Chemical Contaminants from Plastics in the Animal Environment

Galen J Gorence,¹ Hannah C Pulcastro,² Crystal A Lawson,² Roy R Gerona,³ Matthew Friesen,³ Tegan S Horan,² Mary C Gieske,² Caroline V Sartain,² and Patricia A Hunt^{2*}

Accidental exposure of our mice to bisphenol A (BPA) from damaged polycarbonate cages 20 y ago provided some of the first evidence of the harmful effects of exposure to this common chemical. Recently we found that housing mice in damaged polysulfone cages resulted in similar harmful effects due to exposure to bisphenol S (BPS). This problem was unexpected for 2 reasons. First, polysulfone is a far more chemically resistant polymer than polycarbonate. Second, BPS is not a component in the manufacture of polysulfone. We report here our efforts to verify the source of the BPS and eliminate the exposure. Our analysis of new polysulfone caging materials confirmed that BPS is a breakdown product of damaged polysulfone plastic. Furthermore, we found that BPS can cross-contaminate new or undamaged cages in facilities that process damaged caging materials. Neither the use of disposable cages nor replacement of caging materials used solely for our colony was sufficient to eliminate exposure effects. Only the replacement of all cages and water bottles in the facility corrected the problem and allowed us to resume our studies. Taken together, our previous and current findings underscore the concern that chemicals from plastics are harmful environmental contaminants for both humans and animals. Furthermore, our results provide strong evidence that the presence of damaged plastic in a facility may be sufficient to affect research results and, by extension, animal health.

Abbreviations: BPA, bisphenol A; BPS, bisphenol S; cps, counts per second; dpc, days post coitum; EDC, endocrine disrupting chemical; LC-MS/MS, liquid chromatography–tandem mass spectrometry

DOI: 10.30802/AALAS-JAALAS-18-000074

Controlling the environment to minimize variability is an important aspect of animal husbandry for both livestock and research animals. Because pathogen control is critical in ensuring optimal animal health, it has been a major focus of attention. However, breeders and scientists who study reproduction, brain development, and behavior have recognized for decades that subtle environmental changes (for example, light, temperature, humidity) can significantly affect breeding and study outcomes. The 1946 report of infertility in Western Australian sheep grazing on clover is, to our knowledge, the first report of breeding effects induced by high levels of phytoestrogens, but similar effects can occur in experimental animals on a soy-based diet (reviewed in reference 8). In addition to naturally occurring phytoestrogens, a variety of common man-made chemicals can affect the endocrine system (reviewed in reference 14). These have been termed endocrine-disrupting chemicals (EDCs) and, because many are common environmental contaminants, their effects on human and animal health and fertility is a growing area of research attention.

We recently reported the results of studies in male and female mice that we conducted after the accidental exposure of our colony to the bisphenol A (BPA) replacement chemical bisphenol S (BPS).¹ As detailed in that report, in the course of routine experiments, we noted increased variability in control data, with some litters providing data consistent with expected laboratory

values and others yielding data similar to those of mice exposed to BPA. We were able to correlate these data discrepancies with the appearance of physical changes in polysulfone cages that had been used in our facility without incident for years. Inconsistencies in data obtained from different litters of male mice, coupled with physical changes on only a subset of cages, made damaged caging materials a plausible source of contamination.

To determine whether chemicals were leaching from cages with physical signs of damage, we used a cage-monitoring technique devised to resolve a previous contamination issue in our facility.⁷ Briefly, this method involved sampling a group of damaged cages by sequentially rinsing a small amount of methanol over the inside surface of each to extract a pooled sample of surface contaminants and analyzing those extracts by using liquid chromatography–tandem mass spectrometry (LC-MS/MS). This analysis suggested that the damaged cages were exposing our mice to BPS.¹

We summarize here the difficulties we encountered in trying to eliminate this exposure. Our experience underscores the inherent difficulty of controlling environmental contaminants and suggests that, for bisphenols, cross-contamination from damaged materials that remain in use may be sufficient to affect experimental results. Given the growing number of environmental contaminants and their ability to affect animal health and fertility, devising strategies to reduce environmental contamination is essential, and we offer recommendations to this end.

Received: 20 Jun 2018. Revision requested: 21 Aug 2018. Accepted: 16 Oct 2018.

