Current Biology, Vol. 13, 546–553, April 1, 2003, ©2003 Elsevier Science Ltd. All rights reserved. DOI 10.1016/S0960-9822(03)00189-1

Bisphenol A Exposure Causes Meiotic Aneuploidy in the Female Mouse

Patricia A. Hunt,[1,*] Kara E. Koehler,[1] Martha Susiarjo,[1] Craig A. Hodges,[1] Arlene Ilagan,[1] Robert C. Voigt,[2,5] Sally Thomas,[3] Brian F. Thomas,[4] and Terry J. Hassold[1]
[1]Department of Genetics
[2]Animal Resource Center
Case Western Reserve University
Cleveland, Ohio 44106-4955
[3]Thoren Caging Systems
Hazleton, Pennsylvania 18201
[4]RTI International
Research Triangle Park, North Carolina 27709-2194

Summary

Background: There is increasing concern that exposure to man-made substances that mimic endogenous hormones may adversely affect mammalian reproduction. Although a variety of reproductive complications have been ascribed to compounds with androgenic or estrogenic properties, little attention has been directed at the potential consequences of such exposures to the genetic quality of the gamete.
Results: A sudden, spontaneous increase in meiotic disturbances, including aneuploidy, in studies of oocytes from control female mice in our laboratory coincided with the accidental exposure of our animals to an environmental source of bisphenol A (BPA). BPA is an estrogenic compound widely used in the production of polycarbonate plastics and epoxy resins. We identified damaged caging material as the source of the exposure, as we were able to recapitulate the meiotic abnormalities by intentionally damaging cages and water bottles. In subsequent studies of female mice, we administered daily oral doses of BPA to directly test the hypothesis that low levels of BPA disrupt female meiosis. Our results demonstrated that the meiotic effects were dose dependent and could be induced by environmentally relevant doses of BPA.
Conclusions: Both the initial inadvertent exposure and subsequent experimental studies suggest that BPA is a potent meiotic aneugen. Specifically, in the female mouse, short-term, low-dose exposure during the final stages of oocyte growth is sufficient to elicit detectable meiotic effects. These results provide the first unequivocal link between mammalian meiotic aneuploidy and an accidental environmental exposure and suggest that the oocyte and its meiotic spindle will provide a sensitive assay system for the study of reproductive toxins.

Introduction

An estimated 10%–25% of fertilized human oocytes are aneuploid; thus, numerical chromosome abnormalities are the leading cause of miscarriage, congenital defects, and mental retardation [1]. Because almost all such aneuploidy derives from meiotic errors, considerable effort has been directed at identifying factors that increase meiotic nondisjunction. A number of potential risk factors, including irradiation (e.g., [2, 3]), smoking or drinking (e.g., [4, 5]), oral contraceptives and fertility drugs (e.g., [4, 6]), and environmental pollutants/pesticides (e.g., [7]), have been suggested. However, significant effects have been small and difficult to verify or disputed, making positive associations hard to establish. In part, this may reflect difficulties in detection. For example, the extraordinary effect of maternal age on aneuploidy may obscure less obvious associations. Further, previous studies may have focused on the "wrong" population; that is, most utilized liveborns, although virtually all aneuploidy terminates in miscarriage. Thus, the contribution of environmental insults to meiotic chromosome errors remains unknown.

We recently experienced an inadvertent environmental exposure in our mouse colony to 2,2-(4,4-dihydroxydiphenol)propane, or bisphenol A. Bisphenol A (BPA) is the monomer that is polymerized to manufacture polycarbonate plastic products and resins, such as those used to line cans containing food and beverages and those found in dental sealents. The exposure was accompanied by highly significant increases in meiotic chromosome abnormalities, including nondisjunction; thus, bisphenol A was implicated as a potent disruptor of meiosis. The ability to experimentally recreate the exposure has allowed us to verify our initial observations and conduct dose-response studies.

Results

A Sudden Increase in Meiotic Abnormalities Is Correlated with Damage to Caging Materials

We recently reported meiotic studies of mouse mutants with defects in the alignment of the chromosomes on the first meiotic (MI) spindle [8]. This meiotic abnormality, which we have termed congression failure (Figure 1), is of particular relevance to humans because it is an age-related feature of human oocytes and has been postulated to be causally related to the well-known increase in aneuploidy associated with advancing maternal age [9].

In the course of meiotic studies of mouse oocytes conducted in 1998, we observed a sudden and dramatic change in congression failure levels. The first wave of follicles that initiate growth in the sexually immature ovary provides access to a large cohort of oocytes, and, typically, only 1%–2% of oocytes from control females exhibit congression failure at metaphase I [8]. However, in experiments conducted in August 1998, congression failure levels suddenly spiked, and approximately 40% of control oocytes exhibited this phenotype or more severe aberrations (Figures 1 and 2).

