Am J Clin Exp Urol 2023;11(5):367-384
www.ajceu.us /ISSN:2330-1910/AJCEU0151314

*Original Article*

# Polychlorinated biphenyl (PCB) exposure in adult female mice can influence bladder contractility

Thomas CM Lavery, Audrey Spiegelhoff, Kathy Wang, Conner L Kennedy, Monica Ridlon, Kimberly P Keil Stietz

*Department of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, Wisconsin 53706, USA*

Received May 19, 2023; Accepted July 21, 2023; Epub October 15, 2023; Published October 30, 2023

**Abstract:** Lower urinary tract symptoms (LUTS) greatly reduce quality of life. While LUTS etiology is not completely understood, it is plausible that environmental contaminants could play a role. Polychlorinated biphenyls (PCBs), are a group of persistent environmental toxicants frequently documented in animal and human tissues. PCBs are capable of influencing voiding function in mouse offspring exposed developmentally, however whether PCB exposure during adulthood can also influence voiding dynamics is unknown. Therefore, the purpose of this study was to determine whether PCB exposure in adult female mice can impact voiding function. As part of a larger study to generate developmentally exposed offspring, adult female C57Bl/6J mice were dosed orally with the MARBLES PCB mixture (0.1, 1, or 6 mg/kg/day) or vehicle control beginning two weeks before mating and throughout gestation and lactation (9 weeks). Adult dosed female dams then underwent void spot assay, uroflowmetry, and anesthetized cystometry to assess voiding function. Bladder contractility was assessed in *ex vivo* bladder bath assays, and bladders were collected for morphology and histology assessments. While voiding behavior endpoints were minimally impacted, alterations to bladder contractility dynamics were more pronounced. Adult female mice dosed with 1 mg/kg/d PCB showed an increase in urine spots 2-3 $cm^2$ in size, increased bladder contractility in response to electrical field stimulation, and decreased bladder wall thickness compared to vehicle control. PCBs also altered contractile response to cholinergic agonist in a dose-dependent manner. Overall, these results indicate that exposure to PCBs in adult female mice is sufficient to produce changes in bladder physiology. These results also highlight the critical role of timing of exposure in influencing voiding function.

**Keywords:** Polychlorinated biphenyls, bladder, POPs, adult exposure, voiding function

## Introduction

Lower urinary tract symptoms (LUTS), such as overactive bladder, nocturia, and incontinence, are prevalent conditions that negatively affect quality of life [1, 2]. LUTS are prevalent in the aging population and are common comorbidities with sleep disturbances and hip fracture risk [3-6]. LUTS are also present in individuals with neurodevelopmental disorders [3-6]. The etiology of LUTS is complex, and while some risk factors, such as aging [3, 4, 7], diabetes [8-10] and urinary tract infections [11-13], have been identified, the complex factors leading to LUTS risk are not fully understood. There is evidence to suggest that environmental contaminants may also play a role in lower urinary tract development and disease [14-19].

Polychlorinated biphenyls (PCBs) are a group of persistent organic pollutants that are extremely resistant to environmental degradation [20-25]. Despite production bans, PCB exposure remains a health concern due to legacy sources from prior to the ban, and new contemporary sources of PCBs produced as unintentional byproducts of industrial processes such as paint pigment production [21, 23, 24, 26, 27]. Diet is a major route of exposure, as PCBs are highly lipophilic, allowing them to accumulate in fatty tissues [27-29]. Example sources of PCBs in the human diet include meat, dairy, and fish [20, 27-33]. The present study uses a mixture of PCBs termed the MARBLES PCB mixture, which mimics the most abundant PCB congeners and their percentages found in the serum of a contemporary cohort of pregnant women at

risk of having a child with a neurodevelopmental disorder [34, 35]. This mixture contains both legacy and contemporary PCB congeners, thus allowing for the assessment of a contemporary, environmentally relevant mixture of PCBs on lower urinary tract function.

Numerous studies on the effects of PCB exposure, during both pre- and postnatal development, have described negative consequences for neurological function and development of the central nervous system [36-45] as well as other organ systems including the liver, intestine, gut microbiome and immune system [46-53]. Gestational and lactational exposure to PCBs has also been shown to influence voiding function of mice once they reach young adulthood [17]. However, whether PCB exposure contributes to voiding function when exposed in later life stages is not known. Understanding the impacts of PCB exposure at all ages could help preventative or therapeutic strategies for all ages of patients suffering from LUTS. Therefore, here we test whether adulthood PCB exposure in female mice alters voiding function.

## Materials and methods

### *Animals*

All procedures involving animals were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the University of Wisconsin-Madison Animal Care and Use Committee. The study used wild type C57Bl/6J mice (#000664, Jackson Labs, Bar Harbor, ME). All mice were kept in clear plastic cages with corn cob bedding, kept at 22 ± 2°C and maintained on a 12 h light and dark cycle. Feed (Diet 2020x, Teklad, Indianapolis, IN) and water were provided *ad libitum*.

### *PCB exposures*

The MARBLES PCB congener mixture was used to simulate environmental exposure levels of PCBs found in a contemporary cohort of pregnant women at risk of having a child with a neurodevelopmental disorder [35]. PCBs were ordered from Accustandard (Accustandard, New Haven, CT) or synthesized and authenticated by the Synthesis Core of the University of Iowa Superfund Research Program with > 99% purity as previously reported [35]. Mice were dosed as described [17]. Briefly, adult female nulliparous mice of an average age of 7 weeks old were dosed orally daily with 0, 0.1, 1 or 6 mg/kg MARBLES PCB mixture dissolved in peanut oil (Spectrum Organic Products, LLC, Mellville, NY) and mixed into organic peanut butter (Trader Joe's, Monrovia, CA). These doses were chosen as they have been used previously to generate PCB levels in offspring and dams at levels relevant to human exposures [54] and have been shown to induce behavioral deficits in developmentally exposed offspring [40, 41, 54-56]. In order to refine and reduce animal numbers, the adult female mice used in this study were generated as part of a larger study to generate offspring [17]. As such, the female mice began the PCB dosing regimen two weeks before mating and continued throughout gestation and lactation and until voiding function assessment was completed following weaning. If female mice failed to maintain pregnancy or care for their litters, they continued to be dosed such that all female mice used in this study were exposed to MARBLES PCBs/vehicle control for an average of 9 weeks. A subset of mice underwent void spot assays (VSA), uroflowmetry and then anesthetized cystometry, while others were euthanized and bladders collected for *ex vivo* bladder tissue bath assays or morphology and histology. N values for each endpoint are detailed below for each assay and in the figure legends.

### *Void spot assay (VSA)*

Void spot assays were conducted according to best practices as described [57-59]. Briefly, after a 1-hour acclimation to the room, mice (n=13-15 mice per treatment group) were placed singly in an empty cage containing 3MM chromatography paper (057163E, Thermo Fisher Scientific, Waltham, MA). The mice had access to food, but not water, during the 4 hour testing period. After the testing period, chromatography papers were allowed to dry and then imaged under UV light using a UVP ChemStudio Plus UV imager (Analytik Jena, Beverly, MA). VSA analysis was conducted in ImageJ using the open access Void Whizzard analysis software [58] by an individual blinded to treatment conditions.

### *Uroflowmetry*

Uroflowmetry was conducted as previously described [17, 60]. At least one day following

VSA testing, mice (n=13-15 mice per treatment group) were acclimated to the room for 1 hour and then placed into uroflowmetry chambers for 4 hours with access to water, but not food. Uroflow data was collected using Raspberry Pi cameras and processors (RS Components Limited, Corby, UK) as described [60]. Data was analyzed by an individual blinded to treatment conditions. Urine events in which urine hit the bars of the metabolic chamber's floor were excluded from analysis. In order to determine a numerical value for stream rating, a scale from 1-3 was used, in which 1 was a void made up of individual drops and 3 was a void made up of a strong stream of urine.

*Anesthetized cystometry*

The terminal procedure of anesthetized cystometry was conducted as described [17, 61]. Briefly, mice (n=8-10 mice per treatment group) were anesthetized using a subcutaneous injection of urethane (AC325540500, Thermo Fisher Scientific) at a dosage of 1.43 g/kg mouse. A PE-50 tubing catheter (NC9140178, Thermo Fisher Scientific) was placed in the dome of the bladder. Following surgery, mice were allowed to recover for approximately 60 minutes on a heated pad, and then connected via the catheter to an in-line pressure transducer and infusion pump. Saline was infused into the bladder at a rate of 0.8 mL/hr. Bladder pressure was recorded using an MLT844 physiological pressure transducer (ADInstruments, Colorado Springs, CO, USA) connected to an FE221 Bridge Amp (ADInstruments) with a PowerLab 2/26 (PL2602) data acquisition system. Cystometrograms were recorded and analyzed using Labchart software (ADInstruments). Cystometrograms were recorded for approximately 1 hour, or until a consistent pattern of voiding was achieved. 5 consecutive voids for each mouse were averaged and analyzed for data collection and selected by an individual blinded to treatment conditions. Measured parameters include void interval, void duration, normalized threshold pressure, normalized peak void pressure, non-voiding contractions, and compliance.

*Bladder bath analysis*

Bladder bath assays were conducted as described [62, 63]. Mice (n=5-6 mice per treatment group) were euthanized via $CO_2$ and the bladder removed and bifurcated longitudinally. Knots were placed on the top and bottom of each bladder half (5-0 silk, Fine Science Tools, Foster City, CA) and tied to an arm and a force pressure transducer (FT-03, Grass Instruments, Quincy, MA). Tissues were submerged in a 37°C water jacketed tissue chamber filled with Krebs solution (133 mM NaCl, 16 mM $NaHCO_3$, 5 mM KCl, 1 mM $MgCl_2$, 1.4 mM $NaH_2PO_4$, 2.25 mM $CaCl_2$ $2H_2O$, 7.8 mM d-glucose, pH 7.2) and aerated with 95% $O_2$-5% $CO_2$. Bladder halves were maintained at a baseline of 1 gram of tension for 60 minutes before experimentation began, with Krebs solution changed every 15 minutes. For electrical field stimulation (EFS) bladders were stimulated with 10 V, duration of 0.5 ms, and frequencies of 1 Hz, 2 Hz, 5 Hz, 10 Hz, 20 Hz, and 50 Hz (Grass Instruments, West Warwick, RI). Bladders were stimulated at each frequency for 5 seconds, with 3 minutes recovery between each stimulation. Once completed, bladders recovered for 30 minutes, with Krebs changed at 15 minutes and tension readjusted to 1 gram as needed. Increasing concentrations (0-10 μM final bath concentration, added from 1000× fresh stocks) of the cholinergic agonist carbachol (AAL0667403, Thermo Fisher Scientific) were then added to baths, with each addition occurring as the response plateaued to the previous addition to elicit a stepwise response. After final application of carbachol, bladders were washed with Krebs solution 3 times and tension readjusted to 1 gram as necessary. Bladders recovered for 30 minutes, with Krebs changed after 15 minutes. ATP (A1852-1VL, Sigma) was then applied to baths at increasing concentrations (0.1 to 1000 μM final bath concentration, added from 1000× fresh stocks) in a similar manner to carbachol. After final addition of ATP, bladders were washed with Krebs solution 3 times, and tension readjusted to 1 gram as necessary. Bladders recovered for 30 minutes, with Krebs solution changed after 15 minutes. Following this period, 60 mM KCl was added to the baths to elicit a maximum response. Bladder contractility was recorded and analyzed by an individual blinded to treatment conditions using Labchart software (ADInstruments). Baseline values were subtracted from each response, and each response normalized to KCl response and expressed as percent. Bladder halves were

averaged, if a bladder half failed to respond to KCl or electrical field stimulation that half was excluded from analysis.