¹Vivarium Services, Office of the Campus Veterinarian, and ²School of Molecular Biosciences, Center for Reproductive Biology, Washington State University, Pullman, Washington; and ³School of Medicine, University of California, San Francisco, California

*Corresponding author. Email: pathunt@wsu.edu

Materials and Methods

Husbandry. Inbred C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME) were maintained through brother–sister mating

and housed in polysulfone cages on ventilated racks (Allentown, Allentown, NJ) in an SPF facility monitored by sentinel testing for mouse hepatitis virus, minute virus of mice, pneumonia virus of mice, NS1 virus, mouse parvovirus types 1 through 5, mouse norovirus, Theiler murine encephalomyelitis virus, epizootic diarrhea of infant mice virus, reovirus 3, Sendai virus, *Mycoplasma pulmonis*, lymphocytic choriocytic meningitis virus, ectromelia virus, *Helicobacter* spp., *Aspiculuris tetraptera*, *Syphacia muris*, *Syphacia obvelata*, *Myocoptes* spp., and *Radfordia* (*Myobia*) spp. Cages contained hardwood bedding (Sanichip 7090A, Harlan Laboratories, Indianapolis, ID) and a cotton square (Ancare, Bellmore, NY) for enrichment. Drinking water in polysulfone bottles and food (Purina Lab Diet 5K52, LabDiet, St Louis, MO) were provided without restriction for all animals except new breeding stock, which received Envigo Teklad 2920 (Huntingdon, United Kingdom). Cages were washed (model 9700 Cage and Rack Washer, Steris, Mentor, OH) by using an alkaline wash (CK180, Steris) followed by an acid wash (CK200, Steris). Final rinse sanitation was completed at a minimum of 180 °F (82 °C) for at least 3 min. All caging and water bottles were bulk-sterilized (Getinge, Wayne, NJ) by using a prevac cycle at 270 °F (132 °C) for 6 min for cages and a liquid cycle at 250 °F (121 °C) for 30 min for bottles. In initial studies to eliminate contaminants, a subset of animals was housed in disposable cages (Inovive, San Diego, CA) on a static rack, and cages were changed every 2 wk. All protocols were approved by the Washington State University IACUC and followed the NIH standards established by the *Guide for the Care and Use of Laboratory Animals*.⁵ Washington State University is fully AAALAC-accredited.

Caging experiments. To identify unpolymerized bisphenols present on new cages, we conducted an extraction on a group of 5 new mouse cages by using a previously described method.⁷ This initial sample and extractions of the same cages obtained after each of the first 5 washes were analyzed by LC-MS/MS (LC1260 System, Agilent, Santa Cruz, CA; 5500 Triple Quadrupole MS, AB Sciex, Foster City, CA) at the University of California–San Francisco. Aliquots of the methanol extraction medium served as a negative control.

Meiotic studies of female mice. We used a sensitive and quantitative variable, levels of meiotic recombination in oocytes from fetal ovaries, to monitor exposure. This assay is a standard technique in our laboratory, and the data provided in the current manuscript were collected as described previously.^{1,15} Briefly, this assay involves daily oral dosing of dams with 1 µL of BPA (500 ng/g body weight in the present experiments) or ethanol–corn oil vehicle from 11 to 17 d post coitum (dpc), killing dams by exposure to concentrated carbon dioxide on 17.5 dpc, and collecting fetuses. Meiotic preparations from fetal ovaries were made by using the method of spreading cell suspensions on slides and fixing in paraformaldehyde as described previously.¹³ Fixed slides were immunostained with antibodies to the mismatch repair protein MLH1 (dilution, 1:60; BD Pharmingen, San Jose, CA) and the synaptonemal complex protein SYCP3 (1:200, Novus Biologicals, Littleton, CO), imaged by using an epifluorescence microscope (Axio Imager, Zeiss, Thornwood, NY), and scored by 2 independent observers blind to animal treatment, as described previously.^{15,16} This allowed us to obtain average MLH1 counts (a measure of meiotic recombination) for each dam and pooled averages for treatment group; statistical analyses were conducted by using standard Student *t* tests (GraphPad Software, <https://www.graphpad.com/quickcalcs/ttest1>).

