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Gestational and lactational exposure to potassium perfluorooctanesulfonate (K^+PFOS) in rats: Developmental neurotoxicity

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ABSTRACT

Perfluorooctanesulfonate (PFOS), a persistent and bioaccumulative compound, is widely distributed in humans and wildlife. Exposure of the human fetus and neonate to PFOS can occur *in utero* and via the mother's milk, respectively. Developmental studies have been conducted with PFOS in the past, including some developmental neurotoxicity endpoints. The objective of this study was to evaluate the functional and morphological changes to the nervous system in rats having gestational and lactational exposures to PFOS per current test guidelines (EPA OPPTS 870.6300 and OECD 426). Female SD rats (25/dosage group) were given daily oral doses of either 0.0, 0.1, 0.3, or 1.0 mg/kg-d potassium PFOS (K^+PFOS) from gestation day (GD) 0 through postnatal day (PND) 20. Offspring were observed through PND 72 for growth, maturation, motor activity, learning and memory, acoustic startle reflex, various behavioral manifestations, and brain weight. Specimens were taken from dams, fetuses, and pups for serum and tissue PFOS concentration, thyroid status endpoints, and liver mRNA transcript analysis, and those results are reported in a companion article. No significant effect was noted on maternal health or reproductive outcomes from dosing of maternal rats with K^+PFOS throughout gestation. Maternal body weights were statistically significantly lower in the 1.0 mg/kg-d dosage group from PND 4 through the end of lactation. Offspring from K^+PFOS-treated maternal groups did not differ significantly from controls with respect to birth weight, growth, age and weight at attainment of sexual maturation, learning and memory, acoustic startle, various behavioral endpoints, and brain weight. Male offspring from the 1.0 mg/kg-d maternal treatment group displayed increased motor activity and reduced habituation on PND 17 but not on PND 13, 21, and 61. The maternal no-observed-adverse-effect-level (NOAEL) was 0.3 mg/kg-d based on decreased body weights observed in lactation. The maternal dose associated with the NOAEL for male offspring was 0.3 mg/kg-d based on increased motor activity and reduced habituation in the 1.0 mg/kg-d maternal dose-group male offspring on PND 17. The maternal dose associated with the NOAEL for female offspring was >1.0 mg/kg-d. Mean serum concentrations of PFOS reported in a companion article for the 0.3 mg/kg-d group maternal rats are several hundred times higher than those reported for females in the United States general population.

1. Introduction

Perfluorooctanesulfonate (PFOS) is an environmentally stable and accumulative compound that has been found to be distributed worldwide in humans and wildlife [1–3]. Due to its observed widespread presence in the environment and accumulative nature, the major manufacturer of PFOS and materials that could generate PFOS through metabolic and environmental degradation ceased manufacturing of these materials between May of 2000 and December of 2002. There has been regulatory interest in controlling these materials on an international scale [4–7].

Although a majority of the biomonitoring studies conducted to determine the presence and amount of PFOS in humans have involved blood-based (whole blood, serum, or plasma) samples from adult populations, exposure of children has been demonstrated [8–15]. These children exposures can begin very early through placental transfer of PFOS from human mothers [11–15] and through milk [16–19]. Previous studies in laboratory animal species have shown that *in utero* exposure has a stronger effect on developmental endpoints than lactational exposure [20,21].

The association of PFOS exposure with developmental outcomes has been extensively studied in laboratory animals [2,22–25] and, to an extent, in humans [15,26–32] (reviewed by Olsen et

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al. [33], this issue); however, the available information on the potential association of PFOS exposure with developmental neurotoxicity has been limited [20,21,24,25,34]. PFOS uptake by the brain may occur prior to formation of the blood–brain barrier in rats based on a comparison of serum and perfused brain tissue PFOS concentrations between rat dams given 3 mg/kg-d PFOS from gestation day (GD) 2 to GD 21 and their pups on postnatal day (PND) 7 (http://www.chem.utoronto.ca/symposium/fluoros/pdfs/TOX020Lau.pdf). A similar observation was made from analysis of fetal and pup non-perfused brain samples taken from this study and published in a companion paper [35, this issue]. Several prior reproduction and developmental studies have included assessments of the developing nervous system in rats [20,21] and mice [24,25], and one recent study evaluated development in PC12 cells exposed to PFOS in culture [34]. Although these prior studies have contributed to knowledge of the potential developmental neurotoxicity of PFOS, generally showing no effect on learning and memory (rats) and some effect on motor activity and habituation (mice), a complete study based on current guidelines for developmental neurotoxicity has been lacking.

Due to its resistance to environmental and metabolic degradation and widespread exposure to children beginning *in utero*, we undertook this study to evaluate the potential of PFOS *in utero* and through lactation to cause disturbances in functional and morphological development of the nervous system in rats, with special emphasis on acoustic startle response, locomotor activity, learning and memory, and brain morphology. Current test guidelines (EPA OPPTS 870.6300 and OECD 426) were employed in this evaluation.

2. Materials and methods

2.1. Test article (K^+PFOS), vehicle, and preparation of dosing solutions

K^+PFOS (lot no. 217, 86.9% purity) was provided by 3M Company (St. Paul, MN). The vehicle used to prepare the test article dosing solution was 0.5% Tween® 20 (SigmaUltra, lot no. 014K0104, Sigma–Aldrich, St. Louis, MO) in deionized water. K^+PFOS dosing solutions were prepared as 0.02, 0.06, and 0.20 mg/mL (not adjusted for purity). Prior to the initial dosing, aliquots of dosing solutions were collected for homogeneity and stability assessments by determining PFOS concentration with a high performance liquid chromatography/tandem mass spectrometry (LC-MS/MS) method.

2.2. Animal husbandry, assignment, breeding, and study design

Sexually mature, virgin female Crl:CD (SD) rats (70 days old at receipt) were obtained from Charles River Laboratories, Inc. (Raleigh, NC). After acclimatization and at approximately 12 weeks of age, each female rat was paired and mated with a resident male Crl:CD (SD) rat from the same source. GD 0 denoted the day when evidence of positive mating was noted.

Mated female rats were randomly divided into four groups of 25, constituting control, 0.1, 0.3, and 1.0 mg/kg-d dose-groups in the main study. Dosage levels were selected carefully based on results of previous studies in rats with the objective of avoiding significant neonatal toxicity. An additional 10 mated females were assigned as satellite phase rats to each of the four groups in order to collect additional maternal and fetal blood and tissue samples for evaluation of pharmacokinetic, thyroid hormone and morphology, and hepatic gene-expression endpoints on GD 20 (reported in a companion article [35]). Doses were given once daily from GD 0 to PND 20 for the main study phase rats and from GD 0 to GD 19 for the satellite phase rats. The test and vehicle control solutions were administered orally by gavage at a volume of 5 mL/kg. All maternal rats were individually housed in plastic maternity cages with their litters through PND 21 (main study group) or through GD 20 (satellite group) with ground corncob bedding nesting material (Bed O'Cobs®; The Andersons, Cob Products Division, Maumee, OH). Following weaning on PND 21, offspring were housed by litter in plastic maternity cages with nesting material until PND 27. On PND 28, surviving offspring were housed individually in suspended wire-mesh cages until scheduled euthanasia. All procedures involving rats were reviewed and approved by the testing facility's Institutional Animal Care and Use Committee. Animals were housed in accordance with the *Guide for the Care and Use of Laboratory Animals* [36]. The animal facilities were accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

Rats were given access to basal diet (Certified Rodent LabDiet® 5002, PMI Nutrition International, LLC) and reverse osmosis-purified (on-site) municipal drinking water *ad libitum*. The room temperature and humidity controls were set to maintain daily averages of $22 \pm 3\,^{\circ}C$ and $50 \pm 20\%$ relative humidity. Light timers were calibrated to provide a 12-h light/12-h dark photoperiod. Air handling units were set to provide a minimum of 10 room air changes per hour, 100% fresh air.

2.3. Clinical observations

2.3.1. Maternal rats

All rats were observed twice daily for moribundity and mortality. Main study (not satellite-group) rats were also observed daily for signs of toxicity approximately 1 h following dose administration. Group-mean maternal body weights and food consumption were calculated on GD 0, 3, 6, 9, 12, 15, 18 and 20 (both main study and satellite-group maternal rats) as well as on PND 1, 4, 7, 10, 14, 17 and 21 (main study maternal rats).

