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Minireview

The Bisphenol A Experience: A Primer for the Analysis of Environmental Effects on Mammalian Reproduction[1]

Patricia A. Hunt,[2,4] Martha Susiarjo,[3,4] Carmen Rubio,[5] and Terry J. Hassold[4]

School of Molecular Biosciences and Center for Reproductive Biology,[4] Washington State University, Pullman, Washington
Instituto Valenciano de Infertilidad,[5] Universidad de Valencia, Valencia, Spain

ABSTRACT

It is increasingly evident that environmental factors are a veritable Pandora's box from which new concerns and complications continue to emerge. Although previously considered the domain of toxicologists, it is now clear that an understanding of the effects of the environment on reproduction requires a far broader range of expertise and that, at least for endocrine-disrupting chemicals, many of the tenets of classical toxicology need to be revisited. Indeed, because of the wide range of reproductive effects induced by these chemicals, interest among reproductive biologists has grown rapidly: in 2000, the program for the annual Society for the Study of Reproduction meeting included a single minisymposium on the fetal origins of adult disease, one platform session on endocrine disruption, and 23 toxicology poster presentations. In contrast, environmental factors featured prominently at the 2009 meeting, with strong representation in the plenary, minisymposia, platform, and poster sessions. Clearly, a lot has happened in a decade, and environmental issues have become an increasingly important research focus for reproductive biologists. In this review, we summarize some of the inherent difficulties in assessing environmental effects on reproductive performance, focusing on the endocrine disruptor bisphenol A (BPA) to illustrate important emerging concerns. In addition, because the BPA experience serves as a prototype for scientific activism, public education, and advocacy, these issues are also discussed.

aneuploidy, bisphenol A, BPA, diet, endocrine disruptors, meiosis, oocyte, phytoestrogens, toxicology

INTRODUCTION

Endocrine-disrupting chemicals (EDCs), man-made compounds that can impair normal hormonal function, have been implicated in a variety of reproductive disorders. Our own laboratory's entry into this field illustrates the unexpected way in which environmental EDCs can intrude on reproductive research. An accident in our mouse facility led to leaching of the plasticizer bisphenol A (BPA) from caging material and water bottles, causing a sudden change in the data of several ongoing studies of female meiosis [1]. Because the onset of leaching was abrupt—the result of inadvertent damaging of caging materials through the use of the wrong detergent—we were quickly able to detect changes in the results of individual experiments and determine the cause. Our studies involved meiotic analyses of periovulatory eggs, and the sudden exposure of our animals to the estrogenic chemical BPA caused a spike in meiotic disturbances in eggs from control females. Indeed, we observed changes in two separate sets of studies: an increase in chromosome alignment defects in cells undergoing the first meiotic division, and an increased level of aneuploidy among metaphase II arrested eggs. The changes in the data sets for both studies suggested that BPA exposure had the potential to disrupt the periovulatory follicle.

In addition to our findings, numerous other groups—typically studying rodent models—have also identified adverse reproductive consequences of low-dose BPA exposure [2, 3, 4]. Exposures during prenatal and neonatal development have been linked to a wide variety of effects, including defects in the male and female reproductive tracts [3], meiotic abnormalities in fetal oocytes [5], complications of pregnancy [6], changes in the morphology of the mammary [7] and prostate glands [8] and associated increases in malignancies in the adult [9, 10], and alterations in brain sexual differentiation [11], among others. Importantly, many of these studies have reported BPA-related effects at very low doses, e.g., at exposure levels less than 50 μg kg^{-1} day^{-1}, the current "safe" dose that is considered acceptable for daily intake by the U.S. Food and Drug Administration (FDA) and that is also the reference dose established by the U.S. Environmental Protection Agency. This suggests that, like endogenous hormones, environmental estrogens can elicit biological effects at extremely low concentrations.

Not all studies have found an effect. Notably, several reproductive toxicity studies in mice or rats [12–15] have failed to find any important reproductive consequences associated with BPA exposure. Importantly, these studies used standard in vivo multi-generation test protocols developed by federal

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[2]Correspondence: Patricia A. Hunt, School of Molecular Biosciences, Fulmer Hall 539, Washington State University, Mail Drop 644660, Pullman, WA 99164-4660. FAX: 509 335 9688;
e-mail: pathunt@wsu.edu
[3]Current address: Department of Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6148.