At the same time that these studies were being conducted, we were also using the animal facility to house

*Correspondence: pah13@po.cwru.edu
[5]Present address: Lab Products, 742 Sussex Avenue, P.O. Box 639, Seaford, Delaware 19973.

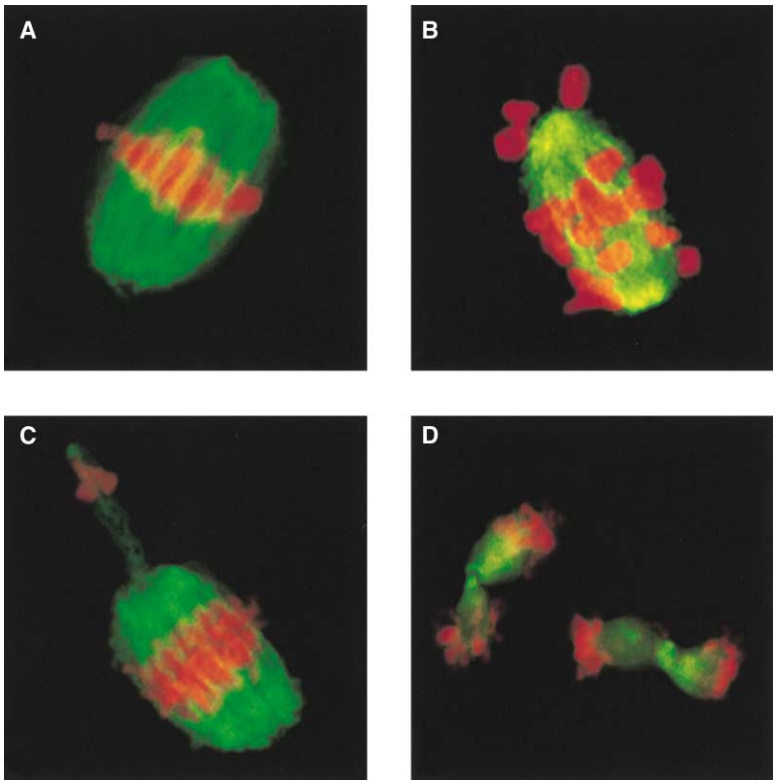

Figure 1. Normal and Abnormal Meiotic Metaphase Configurations

Confocal images of intact mouse oocytes immunostained with an antibody to β-tublin to visualize the meiotic spindle (green) and counterstained with propidium iodide to visualize the chromosomes (red).

(A) Normal metaphase I configuration.

(B–D) Representative meiotic abnormalities from exposed females. (B) Congression failure in an MII-arrested oocyte. Most abnormalities were of this type, but, at the time of maximal exposure, others were observed (e.g., [C] metaphase I cell with chromosomes that have been ejected from the spindle; [D] a cell that should be undergoing the first meiotic division but appears to have two separate groups of chromosomes in a telophase-like configuration).

mice for another analysis of meiotic chromosome segregation. This second set of studies involved cytogenetic analyses of meiosis II preparations in paracentric inversion-carrying female mice and controls and was designed to ask whether meiotic nondisjunction might be increased in inversion heterozygotes. In total, we analyzed five different inversions involving either chromosome 2, 19, or the X chromosome (In(2)2H, In(2)5Rk, In(2)40Rk, In(19)37Rk, and In(X)1H) [10]. However, in the context of the present report, the data on inversion heterozygotes were unhelpful, since data were not collected at all relevant exposure time points; thus, the following discussion pertains only to control animals.

Data on aneuploidy levels were collected for three different types of control animals: inversion homozygotes, chromosomally normal sibs of inversion heterozygotes, and unrelated chromosomally normal (C57BL/6) animals. A dramatic increase in aneuploidy levels in these animals coincided with the increase in congression failure levels. Meiotic aneuploidy is infrequent in the mouse, and baseline rates of hyperploidy (i.e., cells with >20 chromosomes, representing one-half of all nondisjunctional events) are approximately 0.5%–1.0% [11]. Our initial studies were consistent with this expectation; the level of hyperploidy in 415 metaphase II control oocytes was 0.7%. However, beginning in August of 1998 and continuing for several months, analyses of control oocytes indicated an extraordinary increase in aneuploidy (Figure 3; Table 1). Overall, 20/345 (5.8%) oocytes were hyperploid, a highly significant increase ($\chi^2 = 16.53$, $p < 0.001$).

