ETX 298 – Summer/Fall 2014

"Emerging Concepts in Environmental Health Science"

Bisphenol A and Plastics: How safe are they, and how concerned should we really be?

Participants: D. Banda, H. Chen, K. Dunaway, E. Karey, S. Tang, R. Zhang

Introduction

The central topic of debate in this class revolved around the safety of plastics, particularly whether plastic monomers like bisphenol A (BPA) and plasticizers such as phthalates were of concern to human health. The discussion was engaged through two antithetical points of view: that BPA and phthalates are safe, and that these chemicals are indeed harmful to human health. These perspectives reflect the muddled public opinion regarding these chemicals, and scientific research has done little to quell the controversy around BPA and phthalates. The goal of this class was to provide a forum for an evidenced-based discussion on BPA and phthalates, and consequent of that, provide insight into the strengths and pitfalls for the two divergent perspectives regarding these chemicals. From this debate, participants were able to develop consensus points as well as identify critical areas of scientific research necessary for moving the debate forward.

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Final Report – Group One

Indicting Plastics: How Bisphenol A and Phthalates Impact Human Health

H. Chen, S. Tang, R. Zhang

Preamble

Countless studies have explored the toxicological effects of bisphenol A (BPA) and phthalates, but many publications over-report these effects by investigating concentrations irrelevant to human exposure levels. The reported toxicological endpoints of BPA and phthalates, both widely-used plasticizers, are many fold, and while it is difficult to argue these chemicals to be toxic to the average healthy individual, we recognize that there exist subpopulations (whether based on age, sex, region, race, socioeconomic status, employment, or concomitant diseases) in which BPA and phthalates can elicit serious untoward effects. Below, we 1) address certain health effects of BPA and phthalates deemed to be of concern, 2) explore potential susceptible sub-populations, and 3) provide recommendations moving forward regarding plastic monomers and additives.

Bisphenol A

A primary monomer of concern is BPA, which is utilized in polycarbonate plastics, epoxy resins, and in package linings. Studies have shown that levels of unconjugated BPA in human serum can range from 0.1-10 μg/L, with higher levels generally found in occupational settings (1, 2). The US Environmental Protection Agency (EPA) has calculated a reference dose of 50 μg/kg (including a safety factor of 1000), with a maximum tolerated dose at 1000 mg/kg. However, these values are of debate due to the assumption that BPA's effects exhibit a monotonic increase (3).

Phthalates

Phthalates are ubiquitous plasticizer additives that are incorporated to give plastics flexibility and elasticity (4). There are over 25 phthalate esters (abbreviations for some can be found in Table 1) and a 2004 NHANES study of urinary levels documented geometric mean concentrations for several phthalates of 5.1 μg/L for DEHP, 91.3 μg/L for DEP, 41.4 μg/L for DBP, and 39.4 μg/L for BBP from a population of 324 US children aged 6-11 (5). Retrospective studies have shown median daily intakes of 4 mg/kg of DEHP in a European population (6). Reference doses by the EPA vary according to ester, but for DEHP, chronic oral exposure is set at 0.02 mg/kg·day whereas the European Food Safety Authority value is 0.05 mg/kg·day (7). The phthalates are of particular concern due to their widespread occurrence, including medical settings, where plasticizer levels are found in neonates at levels higher than tolerable daily intake (8).

Table 1 – Common phthalates and their abbreviations

Phthalate Name	**Abbreviation**
Di-2-ethylhexyl phthalate	DEHP
Diethyl phthalate	DEP
Dibutyl phthalates	DBP
Benzylbutyl phthalate	BBP

Health Effects

Neurobehavioral

Evidence suggests that children exposed *in utero* or before puberty to BPA exhibit altered neurobehavioral characteristics. Gestational BPA exposure has been linked to increased hyperactivity, anxiety and emotional and behavioral control in tested 3 year olds, with the geometric mean of urinary BPA of the mothers being 2.0 μg/L (9). Another report details a significant negative association between maternal urinary BPA levels (geometric median of 1.2 μg/L) and a 7-9 year old child's performance on an autistic behavior test when outliers are accounted for (10).

Phthalates likewise show a similar effect on children. A sample of children in Korea between 8-11 years old showed a correlation between urinary phthalate levels and both teacher-rated ADHD symptoms and performance on an ADHD continuous performance test, with mean concentrations of urinary phthalate metabolites of mono-2-ethylhexyl phthalate (MEHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono-*n*-butyl phthalate (MNBP) being 34 μg/dL, 23.4 μg/dL, and 46.7 μg/L respectively (11). A prospective Mother's and Children Environmental Health study by the same group revealed prenatal exposure to phthalates inversely correlates with Mental and Psychomotor Developmental Indices, particularly in males, when assessed at 6 months of age(12). Furthermore, levels of urinary MEHP, MEOP, and MBP were detected at slightly lower levels (8.9 μg/L, 7.4 μg/L, and 12.4 μg/L) than those seen in an earlier NHANES study amongst a Norwegian cohort (13). A 2014 study showed similar results, with 6-10 year old boys testing higher for measures of inattention, aggression, and poor conduct when correlated with maternal urinary phthalate levels (composed of DEHP and DBP metabolites) (14).

Reproductive Effects

There is accumulating evidence to show that BPA affects adversely on reproduction systems from animals to humans, especially for ovary's function. BPA's effects on female meiosis were discovered accidentally by Patricia Hunt's group in 2003. What they found was that polycarbonate cages and water bottles that had been damaged by detergent released BPA and this led to a higher incidence of chromosome congression failure and aneuploidy in female mouse. In that study, they also determined that relatively low dose (20 μg/kg body weight/day) and short exposure (7 days) of BPA can elicit significant meiotic defects (15). Later, this research group further found out that BPA (20 μg/kg body weight/day) can affect early stage of oocyte development by interrupting meiotic recombination, such as synapsis between homologs and increase in recombination. Interestingly, homozygous knockout of estrogen receptor β (ERβ) in mice resulted in the same early meiotic defects; BPA exposure elicited no additional effects in ERβ null females (16). Based on these results, the authors proposed that an environmentally relevant level of BPA can disrupt oocyte development by interfering with the estrogen signal pathway in vivo. BPA's negative effect on oocyte development was also confirmed in other organism such as *C. elegans* and rhesus monkey. In *C.elegans*, a relatively higher level of BPA (2 μg/g worm extract) leads to lower fertility and higher embryonic lethality. In addition to the defects in synapsis of homologs and changes in chromosome architecture, down expression of repair factors might explain persistent DNA double strand breaks observed after exposure to BPA (17). Highly consistent with

the mouse data, at a level similar to human exposure (2-5 ng/mL), BPA results in higher recombination events between homologs and defects in synapsis. In addition, a single daily oral dose of BPA on later stage of fetal ovary disrupts the follicle development, leading to higher incidence of multioocyte follicles (18). Therefore *in vivo* experiments in mouse and rhesus monkey have shown that at exposure concentrations similar to those of the general human population, that BPA can disrupt female meiotic recombination and follicle formation. Data from studies of human oocyte cultures also show that concentrations of BPA as low as 1 μM can disrupt the meiotic recombination, leading to higher levels of oocyte degeneration.

Since BPA has been detected in follicular fluid in women who undergo IVF procedures (19), this raises the concern about the potential effect of BPA exposure on human oocyte development. Similar to mammals, several in vitro studies have shown that BPA exposure affects oocyte development at different stages. Machtinger et al. also found a dose-dependent association of BPA exposure with a delay of human oocyte maturation (20). With the increase concentration of BPA (20 ng/mL, 200 ng/mL and 20 μg/mL), less oocytes reach MII phase with higher percentage of chromosome alignment defects, self-activation as well as degeneration. Similarly, data from Brieno-Enriquez and coworkers also showed that BPA (230 ng/mL) disrupts meiotic progress and leads to oocyte degeneration on fetal ovary culture (21).

So far the above evidence strongly supports the idea that BPA is an ovarian toxicant that disrupts this meiosis at the early stage and follicle formation at later stage during ovary development. In other words, pregnant women are more vulnerable to BPA exposure.

Cardiovascular Effects

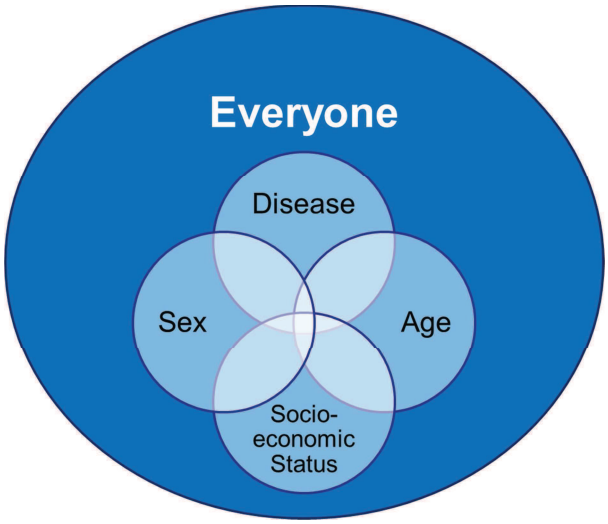
A cross-sectional analysis of 2003-04 NHANES data, and published in 2008 in the *Journal of the American Medical Association*, reported significant associations between higher urinary BPA concentrations and cardiovascular diagnoses (angina, coronary heart disease, heart attack) (22). Since this report, several studies have explored possible electrophysiological mechanisms by which BPA could alter cardiac physiology, including 1) activating the large conductance Ca^{2+}-activated K^{+} channel (23), 2) inhibiting various voltage-sensitive Ca^{2+} channels, including the cardiac L-type isoform (24), and 3) inhibiting the cardiac sodium channel (25). Despite these revelations that BPA may interact with ion channels, they share a common flaw: the EC_{50}/IC_{50} for each reported effect was >10 μM, well above typical human exposure levels. Several studies reporting on the cardiovascular effects of DEHP and MEHP exhibit the same pitfall.

One group, however, has published findings regarding the arrhythmogenic potential of BPA at concentrations well within typical human serum BPA levels. Specifically, the authors reported a potentiating effect between 17β-estradiol (E2, 1 nM) and BPA (1 nM) in producing ectopic Ca^{2+} transients in female murine cardiac myocytes in vitro, but not in male myocytes nor in female ERβ knockout myocytes (26). Moreover, BPA alone (1 nM) or in combination with E2 (1 nM) dramatically exacerbated ventricular arrhythmias during reperfusion following an ischemic event in female, but not male, murine hearts (27). The potential for simple arrhythmic events to devolve into sustained, and

lethal, ventricular fibrillation presents a novel toxicological endpoint for BPA, one that does not necessarily require chronic exposure and time to manifest, but one that can precipitate immediately upon exposure. Based on these studies, it is evident that, in terms of arrhythmogenic risk, BPA's effects are vastly skewed towards the female sex, which essentially creates a risk group comprised of one half of the human population (*vide infra* regarding sex-based risks). Furthermore, while these studies suggest that BPA alone may be sufficient to precipitate acute arrhythmic events, especially in females, the absolute risk of cardiac arrhythmias is heavily confounded by acquired diseases such as congestive heart failure (28), genetic channelopathies such as long QT syndrome (29), and consumption of certain medications (30, 31). Examining how BPA's arrhythmogenic potential interacts with these conditions will reveal potential subpopulations at greater risk of BPA intoxication.

Populations at Risk

The adverse effects of plasticizers may be influenced by a number of factors and by the endpoint being investigated, with certain individuals amongst the general population being particularly susceptible.



Age

Like all chemical toxicants, the timing of BPA and phthalate exposure plays a significant role in determining a toxicological effect. In studies related to neurobehavioral outcomes, behavioral endpoints *in vivo* have shown different results based on the timing of exposure of BPA and even duration (32, 33). Furthermore, most literature suggests that it is primarily prenatal exposure that is associated with adverse neurodevelopmental outcomes. Beyond behavioral endpoints, perinatal exposure paradigms in rodents have also revealed additional toxicological endpoints such as liver injury (34), food intolerance (35), increased pro-inflammatory mediators (36), and predisposition to metabolic syndrome (37) in the offspring. While there have been reports of *in vivo* effects on the rat brain, NHANES studies in the elderly have shown there is no correlation between declines in cognition and circulating levels of phthalates (38, 39).

Analysis of NHANES data has revealed a negative correlation between age and urinary BPA concentrations (40), and premature infants in neonatal intensive care units can often have more than ten times the urinary BPA concentration (geometric mean of 30.3 μg/L) as teenagers and adults (41). While premature infants in a hospital setting appear to be the most susceptible to BPA intoxication due to their exposure to plastic medical products, neonates in general are at higher risk owing to their decreased capacity for drug metabolism and clearance. Specifically, isoforms of UDP-glucuronosyltransferase (UGT), the primary enzyme responsible for metabolizing BPA and certain phthalates, do not reach adult levels until at least 1 year after birth (42). In this respect, infants and young children would benefit greatest from abstaining from excessive exposure to plastics in both domestic and medical environments.

Sex

As BPA is classified as an estrogenic chemical, it appears to have a sex-specific influence (43). While certain phthalates are generally classified as having estrogenic properties. Some of the data is conflicting and neurobehavioral endpoints suggest that phthalates affect sexes in a different manner than BPA, with BPA having a more adverse outcome on girls and phthalates on boys according to neurobehavioral measurements (9, 12, 44, 45). Moreover, as elaborated above, BPA appears to differentially predispose individuals to cardiac arrhythmias based on sex. However, BPA's effect on female cardiovascular health is not expected to be monolithic, but furthermore stratified by age (pre- or post-menopausal) and disease (coronary heart disease). This is based on well-established observations that while women have a significantly lower risk of coronary heart disease versus men, prognosis following acute myocardial infarction is significantly worse for women (46). Since cardiac arrhythmias often occur following myocardial infarction, BPA exposure (perhaps through medical equipment) may become an additional risk factor in women being treated for this condition.

Region and Race

Multiple studies have shown that non-Hispanic blacks have a higher burden of BPA and phthalate metabolites than whites, while Mexican Americans have lower levels than both (40, 47-49).

Socioeconomic Status

Evidence suggests that individuals with lower incomes have a higher burden of BPA (50). However while social class and education also appear to be positively correlated with certain phthalate metabolite exposures, there are also some studies that show that there is a negative correlation between exposure and poverty income ratio and these burdens depend on the metabolite being accounted for (47, 51-53).

Disease

Human exposure to BPA can result in blood levels comparable within the biologically active range in over 95% of people sampled (54). Given the wide range of adverse effects of BPA and chronic low level exposure of individuals in developed countries, certain populations are likely uniquely susceptible to

disease risks by this compound. Both BPA and phthalates may have epigenetic effects, thus serving as a potential risk factor in disease susceptibility (55, 56).

BPA has been shown to adversely affect glucose metabolism, with environmentally relevant concentrations (1 nM) sensitizing glucose-induced insulin secretion from islets from human donors and causing closure of K_{ATP} channels (57). Indeed, urinary BPA concentrations in adults (20 and over) have shown positive correlation with cardiovascular diseases and diabetes (22, 58). Associations between obesity and high BPA exposure have been made both in children and in adults (59, 60). BPA has also been shown to induce adipocyte differentiation of preadipocytes at higher concentrations (25 µM and up) (61). Phthalates have also been linked to insulin resistance, and certain metabolites with diabetes (22, 62).