Results

Source of BPS. Our previously reported identification of BPS exposure of mice housed in damaged polysulfone caging¹ was surprising initially because, according to the manufacturer (Solvay, Princeton, NJ), the polysulfone used in cages is produced through the polymerization of bisphenol A and 4,4'-dihalodiphenylsulfone monomers. However, although BPS is not a component of polysulfone, breakage of the bond between these monomers is most likely to result in the release of BPS (Figure 1 A), as we reported previously.¹

Physical changes that were initially mild and limited to a subset of cages in the facility progressively worsened as the cages continued to be used, washed, and autoclaved (Figure 1 B). This outcome is consistent with polymer damage and progressive deterioration, as compared with a surface residue contaminant from detergent or hard water. Because newly manufactured polycarbonate and polysulfone caging both have a surface residue of unpolymerized BPA,² we reasoned that unpolymerized BPS residue might also be detectable on new polysulfone cages. To test this notion, we identified a set of 5 new cages, conducted a pooled methanol extraction on them, washed the cages by hand, and repeated the extraction and wash procedure. After the third postwash extraction, the cages were placed into use in our colony, but samples were extracted from them after their 4th and 5th washings (Figure 1 C). As expected, unpolymerized BPA was present on the surface of new cages, remained present after one wash, but was undetectable after subsequent washes. BPS was not detected on new cages or after the first 3 washes, consistent with the fact that it is not a component of polysulfone cages. However, after these cages were placed into use in the facility, BPS became detectable on them after the 5th wash. Because visible signs of damage were not evident on the cages being monitored, we suspected that exposure was due to cross-contamination during washing, autoclaving, or both rather than damage or degradation.

Evidence of cross-contamination. A critical aspect of meiosis, the repair of DNA double-strand breaks as sites of exchange between the maternal and paternal chromosomes, is sensitive to environmental influences. This repair can be quantified, shows strain-specific variation and, in females, is reproducibly increased by maternal BPA exposure coinciding with the occurrence of these events in the developing female ovary.¹⁵ Concurrent with changes in control data that we reported previously for male mice exposed to replacement bisphenols,¹ we also observed higher control values in females, consistent with exposure (Figure 2 A, compare vehicle control values in left panel [obtained prior to exposure] with those in middle panel [obtained during exposure]).

Disposable cages. We first attempted to alleviate environmental exposure by purchasing new breeders and housing them in disposable cages. Neither group (BPA-exposed nor vehicle control) had levels of MLH1 foci in the range expected on the basis of our previous studies, with no difference between the 2 groups (Figure 2 A, right panel).

New cages. Next, we replaced the cages and water bottles for mice in our colony with new prewashed polysulfone materials. Because old cages also remained in use in the facility, new cages were engraved to distinguish them, and they were washed and processed outside the facility. We conducted 2 sets of experiments on mice housed in new cages.

First, we examined effects on the descendants of the contaminated mice by weaning offspring from the exposed colony of C57BL/6J animals into new cages, mating them at sexual

Figure 1. Bisphenol S (BPS) exposure from cages with physical changes. (A) Polysulfone is produced from the dimerization of BPA (blue) and diphenyl sulfone (black), but breakage of the bonds between them (red X) generates BPS (red). (B) Top images show new (left) and in use (right) cages. Bottom images show cages exhibiting mild (left) and more severe (right) signs of damage. (C) LC-MS/MS analysis of a pooled methanol extraction from 5 cages with visible signs of damage provided evidence of BPS, measured as counts per second (cps). Subsequent sequential analysis of extracts from a group of 5 new cages demonstrated detectable levels of BPA prior to the first wash and lower but detectable levels after the first wash (the low levels of BPS detected in unwashed cages in this analysis were considered below the level of detection). Neither BPA nor BPS was detected after wash 3 or 4. Cages were used to house animals after the 3rd wash, and low levels of BPS were detected after the 5th wash.