*Bladder metrics and histology*

Whole bladders were collected for bladder metrics and histology from mice that did not undergo anesthetized cystometry, as cystometry cannot preserve the architecture of the bladder. Mice were euthanized with $CO_2$ and bladder length, width, and height measured using a digital caliper to calculate relative volume for an ellipsoid (n=5-11 mice per treatment group). For histology, bladders were removed, weighed, and fixed in 4% paraformaldehyde (Thermo Fisher Scientific, w/v diluted in PBS) overnight, dehydrated into methanol and stored at -20°C. Bladders were then rehydrated, embedded in low melt agarose so all treatment groups were present in one block, dehydrated in ethanol, embedded in paraffin, sectioned and stained with hematoxylin and eosin as previously described [19]. Bladder sections were imaged using an Eclipse Ci compound microscope (Nikon Instruments, Inc., Melville, NY) and DS Ri2 camera (Nikon Instruments, Inc.) interfaced to NIS elements imaging software (Nikon instruments, Inc.). At least 3 independent sections were imaged of each bladder. ImageJ was used to measure bladder muscle width in several locations from each section by an individual blinded to treatment conditions. Bladder muscle segments were selected for measure if they were intact. Some images were eliminated post hoc due to tearing or insufficient intact muscle. All muscle width measurements were averaged for each image, and all image measurements were then averaged to create an average for each bladder with an n=3-6 bladders per treatment group.

*Statistics*

Statistics were run with GraphPad Prism software version 9.2.0. Data were analyzed for normality using Shapiro-Wilk and Kolmogorov-Smirnov normality tests, and variance using Brown-Forsythe and Bartlett's tests. Data that passed normality and variance tests were analyzed using a one-way ANOVA followed by Dunnett's multiple comparisons test or one-way repeated measures ANOVA with Dunnett's multiple comparison test. Data that did not pass normality underwent Kruskal-Wallis test followed by Dunn's multiple comparisons test or for repeated measures, Friedman test followed by Dunn's multiple comparison test. Data that did not have equal variances underwent Welch's ANOVA. The EC50 best fit values from bladder bath assays were generated from nonlinear fit analysis for log agonist vs. response curves within Prism. Results with $P \leq 0.05$ were considered statistically significant. N values for each treatment group and parameter, along with the statistical test run, are indicated in the figure legends.

## Results

*PCB exposure in adult female mice results in an increase in medium-sized urine spots*

While developmental exposure to PCBs can alter mouse voiding physiology [17], the effects of adulthood exposure to PCBs on mouse voiding function in the dams is unknown. The goal of this study was to determine whether exposure to a mixture of PCBs (MARBLES PCB mixture) for 9 weeks in adulthood was sufficient to alter voiding physiology in female mice. To determine PCB effects on voiding function, female mice were first tested using the noninvasive void spot assay (VSA) (**Figure 1A-F**). There were no statistically significant differences in the total number of urine spots, total urine area, or percent urine area in the center or corners of the paper after the 4 hour testing period (**Figure 1B-E**). Mice typically void in large (4+ $cm^2$) or very small urine spots (0-0.1 $cm^2$) as assessed by VSA. When urine spot sizes were examined individually, there was a significant increase in the number of urine spots in the 2-3 $cm^2$ size range in the 1 mg/kg/d PCB group compared to vehicle control (**Figure 1F**).

*PCB exposure in adult female mice causes no significant differences in uroflowmetry or anesthetized cystometry parameters*

Uroflowmetry was used to assess individual void events in real time, which is not possible with VSA. This method allows for the quantification of average void mass, void duration, flow rate, stream quality, number of void and fecal events as well as the interval between voids. PCBs did not significantly alter any of these parameters in adult female mice (**Figure 2**).

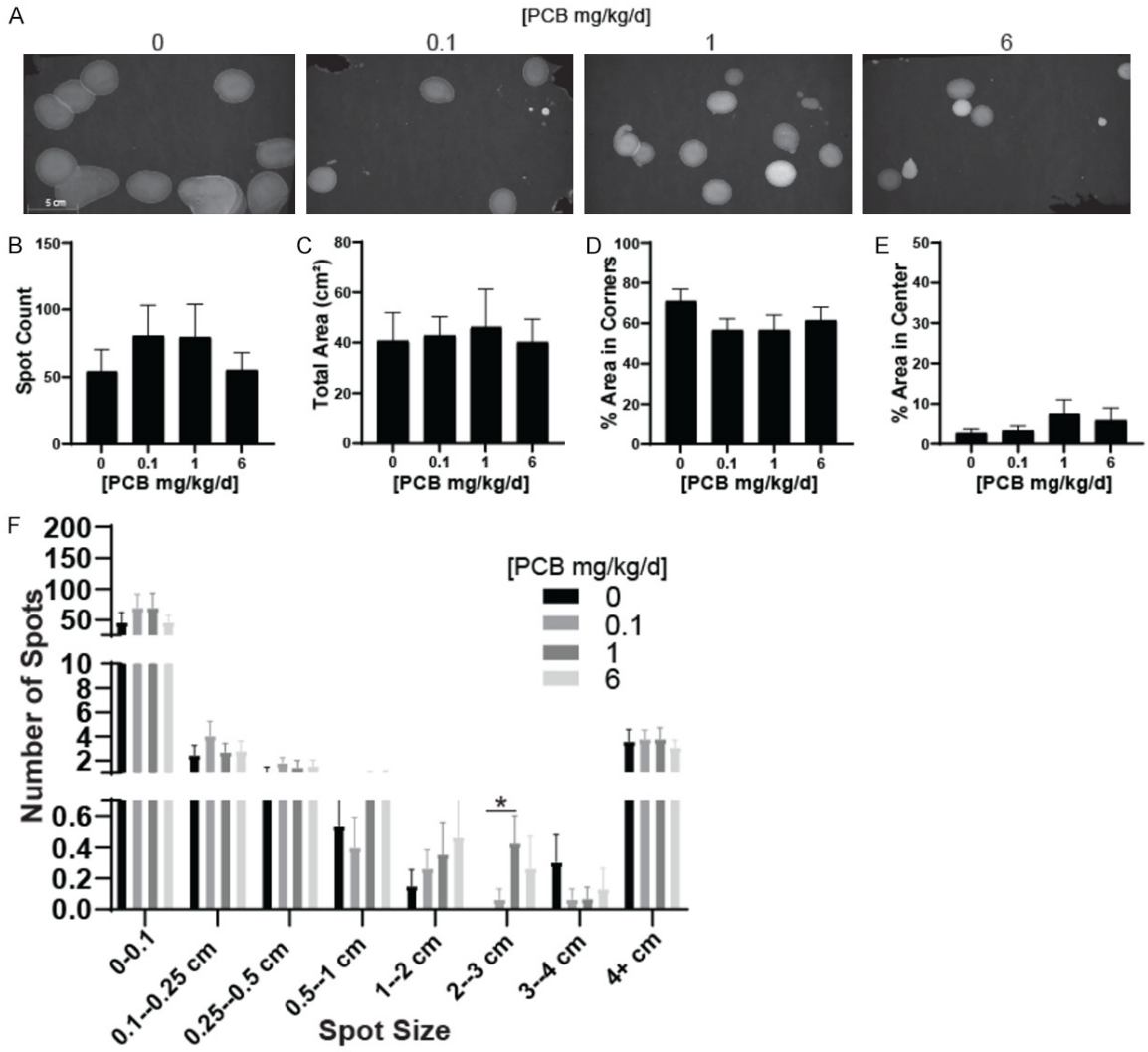


**Figure 1.** PCB exposure during adulthood causes an increase in urine spots in the 2-3-centimeter size group. Void spot assay (VSA) testing was conducted in adult female mice. (A) Representative VSA images. Quantification of (B) total urine spot number, (C) total urine area on VSA paper, (D) percentage of urine area in corners, (E) percentage of urine area in center. (F) Number of urine spots within defined sizes. Results are mean ± SEM n=13-15 females per group. * indicates significant difference from vehicle control within the 2-3 cm spot size category, $P \leq 0.05$ as determined by Kruskal-Wallis test (B, C, E, F) followed by Dunn's multiple comparisons test or one-way ANOVA (D).

VSA and uroflowmetry are non-invasive and performed in awake, freely moving mice. To complement these assays we also assessed voiding function via anesthetized cystometry, which can be used to investigate differences in voiding physiology without confounding variables stemming from behavior or environmental stimuli and allows for the assessment of pressure and volume relationships during voiding cycles [57, 58]. No statistically significant differences were found in intervoid interval, threshold pressure, maximum pressure, compliance or number of non-voiding contractions between PCB dose groups (**Figure 3**).