Thus, two independent meiotic studies indicated surprising increases in abnormalities at exactly the same time. Further, the concomitant increases in congression

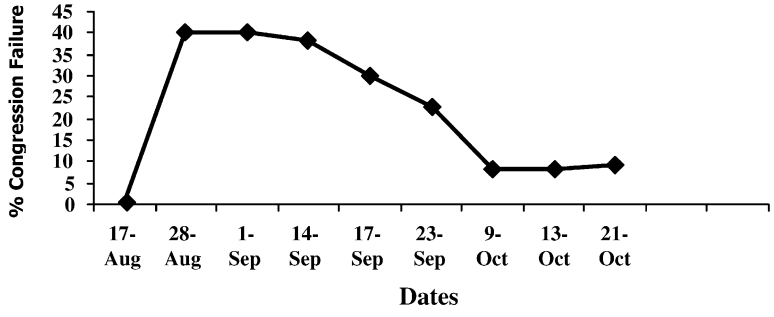

Figure 2. Levels of Congression Failure at Metaphase I in Control Oocytes before and during the Exposure

Our previous studies have established a consistent level of 1%–2% congression failure at metaphase I in ooyctes from control females [8]. Experiments run 2 weeks apart in August 1998 revealed a highly significant increase ($p < 0.001$) in congression failure in oocytes from control females on the C57BL/6 background. In subsequent weekly experiments, values remained high until the end of September (all time points were significantly higher than expectation at $p < 0.01$), when the most visibly damaged cages were replaced by new polycarbonate cages. While the level of congression failure declined to approximately 10% during October, all values remained significantly higher than expected ($p < 0.05$). Similar results were obtained for females on the C3H inbred background (data not shown).

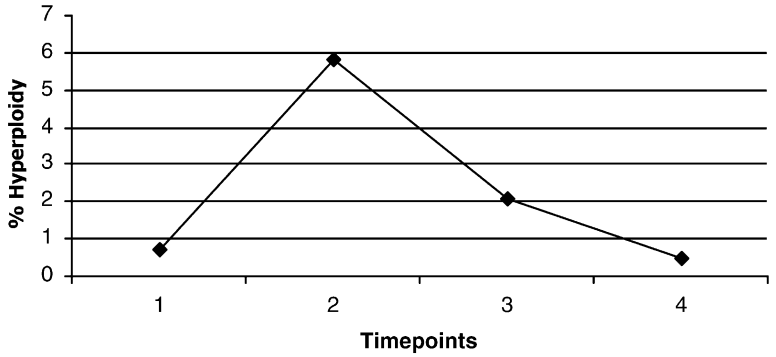

Figure 3. Levels of Hyperploidy in Oocytes before, during, and following Exposure

Levels of hyperploidy (one half of all nondisjunction events) in control oocytes before (1/98–7/98), during (8/98–4/99), and after (5/99–12/00) the exposure (time points 1, 2, and, 3, respectively) and following transfer of the mouse colony to a "clean" facility (time point 4). The numbers of control oocytes analyzed during time points 1, 2, 3, and 4 were 415, 345, 959, and 204, respectively.

failure and aneuploidy were consistent with earlier studies suggesting that congression failure at metaphase I is often "translated" into nondisjunction [8].

Subsequent studies allowed us to rule out an effect of medium constituents or culture conditions as the source of the meiotic disturbances and allowed us to determine that the changes coincided with damage to caging materials caused by the inadvertent use of a harsh alkaline detergent, A33 (Airkem Professional Products, Ecolab; see [12] for further details). Both cages and water bottles were comprised of polycarbonate plastic. A monomer used in the production of polycarbonate, bisphenol A (BPA), is weakly estrogenic in some assay systems (e.g., uterus [13, 14] and human MCF-7 breast cancer cells [15]) and is known to leach from polycarbonate [16]; thus, it seemed possible that the meiotic disturbances were the result of BPA exposure.

Cause and Effect: Confirmation that the Meiotic Effects Are Mediated by Caging Materials

As the most severely damaged cages were pulled from use, a corresponding drop in the level of congression failure was observed (Figure 2), providing indirect evidence that the effect was mediated by the damaged caging materials. Similarly, a third round of cytogenetic studies conducted after the elimination of all damaged caging from the animal facility (May 1999–November 2000) demonstrated a decrease in hyperploidy levels to 2.2% (Figure 3). Because this value was still 3-fold higher than values obtained prior to the exposure, raising the possibility of a residual effect, the colony was moved to a separate housing facility outfitted with new polysulfone cages and glass water bottles, and new breeding stock was purchased. Nondisjunction studies of control females born and raised in the facility confirmed a return to pre-exposure levels of hyperploidy (Figure 3), and the level of congression failure in several hundred oocytes analyzed from offspring of different mating cages demonstrated a return to typical control levels (i.e., 1%–2%; data not shown).