While BPA and phthalates may exacerbate certain disease conditions, conversely certain diseases may magnify the effects of BPA by altering its pharmacokinetics in the body. Genetic disorders typically associated with hyperbilirubinemia such as Gilbert-Meulengracht syndrome, which affects 5-10% of the population, and Crigler-Najjar syndrome can lead to various degrees of decreased UGT activity (63), and ultimately impair the capacity to clear BPA and phthalates from systemic circulation. Liver diseases such as hepatitis and cirrhosis, as well as hypothyroidism may similarly impact glucuronidation capacity. Concomitant exposure to medications, or other environmental pollutants, can also potentially influence the metabolism of BPA and phthalates (64). This final notion perhaps best reflects actual human exposure to these chemicals: not one at a time, but in a mixture with complex, unknown, interactions.

Potential Action Plan

Even though there have been increasing studies of BPA on human health issue, a number of things can be done to make the results are more comparable or relevant to human exposure. For examples, measurement of the free but not the conjugated form of BPA at multiple time points (serum or urinal samples) may more directly indicate the relationship between the active BPA and certain effect. Using more reliable method such as isotope-dilution HPLC-mass spectrometry can avoid sample contamination (65).

Based on the results from increasing publications, the negative effects of BPA on human health become more obvious, especially on neurodevelopment on children, female reproduction system, cardiovascular diseases, type-2 diabetes as discussed above. More importantly, most of these negative effects are correlated to environmental exposure level of BPA. Given the high volume production of BPA and its wild use in daily life, we need to take some measures to reduce BPA's effect on human health issues in the future. For example, subpopulations of people who are more vulnerable than those such as pregnant women, neonatal and postnatal individuals should be limiting their exposure, in order to minimize to minimize or reduce BPA's negative effects.

Another way is to look for "safer" alternative of BPA. However, it may not be so simple. Since some structural analogues to BPA also have been shown to have the same qualitative effects on estrogen receptor and androgen receptor activities, it will risky to simple replace BPA with other similar analogues without comprehensive safety evaluation studies (66).

Although there are accumulating studies of BPA on human health, a lot of studies only show the correlation between BPA level and some kinds of health effects. But in reality, there are many other factors that may contribute to the same effects. It will be important to test whether other factors such as other chemical will show synergistic effects when combined with BPA.

Further research regarding the effects of BPA and phthalates are indeed needed to better understand how these chemicals impact human health. Perhaps, a new approach to this research is needed. Most toxicological research thus far have focused on exploring the effects of BPA and phthalates *per se*, and often involve dosing beyond typical exposure levels in order to observe an adverse endpoint. However, since BPA has been shown to interact with a variety of targets, research should instead focus on whether these chemicals potentiate endogenous (patho)physiological processes in order bring about untoward outcomes. The previously described cardiovascular studies in female hearts with BPA are a prime example of this sort of research question.

The ideal solution for preventing BPA- and phthalate-induced toxicity in specific subpopulations would be to completely abstain from using plastics. However, this would be infeasible since plastics have become an essential component of modern technologies and standards of living. As plastics will never be eliminated, the onus is on the consumer to exercise vigilance and avoid certain monomers and additives like BPA and phthalates. However, even this option is fraught with imprecision, as several BPA-free plastics have been shown to nonetheless leach estrogenic chemicals (67). Estrogenicity does not necessarily equate to endocrine disruption, but public opinion often assumes endocrine disruption potential from estrogenic activity. A better understanding of these related, but distinct, definitions would allow the public to adequately appreciate the dangers of certain plastics without overreacting. Ultimately, BPA and phthalates should be viewed as highly modifiable risk factors in the milieu of human health and disease, and that by banning or severely restricting these chemicals from consumer exposure would greatly improve health outcomes.

Overall Conclusions

There is a significant body of evidence that BPA and phthalates can negatively impact neurobehavioral, reproductive, and cardiovascular endpoints. However at relevant human exposure levels, these effects may not be consequential to a healthy adult. The timing of exposure is critical and infants and young children may be most at risk due to their reduced capacity to metabolize toxicants as well as ongoing development. Thus the toxicological impact of BPA and phthalates should be evaluated based on both the timing of the exposure and the specific subpopulations that may be more susceptible or have higher body burdens of these compounds. We also recommend further research into how BPA and phthalates may potentially exacerbate existing diseases and how similarly structured compounds could impact human health.

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Final Report – Group Two

BPA: Big threat or much ado about nothing?

Introduction

Bisphenol A (BPA) was first synthesized in the late 1800s[1], but it wasn't until the mid-1950s, when the plastics industry exploded, that it began flooding the consumer marketplace. When BPA reacts with phosgene ($COCl_2$), the result is a durable polycarbonate that can be used in a wide array of applications ranging from electrical insulation to lightweight construction materials and food contact surfaces.[2] The resulting ubiquitous crystal-clear BPA-based plastics offered a much welcomed shatterproof substitute for glass for more than half a century. Advertised as safe, strong, and inexpensive, these materials were presumed to be as benign as glass. However, an accidental discovery in 1992 turned up evidence of estrogenic activity associated with polycarbonate plastic flasks, but not glass ones. This implicated BPA as a synthetic estrogen.[3] The discovery — coupled with BPA's now widespread use in food storage containers, including baby bottles — raised concerns that BPA might pose a significant risk to human health throughout the developed world.

The World Health Organization defines endocrine disrupting chemicals (EDCs) as "compounds either natural or synthetic, which through environmental or inappropriate developmental exposures alters the hormonal and homeostatic systems that enable the organism to communicate with and respond to its environment."[4] The endocrine system includes more than ten tissues that influence almost every cellular function.[5] Responsive tissues are exquisitely sensitive to low levels of circulating hormones. Indeed, very low levels can regulate a wide variety of functions including growth, development, sexual processes, reproduction, metabolism and mood.[6] While the endocrine system is responsible for regulation of critical physiological activities throughout the day, such as circadian behavior and metabolism, the most sensitive windows of exposure are during development and sexual maturation. This is when tissue signaling and differentiation are particularly vulnerable to environmental perturbations.[7]

BPA's potential to alter normal physiology through chronic exposure to an exogenous estrogen mimic has been a contentious area of concern.[8] A growing body of literature supports the argument that BPA can act as an EDC, triggering myriad negative health impacts.[9,10]

However, the experiments that turned up such impacts have been conducted using a wide variety of species, ages, doses, and routes of exposure. This broad variability has made comparisons of apparent or potential toxicity between experiments difficult to assess.

To ascertain BPA's toxicity, we attempt to identify benchmarks across studies that represent relevant dosing ranges, model species, and windows of peak susceptibility. We hope that by better defining these parameters, potential alternatives to BPA can be tested with the same rigor and quality so that EDCs can be more definitively characterized in the future.

Requisite Experimental Considerations

Abundant information about BPA's alleged toxicity exists. But how useful is it? While many studies provide evidence suggesting health risks associated with BPA exposure, few definitively indict BPA to adverse health outcomes. Screenings and correlation are useful for initially flagging potentially dangerous chemicals. But thorough follow-up testing is then needed to establish the physiological mechanisms that might explain how exposures could lead to the witnessed adverse impacts. We have therefore identified three benchmarks that must be met to establish the toxicological relevance of a chemical-specific adverse effect, namely: use of

relevant human-exposure levels, an appropriate animal model, and an appropriate routes of exposure.

1. **Relevant human exposure**. Although an extensive array of studies indicate BPA might adversely affect human health, one must consider the dose of BPA used in these experiments in order to accurately assess the potential risk of a given population. To accurately gauge human exposures to BPA, Teeguarden and Hanson-Drury have evaluated urine-based biomonitoring data from 18 studies, including two national-scale studies[11]. Across all age groups, mean human exposure was determined to be 0.07 ug/kg/day, with an upper exposure limit of 0.5 ug/kg/day. The authors then conducted an analysis of reported "low dose" exposure studies from the published literature in order to assess exposure levels used in mouse and/or rat models. They identified 123 *in vivo* studies that met the criteria for inclusion. Data from those studies reported "low dose" exposures that spanned more than 9 orders of magnitude (from 0.005 to 10,000,000 µg/kg/day), see *Figure 1*, below. Shockingly, only 6% of the 342 different concentrations tested in these studies were actually in the range of adult human exposure of which a mere 1% exposed animals orally. Similarly, only nine percent of the 342 experiments exposed animals to BPA in ranges that fell within observed ranges reflective of infant and child exposures; of those studies, only 1% reflected oral exposure. More importantly, no studies examined more than two exposure concentrations that fell within the range of likely human exposures. When considering this information, it is clear that guidelines must be established for *in vivo* BPA dosing if we are to estimate the risk of BPA toxicity based on body-burdens measured in human populations.

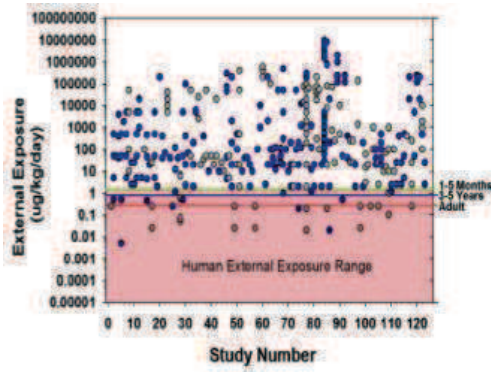

Figure 1. Comparison of BPA exposures from "low dose" *in vivo* animal studies to human aggregate external exposure levels (based on blood serum concentrations). Blue circles represent oral route of exposure and grey circles represent non-oral exposures. Borrowed from Teeguarden & Hanson-Drury, 2013.

2. **Appropriate rodent models**. To understand the effects on human health, a chemical needs to be tested *in vivo*. Since purposefully exposing humans to potentially harmful chemicals is morally and ethically wrong, we rely on animal models as a proxy. However, not every rodent model yields data physiologically relevant to humans. For instance, Sprague-Dawley-derived CD rats are much more resistant to estrogenic disruption of reproduction than are F344 rats[12,13,1415]. Similarly, C57BL/6J mice are than 16-fold more sensitive to estrogenic impairment of male gonadal development by estradiol than are three more sensitive strains (B6, C17/JI, and S15/JI)[16]. Estrogen-resistant rodent strains often are chosen because of their large litter sizes, making them especially useful in research.[17] Their larger litters allow for littermate replicates. However, these resistant strains likely underestimate the effects of EDCs while simultaneously supporting the safety of higher environmental doses of these chemicals. It's unclear which, if any, at-risk human populations these inbred rodent strains might best serve as surrogate model

systems. Another factor complicating the use of rodents in modeling human health is the influence that fetal neighbors have on littermates. A life-long influence of intrauterine hormone signaling has been observed in litter-bearing species as well as in singleton/twin births.[18] However, the fixed position of fetuses within rodent uterine horns guarantees that embryos will be exposed to differential hormone signaling based on the sex of their immediate neighbors — the effects of which are often masked until animals have reached puberty.

3. **Route of exposure**. Human exposure to BPA can occur through various routes including indoor inhalation of contaminated dust and through contact with soil or bathing water that has been contaminated with free BPA from landfills.[19] However, when concerned with BPA exposure, the most significant route is oral exposure[20]. Furthermore, oral exposure in *in vivo* models should reflect exposures from the diet and dental sealants rather than oral gavage. Contact with chemicals such as BPA encountered via the diet can be absorbed more efficiently through oral cavity surfaces, thus evading first-pass metabolism by the intestine and liver[21]. And first pass metabolism via the gut wall is relevant in rats exposed to BPA, but not in humans, where liver first-pass metabolism is more important. Therefore, appropriate *in vivo* studies should accurately reflect this by oral route of exposure through diet or drinking water. Of the 19 *in vivo* studies reviewed by Teeguarden and Hanson-Drury that used BPA exposures in the range of human external exposure — and reported statistically significant effects — two studies exposed animals through drinking water and one study used oral exposure through diet. Fifteen other studies exposed animals through oral gavage or subcutaneous injection and one study injected BPA directly into the brain of rats[11]. From this information alone, it is clear that guidelines for routes of exposure must be established in order to accurately assess relevant human exposures to BPA. Indeed, these data would seem to support the need for further experiments to be conducted on BPA using *in vivo* models.

Overwhelming a system with a foreign chemical is bound to alter homeostasis and normal physiology. Therefore, it's essential to design experiments that mimic actual human exposures with regard to dose, routes of exposure, and windows of vulnerability. In this way, we can better identify whether potential EDCs, including BPA, are toxic to the entire population or just to some especially vulnerable subpopulations. To identify susceptible populations only doses reflective of actual human exposures administered via the primary route of exposure (oral) should be tested. Because inbred strains can vary with regard to toxicant sensitivity, care should be taken when choosing model species and strains to ensure that a study isn't biased toward a resistant or susceptible genetic background that would mask — or exaggerate — findings.

Is BPA Associated with Adverse Health Outcomes?

Now that we have outlined experimental standards for examining BPA toxicity, we will only consider experiments that have met these criteria. Interestingly, the single factor that eliminated most studies was the use of excessively high doses of BPA in order to elicit negative health effects. If we restrict studies to doses of BPA reflective of what occurs in people, 80% of published studies must be discarded: Their administered doses were several-fold too high. In fact, only nine percent of the doses included fall into the highest anticipated dose ranges of human exposures. We further eliminated experiments that used non-oral routes of exposure. That left us with only nine experiments. Of those, only three exposed animals by adding BPA to food or water. These three studies, summarized below, met our criteria for relevant human exposure levels via oral administration.

One study suggested that adult exposure to BPA in drinking water could accelerate the onset of mammary tumorigenesis in genetically engineered mice with a predisposition for developing mammary tumors (MMTV-erbB2 mice).[22] Both low and moderate doses of BPA (2.5 and 25 μg BPA/L drinking water) resulted in mice developing tumors earlier than controls. However, extremely high doses of BPA (250 and 2,500 μg BPA/L) did not differentially alter mammary tumorigenesis. Non-linear dose responses are often observed in EDC studies; endocrine effects occur preferentially at low doses and disappear at higher ones. Another study found that perinatal BPA exposure caused male rats to develop a "sweet tooth".[23] Pregnant mice were exposed to 0.1 and 1.0 mg BPA/L drinking water; F1 males exposed to BPA perinatally displayed a preference to sweeter water and increased body weight compared to male mice without earlier life exposure to BPA. However, F1 female littermates showed an opposite response: their sucrose preference was suppressed compared to female controls. Sexually dimorphic findings are common in endocrine disruption studies, suggesting that EDCs may produce sex-dependent phenotypes through multiple mechanisms.[24,25,26]

The third study confirmed that the initial weight gain observed from perinatal BPA in F1 males was actually due to premature maturation, not acute obesity.[27] These differences in weight from an accelerated maturation were not sustained into adulthood, suggesting that perinatal BPA does not produce adult obesity. However, it is unclear whether this accelerated maturation produces any adverse reproductive effects in the offspring.

BPA Analogs

To avoid potential toxicological risks of BPA, industry has been quick to switch to BPA analogs, like bisphenol S (BPS). In fact, Liao et al. detected BPS in 81% of urine samples from the general population of the United States and of seven Asian countries. This analysis estimated human BPS exposure at 0.930 ± 2.48 μg/day (n=315)[28]. Despite this finding, there is little or no toxicological information on BPA analogs, such as BPS. Yet due to their structural similarities to BPA, these substitutes are predicted to produce a spectrum of adverse effects similar to those caused by BPA. To address this, Rosenmai et al. compared the hazards of BPS, BPB, BPE, BPF and 4-cumylphenol (HPP) to BPA through use of a series of *in vitro* assays [29]. The authors used several cell-based assays to test a given compound's potential to affect steroid hormone synthesis and stimulate hormone-receptor-dependent gene expression. BPA and its analogs showed an effect on estrogen-receptor (ER) and androgen-receptor (AR) signaling and steroid hormone synthesis activity in cells. These cellular-activity assays suggest that all BPA analogs may interfere with the endocrine system to varying degrees and *by different modes of action*. In the H295R assay, BPS was the only analog that did not affect estrogen levels. Overall, BPB and HPP treatment caused the highest increase in estrogen levels when compared to all other analogs.