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agencies worldwide to evaluate the effects of chemicals and pharmaceuticals on reproduction and fertility (e.g., http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/Series/870-3800.pdf). These multi-generation tests have the advantage of being conducted under strict quality control guidelines for Good Laboratory Practices (GLP), enabling regulators to document data quality in courts of law. However, as detailed below, adherence to GLP guidelines, though ensuring the quality of the data collected, provides no assurance of the soundness of the data analysis and interpretation. Importantly, for testing of EDCs, standard multi-generation test protocols have well-recognized limitations. Although designed to detect the impact of fetal exposure on reproductive development and adult reproductive function, these test protocols typically involve methodologies developed many decades ago, including conventional histochemistry, gross anatomical examinations, and comparisons of tissue weights. Thus, a variety of biological effects detectable by contemporary techniques (e.g., immunofluorescence, RT-PCR, and methylation assays) would likely go unnoticed in these analyses. The original multi-generation protocols that relied almost exclusively on fertility and pregnancy outcome measures have been updated to include some endocrine-sensitive outcomes (e.g., estrous cyclicity, pubertal landmarks, sperm measures, and more detailed histopathologic examination specific to reproductive effects; see EPA guidelines updated in 1998 [U.S. Environmental Protection Agency. Health Effects Test Guidelines, OPPTS 870.3800, Reproduction and Fertility Effects, August 1998. http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/Series/870-3800.pdf]). Nevertheless, even with these updates the protocols remain inadequate, and recognition of the limitations in the multi-generation tests and the need for more specific tests for endocrine-active compounds led to the congressionally mandated endocrine disruption screening and testing program initiated in 1998 (United States Environmental Protection Agency [US EPA], Endocrine Disruptor Screening Program [EDSP], http://epa.gov/endo/index.htm).

Discrepancies between the results of standard in vivo multi-generation studies and basic research studies evaluating a variety of outcomes have created controversy over the reproductive effects of BPA. Further, even among studies that have reported BPA-induced reproductive abnormalities, there are disagreements about the type and/or severity of the defects. For example, following our initial report on meiotic abnormalities, four other studies examined the effect of BPA on the periovulatory oocyte [16–19]. Though each reported meiotic disturbances, the types of abnormalities varied among the studies, and none reported an increase in aneuploidy.

This has led some to question the validity of our initial observations and, more generally, the validity of any reports of BPA-induced reproductive defects [20]. Further, it has allowed BPA manufacturers to suggest that concerns about the safety of BPA are unfounded (e.g., see www.bisphenol-a.org), since standard multi-generation test protocols have failed to find BPA-related abnormalities and some effects from other studies cannot be replicated verbatim. In the sections that follow, we discuss three complex issues surrounding the testing of chemicals like BPA with endocrine-disrupting properties, specifically: 1) limitations in the ability of standard multi-generation test protocols to detect the effects of EDCs on reproduction, especially at low doses; 2) confounding variables that can significantly affect the results of studies of EDCs; and 3) the inherent difficulties of study replication in this field.

EDCs AND TOXICOLOGY: URGENT NEED FOR NEW TESTING PARADIGMS

Recent reviews of BPA by both the FDA and the European Food Safety Authority have concluded that current human exposure levels are safe. These conclusions, however, are based largely on data from a few standard multi-generation studies, some of which were conducted under outdated guidelines. Because these studies were supported by industry, the implication has been made that they may be less than objective [2]. Despite this criticism and the fact that these studies are at odds with the results of hundreds of studies conducted in academic settings [3], the data from standard multi-generation protocols have been perceived by regulatory agencies as definitive. As detailed in a recent commentary, however, these "definitive" studies have major flaws [21]. Specifically, in addition to the deficiencies in standard multi-generation protocols detailed above (e.g., the use of outdated protocols that do not consider contemporary endpoints, such as analyses of effects on meiotic chromosome behavior in germ cells, epigenetic programming, and neurobehavior), Myers et al. detail significant flaws in each of the previous standard multi-generation studies of BPA. For example, one of the studies failed to include a positive control to demonstrate the sensitivity of the system to detecting estrogenic effects [14]. Although positive controls were included in each of the other three multi-generation studies, in two the authors were unable to identify effects in the positive estrogenic control (diethylstilbestrol [DES]) for any endpoint [12, 13], and in the third, the positive control demonstrated that the mice were surprisingly insensitive to estrogen by comparison with other studies using the same strain [15]. In short, none of the multi-generation studies has been able to demonstrate that the test system being used is capable of detecting effects of estrogenic compounds, and this casts doubt on the conclusions of these studies regarding the biological consequences of the test estrogenic compound, BPA.

