and 2) the mechanism of BPA action that would act to further increase the biological activity of BPA in the fetus relative to its apparent weak estrogenic activity in the adult, and these issues will be discussed in detail below in *Molecular Mechanisms of BPA Action*.

There is evidence that 1) changes in the metabolism of BPA during pregnancy can lead to higher levels of BPA in the mother, 2) in animal studies, the fetus is rapidly exposed when the mother is fed BPA, 3) the fetus and newborn have very limited capacity to metabolize BPA and other related compounds such as DES, and 4) current exposure of human adults and fetuses to BPA is within a biologically active range. This last issue has great significance with regard to the potential for adverse effects of BPA on human fetal development because, as discussed in below in *Sources and Levels of Exposure of Animals and Humans to BPA*, unconjugated BPA levels in human fetal blood are greater than blood levels of BPA that cause adverse effects in animals and that have been shown to stimulate human tissues in culture. In addition, BPA has been shown to act as a SERM and to have activities that include actions through nonclassical estrogen signaling pathways. This can lead to substantially increased estrogenic activity, relative to its weak activity in some bioassays conducted only in adults, as well as unique effects.

It is important to indicate that some laboratories have not reproduced the initial *in vivo* findings that very low doses of BPA altered development of the fetal reproductive system in male mice, although we and now many others have replicated our findings. However, an analysis of the outcome of low-dose *in vivo* studies with BPA in relation to source of funding provides strong evidence of conflict of interest and bias in reporting findings on BPA; we have discussed this issue in detail in other recent reviews (22, 35, 44). Specifically, the majority of published reports that our findings are not valid or reliable emanate from corporate-funded publications, 100% of which report that BPA causes no significant effects (Table 2 in Ref.35), not just effects on the male reproductive system.

In sharp contrast, there are now over 125 published studies funded by government agencies such as the National Institutes of Health documenting that BPA has a wide range of significant effects including structural and neurochemical changes throughout the brain associated with behavioral changes, such as hyperactivity, learning deficits, increased aggression, and increased likelihood of drug dependency; abnormalities in sperm production in males and oocytes in females; disruption of hormone production and fertility in both males and females; immune disorders, increased growth rate; and early sexual maturation (listed in Table 1 in Ref. 35). Most of the small number of studies funded by government agencies that report no significant effects of BPA used one model animal (the CD-SD rat) that after being subjected to selective breeding for over 1000 generations has become extremely insensitive to any estrogenic chemical or drug, thus revealing the importance of determining the appropriateness of the animal model being used by including a positive control, such as DES, in studies of the estrogenic effects of BPA (22, 35). Endocrinologists are well aware of the issue of corporate bias in research, and this issue has recently received considerable attention in articles published in special issues of journals (45–49), in a letter we have published (44), as well as in a review in Scientific American (50).

## Molecular Mechanisms of BPA Action

### *BPA is a SERM*

There is now evidence that BPA acts as a SERM and, relative to estradiol, 1) interacts differently within the ligand-binding domain of ERs (51), 2) shows a different binding affinity for and regulation of ER$\alpha$ and ER$\beta$ in target cells (25, 52), and 3) interacts differently with transcriptional coregu-

lators (25). In addition, there is evidence that similar to estradiol, BPA can elicit rapid responses in cells through nongenomic signaling systems (9, 53–59).

After binding hormone, ER regulates the rate of gene transcription through its association with coregulators. Findings from a number of studies (25, 51, 52, 60) suggest that at a molecular level, the interaction of BPA and estradiol with ERs is different, and it is likely that BPA induces a unique ER conformation. This provides the basis for a difference in the interaction of ER with coregulatory proteins that act as coactivators or corepressors of ER-mediated transcription. The recruitment of some coregulators by the BPA-ER complex has been shown to be disproportionate to BPA's binding affinity for each ER subtype. Specifically, although BPA displayed a 10-fold higher binding affinity for ER$\beta$ over ER$\alpha$, the BPA-ER$\beta$ complex had over 500-fold greater potency than the BPA-ER$\alpha$ complex in recruiting the coactivator TIF2 (25). It is hypothesized that the overall balance of the relative expression levels of ER subtype and ER coregulators is an important determinant of the tissue specificity of SERMs such as BPA.

Additionally, within the same tissue, different cell types have unique profiles of estrogen-stimulated gene expression, and thus individual SERMs can have a mix of agonist and antagonist activity within the same tissue. For example, stimulation of uterine cell proliferation and water imbibition in the uterus of rodents is a standard assay for assessing the potency of xenobiotic estrogens. BPA was a weak partial agonist for stimulation of uterine wet weight gain. However, in the same tissue, BPA significantly stimulated an estrogen-responsive reporter gene (three copies of the vitellogenin estrogen response element linked to the lac Z gene) *in uteri* from ER action indicator (ERIN) transgenic mice (61). Conversely, tamoxifen is a potent stimulator of uterine wet weight gain in mice but did not stimulate ERIN reporter gene activity in this tissue (61).

It is not always possible to predict the dose required for BPA to elicit specific effects in one tissue based solely on the dose of the chemical that will elicit responses in other tissues. This, of course, is not specific to BPA. Numerous articles now describe marked differences in BPA potency between different estrogen-responsive tissues within the same animals (consistent with SERM activity) at doses far below those previously predicted to cause biological effects based on traditional high-dose toxicological studies (19). As a result, BPA has become one of the primary EDCs being chosen for study by researchers in many different disciplines outside of the field of toxicology who have an interest in chemicals that interact in unique ways with the various components of genomic and nongenomic hormone-response systems.

Although the term SERM was originally applied to chemicals, such as the drug tamoxifen, that act as both ER agonists and antagonists, depending on the tissue being examined, SERM is now also applied to chemicals, such as BPA, that have tissue-specific and species-specific effects. With regard to tissue-specific effects, there are published studies showing that the effects of BPA are virtually identical to estradiol, ethinyl estradiol, and DES in the fetal mouse prostate (33, 38, 41) but markedly different from estradiol in the uterus (61, 62). For example, administering pregnant mice a daily dose of 10 $\mu$g/kg·d BPA produced permanent changes in reproductive organs in male offspring in CD-1 mice (41). In contrast, effects of BPA in the uterus of developing CD-1 female mice occur only at maternal doses dramatically higher (100 mg/kg·d) than those required to alter reproductive organ function in males of the same mouse strain (62). However, the extremely low dose of 0.025 $\mu$g/kg·d BPA was administered to pregnant and lactating female CD-1 mice via an implanted Alzet mini-pump, and mammary gland duct development was stimulated in female offspring (similar to the effect of BPA on prostate ducts in CD-1 male mice), and there was also altered postnatal growth, rate of sexual maturation, and estrous cycles during later adulthood (63–65). Similar findings that prenatal exposure to BPA accelerated postnatal growth and caused an early onset of puberty were also reported after oral administration one time per day of 2.4 $\mu$g/kg·d BPA to pregnant mice (66). In this regard, it is interesting that BPA stimulates insulin secretion in mice (58, 59).

Not all responses to BPA are predicted by effects of estradiol. BPA has been shown to antagonize the action of estradiol in the rat hippocampus by blocking the stimulatory effect of estradiol on synaptogenesis (26). In another study, BPA was shown to act as a highly potent estradiol mimetic and also to disrupt the rapid actions of estradiol at very low concentrations during cerebellar development via rapid nongenomic signaling systems (57).

With regard to different effects in different strains of animals, BPA stimulates responses in the pituitary and reproductive organs of female Fischer 344 rats at doses much lower than those required to stimulate responses in Sprague Dawley rats, although these two different types of rat did not show differences in the metabolic clearance of BPA (67) or response to estradiol, and binding of BPA in target tissues to ER$\alpha$ was similar for the two strains (67–69). We have discussed in detail elsewhere the insensitivity of the CD-SD rat to estrogenic drugs as well as BPA (22).

### *Effects of BPA mediated by nongenomic response systems*

There is now extensive evidence that some effects of estradiol occur through the activation of cell signaling systems associated with receptors that are not located in the cell nucleus and, instead, may be associated with the cell membrane. These effects are very rapid and occur in addition to the well studied effects mediated by receptors located in the cell nucleus, which take longer to occur (70). A characteristic of cell signaling systems is a very high level of amplification, with the result that a very low concentration of a compound can activate large changes in cell function.

Recent studies have shown that BPA can act via nongenomic (nonnuclear) receptors to activate cell-signaling pathways at very low concentrations. In rat pituitary tumor cells, BPA significantly stimulated a rapid (within 30 sec) influx of calcium at a dose of 1 pM, and prolactin release, which is triggered by calcium influx in these cells, was also detected within 1 min, similar to the response to estradiol (9). In mouse pancreatic $\beta$-cells, phosphorylation of the transcription factor cAMP response element-binding protein associated with rapid induction of calcium influx was reported at a BPA dose of 1 nM, which was equal in magnitude to the

response caused by the same dose of estradiol (55). In addition, rapid (within 1.5 min) influx of calcium was observed in human MCF-7 breast cancer cells in response to estradiol and BPA that was significant at the lowest dose tested, which was 0.1 nM BPA; for estradiol, the $EC_{50}$ was 0.11 nM, and for BPA the $EC_{50}$ was 0.15 nM (56).

BPA also induces expression of the nuclear transcription factor Nur77 in mouse Leydig cells, which is involved in LH-mediated testosterone synthesis (71). BPA-induced expression of Nur77 in Leydig cells is mediated by activation of protein kinase A and MAPK, with phosphorylation of MAPK being detected within 5 min after administration of BPA and reaching a maximum at 10 min; this results in altered steroidogenesis. This response is too rapid to be mediated by activation of transcription factors, which includes the classical nuclear ERs. Nur77 mRNA levels were increased above baseline at 1 nM BPA (71).