All main study maternal rats were allowed to deliver naturally. They were observed twice daily for initiation and completion of parturition and for any signs of dystocia (prolonged labor, delayed labor or other difficulties). On PND 0, the day that parturition was initiated, all pups were sexed and examined for gross malformations, and the numbers of stillbirths and live pups were recorded.

All satellite dams and their fetuses and 10 randomly selected main study phase dams were euthanized by decapitation on GD 20 and PND 21, respectively. Blood and tissue samples were collected and evaluated for pharmacokinetic, thyroid hormone and morphology, and liver gene-expression endpoints, as described in the companion article [35, this issue].

All remaining main study phase dams were euthanized and subjected to a gross examination on PND 21. The numbers of implantation sites were determined for dams that delivered, and a pregnancy status was determined for each dam that failed to deliver. The satellite-group dams did not receive a gross examination, but were examined for pregnancy status. Uteri with no macroscopic evidence of implantation were opened and subsequently placed in a 10% ammonium sulfide solution for detection of early implantation loss [37].

2.3.2. Offspring

Each litter was examined daily for survival and any changes in appearance or behavior, including nursing habit. A detailed physical examination was performed and body weight was recorded for each pup on PND 1, 4, 7, 11, 14, 17 and 21 and weekly thereafter until necropsy on PND 72. All pups were sexed on PND 0, 4, 11 and 21. Intact offspring dying from PND 0 to 4 were necropsied using a fresh dissection technique [38]. A detailed gross necropsy was performed on any pup that died after delivery.

To minimize the biological variability among the litters, 8 pups/litter (4 pups/sex when possible) were randomly selected and grouped on PND 4 into Subset A and Subset B for further observation and evaluation. Ten culled pups (randomly selected per litter per group) from dams that were used for blood and tissue collection (as described above) were euthanized on PND 4 by decapitation. Blood and tissue samples were collected for evaluation as described in a companion article [35, this issue].

Subset A consisted of 20 pups/sex/group (1 rat/sex/litter from 20 litters/group), and it was assigned to the functional observations battery (PND 4, 11, 21, 35, 45 and 60), acoustic startle response (PND 20 and 60), locomotor activity (PND 13, 17, 21 and 61), and learning and memory (PND 22). While all the pups in Subset A were euthanized on PND 72, 15 pups/sex/group were evaluated for brain weights while the remaining 5 pups/sex/group were designated for blood and tissue collection as described in the companion article [35, this issue]. Subset B consisted of 15 pups/sex/group (1 rat/sex/litter from 15 litters/group); these rats were selected for brain-weight evaluations on PND 21. All rats not included in Subset A or Subset B were euthanized and necropsied on PND 21.

2.4. Developmental landmarks, sensory function, and neurobehavioral testing (Subset A)

The various investigations described herein were used to assess the maturation and behavioral development of the selected Subset A rats. These procedures were concluded when the oldest rats were 66 days of age.

2.4.1. Sexual maturation

Each male and female pup was observed for balanopreputial separation beginning on PND 35 [39] or vaginal perforation beginning on PND 25 [40], respectively. The day on which balanopreputial separation or vaginal lumen opening was first observed was recorded for each pup. Individual body weights were recorded on the day of attainment of these landmarks.

2.4.2. Functional observation battery (FOB)

FOB assessments were conducted on all Subset A rats on PND 4, 11, 21, 35, 45 and 60, based on previously developed protocols [41–46]. The same rats were observed at each interval for the following parameters: ease of cage removal; ease of handling in hand; lacrimation/chromodacryorrhea; salivation; piloerection; appearance of fur; palpebral closure; respiratory rate/character; red, crusty deposits; mucous membranes/skin color; eye prominence; eye color; mobility; muscle tone; convulsions/tremors; hindlimb extension; grooming; arousal; bizarre/stereotypic behavior; urination/defecation; papillary response; backing; forelimb/hindlimb grip strength; tail pinch response; gait; and air righting. Piloerection, fur appearance,

palpebral closure, eye prominence, eye color, hindlimb extension, grooming papillary response forelimb/hindlimb grip strength, tail pinch response, and air righting reflex were not assessed on PND 4 and 11 due to stage of development. Mobility, backing and gait were not evaluated on PND 4, also because of stage of development.

2.4.3. Acoustic startle response

An acoustic startle response test was performed for all Subset A rats on PND 20 and 60 using the SR-Lab Startle Response System (San Diego Instruments, San Diego, CA). The same rats were tested at each interval. Acoustic startle response testing was performed in a room equipped with a white-noise generation system set to operate at 70 ± 10 decibels (db). Each test session consisted of a 5-min acclimation period with a 65 ± 5-db broadband background white noise. The startle stimulus for each trial was a 115 ± 5-db mixed-frequency noise burst stimulus, approximately 20 ms in duration. Responses were recorded during the first 100 ms following the onset of the startle stimulus for each trial. The recording of the startle reflex when the rat jumps in response to the startle stimulus results in a waveform that represents the pressure on a piezoelectric sensor accelerometer mounted below a cylindrical animal enclosure mounted on a Plexiglas base. During the calibration process, the gain was adjusted higher for the PND 20 rats than for the PND 60 rats so that the reported maximum response amplitude (V_{max}) and average response amplitude (V_{avg}) for the rats at the two ages were similar. Each test session consisted of 50 trials, with an 8-s inter-trial interval. Startle response data were analyzed in 5 blocks of 10 trials each. Startle response measurements obtained were maximum response amplitude (V_{max}), average response amplitude (V_{avg}) and latency to V_{max} (T_{max}).

2.4.4. Locomotor activity

Locomotor activity was assessed individually on PND 13, 17, 21 and 61 for all Subset A rats. The same rats were tested at each interval. Locomotor activity was measured automatically using the SDI Photobeam Activity System (San Diego Instruments, San Diego, CA). Four-sided black plastic enclosures were used to surround the amber plastic boxes and decrease the potential for distraction from extraneous environmental stimuli or activity by laboratory personnel or adjacent rats. Data were collected in 5-min epochs (print intervals), and the test session duration was 60 min. Data for ambulatory and total locomotor activity were tabulated. Total locomotor activity was defined as a combination of fine locomotor skills (i.e., grooming; interruption of a single photobeam) and ambulatory locomotor activity (e.g., interruption of two or more consecutive photobeams).

2.4.5. Biel maze swimming trials (learning and memory)

Beginning on PND 22, swimming ability and learning and memory were assessed for Subset A rats using a water-filled 8-unit T-maze [47]. Rats were placed in the maze and were required to traverse the maze and escape by locating a submerged platform. For the learning and memory phases, the time required to traverse the maze and the numbers of errors for all trials were recorded. An error was defined as any instance when a rat deviated from the correct channel with all 4 legs.

Each testing interval consisted of three phases that were conducted over 7 consecutive days. Phase 1 was an evaluation of swimming ability and motivation to escape from the maze and was performed on day 1 of the Biel maze procedure (PND 22). For this evaluation, rats were placed in a straight channel opposite the submerged escape platform, and the time required for each rat to climb up on the escape platform was recorded. Once the rat reached the platform, it was immediately placed at the starting position for another trial for a total of four consecutive trials.

Phase 2 of the Biel maze procedure evaluated sequential learning. This evaluation was conducted on days 2–6 of the Biel maze procedure. Rats were allowed two trials per day for 2 days (PND 23 and 24) to solve the maze in path A. Rats were then allowed two trials per day for 3 consecutive days (PND 25, 26, and 27) to solve the maze in path B (reverse of path A). For each trial, rats were allowed 180 s to solve the maze. If a rat did not escape the maze within the allotted 180 s, the rat was placed on the escape platform for approximately 20 s, then removed from the maze. The minimum inter-trial interval was 1 h.

Phase 3 of the Biel maze procedure probed the rat for its memory to solve the maze when challenged in path A. This evaluation was conducted on day 7 of the Biel maze procedure (PND 28). Each rat was allowed two trials to solve the maze in path A.

Biel maze data were evaluated as the mean time to escape over all trials for each of the three phases (i.e., swimming ability and motivation, sequential learning, and memory) of the Biel maze procedure. Also, the numbers of errors committed were evaluated for phases 2 and 3.

