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Delivering Sustainable Solutions to Complex Local Challenges, Worldwide

A Survey of International Regulation and Operational Guidance Related to Aqueous Film Forming Foams and Per- and Polyfluoroalkyl Substances (PFASs)

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Abstract

Per- and polyfluorinated substances (PFASs) related to aqueous film forming foams (AFFF) have been identified at various release sites as compounds of interest and are generally considered emerging contaminants due to their regulatory uncertainty. PFAS contamination has been found to be present at fire fighter training areas, fire stations and related storage facilities (military, civilian aviation and others), at sites where petroleum fires were extinguished or suppressed using foam (automobile and aircraft crashes, petroleum handling facilities, refineries and aircraft hangers) and storage at shore facilities for tankers and military ships. An evolving body of toxicological and risk assessment knowledge emanating from academia and the environmental consulting industry is driving regulatory uncertainty. Regulatory agencies struggle with defining screening levels and cleanup objectives in this ever-changing landscape.

Approach

The present state of policies and guidance related to PFASs manufacture, usage, and disposal was compiled from different Federal or Commonwealth-level regulatory groups. Additionally, a brief survey of representative US state regulators was performed to gather information regarding the range of cleanup standards, requirements and guidance related to PFASs.

Operational Guidance

A number of organizations have provided regulation or guidance related to manufacture, usage and disposal of PFOS/PFOA-laden AFFF.

- In 2006, US EPA led a voluntary PFOA Stewardship Program that invited eight leading US manufacturers Arkema , Asahi, BASF, Clariant, Daikin, 3M/Dyneon, DuPont, Solvay, and Solexis) to phase out production of PFOA and PFOA precursors by the end of 2015. As of January 2015, all had reduced production in the US by between 96 and 100%.
- US EPA issued or amended Significant New Use Rules (SNURs) for PFASs, including long-chain perfluoroalkyl carboxylate (LCPFAC) chemicals (e.g., those greater than 7 carbons long) for treating carpets for stain resistance. These rules govern the importation and manufacture of 88 PFASs being phased out, including fluorotelomers and precursors.
- Many countries worldwide (although notably not the US) have adopted the requirements contained in the Stockholm Convention on Persistent Organic Pollutants (POPs), which restricts production and use of PFOS, its salts, and perfluorooctane sulfonyl fluoride, although specific exemptions are allowed.
- Some specific US states have provided operational guidance for AFFF discharge testing in hangars, such as Florida Department of Environmental Protection, which issued an Operating Instruction for Hurlburt Field requiring: closing floor drain valves, berms and plastic protecting grassy areas, protecting storm drains, and capturing and disposing of released materials from floors and paved areas.
- Public Health England issued guidance in 2009 for incident response which included the following key points: In the event of a fire involving PFOS or PFOA, use fine water spray and liquid tight kit with breathing apparatus, avoid release into the environment, and Inform Environment Agency of substantial incidents.
- Queensland Australia, Queensland Department of Environmental and Heritage Protection issued a policy on Management of Firefighting Foam in September 2013. The policy states that all foams should be selected carefully and used wisely, but PFOS/P{FOA foams are not permitted at all, and stocks should be withdrawn from service immediately and managed as a regulated waste.
- Environment Canada PFOS Regulations prohibit the manufacture, import, sale, offer for sale and use of PFOS or products containing PFOS, unless incidentally present, with certain exemptions.

Groundwater Screening Levels

Representative International Regulations (µg/L)

Country/ Union	PFOS	PFOA	Notes
Denmark	0.1	0.3	
Germany	0.3	0.3	Health risk limit (drinking water criteria)
Netherlands	0.53	-	Environmental risk limit in groundwater
Sweden	0.09	(0.09)	0.9 for mixtures of 7 PFASs
U.K.	0.3	10	Maximum acceptable concentration in drinking water
Canada	0.3/0.6	0.7/0.2	DW Guidance Value, DW Screening Value

Representative US State Regulations/Guidelines (µg/L)

United States	PFOS	PFOA	Notes
EPA	0.07	0.07	FINAL as of May 2016, and additive
Minnesota	0.3	0.3	Health risk limit
Alaska	0.602	0.401	Risk-based cleanup level (revised summer 2015)
New Jersey	-	0.04	Drinking water guidance level
North Carolina	-	2	Interim max allowable concentration
ATSDR	1	0.7	Derived from draft toxicity data (Aug 2015)
Michigan	0.1	0.42	Groundwater Drinking Water Value,
Maine	0.56	0.1	Groundwater remedial action guidelines
Texas	0.56	0.098	Tier 1 Protective Concentration Levels for Residential Ingestion
Delaware	0.2	0.4	MCLG
Vermont	-	0.02	Drinking water health advisory level

Other standards for water:

— Minnesota: PFBA – 7 ug/L, PFBS – 7 ug/L, PFHxS 0.3 ug/L (interim), handled additively

— New Jersey: PFNA – 0.01 ug/L (interim)

— Canada: PFNA, PFHpA, PFHxA, PFPeA–0.2 ug/L, PFHxS–0.6 ug/L, PFBS–15 ug/L, PFBA–30 ug/L

Acknowledgements

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Soil Screening Levels

Residential Soil Guidelines (mg/kg)

Direct Exposure

Agency	PFOS	PFOA	Notes
US EPA	6	16	Region 4 Screening Levels
Texas CEQ	0.53	0.24	Tier I Protective Concentration Levels
Alaska DEQ	3.04	2.03	Proposed standards (December 2015)
US Navy	4.9	12	Derived from noncancer toxicity values

Leaching to Groundwater

Agency	PFOS	PFOA	Notes
US EPA	-	-	No leaching to GW standards
Alaska DEQ	0.57	0.14	Proposed standards (December 2015)

Texas Tier 1 Protective Concentration Levels

PFAS Compound	$^{GW}Soil_{ING}$[1]	
	Residential	Commercial
Perfluorooctane sulfonic acid (PFOS) (C8)	0.0087	0.0260
Perfluoroundecanoic acid (PFUnDA) (C11)	0.0031	0.0091
Perfluoropentanoic acid (PFPA) (C5)	0.0010	0.0029
Perfluorohexanoic acid (PFHxA) (C6)	0.0015	0.0043
Perfluorododecanoic acid (PFDoA) (C12)	0.0056	0.0170
Perfluorooctanoic acid (PFOA) (C8)	0.0005	0.0015
Perfluorodecanoic acid (PFDA) (C10)	0.0037	0.0110
Perfluorodecane sulfonic acid (PFDS) (C10)	0.0067	0.0200
Perfluorohexane sulfonic acid (PFHxS) (C6)	0.0062	0.0180
Perfluorobutyric acid (PFBA) (C4)	0.0320	0.0960
Perfluorobutane sulfonic acid (PFBS) (C5)	0.0160	0.0470
Perfluoroheptanoic acid (PFHA) (C7)	0.0008	0.0024
Perfluorononanoic acid (PFNA) (C9)	0.0005	0.0015
Perfluorotetradecanoic acid (PFTeDA) (C14)	0.0190	0.0560
Perfluorotridecanoic acid (PFTrDA) (C13)	0.0100	0.0300
Perfluorooctane sulfonamide (PFOSA) (C8)	0.1500	0.4600

$^{GW}Soil_{ING}$[1] Soil-to-GW leaching to Class 1 and 2 groundwater, residential, 30-acre source area.

Other standards for soil:

— Minnesota Residential: PFBA – 38 mg/kg, PFBS – 18 mg/kg

— New Jersey: PFNA

Discussion/Conclusions

Regulatory approaches to management of PFASs vary dramatically across states and nations, confirming the difficulty in assessing the potential impacts from this emerging group of compounds. Where cleanup standards are promulgated, they are generally limited to only PFOS and PFOA, as opposed to the dozens of other related PFAS compounds commonly released concurrently in AFFF. Standards are also generally only available for groundwater/drinking water, although standards for direct exposure to soil and soil migration to groundwater can be found in limited jurisdictions. Ecological exposure standards are in their infancy.

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Cc: Duchovnay, Andrew[Duchovnay.Andrew@epa.gov]; Day, Joanna[Day.Joanna@epa.gov]
From: Clark, Jacqueline
Sent: Thur 6/2/2016 3:24:40 PM
Subject: TIME CRITICAL - DuPont order
2016 DuPont Order Draft May 2016 v13.docx

Hi Eric, Jamie, and Joyce – can we have a brief call to discuss paragraphs 16, 32, 33, 35, 42 and 43 in the attached draft order today? I don't think the conversation will take more than 15-20 minutes, but it's critical that we at least are able to talk to Eric at least today if at all possible.

Jacqueline Clark

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To: Donohue, Joyce[Donohue.Joyce@epa.gov]
From: Strong, Jamie
Sent: Wed 6/1/2016 7:31:54 PM
Subject: FW: Suggestions for CDPHE on Perfluorinated Compounds
Butenhoff 2009 PFHxS.pdf
Lieder 2009a PFBS.pdf
Lieder 2009b PFBS.pdf
Memo to CDPHE v2.docx

do not respond directly to bob, send any comments to me.

From: Benson, Bob
Sent: Wednesday, June 01, 2016 3:12 PM
To: Donohue, Joyce <Donohue.Joyce@epa.gov>; Strong, Jamie <Strong.Jamie@epa.gov>; Behl, Betsy <Behl.Betsy@epa.gov>; Southerland, Elizabeth <Southerland.Elizabeth@epa.gov>
Cc: Bahrman, Sarah <Bahrman.Sarah@epa.gov>; Garcia, Bert <Garcia.Bert@epa.gov>; O'Connor, Darcy <oconnor.darcy@epa.gov>
Subject: Suggestions for CDPHE on Perfluorinated Compounds

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Evaluation of potential reproductive and developmental toxicity of potassium perfluorohexanesulfonate in Sprague Dawley rats

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abstract

This study evaluates the potential reproductive and developmental toxicity of perfluorohexanesulfonate (PFHxS), a surfactant found in sera of the general population. In a modified OECD 422 guideline-based design, 15 rats per sex and treatment group (control, 0.3, 1, 3, and 10 mg/kg-d) were dosed by gavage with potassium PFHxS (K^+PFHxS) or vehicle (0.5% carboxymethylcellulose) 14 days prior to cohabitation, during cohabitation, and until the day before sacrifice (21 days of lactation or presumed gestation day 25 (if not pregnant) for females and minimum of 42 days of treatment for males). Offspring were not dosed by gavage but were exposed by placental transfer *in utero* and potentially exposed via milk. Evaluations were made for reproductive success, clinical signs, body weight, food consumption, estrous cycling, neurobehavioral effects, gross and microscopic anatomy of selected organs, sperm, hematology, clinical pathology, and concentration of PFHxS in serum and liver. Additional three rats per sex per group were added to obtain sera and liver samples for PFHxS concentration determinations during the study. No reproductive or developmental effects were observed. There were no treatment-related effects in dams or offspring. K^+PFHxS-induced effects noted in parental males included: (1) at all doses, reductions in serum total cholesterol; (2) at 0.3, 3, and 10 mg/kg-d, decreased prothrombin time; (3) at 3 and 10 mg/kg-d, increased liver-to-body weight and liver-to-brain weight ratios, centrilobular hepatocellular hypertrophy, hyperplasia of thyroid follicular cells, and decreased hematocrit; (4) at 10 mg/kg-d, decreased triglycerides and increased albumin, BUN, ALP, Ca^{2+}, and A/G ratio. Serum and liver concentrations of PFHxS are reported for parents, fetuses, and pups. PFHxS was not a reproductive or developmental toxicant under study conditions.

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1. Introduction

Perfluorohexanesulfonate (PFHxS) is a perfluorinated surfactant in which all hydrogens in carbon–hydrogen bonds have been replaced with fluorine. As such, PFHxS is one of the number of functionalized, polyfluorinated compounds that have been produced for over half a century for use in specialized applications [1] as well as becoming the subject of increasing investigation with regard to environmental and health-related properties [2–5]. The unique properties of this and other perfluorinated surfactants, such as high surface activity, exceptional stability to degradation, density, solubility characteristics, and low intermolecular interactions, have been exploited in numerous industrial and consumer applications [6].

However, these same properties also create challenges for managing these materials in the environment. Exceptional stability to environmental and metabolic degradation together with poor elimination from the body in the case of several perfluorinated surfactants [7–9], including PFHxS, creates a potential for accumulation and biomagnification. The geometric mean serum PFHxS elimination half-life in 26 retired production workers was estimated to be approximately 7.3 years (95% CI = 5.8–9.2) [7]. Spliethoff et al. [10] estimated the serum elimination half-life for PFHxS to be 8.2 years (95% CI = 5.4–16.2) using PFHxS concentration data from neonatal screening blood spots. Although the reported half-life values from the latter two articles are imprecise estimates, they are consistent in suggesting poor elimination of PFHxS in humans. Due to these properties, it may not be surprising that Hansen et al. [11] found PFHxS in pooled serum from the United States general population, and numerous later biomonitoring studies have found PFHxS widely distributed at low ng/mL concentrations in individual samples from the general population [10,12–26].

The presence of PFHxS in umbilical cord blood at birth [19] and neonatal screening program blood spots [10] demonstrates that human exposures can begin *in utero*. The primary sources of exposure are not understood; however, exposure to PFHxS in human

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milk [20–22], house dust [27,28] or from contact with surfaces treated with PFHxS-containing carpet and upholstery protection formulations [14] may provide some contribution as well as potential dietary [24] and water sources [23,26,29]. These sources may contribute to PFHxS exposure to children after birth.

Between the years 2000 and 2002, due to persistence and evidence of widespread exposure of the general population, 3M Company discontinued production of PFHxS along with perfluorooctanoate (PFOA) and chemistries based on perfluorooctanesulfonyl fluoride, including perfluorooctanesulfonate (PFOS). Several recent biomonitoring studies have demonstrated that blood concentrations of PFHxS, PFOA, and PFOS in the general population have declined significantly since 3M Company ceased manufacturing of these materials [10,13,17]

Although its eight-carbon analog, PFOS, has been extensively studied for its potential heath effects [2,30,31], little information on the toxicity potential of PFHxS has been documented. The fact that exposure occurs *in utero* and is present at all life stages together with the known biological effects profile of PFOS from laboratory studies warrants a need to understand potential health risk associated with exposure. The objective of this study was to evaluate the potential reproductive, developmental, and neurological responses of rats to treatment with potassium PFHxS in an assessment based on OECD Method 422 guidelines [32]. Because internal dose metrics have become important in evaluating health risk [2,33–35], collection of serum and liver PFHxS concentration data was included in the study design. One of the earliest clinically measurable indicators of biological response to treatment of rats and non-human primates with PFOS has been reduction in serum cholesterol [36,37]. Accordingly, serum total cholesterol was included in the clinical chemistry profile. We believe that this is the first article to report on the biological response to treatment with PFHxS.

2. Materials and methods

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

K^+PFHxS (purity 99.98%), a white powder, was obtained from 3M Company (St. Paul, MN). The vehicle used in preparation of the test article formulations and for administration to the control group was 0.5% carboxymethylcellulose, medium viscosity (lot number 120K0252, Sigma–Aldrich, St. Louis, MO) in deionized water. Dosing formulations for four K^+PFHxS treatment levels of 0.3, 1, 3, and 10 mg/kg-d were prepared as K^+PFHxS concentrations in vehicle of 0.03, 0.1, 0.3, and 1 mg/mL, respectively, based on a dosing volume of 10 mL/kg and were not adjusted for purity. The purity and stability of the chemical were verified by analysis before the study. All samples were analyzed for PFHxS concentration using a high performance liquid chromatography/tandem mass spectrometry (LC–MS/MS) method.