### *Anti-thyroid-hormone effect of BPA*

BPA has been reported to antagonize $T_3$-stimulated transcription of genes in human TSA201 cells at concentrations between 100 nM and 1 $\mu$M. BPA recruited the nuclear corepressor to the thyroid hormone receptor. This provides the first evidence for direct effects of low doses of BPA on disruption of thyroid hormone action within cells by competitively displacing $T_3$ from the receptor and by recruiting a corepressor to the thyroid receptor, thus suppressing activation of transcription of thyroid hormone-regulated genes (4). Zoeller and colleagues (72) have also reported antithyroid effects of BPA *in vivo* in rats.

## Sources and Levels of Exposure of Animals and Humans to BPA

A critical issue with regard to EDCs that are components of commonly used products is whether or not it is likely that normal use of the products will result in exposure of humans and/or wildlife because of manufacturing practices, degradation of products after disposal, or leaching from the products during use. The large and rapidly expanding literature on BPA reveals that there are a multitude of sources of exposure to this very-high-volume chemical that is now ubiquitous in our environment. For example, BPA accounts for the majority of estrogenic activity detected leaching out of landfills in both the United States and Japan (73, 74).

There appear to be extensive sources of exposure to BPA (22). For example, it is known that human exposure to BPA occurs as a result of the use of BPA to manufacture food-storage containers, water bottles, baby bottles, and the resin lining of food and beverage cans and in dental sealants. Exposure from these products thus accounts for a portion of the high levels of BPA being reported in human blood and tissues in multiple studies. These published findings contrast dramatically with assurances by chemical manufacturers and their surrogates that there is little human exposure to BPA (75–77), which has led to critiques of these claims (22, 35, 44, 78). That there is significant exposure to BPA reported in all regions of the world so far examined is not surprising, given that production capacity for this chemical exceeds 6 billion pounds per year (1).

An important aspect of effects seen in animals within the low-dose range (reviewed in Ref. 22) is that the doses used are within the range of human exposure based on the rate of leaching of BPA from food and beverage containers, other polycarbonate products, and some dental sealants (14–18, 79–84) and as a result of the presence of BPA in rivers and streams (85), in drinking water (86), and in indoor air (87, 88). The consequence of this exposure is that the concentration of unconjugated BPA in men and nonpregnant women, as well as pregnant women and their fetuses, is higher than any chemical industry-sponsored models of exposure predict (75, 77).

The doses of BPA used in the experimental studies described here are within the range of exposure to BPA of laboratory animals housed in polycarbonate cages or provided water in polycarbonate water bottles (16, 17, 89), and these biologically active exposures result in blood levels of BPA (90) that are exceeded by the range currently detected in human adult or fetal blood (Table 1). Given these findings, researchers must be careful to avoid the use of polycarbonate products in studies of the effects of BPA or other estrogenic chemicals. It is also important to avoid the use of polycarbonate or polystyrene products that can leach BPA or other estrogenic additives, such as nonylphenol (14, 91). In our animal studies, for example, we use polypropylene cages and glass water bottles, the drinking water is purified by reverse osmosis and carbon filtration, water flows through copper pipes, and we avoid the use of polycarbonate and modified polystyrene products in our cell culture studies.

### *Human exposure to BPA in relation to adverse effects in animal experiments*

Although it has been maintained that there is little human exposure to BPA from plastics in ordinary use, circulating levels of unconjugated BPA in humans have been reported since 1999 (Table 1). BPA determinations in human serum require sensitive methods with detection limits of less than 1 ng/ml (<1 ppb). This is because 1) the circulating levels of the unconjugated, biologically active chemical in blood of animals responding to low-dose exposures falls in the low picogram through low nanogram per milliliter range, based on low-dose animal studies (90), and 2) specific biological actions of BPA in cell culture have been reported in and below this same low nanogram per milliliter to picogram per milliliter range (Table 2). Therefore, methods to detect environmentally relevant exposures must be sensitive within this range. There have been studies that report no human exposure using insensitive methods of detection (92), with sensitivities in extraction media surveyed limited to 100 ppb (93). Standard UV or fluorescence detection can be limited in serum to 10–150 ng/ml (94), and these older detection techniques are subject to sensitivity limits 200- to 3000-fold weaker than modern techniques (10, 94). Therefore, older studies with poor sensitivity that claimed that there was no human exposure to BPA are not relevant to current understanding of the specific high activities of BPA on many end points within the range of human exposure being detected by current methods (Table 2).

Over a dozen published studies (Table 1) meet or exceed

**TABLE 1.** Published papers reporting BPA exposure in human serum and tissues

| Authors (Ref.) | Year | Detection method (sensitivity in end point) | Sensitivity (ng/ml) | End point(s) | Levels found [ng/ml (ppb), mean ± SEM] | Unit if not ng/ml | Other chemicals examined |
|---|---|---|---|---|---|---|---|
| Sajiki *et al.* (95) | 1999 | Electrochemical detection | 0.2 | Healthy human serum | 0–1.6 | | |
| | | MS/ESI | 0.1 | | | | |
| Inoue *et al.* (94) | 2000 | Electrochemical detection | 0.01 in solvent | Healthy human serum | 0.32 | | |
| | | Coulometric array | 0.05 in serum | | | | |
| Ikezuki *et al.* (96) | 2002 | ELISA | 0.3 in serum (11) | Female nonpregnant serum | 2.0 ± 0.8 | | |
| | | | | Early pregnancy serum | 1.5 ± 1.2 | | |
| | | | | Late pregnancy serum | 1.4 ± 0.9 | | |
| | | | | Fetal (cord) serum | 2.2 ± 1.8 | | |
| | | | | Amniotic fluid (15–18 wk) | 8.3 ± 8.9 | | |
| | | | | Late amniotic fluid | 1.1 ± 1.0 | | |
| | | | | Follicular fluid | 2.4 ± 0.8 | | |
| Schonfelder *et al.* (10) | 2002 | Derivatization-GC/MS | 0.01 in serum | Fetal cord serum | 0.2–9.2 | ng/g tissue | |
| | | | | Maternal serum | 0.3–18.9 | | |
| | | | | Placenta | 1.0–104.9 | | |
| Takeuchi and Tsutsumi (11) | 2002 | ELISA | 0.3 in serum | Normal male serum | 1.49 ± 0.11[a] | | Total and free testosterone |
| | | | | PCOS female serum | 1.04 ± 0.1[a] | | |
| | | | | Normal female serum | 0.64 ± 0.1 | | |
| Todaka and Mori (126) | 2002 | GC-MS | ? | Umbilical cords at birth | Mean, 4.4 ± 1.5; range, 0.11–15.2 | ng/g tissue | |
| Yamada *et al.* (127) | 2002 | ELISA | 0.5 | Normal maternal serum | 2.24[a] | | |
| | | | | Normal fetal amniotic fluid | 0.26 | | |
| | | | | Abnormal fetal karyotype maternal serum | 2.97[a] | | |
| | | | | Abnormal fetal karyotype fetal amniotic fluid | 0 | | |
| Kuroda *et al.* (128) | 2003 | Fluorescence derivatization, column switching | 0.04 | Maternal serum | 0.46 ± 0.20 | | |
| | | | | Fetal cord serum | 0.62 ± 0.13 | | |
| | | | | Sterility female serum | 0.46 ± 0.20 | | |
| | | | | Ascitic (peritoneal) fluid | 0.56 ± 0.19[a] | | |
| Tan and Mohd (129) | 2003 | GC-MS | 0.05 | Fetal cord plasma | ND to 4.05 (88% of samples with positive detection) | | Nonylphenol pesticides, other alkylphenols |
| Hiroi *et al.* (103) | 2004 | ELISA | 0.5 [from Kodaira *et al.* (29)] | Healthy control women, normal endometrium | 2.5 ± 1.5 | | |
| | | | | Simple endometrial hyperplasia, benign | 2.9 ± 2.0 | | |
| | | | | Complex endometrial hyperplasia, malignant potential | 1.4 ± 0.4[a] | | |
| | | | | Postmenopausal endometrial cancer | 1.4 ± 0.5[a] | | |
| Sun *et al.* (130) | 2004 | DIB-Cl derivatization/HPLC | 0.11 | Breast milk | 0.61 ± 0.2 | | |
| Takeuchi *et al.* (101) | 2004 | ELISA | 0.3 in serum (11) | Serum | | | Total and free testosterone, DHEAS, and androstenedione |
| | | | | Nonobese normal | 0.71 ± 0.09 | | |
| | | | | Nonobese PCOS | 1.05 ± 0.10[a] | | |
| | | | | Obese normal | 1.04 ± 0.09[a] | | |
| | | | | Obese PCOS | 1.17 ± 0.16[a] | | |
| Engel *et al.* (131) | 2005 | HPLC/electrochemical detection | 0.5 | Residual amniotic fluid from amniocentesis, <20 wk gestation | 0.55 (10% > 0.5 ng/ml) | | Enterolactone, daidzein, and genistein |
| Sugiura-Ogasawara *et al.* (102) | 2005 | ELISA | 0.5 [from Kodaira *et al.* (29)] | Serum | | | Antinuclear antibodies |
| | | | | Control healthy women | 0.77 ± 0.38 | | |
| | | | | Recurrent miscarriage women | 2.59 ± 5.23[a] | | |

DHEAS, Dehydroepiandrosterone sulfate; ESI, electrospray ionization; ND, none detected; DIB-Cl, 4-(4,5-diphenyl-1H-imidazol-2-yl)benzoyl chloride; PCOS, polycystic ovary syndrome.

