Journal of the American Association for Laboratory Animal Science
Copyright 2017
by the American Association for Laboratory Animal Science

Vol 56, No 3
May 2017
Pages 269-272

Effects of Water Bottle Materials and Filtration on Bisphenol A Content in Laboratory Animal Drinking Water

Jennifer A Honeycutt,[1] Jenny Q T Nguyen,[2] Amanda C Kentner,[2,†] and Heather C Brenhouse,[1,*,†]

Bisphenol A (BPA) is widely used in the polycarbonate plastics and epoxy resins that are found in laboratory animal husbandry materials including cages and water bottles. Concerns about BPA exposure in humans has led to investigations that suggest physiologic health risks including disruptions to the endocrine system and CNS. However, the extent of exposure of laboratory animals to BPA in drinking water is unclear. In the first study, we compared the amount of BPA contamination in water stored in plastic bottles used in research settings with that in glass bottles. The amount of BPA that leached into water was measured across several time points ranging from 24 to 96 h by using a BPA ELISA assay. The results showed that considerable amounts of BPA (approximately 0.15 μg/L) leached from polycarbonate bottles within the first 24 h of storage. In the second study, BPA levels were measured directly from water taken from filtered compared with unfiltered taps. We observed significantly higher BPA levels in water from unfiltered taps (approximately 0.40 μg/L) compared with taps with filtration systems (approximately 0.04 μg/L). Taken together, our findings indicate that the use of different types of water bottles and water sources, combined with the use of different laboratory products (food, caging systems) between laboratories, likely contribute to decreased rigor and reproducibility in research. We suggest that researchers consider reporting the types of water bottles used and that animal care facilities educate staff regarding the importance of flushing nonfiltered water taps when filling animal water bottles.

Abbreviations: BPA, bisphenol A; HTPC: high-temperature polycarbonate

Bisphenol A (BPA) is a synthetic chemical used in the production of epoxy resins, which are often used to join and coat plumbing pipes, and polycarbonate plastics. A variety of laboratory products are produced from polycarbonate plastic, ranging from rodent housing and feeding and watering supplies to food and drink containers.[12] Over the past decade, awareness of the physiologic effects of acute and prolonged BPA exposure stemming from research in both human populations and laboratory animals has increased (see reference 22 for review). Of notable concern are findings indicating that, in sufficient doses, BPA acts as an endocrine-disrupting chemical, functioning similarly to endogenous 17β-estradiol.[15,26]

Although considerable steps have been taken to minimize the exposure of humans to BPA from consumer products, less attention has been given to products used in laboratory animal housing and feeding supplies—many of which are made from inexpensive polycarbonate plastics. One group[23] recently highlighted the potential effect of animal diet and caging and watering supplies on BPA exposure and estrogenic activity. For example, animal diets containing high levels of phytoestrogens decreased the age of vaginal opening and puberty onset in rodents.[23] This finding demonstrated a proof-of concept that standard supplies present in the laboratory environment can affect physiology, and potentially behavior, thereby undermining the results of experiments. However, many laboratories still use various types of plastics containing estrogenic compounds in their husbandry equipment. Indeed, polycarbonate caging, regularly used to house experimental rodents, has been shown to leach BPA into neutral pH water at room temperature.[8] Specifically, the most noteworthy culprit of BPA leaching was polycarbonate caging (previously washed at high temperature in an industrial cage cleaner), yielding levels as high as 310 μg/L BPA over the course of 1 wk.[8] Whereas the US Environmental Protection Agency's recommended maximal dose of BPA is 50 μg/kg per day in humans, mounting evidence derived from rodent research suggests that daily doses as low as 0.25 to 40 μg/kg per day can have physiologic consequences (for review, see reference 25). In contrast, other reports suggest that these same doses do not affect behavior[19] or endocrine regulation, at least in adults.[16] Notably, although BPA has been reported to leach from polycarbonate water bottles intended for human consumption,[15] direct evidence of BPA derived from various types of water bottles for laboratory animals is scant. Given the risk of effects related to BPA exposure on animal physiology and behavior, it is notable that many laboratories use tap water to fill rodent water bottles, which may be compromised by BPA leaching from epoxy resins used in piping.[18] In light of these findings, it is crucial to evaluate the extent to which commonplace laboratory products and procedures confound research studies by determining the product(s) that minimize BPA leaching into the experimental environment.