2.5. Offspring macroscopic observation

On PND 21, all pups not selected for neurobehavioral evaluations, thyroid function assessments or brain weight measurements were euthanized by carbon dioxide inhalation and subjected to gross examinations. On PND 72, rats (5 pups/sex/group) not allocated for brain weight measurements were euthanized by decapitation; blood and tissue samples were collected and rats were subjected to a gross examination. The necropsy included examination of the external surface, all orifices and the cranial, thoracic, abdominal and pelvic cavities, including viscera/contents.

Macroscopic neuropathological examinations were conducted on all Subset B rats (15 rats/sex/group) on PND 21 and selected Subset A rats (15 rats/sex/group) on PND 72. After anesthesia with sodium pentobarbital (via ip injection) and perfusion *in situ* with fixative (4% paraformaldehyde/1.4% glutaraldehyde), the whole brains were removed (including olfactory bulbs) with weight, length, and width recorded. They were also evaluated for any abnormal coloration or lesions of the external brain and spinal cord.

2.6. Statistics

All statistical tests were performed using appropriate computing devices or validated data collection and reporting system in the testing facility. SAS program analyses were conducted using two-tailed tests (except as noted otherwise) for minimum significance levels of 1% and 5%, comparing each test article-treated group to the control group by sex. Where applicable, the litter was used as the experimental unit.

Continuous data variables were subjected to a parametric one-way analysis of variance (ANOVA) [48] to determine intergroup differences. If the ANOVA revealed statistically significant ($p < 0.05$) intergroup variance, Dunnett's test [49] was used to compare the test article-treated groups to the control group. Mean litter proportions (percent per litter) of males at birth and pup viability during the postnatal period were subjected to the Kruskal Wallis [50] non-parametric ANOVA test to determine intergroup differences. If the ANOVA revealed statistically significant ($p < 0.05$) intergroup variance, Dunn's test [51] was used to compare the test article-treated groups to the control group. Functional observational battery parameters that yielded scalar and descriptive data were analyzed by the Fisher's Exact Test [52].

Total count measurements from the locomotor activity assessment, peak response and latency to peak response measurements from the auditory startle response data, learning and memory analyses and the number of errors committed during the learning and memory evaluations were analyzed by sex with a repeated measures analysis of variance (RANOVA) [53]. Factors in the model included ANIMAL, treatment group (TRT), TIME (locomotor activity) or TRIAL (auditory startle response and learning and memory), and the interaction term (TRT × TIME or TRT × TRIAL). The SAS® procedure PROC MIXED was used for the analysis with the random effect of rat included as the repeated measurement. The covariance structure across time was selected by comparing Akaike's Information Criterion (AIC) for compound symmetry (CS) and/or first-order autoregressive (AR(1)) structures.

The locomotor activity and auditory startle response data were analyzed for the entire test session. The learning and memory data were analyzed by phase (learn path A, learn path B, and memory path A). In addition, the time to escape for an rat that did not escape the maze in the allotted time was considered censored and set equal to the maximum allotted time for statistical analysis.

The monotonic dose-response relationship was evaluated using sequential linear trend tests based on ordinal spacing of dosage levels. The linear dose by time interaction (LinTRT × TIME) or linear dose by trial interaction (LinTRT × TRIAL) was evaluated and, if significant at the 0.05 level, trend tests on treatment means were performed at the 0.05 level for each time interval or trial. If the linear dose by interaction term was not significant, the trend test was conducted across the pooled trials of the entire session or phase (as appropriate) only.

Non-monotonic dose responses were evaluated whenever no significant linear trends were detected but the TRT and/or the interaction term (TRT × TIME or TRT × TRIAL) was significant at the 0.01 level. Within the framework of the RANOVA, pairwise comparisons were made for each individual test article-treated group with the control group through linear contrasts. If the interaction term was significant, the comparisons were conducted for each time interval or trial. If only the TRT effect was significant, the comparisons were conducted across the pooled intervals of the entire session or phase (as appropriate). These non-monotonic dose–response comparisons were conducted at the 0.01 significance level.

3. Results

3.1. Analytical

Dosing solutions were found to be stable over an eight-day period (>90% of time 0 concentration) and homogenous (data not shown). Mean PFOS (and inferred K^+PFOS) concentrations for seven sets of dosing solutions were within 2% of the target concentrations and coefficients of variation were less than 10%.

3.2. Maternal rats

3.2.1. Clinical observations and survival

All maternal rats survived to the scheduled necropsy. No test article-related clinical findings were noted at the daily examinations or 1 h following dose administration.

Table 1
Mean (±SD) mating and pregnancy outcomes for maternal female rats.

Endpoint evaluated	Maternal dose (mg/kg-d)			
	0.0	0.1	0.3	1.0
Number of females for mating	25	25	25	25
Number confirmed pregnant	25	23	25	24
Number of litters (*N*)	25	23	25	24
Gestation length (*d*)	21.9 ± 0.5	21.7 ± 0.5	21.7 ± 0.48	21.8 ± 0.44
Implantation sites (*N*)	16.6 ± 3.0	16.1 ± 2.5	15.9 ± 2.2	16.0 ± 1.3
Unaccounted sites (potential resorptions) (*N*)	0.8 ± 0.8	0.9 ± 0.9	0.8 ± 0.8	0.6 ± 0.7

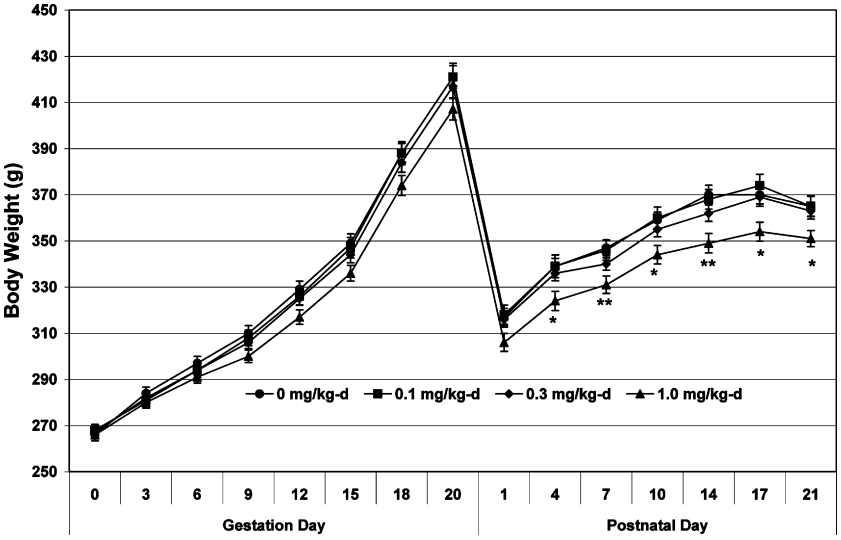

Fig. 1. Maternal weights during gestation and lactation. Data points represent means and error bars represent standard errors of the mean. Statistically significant (*$p < 0.05$, **$p < 0.01$) decreased maternal body weights were evident in the 1.0 mg/kg-d dose-group dams from postnatal day (PND) 4 through PND 21. During gestation days (GD) 9 through 20, these dams showed somewhat decreased body weights but without statistical significance.

3.2.2. Pregnancy status

The percent of rats determined pregnant did not vary significantly between groups (Table 1). The pregnancy percents in the control, 0.1, 0.3 and 1.0 mg/kg-d groups were 25/25 (100.0%), 23/25 (92.0%), 25/25 (100.0%) and 24/25 (96.0%), respectively.

3.2.3. Body weights

Maternal body weights during gestation and lactation are summarized in Fig. 1, and body-weight change data for these time periods are presented in Table 2. While slightly lower (not statistically significant) mean body-weight gains were noted in the 1.0 mg/kg-d group maternal rats during GD 0–15 compared to the control group values (data not shown), mean body-weight gains in the 1.0 mg/kg-d group females were similar to the control group values for the remainder of gestation. As a result of the slightly lower (but not statistically significant) mean body-weight gains during GD 0–15, mean body-weight gain in the 1.0 mg/kg-d group was lower (without statistical significance) when the entire period of gestation (GD 0–20) was evaluated.

While a slightly lower (but not statistically significant) mean body-weight gain was also noted in the 1.0 mg/kg-d group dams during PND 1–4 as compared to the control group, mean body-weight gains in this group were similar to the controls throughout the remainder of lactation. As a result of lower mean body-weight gain during gestation and PND 1–4, overall mean body weights in this group were statistically significantly lower than the control group values from PND 4 through PND 21.