[a] Statistically different from unmarked results within the group at $P < 0.05$.

**TABLE 2.** Published papers reporting biological effects *in vitro* for BPA in the low-dose exposure range

| Authors | Year | Animal | Gender | End points | Exposure, vehicle | Doses tested [ng/ml (ppb)] | Other chemicals tested |
|---|---|---|---|---|---|---|---|
| Steinmetz *et al.* (68) (also *in vivo*) | 1997 | Rat | Female | $GH_3$/*luc* cell prolactin gene expression in $GH_3$ pituitary tumor cells | Cell culture medium | 0.23[a] | Estradiol |
| Gupta (38) (also *in vivo*) | 2000 | Mouse | Male | Prostate weight and prostate AR | Organ culture medium | 0.005, 0.05[a] | DES |
| Takai *et al.* (132) | 2000 | Mouse | Female/male | Rate of embryo development | Oocyte culture medium | 0.023, 0.23,[a] 0.684,[a] 23,000[a] | Inhibited by antiestrogen tamoxifen |
| Takai *et al.* (133) | 2000 | Mouse | Female/male | Embryonic exposure/postnatal growth | Oocyte culture medium | 0.23[a], 23,000[a] | |
| Chun and Gorski (134) | 2000 | Rat | Female Fischer 344 | PR1 cell prolactin secretion<br>Cell proliferation | Culture medium | 2.3,[b] 23,[b] 230[b]<br>$EC_{50}$ 2.3 | Estradiol and DES |
| Moriyama *et al.* (4) | 2002 | Human | | Recruitment of thyroid hormone receptor corepressor | Organ culture medium | 23,[a] 230[a] | Thyroid hormone |
| Quesada *et al.* (55) | 2002 | Mouse | | Activation of CREB in pancreatic islet cells | Cell culture medium | 0.23[a] | Estradiol |
| Sato *et al.* (54) | 2002 | Rat | | Glutamate-induced neuronal damage | Organ culture medium | 0.00023,[a] 0.23,[a] 230,[a] 23,000[a] | Estradiol, DES, ethinyl estradiol, and nonylphenol |
| | | | | NR1/NMDA-R expression | | 0.23[a] | |
| | | | | Spine density | | 0.23[a] | |
| | | | | Mossy fiber sprouting | | 0.23[a] | |
| Song *et al.* (71) | 2002 | Mouse | Male | Leydig cell Nur77 gene expression | Organ culture medium | 0.23[b] to 230[b] | Bisphenol F, -M, -S, and -Z |
| Wetherill *et al.* (6) | 2002 | Human | Male | LNCaP cell proliferation<br>AR activation | Cell culture medium | 0.023 0.23,[b] 2.3[b]<br>230 | DHT |
| Cappelletti *et al.* (135) | 2003 | Human | Female T47D and BT20 breast cancer cells | Up-regulation of ERα and ERβ in T47D cells | Cell culture medium | 2.3,[a] 23,[a] 230,[a] 2300[a] | Estradiol, octylphenol, and 2- and 4-hydroxybiphenyl |
| | | | | Up-regulation of ERα in BT20 cells | | 23,[a] 230,[a] 2300[a] | |
| | | | | Modulation of PgR expression in T47D cells | | 2.3,[a] 23,[a] 230,[a] 2300[a] | |
| Lee *et al.* (5) | 2003 | Human | Female HeLa cells transfected with mouse AR | Inhibition of binding of 5α-DHT to mouse AR | Cell culture medium | 11.4[b] | Testosterone, DHT, CPA, and nonylphenol |
| Negishi *et al.* (136) | 2003 | Rat | | Primary rat hippocampal and cortical neurons; inhibition of caspase-3 increase | Cell culture medium | 2.3[a] | Estradiol and nonylphenol |
| Sawai *et al.* (137) (also *in vivo*) | 2003 | Mice | Female | Inhibition of ConA-induced secretion of IFNγ by splenic mononuclear cells | Cell culture medium | 0.114, 11.4,[a] 1140[a] | |
| Akingbemi *et al.* (117) (also *in vivo*) | 2004 | Rat | Male | Decreased Leydig cell testosterone synthesis | Cell culture medium | 0.0023,[a] 0.023, 0.23, 2.3, 23, 230 | DES and HPTE |
| | | | | Estradiol production and aromatase activity | | 0.0023[a] | |
| Canesi *et al.* (138) (also aquatic) | 2005 | Mussel | | Lysosomal membrane destabilization<br>MAPK and STAT CREB-like transcription factor | Injection | 5.7[a] ng/ml in hemolymph | |
| Walsh *et al.* (56) | 2005 | Human | Female breast cancer cells MCF-7, MDA-MB-231, and SKBR-3 | Intracellular $Ca^{2+}$ increase in MCF-7 cells | Cell culture medium | 0.023,[a] 0.23,[a] 2.3,[a] 23[a] | Estradiol, DES, DDT, and octylphenol |
| | | | | In MDA-MB-231 and SKBR-3 cells | | 0.023,[a] 0.23,[a] 2.3,[a] 23[a]<br>$EC_{50}$ 0.0342 | |
| Wetherill *et al.* (139) | 2005 | Human | Male LNCaP prostate cancer cells | AR activation<br>Enhanced DHT-stimulated transcription<br>Induction of prostate-specific antigen | Cell culture medium | 2.3,[b] 230,[b] 2300[b]<br>2.3,[b] 230,[b] 2300[b]<br>0.23,[b] 2300[b] | Dihydrotestosterone |
| Wozniak *et al.* (9) | 2005 | Rat | Female $GH_3$ pituitary tumor cell line | Rapid $Ca^{2+}$ influx<br>Prolactin release | Cell culture medium | 0.00023,[a] 0.23,[a] 2.3[a]<br>0.00023[a] | Estradiol, DES, coumestrol, nonylphenol, dieldrin, DDE, and endosulfan |
| Yanagihara *et al.* (140) | 2005 | Bovine | Primary adrenal medullary cells | Catecholamine synthesis<br>Tyrosine hydroxylase activity | Cell culture medium | 2.3[a]<br>2.3[a] | Estradiol and nonylphenol |

Low-dose range for *in vitro* studies was less than 50 ng/ml (<50 ppb; <219 nM; 1 nM = 0.228 ng/ml) in organ or tissue culture medium, the estimated circulating level after dosing at the defined cutoff for low-dose animal exposure of 50 mg/kg body weight *in vivo*. AR, Androgen receptor; ConA, concanavalin A; CREB, cAMP response element-binding protein; DHT, dihydrotestosterone; INFγ, interferon-γ; PgR, progesterone receptor; CPA, cyproterone acetate; HPTE, 2,2-bis(p-hydroxyphenyl)-1,1,1-trichloroethane; DDT, 2,2-bis(4-chlorophenyl)-1,1,1-trichloroethane; DDE, 1,1-dichloro-2,2-bis(4-chlorophenyl)-ethene; STAT, signal transducers and activators of transcription.

[a] $P < 0.05$ compared with control.

[b] Indicated as responsive in the publication.

the detection limit above, and, not accidentally, these are the studies that report detection of BPA in human serum and tissues in most or all samples. Beginning in 1999 (95), a number of investigators using a variety of different techniques have reported human circulating levels of unconjugated parent BPA ranging from 0.2–20 ng/ml serum and exceeding 100 ng/g tissue in the placenta. A number of these studies are detailed in Table 1, where the authors, the analytical technique, the sensitivity of the specific technique in application for BPA in human samples, and the resulting levels observed have been compiled, although this is not a comprehensive list.

The techniques used to measure BPA include gas chromatography mass spectrometry (GC-MS), derivatization with at least three different chemical moieties, and ELISA, all with sensitivities for BPA (in serum) ranging from 0.01–0.5 ng/ml. Several of these publications also detailed the precautions that must be undertaken to prevent contamination to achieve the level of detection required for human samples (94, 95); contaminations by BPA appear almost ubiquitous in many lab plastics and may indicate sources of human exposures leading to the high tissue levels being detected. Human exposures are most likely through the oral route, although transdermal exposure by bathing in BPA-contaminated water is also of concern, as is exposure via inhalation; both of these latter routes of exposure would escape the extensive first-pass conjugation that occurs with oral administration.

Of particular concern are the high levels of BPA detected in many studies in fetal cord serum, maternal serum during pregnancy, and fetal amniotic fluid at developmental stages of perhaps greatest sensitivity to BPA (10). In one report (96), the human maternal sera showed average BPA at 1.4–2.4 ng/ml levels, whereas the 15- to 18-wk fetal amniotic fluid showed higher levels averaging 8.3 ng/ml. The highest level of fetal exposure thus unfortunately coincided with the period of greatest fetal sensitivity to disruption of development by estrogenic chemicals (97).

In complementary studies, BPA has been measured in human urine (13, 98–100), which confirms wide human exposure to BPA; the study conducted by the CDC found BPA in 95% of samples at levels greater than 0.1 $\mu$g/liter urine (>0.1 ng/ml urine) (13) and concluded that "the frequent detection of BPA suggests widespread exposure to this compound in residents of the United States." Based on the studies detecting BPA in human serum above, many as yet unidentified sources may contribute to the total body burden. It is not unexpected that the range, median, and mean for BPA in urine reported in the CDC study would be very similar to these statistics reported in human blood (10, 11, 96).