To determine whether commonly used rodent watering supplies leach BPA into water intended for animal consumption, the present study evaluated the level of BPA leaching among several common brands and types of water bottles used in animal facilities. We also evaluated the difference in BPA

Received: 13 Sept 2016. Revision requested: 18 Nov 2016. Accepted: 30 Jan 2017.
[1]Psychology Department, Northeastern University, Boston, Massachusetts, and [2]Program in Health Psychology, Massachusetts College of Pharmacy and Health Sciences, Boston, Massachusetts.
[*]Corresponding author. Email: h.brenhouse@neu.edu
[†]These authors contributed equally to the study

contamination between filtered and unfiltered tap water to determine whether simply filtering water prior to filling water bottles may, in itself, reduce the overall amount of BPA exposure from the water source. Using a highly sensitive BPA ELISA kit to evaluate BPA contamination, we found that polycarbonate water bottles leached significantly more BPA into water samples, albeit at low levels (approximately 0.20 µg/L), than do glass bottles (approximately 0.01 µg/L) during a 96-h evaluation period. In addition, we report that filtered tap water had significantly less BPA contamination (approximately 0.04 µg/L) than primary samples of unfiltered tap water (approximately 0.4 µg/L). Taken together, these findings support the need for increased oversight and reporting of the choice of materials for laboratory animal care and that BPA exposure through drinking water can be mitigated by using a filtered water source and BPA-free water bottles.

Materials and Methods

Materials. HPLC-grade water (Pierce LC-MS grade, product no. 51140, Thermo Fisher Scientific, Waltham, MA) was used for washes and sample preparations. For comparison, we obtained 3 glass water bottles (16 oz., product no. FS-101, Ancare, Bellmore, NY), 3 polysulfone bottles (16 oz. [500 mL]; product no. 30020ZF; Lab Products, Seaford, DE), 3 polypropylene bottles (16 oz. [500 mL]; product no. FSPC16PP, Ancare), 5 polycarbonate bottles (16 oz. [500 mL]; product no. 30020, Lab Products), and 5 high-temperature polycarbonate (HTPC) bottles (16 oz. [500 mL]; product no. FSPC16HT, Ancare) from 3 different animal care facilities. All bottles were used with standard husbandry treatment for 1 to 5 y. The bottles had minimal to no visible scratches in the plastic.

Sample collection. In the first study, all water bottles ($n = 3$ to 5 per bottle type) were washed through a standardized washing–rinsing protocol using an alkaline base detergent in the automated cage washing system and allowed to air dry. Each bottle was filled with 400 mL of HPLC-grade water on day 1 to ensure that concentrations from potential BPA leaching would be comparable. Bottles were left to incubate at room temperature (approximately 22 °C). At 24, 48, 72, and 96 h, a glass Pasteur pipette was used to remove 20 mL of water for transfer to a glass scintillation vial for later analysis. An additional 80 mL of water was removed from each bottle at each time point, to mimic the decrease in volume due to 2 rats per cage drinking from each bottle (that is, 10 to 12 mL per 100 g of body weight per rat). In a second study, glass scintillation vials were directly filled from reverse-osmosis–filtered ($n = 3$; EMD Millipore, Billerica, MA) or unfiltered ($n = 4$) water taps from wet labs and animal care facilities at 2 different institutions (Northeastern University, Boston, MA [1 filtered, 2 unfiltered], and MCPHS University, Boston, MA [2 filtered, 2 unfiltered]) to assess BPA exposure levels in untreated tap water. Water was collected as primary samples (water samples collected directly from a tap without flushing) from each regularly used tap. The water source of the filtered and unfiltered taps was a reservoir. Lastly, HPLC-grade water was collected in a glass scintillation vial as a negative control.