2.2. Animal husbandry, assignment, and breeding

Crl:CD®(SD)IGS BR VAF/Plus® rats (n = 90/sex) supplied by Charles River Laboratories from St. Constant, Quebec, Canada (male rats) and Raleigh, NC, US (female rats) were used in this study. All rats were individually housed in stainless steel, wire-bottomed cages except during cohabitation and postpartum periods. During cohabitation, each pair of male and female rats was housed in the male rat's home cage. F_0 generation female rats were individually housed in nesting boxes (with Bed-o'cobs® bedding, The Andersons Industrial Products Group, Maumee, OH) until they either naturally delivered litters or were sacrificed. Each dam and her delivered litter were housed in a common nesting box during the postpartum period. All cage sizes and housing conditions were in compliance with the *Guide for the Care and Use of Laboratory Animals* [38].

Rats were given *ad libitum* access to Certified Rodent Diet #5002 (PMI Nutrition International, Inc., St. Louis, MO) except for the evening prior to sacrifice. Municipal water, processed through reverse osmosis membrane and fortified with chlorine, was given to the rats *ad libitum*. The study room was maintained under conditions of positive airflow with a minimum of ten changes per hour of air that passed through 99.97% HEPA filters. Room temperatures were maintained between 18 °C and 26 °C and relative humidity maintained at 30–70%. Lighting was maintained at 12-h light and 12-h dark cycles. The testing facility (Charles River Preclinical Services, Horsham, PA) is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. All procedures involving animals were approved by the testing facility's Institutional Animal Care and Use Committee.

2.3. Study design

This study was conducted according to OECD test guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test [32]). Male and female rats (n = 18/sex/dose) were allocated randomly into five oral gavage treatment groups consisting of the vehicle control, and 0.3, 1, 3, and 10 mg/kg-d K^+PFHxS. In each treatment group, rats were assigned to either the main study (15 rats/sex) or for collection of sera and liver samples for PFHxS concentration determination (3 rats/sex). The dosage volume (10 mL/kg) was adjusted daily on the basis of the individual body weights recorded before intubation. Rats were dosed at approximately the same time each day. Daily dosing of all rats began 14 days prior to cohabitation and continued until the day prior to scheduled sacrifice. F_1 generation pups were not dosed directly but were exposed to PFHxS *in utero* and were potentially exposed via the milk during the lactation period.

Male rats were sacrificed on study day (SD) 42 (3 males assigned for PFHxS concentration determinations) and SD 44 (main-study males). For main-study female rats, scheduled sacrifice was either through postnatal day (PND) 22 or presumed gestation day (GD) 25 for rats that did not deliver a litter. Female rats assigned for collection of sera and liver samples for PFHxS concentration determination were sacrificed on GD 21. F_1 pups were sacrificed on PND 22. Carbon dioxide asphyxiation was used for sacrifice.

2.4. Observations and evaluations (F_0 parental rats)

All main study rats were observed daily for body weight (except during cohabitation) and clinical signs of toxicity (before and approximately 60 min after dosing), and detailed clinical observations were conducted on a weekly basis. Feed consumption was evaluated weekly for males except during cohabitation. Feed consumption for females was evaluated up to cohabitation and on GD 0, 7, 10, 12, 15, 18, and 20 and PND 1, 5, 8 and 15. During parturition, female rats were evaluated for adverse clinical signs, duration of gestation, litter sizes (all pups delivered), and pup viability at birth. Maternal behavior was evaluated on PND 1, 5, 8, 15, and 22.

Estrous cycling was evaluated in all female rats (including rats assigned to sera and liver sample collection) by examination of vaginal cytology after the first dose and daily until spermatozoa were observed in a vaginal smear and/or a copulatory plug was observed *in situ* during the cohabitation period.

In each treatment group, functional observation battery (FOB) and motor activity assessments were conducted on 10 male rats (prior to scheduled sacrifice and before blood sample collection) and 10 female rats (prior to scheduled sacrifice during lactation period) [39–42]. The FOB evaluations were conducted in a blinded manner with the following parameters assessed: lacrimation, salivation, palpebral closure, prominence of the eye, pupillary reaction to light, piloerection, respiration, and urination and defecation (autonomic functions); sensorimotor responses to visual, auditory, tactile and painful stimuli (reactivity and sensitivity); reactions to handling and behavior in the open field (excitability); gait pattern in the open field, severity of gait abnormalities, air righting reaction and landing foot splay (gait and sensorimotor coordination); forelimb and hindlimb grip strength; and abnormal clinical signs including convulsions, tremors and other unusual behavior, hypotonia or hypertonia, emaciation, dehydration, unkempt appearance and deposits around the eyes, nose or mouth. Motor activity was evaluated by a passive infrared sensor. Each test session was 1.5 h in duration with the number of movements and time spent in movement tabulated at each 5-min interval.

Gross necropsy of all male and female rats included macroscopic evaluation of external surfaces and all orifices, as well as cranial, thoracic and abdominal cavities, and their contents for gross lesions. The reproductive organs were closely examined (see below).

Ten rats per sex from each treatment group that were assigned to FOB and motor activity testing were used for evaluation of organ weights, microscopic histological examination of tissues, detailed examination or sexual organs and related parameters, clinical chemistry, and hematology. Procedural descriptions follow.

Organ weights, including sexual organ weights, were determined, and microscopic histological samples were taken for potential examination. Liver, kidneys, adrenals, thymus, testes (left and right), epididymis (left and right), seminal vesicles (with and without fluid), prostate, spleen, brain, heart, ovaries (left and right) and uterus were weighed. Brain, small and large intestines, lungs, lymph nodes, peripheral nerve, stomach, kidneys, spleen, thymus, trachea, urinary bladder, spinal cord, liver, adrenals, heart, thyroid/parathyroid, bone marrow, testes, prostate, seminal vesicles, the remaining portion of the left epididymis, the right epididymis, ovaries, uterus, vagina, mammary gland and any gross lesions were retained in neutral buffered 10% formalin and evaluated histologically by Research Pathology Services, Inc. (New Britain, PA). The control and 10 mg/kg-d treatment groups were evaluated first. If differences were found between control and the high treatment group, the remaining three treatment groups were to be evaluated.

In addition to organ weights, potential toxicity to male and female reproductive systems was closely evaluated. For male rats, sperm concentration (per gram of tissue weight), sperm motility (by integrated visual optical system, or IVOS, Hamilton Thorne, Inc., Beverly, MA), and sperm morphology (by determining the percentage of normal and abnormal sperms) were assessed. Uteri of both pregnant and non-pregnant rats were examined for the presence/absence of and the number (if

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EPA-HQ-2016-005679 06/14/2017

present) of implantation sites. A quantitative evaluation of primordial follicles was also conducted for F_0 generation female rats.

Hematology and clinical chemistry samples were collected from the inferior vena cava of fasted rats assigned to the FOB assessment in the main study during the scheduled sacrifice. All samples were analyzed by Redfield Laboratories, Redfield, AK (a division of Charles River Laboratories).

Analyses of hematologic parameters included erythrocyte count (RBC), hematocrit (HCT), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), total leukocyte count (WBC), and differential leukocyte count, platelet count (PLAT), mean platelet volume (MPV), cell morphology, prothrombin time (PT), and activated partial thromboplastin time (APTT).

The evaluated clinical chemistry parameters were: total protein (TP), triglycerides (TRI), albumin (A), globulin (G), albumin/globulin ratio (A/G), glucose (GLU), cholesterol (CHOL), total bilirubin (TBILI), urea nitrogen (BUN), creatinine (CREAT), creatinine kinase (CK), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALK), calcium (CA), phosphorus (PHOS), sodium (NA), potassium (K), and chloride (CL).

Blood samples (processed to plasma) were collected from three male rats per treatment group on SD 14 and 42. Blood samples were also collected from the three female rats per treatment group on SD 14 and GD 21. Samples were collected from the orbital sinus on SD 14 and from the vena cava on SD 42. After the last blood collection on SD 42, all three male rats were euthanized and liver samples were harvested and weighed. Liver samples were also collected and weighed from the three female rats euthanized on GD 21 followed by the subsequent collection of fetal blood and liver (pooled by litter) samples. All samples were stored frozen until PFHxS concentration analysis (Exygen Research, State College, PA).

2.5. Observations and Evaluations (F_1 Generation)

The F_1 pups in each litter were counted once daily and clinical observations were recorded daily. Pup body weights were recorded on PND 1, 8, 15 and 22. No feed consumption or interim body-weight data were recorded prior to the scheduled sacrifice on PND 22. Pups were examined for gross lesions at sacrifice, and the necropsy also included a single cross-section of the head at the level of the frontal-parietal structure and examination of the cross-sectioned brain for apparent hydrocephaly.

At study termination, liver samples were excised and weighed and blood samples were collected and processed for serum from five pups per sex per litter from the 10 dams selected for FOB assessments. Serum samples were pooled by litter. The median liver lobe was stored frozen, and the remaining liver portion was retained in neutral buffered 10% formalin for possible histological evaluation. Samples remained frozen until PFHxS concentration analysis (Exygen Research, State College, PA).

2.6. Determination of PFHxS Concentrations in Serum and Liver

PFHxS concentrations were determined by LC–MS/MS. All samples underwent extraction procedures prior to chromatographic analyses. Blank serum and liver samples were purchased from Pel Freeze Biologicals (Rogers, AR) and these blank matrices were used to prepare the appropriate matrix-matched PFHxS standard curves with the addition of known amounts of PFHxS.

Serum (100 ffiL) and liver (100 mg) samples were diluted with deionized water so that the final volumes were 20 mL and 10 mL, respectively. After vortexing (serum and plasma) and homogenization (liver), 1 mL of the aliquot was mixed with 5 mL acetonitrile using a mechanical shaker (20 min, room temperature) and supernatant was obtained after centrifugation (3000 rpm, 5 min). After adding 35 mL of deionized water to the supernatant, the entire mixture was loaded onto Waters Sep-Pak® solid phase extraction (SPE) column (6 mL with 1 g C18 sorbent). The SPE columns were pre-conditioned with 10 mL methanol followed by 5 mL deionized water. Samples were eluted with 2 mL methanol.

The instrument used for PFHxS analysis was a Micromass Quattro Ultima mass spectrometer configured with electrospray ionization source (ESI) in negative ion mode. A Genesis® C8, 4 ffim, 50 mm × 2.1 mm i.d. HPLC column with a flow rate of 0.3 mL/min was used for PFHxS analysis. The gradient condition for the mobile phase started with 90% 2 mM ammonium acetate and 10% methanol to 100% methanol for 9.5 min. All source parameters were optimized under these conditions according to manufacturer's guidelines. Transition ions monitored for PFHxS were from 399 → 80 atomic mass units.

2.7. Statistical analysis

F_0 parental rat data were evaluated with the individual rat as the unit measured. Litter values were used in evaluation of pup data, as appropriate. Variables with interval or ratio scales of measurement, such as body weights, feed consumption values, latency and errors per trial scores in behavioral tests, and percent mortality per litter were analyzed as parametric data. Bartlett's Test of Homogeneity of Variances [43] was used to estimate the probability that the dosage groups have different variances. A non-significant result ($p > 0.001$) indicated that an assumption of homogeneity of variance was appropriate, and the data were compared using the Analysis of Variance (ANOVA) [44]. If ANOVA test was significant ($p \leq 0.05$), the groups given the test substance were compared with the control group using Dunnett's Test [45]. If Bartlett's Test was significant ($p \leq 0.001$), the ANOVA was not appropriate, and the data were analyzed as nonparametric data. When 75% or fewer of the scores in all the groups were tied, the Kruskal–Wallis Test [46] was used to analyze the data, and in the event of a significant result ($p \leq 0.05$), Dunn's Test [47] was used to compare the groups given the test substance with the control group. When more than 75% of the scores in any dosage group were tied, Fisher's Exact Test [48] was used to compare the proportion of ties in the groups.

Data from the motor activity test, with measurements recorded at intervals (blocks) throughout each test session, were analyzed using an ANOVA with repeated measures [49]. A significant result ($p \leq 0.05$) in that test could have appeared as effect of concentration (differences among dosage groups in the totals of all measurements in a session) or as an interaction between concentration and block (differences in the patterns of dosage group values across the measurement periods). If the concentration effect was significant, the totals for the control group and the groups given the test substance were compared using Dunnett's Test [45]. If the concentration × block interaction was significant, an ANOVA [44] was used to evaluate the data at each measurement period, and a significant result ($p \leq 0.05$) was followed by a comparison of the dosage groups using Dunnett's Test [45].

3. Results

All data reported in this study are presented as mean ± standard deviation (SD) unless specified otherwise.

3.1. Stability and homogeneity

Dosing solutions were found to be stable and homogenous. Mean PFHxS (and inferred K^+PFHxS) concentrations for seven sets of dosing solutions were within 70–125% of the target concentrations. The wide range of PFHxS concentrations obtained was likely due to the unavailability of appropriate internal standard at the time during which the analysis was conducted. (While the LC–MS/MS analytical coefficient of variation for PFHxS analysis typically ranges from 20 to 30% without employing an internal standard, it has been reduced significantly to less than 10% when an internal standard such as stable isotope ^{18}O-labeled PFHxS is used.)

3.2. F_0 parental rat results

All F_0 rats survived to the scheduled necropsy and no K^+PFHxS-treatment related mortalities occurred. No abnormal clinical findings were noted in any of the rats receiving either control or K^+PFHxS doses at the daily examinations or 1 h following dose administration.

Body weight and body-weight change data for F_0 males are summarized in Table 1. In males given K^+PFHxS, mean body weights by K^+PFHxS treatment level were not statistically significantly different than male control body weights at any time during the study. Statistically significant decreases in mean body-weight gains relative to mean control body-weight gains were noted in 0.3, 3, and 10 mg/kg-d K^+PFHxS-treated male groups between SD 29 (first value recorded after cohabitation) and 36, resulting in an overall decrease in body-weight gains through termination on SD 43, even though no differences from mean control values were noted between SD 36 and 43. Mean body-weight gain in the 10 mg/kg-d treatment group was decreased over the entire study period (SD 1–43) with statistical significance as compared to the mean for controls. Regardless of these variations in body-weight gain, mean terminal body weights were not statistically significantly different between K^+PFHxS-treated males and controls.

Body weight and body-weight change data for F_0 females also are summarized in Table 1. During pre-cohabitation and gestation, no statistically significant differences were noted in mean body weights or body-weight changes in K^+PFHxS-treated female groups when compared to the mean for controls for the same time periods. During lactation, there were no statistically significant differences between mean body-weight changes in K^+PFHxS-treated

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EPA-HQ-2016-005679 06/14/2017

Table 1
Body weights and body-weight changes summary for male and female parental rats during specified study periods (data in grams, $N = 15$ unless otherwise noted).