These and other considerations led Myers et al. [21] to conclude that the industry-sponsored multi-generation studies are outmoded by comparison with more sensitive, modern methodologies and that the multi-generation studies—long regarded as the gold standard in the field—are actually incapable of detecting low-dose effects of BPA and other EDCs. The recognition that current testing guidelines are inadequate for chemicals with hormone-like action is not new. In 1996, the Food Quality Protection Act passed by Congress mandated the development of new screening and testing procedures for EDCs. Ten years later, despite progress toward the goal of developing and validating a screening battery for EDCs (US EPA, EDSP, http://epa.gov/endo/index.htm), no chemicals have been tested. This reflects, at least in part, the fact that the U.S. regulatory system includes rigorous peer review by the public and is a slow and iterative process. As a result, new and better tests are often developed before the first round of improvements is implemented. In the interim, despite the evidence that current testing protocols are inefficient and the direct criticisms of standard multi-generation protocols used in safety assessments of BPA, regulatory decisions continue to be made on the basis of this assessment tool, while more modern technologies are either discounted or ignored.

The solution seems simple: federal agencies need to use all available, peer-reviewed data in their risk assessments, and reproductive biologists need to be more vocal and comprehensible in explaining their results and advocating for the inclusion of their findings in weight-of-evidence risk assessments. The

reality, however, is far more complex and boils down to issues of legality. Regulatory agencies place the greatest emphasis on studies conducted under traditional multi-generation testing paradigms because the good laboratory practices (GLP) guidelines used ensure that data quality is documented, and this, in turn, ensures that it will hold up in a court of law. Though industries submitting data to regulatory agencies are *required* to use GLP, these studies are very expensive and can rarely be performed in academic laboratories. As a result, almost all standard multi-generation tests on BPA have been conducted by industry. In contrast, funding agencies that support academic research typically strive to advance knowledge by funding innovative studies, like the cellular and molecular studies that have provided non-traditional evidence for low-dose effects of BPA. Regulators may not understand the predictive power of these test systems, and the sobering reality is, in the absence of definitive links, regulatory decisions based on these innovative and sensitive test systems may not stand up in a court of law.

AN EVER-GROWING LIST OF CONFOUNDERS COMPLICATES THE STUDY OF EDCs

Studies of hormones and hormone-like chemicals are, by their very nature, vulnerable to the effects of confounding variables. As discussed above, studies designed to replicate some BPA low-dose effects have yielded variable results, and this variation has been used by industry to diminish the significance of BPA-induced effects. Indeed, controversy has clouded the field of BPA research from the outset. Nagel et al. [22] were the first to report a low-dose BPA effect: When an experimental increase in levels of free serum estradiol resulted in an increase in the size of the fetal prostate [23], they reasoned that estrogenic chemicals like BPA would produce a similar effect. Using a CF1 mouse model, they found a significant increase in adult prostate size in male offspring of females fed 2 or 20 μg/kg doses of BPA during gestation [22]. These findings were extended in a paper published a year later that reported not only an effect on prostate size, but also a drop in testis size and a decrease in sperm production [24]. These three academic studies were quickly followed by two industry-funded studies that reported no effect of fetal BPA exposure on the size of the prostate or testis or on sperm production [12, 13]. The inability of independent laboratories to replicate the findings raised concerns about the validity of the effects reported by vom Saal and his colleagues.

In the intervening decade, the effect of BPA on the developing prostate has been confirmed and extended by a host of investigators, with recent studies demonstrating that BPA exposure during prostate development alters the prostate epigenome and increases the risk of prostate cancer [25]. But why was there a controversy at the outset, and how does one account for the negative findings reported in the industry-funded studies? An extensive review of low-dose BPA effects published through December of 2004 revealed a disturbing trend [2]. Of a total of 115 in vivo studies, 94 (81.7%) reported significant adverse effects as a result of BPA exposure. However, when the studies were divided into those that were funded by industry sources and those that were government-supported, there was a remarkable difference: no fewer than 94/104 (90.4%) of the government-funded studies found adverse effects, whereas none of the 11 industry-supported studies reported effects. A few of the industry studies were multi-generational GLP studies and, as discussed above, the sensitivity and relevance of this approach for the study of EDCs has been questioned.