Measurement of BPA concentrations in water samples. A highly sensitive ELISA kit (Ecologiena, Japan Environchemicals, Tokyo, Japan) was used to evaluate BPA concentrations in samples collected from different water taps and bottles. Briefly, according to the manufacturer's instructions, the competitive ELISA was performed according to the standard test protocol that achieved minimal and maximal quantitative detection limits of 0.05 ng/mL and 10 ng/mL, respectively. This assay has been shown to have high correlation ($R^2 = 0.91$) with traditional gas chromatography–dual mass spectroscopy of BPA analysis (http://www.abraxiskits.com/moreinfo/PN590023USER.pdf)[1]. The 96-wells were coated with monoclonal antibodies that exclusively bind with BPA, thus limiting cross-reaction with other chemicals of similar structure and making the assay highly reproducible. All liquid transfers of 1 mL or less were performed using new, sterile micropipette tips. Water samples for the ELISA were prepared by using 10% HPLC-grade methanol in new polypropylene test tubes. A standard curve was created by using samples of known BPA concentrations (0.01, 0.05, 0.3, 1.0, and 10.0 µg/L), and nonlinear regression analyses to determine BPA concentrations in all samples were conducted for each test plate. In addition to the experimental samples and known BPA concentrations, blank (buffer solution alone) and HPLC-grade water controls were analyzed for comparison and to ensure correct assay performance. All control and experimental samples were run in triplicates and measured at 450 nm by using a microprocessor-controlled microplate reader (SpectraFluor PLUS, Tecan, Mannedorf, Switzerland).

Data were exported and fitted to a 4-parameter logistic curve, and statistical analysis was conducted by using MyAssays (MyAssays Limited; Brighton, UK). In study 1, BPA concentrations were evaluated using a 2-way repeated measures ANOVA (bottle type × time), and multiple comparisons were performed by using Tukey posthoc tests. In study 2, a one-way ANOVA compared BPA levels between different taps, with Tukey posthoc tests for multiple comparisons. A P value less than 0.05 was used to determine statistical significance.

Results

To determine the level of BPA exposure from standard plastic water bottles, we quantified the amount of BPA leakage over time from different types of water bottles (plastic compared with glass) obtained from 2 different animal care facilities.

Study 1 revealed a significant main effect of time with regard to the amount of BPA that leached into the water bottles ($F_{3,77} = 3.254$; $P = 0.0262$; Figure 1 A), however post hoc tests did not reveal further significant differences across time. Notably, a significant main effect of bottle type ($F_{5,77} = 34.55$; $P < 0.0001$; Figure 1 A and B) emerged. Overall, water stored in polycarbonate bottles had a higher BPA concentration (approximately 0.2 µg/L) compared with control HPLC-grade water (approximately 0.03 µg/L; $q_{15} = 7.573$; $P < 0.05$) and compared with water stored in glass bottles (approximately 0.01 µg/L; $q_{15} = 13.37$; $P < 0.05$). The HTPC bottles leached significantly less BPA (approximately 0.10 µg/L) than the polycarbonate bottles ($q_{15} = 9.875$; $P < 0.05$; Figure 1 A and B) but more than glass ($q_{15} = 4.822$; $P < 0.05$). Water stored in all other bottle types had BPA concentrations that were similar to control HPLC-grade water. Two-way repeated-measures ANOVA did not reveal any significant interactions between water bottle type and time.