	Potassium perfluorohexanesulfonate dose (mg/kg-day)				
	0 (Control)	0.3	1	3	10
Male body-weight study day (SD) 1	342.7 ± 12.6	338.9 ± 13.1	341.9 ± 14.7	345.6 ± 11.2	343.3 ± 12.4
Male body-weight SD 43[a]	490.5 ± 37.2	475.1 ± 38.3	482.5 ± 53.1	490.5 ± 40.2	462.1 ± 22.0
Male body-weight SD 44[b]	472.5 ± 33.9	453.7 ± 34.3	463.1 ± 48.2	468.3 ± 37.1	441.5 ± 16.5
Male body-weight gain SDs 1–44[b,c]	129.8 ± 25.5	114.8 ± 27.5	121.2 ± 37.7	122.7 ± 30.0	98.2 ± 13.9**
Female body-weight SD 1	228.6 ± 8.0	224.7 ± 8.1	226.3 ± 7.7	226.8 ± 7.9	226.9 ± 8.0
Female body-weight SD 14	258.5 ± 16.1	250.7 ± 12.6	257.9 ± 13.6	253.0 ± 13.2	251.1 ± 18.0
Female body-weight gain SD 1–14[d]	29.9 ± 13.7	25.9 ± 7.2	31.6 ± 8.4	26.2 ± 8.3	24.2 ± 14.4
Maternal body-weight gestation day (GD) 0	270.1 ± 14.3	259.4 ± 12.0	270.9 ± 15.6[e]	265.6 ± 15.4[e]	260.9 ± 18.2
Maternal body-weight GD 20	412.0 ± 35.0	397.4 ± 27.8	412.1 ± 21.7[e]	398.8 ± 27.6[e]	395.1 ± 28.1
Maternal body-weight gain GD 0–20[d]	141.9 ± 29.0	138.0 ± 27.4	141.2 ± 14.1[e]	133.2 ± 21.9[e]	134.2 ± 16.9
Maternal body-weight postnatal day (PND) 1	313.1 ± 22.6	302.2 ± 16.8	309.5 ± 20.6[e]	304.7 ± 23.2[e]	296.3 ± 19.9
Maternal body-weight PND 8	342.3 ± 19.8	320.7 ± 12.6*	333.6 ± 19.6[e,f]	322.2 ± 21.5*	318.1 ± 26.0**
Maternal body-weight PND 15	355.3 ± 31.2	342.4 ± 10.4[g]	343.2 ± 17.2[e,f]	348.7 ± 21.4[e,f]	333.4 ± 33.6[g]
Maternal body-weight PND 21[a]	350.1 ± 26.9[g]	335.7 ± 18.7[g]	343.4 ± 19.8[e]	344.4 ± 25.8[e]	334.4 ± 35.9
Maternal body-weight PND 22[b]	306.3 ± 26.0	324.3 ± 27.1	311.2 ± 35.0[e]	319.6 ± 40.4[e]	303.5 ± 37.9
Maternal body-weight change (+/−) PND 1–22[h]	−6.9 ± 28.4	+22.1 ± 26.0	+1.7 ± 28.0[e]	+14.9 ± 31.8[e]	+7.3 ± 29.3

[a] Non-fasted body weight on last day before termination.
[b] Fasted terminal body weight.
[c] In males given potassium perfluorohexanesulfonate (K^+PFHxS), mean body weights by treatment level were not statistically significantly different than male control body weights at any time during the study. Statistically significant decreases in mean body-weight gains relative to mean control body-weight gains were noted in 0.3, 3, and 10 mg/kg-d K^+PFHxS-treated male groups between SD 29 (first value recorded after cohabitation) and 36 and from SD 29 through termination on SD 43; however, no differences from mean control values were noted between study days 36 and 43.
[d] During pre-cohabitation and gestation, no statistically significant differences were noted in mean body weights or body-weight changes in K^+PFHxS-treated female groups when compared to the mean for controls for the same time periods.
[e] $N = 13$ due to 2 nonpregnant females.
[f] $N = 12$ due to exclusion of 2 body-weight determinations that appeared to be incorrect as a result of interrupted water access.
[g] $N = 14$ due to exclusion of 1 body-weight determination that appeared to be incorrect as a result of interrupted water access.
[h] During lactation, there were no statistically significant differences between mean body-weight changes in K^+PFHxS-treated groups when compared to mean control values over the same time period. There were occasional periods of statistically significant differences in mean body weights among 0.3, 3, and 10 mg/kg-d K^+PFHxS-treated groups and controls from PND 4–14. Statistically significant reductions in mean body weight occurred in K^+PFHxS-treated females as compared to control mean values from PND 4 to PND 14 as follows: PND 4, 6–8, 11, and 13 in the 0.3 mg/kg-d group; PND 7 and 8 in the 3 mg/kg-d; and PND 4, 6–9, 11, 13, and 14 in the 10 mg/kg-d group. Mean body weights on PND 15 through term on PND 22 in K^+PFHxS-treated groups were not statistically significantly different than control means.
* Statistically significantly different from control value ($p \leq 0.05$).
** Statistically significantly different from control value ($p \leq 0.01$).

groups when compared to mean control values over the same time period. There were occasional periods of statistically significant differences in mean body weights among 0.3, 3, and 10 mg/kg-d K^+PFHxS-treated groups and controls from PND 4–14. Statistically significant reductions in mean body weight occurred in K^+PFHxS-treated females as compared to control mean values from PND 4 to PND 14 as follows: PND 4, 6–8, 11, and 13 in the 0.3 mg/kg-d group; PND 7 and 8 in the 3 mg/kg-d; and PND 4, 6–9, 11, 13, and 14 in the 10 mg/kg-d group. Mean body weights on PND 15 through term on PND 22 in K^+PFHxS-treated groups were not statistically significantly different than control means.

Treatment of male and female rats with K^+PFHxS at doses up to 10 mg/kg-d did not affect mean absolute and relative feed consumption when compared to controls.

Mating and fertility data are presented in Table 2. K^+PFHxS treatment did not affect any mating or fertility parameters investigated. Estrous cycling and all mating and fertility indices, including days in cohabitation, were unaffected by the K^+PFHxS treatments up to 10 mg/kg-d.

Pregnancy status, gestation length, and pregnancy outcome are summarized in Table 3. Pregnancy occurred in 88.7–100% of rats in all treatment groups and all pregnant F_0 dams delivered lit-

Table 2
Mating and fertility values (mean ± SD) of F_0 male and female rats.

	K^+PFHxS dose (mg/kg-d)				
	0	0.3	1.0	3.0	10.0
Male					
Number of animals	15	15	15	15	15
Cohabitation length (days)	3.2 ± 1.4	1.9 ± 1.2	2.8 ± 1.2	3.1 ± 2.0	2.5 ± 1.4
Mating index[a] (%)	14/15 (93.3%)	15/15 (100%)	13/15 (86.7%)	12/15 (80.0%)	15/15 (100%)
Fertility index[b] (%)	14/14 (100%)	15/15 (100%)	13/15 (86.7%)	12/13 (92.3%)	15/15 (100%)
Female					
Number of animals	15	15	15	15	15
Cohabitation length (days)	3.7 ± 3.0	1.9 ± 1.2	2.8 ± 1.2	4.0 ± 4.0	2.5 ± 1.4
Estrous cycle per 13-day	2.7 ± 0.9	2.8 ± 0.9	3.3 ± 0.6	2.9 ± 0.8	2.1 ± 1.2
Mating index (%)	15/15 (100%)	15/15 (100%)	13/15 (86.7%)	13/15 (86.7%)	15/15 (100%)
Fertility index (%)	15/15 (100%)	15/15 (100%)	13/15 (86.7%)	13/14 (92.8%)	15/15 (100%)

[a] Rats pregnant/number of rats in cohabitation.
[b] Pregnancies/number of rats that mated.

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EPA-HQ-2016-005679 06/14/2017

Table 3
Pregnancy outcome in F_0 female rats.

	K^+PFHxS dose (mg/kg-d)				
	0	0.3	1.0	3.0	10.0
Number of rats assigned for mating	15	15	15	15	15
Number of rats successfully mated	15	15	15	14	15
Number of rats became pregnant	15	15	13	13	15
Pregnancy rates[a] (%)	100	100	86.7	92.9	100
Duration of gestation (days)	22.5 ± 0.5	22.7 ± 0.4	22.7 ± 0.5	22.8 ± 0.4	22.6 ± 0.5
Total implants	251	230	206	192	231
Litters delivered	15	15	13	13	15
Implants/litter	16.7 ± 1.3	15.3 ± 2.9	15.8 ± 1.3	14.8 ± 2.8	15.4 ± 2.3
Gestation index (%)	15/15 (100%)	15/15 (100%)	13/13 (100%)	13/13 (100%)	15/15 (100%)
Total number of pups delivered	235	214	201	181	220
Liveborn	235	214	200	177	218
Stillborn	0	0	1	3	2
Unknown vital status	0	0	0	1	0

[a] Pregnancy rate = number of rats became pregnant relative to the total number assigned (for mating).

ters. Pregnancy status and outcomes were unaffected by K^+PFHxS treatments up to 10 mg/kg-d, the highest dose used in the study.

No test substance-related internal findings were observed for F_0 males and females at scheduled necropsies at any dose level. Macroscopic findings observed in the test article-treated groups occurred infrequently, at similar frequencies in the control group and/or in a manner that was not dose-related.

With the exception of liver weights in male rats, K^+PFHxS treatment did not affect the mean absolute or relative weights of organs when compared to control. K^+PFHxS treatment caused a statistically significant ($p < 0.05$) dose-related increase in mean absolute liver weight in male rats at doses of 3.0 and 10 mg/kg-d, which resulted in increases in mean liver weight of 20 and 56% over control, respectively. As a result, mean liver weight as a percent of body weight was also increased with statistical significance in male rats in the 3.0 and 10 mg/kg-d treatment groups (Fig. 1) as well as liver weight to brain weight ratio (data not shown). For female rats, K^+PFHxS did not result in treatment-related changes in absolute or relative liver weights.

With the exception of liver and thyroid tissues in male rats, there were no differences in tissue histology between control and K^+PFHxS-treatment groups. There were increased incidences of minimal to moderate hypertrophy seen in the liver and thyroid gland of male rats receiving 3.0 and 10 mg/kg-d K^+PFHxS doses (Table 4). The affected centrilobular hepatocytes were enlarged with an increased amount of dense eosinophilic granular cytoplasm. The changes in the thyroid gland consisted of hypertrophy and hyperplasia of the follicular epithelium cells. These microscopic changes in the liver and thyroid were consistent with the known effects of compounds that cause microsomal enzyme induction where the hepatocellular hypertrophy results in a compensatory hypertrophy and hyperplasia of the thyroid due to increased plasma turnover of thyroxine and associated stimulation of thyroid-stimulating hormone in rats [50]. No treatment-related microscopic changes were observed in any of the female rats or male rats administered 0.3 or 1.0 mg/kg-d K^+PFHxS doses.

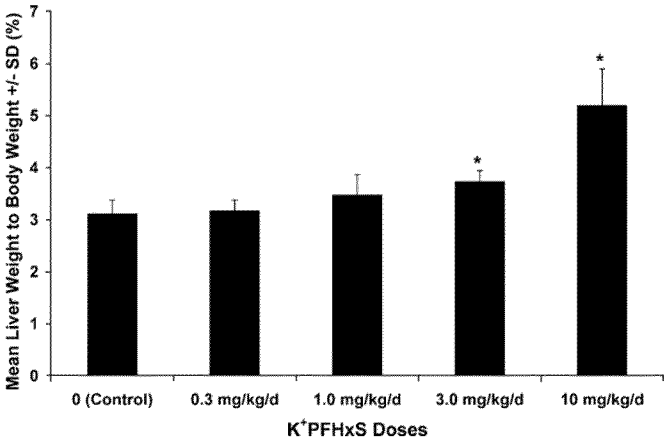

Fig. 1. Mean liver weight to body-weight percent data for F_0 male rats. Rats receiving daily K^+PFHxS treatments at 3.0 mg/kg-d and 10.0 mg/kg-d had statistically significant increased liver weight to body-weight percent than controls. Asterisk (*) denotes significant difference from control ($p < 0.05$).

Sperm motility, count, density, and morphology values for all F_0 male rats and primordial follicle counts for F_0 female rats (from control and 10 mg/kg-d K^+PFHxS treatment group only) were unaffected by treatment with K^+PFHxS.

Compared to controls, K^+PFHxS treatments did not affect any of the hematology or clinical chemistry in F_0 female rats.

For F_0 male rats, the hematology and clinical chemistry parameters that were statistically different than the values reported for controls are summarized in Table 5. While the hematocrit and RBC counts were significantly decreased in the 3 and 10 mg/kg-d treatment groups, hemoglobin concentrations were significantly decreased in the 1, 3 and 10 mg/kg-d groups. Prothrombin time was significantly increased in the 0.3, 3 and 10 mg/kg-d dosage groups but not the 1.0 mg/kg-d group. While albumin, albumin/globulin ratio, blood urea nitrogen, alkaline phosphatase, and calcium were significantly increased in the 10 mg/kg-d treatment group, cholesterol values were significantly decreased in all treatment groups and triglycerides was significantly decreased in the 10 mg/kg-d treatment group only.

Table 4
Histopathology of the F_0 generation male rats.

	K^+PFHxS dose (mg/kg-d)				
	0	0.3	1.0	3.0	10.0
Number of rats evaluated	10	10	10	10	10
Liver hypertrophy (hepatocellular, centrilobular)					
Minimal	0	0	0	8	4
Mild	0	0	0	1	5
Moderate	0	0	0	0	1
Total incidence	0	0	0	9	10
Thyroid hypertrophy/hyperplasia (follicular epithelium)					
Minimal	0	1	1	2	0
Mild	2	2	1	2	3
Moderate	0	0	0	0	4
Total incidence	2	3	2	4	7

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EPA-HQ-2016-005679 06/14/2017

Table 5
Hematological and clinical chemistry observations in male rats treated with K^+PFHxS for at least 42 days.

	K^+PFHxS dose (mg/kg-d)				
	0	0.3	1.0	3.0	10.0
Number of rats	10	10	10	10	10
Hematology					
Red blood cells (10^6/mm^3)	7.51 ± 0.40	7.33 ± 0.40	7.32 ± 0.44	6.93 ± 3.05*	6.99 ± 0.44*
Hemoglobin (g/dL)	16.5 ± 1.2	15.9 ± 0.4	15.7 ± 0.6*	15.4 ± 0.7**	15.6 ± 0.8*
Hematocrit (%)	43.5 ± 3.5	42.2 ± 1.6	42.0 ± 2.2	40.2 ± 2.3**	40.7 ± 1.8*
Prothrombin time (s)	13.4 ± 0.2	14.2 ± 0.3**	13.6 ± 0.2	13.8 ± 0.4*	14.0 ± 0.5**
Clinical chemistry					
Albumin (g/dL)	4.3 ± 0.2	4.1 ± 0.2	4.3 ± 0.2	4.2 ± 0.2	4.5 ± 0.2**
Albumin/globulin	2.1 ± 0.2	2.1 ± 0.2	2.2 ± 0.3	2.2 ± 0.2	2.5 ± 0.2**
BUN (mg/dL)	16.0 ± 1.5	16.0 ± 0.8	16.0 ± 1.8	17.0 ± 1.6	21.0 ± 2.4**
Creatinine (mg/dL)	0.30 ± 0.04	0.30 ± 0.05	0.30 ± 0.06	0.30 ± 0.03	0.30 ± 0.05
Calcium (mg/dL)	10.9 ± 0.5	10.7 ± 0.4	11.1 ± 0.4	11.1 ± 0.3	11.5 ± 0.4**
Sodium (mmol/L)	146.0 ± 1.4	146.0 ± 1.3	147.0 ± 1.6	147.0 ± 1.0	146.0 ± 2.2
Potassium (mmol/L)	6.6 ± 2.1	6.1 ± 0.9	6.5 ± 1.3	6.0 ± 0.6	6.9 ± 1.6
Chloride (mmol/L)	98.0 ± 2.7	99.0 ± 1.5	100.0 ± 2.3	100.0 ± 2.7	100.0 ± 1.2
Cholesterol (mg/dL)	57 ± 8	41 ± 11**	46 ± 12*	43 ± 13**	33 ± 7**
Triglycerides (mg/dL)	52 ± 21	47 ± 17	36 ± 14	36 ± 28	17 ± 8**
Alkaline phosphatase (U/L)	105 ± 14	111 ± 37	100 ± 12	115 ± 25	144 ± 38**
ALT (U/L)	42 ± 6	63 ± 70	60 ± 34	95 ± 124	45 ± 7
AST (U/L)	96 ± 22	121 ± 93	117 ± 41	198 ± 249	97 ± 16

* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group value ($p \leq 0.01$).