Anti-androgenic effects often accompany adverse estrogenic male reproductive system effects.[30] Decreases in androgen levels were observed for all test compounds, causing AR-receptor inhibition and affecting estrogen synthesis. BPA and BPE were the most potent compounds in terms of decreasing androgen levels. All compounds except BPA generally increased progesterone levels, with BPS and BPF producing the greatest activation of hormone synthesis. Lastly, BPE and BPF increased corticosteroid levels, which are associated with the development of both cardio-metabolic and behavioral disorders in adulthood.[31] All other compounds, including BPA, led to decreased corticosteroid levels.

From these data, it seems apparent that current BPA alternatives, which often make modest changes to a single substituent may pose a similar health risk to the general population through a

variety of hormone-receptor-dependent mechanisms. However, follow-up studies should be conducted to further support this claim.

Conclusions

The ubiquitous appeal of polycarbonate plastics makes fully characterizing the potential toxicity of BPA essential before it is banned in favor of supposedly "safer" but little-tested alternatives. While *in vitro* screening of compounds can identify potential estrogenic activity on a cellular level, confirming that estrogenicity in an intact animal is far more complicated and difficult to demonstrate irrefutably. At a minimum, it requires testing of compounds *in vivo* to establish whether they elicit a biological/toxicological effect.

Paracelsus, the father of toxicology, is credited for coming up with the fundamental notion, "the dose makes the poison." This mantra is not universally applicable to endocrine disruptors because they can mimic hormonal signaling at concentrations lower than that of typical environmental exposures. Additionally, the onset of observable effects can be delayed greatly from the initial window of exposure — making it difficult to definitively tie a particular toxic response to a particular exposure. As a result, it is important that EDC toxicity parameters be clearly defined so that appropriate assessments of risk can be assigned.

We have attempted to address several inconsistent areas of experimental design that currently limit our ability to draw firm conclusions about BPA's toxicity. Traditionally, it is easier to replace a known dangerous chemical with a novel, presumably safe one — although "safety" is often analogous to "undefined risk." The problem with rushing to substitutes is that this does not necessarily reduce the risk posed to the public; rather, it just substitutes a known, dangerous exposure with a perceived, "safe" unknown.

Plastics are so pervasive in contemporary society that eliminating them due to their potential, low-grade toxicity is not feasible. However, we do appreciate that certain subpopulations, like pregnant women or those with a genetic propensity for an adverse health outcome linked to hormonal imbalances are potentially more sensitive to chronic exposure to these chemicals. For them, it may be prudent to reduce their exposure, if it cannot be prevented, until more data emerge establishing — or largely refuting — toxicity. Therefore, we propose a novel set of mandatory experimental benchmarks to better ascertain the real risks associated with present polycarbonate plastics. These should lead to more realistic and defensible assessments of whether BPA, its proposed alternatives, or other potential EDCs pose true and substantial health risks. Going forward, it will be necessary to implement well-designed experiments that use appropriately sensitive models, dosing paradigms that reflect relevant human routes of exposure, and that windows of exposure that represent the greatest risks. Only then can data from such experiments confidently be compared. Additionally, necessary positive controls need to included in order to implement legislation that better reflects the true risk of toxicants.

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Summary Statements

Summary

Consensus Statements:

- BPA correlative human studies provide sufficient weight of evidence that BPA likely interferes with hormone signaling, producing adverse health effects.
- Numerous epidemiological studies over-report effects of BPA.
- Few existing *in vivo* studies mimic appropriate route of administration or dose.
- Few existing *in vitro* studies use appropriate concentrations reflective of BPA levels in human tissues.
- Not all populations are equally vulnerable to BPA toxicity.
    - Hospitalized infants are especially vulnerable given exacerbated exposure to clinical plastics.
    - Perinatal exposures during development may reprogram health outcomes of fetus later in life.
    - Adults with predisposition to estrogenic-promoted cancers may accelerate tumorigenesis.
    - Adult populations prone to arrhythmias may be more sensitive to chronic, low-dose exposures.
    - Body burdens of BPA and phthalates are not monolithic across the population, but vary according to several factors, including age, sex, socioeconomic status, race, and disease.
- BPA alternatives exist, but these monomers may not necessarily be safer than BPA.
- More rigorous experimental designs are needed to better extrapolate the risks of BPA exposure to human health.

Points of Disagreement:

Sources of contention between the groups was the use of human correlation studies and mammalian models, the importance of the route of administration, and ultimately the final recommendations as to how to deal with BPA and similar analogs. In regards to human correlation studies, a noted factor that was agreed upon was the need for a weight of evidence assessment to evaluate the toxicological potential of a compound. However a clear consensus was not established about the use of different model systems, with groups differing on their evaluation of the utility of *in vitro* systems for characterizing toxicity. The preferred route of administration in these experimental studies was also not fully agreed upon. Proponents against the use of BPA argued that while the oral route of exposure is important, what ultimately matters is the levels of BPA in circulation and in serum, and as advised by other participants of the discussion, the levels at the tissue of interest. Furthermore, proponents against the use of BPA argued that oral route, while being the primary source of exposure, is not the exclusive exposure route. Finally, there was no clear consensus as to how best to deal with BPA, with proponents against the use of BPA preferring elimination of the compound due to being a highly modifiable risk factor that would incur significant cost to regulate across the numerous susceptible populations.

Recognized Gaps in Knowledge:

- Estimates of leaching potential of various BPA- and BPA-analog-plastics based on aqueous or organic nature of contents.
- *In vivo* studies elucidating adverse health effects of BPA linked to activation of estrogen-related receptor gamma, the estrogen receptor with the greatest affinity for BPA.
- Characterization of BPA's influence on non-estrogenic hormone receptors.
- Characterization of BPA-metabolite toxicity.
- Bridging the gap between *in vitro* estrogenic activity and endocrine disruption *in vivo*. Can we develop a predictive *in vitro* model for endocrine disruption?

Recommendations:

In addition to the aforementioned gaps in knowledge that need to be addressed in BPA research, we have developed, by consensus, a set of experimental criteria which we feel are critical for moving the field of BPA research forward: 1) *in vivo* studies should report not only the administered dose, but also the plasma BPA concentrations, urinary concentrations (both parent and metabolites), as well as levels of BPA in tissues relevant to the site of action being studied; 2) in order to establish better concentration-response relationships, experiments should utilize several doses representing the entire range of relevant human exposure levels; 3) *in vivo* studies should employ rodent models reflective of sensitive populations as opposed to strains that exhibit resistance to endocrine disruption; 4) instead of searching for biological effects of BPA *per se*, studies should focus on how BPA may modulate and enhance physiological or pathophysiological processes.

Appendices: Group One Presentation

Indicting Plastics: How Bisphenol A and Phthalates Impact Human Health

H Chen
S Tang
R Zhang

Over-Reporting of BPA's association with chronic diseases? Limitations…

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PLOS ONE

Disconcordance in Statistical Models of Bisphenol A and Chronic Disease Outcomes in NHANES 2003-08

Martin F. Casey[1,2*], Matthew Neidell[1]

1 Department of Health Policy and Management, Mailman School of Public Health, Columbia University, New York, New York, United States of America, 2 Icahn School of Medicine at Mount Sinai, New York, New York, United States of America

Conclusions: Limitations in the NHANES data and a poor understanding of the mode of action of BPA have made it difficult to develop informative statistical models. Given the sensitivity of effect estimates to functional form, researchers should report results using multiple specifications with different assumptions about BPA measurement, thus allowing for the identification of potential discrepancies in the data.

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PLOS ONE

Use of NHANES Data to Link Chemical Exposures to Chronic Diseases: A Cautionary Tale

Judy S. LaKind[1,2,3*], Michael Goodman[4], Daniel Q. Naiman[5]

1 LaKind Associates, LLC, Catonsville, Maryland, United States of America, 2 Department of Epidemiology and Public Health, University of Maryland School of Medicine, Baltimore, Maryland, United States of America, 3 Department of Pediatrics, Pennsylvania State University College of Medicine, Hershey, Pennsylvania, United States of America, 4 Department of Epidemiology, Emory University, School of Public Health, Atlanta, Georgia, United States of America, 5 Department of Applied Mathematics and Statistics, The Johns Hopkins University, Baltimore, Maryland, United States of America

Conclusions: Using scientifically and clinically supportable exclusion criteria and outcome definitions, we consistently found no associations between urinary BPA and heart disease or diabetes. These results do not support associations and causal inferences reported in previous studies that used different criteria and definitions. We are not drawing conclusions regarding whether BPA is a risk factor for these diseases. We are stating the opposite–that using cross-sectional datasets like NHANES to draw such conclusions about short-lived environmental chemicals and chronic complex diseases is inappropriate. We need to expend resources on appropriately designed epidemiologic studies and toxicological explorations to understand whether these types of chemicals play a causal role in chronic diseases.

Review

Bisphenol A and human health: A review of the literature

Johanna R. Rochester*

The Endocrine Disruption Exchange (TEDX), P.O. Box 1407, Paonia, CO 81428, United States

Phthalate Diesters and Their Metabolites in Human Breast Milk, Blood or Serum, and Urine as Biomarkers of Exposure in Vulnerable Populations

Johan Högberg,[1] Annika Hanberg,[1] Marika Berglund,[1] Staffan Skerfving,[2] Mikael Remberger,[3] Antonia M. Calafat,[4] Agneta Falk Filipsson,[1] Bo Jansson,[5] Niklas Johansson,[1,6] Malin Appelgren,[1] and Helen Håkansson[1]

[1]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; [2]Section of Occupational and Environmental Medicine, University Hospital of Lund, Sweden; [3]IVL Swedish Environmental Research Institute Ltd., Stockholm, Sweden; [4]Division of Laboratory Sciences, National Centre for Environmental Health, Centers for Disease Control and Prevention, Atlanta, Georgia, USA; [5]Department of Applied Environmental Sciences, Stockholm University, Stockholm, Sweden; [6]Swedish Environmental Protection Agency, Stockholm, Sweden

- BPA (serum): Concentrations in serum range between 0.1-10 µg/L (higher in occupational settings, up to 102 µg/L)
- Phthalates (urinary): DEHP 5.1 ug/L, DEP 91.3 ug/L, 41.4 ug/L DBP, 39.4 ug/L BBP.

Review

Similarity of Bisphenol A Pharmacokinetics in Rhesus Monkeys and Mice: Relevance for Human Exposure

Julia A. Taylor,[1] Frederick S. vom Saal,[1] Wade V. Welshons,[2] Bertram Drury,[1] George Rottinghaus,[3] Patricia A. Hunt,[4] Pierre-Louis Toutain,[5,6] Céline M. Laffont,[5,6] and Catherine A. VandeVoort[7]

[1]Division of Biological Sciences, [2]Department of Biomedical Sciences, and [3]Veterinary Medical Diagnostic Laboratory, University of Missouri–Columbia, Columbia, Missouri, USA; [4]School of Molecular Biosciences, Washington State University, Pullman, Washington, USA; [5]INRA, TOXALIM (Research Centre in Food Toxicology), Toulouse, France; [6]Ecole Nationale Veterinaire de Toulouse, Université de Toulouse, Toulouse, France; [7]Department of Obstetrics and Gynecology, California National Primate Research Center, University of California–Davis, Davis, California, USA

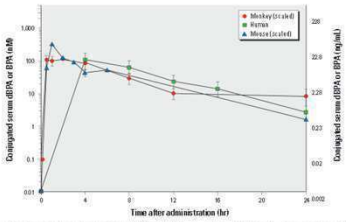
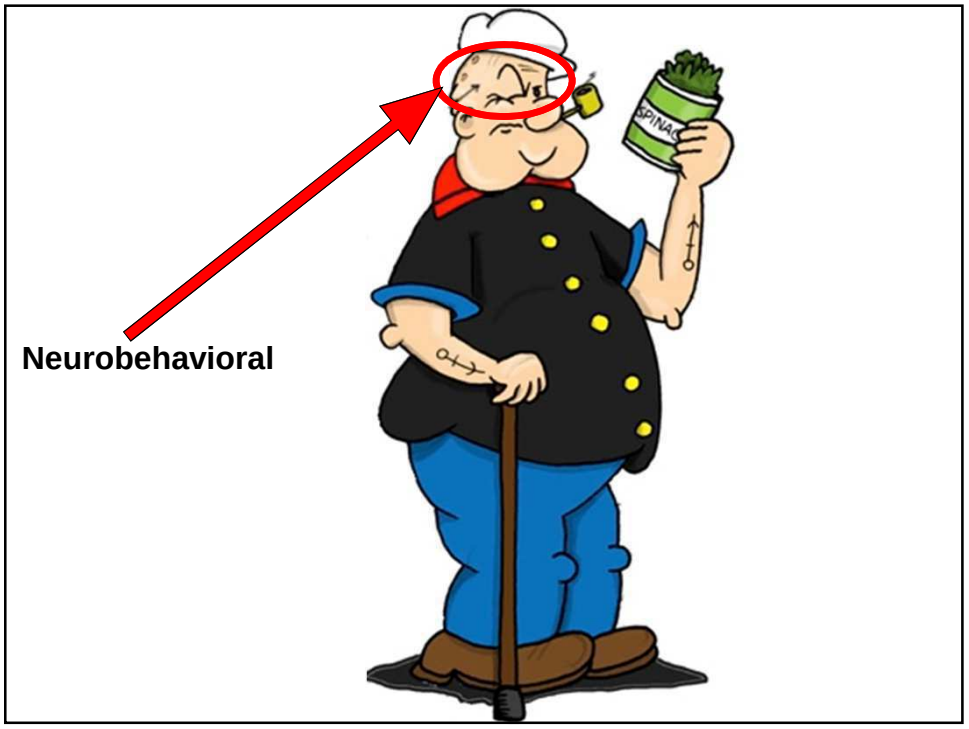


Figure 7. Concentration of conjugated dBPA or BPA in serum from adult female rhesus monkeys, CD-1 mice, and humans during the 24 hr after one oral dose. Women were administered an average dose of 65.3 µg/kg dBPA (Völkel et al. 2002). Rhesus monkeys were administered 400 µg/kg dBPA and mice were administered 100,000 µg/kg BPA; results for monkeys and mice were scaled to 65.3 µg/kg, based on evidence for linear kinetics and because in mice the administered dose was linear with serum BPA between 2 and 100,000 µg/kg (Figure 4). Both nanomolar and nanograms per milliliter data are presented for comparison with the human data of Völkel et al. (2002).

Table 3. Kinetic parameters for conjugated dBPA in serum during the 24 hr after administration of 65.3 µg/kg dBPA to adult women (Völkel et al. 2002), compared with data from rhesus monkeys and CD-1 mice in the present study.