Differences between industry and academia aside, the variation in the results of BPA studies conducted in different laboratories but designed to evaluate the same endpoints is sufficient to raise concern. A number of important variables—some familiar and some whose importance has emerged from studies of BPA and other EDCs—can impact study results and create significant differences in the results of studies designed to replicate previously reported findings. Below we briefly consider several variables that have contributed to the controversy surrounding BPA: 1) the species and strain of animal tested, 2) the route of exposure, 3) sources of exogenous estrogens, 4) EDC-enhancing compounds, and 5) comparisons between in vitro and in vivo studies.

Species and Strain Differences: Are Rodents Accurate Predictors of Human Effects?

Differences among species or strains of animals are always an important consideration—environmental exposures may affect individual species differently and, within species, genetic background differences frequently underlie differences in response. With respect to BPA, this variable has come into play in two ways, first as a concern in translating effects seen in animal models to assessments of human risk and second as a variable that may explain differences in study results.

Perhaps the strongest argument that rodent models are, indeed, pertinent to humans comes from studies of another synthetic estrogen, DES, which was administered to millions of women from the late 1940s to the 1970s in an attempt to prevent miscarriage. Unfortunately, it provided no such benefit and instead had unexpected negative consequences: it is now evident that men and women who were exposed to DES in utero have an increased incidence of reproductive tract aberrations, infertility, and breast and testicular cancer [26]. As studies conducted in rodents *after* the first report of adverse effects in DES-exposed humans attest, in the case of DES the defects are remarkably similar among species [27]. Further, rodent studies have extended the concerns about DES exposure, revealing new aberrations that were subsequently observed in humans (e.g., abnormalities of the oviduct [28] and uterine fibroids [29]) and identifying DES-induced changes that could be transmitted to the offspring of females exposed to DES in utero [30, 31]. Although this latter, transgenerational effect is far more difficult to discern in humans, there is some evidence that the children of DES daughters have an increased incidence of reproductive tract abnormalities and cancers [32–34].

Rodent studies also suggest remarkable consistency in the effects of different exogenous estrogens, with prenatal or perinatal exposures to DES, phytoestrogens, and BPA all evidencing a disruptive effect on the female reproductive tract [29, 35, 36]. Indeed, a recent BPA study demonstrated that even very low doses of BPA increase the incidence of both benign (e.g., ovarian cysts, cystic Wolffian duct remnants, proliferative lesions of the oviduct, uterine adenomyomas) and malignant (e.g., cystadenoma, stromal polyps, stromal sarcoma) reproductive tract lesions [37]. Taken together, these findings suggest that the argument that studies in rodents do not allow us to make predictions about humans has little, if any, validity when it comes to reproductive effects of EDCs.

Against this background, it is important to recognize that within-species variation may affect the ability to make inferences from rodents to humans. For example, many of the studies that have reported no adverse effects of BPA exposure have used the Charles River Sprague-Dawley (CD-SD) rat [14]. Unfortunately, this is not an optimal model for

studies of EDCs: the CD-SD rat is considered to be estrogen insensitive, as it has very low sensitivity to ethinyl estradiol, the potent estrogenic drug used in birth control pills [38]. Indeed, when studies using this animal model were removed from the meta-analysis [2] discussed above, the number of government-funded studies reporting adverse effects as a result of BPA exposure increased from 90% to 96%. Thus, it is critical that appropriate model species—and strains—be used in making comparisons to the human condition.

The Exposure Paradigm—Are Some Approaches "Better" Than Others?

The route of exposure of an EDC is an important variable that may affect the results of individual studies. For BPA, oral exposure has been suggested to most accurately mimic human exposure and, therefore, to be the most appropriate route of exposure [39]. In addition, because oral exposures are subject to first-pass metabolism, concerns have been voiced that other routes of exposure (e.g., injection, subcutaneous pellet implantation) produce inappropriately high levels of biologically active BPA [20, 40]. Indeed, in a recent assessment of the developmental effects of BPA conducted by a panel of the U.S. National Toxicology Program's Center for the Evaluation of Risks to Human Reproduction (CERHR), this rationale was used to eliminate many studies from consideration (November 26, 2007, http://cerhr.niehs.nih.gov/chemicals/bisphenol/BPAFinalEPVF112607.pdf).