3.2.4. Food consumption

Mean overall food consumption data during gestation and lactation are summarized in Table 3. Mean food consumption (evaluated as g/rat-d and g/kg-d) was similar between all treatment groups and control group maternal rats during the entire study duration except the 1.0 mg/kg-d group dams, which had statistically sig-

Table 2
Mean (±SD) maternal weight change during gestation and lactation.

Day of weighing	Maternal dosage group (mg/kg-d) (*N*)			
	0 (25)	0.1 (23)	0.3 (25)	1.0 (24)
Weight on GD[a] 0 (g)	266 ± 13	268 ± 12	267 ± 12	266 ± 13
Weight on GD 20 (g)	421 ± 25	421 ± 29	417 ± 25	407 ± 23
Weight change (GD 0–20) (g)	155 ± 19	153 ± 22	150 ± 20	141 ± 17
Weight on PND[b] 1 (g)	317 ± 19	318 ± 20	316 ± 17	306 ± 19
Weight on PND 21 (g)	365 ± 23	365 ± 21	363 ± 18	351 ± 17*
Weight change (PND 1–21) (g)	48 ± 16	47 ± 18	47 ± 13	45 ± 15

* Statistically significant ($p < 0.05$).
[a] Gestation day.
[b] Postnatal day.

Table 3
Mean (±SD) absolute and relative (to body weight) maternal food consumption through gestation and lactation[a].

Time period	Units	Maternal dosage group (mg/kg-d) (*N*)			
		0 (25)	0.1 (23)	0.3 (25)	1.0 (24)
Absolute food consumption (GD[b] 0–20)	g/rat-d	25 ± 2	24 ± 2	24 ± 2	23 ± 1**
Relative food consumption (GD 0–20)	g/kg-d	74 ± 3	74 ± 3	73 ± 4	72 ± 3
Absolute food consumption (PND[c] 1–21)	g/rat-d	57 ± 6	60 ± 5	58 ± 4	55 ± 9
Relative food consumption (PND 1–21)	g/kg-d	161 ± 18	170 ± 12	165 ± 12	165 ± 10

** Statistically significant ($p < 0.01$).
[a] When food consumption could not be determined for an animal during a given interval (due to a weighing error, food spillage, obvious erroneous value, etc.), group-mean values were calculated for that interval using the available data.
[b] Gestation day.
[c] Postnatal day.

nificant ($p < 0.01$) lower mean food consumption value during GD 6–12. Primarily as a result of the lower food consumption during GD 6–12, overall mean food consumption in the 1.0 mg/kg-d group was lower ($p < 0.01$) on a g/rat-d when the entire period of gestation (GD 0–20) was evaluated. Corresponding slight reductions in mean body-weight gains were noted in these dams, as mentioned previously.

A statistically significant ($p < 0.05$) decrease in food consumption was noted in the 0.3 mg/kg-d group during GD 6–9 on both a g/rat-d and a g/kg-d basis (data not shown). However, this difference was not considered to be K^+PFOS treatment-related because it was transient and not observed with corresponding effects on mean body-weights or body-weight gains.

Mean food consumption in the 1.0 mg/kg-d group dams was also lower than the control group values during PND 1–4 (36 g/rat-d versus 40 g/rat-d in the 1.0 mg/kg-d and control groups, respectively) and corresponded to the previously noted slight reduction in mean body-weight gain noted in these females during PND 1–4. Mean food consumption in the 1.0 mg/kg-d group was similar to the control group values during the remainder of lactation. None of the differences in food consumption at 1.0 mg/kg-d was statistically significant from the control group during the lactation period.

3.2.5. Gestation length

Gestation length and pregnancy outcome data are summarized in Table 1. No K^+PFOS treatment related effects were noted on mean gestation lengths or the process of parturition at any dosage level. No signs of dystocia were noted at any dosage level. At the PND 21 necropsy, no test article-related effects were observed on the number of former implantation sites and the number of unaccounted-for sites (Table 1).

3.2.6. Macroscopic examinations

No K^+PFOS treatment related internal findings were observed for dams that failed to deliver or for dams necropsied on PND 21 at any dosage level. Macroscopic findings observed in the K^+PFOS treated groups occurred infrequently, at similar frequencies as the control group and/or in a manner that was not dose-related.

3.3. Litter results

Litter data on PND 0 and postnatal survival data are summarized in Table 4. The mean number of pups born, live litter size, percentage of males per litter at birth and postnatal survival were unaffected by maternal administration of the test article at all dosage levels.

The numbers of pups found dead, missing, presumed cannibalized, as well as the general physical condition of all pups in this study, were unaffected by maternal test article administration. No internal findings that could be attributed to maternal administration of the test article were noted at the necropsies of pups that were found dead.

Table 4
Mean (±SD) litter parameters.

Parameter evaluated	Maternal dosage group (mg/kg-d)			
	0.0	0.1	0.3	1.0
Delivered litters (*N*)	25	23	25	24
Number of pups born per litter (*N*)	15.8 ± 3.0	15.2 ± 2.2	15.1 ± 2.2	15.4 ± 1.4
Live litter size on PND[a] 0 (*N*)	15.6 ± 3.0	15.2 ± 2.2	15.0 ± 2.2	15.1 ± 1.7
Sex at birth (% males per litter)	48.7 ± 9.9	46.6 ± 12.4	49.1 ± 11.8	48.9 ± 10.4
Birth to PND 4[b] survival (% per litter)	97.5 ± 4.9	97.6 ± 4.0	96.7 ± 4.6	95.9 ± 6.1
PND 4–21[c] survival (% per litter)	98.0 ± 5.9	100 ± 0.0	98.0 ± 5.9	100 ± 0.0
Pup weight (g)[d]				
PND 1: male	7.1 ± 0.6	7.2 ± 0.7	7.0 ± 0.6	6.9 ± 0.7
PND 1: female	6.7 ± 0.6	6.7 ± 0.7	6.7 ± 0.6	6.6 ± 0.6
PND 21: male	52.7 ± 6.5	54.7 ± 5.6	54.0 ± 5.4	50.7 ± 5.9
PND 21: female	50.2 ± 6.6	51.7 ± 5.3	51.8 ± 6.2	50.1 ± 4.8
PND 72: male	413 ± 39	440 ± 47	428 ± 25	420 ± 35
PND 72: female	245 ± 26	255 ± 22	251 ± 22	246 ± 24
Age at vaginal patency (PND)	31.7 ± 1.0	32.7 ± 1.5	32.0 ± 1.6	32.3 ± 0.7
Weight at vaginal patency (g)	106 ± 11	119 ± 12*	111 ± 9	110 ± 10
Age at balanopreputial separation (PND)	46.2 ± 2.1	47.0 ± 3.6	45.8 ± 2.6	45.8 ± 1.8
Weight at balanopreputial separation (g)	248 ± 18	268 ± 39*	253 ± 21	244 ± 21

* Statistically significant ($p < 0.05$).
[a] Postnatal.
[b] Pre-selection.
[c] Post-selection.
[d] $N = 20$ rats per dose-group per sex.

An increased incidence of hair loss (primarily on the limbs) was noted in the 0.3 mg/kg-d group. This finding was not considered to be related to maternal test article administration because it occurred primarily in 1 litter and was not noted at the 1.0 mg/kg-d dosage level.

Pup body weights through PND 72 are summarized in Table 4. Mean pre-weaning pup body weights and body-weight changes (data not shown) in all 0.1, 0.3 and 1.0 mg/kg-d group male and female pups were unaffected by administration of K^+PFOS to the maternal rats compared with the control group. A statistically significant ($p < 0.05$) higher mean body-weight gain was noted in the 0.1 mg/kg-d group males during PND 56–63 compared to the control group; however, because the increase was transient and did not show a dose-response, it was not considered K^+PFOS treatment-related. Otherwise, there were no effects of maternal K^+PFOS treatment on body weight or body-weight gain in offspring.

Balanopreputial separation and vaginal patency data for male and female pups, respectively, are summarized in Table 4. Mean ages of attainment and mean body weights at the age of attainment were unaffected by administration of the test article to the maternal rats. Mean body weight at the age of attainment in the 0.1 mg/kg-d group was statistically significantly increased ($p < 0.05$) compared to the concurrent control group but was not considered to be related to maternal test article administration due to the lack of a dose-response.