Kinetic parameter, day 1	Women	Monkeys	Mice
Concentration at 4 hr [ng/mL (SE)]	24.05 (9.52)	19.82 (7.52)	10.17
$K_{terminal}$ (/hr)	−0.18	−0.07	−0.17
Terminal $t_{1/2}$ (hr)	3.76	10.08	4.07
AUC_{4-24} [ng-hr-mL (SE)]	148.51 (25.42)	96.91 (16.91)	134.1
Average AUC_{4-24} (ng/mL)	7.43	4.85	6.7

The terminal $t_{1/2}$ in women (n = 3) is based on data from Völkel et al. (2002; see their Figure 7) and is expressed in hours instead of minutes. The $K_{terminal}$ was from 16 to 24 hr for women, 12 to 24 hr for monkeys, and from 6 to 24 hr for mice. Data presented here are for between 4 and 24 hr because Völkel et al. (2002) did not report data for women before 4 hr. Monkey and mouse data were scaled to 65.3 µg/kg from the single dose of 400 µg/kg dBPA fed to monkeys and 100,000 µg/kg BPA fed to mice. No variance estimates (SEs) are available from the mouse study (experiment 2C) because serum samples were pooled for each time point.



Neurobehavioral Effects of Plasticizers

Impact of Early-Life Bisphenol A Exposure on Behavior and Executive Function in Children

AUTHORS: Joe M. Braun, MSPH, PhD,[a] Amy E. Kalkbrenner, MPH, PhD,[b] Antonia M. Calafat, PhD,[c] Kimberly Yolton, PhD,[a] Xiaoyun Ye, PhD,[c] Kim N. Dietrich, PhD,[a] and Bruce P. Lanphear, MD, MPH[a,d]

"Each 10-fold increase in gestational BPA concentrations was associated with more anxious and depressed behavior… and poorer emotional control and inhibition (in 3 year olds)."

Endocrine Disruptors and Childhood Social Impairment

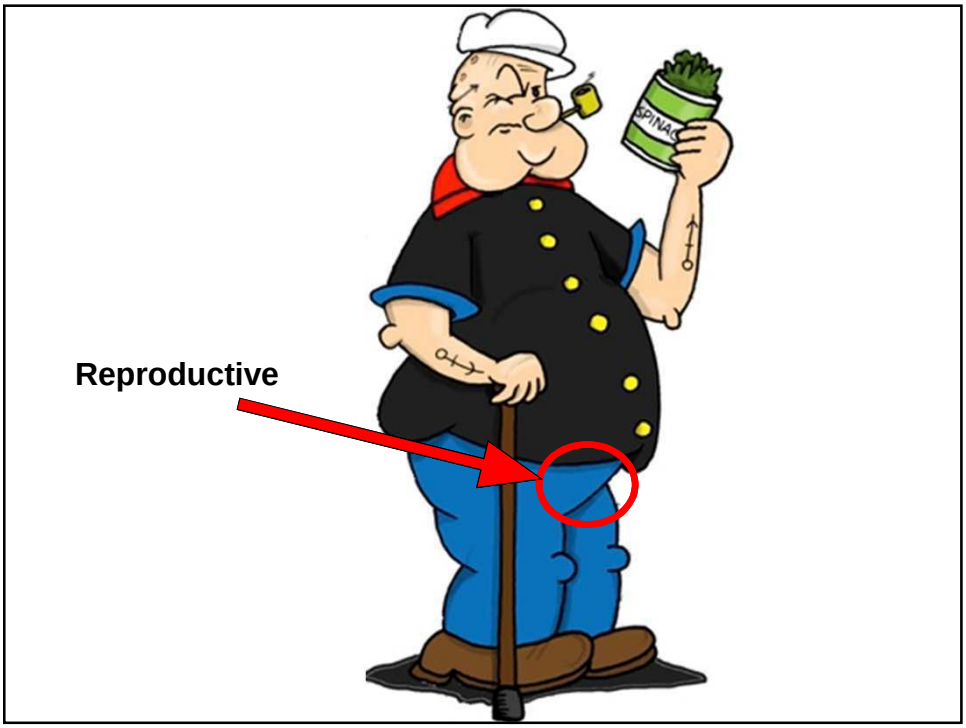
Amir Miodovnik[a], Stephanie M. Engel[a], Chenbo Zhu[a], Xiaoyun Ye[c], Latha V. Soorya[b], Manori J. Silva[c], Antonia M. Calafat[c], and Mary S. Wolff[a]

[a]Department of Preventive Medicine, Mount Sinai School of Medicine, New York, NY, United States

[b]Department of Psychiatry, Mount Sinai School of Medicine, New York, NY, United States

[c]National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta GA, United States

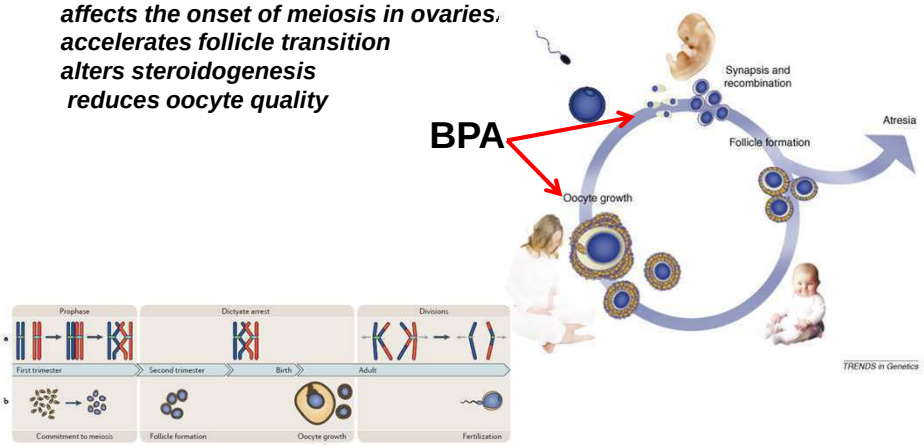
In unadjusted models, we found a positive correlation… reflect(ing) poorer SRS (Social Responsiveness Scale) scores with increasing BPA and phthalate metabolite concentrations."



Bisphenol A and Reproductive Health

BPA is an ovarian toxicant in animal models and women.

- ***affects the onset of meiosis in ovaries***
- ***accelerates follicle transition***
- ***alters steroidogenesis***
- ***reduces oocyte quality***



Patricia A. Hunt , Terry J. Hassold. Trends in Genetics, 2008

Nagaoka et al., Nature review Genetics, 2012

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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,[2,3] 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

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(10)37Rk/In(19)37Rk	4 weeks	89	7 (7.9)
	In(2)5Rk (+/+)*	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 (+/+)*	4 weeks	145	0 (0)
	Pooled		959	21 (2.2)
NEW FACILITY (December 2000–February 2001)	C57BL/6	4 weeks	204	1 (0.5)

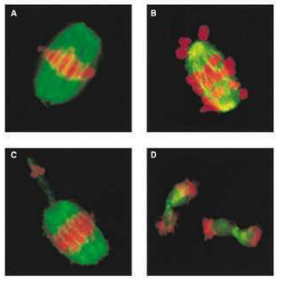
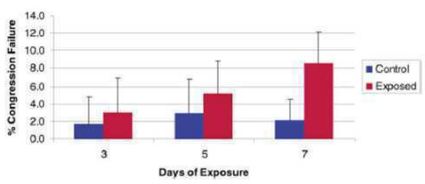


Figure 1. Normal and Abnormal Meiotic Metaphase Configurations

Confocal images of intact mouse oocytes immunostained with an antibody to β-tubulin 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).



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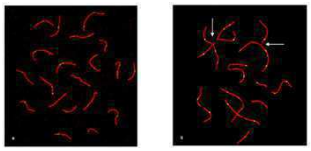
Bisphenol A Exposure In Utero Disrupts Early Oogenesis in the Mouse

Martha Susiarjo[1,2], Terry J. Hassold[2], Edward Freeman[1,3], Patricia A. Hunt[2*]

1 Department of Genetics, Case Western Reserve University, Cleveland, Ohio, United States of America, 2 School of Molecular Biosciences and Center for Reproductive Biology, Washington State University, Pullman, Washington, United States of America, 3 Department of Biology, St. John Fisher College, Rochester, New York, United States of America



Control BPA

BPA implanted pellets, 20ug/kg/day

Table 4. Aneuploidy Analysis

Group	Number of Mice	Number of Cells	Total Chromosomes			
			20	≥21	20.5	19.5
Placebo	10	57	56	1	0	0
BPA	16	56	43	10	2	1

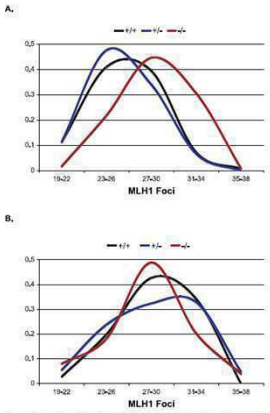


Figure 3. Analysis of Exchanges in Pachytene Oocytes from Unexposed and BPA-Exposed JERKO Females

Defect in synapsis and increase end-to-end association
Increase recombination between homologs via ER β pathway(antagonist).
Increase aneuploidy in eggs and embryos from adult females

PNAS

Bisphenol A alters early oogenesis and follicle formation in the fetal ovary of the rhesus monkey

Patricia A. Hunt[a,1], Crystal Lawson[a], Mary Gieske[a], Brenda Murdoch[a], Helen Smith[a], Alyssa Marre[a], Terry Hassold[a], and Catherine A. VandeVoort[b]

[a]School of Molecular Biosciences and Center for Reproductive Biology, Washington State University, Pullman, WA 99164; and [b]Department of Obstetrics and Gynecology and California National Primate Research Center, University of California, Davis, CA 95616

Edited* by Joan V. Ruderman, Harvard Medical School, Boston, MA, and approved August 24, 2012 (received for review May 9, 2012)

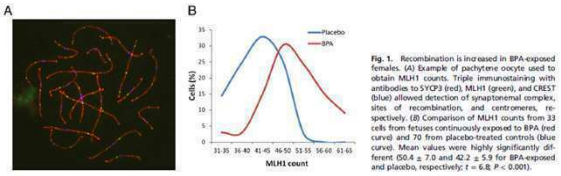
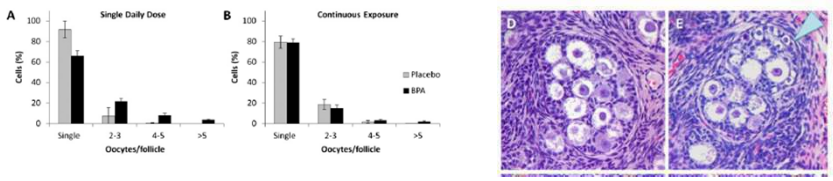


Fig. 1. Recombination is increased in BPA-exposed females. (A) Example of pachytene oocyte used to obtain MLH1 counts. Triple immunostaining with antibodies to SYCP3 (red), MLH1 (green), and CREST (blue) allowed detection of synaptonemal complex, sites of recombination, and centromeres, respectively. (B) Comparison of MLH1 counts from 33 cells from fetuses continuously exposed to BPA (red curve) and 70 from placebo-treated controls (blue curve). Mean values were highly significantly different (50.4 ± 7.0 and 42.2 ± 5.9 for BPA-exposed and placebo, respectively; $t = 6.8$; $P < 0.001$).

BPA serum levels: 0.3-0.9ng/mL (unconjugated)



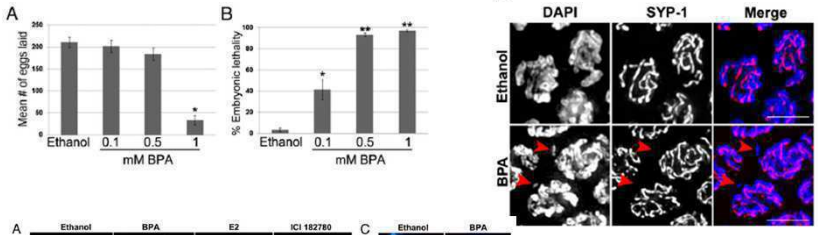
PNAS

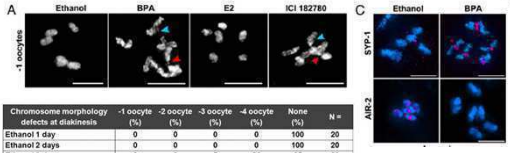
Bisphenol A impairs the double-strand break repair machinery in the germline and causes chromosome abnormalities

Patrick Allard and Monica P. Colaiácovo¹

Department of Genetics, Harvard Medical School, Boston, MA 02115

Edited* by Joan Ruderman, Harvard Medical School, Boston, MA, and approved October 6, 2010 (received for review July 16, 2010)





B

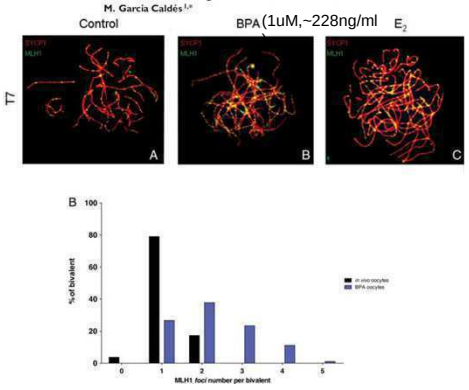
Chromosome morphology defects at diakinesis	-1 oocyte (%)	-2 oocyte (%)	-3 oocyte (%)	-4 oocyte (%)	None (%)	N =
Ethanol 1 day	0	0	0	0	100	20
Ethanol 2 days	0	0	0	0	100	20
Ethanol 3 days	0	0	5	30	65	20
BPA 1 day	10	55	35	0	0	20
BPA 2 days	9.5	71.5	19	0	0	21
BPA 3 days	25	70	5	0	0	20
E2 1 day	0	0	0	0	100	20
ICI 1 day	6.5	22.5	32.2	25.8	13	31
BPA + E2 1 day	0	0	20	20	60	20
Eth. + DMSO 1 day	0	0	0	15	75	20
BPA + ICI 1 day	4.8	57.2	38	0	0	21

Human Reproduction, Vol.26, No.10 pp. 2807–2818, 2011
Advanced Access publication on July 26, 2011 doi:10.1093/humrep/der249

human reproduction ORIGINAL ARTICLE *Reproductive biology*

Human meiotic progression and recombination are affected by Bisphenol A exposure during *in vitro* human oocyte development

M.A. Brieño-Enríquez[1,2], P. Robles[3], N. Camats-Tarruella[4], R. García-Cruz[1], I. Roig[1], L. Cabero[3], F. Martínez[2], and M. García Caldés[1,*]

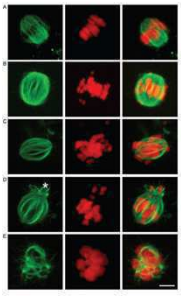


Human Reproduction, Vol.28, No.10 pp. 2735–2745, 2013
Advanced Access publication on July 30, 2013 doi:10.1093/humrep/det312

human reproduction ORIGINAL ARTICLE *Infertility*

Bisphenol-A and human oocyte maturation *in vitro*

Ronit Machtinger[1,†], Catherine M.H. Combelles[2,†], Stacey A. Missmer[1,3,4], Katharine F. Correia[1], Paige Williams[5], Russ Hauser[6,7], and Catherine Racowsky[1,*]