To assess directly the link between damaged polycarbonate caging materials and meiotic disruption, a series of studies involving the assessment of chromosome alignment in metaphase II (MII)-arrested oocytes was initiated. This stage was chosen for two reasons. First,

Table 1. Incidence of Hyperploidy in MII-Arrested Oocytes of Control Animals Analyzed before, during, or after the Environmental Exposure, or in a Separate "Clean" Animal Facility

Time Interval	Strain/Genotype	Age	Number of Cells	Number of (%) Hyperploid
BEFORE (prior to August 1998)	In(2)2H/In(2)2H	4 weeks	415	3 (0.7)
DURING (September 1998–April 1999)	In(2)2H/In(2)2H	4 weeks	104	5 (4.8)
	In(19)37Rk/In(19)37Rk	4 weeks	89	7 (7.9)
	In(2)5Rk (+/+)[a]	4 weeks	29	1 (3.4)
	C57BL/6	8–12 months	32	3 (9.4)
	In(2)2H/In(2)2H	8–12 months	91	4 (4.4)
	Pooled		345	20 (5.8)
AFTER (May 1999–December 2000)	In(19)37Rk/In(19)37Rk	4 weeks	183	5 (2.7)
	In(X)1H/In(X)1H	4 weeks	133	2 (1.5)
	In(2)40Rk/In(2)40Rk	4 weeks	73	2 (2.7)
	C57BL/6	4 weeks	425	12 (2.8)
	In(2)5Rk (+/+)[a]	4 weeks	145	0 (0)
	Pooled		959	21 (2.2)
NEW FACILITY (December 2000–February 2001)	C57BL/6	4 weeks	204	1 (0.5)

Control animals consisted of inversion homozygotes, chromosomally normal sibs of inversion heterozygotes, and unrelated chromosomally normal (C57BL/6) animals.

[a] The inversion In(2)5Rk is homozygous lethal. Stock matings thus generated both inversion heterozygotes (In(2)5Rk/+) and normal sequence (+/+) progeny.

Table 2. Meiotic Effects Mediated by Caging Materials

	Total MII-Arrested Oocytes	Congression Failure	χ^2 Value
Control: New Cages/Glass Bottles	271	5 (1.8%)	
Damaged Cages/Glass Bottles			
Mild	401	35 (8.7%)	13.62, $p < 0.001$
Severe	149	30 (20.1%)	42.20, $p < 0.001$
Damaged Bottles	197	53 (26.9%)	66.05, $p < 0.001$
Damaged Cages/Damaged Bottles	58	24 (41.4%)	93.19, $p < 0.001$
Damaged Bottles (Conventional Polycarbonate)	134	40 (29.9%)	71.12, $p < 0.001$

oocytes that complete the first division remain arrested at MII until fertilization; thus, this time point provides access to a nontransient metaphase stage. Second, the analysis of chromosome alignment is both more rapid and more cost effective than traditional cytogenetic analysis of aneuploidy. New high-temperature polycarbonate (polyphthalate carbonate) cages were exposed to a single contact with dilute (1:64) or full strength A-33 detergent. Control animals were housed in new polysulfone cages, and glass water bottles were used for both treated and control cages. Analysis of MII-arrested oocytes from normal females born and raised in intentionally damaged cages revealed a direct correlation between the degree of damage and the level of meiotic disturbance, and offspring from mildly and severely damaged cages exhibited an $\sim$5-fold and $>$10-fold increase in congression failure, respectively (Table 2). Although both mildly and severely damaged cages exhibited significant increases compared to controls (Table 2), the level of congression failure did not approach that observed during the initial incident. This finding suggests that water bottles were an additional source of exposure. Indeed, subsequent analyses of damaged bottles revealed significant increases in congression failure (Table 2), and experiments involving both intentionally damaged cages and bottles also resulted in a significant increase (Table 2) and produced levels comparable to those at the height of the original exposure.

The high-temperature, autoclavable polycarbonate cages and bottles used in our facility were comprised of a synthetic thermoplastic polymer that is a blend of bisphenol A and polyester carbonate. Thus, it remained possible that the meiotic effects were not mediated by BPA. To test this, we conducted a series of experiments with conventional polycarbonate bottles (i.e., comprised only of BPA) that were intentionally damaged by exposure to dilute (1:64) A-33 detergent and subsequently washed in water but not autoclaved. As shown in Table 2, the congression failure rate for this type of damaged bottle was highly significantly increased over controls and was virtually identical to the level observed for the high-temperature polymer. This set of experiments suggests that, following chemical damage, BPA continues to leach from polycarbonate even in the absence of further harsh treatment (e.g., autoclaving).