Figure 2. Studies of fetal ovaries provide evidence of exposure. (A) Left panel: pregnant female mice were treated orally with BPA (500 ng/g daily) or vehicle only on 11 through 18 dpc. Diamonds, number (mean $\pm$ SEM) of MLH1 foci (a surrogate for recombination) in treated (pink; $n = 94$ cells from 2 litters) and control (black; $n = 79$ cells from a single litter) groups as measured by immunofluorescence staining. The difference between these groups was highly significant ($t = 3.94$; $P < 0.001$) and similar in magnitude to our previously reported observations.^{4,15} Middle panel: data obtained from cells from BPA-treated ($n = 353$ cells from 7 litters) and vehicle-control litters ($n = 332$ cells from 8 litters) in early 2016 (during the contamination event). Values did not differ significantly ($t = 0.66$; $P > 0.05$). Right panel: data obtained for BPA-treated ($n = 136$ cells from 3 litters) and vehicle-control ($n = 248$ cells from 3 litters) animals in disposable cages did not differ significantly ($t = 1.62$; $P > 0.05$). (B) Left panel: data by litter (denoted as black diamonds) in descendants of exposed mice moved to new cages and mated in late 2016. Values for 6 of the 7 litters analyzed were in the exposed range. Middle panel: data by litter from new breeding stocks placed in new cages in late 2016. Values were normal initially, but increasing variability over time suggested persistent contamination. Right panel: replacement of all caging materials in the facility and the purchase of new breeding stock resulted in a return to expected levels within several months.

maturity, and analyzing the ovaries from female fetuses over a 6-mo period (Figure 2 B, left panel). Values from 6 of the 7 litters analyzed remained in the expected range for exposed animals, suggesting the persistence of exposure effects in the daughters of exposed mice.

Second, to eliminate both residual effects of exposure in descendants and as many potential sources of contamination as possible, new C57BL/6J breeders were purchased, housed in new cages, and fed an irradiated diet (Envigo Teklad 2920). The first 3 litters examined from this new colony yielded values at or near control levels (Figure 2 B, middle panel), suggesting that contamination had been eliminated successfully. Accordingly, all animals in the colony were moved into new cages that were

engraved to distinguish them from other cages in the facility. Because replacing all the cages in the colony exceeded our ability to handwash them, cages were washed and processed inside the facility. Although new cages for our colony were washed and processed separately from all other cages in the facility, increasing variability in the data from new breeders became evident over the next several months (Figure 2 B, middle panel), suggesting cross-contamination from damaged materials that remained in use in the facility.

Replacement of all caging materials in the facility. The results of our cage-monitoring experiment and the failure to obtain expected control levels of recombination in studies of mice housed to eliminate as many potential sources of con-

tamination as possible (that is, new mouse stocks housed in new cages, fed irradiated food, and provided cotton squares autoclaved outside the facility) suggested continued exposure. Taken together, the results suggested that the presence of damaged plastic caging materials still in use in the facility was sufficient to contaminate new caging materials used for our colony when the cages were washed and autoclaved in the same facility. This outcome suggested that the only means of eliminating contamination completely was the replacement of all caging materials used in the facility. Although this strategy was expensive and required careful coordination, it ultimately was achieved in Fall 2017, and new C57BL/6 breeding stock was purchased again. Variability was evident in the data obtained from the first few control litters produced in this new colony (Figure 2 B, right panel), raising concern about lingering contamination. However, after several months, data from all litters examined returned to historic control levels, allowing us to reproduce our previously published findings for the first time in 2 y.

Discussion

Environmental contamination disrupted a major portion of our research for more than 2 y, and the studies conducted to identify and eliminate the source and verify that normal conditions had been restored were expensive and frustrating. Although the cause of the damage to caging materials remains unknown, we were able to resume our studies after the replacement of all caging materials in the facility and the purchase of new breeding stock. From an animal husbandry perspective, 3 important conclusions can be derived from our experience. First, our data demonstrate that plastic animal-contact materials must be considered a potential source of contamination. Second, data from our studies of both new cages and of animals during attempts to eliminate exposure provide evidence of cross-contamination, suggesting that replacing animals and caging materials may be insufficient to eliminate animal contamination if damaged materials remain in the facility. Lastly, this contamination experience—coupled with previous environmental effects encountered in the course of our studies—suggests that controlling the environment for both livestock and research animals requires scrupulous attention to detail. Accordingly, we provide a summary of the factors we think are most critical in ensuring a stable animal environment.

First, our experience underscores the importance of viewing all animal-contact materials as potential sources of contaminants. Our laboratory studies sensitive reproductive endpoints that are susceptible to environmental influences, and environmental contaminants are immediately evident as changes in control data. We experienced the first such data shift in 1998, when chemical damage induced by the use of a high pH floor detergent caused polycarbonate cages and water bottles in our animal facility to leach BPA.³ This environmental contamination was evident as a sudden, dramatic change in eggs from control female mice. In the intervening 20 y, we also experienced environmental effects induced by the use of quaternary ammonium disinfectants⁶ and variable levels of phytoestrogens in rodent diet.⁹ These experiences have increased our awareness of the need to maintain a controlled animal environment but, despite our increased vigilance, we again encountered environmental contamination from damaged caging.