BPA:20, 200 ng/ml or 20ug/ml

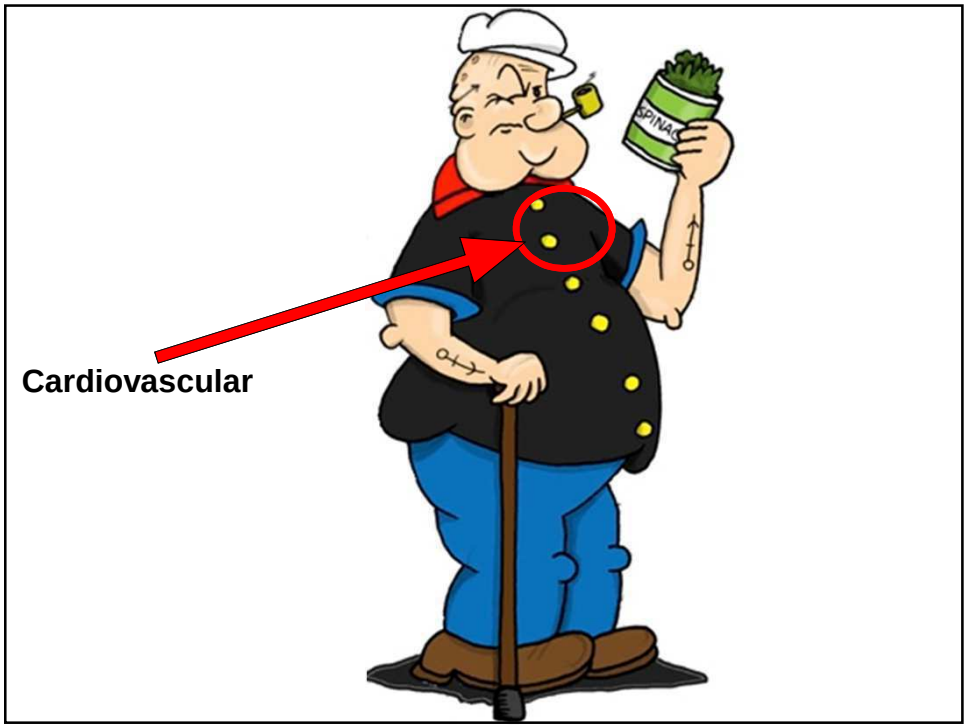


Table 5 Experimental studies examining the effect of BPA on cardiac physiology

Reference	Concentration	Model and exposure length	Results
Asano et al. [136]	10–100 μM	Human and canine coronary smooth muscle cells; 1 min	BPA activates maxi-K channels
Belcher et al. [121]	1 pM–1 nM	Female ventricular myocyte; 7 min	BPA ↑ contractility. Effect abolished in myocytes from ovariectomized females, and ERβ knockout mice. Effect not inhibited by L-Name pretreatment
Deutschmann et al. (2013)	1 μM–1 mM	Mouse cardiomyocytes and dorsal root ganglion neurons, rat endocrine GH3 cells, human HEK cells; 1 min	BPA reversibly blocks multiple calcium channels. Effect not due to intracellular signaling (PKA, PKC pathways). EC_{50} = 26–35 μM
Gao et al. [132]	1 nM	Female ventricular myocyte; 15 min	BPA transiently alters ryanodine receptor phosphorylation at PKA site and phospholamban at CAMKII site. Effects abolished with ERβ or PKA blocker.
Lee et al. [125]	1–100 ng/mL	Rice fish embryo; 2–4 days	BPA ↓ heart rate
O'Reilly et al. [134]	0.1–1 mM	HEK cells; 2 min	BPA blocks hNav1.5 sodium channel in closed/resting-state. NOAEL = 100 nM, LOAEL = 1 μM, K_d = 25 μM
Pant et al. [127]	0.1–100 μM	Rat right atria; 10 min	BPA ↓ heart rate, ↓ force of contraction. Effect abolished with L-Name pretreatment or methylene blue. Effect not inhibited by atropine pretreatment.
Pant et al. [126]	LD_{50} = 841 mg/kg bw (i.p.), 35 mg/kg bw (i.v.).	Female rat; 7 min	Lethal BPA dose (40 mg/kg bw) produced respiratory arrest, hypotension, and bradycardia.
Patel et al. [128]	0.5–200 μg/kg/day	Male mice; 30 days–4 months	Prenatal and postnatal BPA exposure resulted in concentric remodeling, ↑ velocity circumferential shortening, ↑ascending aorta velocity, and ↑ calcium mobility
	0.5–200 μg/kg/day	Female mice; 30 days–4 months	Prenatal and postnatal BPA ↓ LV mass and wall thickness, ↑ blood pressure, ↓ calcium mobility
Posnack et al. [123]	0.1–100 μM	Female excised whole heart; 15 min	BPA ↓ epicardial conduction velocity, ↑ atrioventricular delay, ↑ PR segment time, ↑ action potential duration, heart block at high doses
Schirling et al. [124]	50–100 μg/L	Snail embryo; 9 days	BPA ↓ heart rate
Yan et al. [122]	1 nM	Female excised whole heart; 60 min	BPA ↑ arrhythmia duration (ischemia reperfusion model). Effects abolished with ERα ± ERβ blocker
	1 nM	Female ventricular myocyte; 2 h	BPA ↑ spontaneous after contractions
Yan et al. [130]	1 nM	Female ventricular myocyte; 7 min	BPA ↑ spontaneous after contractions, ↑ calcium leak, and load in sarcoplasmic reticulum. Effects abolished in ERβ knockout mice

Table 2 Experimental studies examining the effect of DEHP or MEHP on cardiac physiology

Reference	Concentration	Model and approximate exposure length	Result
Aronson et al. [72]	250 μM DEHP	Excised rat heart; 60 min	DEHP ↓ heart rate, ↓ coronary flow, ↓ systolic tension, and ↑ diastolic tension, prolonged PR and QT intervals. DEHP ↑ lactate levels in tissue and perfusate media
Barry et al. [42, 75]	15–300 μg/mL MEHP	Human atrial trabeculae; 10–120 min	MEHP reversibly ↓ contractility (IC_{50} = 85 μg/mL), and ↑ arrhythmia incidence. MEHP may act via cholinergic receptors (atropine shifts IC_{50} = 120 μg/mL)
Calley et al. [79]	350 mg/kg DEHP	Rabbit; 3 min	DEHP ↓ blood pressure
Gillum et al. [71]	1–50 μg/mL DEHP	Neonatal rat cardiomyocytes; 24–72 h	DEHP ↓ conduction velocity, ↓ cell synchronicity, ↓ gap-junctional connexin-43 expression, and modified mechanical movement of cell layers
Mangala et al. [82]	0–100 mg/kg/day	Female rat; 21 days	Postnatal DEHP exposure (via lactation) impaired insulin signal transduction and glucose oxidation in cardiac muscle in female progeny
Martinez-Arguelles et al. [77]	300 mg/kg/day DEHP	Male rat; 7 days	Prenatal DEHP exposure ↓ heart rate, ↓ systolic and diastolic blood pressure in adult males
Posnack et al. [81]	50–100 μg/mL DEHP	Neonatal rat cardiomyocytes; 72 h	DEHP ↑ fatty acid substrate utilization, ↑ oxygen consumption, ↑ mitochondrial mass, ↑ PPARa expression, and ↑ extracellular acidosis. Effects partially mimicked by PPARa agonist
Posnack et al. [80]	1–50 μg/mL DEHP	Neonatal rat cardiomyocytes; 24–72 h	DEHP modified gene expression related to cell electrical activity, calcium handling, adhesion, and microtubular transport. DEHP also modified adhesion and transport proteins
Rock et al. [74]	0–100 mg MEHP	Rat; < 15 min	MEHP ↓ heart rate (LOAEL = 10 mg) and ↓ blood pressure (LOAEL = 55 mg)
Rubin et al. [28]	4 μg/mL DEHP	Chick embryonic heart cells; 30 min–24 h	DEHP stopped cell contractions (30 min) and resulted in 97–98 % cell death within 24 h
Schulpen et al. [73]	0.004–4.4 mM MEHP	Embryonic stem cells; 5–7 days	MEHP ↓ cell viability and cardiac differentiation, and genes associated with these endpoints
Wei et al. [78]	0.25–6.25 mg/kg/day DEHP	Rat; 40 days	Prenatal and postnatal DEHP exposure ↓ renal protein expression, ↑ blood pressure

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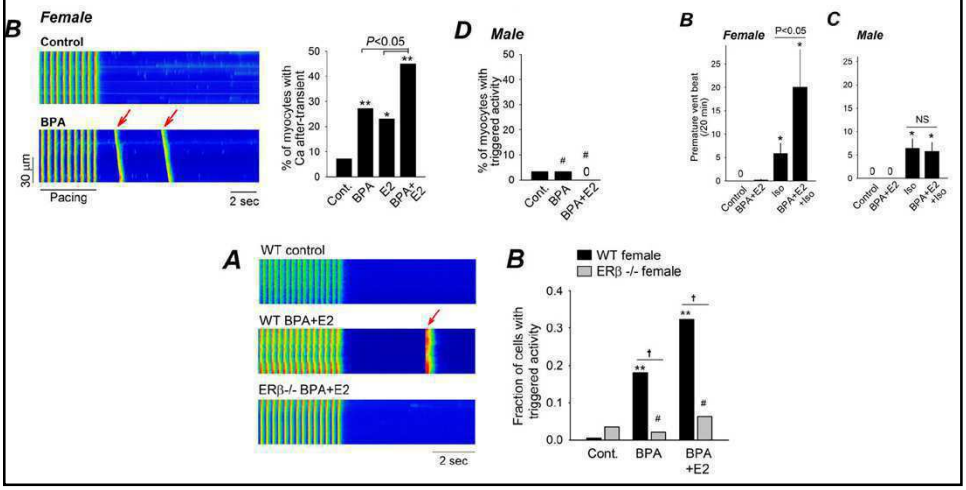
PLoS one

Bisphenol A and 17β-Estradiol Promote Arrhythmia in the Female Heart via Alteration of Calcium Handling

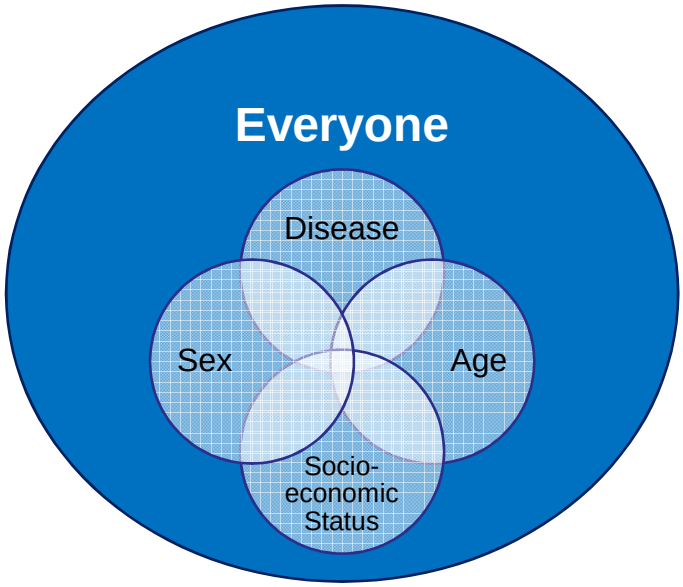
Sujuan Yan[¤], Yamei Chen, Min Dong, Weizhong Song, Scott M. Belcher, Hong-Sheng Wang*

Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, Cincinnati, Ohio, United States of America



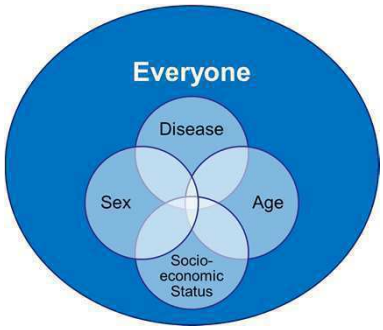
- Disrupting cardiovascular function presents both an immediate concern (cardiac arrhythmias, sudden death), as well as promotes remodeling leading to irreparable damage that manifests as chronic heart disease (congestive heart failure, cardiomyopathy, cardiac hypertrophy)
- These effects are indeed tangible, although not all are immediately evident

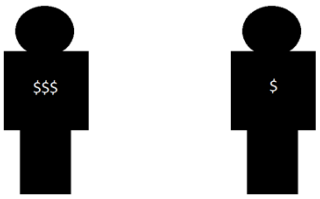
Who is at Risk?



Populations at Risk

- Age
  - Shiue I, Starr J. Circulating urine phthalates are not associated with a decline in cognition in adults and the elderly: NHANES, 1999-2002. Neuroepidemiology. 2012
- Sex of individual
- SES
  - Associations between socioeconomic status and environmental toxicant concentrations in adults in the USA: NHANES 2001-2010. Tyrrell et al Environ Int. 2013
  - Perera F et al Prenatal Bisphenol A Exposure and Child Behavior in an Inner-City Cohort. Environ Health Perspect. 2012 Aug;120(8):1190-4
- Race
  - Trasande L et al 2013 Race/ethnicity-specific associations of urinary phthalates with childhood body mass in a nationally representative sample. Environ Health. 2013
  - Huang T et al Gender and racial/ethnic differences in the associations of urinary phthalate metabolites with markers of diabetes risk: national health and nutrition examination survey 2001 – 2008 Environ Health. 2014 Feb 5;13(1):6.
- Disease
  - Soriano S et al. Rapid insulinotropic action of low doses of bisphenol-A on mouse and human islets of Langerhans: role of estrogen receptor β. PLoS One. 2012;7(2):e31109.
  - Lang IA et al. Association of urinary bisphenol A concentration with medical disorders and laboratory abnormalities in adults. JAMA. 2008 Sep 17;300(11):1303-10.