*PCB exposure in adult female mice results in increased bladder contractile strength to electrical field stimulation and dose dependent changes in sensitivity to cholinergic stimulation*

Despite minimal changes in voiding behavior, it remains possible that subtle alterations at the tissue level are present and could be hypothesized to manifest later in life or interact with other factors to increase severity or latency to develop LUTS. Therefore, we next examined bladder contractility in *ex vivo* bladder tissue bath preparations. Bladder contractility was

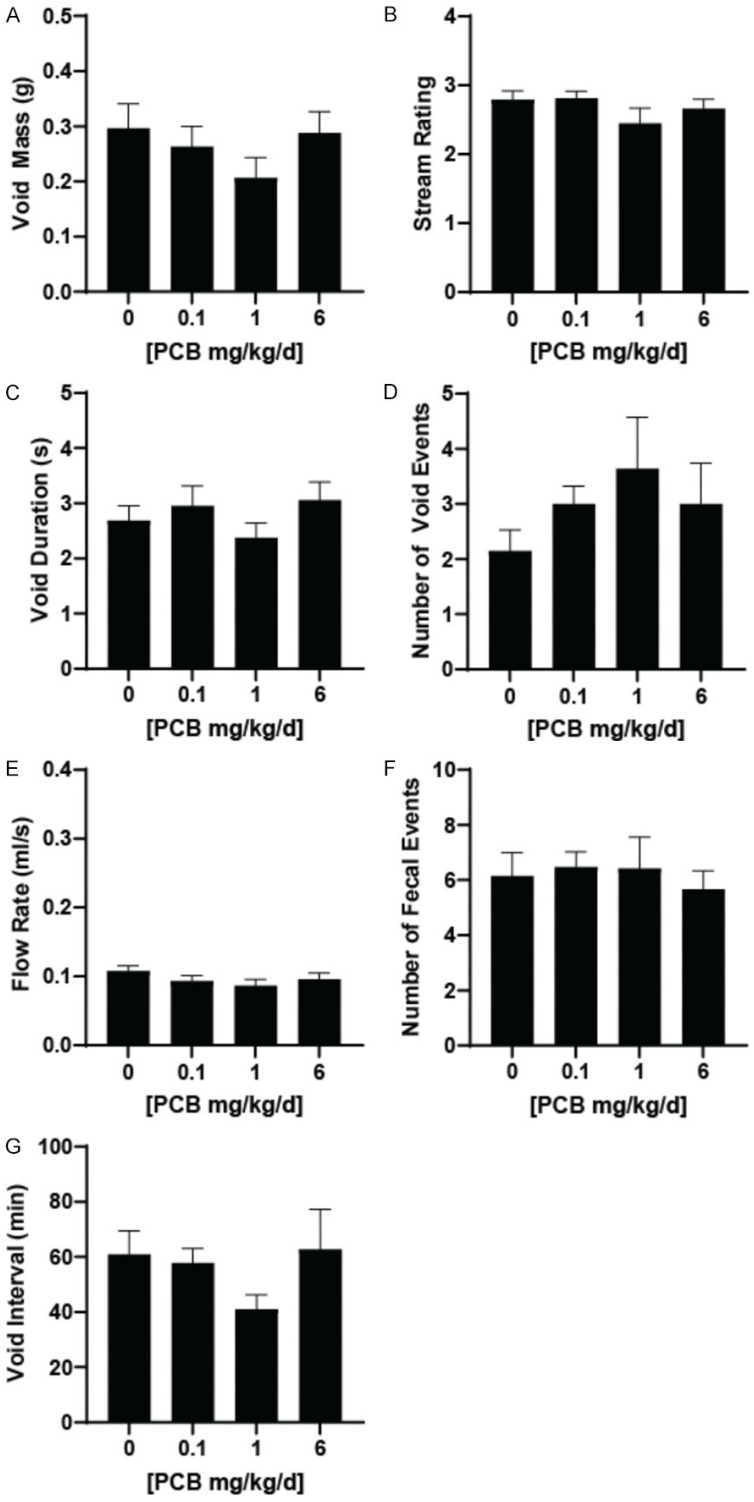


**Figure 2.** PCB exposure during adulthood does not alter uroflowmetry parameters. Uroflowmetry was conducted in adult female mice at least one day following VSA testing. Quantification of (A) void mass, (B) stream rating, (C) void duration, (D) number of void events, (E) flow rate, (F) number of fecal events, (G) interval between voids. Results are mean ± SEM n=8-13 (A-C, E, G) n=13-15 (D, F) females per group. No significant difference from vehicle control $P \leq 0.05$ as determined by Kruskal-Wallis (A-D, F, G) or one-way ANOVA (E).

assessed in response to electrical field stimulation (EFS) and agonists of the cholinergic (carbachol) and purinergic (ATP) pathways essential in mediating bladder contractions [64, 65]. We first confirmed that PCBs did not significantly alter baseline tension prior to addition of stimuli, or the ability of bladders to elicit a contraction in response to KCl (**Figure 4A-E**). Mass of bladder strips used for assays also did not differ between treatment groups (**Figure 4F**). In response to EFS, bladders from adult female mice dosed with 1 mg/kg/d PCB had a significantly greater contractile response at frequencies of 10 Hz and 20 Hz compared to bladders from vehicle control mice (**Figure 5A**). There was also a trend for increased contractility at the 50 Hz stimuli driven by elevated response in the 1 mg/kg/d PCB group compared to control (**Figure 5A**). EC50 values calculated from the dose response curve were lower in all PCB treatment groups compared to control, suggesting increased sensitivity (**Figure 5A**, inset). To examine receptor-mediated contractility, bladder strips were incubated with increasing concentrations of the cholinergic agonist carbachol. There was a significant increase in the maximum contractile response to the lowest concentrations of 0.01 and 0.03 μM carbachol in the 6 mg/kg/d PCB treatment group compared to vehicle control (**Figure 5B**). Conversely, in response to 0.3 μM carbachol, there was a significant decrease in contractile response in the 1 mg/kg/d PCB group compared to vehicle

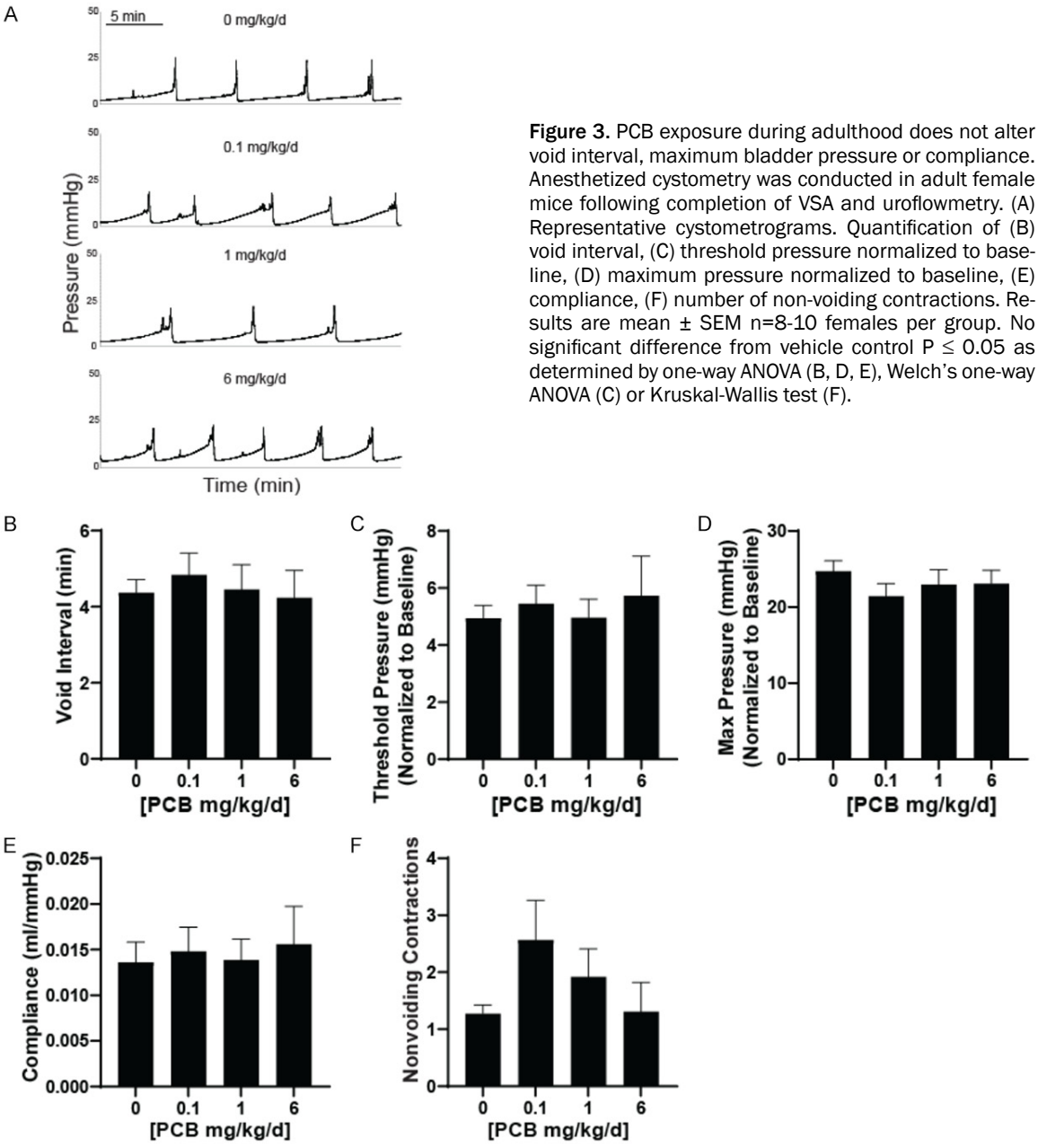


**Figure 3.** PCB exposure during adulthood does not alter void interval, maximum bladder pressure or compliance. Anesthetized cystometry was conducted in adult female mice following completion of VSA and uroflowmetry. (A) Representative cystometrograms. Quantification of (B) void interval, (C) threshold pressure normalized to baseline, (D) maximum pressure normalized to baseline, (E) compliance, (F) number of non-voiding contractions. Results are mean ± SEM n=8-10 females per group. No significant difference from vehicle control $P \leq 0.05$ as determined by one-way ANOVA (B, D, E), Welch's one-way ANOVA (C) or Kruskal-Wallis test (F).

control (**Figure 5B**). EC50 values were greater in all PCB groups compared to control and greatest for the 1 mg/kg/d PCB group, indicating decreased sensitivity (**Figure 5B**, inset). There were no significant PCB effects in contractile response to ATP at any of the concentrations tested (**Figure 5C**). These data suggest that PCBs are capable of influencing the strength of bladder contractions in response to stimuli. Finally, we determined the strength of stimuli required within each PCB treatment group to significantly increase the response above that of the first stimuli, to measure the tolerance to repeated doses or in other words the lowest observed adverse effect level (**Figure 6**). Bladders from the 1 and 6 mg/kg/d PCB groups showed increased contractility compared to 1 Hz at higher frequencies than control or 0.1 mg/kg/d PCB groups (**Figure 6A**). Similarly, compared to control, PCB treated bladders also required higher concentrations of carbachol before contractility was greater than the first applied concentration (**Figure 6B**). ATP response was elevated at the highest concen-