Current metabolic data in animals are based on single-dose exposures, whereas human exposure data follow what appear to be long-term, steady-state exposures. In fact, an approach to administering BPA that may best mimic what appears to be tonic exposure by humans is by administration via SILASTIC brand silicon tubing or osmotic pumps (65). Based on the available human exposure data, we predict that there are already extensive human biological actions of BPA within the range of current human exposures (Fig. 2). Clearly, research is urgently needed on repeated-exposure

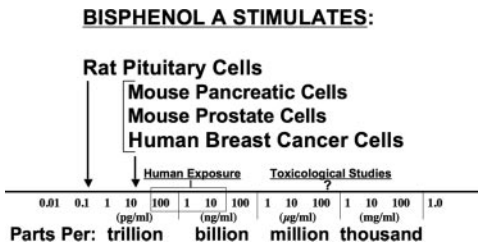


FIG. 2. Schematic diagram showing the bioactive (parts per trillion) range in culture medium for most sensitive reported effects of BPA in rat, mouse, and human tissues in relation to the parts-per-trillion to parts-per-billion range for unconjugated (parent) BPA in human blood determined in many studies (Table 1). The detection of BPA in virtually all people within the relatively high 0.1- to 10-ppb range suggests multiple exposures from many sources is occurring, which would be predicted for a chemical that is produced in excess of 6 billion pounds per year for use in a wide range of products. Studies involving continuous exposure (64) or multiple exposures per day are required to address this data gap, whereas it is common in a traditional toxicological animal study conducted for regulatory purposes to administer one dose per day and to examine only a few very high doses.

data in animal studies and to evaluate the most likely sources of a level of human exposure from the products manufactured by over 6 billion pounds per year of BPA. We have described in detail elsewhere (35) that even in humans in the lowest fifth percentile of exposure to BPA (~0.1 ppb), the amount of BPA detected in blood, urine, and other tissues exceeds adverse-effect levels in a multitude of animal studies.

### *Epidemiological studies of BPA*

Only recently have the first epidemiological studies been published concerning the relationship of blood levels of BPA and disease in humans. There is a relationship between blood levels of BPA, obesity, polycystic ovary syndrome, and circulating androgens (11, 101) as well as BPA and repeated miscarriage (102). There is an inverse relationship between serum BPA and endometrial hyperplasia in women (103). Additional epidemiological studies are clearly warranted based on the extensive literature that now exists for adverse effects of BPA in animals at very low doses.

### *BPA distribution in mother and transport to the fetus and neonate*

Although the fetus is acutely sensitive to chemical derangement of development, the fetus had initially been presumed to be protected by the placental barrier between the maternal circulation and the fetal circulation. However, recent work has shown that BPA has rapid access to the fetus after maternal exposure, facilitated by accidents of metabolism that may lead to increased levels of BPA in the pregnant female as well as accumulation in the fetus. Specifically, BPA is retained in the fetal circulation and increases between 12 and 50 h after a single dose to pregnant rats (104, 105), which suggests a depot or an enterohepatic circulation of BPA and that multiple doses may result in accumulation of circulating levels. However, even in acute single exposures, BPA accumulates in the fetus, such that fetal levels exceed maternal levels by only 40 min after the maternal dose (105). BPA levels in the fetus above maternal plasma levels are thus

achieved (104, 105). Interestingly, the appropriateness of DES as a positive control for experiments involving exposure during fetal life to BPA is supported by the finding that DES accumulates in the fetal circulation at higher levels than in the treated mother, with similar time course and kinetics when compared with BPA (37, 104, 105).

Yoo *et al.* (106) have also described BPA in milk of lactating Sprague Dawley rats. They found in an infusion study (to obtain a steady-state circulating level of the chemical) that BPA was present in milk at 2.4–2.7 times the level in maternal serum, indicating that BPA accumulates in breast milk and that transfer of BPA from an exposed mother thus continues to the pup after birth through weaning.

A study was conducted that directly measured BPA levels in pregnant mice and fetuses after administration of a low, environmentally relevant dose of radiolabeled BPA (90). Zalko and colleagues (90) injected pregnant CD-1 mice sc on gestation d 17 with a 25 $\mu$g/kg dose of tritiated BPA. Parent (unconjugated) BPA levels in fetuses at 0.5, 2, and 24 h after administration were 4.20, 0.48, and 0.13 ng/g, respectively. In addition, we have measured 10 and 100 pg/ml in our pregnant mice at biologically active low doses of BPA of 2 and 20 $\mu$g/kg (Welshons, W. V., and J. A. Taylor, unpublished observation). Numerous published findings have shown that these doses (and lower doses) of BPA administered to pregnant mice and rats caused permanent changes in reproductive organs of male and female offspring (reviewed in Ref. 22).

A critical aspect of the findings reported by Zalko is that it has been reported that the levels of unconjugated BPA in human fetal serum collected at parturition is in the range of 0.1–10 ng/ml (0.1–10 ppb), and the mean BPA concentration in human male fetuses is 3.5 ng/ml (10). The levels of BPA found in human fetuses are thus similar to levels in fetal mice shortly after pregnant mice are exposed to BPA (in the low parts per billion range). Between 2 and 24 h after treatment of pregnant mice with this very low dose of BPA, human fetal levels exceed the levels in fetal mice.

Significant effects caused in rats and mice by exposure during development to doses of BPA at and below the level studied by Zalko include structural and neurochemical changes throughout the brain associated with behavioral changes, such as hyperactivity, learning deficits, increased aggression, and increased likelihood of drug dependency; abnormalities in sperm production in males and oocytes and meiosis in females; disruption of hormone production; changes in nuclear superfamily gene expression, including aryl hydride receptor, RAR and RXR; changes in all reproductive organs and in fertility in both males and females; immune disorders; increased growth rate; and early sexual maturation (reviewed in Ref. 35). A document is posted on the web that is periodically updated that contains a comprehensive list of references concerning research conducted with BPA (23).

The appearance of BPA in the mouse fetus within the range of 0.1–5 ng/g (parts per billion) after maternal administration of a 25 $\mu$g/kg dose indicates substantial transport through the placenta and thus significant fetal exposure after maternal exposure to a very low dose of BPA (90). Maternal levels of unconjugated, bioavailable BPA were also measured directly in this experiment. Maternal blood levels of parent BPA were 1.06 and 0.15 ng/ml at 0.5 and 3 h after exposure, respectively, whereas the long-term residual exposure at 24 h was below the quantifiable level. Zalko and colleagues (90) reported that there was significant variability within and between replicate experiments, which may indicate substantial individual variation in metabolism of BPA. Our own findings show that individual CD-1 pregnant mice differ by as much as 20-fold in the levels of BPA detected during the 24 h after oral administration of a 20 $\mu$g/kg dose (Welshons, W. V., and J. A. Taylor, unpublished observation).

### *Metabolism of BPA*

The main metabolism of BPA is to the monoglucuronide for excretion, although smaller amounts of other conjugates are also produced. BPA glucuronide itself does not show estrogenic activity based on several studies (8, 90, 107), so only unconjugated BPA is considered bioavailable and biologically active, although there is now evidence that some metabolites of BPA are as much as 250-fold more potent than parent BPA (108). Glucuronidation enzymes are hepatic UDP-glucuronosyltransferases, specifically the BPA-active isoform UGT2B1 (109). Interestingly, Matsumoto *et al.* (109) have reported that this UGT2B1 enzymatic activity with BPA as the substrate is reduced in the pregnant mother compared with the nonpregnant female rat. The enzyme is absent in the rat fetus, and activity appears slowly after birth, with nonpregnant adult levels attained by 3 wk. Therefore, the pregnant mother, the fetus, and the newborn rat pup may show heightened sensitivity to BPA because of a reduced rate of clearance and increased half-life of unconjugated, estrogenically active BPA. There are similar data for DES, which is commonly used as a positive control in studies of the estrogenic activity of BPA. In a study of rats from birth to 50 d of age, the clearance of [$^{14}$C]DES increased by 10-fold between birth and 25 d of age. Intestinal hydrolysis of DES conjugates was minimal at birth (because of the lack of intestinal bacteria) but fully developed by 25 d of age, and there was also a deficiency in liver enzymes required for conjugation in newborn rats (110).

BPA has also been reported to interact with drug- and steroid-metabolizing activity of rat (111), pig (112), and human hepatic cytochrome P450s (113, 114) by inhibition of activities, although these were measured at high micromolar concentrations of BPA. For example, testosterone 16$\beta$-hydroxylase and testosterone 2$\alpha$-hydroxylase activities were inhibited by BPA at 100 $\mu$M (69 and 74%, respectively) (111). UDP-glucuronosyltransferase activities toward BPA and testosterone as well as estradiol were significantly decreased in liver microsomes prepared from adult male Wistar rats administered a 1 mg/kg dose of BPA (115). Whether inhibition of metabolizing enzymes may act to modulate BPA exposures at environmentally relevant doses remains to be determined. However, there are a number of studies that report higher blood levels of BPA associated with elevated levels of testosterone in humans (10, 11, 101). This is quite interesting in that exposure to very low doses of BPA during development (116, 117) or after weaning (118, 119) results in a decrease in testosterone in male rats and mice. *In vitro* studies

indicate that this occurs via an inhibition by BPA of the androgen-synthesizing enzyme 17$\alpha$-hydroxylase in both rats (117) and humans (114).