In study 2, nonfiltered taps were associated with significantly higher BPA levels in water (approximately 0.40 µg/L) compared with filtered tap locations (approximately 0.04 µg/L; $F_{2,7} = 11.18$; $P < 0.05$; Figure 2). It is important to note, however, that these samples were taken from the tap without prior flushing. In a posthoc pilot experiment, we compared the first sample from the tap, stored 24 h in a polycarbonate bottle, with samples taken after running the water for approximately 10 s, which then were stored for 24 h in a polycarbonate bottle. The BPA concentration of the first sample (without flushing) was 0.677 µg/L, whereas that of the 3 samples taken after flushing ranged from 0.013 to 0.31 µg/L. Although these data require further empirical

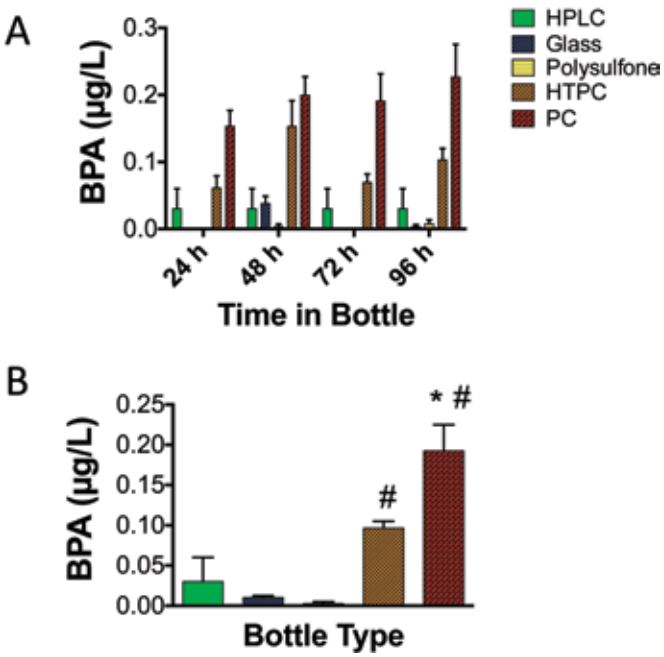

Figure 1. BPA leached from various types of water bottles used in animal care facilities. (A) BPA levels (µg/mL) in water stored in animal water bottles over time. (B) Because no interaction with time occurred, BPA (µg/mL) levels in water stored in each bottle type are presented collapsed over time. HTPC, high-temperature polycarbonate; PC, polycarbonate. Data are presented as mean ± SEM; *, value is significantly ($P < 0.05$) different from that for fresh HPLC-grade water control; #, value is significantly ($P < 0.05$) different from that from glass. Note that BPA levels in samples from polysulfone and glass bottles at the 24- and 72-h time points were below the level of detection.

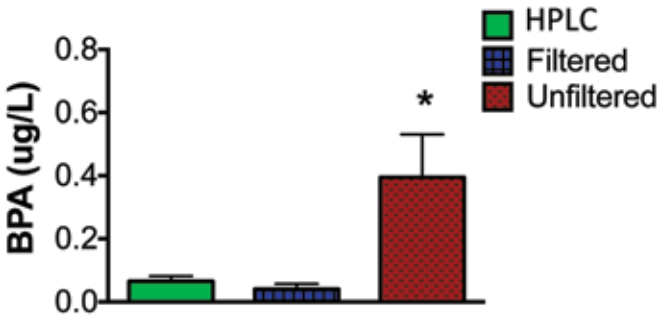

Figure 2. Comparison of BPA levels in water from filtered or unfiltered taps with that from fresh HPLC-grade water. *, value significantly ($P < 0.05$) different from that for HPLC-grade water.

confirmation, it appears likely that simply flushing the tap might significantly reduce the level of BPA attributed to the plumbing.

Discussion

Here, we report findings indicating significantly higher levels of BPA in water samples stored in polycarbonate bottles compared with glass. Moreover, we found increased levels of BPA in primary samples of nonfiltered water compared with filtered water samples. Therefore, animals in laboratory facilities might be exposed to low levels of BPA from the use of polycarbonate water bottles as well as from unfiltered tap water, which is commonly used to fill watering bottles.