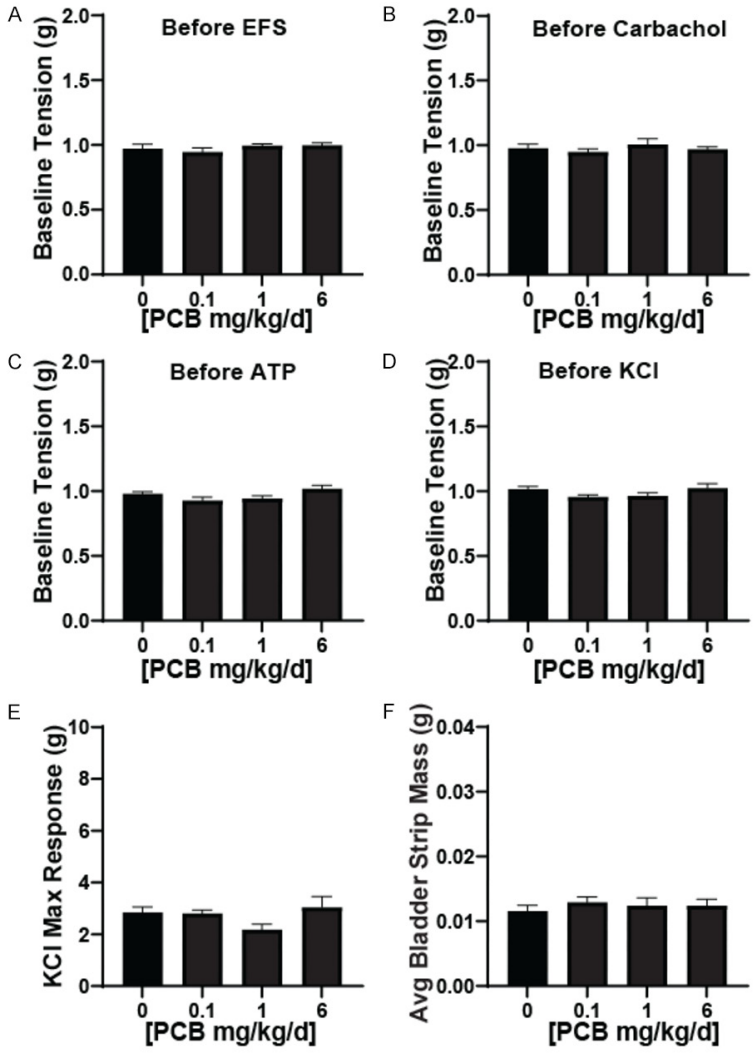


**Figure 4.** PCB exposure during adulthood does not significantly alter baseline tension prior to electrical field stimulation, carbachol or ATP addition or alter maximal contractility to KCl. *Ex vivo* bladder bath analysis was conducted on bladders from adult female mice. Baseline bladder strip tension (g) prior to (A) EFS stimulation, (B) carbachol addition, (C) ATP addition, (D) KCl addition. (E) Raw maximum response to KCl. (F) Average mass of bladder strips used in *ex vivo* contractility assays. Results are mean ± SEM n=5-6 females/group. No significant difference from vehicle control $P \leq 0.05$ as determined by one-way ANOVA (A-C, E) or Kruskal Wallis test (D, F).

trations of ATP versus the lowest concentration of ATP only in the control and 0.1 mg/kg/d PCB groups (**Figure 6C**). These data suggest that PCBs can also influence the magnitude of change upon increasing strength of additive stimuli, generally with the 1 and 6 mg/kg/d PCB bladders requiring greater stimuli to produce a change from the lowest stimuli. Thus, these bladders were more tolerant to increasing concentrations or in other words had a higher lowest observed adverse effect level. Taken together these data provide evidence that PCBs influence the strength of contraction to a given stimuli and also influence the magnitude of change upon increasing strength of stimuli. Further, these data provide evidence that sensation to bladder contractile stimuli may be disrupted by PCB exposure in a dose dependent manner and further may be influenced by the type and concentration of contractile stimuli.

*PCB exposure in adult female mice causes no significant differences in bladder volume or mass but does decrease bladder muscle thickness*

To investigate morphological differences due to PCB exposure, bladder volume and mass were assessed in adult female mice. No statistically significant differences were found in bladder volume or mass between the dosage groups, both before and after being normalized to body mass (**Figure 7**). To investigate anatomical differences at the tissue level, smooth muscle thickness was assessed in bladder sections (**Figure 8A**). A statistically significant decrease in bladder smooth muscle thickness was observed in the 1 mg/kg/d PCB group in comparison to control group (**Figure 8B**). There was also a trend for decreased bladder smooth muscle thickness in the 0.1 mg/kg/d PCB group in comparison to control group (P=0.06, **Figure 8B**).

## Discussion

Adulthood exposure to PCBs in female mice led to changes in voiding physiology and bladder contractility compared to untreated controls. While uroflowmetry and cystometry endpoints

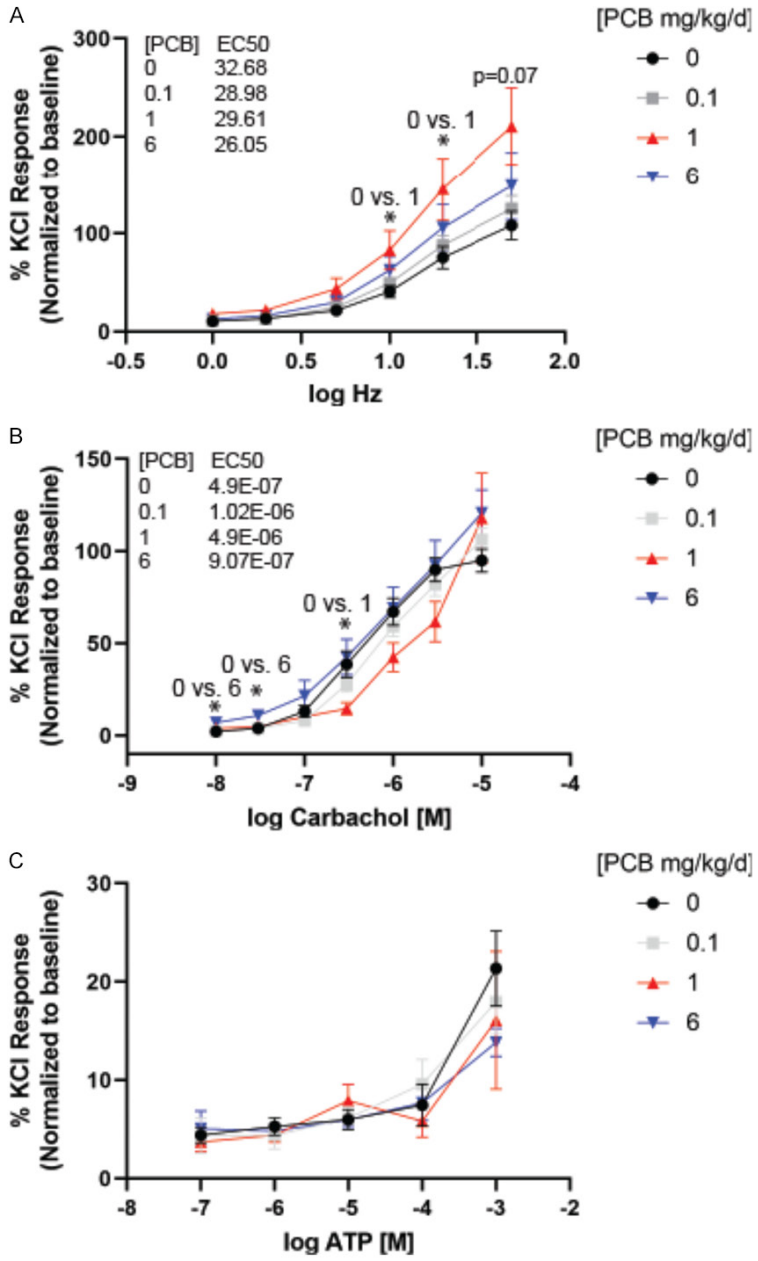


**Figure 5.** PCB exposure during adulthood increases contractility in response to electrical field stimulation at 1 mg/kg/d dose. *Ex vivo* bladder bath analysis was conducted on bladders from adult female mice. (A) Percent maximal contractile response normalized to baseline for applied electrical field stimulation at 1, 2, 5, 10, 20 and 50 hz. (B) Percent maximal contractile response normalized to baseline for applied carbachol concentrations (0.01, 0.03, 0.1, 0.3, 1, 3, 10 uM final bath concentration). (C) Percent maximal contractile response normalized to baseline for applied ATP concentrations (0.1, 1, 10, 100, 1000 uM final bath concentration). Results are mean ± SEM n=5-6 females per group. Bar and * indicate significant differences as determined by Kruskal-Wallis test followed by Dunn's multiple comparisons (A; B: 0.01, 0.03, 10; C: 0.1, 1, 1000), one-way ANOVA followed by Dunnett's multiple comparisons test (B: 0.3, 1, 3; C: 10, 100), Welch's one-way ANOVA (B: 0.1). EC50 values were generated from nonlinear fit best fit values from log agonist vs. response curves within Prism.

were unchanged, VSA analysis revealed an increase in medium 2-3 $cm^2$ sized spots in the 1 mg/kg/d PCB mice compared to vehicle control mice. The number of PCB induced changes observed in bladder contractility in an *ex vivo* preparation were more pronounced compared to *in vivo* voiding physiology assays. PCBs influenced both the strength of contraction and sensitivity to contractile stimuli in a dose- and stimulus-dependent manner. In addition, PCBs decreased bladder smooth muscle thickness in adult female mice of the 1 mg/kg/d PCB group compared to vehicle control. Overall, these studies are the first to identify alterations to voiding function and bladder contractility following adult exposure to PCBs in female mice.