Following weaning, all selected male and female pups in the control, 0.1, 0.3 and 1.0 mg/kg-d groups survived to the scheduled necropsy. No K^+PFOS treatment-related clinical findings were noted at the daily examinations. Findings noted in the K^+PFOS-treated groups, including hair loss and scabbing on various body surfaces, occurred infrequently, at similar frequencies as the control group and/or in a manner that was not dose-related.

On PND 72 necropsies, no internal gross findings related to maternal administration to the test article were observed in offspring. Incidental internal findings included dilated renal pelvis (unilateral or bilateral) in one female, one female and two males, and two males in the control, 0.1, and 1.0 mg/kg-d groups, respectively, clear fluid in the uterus in one female in each of the control and 1.0 mg/kg-d groups, opacity of the eyes in one male in the 1.0 mg/kg-d group, and dark red areas on the thymus of one female in the control group. No other internal findings were noted.

3.4. Sensory and behavioral testing—Subset A

3.4.1. Functional observation battery (FOB)

There were no K^+PFOS treatment-related findings noted for the male or female rats during the FOB assessments on PND 4, 11, 21, 35, 45 and 60. Although a statistically significant ($p < 0.05$) decrease in hindlimb grip strength was noted in the 1.0 mg/kg-d maternal dose-group males (66.7 g) when compared to the control group (79.8 g) on PND 21, the mean value in the 1.0 mg/kg-d group was within the mean value for the laboratory's historical control data, which was 70 ± 26 g (approximated ±37% difference). This isolated observation occurred on PND 21 only, and the difference in hindlimb grip strength was only 16% less than that of the control. Because there were no effects on other parameters such as forelimb grip strength, hindlimb extensor strength, gait, mobility and muscle tone, the decrease in hindlimb grip strength was not attributed to maternal treatment with K^+PFOS.

3.4.2. Locomotor activity

Fig. 2 illustrates 60-min cumulative counts for male and female pups on PND 13, 17, 21, and 61. In addition, Fig. 3 shows 15-min sequential time locomotor activity data for male pups. All test article-treated groups displayed the expected "inverted U-shaped pattern" during the pre-weaning period, with lower levels of activity on PND 13, highest levels of activity on PND 17, and somewhat lower levels on PND 21.

For both male and female pups, there was no difference in total cumulative activities between K^+PFOS-treatment groups and controls during all time periods evaluated except for 0.3 and 1.0 mg/kg-d group males on PND 17 and 1.0 mg/kg-d group females on PND 21.

On PND 17, total cumulative activities were increased with statistical significance ($p < 0.05$) in the 0.3 and 1.0 mg/kg-d maternal dose-group males compared to the concurrent control group

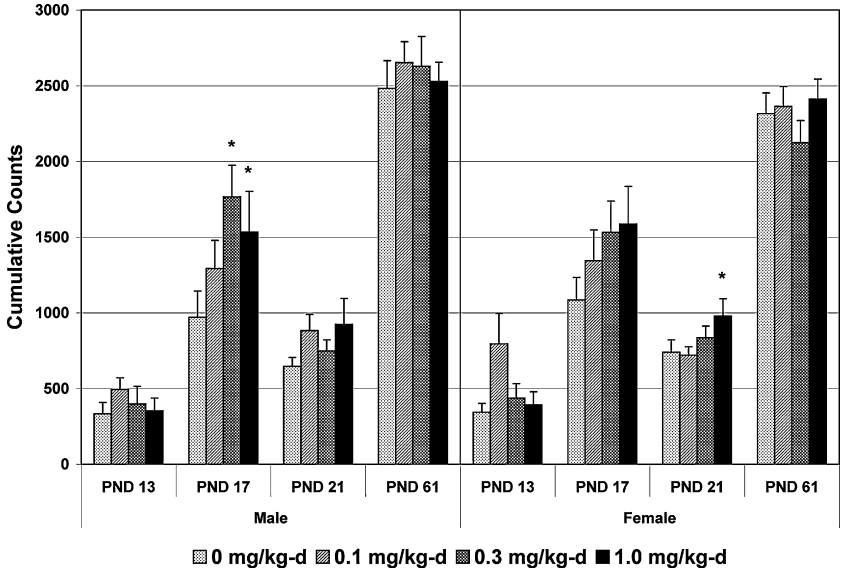

Fig. 2. Motor activity cumulative counts by postnatal day (PND) for males and females. Males in the 1.0 mg/kg-d maternal dose-group showed increased motor activity and lack of habituation on PND 17. Increased activity was also noted on PND 17 in males from the 0.3 mg/kg-d maternal dose-group and was largely driven by increased activity in the 16–30 min test session (see Fig. 3); however, these males displayed expected habituation response. Females in the 1.0 mg/kg-d maternal dose-group had statistically significant higher total activity on PND 21; however, habituation to the test environment in these females was similar to the concurrent control group on PND 21 and there were no differences between the controls and the 1.0 mg/kg-d maternal dose-group females for the individual evaluation time periods (data not shown).

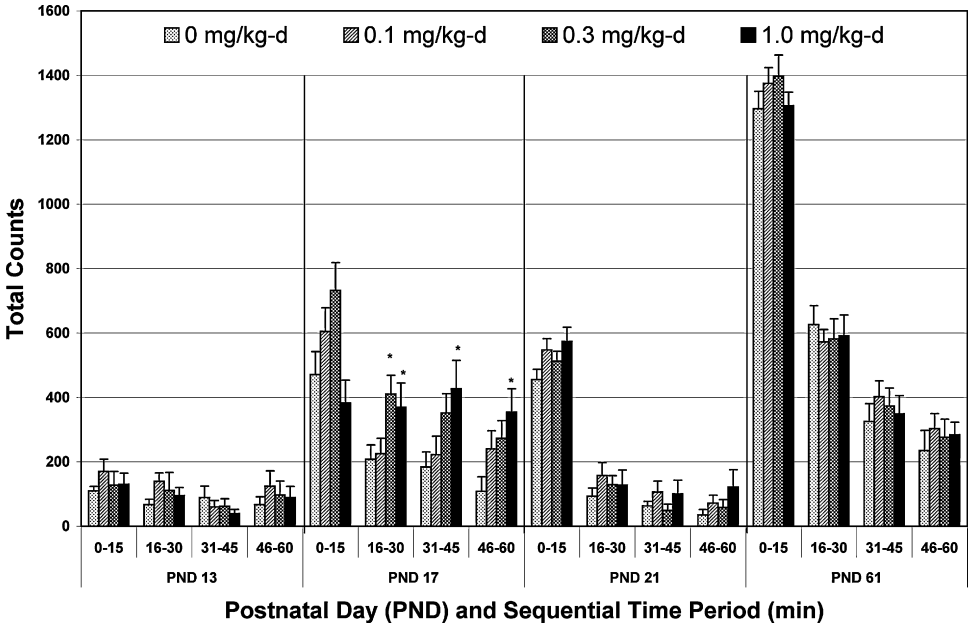

Fig. 3. Male motor activity counts by time period and postnatal day (PND). Rats from the 1.0 mg/kg-d maternal dose-group displayed increased motor activity compared to controls on PND 17 and failed to show habituation. Rats in the 0.3 mg/kg-d maternal dose-group showed increased activity in the 16–30 min period, and total activity was increased (see Fig. 2); however, these rats showed a normal habituation pattern.

(Fig. 2). The increased locomotor activity in the 1.0 mg/kg-d maternal dose-group males was also accompanied by slightly increased ambulatory activity (without statistical significance, data not shown). By analyzing the activity per 15-min sequential time period, total activities during three of the four session intervals (16–30 min, 31–45 min and 46–60 min) were increased with statistical significance ($p < 0.05$) in the 1.0 mg/kg-d maternal dose-group males compared to the concurrent control group (Fig. 3). Eight males in the 1.0 mg/kg-d maternal dose-group had higher total activity counts during the 46–60 min interval than the 0–15 min interval, indicating an absence of habituation to the test environment on PND 17 (an age when rats should begin to show habituation). Because these eight males failed to habituate, the increased motor activity on PND 17 was considered K^+PFOS treatment related.

A statistically significant ($p < 0.05$) increase in total and cumulative activity was noted in the 0.3 mg/kg-d group males on PND 17 and 1.0 mg/kg-d group females on PND 21 (Fig. 2). Because habituation to the test environment in these two groups of rats was similar to the concurrent control group and no other differences from the concurrent control group in total and ambulatory activity were noted during other time periods evaluated, the increased motor activity noted in these two groups was not considered K^+PFOS treatment-related.