The current results support previous reports that polycarbonate bottles intended for humans leach BPA into water at room temperature. For example, 2 groups have reported concentrations of approximately 0.3 µg/L in water stored at room temperature in polycarbonate bottles.[6,15] Here, we compared standard-grade polycarbonate with HTPC, which has a higher temperature rating and considerably better resistance to alkaline chemicals. We found that standard-grade polycarbonate bottles leached the most BPA into water (approximately 0.2 to 0.3µg/L) compared with negligible leaching from HTPC. Previous repeated washing of these bottles, according to the minimal standards outlined in the *Guide for the Care and Use of Laboratory Animals*,[9] which specify washing with hot water of at least 180 °F (82.2 °C) along with common alkaline detergents, might lead to the elevated BPA release we observed.

In addition, we detected BPA levels of approximately 0.4 µg/L in unfiltered compared with reverse-osmosis–filtered tap water. Although we did not investigate the source of this BPA, the levels we observed were likely due to leaching from epoxy resin used in the joints and coatings of plumbing pipes. These findings confirm earlier warnings that unfiltered tap water might be a potential source of BPA to animals.[20] Notably, however, our samples were collected directly as small primary samples from the tap, and flushing water through the pipes may minimize BPA levels that accumulate between uses.

The levels of BPA observed here yield a potential for exposure to laboratory rats of approximately 0.04 to 0.08 µg/kg per day, given that adult rats drink approximately 50 mL daily. This result can be compared with studies showing that typical rat feed contains approximately 0.7 to 2.2 parts per billion BPA,[5] which would yield an exposure of approximately 0.035 to 0.11 µg/kg per day, given that an adult rat eats approximately 20 g daily. Therefore, water is only one of several potential sources of low-dose exposure to BPA, and it may be useful to take all sources into account. To our knowledge, few investigations have directly evaluated whether BPA concentrations comparable to those we measured in our water samples are biologically active in vivo. Water with as little as 0.0023 ng/mL BPA reportedly have estrogen-like activity on cultured developing cerebellar neurons,[15] whereas other in vivo studies report that estrogenic activity is altered only after much higher doses of BPA. For example, a 3-generation study conducted in 2002[24] investigated Sprague–Dawley rats fed a diet containing BPA at levels from 0 to 7500 parts per million, yielding approximate intakes of 0, 0.001, 0.02, 0.3, 5, 50, or 500 µg/kg daily. Analysis of several endocrine-related end points including fertility, reproductive behavior, and estrous cyclicity for the parental and 3 progeny generations revealed no evidence of a low-dose effect of BPA. A similar study[7] investigated rats that were dosed orally by stomach tube over 2 generations at doses of 0, 0.2, 2.0, 20, or 200 µg/kg daily revealed no evidence of a low-dose effect of BPA. Several other groups have investigated the developmental consequences of exposure to doses of BPA between 2 and 200 µg/kg daily and have reported effects including abnormal neurogenesis and hyperplasia;[11,14] disrupted maternal care;[17] decreased plasma testosterone in males and increased aggressiveness;[13] altered immune functioning;[27] hyperactivity;[10] changes in pain reactivity;[2] and decreased pyramidal cell dendritic spine density.[4] Moreover, these effects of early or lifetime BPA exposure have been shown to be transmitted intergenerationally.[3] Interestingly, sensitivity to the biologic effects of BPA differs between species and strain,[21] therefore these housing conditions will differentially affect laboratory animal wellbeing depending on the model used. Therefore, although large-scale studies indicate that many end points are not grossly affected by doses of BPA due to exposure through drinking water, some evidence exists for discrete effects of higher BPA doses, but those effects have not been tested at the doses we report here. It is therefore

important to consider that minimal exposure to BPA from tap water or from polycarbonate water bottles has the potential for physiologic consequences that might add to variability between laboratories. The effects of low-dose BPA could be particularly important over the course of early development and should be considered by developmental labs using polycarbonate bottles. Taken together, the use of different types of water bottles and water sources and different laboratory products (that is, food, caging systems), species, and strains between laboratories likely contributes to decreased rigor and reproducibility in research. Therefore, these variables require increased attention and vigilance in reporting from investigators and animal facility staff.