There were no statistically significant differences between control and K^+PFHxS treatments on the assessments of FOB parameters (autonomic functions, sensorimotor functions, excitability, gait and sensorimotor coordination and forelimb and hindlimb grip strength and abnormal clinical observations) and motor activity (data not shown).

3.3. F_1 Litter Results

In all K^+PFHxS-treated groups, there were no statistically significant differences in litter outcomes when compared to the control values (given in parentheses) for the numbers of F_1 pups delivered (235 per 15 litters), liveborn pups (235), stillborn pups (0), live pups on PND 1 (234), 8 (228), and 22 (228), and pups per litter on PND 22 (15.2 ± 1.8). The percent of pups that were male (45.2 ± 13.0), pup body weights (38.17 ± 4.72 and 36.67 ± 4.44 g for males and females, respectively), pup liver weights (1.48 ± 0.26 and 1.59 ± 0.24 g for males and females, respectively), liver-to-body-weight ratios (3.86 ± 0.27 and 4.33 ± 0.24 for males and females, respectively) on PND 22 also did not differ with statistical significance between control pups and those from K^+PFHxS-treated groups. The viability indices (number of live pups on PND 8 relative to number of live pups on PND 1 as a percent) and lactational indices (number of live pups on PND 22 relative to number of live pups on PND 8 as a percent) were not statistically significantly different between pups from K^+PFHxS-

Table 6
Mean serum/plasma and liver PFHxS concentration ± SD (ug/mL or ug/g) data summary.

		K^+PFHxS dose (mg/kg-d)				
		0	0.3	1.0	3.0	10.0
Serum PFHxS concentrations (ug/mL)						
SD 14	F_0 male[a]	0.14 ± 0.05	18.18 ± 2.42	80.97 ± 30.83	143.05 ± 82.09	182.67 ± 8.25
	F_0 female[b]	0.39[c]	2.78 ± 0.81	9.85 ± 3.91	20.67 ± 3.91	42.02 ± 6.47
SD 42	F_0 male	0.32 ± 0.09	44.22 ± 12.66	89.12 ± 0.80	128.67 ± 10.30	201.50 ± 20.02
GD 21	F_0 female	<LLOQ[d]	3.32 ± 0.71	10.65 ± 6.41	32.75 ± 7.83	59.80 ± 11.54
	F_1, pooled[e]	<LLOQ	5.32 ± 1.32	13.47 ± 2.06	37.10 ± 2.89	44.33 ± 6.50
PND 22	F_1, pooled	<LLOQ	8.57 ± 2.41	34.34 ± 10.86	32.35 ± 8.20	93.55 ± 55.79
Liver PFHxS concentrations (ug/g)						
SD 42	F_0 male	0.35 ± 0.23	43.80 ± 8.07	149.50 ± 26.06	338.67 ± 128.42	593.50 ± 81.41
GD 21	F_0 female	<LLOQ[f]	0.79 ± 0.19	2.61 ± 0.54	7.80 ± 1.58	16.53 ± 2.57
	F_1 fetus[g]	<LLOQ	1.37 ± 0.53	3.29 ± 1.17	7.19 ± 1.39	18.87 ± 4.28
PND 22	F_1 male[h]	<LLOQ	1.13 ± 0.31	3.86 ± 0.94	8.73 ± 1.65	16.22 ± 4.41
	F_1 female[i]	<LLOQ	1.04 ± 0.24	3.91 ± 1.05	9.96 ± 2.69	18.39 ± 2.32

[a] Parental male rats.
[b] Parental female rats (dams).
[c] Excludes data for two females that had serum measured below lower limit of quantification (LLOQ) of 0.1 ffig/mL.
[d] Serum LLOQ = 0.1 ffig/mL.
[e] GD 21 fetuses pooled by litter.
[f] Liver LLOQ = 0.ffig/g.
[g] Individual GD 21 fetuses.
[h] Male pups.
[i] Female pups.

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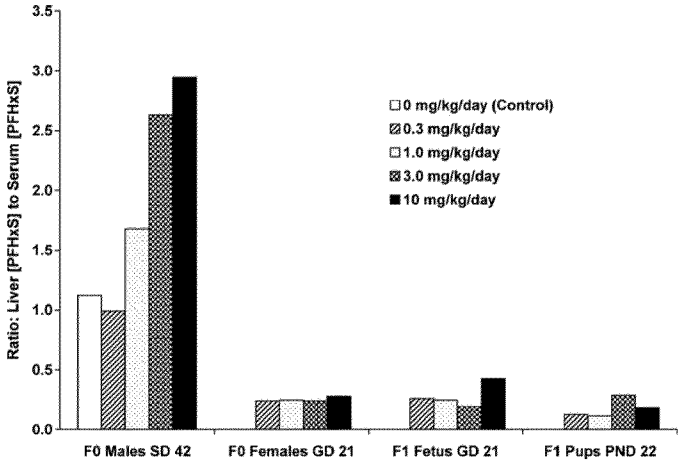

Fig. 2. Mean liver to serum PFHxS concentration ratio for F_0 males on study day (SD) 42, F_0 females on gestation day (GD) 21, F_1 fetus on GD 21, and F_1 pups on postnatal day 22. The F_1 generation rats did not directly receive K^+PFHxS treatment; therefore, presence of PFHxS in serum and liver was the result of *in utero* and lactational exposure.

treated and control groups (viability index = 97.4% and lactation index = 100%).

3.4. PFHxS concentrations in serum and liver

Mean PFHxS concentrations (±SD) in serum (or plasma) and livers are summarized in Table 6. The mean ratio between liver and serum PFHxS concentrations for each treatment groups are illustrated as Fig. 2.

4. Discussion

The purpose of this study was to provide information on the possible health hazards that may result from repeated exposure of Crl:CD®(SD)IGS BR VAF/Plus® male and female rats to K^+PFHxS beginning 14 days before cohabitation, through mating and continuing for at least 42 days (male rats) or through parturition until PND 21 (female rats). This repeated dose study incorporated a reproduction/developmental toxicity test that provided initial information on possible effects on male and female reproductive performance (e.g., gonadal function, mating behavior, conception, development of the conceptus and parturition). The study also included an evaluation of potential neurological effects in parental rats.

The overall body-weight gain from 10 mg/kg-d-dosed group F_0 males was statistically significantly lower than controls between SD 1 and SD 44, however, the terminal mean body weight was not statistically significantly different than control. The effect on body weight in 10 mg/kg-d dose-group males did not appear to be related to decreased feed consumption. In a two-generation reproduction and developmental study with the PFHxS congener, PFOS, with daily gavage dosing, F_0 male body weights were lower than controls at doses of 1.6 and 3.2 mg K^+PFOS/kg/d, becoming statistically significant by days 56 and 36 in these treatment groups, respectively [51]. Feed consumption and body-weight gain were also reduced with statistical significance by K^+PFOS treatment at doses ≥0.4 mg/kg-d. These data suggest that PFOS may be more effective in adversely affecting body weight in male SD rats than PFHxS at equivalent doses. This greater potency could be due to pharmacokinetic differences, pharmacodynamic differences, or both of these factors. Based on data presented by Luebker et al., the mean ffiM concentrations of PFOS in serum by dose were 91, 305, and 547 for the 0.4, 1.6, and 3.2 mg/kg-d dose F_0 male treatment groups, respectively. Mean liver PFOS concentrations in ffiM for the 0.4, 1.6, and 3.2 mg/kg-d dose F_0 male treatment groups from Luebker et al. were 353, 647, and 2719, respectively. Based on data presented in this article, for the 10 mg/kg-d K^+PFHxS-treated F_0 males, the mean ffiM PFHxS concentrations in serum and liver were 594 and 1487, respectively. Considering that the K^+PFOS-treated F_0 males in the Luebker et al. study had been dosed approximately three weeks longer than those from the study reported herein, it is apparent that PFOS may be more potent than PFHxS. At doses of 1.0 mg/kg-d or higher, K^+PFHxS-treated F_0 male rats appeared to have reached serum steady state by SD 14 as their serum PFHxS concentrations were not statistically different between SD 14 and SD 42. In contrast, serum PFHxS concentrations in F_0 females were 12–23% of the F_0 males on SD 14. This gender difference in body burden may have contributed to the absence in body-weight effect in F_0 females as compared to males in each K^+PFHxS treatment group. In addition, differences in pharmacodynamic response cannot be ruled out.

The only effect of treatment with K^+PFHxS on organ weights observed were 20 and 56% increases in mean liver weight in F_0 male rats in the 3 and 10 mg/kg-d treatment groups, respectively, when compared to F_0 male control mean liver weight. Liver weight relative to either body weight or brain weight was also increased to a similar extent in the 3 and 10 mg/kg-d dose-group males as compared to controls. The incidence and severity of centrilobular hepatocellular hypertrophy observed correlated well with liver weight observations.

In the study reported herein, the mean serum and liver concentrations in F_0 males associated with no effect on liver weight at the 1 mg/kg-d dose were 89 ffig/mL and 150 ffig/g, respectively, after six weeks of K^+PFHxS treatment. At the effective K^+PFHxS dose of 3 mg/kg-d, mean serum and liver PFHxS concentrations in males were 129 ffig/mL and 339 ffig/g, respectively. It is interesting to note that, in data from a currently unpublished 3M study, daily gavage dosing of male and female SD rats with potassium PFOS at a dose of 1 mg/kg-d for 28 days resulted in increased liver-weight-to-body-weight ratios in the absence of a statistically significant effect on body weight. This effect was noted at mean serum and liver PFOS concentrations of 46 ffig/mL and 107 ffig/g for males, respectively, and 74 ffig/mL and 126 ffig/g for females, respectively. This observation would suggest that PFOS may be more potent in inducing hepatic hypertrophy than PFHxS.

The observed increase in mild to moderate thyroid follicular epithelial hypertrophy/hyperplasia in the 10 mg/kg-d treatment group males was consistent with the increase in centrilobular hepatocellular hypertrophy [50]. Thyroid hormones were not measured, but prior work with PFOS has demonstrated that competition for binding resulting in increased displacement of thyroid hormones as well as potential induction of increased metabolism of thyroid hormones by liver results in hypothyroxinemia without a strong compensatory increase in pituitary thyrotropin (thyroid-stimulating hormone or TSH) [52]. This is not the result of decreased pituitary release of TSH or response to hypothalamic thyrotropin releasing hormone. Increased hepatocellular hypertrophy in rats is often accompanied by increased thyroid follicular epithelial hypertrophy/hyperplasia as a consequence of induction of thyroid hormone metabolism [50,53]. A two-year dietary study conducted with K^+PFOS in Sprague Dawley rats did not produce an increase in thyroid follicular cell tumors in rats given K^+PFOS continuously throughout the study. However, male rats at the high dose level (20 ppm in diet) that were given potassium PFOS for the first year of the study and control diet thereafter did have an increase in thyroid follicular cell adenoma [54]. Whether the latter observation was a chance occurrence or represents a true effect remains to be determined.

K^+PFHxS treatment resulted in mild but statistically significant decreases in RBC count, hematocrit, and hemoglobin in male rats in the 3 and 10 mg/kg-d treatment groups. Histological examination

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EPA-HQ-2016-005679 06/14/2017

of bone marrow in the 10 mg/kg-d dose-group males did not reveal any abnormalities as compared to controls. Taking the hematology data from the study as a whole, this pattern would suggest slight to mild plasma volume expansion; although other potential etiologies cannot be ruled out with the available data. A similar pattern has observed with the PFHxS homolog, perfluorobutanesulfonate (PFBS) [55]. The PFHxS homolog, PFOS, has been demonstrated to be both a PPARffl [56–59] and a PPAR[56] agonist; although Takacs and Abbott did not observe activation of PPAR with PFOS [60]. Plasma volume expansion is a known effect of thiazolidinediones mediated via PPAR stimulation of the epithelial sodium channel (ENaC) of the collecting duct in kidneys [61]. Typically, weight gain is a consequence of fluid retention with thiazolidinediones, and this was not observed with PFHxS. Indeed, K^+PFHxS treatment caused decreased weight gain at the highest dose in males. In addition, no increases in sodium levels were noted. Dual activation of PPAR and PPARffl could mask a weight gain effect due to the increased -oxidation associated with PPARffl activation. Additional investigation would be required to develop a better understanding of the etiology of the reduced RBC count, hematocrit, and hemoglobin occurring in male rats given PFHxS.

The statistically significant increase in serum urea nitrogen (BUN) in 10 mg/kg-d dose-group parental males without a change in serum creatinine is suggestive of mild dehydration. However, this would be inconsistent with the observed small but significant decreases in RBC count, hematocrit, and hemoglobin, which are suggestive of volume expansion. The mean control value for BUN was consistent with the reported control values for the sex and strain for fasted rats [62]. BUN could also be increased as a result of increased protein catabolism [63]. With the information available from the study, it is difficult to establish the cause of the apparent increase in BUN.

The statistically significant effects observed on prothrombin time in male rats given K^+PFHxS were slight and did not follow a specific trend. These may represent normal variation in this parameter, as the values were within the range of normal (mean 13.6 ± 2.7 s) for 10–20-week-old Sprague Dawley rats as reported by Matsuzawa et al. [62].

The mild hypercalcemia observed in male rats at the 10 mg/kg-d dose is likely due to the increase in serum albumin also occurring at this dose. The ratio of serum albumin to calcium concentration is relatively constant across doses for males. Serum calcium should be corrected for serum albumin in cases where serum albumin is known to be altered [63]. In 10 mg/kg-d dose-group parental males, serum albumin increased over controls by about 5%, with serum calcium increasing by about 6%. Less likely potential etiologies of the mild increase in serum calcium would be an effect on parathyroid function or slight hyperthyroid state. The 37% increase in mean serum alkaline phosphatase in 10 mg/kg-d dose-group males compared to controls, while of unknown origin, would support either of these scenarios. However, increased serum albumin is the simplest and most supportable explanation.

