ENDOCRINE DISRUPTORS

Common bisphenol A replacements are reproductive toxicants

Genoa R. Warner and Jodi A. Flaws

Replacements for the chemical bisphenol A (BPA) have become commonplace in plastics labelled BPA free. However, many of these chemicals have similar structures and properties to BPA. A new study reports that replacement bisphenols, which were discovered as laboratory contaminants, are reproductive toxicants and that their effects might persist for multiple generations.

Refers to Horan, T. S. et al. Replacement bisphenols adversely affect mouse gametogenesis with consequences for subsequent generations. *Curr. Biol.* **28**, 2948–2954 (2018).

Bisphenol A (BPA) is a widely studied chemical that is associated with many negative health effects at exposure levels measured in the general population[1]. As a result, manufacturers have developed replacement chemicals to remove BPA from consumer products. However, most replacements have only slightly altered structures, retaining the bisphenol backbone and therefore the properties of BPA, including toxicity. BPA analogues were not effectively tested for safety, and human exposure is now widespread as consumers have sought products labelled BPA free under the assumption that they are safer than BPA-containing products[2].

A recent study[3] has identified BPA replacements as contaminants in scientific research. Horan and co-workers were studying the effects of exposure to endocrine-disrupting chemicals and found chromosomal abnormalities in control mouse oocytes (that is, mice not experimentally exposed to endocrine-disrupting chemicals) at levels similar to those found in groups from previous studies that were treated with BPA. Further analysis identified that both BPA and the BPA analogue bisphenol S (BPS) were leaching from polysulfone mouse cages. BPS is not a constituent monomer of polysulfone, but is produced during environmental degradation of the polymer. BPS, as well as bisphenols F and AF (BPF and BPAF, respectively), were confirmed as germline toxicants in further dosing studies[3].

Tracking the long-term effects of exposure to bisphenols in humans is nearly impossible owing to the ubiquity of these chemicals. Bisphenols are not persistent in the body and are metabolized and excreted within hours to days, a fact that has been used to argue that BPA is safe[1]. However, Horan and colleagues demonstrate that the meiotic effects due to contamination persisted for multiple generations in the males after the contamination was eliminated. Our laboratory has also demonstrated effects from BPA on reproductive outcomes in female mice that persisted into the third generation of offspring, who were not directly exposed to BPA[4].

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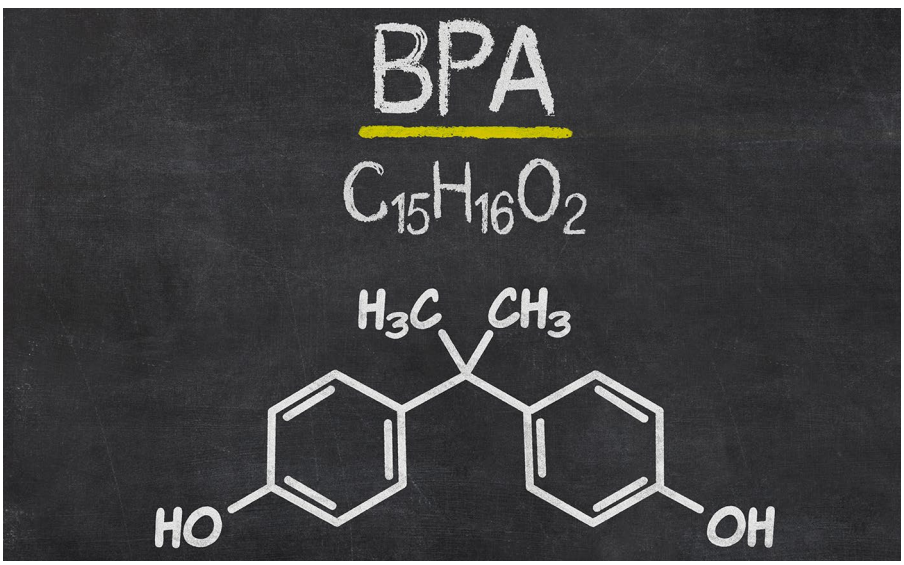
The presence of bisphenol contaminants in the research laboratory is especially troublesome for scientific studies on endocrine and reproductive end points. The contamination reported by Horan and colleagues was not uniform across all animals. Only the damaged cages leached BPS and BPA, resulting in some normal controls and some with meiotic effects. If the data had only been reviewed in aggregate as a mean and standard error, the contamination might not have been discovered. If this had occurred in a study on the effects of BPA, unnoticed contaminated controls would have led to the conclusion that BPA had no effect, when in fact there was no true control with which to draw any conclusions. This scenario might be one explanation for the difficultly of reproducing results in the BPA literature and in endocrine-disruption studies in general.

The ubiquity of bisphenol contamination might be worrisome for the Consortium Linking Academic and Regulatory Insights on BPA Toxicity (CLARITY-BPA) study, a collaboration between government and academic researchers that was designed to surmount the divide in study methods and reproducibility



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that has led to a regulatory standstill on BPA. Animals in the CLARITY-BPA study were housed and dosed at the FDA's National Center for Toxicological Research (NCTR). A portion of the animals stayed in the government facilities for analysis and others were sent to academic grantees. In preliminary studies performed at the NCTR, the naive and vehicle control animals were reported to have serum levels of BPA that were similar to that in the mice exposed to the lowest dose in the study[5]. The source of contamination was not confirmed, and the grantee study was probably performed without the contaminant being eliminated. Additionally, neither the preliminary study nor the official CLARITY study were monitored for bisphenols other than BPA. The difficult, months-long process of removing bisphenol contamination reported by Horan and colleagues combined with the reported preliminary CLARITY study contamination calls into question the rigor of some of the controls in the CLARITY study. For example, Horan and co-workers identified effects from BPA, BPS, BPF and BPAF in the male germline, but analysis of sensitive testicular end points from CLARITY found no effect at a similar dose[6]. This discrepancy could be due to a number of different factors, including the animal model used or contamination, but it serves to highlight the point that negative results should not be considered proof of no effect under conditions in which reproducibility is challenging.

In its 2018 draft report[7] on the government branch of the CLARITY study, the FDA compares rates of female mammary gland lesions in the group receiving the lowest dose of BPA with rates in historical control animals from the NCTR. Given the ubiquity of BPA and replacement bisphenols, it is problematic to compare the CLARITY results to historical controls that might not have been examined for BPA exposure and might even date back to the days before polycarbonate cages and water bottles were known to leach BPA. We suspect that historical control mammary gland lesion rates are more indicative of low dose exposure than no exposure.

> “Manufacturers need to look to green and sustainable chemistry methods”

The study by Horan and colleagues reinforces the necessity of testing all replacement chemicals for the toxic properties of the original compounds. Methods to develop useful chemicals that are also safe for the endocrine system exist, but have not been effectively utilized[8,9]. Manufacturers need to look to green and sustainable chemistry methods to avoid regrettable replacements for bisphenols, phthalates, perfluorinated chemicals and other classes of endocrine disruptors. Substituting structurally similar chemicals without adequately testing their safety is nothing more than a marketing ploy with lasting consequences for human health and a multi-billion dollar price tag[10].

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Competing interests
The authors declare no competing interests.