Thus, meiotic analyses both at the time of the initial exposure and in subsequent studies using intentionally damaged caging materials provided strong circumstantial evidence of a link between BPA exposure and the meiotic disturbances.

BPA Exposure: Determining the Dosage and Timing Necessary to Induce Meiotic Effects

To characterize the timing and exposure levels necessary to induce meiotic defects, a series of studies involving the treatment of females with daily oral doses of BPA was initiated. As a first step, we estimated the constant exposure levels that elicited a demonstrable effect in the water bottle experiments (Table 2) by determining BPA levels in water from damaged bottles. Gas chromatography-mass spectrometry analysis with electron impact ionization methodology indicated approximate BPA levels of 100 and 360 ng/ml (Figure 4). Assuming an oral intake of 4–5 ml water per day and an average weight of 25–28 g/mouse, daily exposure levels from water were estimated to be 14–72 ng/g body weight. Using these doses as a guideline, we directly tested the meiotic effects of BPA by treating juvenile females (20- to 22-day-old mice) with 20, 40, or 100 ng/g body weight/day oral doses of BPA for 6–8 days preceding oocyte analysis. Low oral doses of BPA have been suggested to be subject to rapid first-pass elimination by the liver and efficient metabolic clearance [17, 18]. Nevertheless, a significant increase in congression failure (Table 3; [$\chi^2 = 4.79$; $p < 0.05$]), and a dose-related increase in the level of abnormalities, was observed among the treated animals. Thus, it appears that low-dose BPA exposure during the final stages of follicle growth is sufficient to cause meiotic abnormalities. To determine the shortest exposure that produced detectable effects, an additional set of experiments using a dose of 20 ng/g for 3, 5, or 7 days prior to oocyte analysis was conducted. All three exposures resulted in increased levels of congression failure, although only the 7-day treatment was significantly elevated over control values ($\chi^2 = 6.21$; $p < 0.05$; Figure 5)

Discussion

Based on previous studies of human oocytes and of mouse mutants, we hypothesized that congression failure in the mammalian oocyte is causally related to aneuploidy and that it results from endocrine disturbances that affect the final stages of oocyte growth [8, 9]. Thus, the unexpected finding that exposure to BPA, a man-made substance with estrogenic properties, induces both a dramatic increase in congression failure and meiotic aneuploidy in mouse oocytes provides serendipitous validation of this hypothesis.

Many previous studies have suggested that BPA and other man-made substances that mimic estrogens have

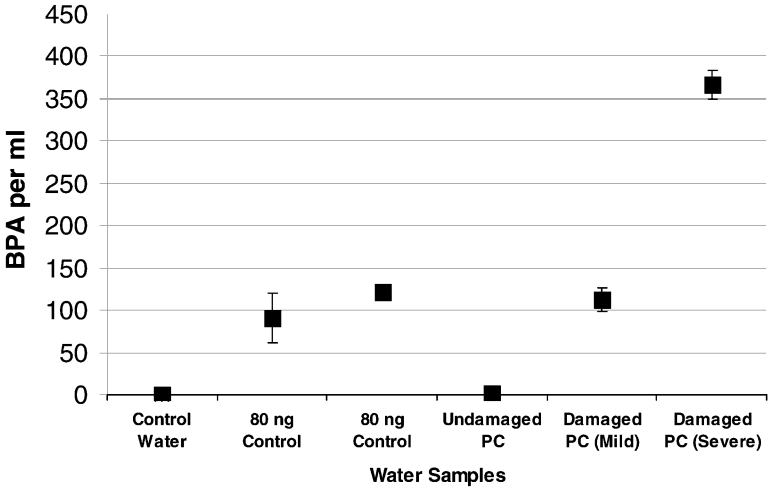

Figure 4. Measurement of BPA in Water Samples

BPA concentrations in water from damaged bottles were determined by gas chromatography-mass spectrometry using electron impact ionization [37]. Test samples included a distilled water control (control water), two samples spiked with 80 ng/ml BPA (80 ng control), one sample from an undamaged polycarbonate bottle (Undamaged PC), one sample from a damaged polycarbonate bottle (Damaged PC [mild]), and one sample from a damaged and leaking polycarbonate bottle (Damaged PC [severe]). To approximate normal operating conditions, undamaged and damaged bottles were filled with 500 ml water, autoclaved by using standard facility procedures (i.e., 60 min on a liquid setting), and allowed to sit at room temperature for 24 hr before samples were acquired. The average concentration and standard deviation for two replicates of each sample type have been plotted on a ng/ml scale, and the level in the control water sample was set to zero. Note that the standard deviation is not apparent for one of the two 80 ng samples, as the replicate values were virtually identical. A slight variation in the 80 ng/ml control values likely reflects the fact that BPA is only moderately soluble in water at neutral pH [37].