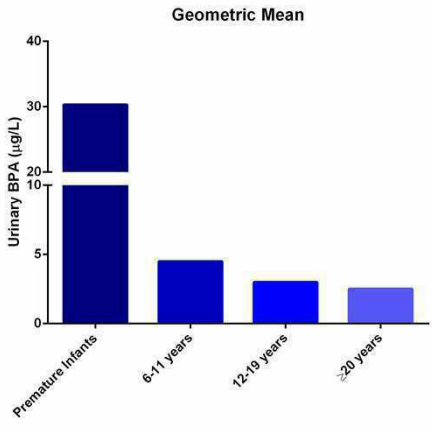
Higher BPA exposure

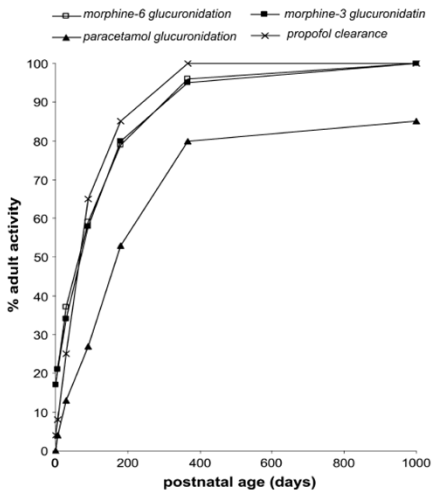
Risk Conferred by Altered Pharmacokinetics

- Diseases that may alter the ADME of BPA, either as a consequence of the disease itself, or through interactions with chronic medications
- Diseases that decrease glucuronidation potential:
  - Hepatitis, cirrhosis
  - Hypothyroidism
  - HIV
  - Gilbert-Meulengracht syndrome
  - Crigler-Najjar syndrome

Perinatal/Neonatal Exposures

- Timing is key! Toxicant exposure during development can have far reaching consequences (e.g. diethylstilbestrol)
- Neonatal patients in NICU can have 10x urinary BPA vs children and adults
- Neonatal drug clearance is low vs adults
  - Lower glomerular filtration
  - Less tubular absorption and excretion
  - Lower capacity to metabolize drugs





Journal of Developmental Origins of Health and Disease (2012), 3(4), 287–292.

doi:10.1017/S2040174412000153

Developmental exposure to bisphenol A leads to cardiometabolic dysfunction in adult mouse offspring

F. R. Cagampang*, C. Torrens, F. W. Anthony and M. A. Hanson

Human Development and Health, Institute of Developmental Sciences, University of Southampton Faculty of Medicine, Southampton General Hospital, Southampton, UK

Bisphenol A Exposure during Pregnancy Disrupts Glucose Homeostasis in Mothers and Adult Male Offspring

Paloma Alonso-Magdalena,[1,2] Elaine Vieira,[1,2] Sergi Soriano,[1,2] Lorena Menes,[1,3] Deborah Burks,[1,3] Ivan Quesada,[1,2] and Angel Nadal[1,2]

[1]Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), and [2]Instituto de Bioingeniería, Universidad Miguel Hernández de Elche, Elche, Spain; [3]Instituto Principe Felipe, Consejo Superior de Investigaciones Científicas, Valencia, Spain

OPEN ACCESS Freely available online

PLOS | ONE

Early-Life Exposure to Bisphenol A Induces Liver Injury in Rats Involvement of Mitochondria-Mediated Apoptosis

Wei Xia*, Ying Jiang, Yuanyuan Li, Yanjian Wan, Juan Liu, Yue Ma, Zhenxing Mao, Huailong Chang, Gengqi Li, Bing Xu, Xi Chen, Shunqing Xu*

Key Laboratory of Environment and Health, Ministry of Education and Ministry of Environmental Protection, and State Key Laboratory of Environmental Health (Incubating), School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Perinatal exposure to low-dose bisphenol A affects the neuroendocrine stress response in rats

Emily Panagiotidou[1], Sophia Zerva[2], Dimitra J Mitsiou[2], Michael N Alexis[2] and Efthymia Kitraki[1]

[1]Department of Basic Sciences and Oral Biology, School of Dentistry, National and Kapodistrian University of Athens, Thivon 2, 11527 Athens, Greece
[2]Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation, 48, Vassileos Constantinou Avenue, 11635 Athens, Greece

The FASEB Journal • Research Communication

Food intolerance at adulthood after perinatal exposure to the endocrine disruptor bisphenol A

Sandrine Menard,*,[1] Laurence Guzylack-Piriou,[†] Mathilde Leveque,* Viorica Braniste,[†] Corinne Lencina,* Manon Naturel,* Lara Moussa,* Soraya Sekkal,[†] Cherryl Harkat,* Eric Gaultier,[†] Vassilia Theodorou,* and Eric Houdeau[†]

*Department of Neurogastroenterology and Nutrition and [†]Department of Intestinal Development, Xenobiotics, and Immunotoxicology, Institut National de la Recherche Agronomique (INRA), Unité Mixte de Recherche (UMR) 1331 Toxalim, Research Centre in Food Toxicology. Toulouse, France

Bittner *et al. Environmental Health* 2014, **13**:41
http://www.ehjournal.net/content/13/1/41

ENVIRONMENTAL HEALTH

RESEARCH **Open Access**

Estrogenic chemicals often leach from BPA-free plastic products that are replacements for BPA-containing polycarbonate products

George D Bittner[1,2*], Chun Z Yang[1] and Matthew A Stoner[1]

Methods: We used two, well-established, mammalian cell-based, assays (MCF-7 and BG1Luc) to assess the EA of chemicals that leached into over 1000 saline or ethanol extracts of 50 unstressed or stressed (autoclaving, microwaving, and UV radiation) BPA-free PC-replacement products. An EA antagonist, ICI 182,780, was used to confirm that agonist activity in leachates was due to chemicals that activated the mammalian estrogen receptor.

Conclusions: This hazard assessment survey showed that many BPA-free PC- replacement products still leached chemicals having significant levels of EA, as did BPA-containing PC counterparts they were meant to replace. That is, BPA-free did not mean EA-free. However, this study also showed that some PC-replacement products did not leach chemicals having significant levels of EA. That is, EA-free PC-replacement products could be made in commercial quantities at prices that compete with PC-replacement products that were not BPA-free. Since plastic products often have advantages (price, weight, shatter-resistance, etc.) compared to other materials such as steel or glass, it is not necessary to forgo those advantages to avoid release into foodstuffs or the environment of chemicals having EA that may have potential adverse effects on our health or the health of future generations.

Most Plastic Products Release Estrogenic Chemicals: A Potential Health Problem that Can Be Solved

Chun Z. Yang,[1] Stuart I. Yaniger,[2] V. Craig Jordan,[3] Daniel J. Klein,[2] and George D. Bittner[1,2,4]

[1]CertiChem Inc., Austin, Texas, USA; [2]PlastiPure Inc., Austin, Texas, USA; [3]Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, DC, USA; [4]Neurobiology Section, School of Biology, University of Texas, Austin, Texas, USA

Table 1. Percentage of unstressed plastic products having EA in at least one extract.

	Extraction solvent							
	EtOH		Concentrated EtOH		Saline		Any extract	
Plastic product	*n*	%D	*n*	%D	*n*	%D	*n*	%D
Resin type								
HDPE	13	69	11	55	18	56	30	70
PP	23	52	6	33	16	81	37	68
PET	30	40	17	94	34	76	57	75
PS	13	62	—	—	16	38	28	50
PLA	10	70	1	100	8	100	11	91
PC	1	0	1	100	2	100	2	100
Product type								
Flexible packaging	82	66	6	33	35	74	121	67
Food wrap	9	100	—	—	9	78	9	100
Rigid packaging	57	56	18	67	31	45	83	64
Baby bottle component	13	69	—	—	16	94	19	89
Deli containers	11	36	—	—	7	7	16	44
Plastic bags	33	97	1	100	23	96	43	98
Product retailer								
Large retailer 1	31	81	2	100	4	75	36	81
Large retailer 2	4	50	4	0	50	54	53	53
Large retailer 3	18	83	2	100	7	29	25	72
Large retailer 4	37	51	—	—	—	—	37	51
Large retailer 5	20	50	3	100	4	100	23	70
Organic retailer 1	28	71	5	60	5	80	32	81
Organic retailer 2	33	88	1	100	10	80	35	89
Total for extract	308	68	51	73	214	69	455	72

Abbreviations: —, not tested; %D, percent detectable fextract produced cell proliferation > 15% RME2; see "Materials and Methods"); *n*, total number of samples purchased (less than the sum of *n* values for individual extracts if some items were tested by more than one extraction protocol); PLA, polylactic acid. Data are percentages of samples for which EA was detected using a standard or concentrated EtOH extract, a saline extract, or one or more such extracts (any extract). Some individual items are listed in two or three categories (e.g., PET and baby bottles) but were counted only once for the extract total. Baby bottle components comprised 11 bottles and 2 sealant ring components.

ORIGINAL ARTICLE

Consumer product exposures associated with urinary phthalate levels in pregnant women

Jessie P. Buckley[1], Rachel T. Palmieri[1], Jeanine M. Matuszewski[2], Amy H. Herring[2,3], Donna D. Baird[4], Katherine E. Hartmann[1,5] and Jane A. Hoppin[4]

Table 4. Frequencies of product use reported in the 48-h recall questionnaire and associations with phthalate metabolites.[a]

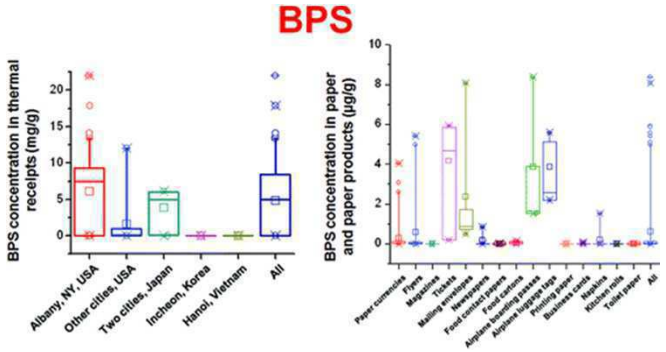
Exposure group/product	*n (%)*	*MBP*	*MBZP*	*MiBP*	*MEP*	*MMP*	*Any DEHP metabolite*[b]
Global items							
Makeup most days of the week	29 (58)	+	+*				
Try to buy fragrance-free products	19 (38)						*
Deli or "to go" foods 3+ times a week	16 (32)						
Household cleaners at least 2 h a week	30 (60)						
In the last 2 days (48 h)							
Cleaning products							
Laundry detergent with fragrance	30 (60)						+
Fabric softener or dryer sheet with fragrance	29 (58)						
Fabric starch	8 (16)						
Spot cleaners	12 (24)						
Carpet cleaners	2 (4)						
Furniture polish or wax	9 (18)						
Shoe cleaner or polish	2 (4)						
Interior car cleaner	1 (2)						
Creams and lotions							
Anti-aging or overnight cream	10 (20)						
Cleansing cream	21 (42)						
Facial masks	7 (14)						+
Sunscreen	5 (10)	+*				+*	
Baby oil	4 (8)						
Petroleum jelly or diaper ointment	18 (36)						
Hand cream or lotion	43 (86)						+
Other cream or lotion, including shaving cream	33 (66)	+					
Toiletries and cosmetics							
Lipstick, chapstick, or lip balm	42 (84)						
Foundation makeup	27 (54)						
Eye shadow, liner, or mascara	33 (66)	+*	+*	+*			+*
Powder	25 (50)						
Cologne or perfume	28 (56)			+*			+*
Bath oil, bath gel, or bubble bath	15 (30)	+				+*	
Hair conditioner	29 (58)						
Hair nutrient product	6 (12)				+*	+*	
Hair spray	23 (46)						
Hair styling gel, mousse, pomade, or grease	33 (66)					+	

Bisphenol S, a New Bisphenol Analogue, in Paper Products and Currency Bills and Its Association with Bisphenol A Residues

Chunyang Liao,[†,‡] Fang Liu,[†] and Kurunthachalam Kannan*[,†]

[†]Wadsworth Center, New York State Department of Health, and Department of Environmental Health Sciences, School of Public Health, State University of New York at Albany, Empire State Plaza, P.O. Box 509, Albany, New York 12201-0509, United States

[‡]Key Laboratory of Coastal Zone Environmental Processes, Yantai Institute of Coastal Zone Research (YIC), Chinese Academy of Sciences (CAS), Shandong Provincial Key Laboratory of Coastal Zone Environmental Processes, YICCAS, Yantai, Shandong 264003, China



Mixtures: Interactions with other pollutants

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Triclosan exacerbates the presence of ^{14}C-bisphenol A in tissues of female and male mice

Tyler Pollock, Brandon Tang, Denys deCatanzaro *

Department of Psychology, Neuroscience & Behaviour, McMaster University, Hamilton, Ontario L8S 4K1, Canada

Overall Conclusions

- At relevant human exposure levels, BPA and phthalates can negatively impact neurobehavioral, reproductive, and cardiovascular endpoints
- These effects may not be consequential to a healthy adult
- Most susceptible to these toxic effects are special subpopulations defined by: Age, Sex, Socioeconomic status, Race, Disease
- Timing of exposure is critical, especially considering the reduced capacity to metabolize toxicants by infants and young children
- BPA and phthalates may potentially exacerbate existing diseases

Recommendations

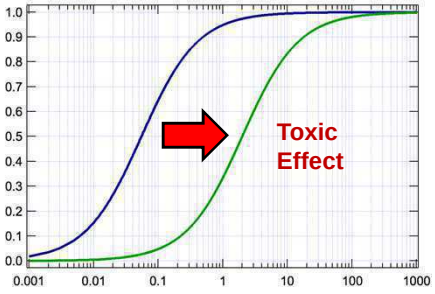
- Exposure to BPA and phthalates should be severely limited in susceptible individuals (e.g. pregnant women, infants, patients with chronic conditions such as heart disease)

Recommendations

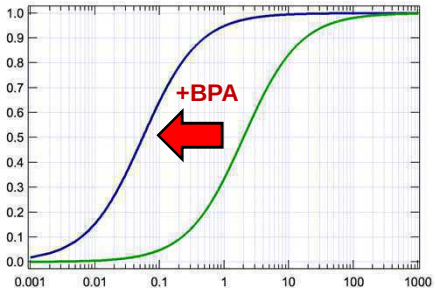
- Exposure to BPA and phthalates should be severely limited in susceptible individuals (e.g. pregnant women, infants, patients with chronic conditions such as heart disease)
- Better research to bridge the gap between estrogenicity/androgenicity and endocrine disruption
- Stronger regulations regarding plastic monomers and additives, and better understanding of how these similarly-structured compounds impact human health endpoints (structure-activity relationships)

Recommendations

- Contributions of BPA and phthalates to disease
- Much research has been on the effects of BPA *per se*
- How does BPA interact with (patho)physiological processes to bring about synergistic effects?



[BPA]



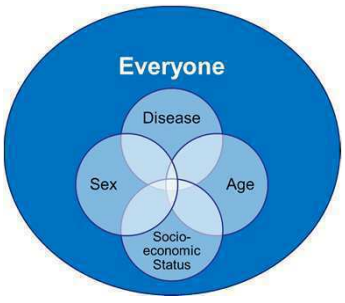
[endogenous ligand]

Bisphenol A and Diabetes, Insulin Resistance, Cardiovascular Disease and Obesity: Controversy in a (Plastic) Cup?

Dianna J. Magliano and Jasmine G. Lyons

Clinical Diabetes and Epidemiology, Baker IDI Heart and Diabetes Institute, Melbourne, Australia 3004

As a potentially highly modifiable risk factor (if in fact the hypothesis can be proven), the public health implications may be vast. In terms of intervention, it will be easier and more cost effective to the health care system to ban BPA rather than implement population-wide, behavior/lifestyle change-based interventions to tackle obesity, cardiovascular disease, and diabetes, all of which we know have limited long-term efficacy. Until more large-scale, well-designed, prospective studies are conducted, controversy is only set to continue.



Appendices: Group Two Presentation

BPA: As good as it gets

ETX 298

Emma, Doug, and Keith

Outline

- Background
- Three Criteria of Reasonable Experiments:
  1. Equivalent Models
  2. Human Exposure Levels
  3. Route of Exposure
- Experiments that meet criteria
- Alternative chemicals
- Conclusions

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Bisphenol A (BPA)

- High-volume chemical used for production of polycarbonate plastics and epoxy resins
- Commonly used for food/drink packaging, some dental sealants/composites
- Majority of human exposure occurs through diet
- Ubiquitous in US population: detectible in 93% of Americans (2003-2004 NHANES)

http://www.niehs.nih.gov/health/topics/agents/sya-bpa/

BPA as Estrogenic Risk

- Initially identified as synthetic estrogen in 1930s
- Rediscovered as estrogenic compound in 1990s
- As unstable ester bond linking BPA-based polymers decays, BPA can be released into surrounding material

Dodds, EC and W Lawson 1938. **Molecular structure**… Proceedings of the Royal Society. London B. 125:222-232.