The effect of K^+PFHxS treatment on decreasing serum total cholesterol in male rats was the most pronounced effect observed, occurring at all dose levels. Although not reaching statistical significance until the 10 mg/kg-d dose, mean serum triglycerides were also lower in all K^+PFHxS-treated male groups compared to the male control group. This observation is consistent with cholesterol reduction as a sensitive clinical endpoint for response to the PFHxS congener, PFOS [36,37]. The mechanism resulting in the reduction in serum total cholesterol with PFOS remains to be elucidated. In Wistar rats fed PFOS, decreased activities of hydroxymethylglutaryl CoA reductase and acyl-CoA:cholesterol acyl transferase and increased fatty acid oxidation leading to decreased lipoprotein particle production due reduced synthesis and esterification of cholesterol have been suggested [64]. Stimulation of PPARffl is likely involved in the case of PFOS [56,59,60]. These modes of action are currently being further investigated with PFHxS. Wolf et al. have demonstrated that PFHxS may actually be a more potent activator of mouse and human PPARffl than either the four-carbon PFBS or eight-carbon PFOS in transiently transfected COS-1 cells [58].

The lack of effect of K^+PFHxS treatment on mating and fertility is consistent with prior observations with the congener, PFOS [51,65], and the perfluoroalkyl carboxylate, PFOA [66]. The dosing period did not allow for a complete cycle of spermatogenesis in the males.

In F_0 rats, the PFHxS concentrations in plasma and liver correlated well with the daily K^+PFHxS doses given. Prior to mating, the body burden (represented as plasma PFHxS concentration) in F_0 rats appeared to be sex-dependent: the plasma PFHxS concentrations in F_0 males were 4 to 8 times higher than that measured in F_0 females on SD 14.

Even though none of the F_1 rats received K^+PFHxS treatment directly, it was evident that neonates can receive PFHxS via *in utero* and lactational exposures. The same dose-dependent increases in serum and liver PFHxS concentrations were also observed in F_1 rats per daily K^+PFHxS doses given to the litter-matched F_0 female rats. The finding that serum and liver PFHxS concentrations in pups at PND 22 were higher than GD 21 indicated that exposure of F_1 progeny to PFHxS via the breast milk is as robust as that which occurs *in utero* across the placenta. Fetal serum PFHxS concentrations were slightly higher than maternal serum PFHxS concentrations on GD 21 in all treatment groups except for the highest treatment group. In both F_0 females and F_1 rats, liver PFHxS concentrations were lower than the respective serum PFHxS concentrations at all times.

While the ratio between F_0 male liver to serum PFHxS concentrations ranged from 1 to 3 on SD 42, the corresponding liver to serum PFHxS concentration ratios were approximately 0.5 or lower for all F_0 females on GD 21 and all F_1 pups on GD 21 and PND 22. Although there appeared to be a sex difference in serum and liver PFHxS concentrations in adult rats, there did not appear to be a sex difference between male and female F_1 rats throughout PND 22. The liver PFHxS concentrations remained similar between F_1 male and female rats in all treatment groups on PND 22. It is interesting that lack of a sex difference in PFOS serum and liver concentrations through PND 22 in rat pups from dams treated with K^+PFOS through gestation and lactation has also been reported [67], however, sex differences in serum PFOS concentrations became apparent after sexual maturation. Further study of the effect of sexual maturation on pharmacokinetic handling of PFHxS and PFOS would be of value.

The lack of significant postnatal effects in F_1 generation pups from K^+PFHxS-treated parents is quite different from the increased neonatal mortality and decreased postnatal growth observed for PFOS in Sprague Dawley rats and in mice (CD1 and SV129 PPARffl wild-type and null) [51,65,68,69] and in CD1 and SV129 PPARffl wild-type mice with PFOA [70–72]. The mean GD 21 maternal and fetal serum PFOS concentrations for the 10 mg/kg-d K^+PFHxS-treated dams (60 ffig/mL and 44 ffig/mL) approximated those of K^+PFOS-treated dams and their fetuses on GD 21 that were associated with significant reductions in postnatal pup survival (62 and 101 ffig/mL, respectively) [65]. This observation suggests that K^+PFHxS treatment in rats is either not capable of producing neonatal mortality or requires higher body burdens in the fetal compartment.

PFHxS was first reported to be present at low ng/mL (ppb) concentrations in human serum in 2001 by Hansen et al. [11]. A number of biomonitoring studies of human populations subsequently have confirmed the widespread presence of PFHxS in samples from non-occupationally exposed populations [10,12–14,19–23] These studies demonstrate that PFHxS can be found in umbilical cord blood serum collected in Canada [19], neonatal screening program blood spots from New York [10], and human milk samples from the

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EPA-HQ-2016-005679 06/14/2017

United States [20], China [21], and Sweden [22]. In addition, PFHxS has been found in plasma from children aged 5–6 from Bavaria [23], serum from children aged 2–12 from the United States [14], and serum from adolescents aged 12–19 from the United States [12,13] as well as adults [12,13,15–18]

The serum PFHxS concentrations in children from the United States are greater at the upper tails of the distribution than those of adults [14]. Olsen et al. [14] found that geometric mean serum PFHxS values among 598 children aged 2–12 from samples collected between 1995 and 1996 were not appreciably different than those of 645 American Red Cross blood donors taken between 2000 and 2001 [16] or 238 elderly blood donor serum samples from 1996 [15]. However, 67 of the 598 children (11%) had serum PFHxS concentrations above 30 ng/mL, as opposed to just one adult in each of the adult studies just cited. Similarly, Calafat et al., in reporting data from serum samples taken during 1999–2000 [12] and 2003–2004 [13] as part of the National Health and Nutrition Examination Survey (NHANES), demonstrated that, during both time periods, adolescents aged 12–19 years had the higher geometric mean serum PFHxS concentrations than the other three age groups (20–39 years, 40–59 years, and ≥60 years) and that the estimated 95th percentile serum PFHxS concentration of these adolescents was significantly greater than that of the three other age groups.

Exposure of children to PFHxS may begin very early in life. Monroy et al [19] studied PFHxS serum concentrations in 101 pregnant women and their matched umbilical cord serum at birth. They found that umbilical cord blood serum at birth had a slightly greater mean PFHxS concentration than the respective mean for maternal serum at delivery (5.05 ± 12.92 ng/mL for umbilical cord blood serum versus 4.05 ± 12.30 ng/mL for mothers' serum); however, PFHxS was detectable in 46% of mothers' serum at birth versus 21% of umbilical cord blood serum samples. Lactational exposure to PFHxS has also been demonstrated in human populations. Human milk concentrations have ranged from a median of 0.011 ng/mL in 19 samples from China [21], a mean of 0.0145 ng/mL in 45 samples from Massachusetts [20], and a mean of 0.085 ng/mL in 12 samples from Sweden [22]. In the Swedish study, the respective mean maternal serum concentration was 4.7 ng/mL.

Reflecting on the larger proportion of children with serum PFHxS concentrations greater than 30 ng/mL as compared to adults, Olsen et al. [14] suggested that differences in the exposure patterns of children as well as potential exposure to surfaces in the home treated with materials that may contain or release PFHxS may account for the greater PFHxS serum concentrations in the tails of the distribution. Kubwabo et al. [27] and Strynar and Lindstrom [28] demonstrated the presence of PFHxS in house dust. In the Kubwabo et al. study, PFHxS concentrations in house dust were positively associated with the presence and extent of carpeting in homes and negatively associated with the age of the homes, which was negatively associated with the presence of carpet. Because children may spend more time in the home and in contact with treated surfaces, and boys may be somewhat more prone to these types of exposures (boys were more likely to have higher serum PFHxS concentrations than girls in the studies reported by Olsen et al. [14] and Hölzer et al. [23]), these data combined with the biomonitoring data provide support for the suggestion made by Olsen et al. to explain the differences in the distributions of adults and children at the upper end.

Sources other than house dust or treated surfaces in the home may include exposure via water, as suggested by Hölzer et al. [23] and Ericson et al. [29]. In particular, Hölzer et al. found that maximum children's plasma PFHxS concentrations were greater than those of adults in two German towns, one with potential exposure via drinking water. PFHxS has been found, less frequently, in samples from various wildlife [73–77], and Falandysz et al. [24] found an association of PFHxS serum concentrations with consumption of fish in the Baltic. However, Ericson et al. [78] were not able to detect PFHxS in market food sources from the Catalan area of Spain.

Although a complete quantitative description of PFHxS exposure from various sources is not feasible at this time, it has become evident that PFHxS blood concentrations in the general public in the United States are declining since 2000–2002 when the principal manufacturer, 3M Company, discontinued production of PFHxS based on evidence of widespread distribution in non-occupationally-exposed populations [10,13,17] In analyzing data from the National Health and Nutrition Examination Survey (NHANES), Calafat et al. [13] reported a 10% decline in PFHxS serum concentrations between 1999–2000 and 2003–2004. Using newborn screening blood spots for the same time period, Spliethoff et al. [10] reported a 23% decline. Similarly, Olsen et al. [17] showed a 30% decline in measured serum PFHxS concentration with American Red Cross blood donors between 2000–2001 and 2006. These declines appear to be consistent with the reported geometric mean serum PFHxS elimination half-life for 26 retired fluorochemical workers of approximately 7 years [7]. However, in Germany, PFHxS serum concentrations have been shown to be rising between 1977 and 2004 [26].

5. Conclusion

Treatment of mature adult Sprague Dawley rats with K^+PFHxS by daily oral gavage at doses up to 10 mg/kg-d for two weeks prior to mating, and for females, through gestation and lactation, and, for males, for a minimum of 42 days, did not produce major detrimental effects on mating, fertility, birth outcome, and development of offspring. The most sensitive effect, observed in parental males, was a decrease in total serum cholesterol, observed at all treatment levels.

Conflict of interest statement

John L. Butenhoff, Shu-Ching Chang, and David J. Ehresman are employees of 3M Company, a former manufacturer of K^+PFHxS and the company supporting the work reported on in the article. Raymond G. York does not have competing interests other than employment in contract research facilities (Charles River Preclinical Services) conducting parts of the work.

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Toxicological evaluation of potassium perfluorobutanesulfonate in a 90-day oral gavage study with Sprague–Dawley rats

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abstract

Perfluorobutanesulfonate (PFBS) is a surfactant and degradation product of substances synthesized using perfluorobutanesulfonyl fluoride. A 90-day rat oral gavage study has been conducted with potassium PFBS (K^+PFBS). Rats were dosed with K^+PFBS at doses of 60, 200, and 600 mg/kg-day body weight. The following endpoints were evaluated: clinical observations, food consumption, body weight, gross and microscopic pathology, clinical chemistry, and hematology. In addition, functional observation battery and motor activity assessments were made. Histological examination included tissues in control and 600 mg/kg-day groups. Additional histological examinations were performed on nasal cavities and turbinates, stomachs, and kidneys in the 60 and 200 mg/kg-day groups. No treatment-related mortality, body weight, or neurological effects were noted. Chromorhinorrhea (perioral) and urine-stained abdominal fur were observed in males at 600 mg/kg-day. Red blood cell counts, hemoglobin, and hematocrit values were reduced in males receiving 200 and 600 mg/kg-day; however, there were no adverse histopathological findings in bone marrow. Total protein and albumin were lower in females at 600 mg/kg-day. There were no significant changes in clinical chemistry in either sex. All rats appeared normal at sacrifice. Microscopic changes were observed only at the highest dose in the stomach. These changes consisted of hyperplasia with some necrosis of the mucosa with some squamous metaplasia. These effects likely were due to a cumulative direct irritation effect resulting from oral dosing with K^+PFBS. Histopathological changes were also observed in the kidneys. The changes observed were minimal-to-mild hyperplasia of the epithelial cells of the medullary and papillary tubules and the ducts in the inner medullary region. There were no corresponding changes in kidney weights. Clinical chemistry parameters related to kidney function were unchanged. These kidney findings are likely due to a response to high concentration of K^+PFBS in tubules and ducts and represent a minimal-to-mild effect. Microscopic changes of an equivocal and uncertain nature were observed in the nasal mucosa and were likely attributable to the route of dosing (oral gavage). The NOAEL for the female rat in this study was 600 mg/kg-day (highest dose of study). The NOAEL for the male rat was 60 mg/kg-day based on hematological effects.

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1. Introduction

In the 1990s, industry (3M) began developing new technologies to augment and eventually replace a chemical class referred to "perfluorooctanyl" chemicals. This chemical class includes perfluorooctanesulfonyl fluoride (POSF), N-alkyl derivatives of POSF, perfluorooctanoate (PFOA), and perfluorooctanesulfonate (PFOS). In May 2000, 3M announced the voluntary manufacturing phase out of this chemical class due to its persistence and widespread distribution in the environment. Subsequently, the United States Environmental Protection Agency (USEPA) issued a Significant New Use Rule (USEPA, 2002), or SNUR, that more broadly regulates the production and import of many perfluorooctanyl-based chemicals. The USEPA subsequently proposed to amend the perfluoroalkyl substances SNUR (USEPA, 2006) to include additional perfluorooctanyl-based chemicals. Canada banned the use and importation of a number of long chain perfluorinated carboxylic acids because of concerns over potential adverse human and environmental effects (CGDE, 2008). In January 2006, the USEPA announced a voluntary 2010/2015 PFOA Stewardship Program to eliminate emissions and product content of PFOA and related chemicals (http://www.epa.gov/opptintr/pfoa/pubs/pfoastewardship). One of the new technologies devel-

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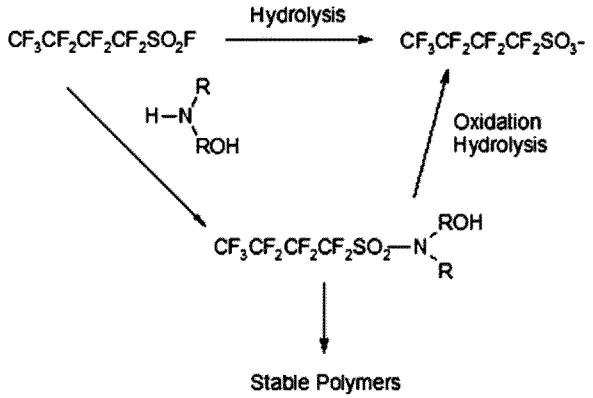

Fig. 1. Degradation pathways of N-alkyl-substituted perfluorobutanesulfonamide.

oped by 3M is based on products made from perfluorobutanesulfonyl fluoride (PBSF). The ultimate degradation and metabolic product from materials based on this chemistry is perfluorobutanesulfonate (PFBS). While PFBS can be formed from the hydrolysis of PBSF, it can also be formed from the metabolic and environmental degradation of certain derivatized perfluorobutanesulfonamides (Fig. 1). Derivatized perfluorobutanesulfonamides have been developed for use in numerous applications including fabric, carpet and upholstery protectants, and surfactants. Extensive testing has been performed on this material which, compared to the perfluorooctanyl-based chemicals, has a low potential to bioaccumulate (NICNAS, 2005; Olsen et al., 2008). We undertook this study to further develop the basic descriptive toxicological profile of PFBS.

2. Materials and methods

2.1. Animals

Crl:CD@(SD)IGS BR VAF/PlusTM rats were supplied by Charles Rivers Laboratory. Rats were assigned to individual housing in stainless steel, wire-bottomed cages. Animal care, cages, and housing conditions were in accordance with the Guide for the Care and Use of Laboratory Animals (ILAR, 1996). Rats were given *ad libitum* access to Certified Rodent Diet R #5002 (PMI Nutrition International Inc., St. Louis, MO) in individual feeders. Water (processed by reverses osmosis) was available to the rats *ad libitum* from individual bottles or an automatic watering system. Lighting was maintained at 12-h light and 12-h dark cycles. The study was conducted in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. All procedures involving laboratory animals were reviewed and approved by the Institutional Animal Care and Use Committee associated with the facility in which the laboratory animals were housed.