adverse effects on mammalian reproduction. However, unlike previous studies that have utilized a prospective toxicological approach (i.e., determining the level of exposure necessary to produce a measurable effect), our studies were a retrospective attempt to understand a sudden change in experimental data. Indeed, the type of environmental accident that precipitated the present studies is, to our knowledge, unprecedented in animal husbandry. In addition to the difference in impetus, the present study differs in two other important respects. First, the abrupt changes in experimental data in two separate studies in our laboratory provide evidence of a striking new reproductive effect; namely, immediate and extreme alterations in the meiotic process in intact animals inadvertently exposed to BPA. Second, unlike many previously reported effects of BPA on mammalian reproduction (e.g., morphological alterations of the reproductive tract [13, 19–22], accelerated onset of puberty [23], disruption of estrus cycles [24], or reduced sperm counts [19, 25]), a defect in meiosis directly influences the genetic quality of the gametes and thus impacts the next generation. Indeed, other data from our laboratory provide evidence that BPA elicits just such a transgenerational effect. In independent studies conducted in the same mouse facility, two nonmosaic chromosome abnormalities (one autosomal trisomy and one unbalanced structural rearrangement) were observed among 16 fetuses karyotyped during the time of maximal exposure. Such abnormalities are extremely rare in our colony and in the literature and seem unlikely to have arisen coincidentally.

Thus, these lines of evidence suggest that, in the mouse, BPA adversely affects chromosome segregation. Clearly, we do not know if this liability extends to humans. Nevertheless, the meiotic program is extraordinarily conserved, and the results of our studies in mice are disturbing because brief exposures during the final stages of oocyte growth were sufficient to cause significant increases in meiotic abnormalities. Further, relatively modest BPA concentrations (i.e., 0.02–0.04 mg/kg body weight/day) elicited significant meiotic effects. Moreover, although the vast majority of our studies were conducted on the first wave of growing follicles in the sexually immature female, a dramatic increase in nondisjunction levels was observed in oocytes from 6- to 8-month-old females during the initial exposure. Thus, sensitivity does not appear to be limited to the prepuberal female.

A number of important questions remain. For example, although our studies suggest that exposure during the final stages of oocyte growth induces meiotic abnormalities, they also raise questions about the critical exposure period. For example, daily oral doses of BPA yielded comparatively low levels of aneuploidy (Table 3; Figure 5), despite our attempts to mimic the exposure dose from damaged caging materials by measuring BPA levels in water from damaged bottles. Possibly, our estimate of initial exposure levels was in error. However, since the level of congression failure in the oral dosing experiments increased both with dose and with exposure time (e.g., Table 3; Figure 5), it seems more likely that the difference is a pharmacokinetic one; i.e., single low oral doses have been reported to be subject to rapid first-pass elimination [17, 18]. Thus, the effect of a more evenly distributed exposure from drinking BPA-contaminated water throughout the day and in association with food intake may be markedly different. It is also possible that BPA acts on meiosis at different developmental stages. For example, an effect that influences the prenatal events of meiotic prophase (i.e., pairing, synapsis, and recombination) might also be evident as an increase in aneuploidy. Alternatively, neonatal exposure might

Table 3. Meiotic Effects of Oral BPA Administration

BPA Dose	Congression Failure
Control: 0 ng/g	2/115 (1.7%)
20 ng/g	10/172 (5.8%)
40 ng/g	19/255 (7.5%)
100 ng/g	5/46 (10.9%)

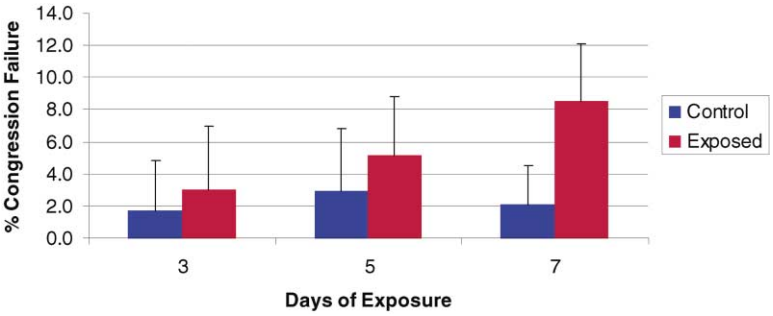

Figure 5. Congression Failure following Oral BPA Administration

Congression failure levels (and 95% confidence intervals) among MII-arrested oocytes from control and BPA-exposed females treated with 20 ng/g body weight BPA in an oil carrier for 3–7 days prior to oocyte collection. The numbers of oocytes examined for the 3-, 5-, 7-day exposures, respectively, were 67, 138, and 234 for treated females and 61, 70, and 140 for control females. While congression failure levels were increased among treated animals for all time points, only the 7-day exposure comparison reached statistical significance ($p < 0.05$).

act to alter the hypothalamic-pituitary-gonadal axis and thus may provide another route to aneuploidy.