## Implications for Public Health

The findings summarized in Fig. 2 show that BPA stimulates human and rodent tissues at concentrations below those detected in human blood. This has to be viewed from the perspective that unconjugated BPA in blood shows very limited binding to SHBG and thus a higher free (~8%) concentration in blood relative to estradiol (34); most BPA would thus be bound only weakly to albumin in blood. The consistent finding that unconjugated BPA is detected in virtually everyone at biologically active concentrations implies virtually continuous exposure, based on the assumption that there is relatively rapid metabolism, although metabolism after exposure via respiration or dermal contact would not be as rapid as after oral exposure. However, we do not have good information about all of the ways that the over 6 billion pounds of BPA produced annually can enter the human body, and no data exist from studies that provide pharmacokinetic information about the levels of BPA in humans or experimental animals as a result of multiple exposures throughout the day.

It is well understood in endocrinology that doses millions of times higher than the physiologically relevant range of a hormone do not produce effects predictive of what would be seen within the physiologically relevant dose range. In contrast, in addressing this issue for the toxicological community and regulatory agencies such as the U.S. EPA and FDA, the response by chemical industry trade organizations has been to reject the possibility of nonmonotonic dose-response curves and that effects could occur at low doses that would not be predicted by experiments that examined only a few very high doses. For example, the Association of Plastics Manufacturers in Europe (APM) stated that "the fundamental principle of toxicology assumes that biological effects increase as the dose increases" (77) (see also the response to this commentary in Ref. 78). It is disturbing to discover that this statement by the APM is actually a fundamental assumption upon which regulatory agencies around the world have designed the methods used to test for the hazards caused by exposure to EDCs in commerce. The assumptions that form the basis for chemical risk assessments have led to the use of experiments that examine only very high doses of chemicals, based on the assumption that all dose-response curves for environmental chemicals are monotonic. The results from these high-dose toxicity studies are then used by regulators to predict doses of chemicals, such as BPA, that the public is assured are safe, even though the putative safe doses are never directly tested in experiments. This process has remained in place even though scientists studying EDCs have been warning regulators at the U.S. EPA and FDA over the last decade that this approach is falsified as a basis for estimating acceptable human exposures to EDCs by thousands of published findings by endocrinologists concerning hormone action (22, 120).

In experiments with hormones, drugs, and other chemicals that act via hormonal, receptor-mediated mechanisms, it is very common for the dose-response curve to be nonmonotonic and form an inverted U, which endocrinologists typically refer to as a biphasic dose-response curve. In contrast, there appears to be a lack of awareness of this phenomenon in toxicology, because many toxicological studies in which effects occur only in a restricted low-dose range, although the effect is not seen at lower or higher doses, conclude that there was no relationship between dose and response. For example, in referring to the results of a study by Gupta (38) in which a low and high dose of DES were tested and stimulating effects on the prostate occurred at the low dose and inhibition of prostate development occurred at the high dose, Tyl (121) stated that "the effects of DES (the only chemical tested at more than one dose) were not dose related, with greater effects at 0.1 microgram/kg/day than at 200 micrograms/kg/day."

There are thus toxicologists that consider only monotonic dose-response relationships to be valid, although Gupta's replication of our inverted-U dose-response relationship for DES and prostate development, where very low doses stimulate growth and very high doses completely inhibit development (33, 41), is essentially regarded as indicating no effect of DES on the prostate. This simplistic approach would eliminate virtually all hormones from consideration by regulatory agencies as a concern for public health, because there is evidence from the endocrine literature of nonmonotonic effects for virtually all hormones (7). A common, but inappropriate, response to nonmonotonic dose-response relationships in toxicological studies is thus to declare that because no monotonic dose-response relationship was observed, any effects caused by the low doses can be ignored. Depending on the response being examined, the dose-response relationship may or may not be nonmonotonic, but the fact that nonmonotonic dose-response relationships do commonly occur in endocrinology has not been incorporated into the process of assessing risk of exposure to environmental chemicals that disrupt the endocrine system.

For studies examining the biological effects of BPA, there are currently 18 published reports of inverted-U dose-response curves (23). One recent example is in rat pituitary tumor cells, where BPA significantly stimulated a rapid (within 30 sec) influx of calcium at a dose of 1 pM; the greatest response occurred at 1 nM, although the magnitude of the response decreased at 10 nM. The calcium influx response to BPA at 1 nM was actually greater than that for estradiol or DES (9). Other examples of unique low-dose effects not predicted by high-dose studies are 1) the finding of greater reduction in fertility in mice at low relative to high doses of pesticides (122), 2) increased DNA damage at low relative to high doses of x-rays (123), and 3) stimulation of human prostate cancer cell growth by low but not high doses of BPA (6). Now that studies of BPA and other chemicals are incorporating a much wider dose range than had traditionally been used (24, 124, 125), unpredicted, unique low-dose effects are being routinely reported.

In summary, current analytical techniques have detected BPA in over 95% of human samples (including serum, tissues, and urine). The techniques include GC-MS, tandem mass spectrometry, coulometric array electrochemical detection, derivatization techniques with multiple different moieties, and ELISA.

These different techniques all detect BPA in human tissues in similar nanogram per milliliter ranges and indicate widespread exposure. This is of particular concern with regard to the levels of BPA being reported in pregnant women and fetuses. The proposed rapid clearance of BPA described in a number of chemical industry-sponsored studies, combined with the high, nanogram per milliliter ranges reported in human circulation and urine by government-funded studies, would indicate 1) that the human intake of BPA is actually very much higher than is calculated by industry estimates and/or 2) that a daily long-term intake of BPA leads to a bioaccumulation and steady-state levels that are not represented in any current pharmacokinetic models for BPA. Taken together, these findings provide a clear basis for an immediate reevaluation by the U.S. EPA and FDA of current estimates of safe daily exposure levels of BPA. A new risk assessment is required that incorporates current published findings, because the current safe exposure levels are based on research conducted in the 1980s (20).