2.2. Test substance

The potassium salt of perfluorobutanesulfonate (K^+PFBS, Lot # 120K0252, 98.2% pure) was provided by 3M Company. Solutions of K^+PFBS were prepared in aqueous carboxymethyl cellulose (0.1% CMC, medium viscosity) using reverse osmosis membrane processed deionized water.

2.3. Study design

This study was performed according to Good Laboratory Practices (USEPA, 2000) and based on the test guidelines, OECD 408 and OPPTS 870.3100 (USEPA, 1998). Dose levels were selected based on the results of a 28-day toxicology study (unpublished) conducted with the test substance. In that study, 900 mg/kg-day produced significant increases in the liver weight of male rats and significant increases in kidney weights of female rats. The no-observable-adverse-effect level (NOAEL) in the 28-day study was 300 mg/kg-day. In this 90-day study, rats (10/(sexgroup)) received daily oral doses by gastric lavage of 0, 60, 200, and 600 mg/kg-day. The doses were prepared using a constant dosing volume of 10 mL/kg and were administered for approximately 90 days (90–93 days). Doses were adjusted for the most recently recorded body weight and administered at approximately the same time each day.

Rats were observed twice daily during the study. Observations for clinical signs were made daily before dosing and approximately 1 h after each dose administration and again on the day of sacrifice. Signs of toxicity were recorded as observed. Detailed clinical observations were conducted for all rats once before the first dose and at least once weekly thereafter. Signs noted included, but were not limited to changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions and autonomic activity (e.g., lacrymation, piloerection, pupil size, and unusual respiratory pattern). Changes in gait, posture, and response to handling, and other behavior patterns were recorded. Ophthalmologic examinations were performed by a veterinary ophthalmologist for all rats prior to the first dose and at termination of the study.

Body weights for male and female rats were recorded weekly during the acclimation and dose periods and at sacrifice. Feed consumption values were recorded weekly during the acclimation and dose periods. A functional observational battery (FOB) and motor activity assessment were conducted on five male and five female rats per group after the 11th week of dosing.

All rats were sacrificed by carbon dioxide asphyxiation on the day following the last administration of the test substance (days 91–94 of the study). Approximately 5 mL of blood was collected for hematological and clinical biochemical evaluations. One milliliter of blood was collected into EDTA-coated tubes and analyzed for the following hematological parameters: erythrocyte count (RBC); hematocrit (HCT); hemoglobin (HGB); mean corpuscular hemoglobin (MCH); mean corpuscular hemoglobin concentration (MCHC); mean corpuscular volume (MCV); total leukocyte count (WBC); differential leukocyte count; platelet count (PLAT); mean platelet volume (MPV); and cell morphology. Blood smears were prepared for each sample for measurements of differential leukocyte count. Another 1.8 mL of blood was added to a tube containing 0.2 mL of sodium citrate for determination of prothrombin time (PT) and activated partial thromboplastin time (APTT).

Two milliliters of blood were processed to obtain serum, immediately frozen on dry ice, and subsequently analyzed for the following parameters: total protein (TP); triglycerides (TRI); albumin (A); globulin (G); albumin/globulin ratio (A/G); glucose (GLU); cholesterol (CHOL); total bilirubin (TBILI); urea nitrogen (BUN); creatinine (CREAT); alanine aminotransferase (ALT); asparatate aminotransferase (AST); alkaline phosphatase (ALK); calcium (CA); phosphorus (PHOS); sodium (NA); potassium (K); and chloride (CL).

A gross necropsy of the thoracic, abdominal, and pelvic viscera was performed. In addition, the cranial, thoracic and abdominal cavities were examined. The lungs were perfused with neutral buffered 10% formalin. The following organs were excised, trimmed and weighed prior to fixation: adrenals; brain; kidneys; heart; thymus; spleen; liver; testes; epididymis; ovaries; and uterus. The following tissues were retained in neutral buffered formalin (10%): adrenals; aorta; bone marrow (sternum); brain; epididymis; esophagus; eyes; femur; heart; large intestines; small intestines; kidneys; larynx; pharynx and nose; liver; lungs; lymph nodes (mandibular and mesenteric); mammary gland; ovaries; pancreas; Peyer's patches; pituitary; prostate; salivary gland (mandibular); sciatic nerve; seminal vesicles; skeletal muscle; skin; spinal cord; spleen; stomach; testes; thymus; thyroid/parathyroid; trachea; uterus; urinary bladder; and vagina.

Tissues for histological examination were processed, embedded in paraffin, and sectioned at 5 ffim and stained with hematoxylin and eosin. Histological examination was performed on all tissues from the 0 (vehicle) and 600 mg/kg-day dose groups. In addition, the nasal cavities, nasal turbinates, stomachs and kidneys of the male and female rats in the 60 and 200 mg/kg-day dose groups were evaluated microscopically.

A 600-mg/kg-day male rat was found dead and examined for the cause of death on day 85 (prior to study termination). The rat was necropsied and examined to the extent possible as described for rats sacrificed according to schedule.

2.4. Statistical analysis

Data generated during the course of this study were recorded either by hand or by using the Argus Automated Data Collection and Management System, the Vivarium Temperature and Relative Humidity Monitoring System, the Coulbourn Instruments Passive Infrared Motor Activity System, the Coulbourn Instruments Auditory Startle System, the Coulbourn Instruments Spatial Delayed Alternation System, and/or the passive avoidance software. All data were tabulated, summarized and/or statistically analyzed using the Argus Automated Data Collection and Management System, the Vivarium Temperature and Relative Humidity Monitoring System, Microsoft Excel (part of Microsoft Office 97 (version SR-2)) and/or The SAS System (version 6.12).

Parameters in the FOB that used interval scales, such as the grip-strength tests and the landing foot splay test, as well as body weight data and feed consumption values, were analyzed initially as parametric data. Bartlett's test of homogeneity of variances (Sokol and Rohlf, 1969a) was used to estimate the probability that the groups had different variances. A non-statistically significant result ($p > 0.001$) indicated that an assumption of homogeneity of variance was appropriate, and the data were compared using the analysis of variance test (Snedecor and Cochron, 1967a,b). If that test was significant ($p < 0.05$), the groups exposed to the test substance were compared with the control group using Dunnett's test (Dunnett, 1955). If Bartlett's test was significant ($p < 0.001$), the analysis of variance test was not appropriate, and the data were analyzed using non-parametric methods. When 75% or fewer of the scores in all the groups were tied, the Kruskal–Wallis test (Sokol and Rohlf, 1969b) was used to analyze the data, and in the event of a significant result ($p < 0.05$), Dunn's test (Dunn, 1964) was used to compare the groups exposed to the test sub-

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EPA-HQ-2016-005679 06/14/2017

Table 1
Mean (±S.D.) body weights in grams by dose group in male and female rats.

Dosage (mg/kg-day)	Male				Female			
	Control	60	200	600	Control	60	200	600
N	10	10	10	10[a]	10	10	10	10
Day 1	192.1 ± 7.3	192.9 ± 6.8	190.0 ± 7.2	193.1 ± 7.2	161.5 ± 9.6	161.0 ± 7.7	157.9 ± 11.2	158.1 ± 10.9
Termination	510.9 ± 47.0	482.6 ± 60.4	479.6 ± 30.3	485.3 ± 49.4[a]	276.5 ± 24.0	286.0 ± 21.5	284.8 ± 30.1	264.6 ± 19.5

[a] Terminal body weights at this dose for males exclude the value for the rat which was found dead on day 85 of study.

stance with the control group. When more than 75% of the scores in any group were tied, Fisher's exact test (Siegel, 1956) was used to compare the proportion of ties in the groups.

Data from the motor activity test, with repeated measurements within a session, were analyzed using an analysis of variance with repeated measures (SAS Institute, 1988). A significant effect ($p < 0.05$) in that test can appear as effect of Concentration (a difference between groups in the total across all measurements in a session) or as an interaction between Concentration and Block (a difference between groups at specific measurement periods). If the Concentration effect was significant, the totals for the control group and the groups given the test substance were compared using Dunnett's test. If the Concentration × Block interaction was significant, an analysis of variance test was used to evaluate the data at each measurement period, and a significant result ($p < 0.05$) was followed by a comparison of the groups using Dunnett's test.

Parameters in the FOB that had graded or count scores were analyzed using non-parametric procedures.

Clinical observation incidence data, as well as the descriptive and quantal data from the FOB, were analyzed as contingency tables using the variance test for homogeneity of the binomial distribution (Snedecor and Cochron, 1967a,b).

3. Results

3.1. Mortality and clinical observations

3.1.1. Male rats

One male rat in the high dose group was found dead on study day 85. Clinical observations on day 84 included red perioral substance, urine-stained abdominal fur, decreased motor activity, cold to touch, ptosis, dehydration, brown substance around mouth, and ungroomed coat. This death was considered unrelated to administration of K^+PFBS. This was a single incident, and the sudden onset of adverse clinical observations indicates a possible injury. All other male rats survived until scheduled sacrifice.

In male rats in the 600 mg/kg-day dose group, the 9 incidences of a red perioral substance (slight to extreme in degree) occurring in 6 of 10 rats and the 27 incidences of urine-stained abdominal fur occurring in 3 of 10 rats were significantly different than control incidences ($p < 0.01$). Chromorhinorrhea occurred in 2 of 10 and 3 of 10 rats in the 200 and 600 mg/kg-day dose groups, respectively. All other clinical observations were considered unrelated to the test substance, because the incidences were not dose-dependent and/or the observations only occurred in one rat in a group. These clinical observations included localized alopecia of the limbs and neck, discolored fur, swollen ears or snout, excess salivation, red substance in cage pan, decreased motor activity, dehydration, ptosis, brown substance around mouth, ungroomed coat, cold to touch, soft or liquid feces, scab on the neck, head or forelimb, ulceration on neck, chromodacryorrhea, missing/broken incisors, and abrasion on neck or head. Ophthalmologic examination of the male rats at study termination revealed one male rat in the 200 mg/kg-day dose group with chromodacryorrhea of both eyes.

3.1.2. Female rats

All female rats survived to scheduled sacrifice. All clinical observations in the female rats were considered unrelated to K^+PFBS, because either the observations were not dose-dependent, and/or the observation only occurred in one or two rats in a group.

3.2. Effects on body weight

Body weights and body weight gains (data not shown) for both the male and female rats were similar in all four dose groups throughout the study period (Table 1). Body weight gain was significantly reduced ($p < 0.01$) in male rats on study days 15–22 in the 60 and 200 mg/kg-day dose groups and in the female rats on study days 43–50 in the 60 mg/kg-day dose group. These effects were not considered treatment related, because they were not dose-dependent and occurred only once.

3.2.1. Organ to body weight data

In male rats, the absolute weights of the spleen and the ratios of the weight of the spleen to terminal body weight and brain weight (data not shown) were reduced or significantly reduced in the 60, 200 and 600 mg/kg-day dose groups ($p < 0.05$ or $p < 0.01$) when compared to control values (Table 2). However, there was no trend in the reduction across the 10-fold increase in dose range (60–600 mg/kg/day), and reductions in spleen weights were not present among treated female rats when compared to controls. All other organ weights and organ weights to terminal body weights and brain weights for male rats were unaffected by doses of K^+PFBS up to 600 mg/kg-day.

In female rats, all organ weights and organ weights to terminal body weights and brain weights were unaffected in the female rats by dose of K^+PFBS up to 600 mg/kg-day (Table 3).

3.3. Feed consumption

There were no notable differences in feed consumption between K^+PFBS-treated male and female rats and their respective control rats.

3.4. Motor activity and functional observation battery

There were no statistically significant or biologically important differences between K^+PFBS-treated male and female rats and their respective control rats in any of the measured parameters. Parameters evaluated included: home cage behavior; autonomic functions (lacrymation, salivation, palpebral closure, prominence of the eye, papillary reaction to light, piloerection, respiration, defecation, and urination); sensorimotor functions; excitability; gait and sensorimotor coordination; forelimb and hindlimb grip strength; and abnormal clinical observations (including but not limited to: convulsions; tremors; unusual behavior; hypotonia; hypertonia; emaciation; dehydration; unkempt appearance; and deposits around the eyes nose or mouth).

3.5. Macroscopic observations at necropsy

All necropsy observations for animals sacrificed at the end of the study were normal.

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EPA-HQ-2016-005679 06/14/2017

Table 2
Mean (±S.D.) organ weights (g) and organ weight to terminal body weight ratios (%) in male rats.

Organ	Measure	Dose group (mg/kg-day)[a]			
		0 (*N*=10)	60 (*N*=10)	200 (*N*=10)	600 (*N*=9[b])
Epididymides	Weight (g)	1.65 ± 0.18	1.46 ± 0.10	1.58 ± 0.17	1.59 ± 0.16
	Ratio (%)	0.325 ± 0.043	0.306 ± 0.037	0.329 ± 0.037	0.332 ± 0.054
Testes	Weight (g)	3.54 ± 0.68	3.38 ± 0.22	3.56 ± 0.24	3.53 ± 0.57
	Ratio (%)	0.689 ± 0.118	0.708 ± 0.086	0.746 ± 0.074	0.733 ± 0.142
Brain	Weight (g)	2.28 ± 0.13	2.28 ± 0.11	2.25 ± 0.09	2.29 ± 0.10
	Ratio (%)	0.451 ± 0.046	0.479 ± 0.054	0.473 ± 0.042	0.474 ± 0.060
Liver	Weight (g)	14.48 ± 1.76	13.83 ± 2.67	13.50 ± 1.28	14.78 ± 1.78
	Ratio (%)	2.832 ± 0.198	2.846 ± 0.273	2.814 ± 0.175	3.049 ± 0.220
Kidneys	Weight (g)	4.18 ± 0.48	4.15 ± 0.55	3.93 ± 0.30	4.18 ± 0.33
	Ratio (%)	0.818 ± 0.058	0.859 ± 0.053	0.818 ± 0.053	0.864 ± 0.049
Adrenals	Weight (g)	0.066 ± 0.014	0.057 ± 0.016	0.068 ± 0.013	0.061 ± 0.010
	Ratio (%)	0.013 ± 0.005	0.012 ± 0.004	0.013 ± 0.005	0.011 ± 0.003
Spleen	Weight (g)	0.93 ± 0.13	0.77 ± 0.10**	0.83 ± 0.06*	0.80 ± 0.11**
	Ratio (%)	0.181 ± 0.018	0.158 ± 0.015**	0.172 ± 0.017	0.163 ± 0.020*
Thymus	Weight (g)	0.49 ± 0.19	0.48 ± 0.17	0.38 ± 0.10	0.38 ± 0.14
	Ratio (%)	0.096 ± 0.039	0.097 ± 0.033	0.078 ± 0.021	0.078 ± 0.023
Heart	Weight (g)	1.61 ± 0.19	1.54 ± 0.18	1.60 ± 0.23	1.59 ± 0.18
	Ratio (%)	0.317 ± 0.020	0.320 ± 0.022	0.334 ± 0.057	0.328 ± 0.037

[a] Dosage occurred on days 1 through 90, 91, 92, or 93 of study.
[b] Excludes values for a rat which was found dead on day 85 of study.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

3.6. Clinical chemistries

Results for clinical chemistry are shown in Table 4. The average value for chloride was significantly increased ($p < 0.01$) in male rats in the 600 mg/kg-day dose group. Average total protein and albumin values were significantly reduced ($p < 0.05$) in female rats in the 600 mg/kg-day group. Average values for glucose, cholesterol, total bilirubin, blood urea nitrogen, creatinine, alanine aminotransferase, asparatate aminotransferase, alkaline phosphate, calcium, inorganic phosphorus, triglycerides, sodium, potassium, globulin, and the albumin/globulin ratios were unaffected in either sex by doses of K^+PFBS as high as 600 mg/kg-day.