The mechanism by which BPA exerts its effect on meiosis remains obscure. Studies of somatic cells exposed in vitro to BPA have demonstrated disturbances in microtubule organization, leading to aneuploidy [26–29]. The effect on the oocyte, however, is likely much more complicated. In the growing follicle, multiple layers of granulosa cells completely envelop the oocyte, and only the innermost layer is in direct contact with the oocyte via gap junctions. Thus, the effect on the oocyte is almost certainly an indirect one, acting through estrogen-mediated changes in the somatic cells of the follicle.

The role of estrogens in folliculogenesis is currently the topic of considerable research interest [30]. The theca cells of the late-stage follicle not only convert testosterone to estrogen, but also express both the ERα and ERβ isoforms of the estrogen receptor [31], whereas the granulosa cells express predominantly ERβ receptors [31]. Thus, estrogenic compounds (e.g., BPA) present in the microcirculation of the ovary have the potential to affect both cell types but likely predominantly those expressing ERβ, since BPA has a higher binding affinity for ERβ than ERα [32]. The importance of estrogen during the late stages of follicle growth has been demonstrated by the reproductive phenotype of targeted disruptions of both estrogen receptors and of the aromatase gene necessary for the conversion of androgens to estrogens (reviewed in [30]). Further, the recent report demonstrating that administration of BPA reverses the reproductive phenotype of the aromatase knockout female provides evidence that the somatic cells of the follicle can be influenced by oral doses of BPA [33].

Currently, there is considerable uncertainty about safe levels of BPA exposure. Plastic industry sources suggest no safety issues related to low-level BPA exposure (e.g., http://www.bisphenol-a.org). Nevertheless, governmental agencies continue to revise safe exposure levels on the basis of animal studies. For example, The European Commission's Scientific Committee on Food recently revised its Tolerable Daily Intake (TDI) from the 0.05 mg/kg body weight level set in 1986 to 0.01 mg/kg body weight (http://europa.eu.int/comm/food/fs/sc/scf/index_en.html). Uncertainty about "safe" exposure levels reflects conflicting reports about the effect of low-dose exposures and differences based on genetic background or route and timing of exposure (e.g., [34–36]). In part, this is due to the lack of a sensitive, reliable, and reproducible assay system. The dose-dependent response observed in our studies suggests that the oocyte and its meiotic spindle may provide just such an assay system for the study of reproductive toxins.

Of more immediate concern, however, we have observed meiotic defects in mice at exposure levels close to or even below those considered "safe." Furthermore, a recent study of pregnant women and their fetuses conducted in Germany suggests that current human exposure levels may well be within this range [21]. Clearly, the possibility that BPA exposure increases the likelihood of genetically abnormal offspring is too serious to be dismissed without extensive further study.

Conclusions

The inadvertent exposure of mice in our colony to bisphenol A provided evidence that this estrogen mimic disrupts chromosome behavior in the mammalian oocyte and causes a specific meiotic phenotype at metaphase (congression failure) and an increased risk of nondisjunction at anaphase. Experimental studies conducted to understand this effect indicate that short-term, low-dose exposure to bisphenol A is sufficient to elicit these meiotic abnormalities.

Our studies have obvious relevance to the genesis of meiotic aneuploidy in the human. Specifically, they are consistent with the hypothesis that endocrine changes affecting oocyte growth underlie human age-related increases in nondisjunction. In addition, these findings raise concerns about the potential reproductive impact of environmental substances that mimic the actions of endogenous hormones.

Experimental Procedures

Oocyte Collection and Analysis

Oocytes were collected and cultured by using conventional laboratory techniques as described previously [8]. Unless otherwise specified, germinal vesicle (GV)-stage oocytes were harvested from 28-day-old females; the first wave of oocytes to initiate growth has reached the antral stage by this time, and a large cohort of meiotically competent oocytes can be obtained. For both congression failure and aneuploidy analyses, the scoring of all preparations was conducted by two independent observers who were blinded with respect to the status (control versus treated) of the specimens.