Current BPA Risk Assessment

NTP conclusions regarding the possibilities that human development or reproduction might be adversely affected by exposure to bisphenol A. The NTP uses a five-level scale of concern:

	SERIOUS Concern *for adverse effects*
	CONCERN *for adverse effects*
Developmental toxicity for fetuses, infants, and children (effects on the brain, behavior, and prostate gland)	**SOME Concern** *for adverse effects*
Developmental toxicity for fetuses, infants, and children (effects on the mammary gland and early puberty in females), and reproductive toxicity in workers	**MINIMAL Concern** *for adverse effects*
Reproductive toxicity in adult men and women and malformations in newborns	**NEGLIGIBLE Concern** *for adverse effects*

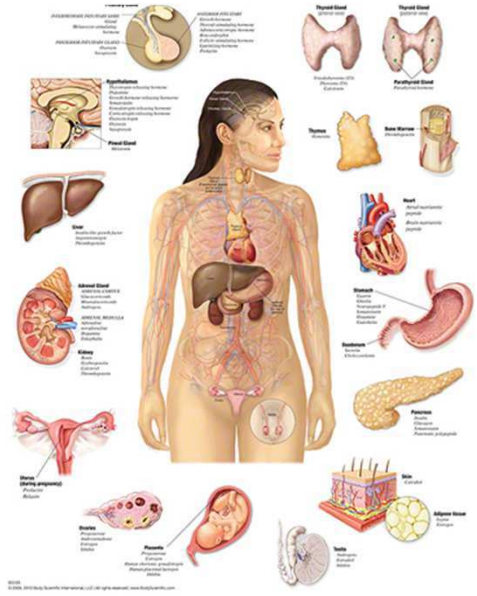
http://www.niehs.nih.gov/health/topics/agents/sya-bpa/

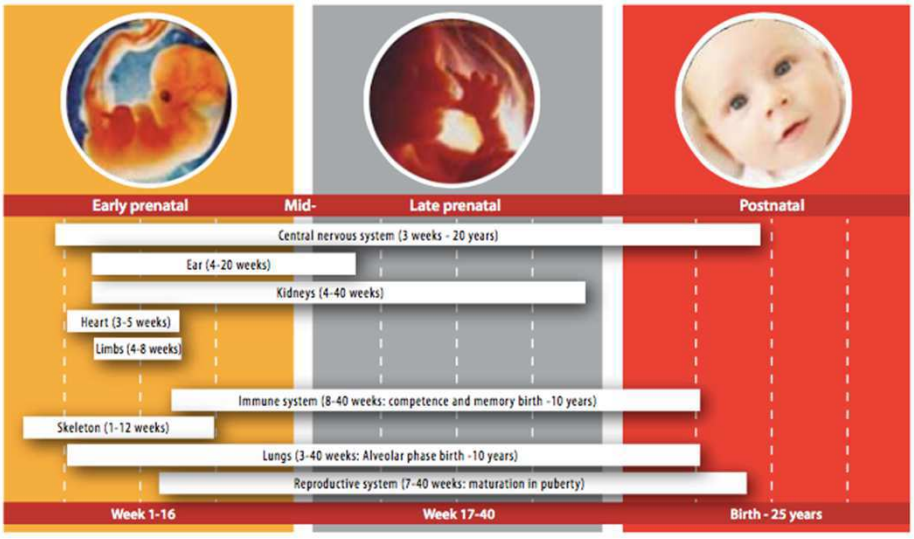
Endocrine Glands/Organs/Tissues

Integrated secretion of hormones dictates several human functions:

- Growth
  - Height, hair, organs
- Metabolism
  - Digestion, absorption
- Immunity
  - Inflammatory responses
- Reproduction
  - Sexual preference, activity, health
- CNS
  - Mood, circadian responses

Hormonal imbalances are linked to numerous adverse health outcomes



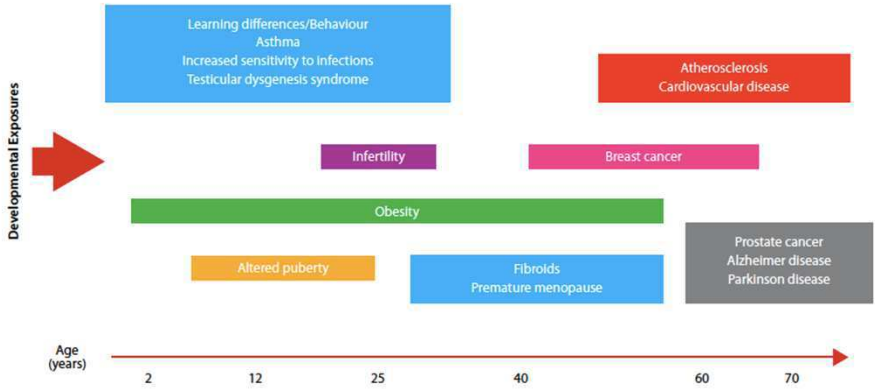


Human Developmental Windows

Figure 3. Sensitive windows of development. Each tissue has a specific window during development when it is forming. That is the sensitive window for effects of EDCs. Notice that some tissues continue developing after birth and into infancy and childhood, providing a longer window for exposures to affect programming.

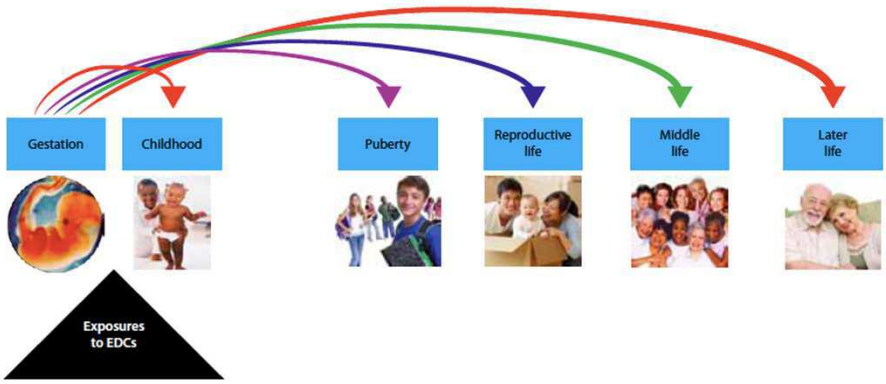
WHO EDC 2013

Late-Onset Adverse Health Outcomes linked to Endocrine Disruption



WHO EDC 2013

Windows of Susceptibility



WHO EDC 2013

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Extreme Routes of EDC Exposure

1. Pre-implantation mouse embryos cultured in 1.0 nm BPA that were transplanted into unexposed females
2. Siliastic Implants of E2 into adolescent mice
3. Intra-cranial injection of BPA

1. **Takai Y, Tsutsumi O, Ikezuki Y, Kamei Y, Osuga Y, Yano T, Taketan Y** 2001 Preimplantation exposure to bisphenol A advances postnatal development. *Reprod Toxicol* 15:71–74
2. PMID: 10455051
3. DOI: 10.1016/j.chemosphere.2009.11.010 **not all pups littermates**

Why we need to define criteria

Ample evidence implicating BPA can produce negative health outcomes. . .but how relevant are these data to human health?

Excluded Studies

- No correlation studies
- No *in vitro* studies

What *can* be considered?

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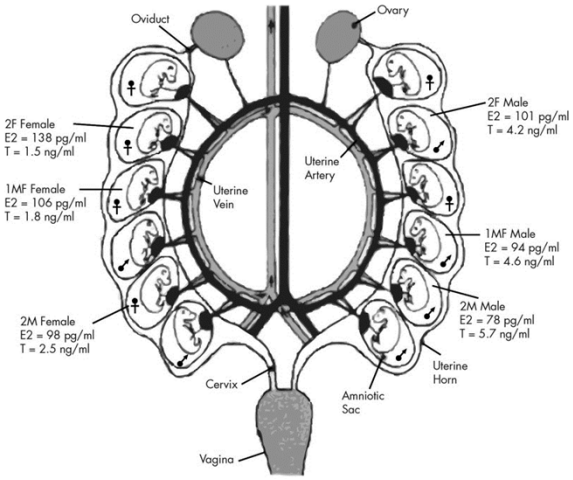
Equivalent Models

- Modeling Human Populations:
  - Species
  - Genotype
  - Sex
  - Age

Intra-Uterine Effects

Female E2
98 -138 pg/ml

Female T
1.5-2.5 ng/ml

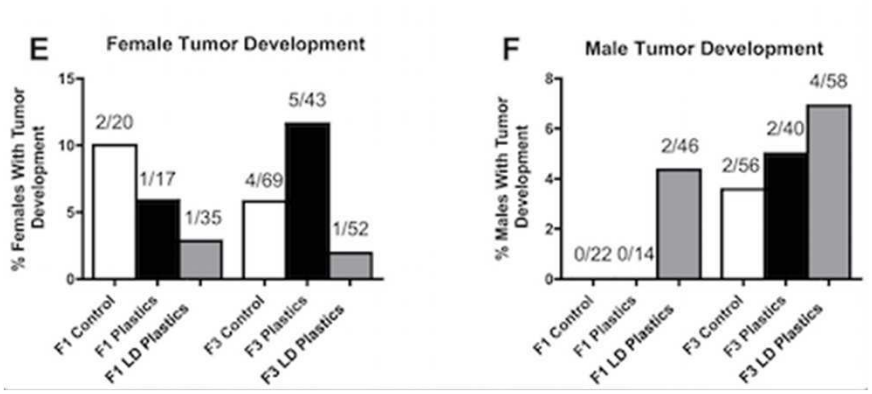


Male E2
78-101 pg/ml

Male T
4.2-5.7 ng/ml

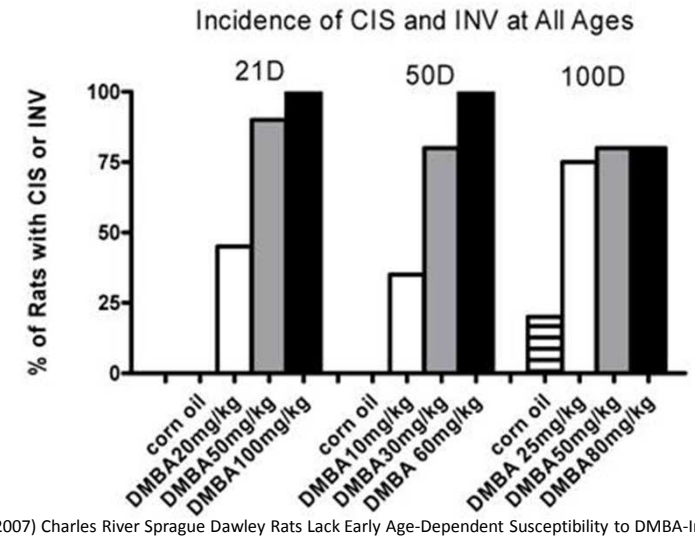
Vandenberg (2012) Hormones and Endocrine-Disrupting Chemicals: Low-Dose Effects... Endocr Rev

Sexual Dimorphic Responses to EDCs



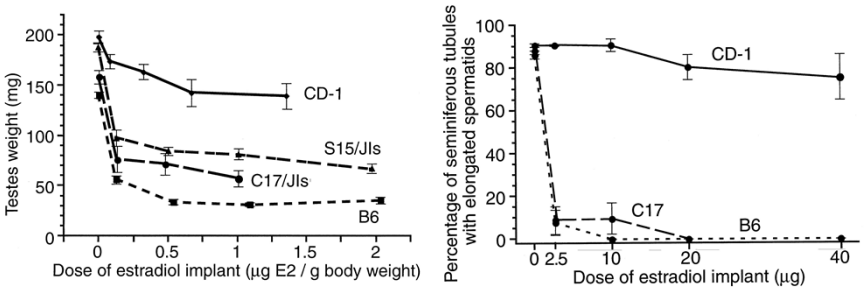
Manikkam M, Tracey R, Guerrero-Bosagna C, Skinner MK (2013) Plastics Derived Endocrine Disruptors (BPA, DEHP and DBP) Induce Epigenetic Transgenerational Inheritance of Obesity, Reproductive Disease and Sperm Epimutations. PLoS ONE 8(1): e55387. doi:10.1371/journal.pone.0055387

SD-Rat Model Limitations



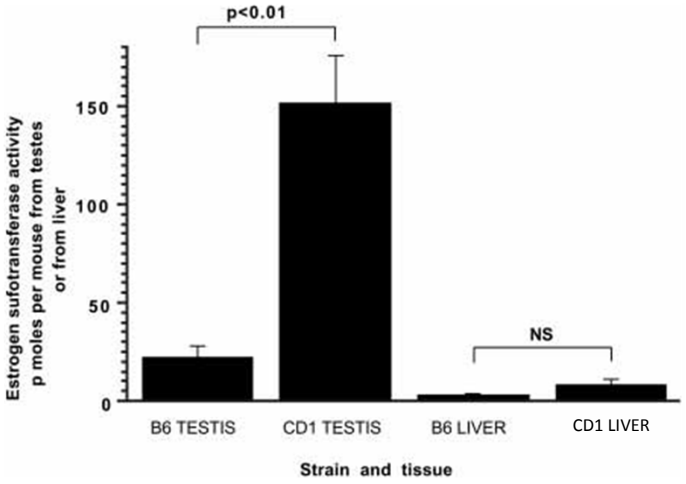
Gear RB (2007) Charles River Sprague Dawley Rats Lack Early Age-Dependent Susceptibility to DMBA-Induced Mammary Carcinogenesis. Int J Biol Sci

Differences in Mouse Susceptibility



Genetic Variation in Susceptibility to Endocrine Disruption by Estrogen in Mice Spearow et al. 1999, Science

Differential Tissue Effects



Genetic variation in physiological sensitivity to estrogen in mice. Spearow et al. 2001, APMIS

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Summary of urine biomonitoring based human BPA exposure measures

Study description	*N*	Country	Citation	(µg/kg/day)		
				Lower	Mean	Upper
Spot urine biomonitoring based exposure assessments						
World Health Organization			a			
1–5 months	47	Germany	b	–	0.07	1.61
3–5 years	599	Germany	c	–	0.12	0.78
6–11 years	314	United States	d	–	0.07	0.31
NHANES 2003–2004	2517	United States	e	0.024	0.047	0.27
NHANES 2005–2006	2548	United States	f	0.017	0.035	0.22
CHMS	5472	Canada	g		0.021	0.089
Japanese adults	22	Japan	i	0.008	0.028	0.075
Japanese adults	36	Japan	j	0.004	0.02	0.23
Males	5	Japan	j	0.01	0.022	0.22
Pregnant	56	Japan	k	–	–	0.16
College students	48	Japan	l	0.002	0.015	0.24
Girls 6–8 years old	90	United States	m	0.012	0.07	2.17
24 h Urine biomonitoring based exposure assessments						
Adult	596	Germany	n	0.0002	0.037	0.171
Adult	8	Germany	o	–	0.07	–
Adult	20	United States	p	0.03	0.27	0.86
Adult	8	United States	q	0.05	0.07	0.09
Pre-school children	81	United States	r	0.057	0.105	0.458

(a) Data for neonatal and childhood exposures were compiled from the following report: (World Health Organization and Food and Agriculture Oranization of the United Nations, 2011). The primary citation and country of origin for each perinatal study population is also provided.(b) Völkel et al. (2011).(c) Becker et al. (2009).(d) Calafat et al. (2008).(e) Lakind and Naiman (2008).(f) Lakind and Naiman (2010).(g) Lakind et al. (2012).(i) Tsukioka et al. (2004).(j) Arakawa et al. (2004).(k) Fujimaki et al. (2004).(l) Ouchi and Watanabe (2002).(m) Wolff et al.(2007).(n) Koch et al. (2012).(o) Moors et al. (2007).(p) Teeguarden et al. (2011).(q) Ye et al. (2011).(r) Morgan et al. (2011).