3.7. Hematology

Results for hematology are shown in Table 5. In male rats in the 200 and 600 mg/kg-day groups, statistically significant reductions in average values for red blood cells (600 mg/kg-day only), hemoglobin concentration, and hematocrit were noted. Average values for leukocytes, mean corpuscu-

Table 3
Mean (±S.D.) organ weights (g) and organ weight to terminal body weight ratios (%) in female rats.

Organ	Measure	Dose group (mg/kg-day)[a]			
		0 (*N*=10)	60 (*N*=10)	200 (*N*=10)	600 (*N*=10)
Ovaries	Weight (g)	0.136 ± 0.019	0.136 ± 0.020	0.139 ± 0.025	0.129 ± 0.018
	Ratio (%)	0.048 ± 0.008	0.046 ± 0.007	0.048 ± 0.009	0.047 ± 0.007
Uterus	Weight (g)	0.64 ± 0.16	0.76 ± 0.26	0.64 ± 0.14	0.80 ± 0.21
	Ratio (%)	0.234 ± 0.064	0.264 ± 0.087	0.228 ± 0.062	0.303 ± 0.077
Brain	Weight (g)	2.07 ± 0.08	2.08 ± 0.09	2.08 ± 0.08	2.06 ± 0.10
	Ratio (%)	0.752 ± 0.060	0.730 ± 0.052	0.736 ± 0.061	0.780 ± 0.080
Liver	Weight (g)	7.71 ± 0.78	8.30 ± 0.72	8.23 ± 0.91	7.79 ± 0.36
	Ratio (%)	2.788 ± 0.152	2.902 ± 0.154	2.890 ± 0.100	2.951 ± 0.204
Kidneys	Weight (g)	2.34 ± 0.22	2.40 ± 0.30	2.40 ± 0.18	2.39 ± 0.41
	Ratio (%)	0.847 ± 0.069	0.838 ± 0.071	0.846 ± 0.059	0.906 ± 0.150
Adrenals	Weight (g)	0.076 ± 0.014	0.078 ± 0.011	0.079 ± 0.013	0.074 ± 0.010
	Ratio (%)	0.026 ± 0.007	0.027 ± 0.005	0.028 ± 0.008	0.027 ± 0.007
Spleen	Weight (g)	0.58 ± 0.09	0.59 ± 0.11	0.57 ± 0.07	0.65 ± 0.11
	Ratio (%)	0.209 ± 0.033	0.208 ± 0.037	0.202 ± 0.030	0.248 ± 0.046
Thymus	Weight (g)	0.35 ± 0.07	0.34 ± 0.13	0.38 ± 0.10	0.36 ± 0.09
	Ratio (%)	0.128 ± 0.033	0.121 ± 0.047	0.131 ± 0.027	0.134 ± 0.031
Heart	Weight (g)	0.97 ± 0.08	1.02 ± 0.07	0.99 ± 0.10	0.98 ± 0.12
	Ratio (%)	0.356 ± 0.050	0.356 ± 0.031	0.348 ± 0.031	0.373 ± 0.046

[a] Dosage occurred on days 1 through 90, 91, 92, or 93 of study.

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EPA-HQ-2016-005679 06/14/2017

Table 4
Mean (±S.D.) clinical chemistry values in male and female rats.

Dosage (mg/kg-day)[a]	Male				Female			
	Control	60	200	600	Control	60	200	600
N	10	10	10	9[b]	10	10	10	9[c]
TP (g/dL)	6.6 ± 0.33	6.5 ± 0.16	6.4 ± 0.19	6.5 ± 0.50	7.2 ± 0.40	7.2 ± 0.34	7.1 ± 0.40	6.7 ± 0.23*
A (g/dL)	4.1 ± 0.17	4.1 ± 0.19	4.0 ± 0.11	4.0 ± 0.26	4.9 ± 0.38	4.8 ± 0.29	4.7 ± 0.31	4.4 ± 0.23*
GLU (mg/dL)	164 ± 29.2	157 ± 12.8	167 ± 21.6	166 ± 13.8	156 ± 16.7	160 ± 21.4	163 ± 30.1	161 ± 13.1
CHOL (mg/dL)	54 ± 12.9	51 ± 14.9	45 ± 8.4	53 ± 14.4	76 ± 11.8	69 ± 10.0	74 ± 18.7	68 ± 16.8
TBILI (mg/dL)	0.1 ± 0.03	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.03	0.1 ± 0.03	0.1 ± 0.03
BUN (mg/dL)	15 ± 1.4	14 ± 1.5	14 ± 2.2	15 ± 1.4	16 ± 1.9	16 ± 1.6	15 ± 1.8	16 ± 2.9
CREAT (mg/dL)	0.3 ± 0.05	0.2 ± 0.05	0.3 ± 0.06	0.3 ± 0.05	0.4 ± 0.05	0.4 ± 0.04	0.3 ± 0.05	0.4 ± 0.05
ALT (U/L)	42 ± 5.8	45 ± 7.1	41 ± 4.6	43 ± 7.8	44 ± 11.6	55 ± 23.6	49 ± 30.7	44 ± 13.3
AST (U/L)	84 ± 11.2	90 ± 10.9	88 ± 7.5	91 ± 12.7	85 ± 15.8	95 ± 25.8	94 ± 51.0	96 ± 19.6
ALK (U/L)	94 ± 15.0	90 ± 12.0	96 ± 12.1	107 ± 12.7	44 ± 8.0	46 ± 13.3	45 ± 12.1	59 ± 18.2
CA (mg/dL)	11.4± 0.37	11.2± 0.27	11.0± 0.24	11.1± 0.32	11.9± 0.50	11.6± 0.39	11.6± 0.53	11.4± 0.16
PHOS (mg/dL)	8.9 ± 0.68	8.7 ± 0.64	8.3 ± 0.71	8.3 ± 0.55	8.2 ± 1.35	8.0 ± 1.39	7.9 ± 1.17	8.4 ± 0.80
TRI (mg/dL)	60 ± 32.3	58 ± 21.0	50 ± 11.6	47 ± 13.7	36 ± 7.7	45 ± 19.0	41 ± 9.2	42 ± 13.5
NA (mmol/L)	148 ± 1.4	148 ± 0.7	149 ± 1.2	149 ± 0.9	146 ± 1.5	146 ± 0.9	146 ± 1.2	145 ± 1.1
K (mmol/L)	5.2 ± 0.59	5.3 ± 0.54	5.1 ± 0.29	5.1 ± 0.38	6.3 ± 0.76	5.9 ± 0.62	6.2 ± 0.60	6.2 ± 0.63
CL (mmol/L)	98 ± 2.0	100 ± 1.2	100 ± 1.4	101 ± 1.7**	101 ± 2.5	101 ± 2.3	102 ± 2.2	102 ± 1.1
G (g/dL)	2.5 ± 0.28	2.5 ± 0.14	2.4 ± 0.16	2.4 ± 0.37	2.4 ± 0.17	2.4 ± 0.22	2.4 ± 0.28	2.3 ± 0.26
A/G	1.7 ± 0.23	1.7 ± 0.14	1.7 ± 0.13	1.7 ± 0.23	2.1 ± 0.21	2.0 ± 0.23	2.0 ± 0.31	1.9 ± 0.26

[a] Dosage occurred on days 1 through 90, 91, 92, or 93 of study.
[b] Excludes data for a rat which was found dead on day 85 of study.
[c] Excludes a rat that did not have sufficient sample volume.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

lar volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, mean platelet volume, prothrombin time, activated partial thromboplastin time, nucleated red blood cell count, segmented neutrophils, bands, monocytes, eosinophils, basophils, abnormal lymphocytes, and other cells were similar to control values among the three K^+PFBS-treated groups of male and female rats. The mean corpuscular hemoglobin concentration was significantly increased ($p < 0.05$) in the 60 mg/kg-day dose-group female rats and was not considered treatment related because it was not dose-dependent.

3.8. Histopathology

Microscopic changes were observed in the kidneys and stomach of the male and female rats of the 600 mg/kg-day dose group (Table 6). In kidneys obtained from rats in the 600 mg/kg-day male and female dose groups, increased incidence of hyperplasia of the epithelial cells of the medullary and papillary tubules and ducts in the inner medullary regions was noted as compared to controls. These tubules had a dark tinctorial appearance with increased amounts of small interstitial cells with prominent dark nuclei. Other treatment-related changes included a lower incidence

Table 5
Mean (±S.D.) hematology values in male and female rats.

Dosage (mg/kg-day)[a]	Male				Female			
	Control	60	200	600	Control	60	200	600
N	10	10	10	9[b]	10	10	10	10
WBC (10^3/mm^3)	17.0 ± 3.66	15.4 ± 3.50	14.7 ± 2.31	15.2 ± 2.80	12.2 ± 3.95	11.5 ± 3.10	12.1 ± 3.77	13.5 ± 3.94
RBC (10^6/mm^3)	7.76 ± 0.469	7.62 ± 0.443	7.55 ± 0.282	7.19 ± 0.481*	7.17 ± 0.315	6.95 ± 0.226	7.16 ± 0.309	6.95 ± 0.297
HGB (g/dL)	16.4 ± 0.96	16.0 ± 0.41	15.6 ± 0.48*	15.5 ± 0.78*	15.9 ± 0.61	15.8 ± 0.57	15.6 ± 0.69	15.3 ± 0.82
HCT (%)	44.2 ± 2.32	42.7 ± 1.44	41.9 ± 1.50*	40.9 ± 2.24**	43.3 ± 1.85	42.1 ± 1.56	42.7 ± 2.14	41.2 ± 1.71
MCV (ffim^3)	57.0 ± 1.25	56.2 ± 2.12	55.6 ± 1.38	57.0 ± 2.08	60.5 ± 1.08	60.6 ± 1.47	59.5 ± 1.50	59.2 ± 1.21
MCH (pg)	21.2 ± 0.53	21.1 ± 1.18	20.7 ± 0.51	21.6 ± 1.01	22.2 ± 0.64	22.7 ± 0.65	21.8 ± 0.61	22.0 ± 0.75
MCHC (%)	37.2 ± 0.50	37.5 ± 1.11	37.2 ± 0.69	37.8 ± 0.96	36.7 ± 0.69	37.4 ± 0.54*	36.7 ± 0.51	37.1 ± 0.70
PLAT (10^3/mm^3)	1235 ± 241.3	1160 ± 175.6	1134 ± 149.4	1202 ± 165.0	1119 ± 88.2	1157 ± 195.8	1181 ± 181.6	1126 ± 283.3
MPV (ffim^3)	7.7 ± 0.35	8.0 ± 0.65	8.0 ± 0.64	7.6 ± 0.51	7.4 ± 0.41	7.2 ± 0.47	7.2 ± 0.45	7.2 ± 0.53
PT (s)	13.7 ± 0.41	14.2 ± 0.45	14.1 ± 0.68	14.2 ± 0.80	13.3 ± 0.29	13.5 ± 0.49	13.1 ± 0.40	13.4 ± 0.16[c]
APTT (s)	24.7 ± 2.80	25.7 ± 3.84	25.6 ± 2.94	25.5 ± 4.43	21.2 ± 2.41	24.3 ± 5.68	24.2 ± 3.27	24.5 ± 5.17[c]
NRBC (count)	0 ± 0.0	0 ± 0.3	0 ± 0.0	0 ± 0.7	0 ± 0.0	0 ± 0.3	0 ± 0.3	0 ± 0.0
Lymphocyte (10^3/mm^3)	13.6 ± 3.93	12.6 ± 3.66	12.2 ± 2.34	13.0 ± 2.62	10.3 ± 3.76	9.6 ± 2.56	9.6 ± 3.87	12.1 ± 3.94
Segmented (10^3/mm^3)	2.7 ± 1.48	2.2 ± 0.90	1.9 ± 0.57	1.6 ± 0.48	1.5 ± 0.59	1.6 ± 0.61	2.1 ± 1.45	1.2 ± 0.47
Bands (10^3/mm^3)	0.0 ± 0.00	0.0 ± 0.06	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Monocytes (10^3/mm^3)	0.5 ± 0.23	0.4 ± 0.26	0.5 ± 0.20	0.5 ± 0.26	0.2 ± 0.10	0.2 ± 0.20	0.2 ± 0.20	0.2 ± 0.18
Eosinophils (10^3/mm^3)	0.2 ± 0.29	0.2 ± 0.19	0.1 ± 0.18	0.1 ± 0.15	0.3 ± 0.15	0.2 ± 0.16	0.2 ± 0.17	0.1 ± 0.10
Basophils (10^3/mm^3)	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.03
Abnormal L (10^3/mm^3)	0.0 ± 0.06	0.0 ± 0.06	0.0 ± 0.07	0.0 ± 0.03	0.0 ± 0.08	0.0 ± 0.00	0.0 ± 0.03	0.0 ± 0.00

[a] Dosage occurred on days 1 through 90, 91, 92, or 93 of study.
[b] Excludes data for a rat which was found dead on day 85 of study.
[c] Excludes values for rats for which there was insufficient sample.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

ED_000954(915)_Processed_PSTs-2_DD_00000882-00005

EPA-HQ-2016-005679 06/14/2017

Table 6
Microscopic incidence of histopathological findings by dose group in male and female rats.

Tissue	Observation	Dose group (mg/kg-day) and sex (M and F)							
		0		60		200		600	
		M	F	M	F	M	F	M	F
Kidney	Hyperplasia, tubular/ductular epithelium papilla	1/10	–[a]	–	–	1/10	1/10	8/10	6/10
	Edema, focal papillary	–	–	–	–	–	–	3/10	3/10
	Necrosis, papillary (both kidneys)	–	–	–	–	–	–	1/10	–
	Basophilia, tubular, multifocal	1/10	–	–	–	–	–	3/10	1/10
	Hyaline droplets, cortical tubules	2/10	–	–	–	–	–	1/10	1/10
	Mineralization, multifocal	–	5/10	–	5/10	–	2/10	–	2/10
	Pyelonephritis chronic	–	–	–	–	–	–	–	2/10
	Mononuclear cell infiltration, focal	1/10	–	2/10	3/10	4/10	–	–	2/10
Stomach	Dilation, mucosal glands	2/10	–	1/10	3/10	2/10	–	1/10	–
	Necrosis, individual cells in limiting ridge	–	1/10	2/10	–	2/10	1/10	8/10	9/10
	Hyperplasia/hyperkeratosis, limiting ridge	–	–	–	–	–	–	5/10	7/10
Liver	Inflammation, chronic, focal/multifocal	6/10	6/10	–	–	–	–	3/10	4/10
Lymph node, mandibular	Hyperplasia, lymphocytic/plasmacytic	5/10	7/10	–	–	–	–	6/10	6/10
Nasal cavity/turbinates	Necrosis, olfactory epithelium, multifocal	–	–	–	–	–	1/10	2/10	2/10
	Hypertosis, turbinate bone	–	–	–	–	–	–	2/10	2/10
	Inflammation, chronic, focal/multifocal	–	–	–	–	2/10	2/10	2/10	1/10

[a] No occurence found.

of focal papillary edema and a single incidence of papillary necrosis in both kidneys of one male rat in the 600 mg/kg-day dose group.