We have collected evidence that PCB exposure in adult female mice influences bladder contractility in a PCB dose-dependent manner. Bladders of the 1 mg/kg/d PCB group showed greater strength of bladder contraction upon electrical stimuli, but no change or diminished contractility in response to cholinergic agonist carbachol. Response to EFS mimics nerve evoked contraction of the bladder, while carbachol targets cholinergic receptor signaling directly [64-66]. Increased contractility in response to EFS without changes to receptor mediated stimulation provides evidence that pre-junctional changes contribute to the effects observed in the 1 mg/kg/d PCB treatment group. On the other hand, bladders of the 6 mg/kg/d PCB treatment

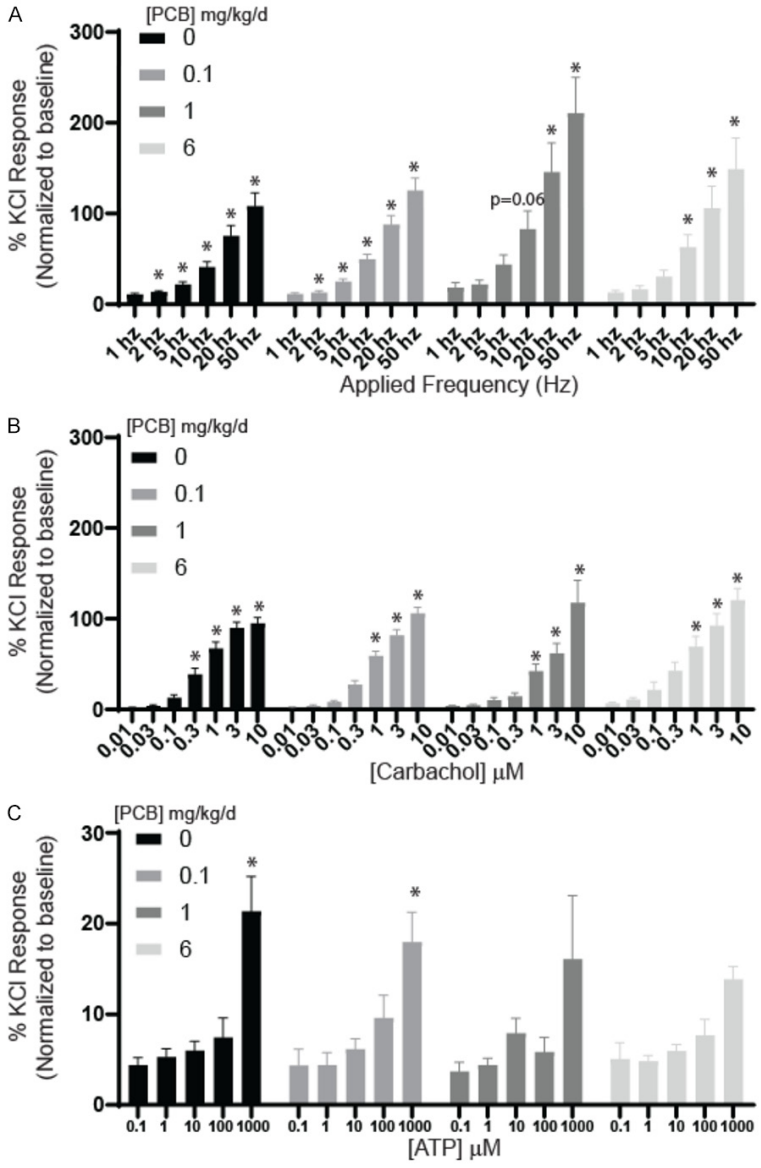


**Figure 6.** PCB exposure during adulthood alters sensitivity to EFS, carbachol and ATP. *Ex vivo* bladder bath analysis was conducted on bladders from adult female mice. (A) Percent maximal contractile response normalized to baseline for applied electrical field stimulation grouped by PCB treatment. (B) Percent maximal contractile response normalized to baseline for carbachol grouped by PCB treatment. (C) Percent maximal contractile response normalized to baseline for ATP grouped by PCB treatment. Results are mean ± SEM n=5-6 females/group. * indicates significant difference from lowest stimuli within each dose group, $P \leq 0.05$ as determined by one-way repeated measures ANOVA with Dunnett's multiple comparison test (A: 0, 0.1, 6; B: 0, 1, 6; C: 0, 1, 6) or Friedman test followed by Dunn's multiple comparison test (A: 1; B: 0.1; C: 0.1).

group displayed increased contractility at low concentrations of carbachol but no changes with EFS. This suggests the 6 mg/kg/d PCB dose may have actions post-junctionally. No changes in contractile strength were present with ATP. These data suggest that PCBs may influence both pre-junctional (nerve) and/or post-junctional (receptor mediated) pathways responsible for contractility and that these effects are dependent upon PCB dose.

Opposite effects in response to EFS and carbachol observed in the 1 mg/kg/d PCB group suggests that PCBs can have differential impacts on the bladder dependent upon whether nerve stimulation is present or whether the nerves are bypassed. This phenomenon has been observed previously in rat bladders, in which activation of protein kinase c (PKC) increased sensitivity to EFS but decreased sensitivity to carbachol, suggesting that PKC activation has different effects dependent upon whether nerves are bypassed or not [67]. While we did not test whether PKC signaling is altered by PCBs it is an intriguing area of future study since PKC signaling and bladder contractility can be greatly influenced by intracellular calcium signaling [65, 68, 69]. PCBs can increase intracellular calcium levels and calcium dependent signaling pathways in the brain [55, 70, 71]. Whether the altered bladder contractility observed here is influenced by fluctuations in calcium dynamics or calcium dependent signaling pathways such as PKC or any of the other calcium dependent channels [66, 72, 73] is an area of future study. Other possibilities exist which may explain the differential effects of PCBs and types of stimuli on bladder contractility. Bladder contraction relies on both myogenic and nerve mediated pathways [73]. Functional changes in the contractile properties of the muscle itself,

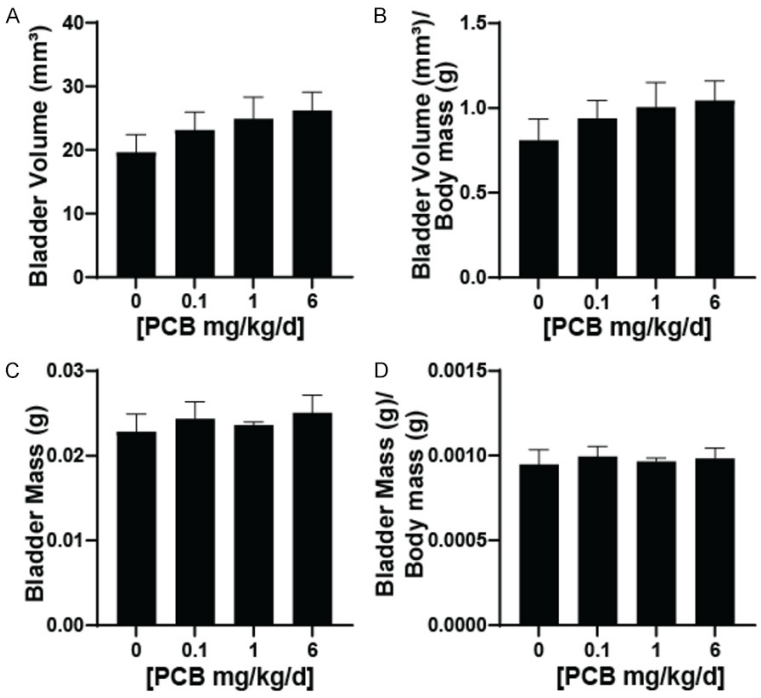


**Figure 7.** PCB exposure during adulthood does not significantly alter bladder mass or volume. Bladder metrics were quantified from adult female mice which did not undergo cystometry and include (A) bladder volume, (B) bladder volume normalized to body mass, (C) bladder mass, (D) bladder mass normalized to body mass. Results are mean ± SEM n=5-11 females/group. No significant differences, $P \leq 0.05$ as determined by one-way ANOVA (A, B) or Welch's one-way ANOVA (C, D).

independent of innervation, could also drive PCB induced changes in contractility. We also cannot exclude the possibility that these types of changes could be responsible for the decrease in bladder smooth muscle thickness observed in the 1 mg/kg/d PCB group. Lastly, other molecules can also influence bladder contractility. Examples include serotonin, which can increase bladder contractility [64, 74, 75] and steroid hormones such as estrogen. Estradiol application to guinea pig bladders reduces contractile response via activation of calcium dependent potassium channels (BK channels) [76] and in rabbit bladder strips can inhibit spontaneous rhythmic contractions of myogenic origin [77]. PCBs can act as endocrine disruptors and are capable of influencing both serotonin and steroid hormone signaling [43, 78-83]. Whether PCB effects on the bladder observed here are mediated via complex endocrine disruption warrants further study.

PCB induced changes in endocrine signaling could also underlie the observed decrease in smooth muscle thickness in 1 mg/kg/d PCB bladders compared to control. For example, ovariectomized rats have decreased bladder wall thickness which is restored upon estrogen supplementation [84]. Thus, whether PCBs reduce estrogens leading to decreased smooth muscle thickness and/or altered contractility is plausible. Changes in bladder smooth muscle thickness can also arise in models of bladder outlet obstruction [85]. However, the observed decrease in bladder muscle thickness observed in the 1 mg/kg/d PCB treatment group does not likely arise from decompensation following obstruction for several reasons. First, bladders showed no indication of increases in volume or mass, which typically arise in obstruction models [85]. Second, decompensated bladders typically would lead to reduced function and changes in parameters such as flow rate, compliance, void interval, and contractile response to KCL. We did not observe any of these changes making obstruction/bladder decompensation unlikely at this timepoint as a possible cause of the observed decreased smooth muscle thickness.

This study reveals an interesting PCB dose-effect in adult female mice. The majority of statistically significant differences were found between the control and the 1 mg/kg/d PCB group, consistent with other studies which found PCBs often elicit biological phenotypes in a non-monotonic dose response or in a dose-dependent manner [40, 41, 55, 56, 86]. While likely multifactorial, the varied outcomes at each dose could arise from differential receptor activation, signaling pathways and/or in metabolizing enzyme abundance which can be differentially regulated by PCB exposure [87]. Previous research has demonstrated that developmental exposure to the A1254 mixture of PCBs at the 1 mg/kg/d dose results in increased ryanodine receptor (RyR1) expres-