In conclusion, results from the current studies showed that potentially biologically relevant levels of BPA occur in unfiltered tap water and in water stored in standard polycarbonate laboratory animal water bottles. We recommend flushing all water taps before filling water bottles and reporting water bottle material in published reports, to protect the reproducibility of future studies using rodent models.

Acknowledgments

We gratefully acknowledge the institutional support provided by the Massachusetts College of Pharmacy and Health Sciences (MCPHS) and the MCPHS Summer Undergraduate Research Fellowship Program (awarded to JN).

References

1. **Abraxis**. [Internet]. 2017. Ecologiena, Supersensitive BPS ELISA Kit (microplate) user's guide. [Cited 01 May 2016]. Available at: http://www.abraxiskits.com/moreinfo/PN590023USER.pdf.
2. **Aloisi AM, Della Seta D, Rendo C, Ceccarelli I, Scaramuzzino A, Farabollini F.** 2002. Exposure to the estrogenic pollutant bisphenol A affects pain behavior induced by subcutaneous formalin injection in male and female rats. Brain Res **937:**1–7.
3. **Boudalia S, Berges R, Chabanet C, Folia M, Decocq L, Pasquis B, Abdennebi-Najar L, Canivenc-Lavier MC.** 2014. A multigenerational study on low-dose BPA exposure in Wistar rats: effects on maternal behavior, flavor intake, and development. Neurotoxicol Teratol **41:**16–26.
4. **Bowman RE, Luine V, Khandaker H, Villafane JJ, Frankfurt M.** 2014. Adolescent bisphenol-A exposure decreases dendritic spine density: role of sex and age. Synapse **68:**498–507.
5. **Camacho L, Lewis SM, Vanlandingham MM, Juliar BE, Olson GR, Patton RE, Gamboa da Costa G, Woodling K, Sepehr E, Bryant MS, Doerge DR, Basavarajappa MS, Felton RP, Delclos KB.** 2016. Comparison of endpoints relevant to toxicity assessments in 3 generations of CD1 mice fed irradiated natural and purified ingredient diets with varying soy protein and isoflavone contents. Food Chem Toxicol **94:**39–56.
6. **Cooper JE, Kendig EL, Belcher SM.** 2011. Assessment of bisphenol A released from reusable plastic, aluminium, and stainless steel water bottles. Chemosphere **85:**943–947.
7. **Ema M, Fujii S, Furukawa M, Kiguchi M, Ikka T, Harazono A.** 2001. Rat 2-generation reproductive toxicity study of bisphenol A. Reprod Toxicol **15:**505–523.
8. **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.
9. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
10. **Ishido M, Masuo Y, Kunimoto M, Oka S, Morita M.** 2004. Bisphenol A causes hyperactivity in the rat concomitantly with impairment of tyrosine hydroxylase immunoreactivity. J Neurosci Res **76:**423–433.
11. **Itoh K, Yaoi T, Fushiki S**. 2012. Bisphenol A, an endocrine-disrupting chemical, and brain development. Neuropathology **32:**447–457.
12. **Kang JH, Katayama Y, Kondo F.** 2006. Biodegradation or metabolism of bisphenol A: from microorganisms to mammals. Toxicology **217:**81–90.
13. **Kawai K, Nozaki T, Nishikata H, Aou S, Takii M, Kubo C.** 2003. Aggressive behavior and serum testosterone concentration during the maturation process of male mice: the effects of fetal exposure to bisphenol A. Environ Health Perspect **111:**175–178.