3.4.3. *Acoustic startle response*

Acoustic startle response data are summarized in Figs. 4 (males) and 5 (females). The acoustic startle response habituation paradigm was conducted as a longitudinal assessment with selected rats evaluated on PND 20 and again on PND 60.

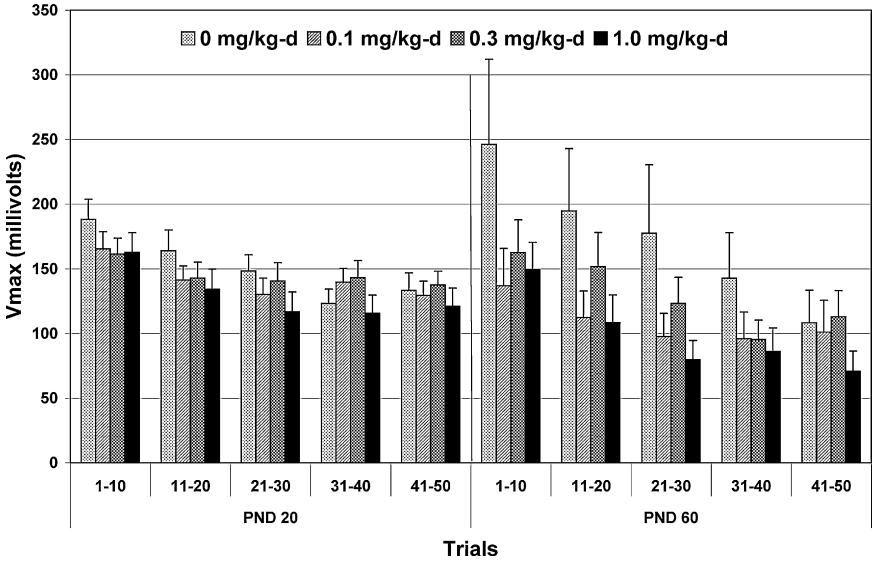

Fig. 4. Male offspring acoustic startle V_{max} on postnatal day (PND) 20 and 60. No statistically significant differences from control rats were noted.

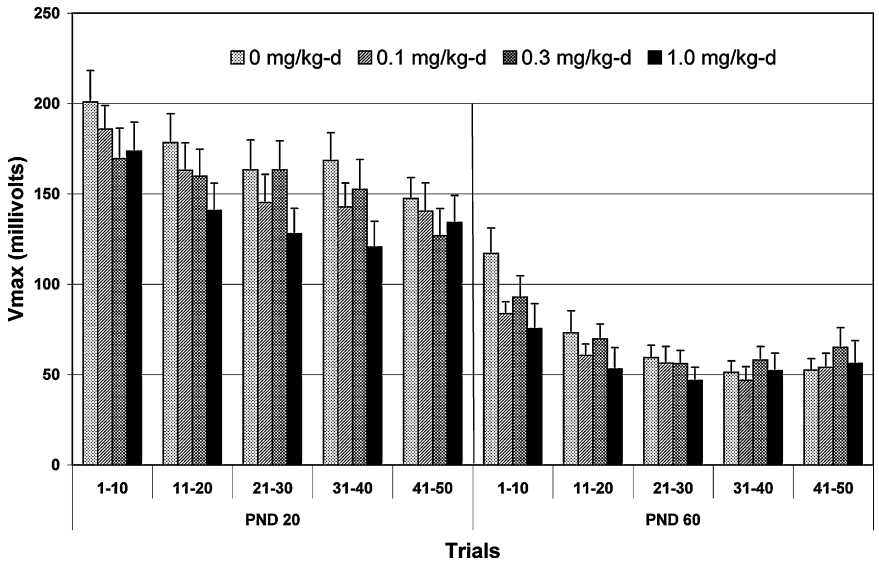

Fig. 5. Female offspring acoustic startle V_{max} on postnatal day (PND) 20 and 60. No statistically significant differences from control rats were noted.

Administration of 0.1, 0.3 and 1.0 mg/kg-d K^+PFOS to the maternal rats had no effect on auditory startle responsiveness. Mean V_{max} for all trials combined in the control group males on PND 60 was at least 26% higher than the values in all K^+PFOS-treated groups. These higher mean control values were attributed to two males in this group with substantially higher values (659.1 and 778.2 mV, respectively) compared to the overall control group mean (173.9 mV). Therefore, the lower V_{max} values in the 0.1, 0.3 and 1.0 mg/kg-d group males on PND 60 were not attributed to maternal treatment with K^+PFOS. Moreover, no statistically significant differences from the control group were noted when analyzed by a repeated measures analysis, and no effects were noted in the pattern of the habituation response over the entire 50-block test session in adult rats.

3.4.4. Biel maze swimming trials

Biel maze swimming trial data are summarized in Figs. 6 (males) and 7 (females). On PND 22, the first day of evaluation for swimming, there were no significant differences or trends between groups in swimming ability or the times to criterion (mean time to locate the submerged platform) during the learning and memory trials. The mean numbers of errors committed during the various phases of evaluation were similar in all groups.

3.5. Brain endpoints

At the necropsies of rat offspring selected for brain measurements on PND 21 and 72, no gross findings attributable to maternal

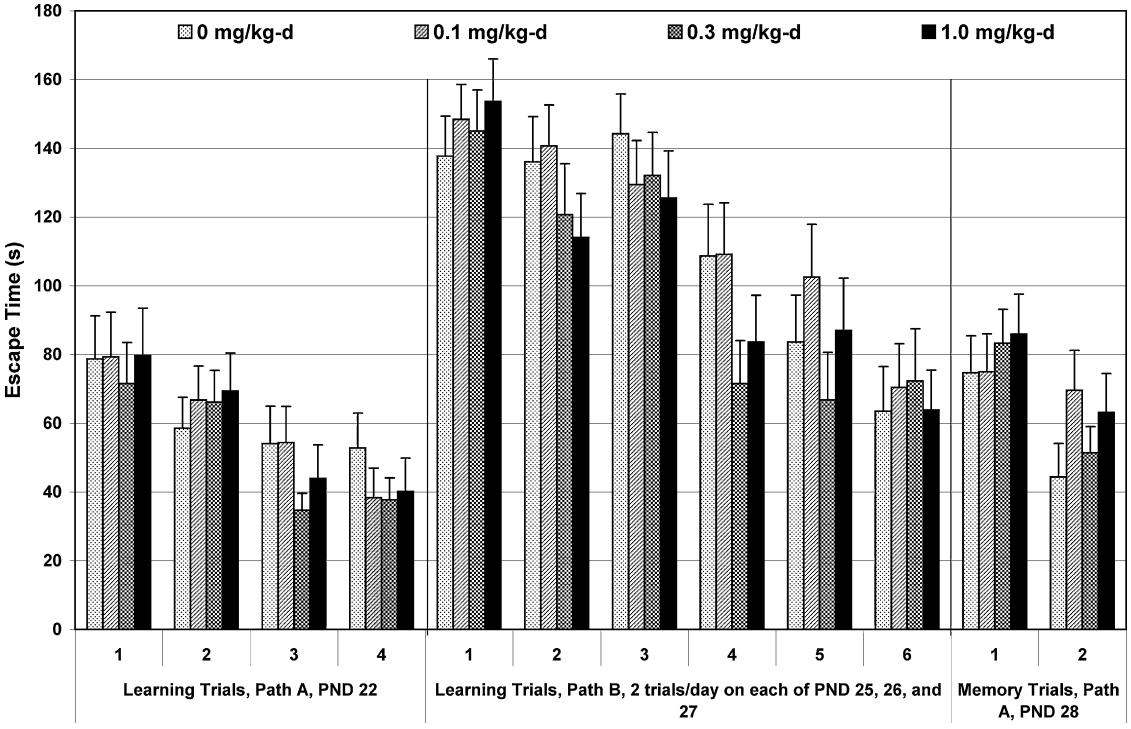

Fig. 6. Male offspring Biel maze trial data. No statistically significant differences from control rats were noted.