•Average "Mean" exposure **= 0.07** µg/kg/day
•Average "Upper" exposure **= 0.5** µg/kg/day

Teeguarden JG & Hanson-Drury S. Food and Chem. Tox. 2013;62:935-948

External exposures and corresponding human serum concentrations

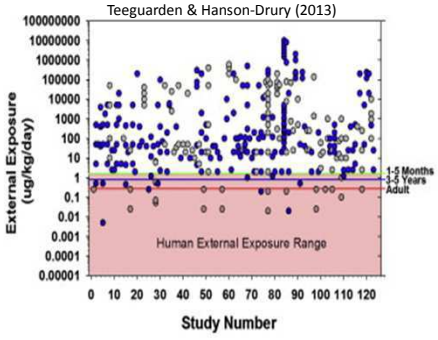
Cohort	Source	Upper bound exposure (μg/kg/day)	Corresponding peak human serum concentrations (nM)
Adult	NHANES 2004-2005[a]	0.27	0.01
Adult	NHANES 2005-2006[a]	0.22	0.01
Child (1-5 months)	World Health Organization[a]	1.61	0.08
Child (3-5 years)	World Health Organization[a]	0.78	0.04
Child (6-11 years)	World Health Organization[a]	0.31	0.02

(a) Data for neonatal and childhood exposures were compiled from the following report: (World Health Organization and Food and Agriculture Oranization of the United Nations, 2011).

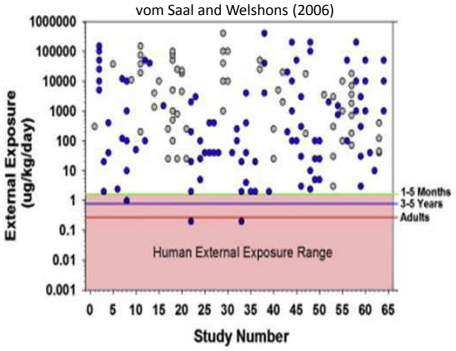
Teeguarden JG & Hanson-Drury S. Food and Chem. Tox. 2013;62:935-948

Comparison of BPA external exposure levels in "low dose" *in vivo* animal studies to human aggregate external exposure levels

Teeguarden & Hanson-Drury (2013)



vom Saal and Welshons (2006)



- 6% of the 342 tested exposures in these studies were in the range of adult human exposure; of these, 1% of studies exposed animals by oral route.
- 9% of the 342 tested exposures in these studies were in the range of infant and child exposure; of these, 1% reflected oral-route exposure.
- No studies had more than two exposures in the range of human exposure.

Teeguarden JG & Hanson-Drury S. Food and Chem. Tox. 2013;62:935-948

Outline

- Background
- Three Criteria of Reasonable Experiments:
  1. Equivalent Models
  2. Human Exposure Levels
  3. Route of Exposure
- Experiments that meet criteria
- Alternative chemicals
- Conclusions

Route of Exposure

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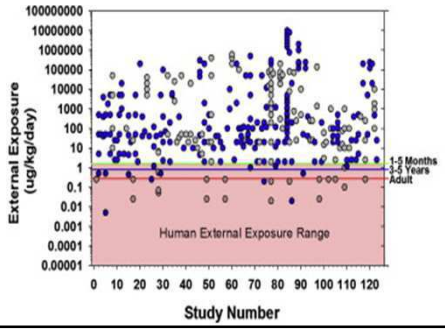
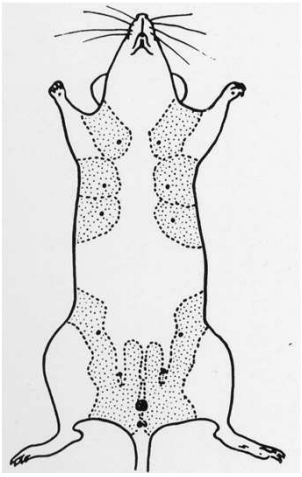
Three criteria for causation

1) Suitable model organism (mammal)
2) Relevant human exposure
3) Appropriate route of exposure



Accelerated Mammary Tumorigenesis



- MMTV-erbB2 mice were genetically designed to develop mammary tumors.
- At 2.5 ug/L and 25 ug/L the mice had earlier onset of mammary tumors.
- However, at 250 ug/L and 2500 ug/L, no accelerated tumorigenesis was observed.

Jenkins, S. et al (2011). Chronic oral exposure to bisphenol A results in a nonmonotonic dose response in mammary carcinogenesis and metastasis in MMTV-erbB2 mice. Environmental Health Perspectives 119, 1604–1609.

Perinatal exposure

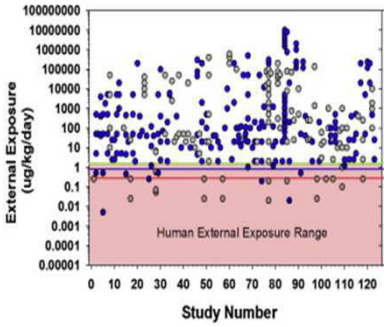
- Two studies exposed mothers to BPA and looked at offspring.
- Both found an increase in weight for the offspring but one looked at length and determined the BPA exposed group grew faster. No changes once adult were observed.
- The other group found sex differences in sweet preference when BPA exposed.

Xu, X., Tan, L., Himi, T., Sadamatsu, M., Tsutsumi, S., Akaike, M., Kato, N., 2011a. Changed preference for sweet taste in adulthood induced by perinatal exposure to bisphenol A–A probable link to overweight and obesity. Neurotoxicology and Teratology 33, 458–463.
Gioiosa, L., Fissore, E., Ghirardelli, G., Parmigiani, S. & Palanza, P. Developmental exposure to low-dose estrogenic endocrine disruptors alters sex differences in exploration and emotional responses in mice. Hormones and behavior 52, 307-316, doi:10.1016/j.yhbeh.2007.05.006 (2007).

Other Group effects

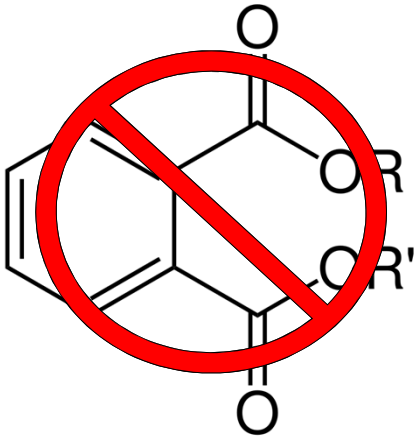
Of the 19 papers presented under “Health Effects”:

- 9 human correlation studies
- 2 in vitro
- 8 inappropriate does and/or route of exposure



Phthalates

Outside the scope of this review.



Summary of Health Effects

- There were no studies that showed a danger of BPA to the general population.
- There could be a danger to:
  - Pregnant women (children show accelerated maturation)
  - High risk for breast cancer (maybe)

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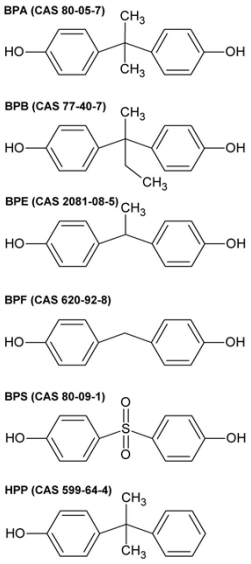
How “safe” are alternative chemicals to BPA?

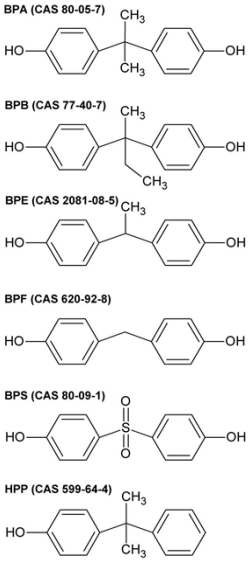


H295R Steroidogenesis Assay - Performed to assess test compounds’ potential to affect steroid hormone synthesis. Add test compounds to cells, incubate, then use solid phase extraction to extract hormone from cell supernatant. Quantify using HPLC-MS/MS

- **Progestogens** – A class of steroid hormones that activate the progesterone receptor. Involved in maintaining pregnancy and are present in phases of the menstrual cycle.
- **Corticosteroids** – A class of steroid hormones involved in a number of responses including stress response, immune response, inflammation, etc.\
- **Androgens**– A class of steroid hormones that control the development and maintenance of male characteristics.
- **Estrogens** – Primary female sex hormones.

Rosenmai A K et al. Toxicol. Sci. 2014;139:35-47

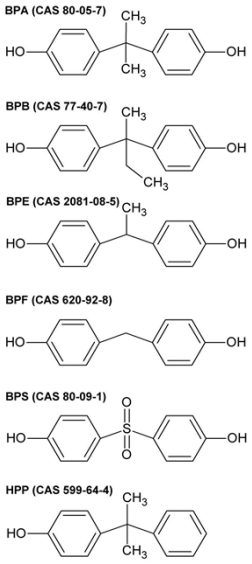
How “safe” are alternative chemicals to BPA?



		BPA
Progesterone	E_{max} (%)	
	CI (%)	
	EC_{50} (μM)	
	CI (μM)	
17α-OH progesterone	E_{max} (%)	22
	CI (%)	17–28
	EC_{50} (μM)	0.004
	CI (μM)	VW
Cortisol	E_{max} (%)	73
	CI (%)	—
	EC_{50} (μM)	11.0
	CI (μM)	2.2–55.3
Corticosterone	E_{max} (%)	
	CI (%)	
	EC_{50} (μM)	
	CI (μM)	
Dehydroandrosterone	E_{max} (%)	44
	CI (%)	38–50
	EC_{50} (μM)	1.4
	CI (μM)	1.0–2.0
Androstenedione	E_{max} (%)	89
	CI (%)	—
	EC_{50} (μM)	3.1
	CI (μM)	2.1–4.7
Testosterone	E_{max} (%)	83
	CI (%)	—
	EC_{50} (μM)	3.2
	CI (μM)	2.1–4.8
Estrone	E_{max} (%)	205[c]
	CI (%)	—
	EC_{50} (μM)	7.2[c]
	CI (μM)	1.7–31.3
17β-Estradiol	E_{max} (%)	209
	SD (%)	31
	EC_{50} (μM)	14.0
	SD (μM)	3.2

No change deactivation activation

How “safe” are alternative chemicals to BPA?



		BPA	BPB	BPE	BPF	BPS	HPP
Progesterone	E_{max} (%)			689	1493	744	387
	CI (%)			—	—	—	240–513
	EC_{50} (μM)			18.2	20.8	14.7	4.8
	CI (μM)			8.8–37.9	18.4–23.5	3.4–63.5	1.9–12.0
17α-OH progesterone	E_{max} (%)	22	74	198	510	1676	298
	CI (%)	17–28	—	—	—	—	249–329
	EC_{50} (μM)	0.004	8.2	23.0	16.0	8.0	6.1
	CI (μM)	VW	4.6–14.4	14.9–35.7	10.8–23.9	5.2–12.3	4.3–8.7
Cortisol	E_{max} (%)	73	72		43	74	78
	CI (%)	—	—		27–59	—	—
	EC_{50} (μM)	11.0	11.8		3.3	4.8	16.3
	CI (μM)	2.2–55.3	5.4–25.5		VW	1.0–22.6	6.5–41.1
Corticosterone	E_{max} (%)		66	292	757	70	30
	CI (%)		—	—	—	—	19–32
	EC_{50} (μM)		4.5	16.1	18.1	4.7	3.4
	CI (μM)		0.1–145.0	10.6–24.6	13.0–25.2	2.4–9.1	VW
Dehydroandrosterone	E_{max} (%)	44	22	68	86	70	
	CI (%)	38–50	16–26	—	74–93	52–77	
	EC_{50} (μM)	1.4	3.8	0.5	4.6	12.5	
	CI (μM)	1.0–2.0	2.2–6.7	0.2–1.6	3.5–5.9	VW	
Androstenedione	E_{max} (%)	89	77	73	64	57	86
	CI (%)	—	—	—	53–75	39–61	—
	EC_{50} (μM)	3.1	16.0	0.3	5.6	14.9	19.0
	CI (μM)	2.1–4.7	12.4–20.7	0.1–0.9	4.1–7.7	9.7–22.8	11.0–32.9
Testosterone	E_{max} (%)	83	78	58	47	62	70
	CI (%)	—	—	—	—	—	—
	EC_{50} (μM)	3.2	10.8	5.0	24.9	14.5	28.0
	CI (μM)	2.1–4.8	7.2–16.2	3.4–7.3	15.0–41.5	4.5–47.0	7.9–98.8
Estrone	E_{max} (%)	205[a]	1592	226	248		845
	CI (%)	—	—	—	—		—
	EC_{50} (μM)	7.2[a]	17.4	13.6	19.7		17.9
	CI (μM)	1.7–31.3	11.0–27.5	6.2–29.7	14.0–27.7		13.0–24.6
17β-Estradiol	E_{max} (%)	209	326	212	481		248
	SD (%)	31	138	29	132		7
	EC_{50} (μM)	14.0	13.6	22.2	17.6		9.7
	SD (μM)	3.2	2.1	0.7	1.3		1.7

No change deactivation activation

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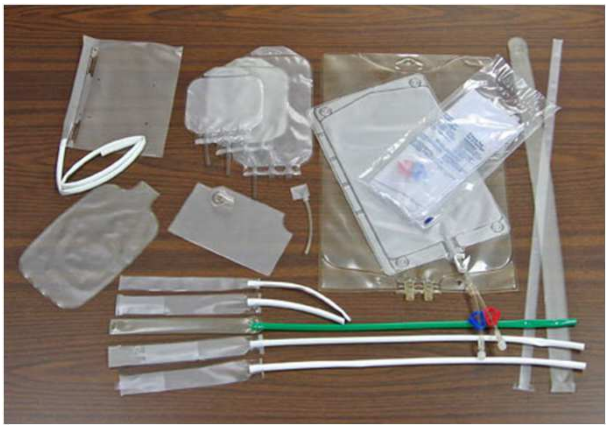
Conclusions

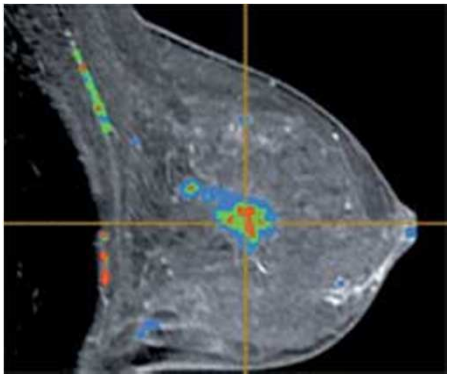
BPA is used in a wide variety of plastics that increase longevity and quality of human life.



Conclusions

Potentially at-risk populations:

Breast Cancer +



Pregnanct

Conclusions

- Hard plastics are an essential part of our daily lives.
- Other chemicals haven't been tested.
- Incorporation of experimental criteria for better designed studies.

Conclusions

Ideal experimental parameters for characterizing EDC toxicity:

- In sensitive mammalian model (mouse/rat)
- Multiple doses
  - Lower dose(s) (0.07 ug/kg/day)
  - Highest dose (0.5 ug/kg/day)
- Oral exposure (water or food)
- No polycarbonate water bottles or cage components
- Sexual Dimorphism?
  - Male vs. Female
- Define windows of susceptibility
  - Adult
  - Gestational exposure → adult phenotypes

Thank You