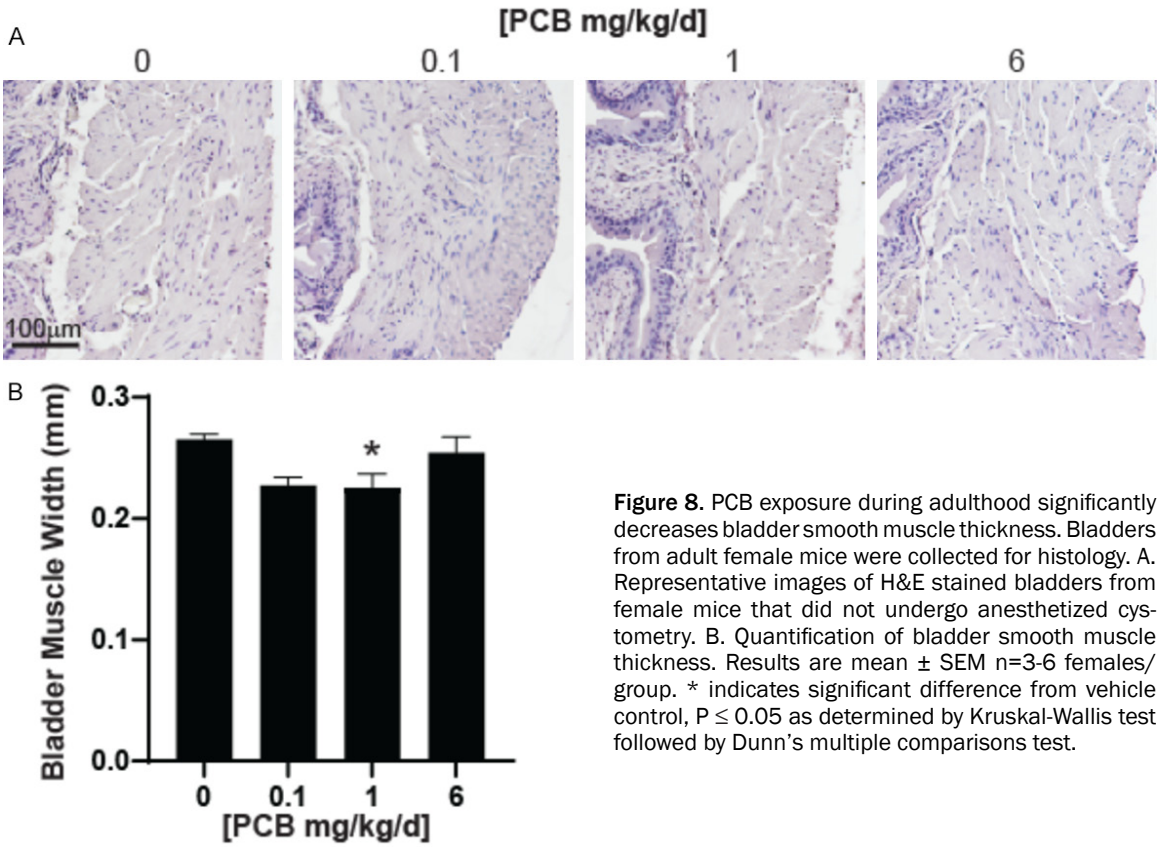


**Figure 8.** PCB exposure during adulthood significantly decreases bladder smooth muscle thickness. Bladders from adult female mice were collected for histology. A. Representative images of H&E stained bladders from female mice that did not undergo anesthetized cystometry. B. Quantification of bladder smooth muscle thickness. Results are mean ± SEM n=3-6 females/group. * indicates significant difference from vehicle control, $P \leq 0.05$ as determined by Kruskal-Wallis test followed by Dunn's multiple comparisons test.

sion in the cerebellum of weanling rats to a greater extent compared to the 6 mg/kg/d treatment group or control [41]. Interestingly, ryanodine receptor upregulation has also been linked to lower urinary tract dysfunction induced by dioxin, testosterone and estradiol exposure in mice [15]. Considering that a subset of PCB congeners can bind ryanodine receptors, and that the MARBLES PCB mixture used in this study has demonstrated affinity for these receptors [35], further research into how ryanodine receptor activation by PCBs affects lower urinary tract function is of significant interest.

Timing of PCB exposure likely impacts phenotypes associated with the lower urinary tract. For example, in utero and lactational exposure to the same doses and mix of PCBs used here, led to young adult mouse offspring with increased number of small urine spots (up to 0.1 $cm^2$), decreased stream rating, decreased intervoid interval or increased voiding pressure in a dose and sex-dependent manner [17]. The current study focusing on PCB exposure only during adulthood in female mice, did not observe any of those effects, instead found an increase in urine spots in the 2-3 $cm^2$ size category. Together, these findings follow an observed trend in which effects of PCBs on adult animals exposed prenatally versus those exposed only after birth can differ or even be modified based on whether animals saw both a developmental and postnatal exposure [43, 44]. While many effects linked to PCB exposure occur in offspring exposed developmentally, effects are also seen in rodent models expos ed in adulthood [88] or upon acute exposure in adult tissues. For example acute application of PCBs to adult rat uterine tissue *ex vivo* can increase contractions [89]. PCB effects on physiology during adulthood exposure are worth examining as they can independently alter physiology in a way that is unique to developmental exposure alone. Furthermore, knowing that humans are typically exposed through all stages of life, the fact that adult exposures can have unique effects on bladder and could interact with developmental exposures in ways that we cannot necessarily predict, places an importance on studying the interactions of developmental versus adult exposures on the bladder in the future. While this study only

focused on a 9 week PCB exposure in adult female mice as part of a larger study in an effort to refine and reduce animal numbers, the consequence of longer adulthood PCB exposure, adulthood PCB exposure in combination with other factors such as stress or obesity, or in adult male mice alone will be beneficial in understanding the consequences of lifetime PCB exposures. While preventing developmental exposures may be beneficial for future generations, for adults currently suffering from LUTS this is not of consequence. However, if alterations to diet or environment to alter PCB exposure in adulthood are feasible to reduce LUTS severity or risk, that is of great potential significance to the aging population.

The PCB induced changes in adult female mouse bladder contractility observed here are of relevance since several human LUTS are associated with bladder activity in adult women, such as overactive bladder/detrusor instability [3, 4, 66, 90-92], or underactive bladder [90, 93-95]. Complications in urinary continence are also prevalent following pregnancy in women [96]. Further research will be necessary to identify whether any associations between PCB exposure and LUTS in women with these conditions exist. Nonetheless, understanding relevant PCB exposure windows and impacts on bladder contractility could help to influence exposure monitoring or mitigate risk in susceptible human populations.

## Acknowledgements

This work was supported by the National Institutes of Health NIEHS R00 ES029537 and R03 ES033304 to KPKS and T32 ES007015 to CLK and MR. This work was also supported by core facilities including the Wisconsin O'Brien Center for Benign Urologic Research U54 DK104310. The synthesis of the MARBLES mix was supported by the Iowa Superfund Research Program at The University of Iowa P42 ES013661. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. Further, the NIH did not endorse the purchase of any commercial products or services mentioned in the publication.

## Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Kimberly P Keil Stietz, Department of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin-Madison, 2015 Linden Drive, Madison, Wisconsin 53706, USA. Tel: 608-265-2879; E-mail: kkeil@wisc.edu

## References

[1] Rubin EB, Buehler AE and Halpern SD. States worse than death among hospitalized patients with serious illnesses. JAMA Intern Med 2016; 176: 1557-1559.

[2] Irwin DE, Kopp ZS, Agatep B, Milsom I and Abrams P. Worldwide prevalence estimates of lower urinary tract symptoms, overactive bladder, urinary incontinence and bladder outlet obstruction. BJU Int 2011; 108: 1132-1138.

[3] Suskind AM. The aging overactive bladder: a review of aging-related changes from the brain to the bladder. Curr Bladder Dysfunct Rep 2017; 12: 42-47.

[4] Wolff GF, Kuchel GA and Smith PP. Overactive bladder in the vulnerable elderly. Res Rep Urol 2014; 6: 131-138.

[5] von Gontard A, Hussong J, Yang SS, Chase J, Franco I and Wright A. Neurodevelopmental disorders and incontinence in children and adolescents: attention-deficit/hyperactivity disorder, autism spectrum disorder, and intellectual disability-a consensus document of the International Children's Continence Society. Neurourol Urodyn 2022; 41: 102-114.

[6] von Gontard A, Pirrung M, Niemczyk J and Equit M. Incontinence in children with autism spectrum disorder. J Pediatr Urol 2015; 11: 264.e261-267.

[7] Brown JS, McGhan WF and Chokroverty S. Comorbidities associated with overactive bladder. Am J Manag Care 2000; 6 Suppl: S574-579.

[8] Gharaee-Kermani M, Rodriguez-Nieves JA, Mehra R, Vezina CA, Sarma AV and Macoska JA. Obesity-induced diabetes and lower urinary tract fibrosis promote urinary voiding dysfunction in a mouse model. Prostate 2013; 73: 1123-1133.

[9] Abler LL, O'Driscoll CA, Colopy SA, Stietz KPK, Wang P, Wang Z, Hartmann F, Crader-Smith SM, Oellete JN, Mehta V, Oakes SR, Grimes MD, Mitchell GS, Baan M, Gallagher SJ, Davis DB, Kimple ME, Bjorling DE, Watters JJ and Vezina CM. The influence of intermittent hypoxia, obesity and diabetes on male genitourinary anatomy and voiding physiology. Am J Physiol Renal Physiol 2021; 321: F82-F92.

[10] Abler LL and Vezina CM. Links between lower urinary tract symptoms, intermittent hypoxia