As a result of our initial experience with BPA exposure, we eliminated all polycarbonate plastic, replacing it with caging

materials made of the more durable polymer, polysulfone. Because we used polysulfone caging for years without incident, we were surprised when changes in control data coincided with the appearance of visible cage damage. In contrast to our experience 20 y earlier,³ the damage was milder and limited to a subset of cages, and analysis of these cages suggested leaching of the replacement bisphenol, BPS.¹ Eliminating this contaminant proved difficult.

A 2nd conclusion from our findings that is relevant to ensuring a consistent animal environment is the risk posed by cross-contamination. Because replacing caging materials is an expensive proposition, we opted for less expensive solutions, first attempting to use disposable cages and then introducing new polysulfone cages to house animals in our colony. Although expected recombination values were not obtained for animals in disposable caging, our analysis does not allow us to determine whether this result was due to the polymer used in these cages or to residual contamination (for example, from feed or cotton squares provided to pregnant female mice, given that both were autoclaved in the facility). We conducted 2 different studies of animals housed in new polysulfone cages: an analysis of offspring from C57BL/6J mice in our BPS-exposed colony that were moved to new cages, and an analysis of offspring from new breeding stock that was purchased and housed in new cages. The rationale for this strategy was as follows: because some exposure effects can be transmitted to subsequent generations (reviewed in reference 1), we wanted to track animals from our exposed colony across generations to determine whether exposure effects persisted in unexposed descendants. In addition, we expected the new breeders in these cages would yield control data equivalent to those of our previous studies, allowing us to resume our experiments. Initially, this goal seemed to have been achieved, but when we switched from hand-washing new cages to washing them in the cage washer in the facility, a shift in the data suggested contamination. Because new cages showed no visible signs of damage, we suspected that this exposure was the result of cross-contamination from old cages that remained in use in the facility. Ultimately, all caging materials in the facility were replaced, and new breeders were purchased, and we finally were able to resume our research. As recently reported,¹ our controlled experiments of replacement bisphenols (including BPS and diphenyl sulfone) demonstrated effects similar to those induced by BPA. Importantly, however, our data suggest that contamination may have lingered in the system for several months after the introduction of new caging materials (Figure 2 B, left panel: compare the variability during the first and last 3 mo). Thus, understanding where and how chemical contaminants can migrate during the washing, autoclaving, and storage of materials is an important direction of investigation for improving animal care.

Third, our experience underscores the fact that maintaining a stable animal environment requires great attention to detail. Our experimental endpoint—meiotic recombination—is sensitive, quantitative, and indicative of the overall health of the process of making eggs or sperm.^{15,16} Our colony is maintained in a facility housing more than 1000 cages of mice for multiple investigators and a transgenic core, typically with 3000 to 4000 cages in circulation. Although the effects of contamination were not evident to other investigators housing animals in the facility, this does not necessarily mean that their studies were unaffected. Data from studies of BPA suggest a wide range of exposure effects in animals exposed during fetal and neonatal development (reviewed in references 10 through 12, 17, and

18), and although few studies of BPS have been conducted, the available evidence suggests that exposure effects are likely similar between BPA and BPS. Thus, we are not merely the victim of a series of unfortunate events but rather the canary in the coal mine.

Human, animal, and environmental studies suggest widespread use of BPA, and the rapid emergence of structurally similar replacement bisphenols has resulted in unavoidable daily exposure to these chemicals. Extensive investigation of BPA (reviewed in references 10 through 12, 17, and 18) and growing consumer concern has driven the rapid introduction of replacement bisphenols in products marketed as 'BPA free.' The rapidity with which this replacement was achieved demonstrates both the marvels of modern chemistry that make possible the rapid development of large chemical families as well as the potential perils of their unregulated release into our daily lives. Our experience demonstrates that these concerns extend to the care and maintenance of animals and the facilities that house them. Furthermore, as our BPS encounter demonstrates, exposure can surprise even the wary. Therefore, the personnel in daily contact with animals and the supervisory, veterinary, and regulatory personnel should be aware of such for potential contaminants and their effects. We offer the following recommendations for reducing environmental contamination:

- 1) All animal-contact materials must be considered potential sources of contamination. Caging, food, bedding, enrichment items, and even building components must be chosen carefully. Decisions regarding the products used should not be based simply on cost but informed by careful component analysis and specific protocols regarding their care, use, and lifespan. For example, any items comprised of or containing recycled materials are likely to contain unwanted chemical contaminants. For these reasons, it is essential to purchase from reputable suppliers who are aware of potential hazards and responsive to consumer concern.
- 2) Any plastic materials are potential sources of contamination, and knowledge of the specific plastic polymer and its potential degradation products is essential and should be provided by the manufacturer on request. Importantly, any visibly damaged animal-contact materials should be immediately removed from use, regardless of their time in service. Replacement is costly, but our experience suggests that the presence of damaged materials provides an opportunity for cross-contamination.
- 3) Disinfectants and cleaning products are important potential sources of contamination. In addition to bisphenols, known components of concern include quaternary ammonium compounds, parabens and other phenolic EDCs, perfluorinated compounds, phthalates, and flame retardants. New 'green' products, such as hydrogen peroxide-based disinfectants, provide safer alternatives. Reducing contamination from cleaning products requires careful analysis of solution components and specific protocols with regard to material contact. As our experience has shown, cleaning solutions of excessively high or low pH may damage plastics, causing them to leach chemicals.³ Finally, the rapid introduction of new chemicals means that it is essential to carefully evaluate any new or reformulated products prior to use.
- 4) For animals used in studies with sensitive endpoints that may be altered by environmental contaminants, routine testing of animal-contact materials should be considered. To differentiate between contaminants introduced during the collection and processing of samples and those to which animals may have been exposed during the experiment, the

inclusion of field blanks (that is, testing of all sample collection, processing, and storage containers and all chemical analysis materials) during this monitoring is essential. In addition, new lots of the product should be monitored, because companies typically do not divulge changes in the sources of raw materials or manufacturing which might significantly influence the end product.

- 5) Cryopreservation of expensive or irreplaceable animals is essential and provides a means of quickly recovering from contamination or other environmental disasters with minimal financial burden. Importantly, cryopreservation not only ensures animal recovery in the event of loss, but is an important means of preserving genetic integrity, that is, the introduction of random mutations over time can cause genetic drift.

The effects of contamination were evident to us because we could not reproduce control data consistent with our previous studies. In the absence of these previous data, we would have erroneously concluded that the EDC we were testing had no effect. This situation raises serious concern because reproducibility is essential in science. Subtle variation in data obtained in different laboratories is common and can be due to a wide range of factors (for example, differences in animal strain, diet, or research protocols). Clearly, eliminating all variation is an unattainable goal, but as our laboratory and others have demonstrated repeatedly, the ability to replicate results can be influenced strongly by animal environment. Therefore, recognizing, addressing, and controlling for emerging environmental contaminants has become an essential aspect of research involving animals. The rapid emergence of new chemicals and their ubiquitous presence in our lives makes environmental control an ever-shifting landscape. Providing a stable animal environment demands awareness and vigilance on the part of the scientific community. In addition, the development of strong partnerships among investigators, support staff, animal suppliers, and vendors of equipment, supplies, and building materials is essential.

Acknowledgments

We gratefully acknowledge the technical support of Hannah Kaiser, Alyssa Marre, and Heather Hall. We also thank Terry Hassold for helpful comments on the manuscript and Linda Toth for helpful discussions of the data. These studies, although not outlined in the original proposals, were supported by NIH grants R01HD083177 (PAH), ES013527 (PAH), and F32 ES026010 (CVS)

References

1. Horan TS, Pulcastro H, Lawson C, Gerona R, Martin S, Gieske MC, Sartain CV, Hunt PA. Replacement bisphenols adversely affect mouse gametogenesis with consequences for subsequent generations. *Curr Biol* 28:2949–2954.e3. <https://doi.org/10.1016/j.cub.2018.06.070>
2. Howdeshell KL, Peterman PH, Judy BM, Taylor JA, Orazio CE, Ruhlen RL, Vom Saal FS, Welshons WV. 2003. Bisphenol A is released from used polycarbonate animal cages into water at room temperature. *Environ Health Perspect* 111:1180–1187. <https://doi.org/10.1289/ehp.5993>.
3. Hunt PA, Koehler KE, Susiarjo M, Hodges CA, Ilagan A, Voigt RC, Thomas S, Thomas BF, Hassold TJ. 2003. Bisphenol A exposure causes meiotic aneuploidy in the female mouse. *Curr Biol* 13:546–553. [https://doi.org/10.1016/S0960-9822\(03\)00189-1](https://doi.org/10.1016/S0960-9822(03)00189-1).
4. Hunt PA, Lawson C, Gieske M, Murdoch B, Smith H, Marre A, Hassold T, VandeVoort CA. 2012. Bisphenol A alters early oogenesis and follicle formation in the fetal ovary of the rhesus monkey. *Proc Natl Acad Sci USA* 109:17525–17530. <https://doi.org/10.1073/pnas.1207854109>.
5. Institute for Laboratory Animal Research. 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.