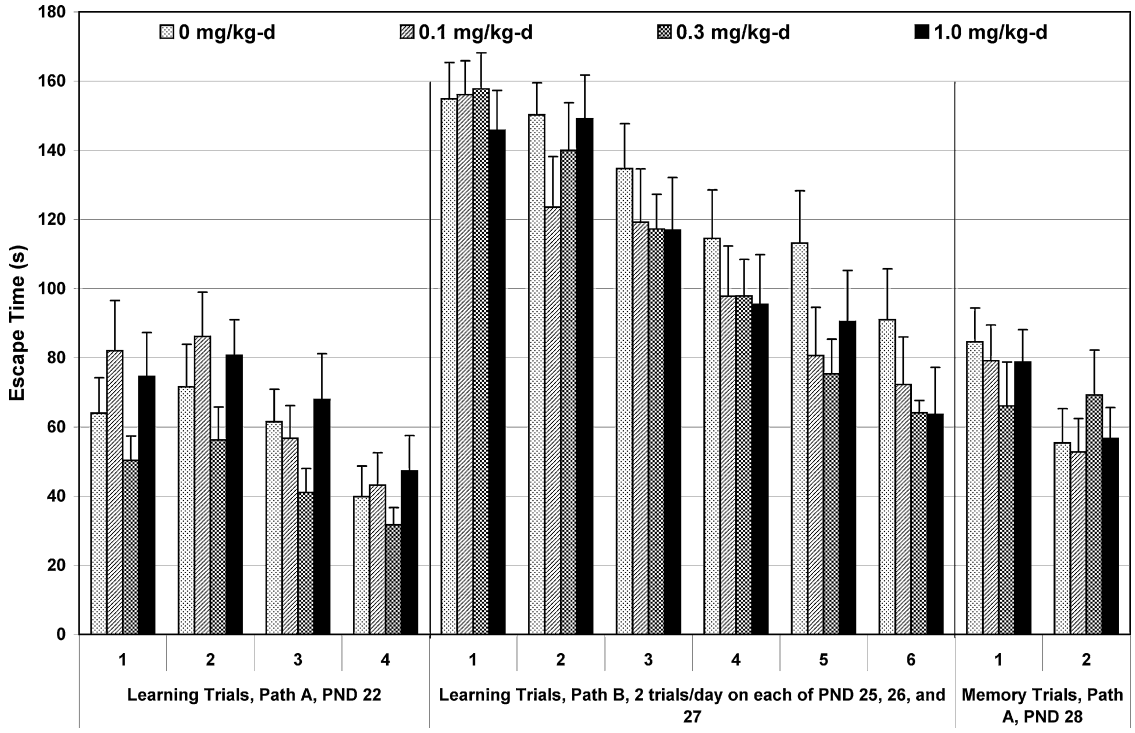

Fig. 7. Female offspring Biel maze trial data. No statistically significant differences from control rats were noted.

dosing with K^+PFOS were noted in the brain or spinal cord. Brain weight and measurement data for PND 21 and 72 rats are summarized in Table 5. There were no K^+PFOS treatment-related effects on brain weight, length or width. Abnormal findings on the brain and spinal cord were limited to a brain cyst in one control group male on the PND 72.

4. Discussion

Oral administration of K^+PFOS to maternal rats at 1.0 mg/kg-d resulted in an effect on motor activity in their respective male pups on PND 17. Total and ambulatory activity counts in these males were increased (generally with statistical significance; $p < 0.05$) compared to the concurrent control group, and a majority of these males failed to habituate to the test environment on PND 17 (an age when rats should begin to habituate), while all control group males showed some indication of habituation. The effect on habituation was transient, occurring only on the PND 17 evaluation and only in the highest dosage group pups. It is noteworthy that increased motor activity and decreased habituation were also observed in mice by Fuentes et al. [24] and Johansson et al. [25].

Care was taken in choosing dosage levels that would obviate complications involving maternal and neonatal weight and neonatal survival that had been observed in previous reproduction and developmental studies with PFOS at doses as low as 0.4 mg/kg-d [20,21]. In previous studies, administration of K^+PFOS to rat dams for 6 weeks prior to mating and through lactation resulted in decreases in pup body weights at dosages of 0.4 mg/kg-d and higher and postnatal survival at dosages of 1.6 mg/kg-d and higher [20] or administration from GD 2 through GD 21 resulted in decreased neonatal body weight and increased postnatal mortality at dosages of 2 mg/kg-d and higher [21]. In the study reported herein, with the exception of some effects on maternal weight gain and food consumption in the 1.0 mg/kg-d dose-group, dams and pups appeared to tolerate PFOS exposures relatively well. Therefore, the results of the developmental neurotoxicity evaluation were not confounded by mortality or other serious systemic effects.

Lau et al. [21] evaluated learning in weanling rat pups from dams exposed from GD 2 through GD 21 to potassium PFOS (K^+PFOS)

Table 5
Summary of mean (±SD) brain weight and brain measurement data for offspring on postnatal days 21 and 72 (N = 15/sex/group).

Brain endpoint	Male maternal dosage groups (mg/kg-d)				Female maternal dosage groups (mg/kg-d)			
	0	0.1	0.3	1.0	0	0.1	0.3	1.0
Postnatal day 21								
Weight (g)	1.61 (0.09)[a]	1.60 (0.07)	1.58 (0.07)	1.62 (0.10)	1.60 (0.10)	1.57 (0.07)	1.56 (0.06)	1.55 (0.09)
Weight (%)[b]	3.04 (0.43)	3.06 (0.35)	3.02 (0.32)	3.07 (0.21)	3.26 (0.44)	3.21 (0.42)	3.06 (0.26)	3.02 (0.22)
Length (mm)	22.3 (0.5)	22.4 (0.3)	22.2 (0.3)	22.3 (0.3)	22.0 (0.6)	21.9 (0.4)	22.2 (0.4)	21.9 (0.4)
Width (mm)	15.1 (0.3)	14.9 (0.3)	14.9 (0.2)	15.0 (0.2)	14.9 (0.4)	14.7 (0.2)	14.9 (0.3)	14.8 (0.3)
Postnatal day 72								
Weight (g)	2.18 (0.09)	2.25 (0.10)	2.16 (0.08)	2.20 (0.08)	1.99 (0.10)	2.02 (0.07)	2.01 (0.05)	2.00 (0.07)
Weight (%)	0.53 (0.05)	0.51 (0.04)	0.51 (0.04)	0.53 (0.04)	0.81 (0.08)	0.79 (0.07)	0.81 (0.06)	0.83 (0.09)
Length (mm)	20.8 (0.4)	20.8 (0.5)	20.6 (0.4)	20.5 (0.4)	19.8 (0.5)	20.0 (0.4)	19.8 (0.4)	19.8 (0.4)
Width (mm)	15.5 (0.3)	15.6 (0.2)	15.4 (0.3)	15.5 (0.3)	14.9 (0.3)	15.0 (0.3)	15.0 (0.2)	14.9 (0.3)

[a] Standard deviation in parentheses.
[b] Brain weight relative to final body weight (g per 100 g final body weight).

doses ranging from 1 to 10 mg/kg-d using a T-maze and delayed alternation. No effects of K^+PFOS exposure *in utero* were noted. Luebker et al. [20], during the course of a two-generation reproduction and developmental study, assessed learning, short-term retention, and memory in rat pups beginning on PND 24 using a passive avoidance apparatus. These rat pups had exposure to PFOS *in utero* and during lactation, with maternal doses of 0.1 and 0.4 mg/kg-d K^+PFOS occurring for 6 weeks prior to mating and through mating, gestation, and lactation. In addition, weaned pups were treated at these dose levels starting on PND 22. Beginning on PND 70, these authors also evaluated neuromuscular coordination, swimming ability, learning, and long-term memory using a water-filled M-maze. No effects of PFOS exposure were noted in these assessments. Fuentes et al. [24] treated pregnant mice with K^+PFOS at 6 mg/kg-d on GD 12–18, restraining half of the pregnant mice through gestation. Postnatal maternal body weights were significantly reduced in restrained groups and pup body weights in the non-restrained PFOS group were reduced on PND 4 and 8. In addition, some decrements in vertical screen pull and climb and forelimb grip strength on PND 11 only were noted in the non-restrained PFOS group. Restraining of PFOS-treated dams during gestation appeared to lead to masking of these effects. A reduction in horizontal movement activity was noted for pups where PFOS exposure and maternal restraint were combined, and maternal restraint was associated with an increased rearing activity. No effects were noted on myelination on PND 22. Johansson et al. [25] gave single oral PFOS doses of 0.75 and 11.3 mg/kg to 10-day-old male mice and evaluated locomotion, rearing, and habituation at 2–4 months of age. They also evaluated nicotine-induced spontaneous behavior at 4 months of age. Reduced habituation and increased activity were reported at 2–4 months, and a hypoactive response, as compared to controls, was noted after nicotine administration.