and diabetes: causes or cures? Respir Physiol Neurobiol 2018; 256: 87-96.
[11] Schwab S, Jobin K and Kurts C. Urinary tract infection: recent insight into the evolutionary arms race between uropathogenic Escherichia coli and our immune system. Nephrol Dial Transplant 2017; 32: 1977-1983.
[12] Lacerda Mariano L, Rousseau M, Varet H, Legendre R, Gentek R, Saenz Coronilla J, Bajenoff M, Gomez Perdiguero E and Ingersoll MA. Functionally distinct resident macrophage subsets differentially shape responses to infection in the bladder. Sci Adv 2020; 6: eabc5739.
[13] Grover S, Srivastava A, Lee R, Tewari AK and Te AE. Role of inflammation in bladder function and interstitial cystitis. Ther Adv Urol 2011; 3: 19-33.
[14] Turco AE, Oakes SR, Keil Stietz KP, Dunham CL, Joseph DB, Chathurvedula TS, Girardi NM, Schneider AJ, Gawdzik J, Sheftel CM, Wang P, Wang Z, Bjorling DE, Ricke WA, Tang W, Hernandez LL, Keast JR, Bonev AD, Grimes MD, Strand DW, Tykocki NR, Tanguay RL, Peterson RE and Vezina CM. A neuroanatomical mechanism linking perinatal TCDD exposure to lower urinary tract dysfunction in adulthood. Dis Model Mech 2021; 14: dmm049068.
[15] Turco AE, Thomas S, Crawford LK, Tang W, Peterson RE, Li L, Ricke WA and Vezina CM. In utero and lactational 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure exacerbates urinary dysfunction in hormone-treated C57BL/6J mice through a non-malignant mechanism involving proteomic changes in the prostate that differ from those elicited by testosterone and estradiol. Am J Clin Exp Urol 2020; 8: 59-72.
[16] Ricke WA, Lee CW, Clapper TR, Schneider AJ, Moore RW, Keil KP, Abler LL, Wynder JL, López Alvarado A, Beaubrun I, Vo J, Bauman TM, Ricke EA, Peterson RE and Vezina CM. In utero and lactational TCDD exposure increases susceptibility to lower urinary tract dysfunction in adulthood. Toxicol Sci 2016; 150: 429-440.
[17] Kennedy CL, Spiegelhoff A, Lavery T, Wang K, Manuel RS, Wang Z, Wildermuth H and Keil Stietz KP. Developmental polychlorinated biphenyl (PCB) exposure alters voiding physiology in young adult male and female mice. Am J Clin Exp Urol 2022; 10: 82-97.
[18] Kennedy CL, Spiegelhoff A, Wang K, Lavery T, Nunez A, Manuel R, Hillers-Ziemer L, Arendt LM and Stietz KPK. The bladder is a novel target of developmental polychlorinated biphenyl exposure linked to increased inflammatory cells in the bladder of young mice. Toxics 2021; 9: 214.
[19] Keil Stietz KP, Kennedy CL, Sethi S, Valenzuela A, Nunez A, Wang K, Wang Z, Wang P, Spiegelhoff A, Puschner B, Bjorling DE and Lein PJ. In utero and lactational PCB exposure drives anatomic changes in the juvenile mouse bladder. Curr Res Toxicol 2021; 2: 1-18.
[20] Vorkamp K. An overlooked environmental issue? A review of the inadvertent formation of PCB-11 and other PCB congeners and their occurrence in consumer products and in the environment. Sci Total Environ 2016; 541: 1463-1476.
[21] Jahnke JC and Hornbuckle KC. PCB emissions from paint colorants. Environ Sci Technol 2019; 53: 5187-5194.
[22] Howell NL, Suarez MP, Rifai HS and Koenig L. Concentrations of polychlorinated biphenyls (PCBs) in water, sediment, and aquatic biota in the Houston Ship Channel, Texas. Chemosphere 2008; 70: 593-606.
[23] Herrick RF. PCBs in school-persistent chemicals, persistent problems. New Solut 2010; 20: 115-126.
[24] Cabrerizo A, Muir DCG, De Silva AO, Wang X, Lamoureux SF and Lafrenière MJ. Legacy and emerging persistent organic pollutants (POPs) in terrestrial compartments in the high arctic: sorption and secondary sources. Environ Sci Technol 2018; 52: 14187-14197.
[25] Alcock RE, Bacon J, Bardget RD, Beck AJ, Haygarth PM, Lee RG, Parker CA and Jones KC. Persistence and fate of polychlorinated biphenyls (PCBs) in sewage sludge-amended agricultural soils. Environ Pollut 1996; 93: 83-92.
[26] Hu D and Hornbuckle KC. Inadvertent polychlorinated biphenyls in commercial paint pigments. Environ Sci Technol 2010; 44: 2822-2827.
[27] Fernández-González R, Yebra-Pimentel I, Martínez-Carballo E and Simal-Gándara J. A critical review about human exposure to polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) through foods. Crit Rev Food Sci Nutr 2015; 55: 1590-1617.
[28] Sethi S, Chen X, Kass PH and Puschner B. Polychlorinated biphenyl and polybrominated diphenyl ether profiles in serum from cattle, sheep, and goats across California. Chemosphere 2017; 181: 63-73.
[29] Barone G, Giacominelli-Stuffler R, Garofalo R, Castiglia D and Storelli MM. PCBs and PCDD/PCDFs in fishery products: occurrence, congener profile and compliance with European Union legislation. Food Chem Toxicol 2014; 74: 200-205.
[30] Barone G, Storelli A, Busco A, Mallamaci R and Storelli MM. Polychlorinated dioxins, furans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (dl-PCBs) in food from Italy: estimates of dietaryintake and assessment. J Food Sci 2021; 86: 4741-4753.

[31] Barone G, Storelli A, Garofalo R, Mallamaci R, Quaglia NC and Storelli MM. PCBs and PCDD/Fs in bluefin tuna: occurrence and dietary intake. Int J Environ Res Public Health 2018; 15: 911.
[32] Chen X, Lin Y, Dang K and Puschner B. Quantification of polychlorinated biphenyls and polybrominated diphenyl ethers in commercial cows' milk from california by gas chromatography-triple quadruple mass spectrometry. PLoS One 2017; 12: e0170129.
[33] Barone G, Storelli A, Quaglia NC, Dambrosio A, Garofalo R, Chiumarulo R and Storelli MM. Dioxin and PCB residues in meats from Italy: consumer dietary exposure. Food Chem Toxicol 2019; 133: 110717.
[34] Hertz-Picciotto I, Schmidt RJ, Walker CK, Bennett DH, Oliver M, Shedd-Wise KM, LaSalle JM, Giulivi C, Puschner B, Thomas J, Roa DL, Pessah IN, Van de Water J, Tancredi DJ and Ozonoff S. A prospective study of environmental exposures and early biomarkers in autism spectrum disorder: design, protocols, and preliminary data from the MARBLES study. Environ Health Perspect 2018; 126: 117004.
[35] Sethi S, Morgan RK, Feng W, Lin Y, Li X, Luna C, Koch M, Bansal R, Duffel MW, Puschner B, Zoeller RT, Lehmler HJ, Pessah IN and Lein PJ. Comparative analyses of the 12 most abundant PCB congeners detected in human maternal serum for activity at the thyroid hormone receptor and ryanodine receptor. Environ Sci Technol 2019; 53: 3948-3958.
[36] Klocke C, Sethi S and Lein PJ. The developmental neurotoxicity of legacy vs. contemporary polychlorinated biphenyls (PCBs): similarities and differences. Environ Sci Pollut Res Int 2020; 27: 8885-8896.
[37] Klocke C and Lein PJ. Evidence implicating non-dioxin-like congeners as the key mediators of polychlorinated biphenyl (PCB) developmental neurotoxicity. Int J Mol Sci 2020; 21: 1013.
[38] Panesar HK, Kennedy CL, Keil Stietz KP and Lein PJ. Polychlorinated biphenyls (PCBs): risk factors for autism spectrum disorder? Toxics 2020; 8: 70.
[39] Pessah IN, Lein PJ, Seegal RF and Sagiv SK. Neurotoxicity of polychlorinated biphenyls and related organohalogens. Acta Neuropathol 2019; 138: 363-387.
[40] Keil Stietz KP, Sethi S, Klocke CR, de Ruyter TE, Wilson MD, Pessah IN and Lein PJ. Sex and genotype modulate the dendritic effects of developmental exposure to a human-relevant polychlorinated biphenyls mixture in the juvenile mouse. Front Neurosci 2021; 15: 766802.
[41] Yang D, Kim KH, Phimister A, Bachstetter AD, Ward TR, Stackman RW, Mervis RF, Wisniewski AB, Klein SL, Kodavanti PR, Anderson KA, Wayman G, Pessah IN and Lein PJ. Developmental exposure to polychlorinated biphenyls interferes with experience-dependent dendritic plasticity and ryanodine receptor expression in weanling rats. Environ Health Perspect 2009; 117: 426-435.
[42] Lesiak A, Zhu M, Chen H, Appleyard SM, Impey S, Lein PJ and Wayman GA. The environmental neurotoxicant PCB 95 promotes synaptogenesis via ryanodine receptor-dependent miR132 upregulation. J Neurosci 2014; 34: 717-725.
[43] Bell MR, Hart BG and Gore AC. Two-hit exposure to polychlorinated biphenyls at gestational and juvenile life stages: 2. Sex-specific neuromolecular effects in the brain. Mol Cell Endocrinol 2016; 420: 125-137.
[44] Bell MR, Thompson LM, Rodriguez K and Gore AC. Two-hit exposure to polychlorinated biphenyls at gestational and juvenile life stages: 1. Sexually dimorphic effects on social and anxiety-like behaviors. Horm Behav 2016; 78: 168-177.
[45] Bullert AJ, Doorn JA, Stevens HE and Lehmler HJ. The effects of polychlorinated biphenyl exposure during adolescence on the nervous system: a comprehensive review. Chem Res Toxicol 2021; 34: 1948-1952.
[46] Matelski L, Keil Stietz KP, Sethi S, Taylor SL, Van de Water J and Lein PJ. The influence of sex, genotype, and dose on serum and hippocampal cytokine levels in juvenile mice developmentally exposed to a human-relevant mixture of polychlorinated biphenyls. Curr Res Toxicol 2020; 1: 85-103.
[47] Rude KM, Pusceddu MM, Keogh CE, Sladek JA, Rabasa G, Miller EN, Sethi S, Keil KP, Pessah IN, Lein PJ and Gareau MG. Developmental exposure to polychlorinated biphenyls (PCBs) in the maternal diet causes host-microbe defects in weanling offspring mice. Environ Pollut 2019; 253: 708-721.
[48] Petriello MC, Hoffman JB, Vsevolozhskaya O, Morris AJ and Hennig B. Dioxin-like PCB 126 increases intestinal inflammation and disrupts gut microbiota and metabolic homeostasis. Environ Pollut 2018; 242: 1022-1032.
[49] Lim JJ, Li X, Lehmler HJ, Wang D, Gu H and Cui JY. Gut microbiome critically impacts PCB-induced changes in metabolic fingerprints and the hepatic transcriptome in mice. Toxicol Sci 2020; 177: 168-187.
[50] Li X, Liu Y, Martin JW, Cui JY and Lehmler HJ. Nontarget analysis reveals gut microbiome-dependent differences in the fecal PCB metabolite profiles of germ-free and conventional mice. Environ Pollut 2021; 268: 115726.
[51] Wahlang B, Song M, Beier JI, Cameron Falkner K, Al-Eryani L, Clair HB, Prough RA, Osborne TS, Malarkey DE, States JC and Cave MC.

Evaluation of Aroclor 1260 exposure in a mouse model of diet-induced obesity and non-alcoholic fatty liver disease. Toxicol Appl Pharmacol 2014; 279: 380-390.

[52] Lehmler HJ, Uwimana E, Dean LE, Kovalchuk N, Zhang QY and Ding X. Probing the role of CYP2 enzymes in the atropselective metabolism of polychlorinated biphenyls using liver microsomes from transgenic mouse models. Chem Res Toxicol 2022; 35: 2310-2323.

[53] Jin J, Wahlang B, Shi H, Hardesty JE, Falkner KC, Head KZ, Srivastava S, Merchant ML, Rai SN, Cave MC and Prough RA. Dioxin-like and non-dioxin-like PCBs differentially regulate the hepatic proteome and modify diet-induced nonalcoholic fatty liver disease severity. Med Chem Res 2020; 29: 1247-1263.

[54] Kania-Korwel I, Lukasiewicz T, Barnhart CD, Stamou M, Chung H, Kelly KM, Bandiera S, Lein PJ and Lehmler HJ. Editor's highlight: congener-specific disposition of chiral polychlorinated biphenyls in lactating mice and their offspring: implications for PCB developmental neurotoxicity. Toxicol Sci 2017; 158: 101-115.

[55] Wayman GA, Yang D, Bose DD, Lesiak A, Ledoux V, Bruun D, Pessah IN and Lein PJ. PCB-95 promotes dendritic growth via ryanodine receptor-dependent mechanisms. Environ Health Perspect 2012; 120: 997-1002.