Congression Failure

Studies conducted at the time of the initial change in control data involved the analysis of the first meiotic division. For these studies,

GV-stage oocytes were liberated from antral follicles, cultured for 8–10 hr, embedded in a fibrin clot attached to a microscope slide, and fixed in a 2% formaldehyde fixative as described previously [8]. Subsequent studies utilized MII-arrested oocytes, since this static arrest phase alleviates the problem of variation due to differences in cell cycle rate. For these studies, GV-stage oocytes were cultured overnight, and only those exhibiting a polar body the following morning were embedded in fibrin clots and fixed. For the analysis of all preparations, slides were immunostained with tubulin antibodies to visualize the spindle and counterstained with DAPI or propidium iodide to visualize the chromosomes as described previously [8].

Aneuploidy Assessment

Studies conducted at the time of the initial change in control data involved the analysis of MII-arrested oocytes from control and inversion carrying females. Air-dried chromosome preparations were made by using a modification of the Tarkowski technique as described previously [8].

Cage and Water Bottle Testing

To assess the effect of damaged polycarbonate caging materials, oocytes were analyzed from normal females born and raised in test and control cages. Siblings were used to establish trio matings (i.e., two females and one male). Test cages consisted of new high-temperature polycarbonate (polyphthalate carbonate) cages exposed to a single contact with either a dilute (1:64) solution ("mild" damage) or full strength ("severe" damage) A-33 detergent (Airkem Professional Products, Ecolab), the detergent responsible for the damage to caging materials in the original animal facility. Following exposure, both mild and severely damaged cages were subjected to 3–4 rounds of thorough rinsing in water, followed by autoclaving before animals were introduced. Subsequently, all caging materials were washed by hand without detergent, and the number of times individual cages were autoclaved for sterilization purposes was recorded by using a cage-marking system. To avoid possible cross-contamination of undamaged caging materials in the course of normal handling, damaged materials were autoclaved separately, and polysulfone cages were used for control matings. In the case of conventional polycarbonate bottles (i.e., nonautoclavable polycarbonate), bottles were simply washed in water following exposure to a dilute (1:64) solution of A-33 detergent. In all cases, test and control matings were run in duplicate.

Measurement of BPA in Water Samples

To determine BPA concentrations in water samples, gas chromatography-mass spectrometry using electron impact ionization was employed [37]. For the assay, the mass spectrometer was run in ion-monitoring mode, and a stock solution of BPA prepared in absolute ethanol at a concentration of 1.01 mg/ml was used to spike 15 ml aliquots of distilled, deionized water to generate a calibration curve. A minimum of two and a maximum of four extractions were run on each test sample by using injection volumes of 1 μl.

Oral BPA Administration

Oocytes were harvested from juvenile (28-day-old) females treated with oral doses of BPA in a corn oil carrier as described previously [19]. In initial studies (Table 3), juvenile females (20- to 22-day-old) were treated with daily doses of 20, 40, or 100 ng/g body weight for 6–8 days preceding oocyte collection and analysis. In subsequent studies (Figure 5), daily doses of 20 ng/g body weight were administered for 3, 5, or 7 days prior to oocyte analysis. Germinal vesicle-stage oocytes were cultured overnight, and oocytes exhibiting a polar body the following morning were fixed, immunostained, and analyzed by using standard laboratory techniques [8]. No difference in the number of oocytes or the rate of polar body extrusion was detected between control and treated females. All scoring was conducted without knowledge of the status (control versus treated) of the female.

Statistical Analysis

Comparisons of levels of aneuploidy or congression failure between controls and exposed/treated animals were conducted by using straightforward goodness of fit tests. In instances in which multiple comparisons were made, Bonferroni corrections were applied to account for the number of statistical tests.

Acknowledgments

The authors express gratitude to A. Vodicka, E. Millie, L. Woods, and J. Cherry for technical assistance and to E. Freeman, F. vom Saal, and R.S. Hawley for helpful discussions. These studies were supported by National Institutes of Health grants ES1172 (P.A.H.) and HD21341 (T.J.H.), ACS postdoctoral fellowship 96994 (K.E.K.), and a Culpepper Foundation Pilot Initiative grant (P.A.H.).

Received: December 11, 2002
Revised: February 3, 2003
Accepted: February 3, 2003
Published: April 1, 2003

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Note Added in Proof

Following the submission of this manuscript, a study describing the release of BPA from new and used polycarbonate caging was published (Howdeshell, K.L., et al. (2003). Bisphenol A is released from used polycarbonate animal cages into water at room temperature. Environ. Health Perspect., in press. Published online February 5, 2003. 10.1289/ehp.5993). This study demonstrates that new polycarbonate cages leach low levels of BPA and that the level of leaching increases markedly under conditions of normal wear.