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## References

1. **Burridge E** April 14–20, 2003 Bisphenol A: product profile. Eur Chem News; 17
2. **Meerts IATM, Letcher RJ, Hoving S, Marsh G, Bergman A, Lemmen JG, van der Burg B, Brouwer A** 2001 In vitro estrogenicity of polybrominated diphenyl ethers, hydroxylated PBDEs and polybrominated bisphenol A compounds. Environ Health Perspect 109:399–407
3. **Dodds EC, Lawson W** 1936 Synthetic oestrogenic agents without the phenanthrene nucleus. Nature 137:996
4. **Moriyama K, Tagami T, Akamizu T, Usui T, Saljo M, Kanamoto N, Hataya Y, Shimatsu A, Kuzuya H, Nakao K** 2002 Thyroid hormone action is disrupted by bisphenol A as an antagonist. J Clin Endocrinol Metab 87:5185–5190
5. **Lee HJ, Chattopadhyay S, Gong EY, Ahn RS, Lee K** 2003 Antiandrogenic effects of bisphenol A and nonylphenol on the function of androgen receptor. Toxicol Sci 75:40–46
6. **Wetherill YB, Petra CE, Monk KR, Puga A, Knudsen KE** 2002 The xenoestrogen bisphenol A induces inappropriate androgen receptor activation and mitogenesis in prostate adenocarcinoma cells. Mol Cancer Ther 7:515–524
7. **Welshons WV, Thayer KA, Judy BM, Taylor JA, Curran EM, vom Saal FS** 2003 Large effects from small exposures. I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity. Environ Health Perspect 111:994–1006
8. **Snyder RW, Maness SC, Guido KW, Welsch F, Summer SCJ, Fennell TR** 2000 Metabolism and disposition of bisphenol A in female rats. Toxicol Appl Pharmacol 168:225–234
9. **Wozniak AL, Bulayeva NN, Watson CS** 2005 Xenoestrogens at picomolar to nanomolar concentrations trigger membrane estrogen receptor-a mediated $Ca^{2+}$ fluxes and prolactin release in GH3/B6 pituitary tumor cells. Environ Health Perspect 113:431–439
10. **Schonfelder G, Wittfoht W, Hopp H, Talsness CE, Paul M, Chahoud I** 2002 Parent bisphenol A accumulation in human maternal-fetal-placental unit. Environ Health Perspect 110:A703–A707
11. **Takeuchi T, Tsutsumi O** 2002 Serum bisphenol A concentrations showed gender differences, possibly linked to androgen levels. Biochem Biophys Res Commun 291:76–78
12. **Domoradzki JY, Pottenger LH, Thornton CM, Hansen SC, Card TL, Markham DA, Dryzga MD, Shiotsuka RN, Waechter Jr JM** 2003 Metabolism and pharmacokinetics of bisphenol A (BPA) and the embryo-fetal distribution of BPA and BPA-monoglucuronide in CD Sprague-Dawley rats at three gestational stages. Toxicol Sci 76:21–34
13. **Calafat AM, Kuklenyik Z, Reidy JA, Caudill SP, Ekong J, Needham LL** 2005 Urinary concentrations of bisphenol A and 4-nonyl phenol in a human reference population. Environ Health Perspect 113:391–395
14. **Krishnan AV, Stathis P, Permuth SF, Tokes L, Feldman D** 1993 Bisphenol-A: an estrogenic substance is released from polycarbonate flasks during autoclaving. Endocrinology 132:2279–2286
15. **Brotons JA, Olea-Serrano MF, Villalobos M, Pedraza V, Olea N** 1995 Xenoestrogens released from lacquer coating in food cans. Environ Health Perspect 103:608–612
16. **Hunt PA, Koehler KE, Susiarjo M, Hodges CA, Hagan A, Voigt RC, Thomas S, Thomas BF, Hassold TJ** 2003 Bisphenol A causes meiotic aneuploidy in the female mouse. Curr Biol 13:546–553
17. **Howdeshell KL, Peterman PH, Judy BM, Taylor JA, Orazio CE, Ruhlen RL, vom Saal FS, Welshons WV** 2003 Bisphenol A is released from used polycarbonate animal cages into water at room temperature. Environ Health Perspect 111:1180–1187
18. **Kang JH, Kito K, Kondo F** 2003 Factors influencing the migration of bisphenol A from cans. J Food Prot 66:1444–1447
19. **Morrissey RE, George JD, Price CJ, Tyl RW, Marr MC, Kimmel CA** 1987 The developmental toxicity of bisphenol A in rats and mice. Fund Appl Toxicol 8:571–582
20. **IRIS** 1988 Bisphenol A (CASRN 80-05-7). In: US-EPA Integrated Risk Information System Substance file. http://www.epa.gov/iris/subst/0356.htm
21. **Nagel SC, vom Saal FS, Thayer KA, Dhar MG, Boechler M, Welshons WV** 1997 Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative in vivo bioactivity of the xenoestrogens bisphenol A and octylphenol. Environ Health Perspect 105:70–76
22. **vom Saal FS, Hughes C** 2005 An extensive new literature concerning low-dose effects of bisphenol A shows the need for a new risk assessment. Environ Health Perspect 113:926–933
23. **vom Saal FS** 2006 Bisphenol A references: list of published articles. In: http://endocrinedisruptors.missouri.edu/vomsaal/vomsaal.html
24. **Welshons WV, Nagel SC, Thayer KA, Judy BM, vom Saal FS** 1999 Low-dose bioactivity of xenoestrogens in animals: fetal exposure to low doses of methoxychlor and other xenoestrogens increases adult prostate size in mice. Toxicol Ind Health 15:12–25
25. **Routledge EJ, White R, Parker MG, Sumpter JP** 2000 Differential effects of xenoestrogens on coactivator recruitment by estrogen receptor (ER) $\alpha$ and ER$\beta$. J Biol Chem 46:35986–35993
26. **MacLusky NJ, Hajszan T, Leranth C** 2005 The environmental estrogen bisphenol A inhibits estrogen-induced hippocampal synaptogenesis. Environ Health Perspect 113:675–679
27. **Jordan VC, Murphy CS** 1990 Endocrine pharmacology of antiestrogens as antitumor agents. Endocr Rev 11:578–610
28. **Yang NN, Venugopalan M, Hardikar S, Glasebrook A** 1996 Identification of an estrogen response element activated by metabolites of 17$\beta$-estradiol and raloxifene. Science 273:1222–1225
29. **Kodaira T, Kato I, Li J, Mochizuki T, Hoshino M, Usuki Y, Oguri H, Yanaihara N** 2000 A novel ELISA for the measurement of immunoreactive bisphenol A. Biomed Res 21:117–121
30. **vom Saal FS** 1989 Sexual differentiation in litter-bearing mammals: influence of sex of adjacent fetuses in utero. J Anim Sci 67:1824–1840
31. **Nonneman DJ, Ganjam VK, Welshons WV, Vom Saal FS** 1992 Intrauterine position effects on steroid metabolism and steroid receptors of reproductive organs in male mice. Biol Reprod 47:723–729
32. **Montano MM, Welshons WV, vom Saal FS** 1995 Free estradiol in serum and brain uptake of estradiol during fetal and neonatal sexual differentiation in female rats. Biol Reprod 53:1198–1207
33. **vom Saal FS, Timms BG, Montano MM, Palanza P, Thayer KA, Nagel SC, Dhar MD, Ganjam VK, Parmigiani S, Welshons WV** 1997 Prostate enlargement in mice due to fetal exposure to low doses of estradiol or diethylstilbestrol and opposite effects at high doses. Proc Natl Acad Sci USA 94:2056–2061
34. **Nagel SC, vom Saal FS, Welshons WV** 1998 The effective free fraction of estradiol and xenoestrogens in human serum measured by whole cell uptake assays: physiology of delivery modifies estrogenic activity. Proc Soc Exp Biol Med 217:300–309
35. **vom Saal FS, Welshons WV** 2006 Large effects from small exposures. II. The importance of positive controls in low-dose research on bisphenol A. Environ Res 100:50–76
36. **National Toxicology Program** 2001 National Toxicology Program: Final Report of the Endocrine Disruptors Low-Dose Peer Review Panel. In: Endocrine Disruptors Low-Dose Peer Review. National Institute of Environment and Health Sciences: Raleigh, NC
37. **Shah HC, McLachlan JA** 1976 The fate of diethylstilbestrol in the pregnant mouse. J Pharmacol Exp Ther 197:687–696
38. **Gupta C** 2000 Reproductive malformation of the male offspring following maternal exposure to estrogenic chemicals. Proc Soc Exp Biol Med 224:61–68
39. **Honma S, Suzuki A, Buchanan DL, Katsu Y, Watanabe H, Iguchi T** 2002 Low dose effect of in utero exposure to bisphenol A and diethylstilbestrol on female mouse reproduction. Reprod Toxicol 16:117–122
40. **Nikaido Y, Yoshizawa K, Danbara N, Tsujita-Kyutoku M, Yuri T, Uehara N, Tsubura A** 2004 Effects of maternal xenoestrogen exposure on develop-