The argument that oral ingestion constitutes the only valid exposure paradigm for BPA studies is flawed for at least two reasons. First, although it is true that, in the adult, levels of biologically active chemical circulating in the blood will be influenced by the route of exposure, it is far less clear that oral exposure is the only—or even the major—route of human exposure [4, 41]. Indeed, a recent study in humans provides compelling evidence that oral ingestion of contaminated food and beverages alone is insufficient to explain BPA levels in humans [42]. Second, recent evidence demonstrates that concerns about variability among developmental studies due to the route of exposure are overstated [39]. Specifically, a comparative analysis of the effect of exposure paradigm (subcutaneous injection vs. oral administration) on circulating levels of biologically active BPA in the neonatal mouse revealed no difference in plasma levels throughout the 24 h following administration. Remarkably, however, circulating levels of biologically active BPA in neonatal mice following oral administration were approximately 10-fold higher than those reported in adults [39]. Although this is perhaps not surprising given the comparatively low level of liver enzyme activity in newborns, it is nevertheless sobering. Indeed, this finding underscores the need for a better understanding of current routes of human exposure to BPA and other EDCs and demonstrates that attention should focus on the fetus and neonate.

Exogenous Estrogens Are Difficult to Control

Although the primary route of human BPA exposure has been assumed to be through contaminated food and beverages, as discussed above, there is growing concern that oral exposure may not be the only—or indeed the major—way that we are exposed. Equally important, it is difficult to control for exposures to other EDCs, since BPA is only one of many EDCs present in our environment. Indeed, in animal studies designed to test the effects of BPA, the inadvertent introduction of other environmental contaminants—most notably environmental estrogens—is a growing concern. Our own experience with damaged caging materials [1] powerfully illustrates the fact that estrogenic exposures can occur in unexpected ways. Unfortunately, in an animal facility the potential sources of environmental estrogens are numerous and represent variables over which the investigator has little or no control. Indeed, laboratory animals are virtually surrounded by sources of exogenous estrogens, including dietary estrogens, mycotoxins, pesticides in their own right or as contaminants in bedding or water, chemicals that may leach from plastics, and compounds that may be present in cleaners and disinfectants [43].

Given the number of potential sources and the seemingly ever-growing list of suspects, controlling for estrogenic contaminants is a moving target. The most obvious source, diet, provides a useful example of a variable that can and should be carefully controlled, but is rarely considered in the design of experiments. Variability in dietary estrogens among commercially available rodent feed was first reported in 1987 [44] and, more recently, the use of a diet low in phytoestrogens has been recommended for studies of EDCs [43]. The issue, however, is not quite so simple for two reasons: First, variability in dietary estrogens is not limited to differences among diets, since considerable variation has been reported among different lots of the same laboratory diet [43, 45, 46]. This has led to the recent recommendation that investigators not only provide information on the diet used in their studies, but also the mill date and estrogenic content [46]. Second, although a diet low in dietary estrogens provides a seemingly logical means of eliminating dietary effects, recent data demonstrate that, paradoxically, animals born to mothers consuming such a diet exhibit "fetal estrogenization syndrome" [47]. Thus, even on a controlled dietary regimen, unanticipated effects may occur.

Given the complexity of this issue, it seems likely that differences in dietary estrogens alone may account for much of the variability in the results of studies of the estrogenic chemical BPA. Indeed, our own recent studies provide evidence that dietary estrogens temper the effect of BPA on the periovulatory oocyte [48]. We compared the frequency of meiotic abnormalities in oocytes from females on diets that were either high or low in dietary estrogens (phytoestrogens) and observed remarkable differences in the effects of BPA exposure. On the low phytoestrogen diet, there was an increase in meiotic defects with increasing BPA dose, as expected. However, the results associated with the high phytoestrogen diet were more surprising: though the effect of BPA was most evident at the highest doses, the level of meiotic abnormalities was actually higher in oocytes from control females than in those exposed to the lowest BPA doses. Thus, at low BPA doses, the high phytoestrogen diet appeared to "protect" oocytes from BPA, suggesting a complex relationship between the two exogenous estrogens. A similar protective effect of dietary estrogens was observed by Dolinoy et al. [49] in studies of the hypomethylating effect of BPA on the A^{vy} metastable epiallele of the mouse Agouti gene.