6. **Maier B.** 2008. Lab disinfectant harms mouse fertility. Cage-cleaning chemicals cause birth defects and fertility problems in mice. *Nature* **453**:964. <https://doi.org/10.1038/453964a>.
7. **Melin VE, Potinini H, Hunt P, Griswold J, Siems B, Werre SR, Hrubec TC.** 2014. Exposure to common quaternary ammonium disinfectants decreases fertility in mice. *Reprod Toxicol* **50**:163–170. <https://doi.org/10.1016/j.reprotox.2014.07.071>.
8. **Messina M.** 2010. A brief historical overview of the past 2 decades of soy and isoflavone research. *J Nutr* **140**:1350S–1354S. <https://doi.org/10.3945/jn.109.118315>.
9. **Muhlhauser A, Susiarjo M, Rubio C, Griswold J, Gorence G, Hassold T, Hunt PA.** 2009. Bisphenol A effects on the growing mouse oocyte are influenced by diet. *Biol Reprod* **80**:1066–1071. <https://doi.org/10.1095/biolreprod.108.074815>.
10. **Murata M, Kang JH.** 2018. Bisphenol A (BPA) and cell signaling pathways. *Biotechnol Adv* **36**:311–327. <https://doi.org/10.1016/j.biotechadv.2017.12.002>.
11. **Nesan D, Sewell LC, Kurrasch DM.** 2018. Opening the black box of endocrine disruption of brain development: lessons from the characterization of bisphenol A. *Horm Behav* **101**:50–58. <https://doi.org/10.1016/j.yhbeh.2017.12.001>.
12. **Peretz J, Vrooman L, Ricke WA, Hunt PA, Ehrlich S, Hauser R, Padmanabhan V, Taylor HS, Swan SH, VandeVoort CA, Flaws JA.** 2014. Bisphenol A and reproductive health: update of experimental and human evidence, 2007–2013. *Environ Health Perspect* **122**:775–786. <https://doi.org/10.1289/ehp.1307728>.
13. **Peters AH, Plug AW, van Vugt MJ, de Boer P.** 1997. A drying-down technique for the spreading of mammalian meiocytes from the male and female germline. *Chromosome Res* **5**:66–68. <https://doi.org/10.1023/A:1018445520117>.
14. **Rochester JR.** 2013. Bisphenol A and human health: a review of the literature. *Reprod Toxicol* **42**:132–155. <https://doi.org/10.1016/j.reprotox.2013.08.008>.
15. **Susiarjo M, Hassold TJ, Freeman E, Hunt PA.** 2007. Bisphenol A exposure in utero disrupts early oogenesis in the mouse. *PLoS Genet* **3**:69–70. <https://doi.org/10.1371/journal.pgen.0030005>.
16. **Vrooman LA, Oatley JM, Griswold JE, Hassold TJ, Hunt PA.** 2015. Estrogenic exposure alters the spermatogonial stem cells in the developing testis, permanently reducing crossover levels in the adult. *PLoS Genet* **11**:1–20. <https://doi.org/10.1371/journal.pgen.1004949>.
17. **Vrooman LA, Xin F, Bartolomei MS.** 2016. Morphologic and molecular changes in the placenta: what we can learn from environmental exposures. *Fertil Steril* **106**:930–940. <https://doi.org/10.1016/j.fertnstert.2016.08.016>.
18. **Wassenaar PNH, Trasande L, Legler J.** 2017. Systematic review and meta-analysis of early-life exposure to bisphenol a and obesity-related outcomes in rodents. *Environ Health Perspect* **125**:106001–106001-15. <https://doi.org/10.1289/EHP1233>.