[56] Sethi S, Keil Stietz KP, Valenzuela AE, Klocke CR, Silverman JL, Puschner B, Pessah IN and Lein PJ. Developmental exposure to a human-relevant polychlorinated biphenyl mixture causes behavioral phenotypes that vary by sex and genotype in juvenile mice expressing human mutations that modulate neuronal calcium. Front Neurosci 2021; 15: 766826.

[57] Keil KP, Abler LL, Altmann HM, Bushman W, Marker PC, Li L, Ricke WA, Bjorling DE and Vezina CM. Influence of animal husbandry practices on void spot assay outcomes in C57BL/6J male mice. Neurourol Urodyn 2016; 35: 192-198.

[58] Wegner KA, Abler LL, Oakes SR, Mehta GS, Ritter KE, Hill WG, Zwaans BM, Lamb LE, Wang Z, Bjorling DE, Ricke WA, Macoska J, Marker PC, Southard-Smith EM, Eliceiri KW and Vezina CM. Void spot assay procedural optimization and software for rapid and objective quantification of rodent voiding function, including overlapping urine spots. Am J Physiol Renal Physiol 2018; 315: F1067-F1080.

[59] Hill WG, Zeidel ML, Bjorling DE and Vezina CM. Void spot assay: recommendations on the use of a simple micturition assay for mice. Am J Physiol Renal Physiol 2018; 315: F1422-F1429.

[60] Wang Z, Guzman EC, Nimunkar A, Keil KP, Vezina CM, Ricke WA, Macoska J and Bjorling DE. Void sorcerer: an open source, open access framework for mouse uroflowmetry. Am J Clin Exp Urol 2019; 7: 170-177.

[61] Ruetten H, Wegner KA, Zhang HL, Wang P, Sandhu J, Sandhu S, Mueller B, Wang Z, Macoska J, Peterson RE, Bjorling DE, Ricke WA, Marker PC and Vezina CM. Impact of sex, androgens, and prostate size on C57BL/6J mouse urinary physiology: functional assessment. Am J Physiol Renal Physiol 2019; 317: F996-F1009.

[62] Keil KP, Abler LL, Altmann HM, Wang Z, Wang P, Ricke WA, Bjorling DE and Vezina CM. Impact of a folic acid-enriched diet on urinary tract function in mice treated with testosterone and estradiol. Am J Physiol Renal Physiol 2015; 308: F1431-1443.

[63] Turco AE, Oakes SR, Keil Stietz KP, Dunham CL, Joseph DB, Chathurvedula TS, Girardi NM, Schneider AJ, Gawdzik J, Sheftel CM, Wang P, Wang Z, Bjorling DE, Ricke WA, Tang W, Hernandez LL, Keast JR, Bonev AD, Grimes MD, Strand DW, Tykocki NR, Tanguay RL, Peterson RE and Vezina CM. A mechanism linking perinatal 2,3,7,8 tetrachlorodibenzo-p-dioxin exposure to lower urinary tract dysfunction in adulthood. Dis Model Mech 2021; 14: dmm049068.

[64] Kullmann FA, Daugherty SL, de Groat WC and Birder LA. Bladder smooth muscle strip contractility as a method to evaluate lower urinary tract pharmacology. J Vis Exp 2014; e51807.

[65] Werner ME, Knorn AM, Meredith AL, Aldrich RW and Nelson MT. Frequency encoding of cholinergic- and purinergic-mediated signaling to mouse urinary bladder smooth muscle: modulation by BK channels. Am J Physiol Regul Integr Comp Physiol 2007; 292: R616-624.

[66] Thorneloe KS, Meredith AL, Knorn AM, Aldrich RW and Nelson MT. Urodynamic properties and neurotransmitter dependence of urinary bladder contractility in the BK channel deletion model of overactive bladder. Am J Physiol Renal Physiol 2005; 289: F604-610.

[67] Hypolite JA, Chang S, Wein AJ, Chacko S and Malykhina AP. Protein kinase C modulates frequency of micturition and non-voiding contractions in the urinary bladder via neuronal and myogenic mechanisms. BMC Urol 2015; 15: 34.

[68] Lipp P and Reither G. Protein kinase C: the "masters" of calcium and lipid. Cold Spring Harb Perspect Biol 2011; 3: a004556.

[69] Petkov GV, Bonev AD, Heppner TJ, Brenner R, Aldrich RW and Nelson MT. Beta1-subunit of the Ca2+-activated K+ channel regulates contractile activity of mouse urinary bladder smooth muscle. J Physiol 2001; 537: 443-452.

[70] Wayman GA, Bose DD, Yang D, Lesiak A, Bruun D, Impey S, Ledoux V, Pessah IN and Lein PJ. PCB-95 modulates the calcium-dependent sig-

naling pathway responsible for activity-dependent dendritic growth. Environ Health Perspect 2012; 120: 1003-1009.
[71] Pessah IN, Cherednichenko G and Lein PJ. Minding the calcium store: ryanodine receptor activation as a convergent mechanism of PCB toxicity. Pharmacol Ther 2010; 125: 260-285.
[72] Meredith AL, Thorneloe KS, Werner ME, Nelson MT and Aldrich RW. Overactive bladder and incontinence in the absence of the BK large conductance Ca2+-activated K+ channel. J Biol Chem 2004; 279: 36746-36752.
[73] Andersson KE and Arner A. Urinary bladder contraction and relaxation: physiology and pathophysiology. Physiol Rev 2004; 84: 935-986.
[74] Hall JD, DeWitte C, Ness TJ and Robbins MT. Serotonin enhances urinary bladder nociceptive processing via a 5-HT3 receptor mechanism. Neurosci Lett 2015; 604: 97-102.
[75] Bolton TB. Mechanisms of action of transmitters and other substances on smooth muscle. Physiol Rev 1979; 59: 606-718.
[76] Yasay GD, Kau ST and Li JH. Mechanoinhibitory effect of estradiol in guinea pig urinary bladder smooth muscles. Pharmacology 1995; 51: 273-280.
[77] Shenfeld OZ, McCammon KA, Blackmore PF and Ratz PH. Rapid effects of estrogen and progesterone on tone and spontaneous rhythmic contractions of the rabbit bladder. Urol Res 1999; 27: 386-392.
[78] Liberman DA, Walker KA, Gore AC and Bell MR. Sex-specific effects of developmental exposure to polychlorinated biphenyls on neuroimmune and dopaminergic endpoints in adolescent rats. Neurotoxicol Teratol 2020; 79: 106880.
[79] Ma R and Sassoon DA. PCBs exert an estrogenic effect through repression of the Wnt7a signaling pathway in the female reproductive tract. Environ Health Perspect 2006; 114: 898-904.
[80] Boix J and Cauli O. Alteration of serotonin system by polychlorinated biphenyls exposure. Neurochem Int 2012; 60: 809-816.
[81] Svobodová K, Placková M, Novotná V and Cajthaml T. Estrogenic and androgenic activity of PCBs, their chlorinated metabolites and other endocrine disruptors estimated with two in vitro yeast assays. Sci Total Environ 2009; 407: 5921-5925.
[82] Takeuchi S, Shiraishi F, Kitamura S, Kuroki H, Jin K and Kojima H. Characterization of steroid hormone receptor activities in 100 hydroxylated polychlorinated biphenyls, including congeners identified in humans. Toxicology 2011; 289: 112-121.
[83] Amir S, Shah STA, Mamoulakis C, Docea AO, Kalantzi OI, Zachariou A, Calina D, Carvalho F, Sofikitis N, Makrigiannakis A and Tsatsakis A. Endocrine disruptors acting on estrogen and androgen pathways cause reproductive disorders through multiple mechanisms: a review. Int J Environ Res Public Health 2021; 18: 1464.
[84] Yu Y, Shen Z, Zhou X and Chen S. Effects of steroid hormones on morphology and vascular endothelial growth factor expression in female bladder. Urology 2009; 73: 1210-1217.
[85] Nicholson TM, Ricke EA, Marker PC, Miano JM, Mayer RD, Timms BG, vom Saal FS, Wood RW and Ricke WA. Testosterone and 17β-estradiol induce glandular prostatic growth, bladder outlet obstruction, and voiding dysfunction in male mice. Endocrinology 2012; 153: 5556-5565.
[86] Sethi S, Keil KP and Lein PJ. Species and sex differences in the morphogenic response of primary rodent neurons to 3,3'-dichlorobiphenyl (PCB 11). Toxics 2017; 6: 4.
[87] Kania-Korwel I, Barnhart CD, Stamou M, Truong KM, El-Komy MH, Lein PJ, Veng-Pedersen P and Lehmler HJ. 2,2',3,5',6-pentachlorobiphenyl (PCB 95) and its hydroxylated metabolites are enantiomerically enriched in female mice. Environ Sci Technol 2012; 46: 11393-11401.
[88] Ramajayam G, Sridhar M, Karthikeyan S, Lavanya R, Veni S, Vignesh RC, Ilangovan R, Djody SS, Gopalakrishnan V, Arunakaran J and Srinivasan N. Effects of Aroclor 1254 on femoral bone metabolism in adult male Wistar rats. Toxicology 2007; 241: 99-105.
[89] Loch-Caruso R. Uterine muscle as a potential target of polychlorinated biphenyls during pregnancy. Int J Hyg Environ Health 2002; 205: 121-130.
[90] Kullmann FA, Birder LA and Andersson KE. Translational research and functional changes in voiding function in older adults. Clin Geriatr Med 2015; 31: 535-548.
[91] Onukwugha E, Zuckerman IH, McNally D, Coyne KS, Vats V and Mullins CD. The total economic burden of overactive bladder in the United States: a disease-specific approach. Am J Manag Care 2009; 15 Suppl: S90-97.
[92] Jiang HH, Song B, Lu GS, Wen QJ and Jin XY. Loss of ryanodine receptor calcium-release channel expression associated with overactive urinary bladder smooth muscle contractions in a detrusor instability model. BJU Int 2005; 96: 428-433.
[93] Gonzalez EJ, Odom MR, Hannan JL and Grill WM. Dysfunctional voiding behavior and impaired muscle contractility in a rat model of

detrusor underactivity. Neurourol Urodyn 2021; 40: 1889-1899.
[94] Chapple CR, Osman NI, Birder L, van Koeveringe GA, Oelke M, Nitti VW, Drake MJ, Yamaguchi O, Abrams P and Smith PP. The underactive bladder: a new clinical concept? Eur Urol 2015; 68: 351-353.
[95] Bhardwaj R and Sinha HK. Underactive urinary bladder - Masquerading as abdominal ascites. J Family Med Prim Care 2022; 11: 790-792.
[96] Rantell A, Veit-Rubin N, Giarenis I, Khullar V, Abrams P and Cardozo L. Recommendations and future research initiative to optimize bladder management in pregnancy and childbirth International Consultation on Incontinence - Research society 2018. Neurourol Urodyn 2019; 38 Suppl 5: S104-S110.