ment of the reproductive tract and mammary gland in female CD-1 mouse offspring. Reprod Toxicol 18:803–811
41. **Timms BG, Howdeshell KL, Barton L, Bradley S, Richter CA, vom Saal FS** 2005 Estrogenic chemicals in plastic and oral contraceptives disrupt development of the mouse prostate and urethra. Proc Natl Acad Sci USA 102:7014–7019
42. **Thayer KA, Ruhlen RL, Howdeshell KL, Buchanan D, Cooke PS, Welshons WV, vom Saal FS** 2001 Altered reproductive organs in male mice exposed prenatally to sub-clinical doses of 17$\alpha$-ethinyl estradiol. Hum Reprod 16:988–996
43. **vom Saal FS, Cooke PS, Buchanan DL, Palanza P, Thayer KA, Nagel SC, Parmigiani S, Welshons WV** 1998 A physiologically based approach to the study of bisphenol A and other estrogenic chemicals on the size of reproductive organs, daily sperm production, and behavior. Toxicol Ind Health 14:239–260
44. **vom Saal FS, Nagel SC, Timms BG, Welshons WV** 2005 Implications for human health of the extensive bisphenol A literature showing adverse effects at low doses: a response to attempts to mislead the public. Toxicology 212: 244–252
45. **Michaels D, Monforton C** 2005 Manufacturing uncertainty: contested science and the protection of the public's health and environment. Am J Public Health 95(Suppl 1):S39–S48
46. **Egilman DS, Bohme SR** 2005 Over a barrel: corporate corruption of science and its effects on workers and the environment. Int J Occup Environ Health 11:331–337
47. **Bohme SR, Zorabedian J, Egilman DS** 2005 Maximizing profit and endangering health: corporate strategies to avoid litigation and regulation. Int J Occup Environ Health 11:338–348
48. 2005 Scientific evidence and public policy. Am J Public Health 95(Suppl 1):1–150
49. 2005 Special issue: corporate corruption of science. Int J Occup Environ Health 11:331–460
50. **Michaels D** 2005 Doubt is their product. Sci Am 292:96–101
51. **Gould JC, Leonard LS, Maness SC, Wagner BL, K. C., Zacharewski T, Safe S, McDonnell DP, Gaido KW** 1998 Bisphenol A interacts with the estrogen receptor $\alpha$ in a distinct manner from estradiol. Mol Cell Endocrinol 142:203–214
52. **Kuiper GG, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S, Gustafsson JA** 1997 Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors $\alpha$ and $\beta$. Endocrinology 138:863–870
53. **Wade CB, Robinson S, Shapiro RA, Dorsa DM** 2001 Estrogen receptor (ER)$\alpha$ and ER$\beta$ exhibit unique pharmacological properties when coupled to activation of the mitogen-activated protein kinase pathway. Endocrinology 142: 2336–2342
54. **Sato K, Matsuki N, Ohno Y, Nakazzawa K** 2002 Effects of 17$\beta$-estradiol and xenoestrogens on the neuronal survival in an organotypic hippocampal culture. Neuroendocrinology 76:223–234
55. **Quesada I, Fuentes E, Viso-Leon MC, Soria B, Ripoll C, Nadal A** 2002 Low doses of the endocrine disruptor bisphenol-A and the native hormone 17$\beta$-estradiol rapidly activate transcription factor CREB. FASEB J 16:1671–1673
56. **Walsh DE, Dockery P, Doolan CM** 2005 Estrogen receptor independent rapid non-genomic effects of environmental estrogens on $[Ca^{2+}]_i$ in human breast cancer cells. Mol Cell Endocrinol 230:23–30
57. **Zsarnovszky A, Le HH, Wang HS, Belcher SM** 2005 Ontogeny of rapid estrogen-mediated extracellular signal-regulated kinase signaling in the rat cerebellar cortex: potent nongenomic agonist and endocrine disrupting activity of the xenoestrogen bisphenol A. Endocrinology 146:5388–5396
58. **Alonso-Magdalena P, Laribi O, Ropero AB, Fuentes E, Ripoll C, Soria B, Nadal A** 2005 Low doses of bisphenol A and diethylstilbestrol impair $Ca^{2+}$ signals in pancreatic $\alpha$-cells through a nonclassical membrane estrogen receptor within intact islets of Langerhans. Environ Health Perspect 113:969–977
59. **Alonso-Magdalena P, Morimoto S, Ripoll C, Fuentes E, Nadal A** 2006 The estrogenic effect of bisphenol A disrupts pancreatic $\beta$-cell function in vivo and induces insulin resistance. Environ Health Perspect 114:106–112
60. **Nikula H, Talonpoika T, Kaleva M, Toppari J** 1999 Inhibition of hCG stimulated steroidogenesis in cultured mouse Leydig cells by bisphenol A and octylphenols. Toxicol Appl Pharmacol 157:166–173
61. **Nagel SC, Hagelbarger JL, McDonnell DP** 2001 Development of an ER action indicator mouse for the study of estrogens, selective ER modulators (SERMs), and xenobiotics. Endocrinology 142:4721–4728
62. **Markey CM, Michaelson CL, Veson EC, Sonnenschein C, Soto AM** 2001 The mouse uterotrophic assay: a reevaluation of its validity in assessing the estrogenicity of bisphenol A. Environ Health Perspect 109:55–60
63. **Markey CM, Luque EH, Munoz De Toro M, Sonnenschein C, Soto AM** 2001 In utero exposure to bisphenol A alters the development and tissue organization of the mouse mammary gland. Biol Reprod [Erratum (2004) 71:1753] 65:1215–1223
64. **Markey CM, Wadia PR, Rubin BS, Sonnenschein C, Soto AM** 2005 Long-term effects of fetal exposure to low doses of the xenoestrogen bisphenol-A in the female mouse genital tract. Biol Reprod 72:1344–1351
65. **Munoz-de-Toro M, Markey C, Wadia PR, Luque EH, Rubin BS, Sonnenschein C, Soto AM** 2005 Perinatal exposure to bisphenol A alters peripubertal mammary gland development in mice. Endocrinology 149:4138–4147
66. **Howdeshell KL, Hotchkiss AK, Thayer KA, Vandenbergh JG, vom Saal FS** 1999 Exposure to bisphenol A advances puberty. Nature 401:763–764
67. **Long X, Steinmetz R, Ben-Jonathan N, Caperell-Grant A, Young PCM, Nephew KP, Bigsby RM** 2000 Strain differences in vaginal responses to the xenoestrogen bisphenol A. Environ Health Perspect 108:243–247
68. **Steinmetz R, Brown NG, Allen DL, Bigsby RM, Ben-Jonathan N** 1997 The environmental estrogen bisphenol A stimulates prolactin release *in vitro* and *in vivo*. Endocrinology 138:1780–1786
69. **Steinmetz R, Mitchner NA, Grant A, Allen DL, Bigsby RM, Ben-Jonathan N** 1998 The xenoestrogen bisphenol A induces growth, differentiation, and c-*fos* gene expression in the female reproductive tract. Endocrinology 139:2741–2747
70. **Judy BM, Welshons WV** 1998 Cellular localization of receptors mediating actions of steroid hormones. In: Conn PM, ed. Handbook of physiology, section 7: the endocrine system. Vol. I: Cellular endocrinology. Bethesda, MD: American Physiological Society; 437–460
71. **Song KH, Lee K, Choi HS** 2002 Endocrine disrupter bisphenol A induces orphan nuclear receptor Nur77 gene expression and steroidogenesis in mouse testicular Leydig cells. Endocrinology 143:2208–2215
72. **Zoeller RT, Bansal R, Parris C** 2005 Bisphenol-A, an environmental contaminant that acts as a thyroid hormone receptor antagonist *in vitro*, increases serum thyroxine, and alters RC3/neurogranin expression in the developing rat brain. Endocrinology 146:607–612
73. **Coors A, Jones PD, Giesy JP, Ratte HT** 2003 Removal of estrogenic activity from municipal waste landfill leachate assessed with a bioassay based on reporter gene expression. Environ Sci Technol 37:3430–3434
74. **Kawagoshi Y, Fujita Y, Kishi I, Fukunaga I** 2003 Estrogenic chemicals and estrogenic activity in leachate from municipal waste landfill determined by yeast two-hybrid assay. J Environ Monit 5:269–274
75. **Gray GM, Cohen JT, Cunha G, Hughes C, McConnell EE, Rhomberg L, Sipes IG, Mattison D** 2004 Weight of the evidence evaluation of low-dose reproductive and developmental effects of bisphenol A. Human Ecol Risk Assess 10:i875–i921
76. **Purchase IFH** 2004 Fraud, errors and gamesmanship in experimental toxicology. Toxicology 202:1–20
77. **APM (Association of Plastics Manufacturers in Europe)** 2005 Hyperbole or common sense? Chem Ind March 7:14–15
78. **vom Saal FS** 2005 Low-dose BPA: confirmed by extensive literature. Chem Ind 7:14–15
79. **Olea N, Pulgar R, Perez P, Olea-Serrano F, Rivas A, Novillo-Fertrell A, Pedraza V, Soto AM, Sonnenschein C** 1996 Estrogenicity of resin-based composites and sealants used in dentistry. Environ Health Perspect 104:298–305
80. May 1999 Baby alert: new findings about plastics. Consum Rep; 28–29
81. **Takao Y, Chul Lee H, Ishibashi Y, Kohra S, Tominaga N, Arizono K** 1999 Fast screening method for bisphenol A in environmental water and in food by solid-phase microextraction (SPME). J Health Sci 45:39
82. **Yoshida T, Horie M, Hoshino Y, Nakazawa H** 2001 Determination of bisphenol A in canned vegetables and fruit by high performance liquid chromatography. Food Addit Contam 18:69–75
83. **Takao Y, Lee HC, Kohra S, Arizono K** 2002 Release of bisphenol A from food can lining upon heating. J Health Sci 48:331–334
84. **Brede C, Fjeldal P, Skjevrak I, Herikstad H** 2003 Increased migration levels of bisphenol A from polycarbonate baby bottles after dishwashing, boiling and brushing. Food Addit Contam 20:684–689
85. **Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT** 2002 Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: a national survey. Environ Sci Technol 36:1202–1211
86. **Kuch HM, Ballschmiter K** 2001 Determination of endocrine-disrupting phenolic compounds and estrogens in surface and drinking water by HRGC-(NCI)-MS in the picogram per liter range. Environ Sci Technol 36:3201–3206
87. **Rudel RA, Brody JG, Spengler JD, Vallarino J, Geno PW, Sun G, Yau A** 2001 Identification of selected hormonally active agents and animal mammary carcinogens in commercial and residential air and dust samples. J Air Waste Manag Assoc 51:499–513
88. **Matsumoto H, Adachi S, Suzuki Y** 2005 Bisphenol A in ambient air particulates responsible for the proliferation of MCF-7 human breast cancer cells and its concentration changes over 6 months. Arch Environ Contam Toxicol 48:459–466
89. **Koehler KE, Voigt RC, Thomas S, Lamb B, Urban C, Hassold TJ, Hunt PA** 2003 When disaster strikes: rethinking caging materials. Lab Animal 32:32–35
90. **Zalko D, Soto AM, Dolo L, Dorio C, Rathahao E, Debrauwer L, Faure R, Cravedi JP** 2003 Biotransformations of bisphenol A in a mammalian model: answers and new questions raised by low-dose metabolic fate studies in pregnant CD-1 mice. Environ Health Perspect 111:309–319
91. **Soto AM, Justicia H, Wray JW, Sonnenschein C** 1991 *p*-Nonyl-phenol: an estrogenic xenobiotic released from "modified" polystyrene. Environ Health Perspect 92:167–173
92. **Biles JE, McNeal TP, Begley TH, Hollifield HC** 1997 Determination of bisphenol A in reusable polycarbonate food-contact plastics and migration to food-simulating liquids. J Agric Food Chem 45:3541–3544
93. **Raloff J** 1999 Food for thought: what's coming out of baby's bottle? Science News Online 156:1–4. In: www.sciencenews.org/sn_arc99/8_7_99/food.htm
94. **Inoue K, Kato K, Yoshimura Y, Makino T, Nakazawa H** 2000 Determination of bisphenol A in human serum by high-performance liquid chromatography