Chemicals That Enhance the Activity of EDCs

The recent recognition that some chemicals enhance the actions of endogenous hormones or EDCs raises concern about confounders that may be introduced through washing and sanitizing procedures in animal facilities. A group of antimicrobial agents (triclosan and a group of carbanilides, including triclocarban [TCC]) that are commonly added to soaps and other personal care products were tested for endocrine-disrupting properties using cell-based screening

assays developed by the University of California, Davis Superfund Basic Research Program [50]. Surprisingly, TCC and its analogs had little or no endocrine activity in estrogen receptor (ER) and androgen receptor (AR) cell bioassays, but were found to markedly enhance the ability of steroid hormones to induce ER- and AR-dependent reporter gene expression in recombinant cell bioassays [51]. In subsequent studies, the amplifying effect of TCC was verified in in vivo studies [51]. The detection of TCC and related compounds in water systems [50], the presence of these compounds in a wide variety of cleaning products, and the potential for bioaccumulation raises the possibility that these compounds or others like them may be serious but unrecognized confounders in studies of EDCs.

In Vitro Systems: A Critical but Incomplete Tool

The use of in vitro models has been critical for the study of EDCs, i.e., for testing the estrogenicity of chemicals and for identifying chemicals that enhance the effects of normal hormones and/or EDCs [50]. However, because disturbances in the endocrine system can affect many different systems, understanding the effects of EDCs is best accomplished by combining in vitro with in vivo approaches.

Analyses of the effect of BPA on the periovulatory oocyte provide a useful example. The potential impact of BPA has been evaluated using both in vitro [16, 17, 19] and in vivo [1, 18, 19] approaches, but differences in the results of these studies have led to considerable confusion about the effects of BPA on the periovulatory oocyte. We have suggested [48] that this among-study variation reflects differing modes of action of BPA, some detectable by in vitro analyses and others by in vivo examination. For example, in vitro exposures of either cumulus-enclosed [16] or denuded [19] oocytes have been associated with meiotic spindle aberrations and cell cycle delays, findings consistent with previous observations of BPA-induced disturbances of microtubule dynamics in somatic cells [52, 53] and sea urchin eggs [54]. In contrast, cell cycle delays have not been observed in studies using in vivo exposures [18, 19]. Presumably, this reflects the fact that in vivo effects of BPA on the preovulatory oocyte are indirect, acting via the granulosa cells rather than directly on the oocyte cytoskeleton, i.e., resumption and completion of the first meiotic division normally occurs within the follicle, with ERβ receptors expressed by the granulosa cells playing a primary role in regulating follicle maturation [55]. Thus, in vitro conditions are more relevant after ovulation, when the egg is suspended in follicular fluid rather than when being influenced by the follicle.

The likelihood that in vitro and in vivo exposure paradigms assess different mechanisms of action precludes direct comparisons of the results of the two types of studies. It does not, however, diminish the importance of either methodology; both have biological relevance, although they provide different insights. The in vitro exposure paradigm, although likely irrelevant or secondary to the first meiotic division, is clearly pertinent to the postovulatory egg (i.e., at the time of fertilization and the completion of the second meiotic division) and to the early cleavage division embryo moving through the female reproductive tract. In contrast, in vivo exposures are relevant to both the resumption and completion of the first meiotic division in the follicle-enclosed oocyte, as well as all divisions that occur as the egg/embryo moves through the female reproductive tract. Thus, in considering the evidence from in vivo and in vitro exposures, it is crucial to remember that the two approaches may uncover different mechanisms of action of EDCs.

REPLICATION OF EXPERIMENTAL DATA: COMPLETE ACCORD OR WEIGHT OF EVIDENCE?

As detailed above, the presence of a variety of confounding factors poses serious problems for studies designed to assess the reproductive effects of EDCs. Consequently, it is not surprising that, in studying the effects of EDCs, similar analyses have often produced different results—indeed, it could be argued that study replication is more difficult in this field. Then how should we interpret differences among studies of EDCs? Should lack of accord lead us to dismiss findings or is the weight of evidence more important in assessing risk to humans?