More recently, Slotkin et al. [34] reported on the potential of PFOS to inhibit DNA synthesis, decease cell numbers and growth, induce oxidative stress, reduce cell viability, and alter differentiation toward or away from the dopamine (DA) and acetylcholine (ACh) neurotransmitter phenotypes in cultured PC12 cell line neurocytes over a concentration range of 10–250 μM. No effects on cell size, number, or neurocyte outgrowth were noted. Cell viability was affected only on day 4 at the 250 μM concentration. PFOS promoted differentiation into the ACh phenotype at the expense of the DA phenotype.

Placental transfer of PFOS has been demonstrated in the current study as well as several human biomonitoring studies. In the study reported herein, near the end of gestation (GD 20), mean serum PFOS concentrations from the 1.0 mg/kg-d group dams and pooled fetus were 26.63 ± 3.94 and 31.46 ± 1.03 μg/mL, respectively [35]. Human biomonitoring studies have shown that neonates can be exposed to PFOS effectively *in utero*. Inoue et al. [12] investigated PFOS concentrations in 15 paired maternal serum and neonatal cord blood serum samples from Japan obtained in 2003. PFOS was detected in all samples, and maternal and neonatal serum PFOS concentrations were highly correlated. Neonatal and maternal serum PFOS ranged from 1.6 to 5.3 ng/mL and 4.7 to 17.6 ng/mL, respectively, and the mean ratio between neonatal and maternal serum concentration was 0.32 (range 0.23–0.41). Midasch et al. [13] also found PFOS in all of 11 paired maternal and cord blood plasma samples obtained in 2003 in Germany. Maternal plasma PFOS concentration was consistently higher than cord blood plasma PFOS concentration, with medians of 13.0 and 7.3 ng/mL, respectively, and a median ratio of cord blood plasma PFOS concentration to maternal plasma PFOS concentration of 0.6. Spliethoff et al. [14] examined the time trend of PFOS concentrations in 110 pooled samples representing 2640 newborn screening program blood spots from the State of New York over the years 1997–2007. Mean PFOS blood concentrations in the pools by year ranged from 0.81 to 2.41 ng/mL. PFOS concentrations were found to decrease exponentially from the year 2000, in which most manufacturing of PFOS and PFOS-generating materials ceased, with a mean half-life of 4.1 years. Apelberg et al. [8] found that PFOS concentrations in 293 cord blood sera samples obtained in 2004 and 2005 from a largely inner city population in Baltimore, Maryland had a geometric mean of 4.9 ng/mL. A recent study from Canada reported by Monroy et al. [15] evaluated PFOS concentrations in 101 maternal samples from weeks 24 to 28 of gestation and birth, paired to cord blood sera samples at birth. At delivery, mean maternal serum PFOS concentration in the 101 samples was 16.2 ng/mL compared to a mean of 7.3 ng/mL for the paired cord blood sera samples. These studies demonstrate that children are exposed to PFOS beginning *in utero* from placental transfer.

Luebker et al. [20] have reported the results of a cross-fostering study with K^+PFOS that provided evidence of lactational exposure to PFOS. In analyzing paired maternal serum and milk samples from the Luebker et al. cross-fostering study, Kuklenyik et al. [54] found that PFOS in two milk samples from dams given 1.6 mg/kg-d through gestation and up to PND 14 were 11% and 51% of the respective maternal serum PFOS concentration. Although the present study did not incorporate a cross-fostering design, analysis of pup serum samples taken during the course of this study and reported in a companion article [35, this issue] have provided evidence suggestive of lactational exposure, even though none of the offspring received K^+PFOS directly.

Lactational exposure to PFOS has also been demonstrated in human populations. So et al. [16] examined human milk PFOS concentrations in 19 samples obtained in 2004 from China. Mean PFOS concentration was 0.121 ng/mL (range 0.045–0.360 ng/mL), and infant daily intake from lactational exposure was estimated to be 30 ng/kg body weight for the infant with the mother with the highest milk PFOS concentration. Kärrman et al. [17] found that PFOS milk concentrations in 12 Swedish mothers were approximately 1% of paired serum concentrations of PFOS and estimated an infant intake of 200 ng PFOS/d from milk. Overall mean milk concentrations were 0.201 ng/mL. Tao et al. [18] evaluated PFOS concentrations in milk samples obtained in 2004 from 45 nursing mothers in Massachusetts. The mean milk concentration among the 45 samples was 0.131 ng/mL, and estimated average and highest daily infant intake of PFOS was 0.015 and 0.065 ng/kg body weight, respectively. Volkel et al. [19] found that 38 archived and 19 fresh human milk samples from Germany had a median PFOS concentration of 0.119 ng/mL (range 0.028–0.309 ng/mL) and that 13 frozen human milk samples from Hungary had higher PFOS concentrations (median 0.330 ng/mL, range 0.096–0.639 ng/mL). They estimated daily infant intake from milk to be 100–270 ng/d.

The presence of PFOS in the blood of older children has also been studied. In a study of 598 serum samples obtained between 1994 and 1995 from children aged 2 through 12 in the United States, Olsen et al. [9] found a geometric mean PFOS concentration of 37.5 ng/mL. Data on PFOS sera concentrations for children aged 12–19 are available from the National Health and Nutrition Examination Survey (NHANES) for the years 1999–2000 ($N = 543$) and 2003–2004 ($N = 640$) [11]. Geometric mean sera concentrations were 29.1 and 19.3 ng/mL for the 1999–2000 and 2003–2004 sampling periods, respectively.

Olsen et al. [33, this issue] have reviewed the small literature on the potential association of PFOS exposure with birth outcomes and development. There is only one article at this writing, a recent (2008) paper by Fei et al. [27] that allows an evaluation of potential neurological development in association with exposure to PFOS. Fei et al. randomly selected 1400 maternal child pairs from 43,045 that were enrolled in the Danish National Birth Cohort (DNBC) between

1996 and 2002. Samples of first trimester maternal blood and cord blood samples at birth were obtained and analyzed for PFOS. In the DNBC, mothers were interviewed at approximately 12 and 30 weeks of gestation, APGAR scores were recorded at birth, and mothers were interviewed again at approximately 6 and 18 months after birth. Developmental questions included those targeted at understanding gross and fine motor development, attention, cognitive function, language development, and social/personal development. No association of maternal serum PFOS was noted with motor or mental developmental parameters. Mean maternal serum PFOS concentrations approximated 35 ng/mL.

In our study, the no observed adverse effect and lowest observed adverse effect dosages for development of the nervous system were 0.3 and 1.0 mg/kg-d, respectively. The no-observed-adverse-effect-level (NOAEL) for maternal toxicity was considered to be 0.3 mg/kg-d based on the slightly lower maternal food consumption and body-weight gains in gestation, and lower maternal body weights through most of lactation at 1.0 mg/kg-d. The NOAEL for offspring developmental neurotoxicity was considered to be 0.3 mg/kg-d based on increased motor activity and a failure to habituate to the test environment noted in the 1.0 mg/kg-d maternal dose-group males on PND 17.

Serum PFOS concentrations in dams and fetuses at GD 21 in the study have been described in detail in a companion paper [35]. At 0.3 and 1.0 mg/kg-d dosage levels, mean maternal serum PFOS concentrations were approximately 6200 and 27,600 ng/mL, respectively. For a representative sample of 1041 women 12 years of age and older from the United States, serum samples taken between 2003 and 2004 had a geometric mean PFOS concentration of 18.4 ng/mL and were 45.7 ng/mL at the 95th percentile. Based on the geometric mean serum PFOS concentration for women as compared to serum concentrations in female rats from our study, the margin of exposure for the no observed adverse effect and lowest observed adverse effect from our study is 340 and 1462, respectively. A similar analysis at the 95th percentile yields respective margins of exposure of 135 and 582.

Conflict of interest

George A. Parker and Donald G. Stump do not have competing interests other than employment in contract research facilities (Biotechnics, LLC and Wil Research, Inc., respectively) conducting parts of the work. This study was funded in its entirety by 3 M Company. John L. Butenhoff, Shu-Ching Chang, and David J. Ehresman are employees of 3 M Company, a former manufacturer of PFOS and the company supporting the work reported on in the article.

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