with multi-electrode electrochemical detection. J Chromatogr B Biomed Sci Appl 749:17–23
95. **Sajiki J, Takahashi K, Yonekubo J** 1999 Sensitive method for the determination of bisphenol-A in serum using two systems of high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 736:255–261
96. **Ikezuki Y, Tsutsumi O, Takai Y, Kamei Y, Taketani Y** 2002 Determination of bisphenol A concentrations in human biological fluids reveals significant early prenatal exposure. Hum Reprod 17:2839–2841
97. **Swan SH, vom Saal FS** 2001 Alterations in male reproductive development: the role of endocrine disrupting chemicals. In: Metzler M, ed. Endocrine disruptors in the environment. Heidelberg: Springer Verlag; 131–170
98. **Matsumoto A, Kunugita N, Kitagawa K, Isse T, Oyama T, Foureman GL, Morita M, Kawamoto T** 2003 Bisphenol A levels in human urine. Environ Health Perspect 111:101–104
99. **Brock JW, Yoshimura Y, Barr JR, Maggio VL, Graiser SR, Nakazawa H, Needham LL** 2001 Measurement of bisphenol A levels in human urine. J Expo Anal Environ Epidemiol 11:323–328
100. **Ouchi K, Watanabe S** 2002 Measurement of bisphenol A in human urine using liquid chromatography with multi-channel coulometric electrochemical detection. J Chromatogr B Analyt Technol Biomed Life Sci 780:365–370
101. **Takeuchi T, Tsutsumi O, Ikezuki Y, Takai Y, Taketani Y** 2004 Positive relationship between androgen and the endocrine disruptor, bisphenol A, in normal women and women with ovarian dysfunction. Endocr J 51:165–169
102. **Sugiura-Ogasawara M, Ozaki Y, Sonta S, Makino T, Suzumori K** 2005 Exposure to bisphenol A is associated with recurrent miscarriage. Hum Reprod 20:2325–2329
103. **Hiroi H, Tsutsumi O, Takeuchi T, Momoeda M, Ikezuki Y, Okamura A, Yokota H, Taketani Y** 2004 Differences in serum bisphenol A concentrations in premenopausal normal women and women with endometrial hyperplasia. Endocr J 51:595–600
104. **Miyakoda H, Tabata M, Onodera S, Takeda K** 1999 Passage of bisphenol A into the fetus of the pregnant rat. J Health Sci 45:318–323
105. **Takahashi O, Oishi S** 2000 Disposition of orally administered 2,2-bis(4-hydroxyphenyl)propane (bisphenol A) in pregnant rats and the placental transfer to fetuses. Environ Health Perspect 108:931–935
106. **Yoo SD, Shin BS, Lee BM, Lee KC, Han SY, Kim HS, Kwack SJ, Park KL** 2001 Bioavailability and mammary excretion of bisphenol A in Sprague-Dawley rats. J Toxicol Environ Health 64:417–426
107. **Matthews JB, Twomey K, Zacharewski TR** 2001 In vitro and in vivo interactions of bisphenol A and its metabolite, bisphenol A glucuronide, with estrogen receptors $\alpha$ and $\beta$. Chem Res Toxicol 14:149–157
108. **Ishibashi H, Watanabe N, Matsumura N, Hirano M, Nagao Y, Shiratsuchi H, Kohra S, Yoshihara S, Arizono K** 2005 Toxicity to early life stages and an estrogenic effect of a bisphenol A metabolite, 4-methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene on the medaka (*Oryzias latipes*). Life Sci 77:2643–2655
109. **Matsumoto J, Yokota H, Yuasa A** 2002 Developmental increases in rat hepatic microsomal UDP-glucuronosyltransferase activities toward xenoestrogens and decreases during pregnancy. Environ Health Perspect 110:193–196
110. **Fischer LJ, Weissinger JL** 1972 Development in the newborn rat of the conjugation and de-conjugation processes involved in the enterohepatic circulation of diethylstilboestrol. Xenobiotica 2:399–412
111. **Hanioka N, Jinno H, Tanaka-Kagawa T, Nishimura T, Ando M** 2000 Interaction of bisphenol A with rat hepatic cytochrome P450 enzymes. Chemosphere 41:973–978
112. **Cannon JM, Kostoryz E, Russo KA, Smith RE, Yourtee DM** 2000 Bisphenol A and its biomaterial monomer derivatives alteration of in vitro cytochrome P450 metabolism in rat, minipig, and human. Biomacromolecules 1:656–664
113. **Niwa T, Tsutsui M, Kishimoto K, Yabusaki Y, Ishibashi F, Katagiri M** 2000 Inhibition of drug-metabolizing enzyme activity in human hepatic cytochrome P450s by bisphenol A. Biol Pharm Bull 23:498–501
114. **Niwa T, Fujimoto M, Kishimoto K, Yabusaki Y, Ishibashi F, Katagiri M** 2001 Metabolism and interaction of bisphenol A in human hepatic cytochrome P450 and steroidogenic CYP17. Biol Pharm Bull 24:1064–1067
115. **Shibata N, Matsumoto J, Nakada K, Yuasa A, Yokota H** 2002 Male-specific suppression of hepatic microsomal UDP-glucuronosyl transferase activities toward sex hormones in the adult male rat administered bisphenol A. Biochem J 368:783–788
116. **Kawai K, Takehiro N, Nishikata H, Aou S, Takii M, Kubo C** 2003 Aggressive behavior and serum testosterone concentration during the maturation process of male mice: the effects of fetal exposure to bisphenol A. Environ Health Perspect 111:175–178
117. **Akingbemi BT, Sottas CM, Koulova AI, Klinefelter GR, Hardy MP** 2004 Inhibition of testicular steroidogenesis by the xenoestrogen bisphenol A is associated with reduced pituitary luteinizing hormone secretion and decreased steroidogenic enzyme gene expression in rat Leydig cells. Endocrinology 145:592–603
118. **Takao T, Nanamiya W, Nagano I, Asaba K, Kawabata K, Hashimoto K** 1999 Exposure with the environmental estrogen bisphenol A disrupts the male reproductive tract in young mice. Life Sci 65:2351–2357
119. **Tohei A, Suda S, Taya K, Hashimoto T, Kogo H** 2001 Bisphenol A inhibits testicular functions and increases luteinizing hormone secretion in adult male rats. Exp Biol Med 226:216–221
120. **Kavlock RJ, Daston GP, DeRosa C, Fenner-Crisp P, Gray LE, Kaattari S, Lucier G, Luster M, Mac MJ, Maczka C, Miller R, Moore J, Rolland R, Scott G, Sheehan DM, Sinks T, Tilson HA** 1996 Research needs for the risk assessment of health and environmental effects of endocrine disruptors: a report of the US EPA sponsored workshop. Environ Health Perspect 104: 715–740
121. **Tyl RW** May 2002 Low dose debate rebuttal. Endocrine/Estrogen Letter; 20
122. **Cavieres MF, Jaeger J, Porter W** 2002 Developmental toxicity of a commercial herbicide mixture in mice. 1. Effects on embryo implantation and litter size. Environ Health Perspect 110:1081–1085
123. **Rothkamm K, Lobrich M** 2003 Evidence for a lack of DNA double-strand break repair in human cells exposed to very low x-ray doses. Proc Natl Acad Sci USA 100:5057–5062
124. **Calabrese EJ, Baldwin LA** 1997 The dose determines the stimulation (and poison): development of a chemical hormesis database. Int J Toxicol 16:545–559
125. **vom Saal FS, Sheehan DM** 1998 Challenging risk assessment. Forum Appl Res Public Pol 13:11–18
126. **Todaka E, Mori C** 2002 Necessity to establish new risk assessment and risk communication for human fetal exposure to multiple endocrine disruptors in Japan. Congenit Anom 42:87–93
127. **Yamada H, Furuta I, Kato EH, Kataoka S, Usuki Y, Kobashi G, Sata F, Kishi R, Fujimoto S** 2002 Maternal serum and amniotic fluid bisphenol A concentrations in the early second trimester. Reprod Toxicol 16:735–739
128. **Kuroda N, Kinoshita Y, Sun Y, Wada M, Kishikawa N, Nakashima K, Makino T, Nakazawa H** 2003 Measurement of bisphenol A levels in human blood serum and ascitic fluid by HPLC using a fluorescent labeling reagent. J Pharm Biomed Anal 30:1743–1749
129. **Tan BLL, Mohd MA** 2003 Analysis of selected pesticides and alkylphenols in human cord blood by gas chromatograph-mass spectrometer. Talanta 61:385–391
130. **Sun Y, Irie M, Kishikawa N, Wada M, Kuroda N, Nakashima K** 2004 Determination of bisphenol A in human breast milk by HPLC with column-switching and fluorescence detection. Biomed Chromatogr 18:501–507
131. **Engel SM, Levy B, Liu Z, Kaplan D, Wolff MS** 2006 Xenobiotic phenols in early pregnancy amniotic fluid. Reprod Toxicol 21:110–112
132. **Takai Y, Tsutsumi O, Ikezuki Y, Hiroi H, Osuga Y, Momoeda M, Yano T, Taketani Y** 2000 Estrogen receptor-mediated effects of a xenoestrogen, bisphenol A, on preimplantation mouse embryos. Biochem Biophys Res Commun 270:918–921
133. **Takai Y, Tsutsumi O, Ikezuki Y, Kamei Y, Osuga Y, Yano T, Taketan Y** 2000 Preimplantation exposure to bisphenol A advances postnatal development. Reprod Toxicol 15:71–74
134. **Chun TY, Gorski J** 2000 High concentrations of bisphenol A induce cell growth and prolactin secretion in an estrogen-responsive pituitary tumor cell line. Toxicol Appl Pharmacol 162:161–165
135. **Cappelletti V, Saturno G, Miodini P, Korner W, Daidone MG** 2003 Selective modulation of ER-$\beta$ by estradiol and xenoestrogens in human breast cancer cell lines. Cell Mol Life Sci 60:567–576
136. **Negishi T, Ishii Y, Kyuwa S, Kuroda Y, Yoshikawa Y** 2003 Inhibition of staurosporine-induced neuronal cell death by bisphenol A and nonylphenol in primary cultured rat hippocampal and cortical neurons. Neurosci Lett 353:99–102
137. **Sawai C, Anderson K, Walser-Kuntz D** 2003 Effect of bisphenol A on murine immune function: modulation of interferon-$\gamma$, IgG2a, and disease symptoms in NZB $\times$ NZW F1 mice. Environ Health Perspect 111:1883–1887
138. **Canesi L, Betti M, Lorusso LC, Ciacci C, Gallo G** 2005 'In vivo' effects of bisphenol A in *Mytilus* hemocytes: modulation of kinase-mediated signalling pathways. Aquat Toxicol 71:73–84
139. **Wetherill YB, Fisher NL, Staubach A, Danielsen M, White RWD, Knudsen KE** 2005 Xenoestrogen action in prostate cancer: pleiotropic effects dependent on androgen receptor status. Cancer Res 65:54–65
140. **Yanagihara N, Toyohira Y, Ueno S, Tsutsui M, Utsunomiya K, Liu M, Tanaka K** 2005 Stimulation of catecholamine synthesis by environmental estrogenic pollutants. Endocrinology 146:265–272

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