Recent studies of the effects of BPA on meiosis in the periovulatory egg provide an instructive case in point for this discussion. As described above, in our initial studies of EDCs [1] we reported an increase in meiotic aneuploidy following exposure of the growing follicle to BPA. Subsequently, four studies have examined various aspects of this same problem, with somewhat different results. For example, following either in vitro or in vivo BPA exposures: Can et al. [16] reported alterations in the centrosome and spindle organization; Lenie et al. [17] observed chromosome alignment abnormalities in a high proportion of oocytes at both meiosis I and II; Eichenlaub-Ritter et al. [19] reported an increase in meiotic arrest; and Pacchierotti et al. [18] reported an increase in premature sister chromatid aberrations at meiosis II. However, notably, none reported an increase in aneuploidy, as we had observed in our original study.

How are we to interpret these observations? One conclusion might be that, since none of the studies reported identical abnormalities, the risk of meiotic defects following BPA exposure has yet to be demonstrated. However, this clearly flies in the face of the evidence. That is, all studies reported that BPA induces detectable meiotic disturbances in the periovulatory egg. On this basis alone, it seems prudent to conclude that BPA has the potential to disrupt the egg. Whether BPA exposure increases the risk of aneuploidy is more difficult to address. None of the recent BPA studies have directly addressed the question of a link between disturbances in chromosome alignment at metaphase I and aneuploidy at metaphase II, although such a link is supported by the results of related non-BPA studies by us [56] and by others [57]. Moreover, the single study that indicated no link between BPA and aneuploidy suggested an equally deleterious BPA effect, i.e., it was postulated that oocytes exhibiting gross disturbances in chromosome behavior at metaphase I are prevented from completing the division by the actions of a checkpoint mechanism. Because neither an arrested oocyte nor an aneuploid egg are compatible with the production of a normal offspring, the finer details of the BPA defect(s) are irrelevant to the larger discussion of the risk of BPA exposure. Thus, to dismiss the meiotic data because there is not complete accord is akin to throwing the baby out with the bathwater: the weight of evidence strongly suggests that BPA disrupts female meiosis.

Similar differences in results plague the early evaluations of prostate and testis development, with some, but not all, studies reporting drops in testis size or sperm counts or changes in prostate size. Subsequent studies have been much more consistent in finding such defects and, further, have established links between fetal exposures and increasing rates of adult cancers and are beginning to unravel the molecular basis of these changes.

This raises an intriguing question: In assessing the effects of environmental chemicals with hormone-like activity, which is more important: strict study replication or general accord? With

respect to BPA, subtle differences in results have been effectively used to diminish the impact of new findings and slow the wheels of regulatory action. The weight of evidence of hundreds of low-dose studies coupled with the willingness of a small group of scientists to voice their opinions [21, 58] has finally begun to shift the balance. This raises our final question: What is the appropriate role of the scientist in the public and regulatory arena?

SUMMARY AND THOUGHTS ABOUT THE FUTURE

The BPA saga provides a tutorial on how one of the world's strongest regulatory agencies, the FDA, works—or possibly, in the case of EDCs, fails to work—to protect the public. For example, although chemicals such as BPA are used in food packaging, they are not subject to the same federal regulations as chemicals that are added to our food. Further, though it has long been recognized that current testing paradigms do not work for EDCs, new testing guidelines still are not available.

Scientists, by the nature of our work, are apolitical. Two decades ago most reproductive biologists were skeptical about reports that sperm counts were dropping, infertility was on the rise, and that the incidence of breast, prostate, and other cancers was increasing. Today, far fewer of us dispute these statements, and many have become convinced that environmental exposures—specifically exposures in utero—contribute to the changes that have occurred in the span of one to two human generations. A growing number of us have also become convinced that speaking to the press and the general public about our findings and our concerns should be a clear part of the job description of the next generation of reproductive biologists. The BPA experience has taught us the value of communicating complex scientific findings to the public in terms they can understand and of sending clear messages about the potential impact of our findings to government decision makers. By refining our communication skills, we can be powerful spokesmen for better test systems and thereby assist government regulators in insuring the preservation of our reproductive health.

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