14. **Komada M, Asai Y, Morii M, Matsuki M, Sato M, Nagao T.** 2012. Maternal bisphenol A oral dosing relates to the acceleration of neurogenesis in the developing neocortex of mouse fetuses. Toxicology **295:**31–38.
15. **Le HH, Carlson EM, Chua JP, Belcher SM.** 2008. Bisphenol A is released from polycarbonate drinking bottles and mimics the neurotoxic actions of estrogen in developing cerebellar neurons. Toxicol Lett **176:**149–156.
16. **Liu J, Yu P, Qian W, Li Y, Zhao J, Huan F, Wang J, Xiao H.** 2013. Perinatal bisphenol A exposure and adult glucose homeostasis: identifying critical windows of exposure. PLoS One **8:**e64143.
17. **Palanza PL, Howdeshell KL, Parmigiani S, vom Saal FS.** 2002. Exposure to a low dose of bisphenol A during fetal life or in adulthood alters maternal behavior in mice. Environ Health Perspect **110 Suppl 3:**415–422.
18. **Rajasärkkä J, Pernica M, Kuta J, Lasnak J, Simek Z, Blaha L.** 2016. Drinking water contaminants from epoxy resin-coated pipes: a field study. Water Res **103:**133–140.
19. **Rebuli ME, Camacho L, Adonay ME, Reif DM, Aylor DL, Patisaul HB**. 2015. Impact of low-dose oral exposure to bisphenol A (BPA) on juvenile and adult rat exploratory and anxiety behavior: a Clarity-BPA Consortium Study. Toxicol Sci **148:**341–354.
20. **Richter CA, Birnbaum LS, Farabollini F, Newbold RR, Rubin BS, Talsness CE, Vandenbergh JG, Walser-Kuntz DR, vom Saal FS.** 2007. In vivo effects of bisphenol A in laboratory rodent studies. Reprod Toxicol **24:**199–224.
21. **Richter CA, Taylor JA, Ruhlen RL, Welshons WV, Vom Saal FS.** 2007. Estradiol and bisphenol A stimulate androgen receptor and estrogen receptor gene expression in fetal mouse prostate mesenchyme cells. Environ Health Perspect **115:**902–908.
22. **Rochester JR.** 2013. Bisphenol A and human health: a review of the literature. Reprod Toxicol **42:**132–155.
23. **Thigpen JE, Setchell KD, Kissling GE, Locklear J, Caviness GF, Whiteside T, Belcher SM, Brown NM, Collins BJ, Lih FB, Tomer KB, Padilla-Banks E, Camacho L, Adsit FG, Grant M.** 2013. The estrogenic content of rodent diets, bedding, cages, and water bottles and its effect on bisphenol A studies. J Am Assoc Lab Anim Sci **52:**130–141.
24. **Tyl RW, Myers CB, Marr MC, Thomas BF, Keimowitz AR, Brine DR, Veselica MM, Fail PA, Chang TY, Seely JC, Joiner RL, Butala JH, Dimond SS, Cagen SZ, Shiotsuka RN, Stropp GD, Waechter JM.** 2002. Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague–Dawley rats. Toxicol Sci **68:**121–146.
25. **vom Saal FS, Hughes C.** 2005. An extensive new literature concerning low-dose effects of bisphenol A shows the need for a new risk assessment. Environ Health Perspect **113:**926–933.
26. **Yoon K, Kwack SJ, Kim HS, Lee BM.** 2014. Estrogenic endocrine-disrupting chemicals: molecular mechanisms of actions on putative human diseases. J Toxicol Environ Health B Crit Rev **17:**127–174.
27. **Yoshino S, Yamaki K, Li X, Sai T, Yanagisawa R, Takano H, Taneda S, Hayashi H, Mori Y.** 2004. Prenatal exposure to bisphenol A upregulates immune responses, including T helper 1 and T helper 2 responses, in mice. Immunology **112:**489–495.