In stomachs obtained from rats in the 600 mg/kg-day male and female dose groups, increased incidence of necrosis of individual squamous epithelial cells in the limiting ridge of the forestomach was noted as compared to controls. This change was characterized by individual squamous epithelial cells with dark pyknotic nuclei surrounded by a clear cytoplasmic halo. This change was seen at a very low incidence in the rats of the other dose groups, including a control female rat, but the increased incidence of this change, along with minimal- or mild thickening of the mucosa of the limiting ridge due to hyperplasia and hyperkeratosis was considered to be treatment related in the 600 mg/kg-day dose group.

Microscopic examination of the nasal cavity and nasal turbinates revealed a few equivocal microscopic changes that occurred at low and sporadic incidences in rats in the 200 and 600 mg/kg-day dose groups. These changes occurred primarily in the posterior nasal cavity/turbinates. These histomorphologic changes included a single- or low incidence of multifocal necrosis or atrophy of the olfactory mucosa, focal acute/sub-acute or chronic inflammation, adhesions of the turbinate to either and adjacent turbinate or to the lateral nasal wall, focal hyperostosis of turbinate bone and/or foci of olfactory epithelia hyperplasia. Foci of inflammation may occur spontaneously in the nasal cavity of rats, but in several of the above-mentioned lesions, the inflammation was associated with these other changes. The lesions in the nasal cavity/turbinates are of uncertain significance and origin mainly because they occurred only in the 200 and 600 mg/kg-day dose groups at very low and sporadic incidence rates and were focal or multifocal in distribution. The nasal cavity/turbinates of most rats of all groups were histologically unremarkable. The varied and focal isomorphic characteristics of these lesions in the nasal cavity/turbinates are not typical or consistent with a systemic toxic effect. Although the mechanism is unknown, many of these lesions are more suggestive of a local irritating effect on the nasal mucosal membranes.

4. Discussion

On the basis of these data, the NOAEL for K^+PFBS in male rats was 60 mg/kg-day. Absolute and relative (to body weight and brain weight) spleen weights were lower than those of control males at all K^+PFBS treatment levels. However, there was no trend in this reduction across the 10-fold dose range and no adverse histopathological effects were noted. Furthermore, the spleen weight to body weight percent values obtained for K^+PFBS-treated males in this study were close to the central estimate of this parameter based on 16–24-week-old male Sprague–Dawley rats historical control data obtained from 19 studies conducted in the same laboratory between September 1994 and April 2008. In the study reported herein, mean spleen weight to body weight percents of 0.181, 0.158 ($p \leq 0.01$), 0.172, and 0.163 ± 0.020 ($p \leq 0.05$) were obtained for the control, 60, 200, and 600 mg/kg-day dose-group males, respectively. The mean (±S.D., range) for this parameter for controls from the 19 historical control studies was 0.159 (±0.015, 0.142–0.207). Therefore, the splenic weight effects were not considered to be of toxicological significance. The 200 and 600 mg/kg-day doses in the male rats were associated with increased adverse clinical observations and reductions in red blood cells, hemoglobin concentration, and hematocrit. Chloride was significantly increased at the 600 mg/kg-day dose.

PFBS treatment resulted in mild but statistically significant decreases in RBC count in male rats in the 600 mg/kg-day dose group and in hematocrit and hemoglobin in the 200 and 600 mg/kg-day dose groups. Histologic examination of bone marrow in the 600 mg/kg-day dose-group males did not reveal any abnormalities as compared to controls. Taking the hematology data from the study as a whole, this pattern would suggest slight to mild plasma volume expansion; although other potential etiologies cannot be ruled out with the available data. It is of interest to note that the same pattern was observed with the potassium salt of the PFBS congener, perfluorohexanesulfonate (PFHxS) in male rats given 3 and 10 mg/kg-day by gavage for at least 42 days (Butenhoff et al., submitted for publication). The PFBS and PFHxS congener, PFOS, has been demonstrated to be both a PPARffl (Shipley et al., 2004; Sohlenius et al., 1993; Vanden Heuvel et al., 2006; Wolf et al., 2008) and a PPAR] agonist (Vanden Heuvel et al., 2006); although Takacs and Abbott did not observe activation of PPAR] with PFOS (Takacs and Abbott, 2007). Plasma volume expansion is a known effect of thiazolidinediones mediated via PPAR] stimulation of the epithelial sodium channel (ENaC]) of the collecting duct in kidneys (Guan et al., 2007). Typically, weight gain is a consequence of fluid retention with thiazolidinediones, and

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EPA-HQ-2016-005679 06/14/2017

this was not observed with either PFBS or PFHxS. Indeed, PFHxS treatment caused decreased weight gain at the highest dose in males (Butenhoff et al., submitted for publication). In addition, no increase in sodium was noted. Dual activation of PPAR| and PPARffl could mask a weight gain effect due to the increased | -oxidation associated with PPARffl activation. Both PFBS and PFHxS have been shown to activate mouse and human PPARffl (Wolf et al., 2008). Additional investigation would be required to develop a better understanding of the etiology of the mild reduction in RBC count, hematocrit, and hemoglobin occurring in male rats given PFBS.

The NOAEL for K^+PFBS in female rats was 600 mg/kg-day. The 600 mg/kg-day dose was associated with significant reductions in average total protein and albumin values, but reductions were minor and were the only changes observed at the 600 mg/kg-day dose in the female rats.

The treatment-related histopathological findings observed in the study were likely related to the presence of high concentrations of the K^+PFBS surfactant, which is a strong surfactant (K^+PFBS is classified as a moderate eye irritant). The nasal and stomach effects may have been related to irritation from repeated exposure to K^+PFBS via gavage dosing. The microscopic kidney effects (mild tubular hyperplasia and papillary edema) were not associated with functional impairment or damage and may have been due to a response to high concentrations of PFBS passing through the kidney and into the urine. Because PFBS is not metabolized and is excreted rapidly in male and female urine with a serum elimination half-life of approximately 4 h in male rats (Olsen et al., 2008), almost 90% of each daily administered dose would be present in the daily urine output of these rats. For example, at the 600 mg/kg-day dose level, a 300-g rat would have received 200 mg of K^+PFBS per day, and excreted most of it in its daily urine output of about 15 mL; giving a urinary PFBS concentration of at least 1.2% or 12,000 ffig/mL. Although we do not understand the full biological consequences of relatively high concentrations of a strong surfactant in direct contact with the kidney tubular cells, it is likely that this was a factor contributing to the histopathological findings. Further work would be needed to differentiate between irritation and other modes of action in understanding the effects that occurred at the high dose of this study.

In recent years, there has been considerable attention on the potential toxicological properties of a higher molecular weight homolog of PFBS, PFOS (Lau et al., 2004, 2007; Organisation for Economic Cooperation and Development, 2002). When comparing the results of a 90-day dietary study with potassium PFOS in Sprague–Dawley rats (Seacat et al., 2003a, b) to the results reported herein for PFBS, it becomes evident that PFOS is more potent in its ability to elicit toxicological responses, both from the much lower doses required and the extent of responses elicited. In the study reported by Seacat et al. (2003a, b), PFOS was administered in the diet at doses of 0.5, 2, 5, and 20 ffig/g (ppm) for 90 days. Most effects were observed in the 20 ppm dose groups, equivalent to 1.33 ± 0.38 and 1.56 ± 0.35 mg/kg for males and females, respectively, based on diet consumption and feed analyses. Liver weight was increased in males, and relative liver weight was increased in males and females. These liver weight increases had histological correlates in increased centrilobular hepatocellular hypertrophy. This was accompanied with vacuolation and cytoplasmic eosinophilic granulation. The effects were also observed in the males given 5 ppm in the diet (equivalent to 0.34 ± 0.09 mg/kg daily dose). At the 20 ppm dose, average blood urea nitrogen concentrations were increased above control levels in males and females. In males, average alanine aminotransferase activity was increased, and serum total cholesterol was decreased when compared to control levels in males. The only hematological alteration was an increase in segmented neutrophiles in males in the 20-ppm dose group.

By contrast, on gavage dosing with K^+PFBS for 90 days at daily doses up to 600 mg/kg, no alterations in liver cell morphology were noted and observed effects were relatively minor. The decreased spleen weights at all treatment levels in males compared to male controls did not show a trend in reduction over a 10-fold dose range. The nasal and stomach effects may have been related to irritation from repeated exposure to K^+PFBS, and the microscopic kidney effects (mild tubular hyperplasia and papillary edema) were not associated with functional impairment or damage and may have been due to a response to high concentrations of PFBS in the urine. Average values for serum total protein and serum albumin were decreased at 600 mg/kg in females when compared to controls, and, at the 600 mg/kg dose level in males, average serum chloride was increased, and mean values for hematocrit, and hemoglobin were decreased at 200 and 600 mg/kg, with red blood cells reduced at 600 mg/kg. Thus, the pattern of effects elicited by treatment with PFBS for 90 days were different than that elicited by PFOS, and minimal doses to produce effects with PFBS were approximately 600 times higher on an administered dose basis (200 mg/kg versus 0.34 mg/kg, for PFBS and PFOS, respectively) and approximately a 1000 times higher on a molar dose basis. This may be due to the combination of more rapid elimination and reduced binding affinities of the PFBS surfactant ligand as compared to PFOS.

Conflict of interest

Paul H. Lieder, Shu-Ching Chang, and John L. Butenhoff are employed by 3M Company, a manufacturer of potassium perfluorobutanesulfonate. Raymond G. York is an employee of Charles River Preclinical Services which was contracted by 3M Company to perform the in-life portion of the study. The 3M Company funded all aspects of the work reported herein.

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A two-generation oral gavage reproduction study with potassium perfluorobutanesulfonate (K^+PFBS) in Sprague Dawley rats

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abstract

Perfluorobutanesulfonate (PFBS) is a surfactant and degradation product of substances based on perfluorobutanesulfonyl fluoride. A two-generation reproductive rat study has been conducted with potassium PFBS (K^+PFBS). Parental-generation (P) rats were dosed orally by gavage with 0, 30, 100, 300 and 1000 mg K^+PFBS/kg/day for 10 weeks prior to and through mating (males and females), as well as during gestation and lactation (females only). First generation (F1) pups were dosed similarly, beginning at weaning. Second generation (F2) pups were not directly dosed but potentially exposed to PFBS through placental transfer and nursing, and the study was terminated 3 weeks after their birth. Endpoints evaluated included body weight, food consumption, clinical signs, estrus cycling, sperm quality, pregnancy, natural delivery, litter outcomes, and developmental landmarks. The no-observable-adverse effect dose level (NOAEL) in the parental generations (P and F1) was 100 mg/kg/day. In the 300 and 1000 mg/kg/day dose group rats, there were (1) increased liver weight (absolute or relative) and corresponding increased incidence of adaptive hepatocellular hypertrophy (male only) and (2) increased incidence of minimal to mild microscopic findings in the medulla and papilla of the kidneys (male and female). There were no K^+PFBS treatment-related effects on fertility or reproduction among the P or the F1 rats. There were no microscopic changes in male or female reproductive organs, and no biologically relevant effects on sperm parameters, mating, estrous cycles, pregnancy, and natural delivery in the P- or F1-generations. There were no K^+PFBS treatment-related effects on survival of pups in the two-generation study. Litter size and average pup birth weight per litter were not statistically significantly different from controls in any dose group. In the F1-generation, terminal body weight was reduced in males at 1000 mg/kg/day. Preputial separation was slightly delayed (approximately 2 days) at this dose, a finding consistent with the body weight reduction. Essentially no effects were observed in the F1 females. F2 pups had normal body weights. The reproductive NOAEL was >1000 mg/kg/day in both generations.

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1. Introduction

In the 1990s, industry (3M) began developing new technologies to augment and eventually replace a chemical class referred to "perfluorooctanyl" compounds. This chemical class includes perfluorooctanoic acid (PFOA), perfluorooctanesulfonyl fluoride (POSF), and POSF-based chemistries, including N-alkyl-substituted perfluorooctanesulfonamides and perfluorooctanesulfonate (PFOS). In May 2000, 3M announced the voluntary manufacturing phase-out of this chemical class. Subsequently, the United States Environmental Protection Agency (USEPA) issued a Significant New Use Rule, or SNUR, that more broadly regulates the production and import of many perfluoroalkyl substances (PFAS) such as perfluorooctanyl-based chemicals (USEPA, 2002). The USEPA subsequently proposed to amend the PFAS SNUR to include additional perfluorooctanyl-based chemicals (USEPA, 2006). Canada banned the use and importation of a number of long-chain perfluorinated carboxylic acids because of concerns over potential adverse human and environmental effects (CanadaGazette, 2004, 2005). In January 2006, the USEPA announced a voluntary 2010/2015 PFOA Stewardship Program (http://www.epa.gov/opptintr/pfoa/pubs/pfoastewardship.htm) to eliminate emissions and product content of PFOA and related chemicals.

One of the new technologies developed by 3M is based on products made from perfluorobutanesulfonylfluoride (PBSF). Based on atmospheric degradation studies of N-methyl-N-(2-hydroxyethane)-perfluorobutanesulfonamide (D'Eon et al., 2006) and mammalian metabolism studies with the analogous materials made from N-ethoxy-N-(2-hydroxyethanol)-perfluorooctanesulfonamide (Xu et al., 2004, 2006), the degradation and metabolic

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product from materials based on this chemistry ultimately will be perfluorobutanesulfonate (PFBS). Consequently, extensive testing has been performed on this material, which, compared to the perfluorooctanyl-based chemicals, has a low potential to bioaccumulate (NICNAS, 2005; Olsen et al., 2009). This paper describes the reproductive effects of PFBS and briefly compares the effects to those of the perfluorooctanyl compounds, PFOA and PFOS, in rats (Butenhoff et al., 2004; Grasty et al., 2006; Lau et al., 2004, 2006, 2003; Luebker et al., 2005a,b; Thibodeaux et al., 2003).

2. Materials and methods

2.1. Animals

Crl:CD(SD)IGS BR VAF/Plus® (Sprague Dawley) rats were supplied by Charles River Laboratory (Kingston, NY – male rats; and Raleigh, NC – female rats). Rats were housed individually except during mating and lactation. Rats were maintained on a 12-h light/12-h dark cycle and fed Certified Rodent Diet® #5002 (PMI Nutrition International, St. Louis, MO) *ad libitum*. Water was provided *ad libitum* from individual bottles and/or from an automatic watering access system. Bed-o'cobs® bedding (Anderson's Industrial Products, Maumee, OH) was used as the nesting material.

The study was conducted in a facility accredited with by the Association for the Assessment and Accreditation of Laboratory Animal Care International. Procedures involving the use of laboratory rats were reviewed and approved by the facility Institutional Animal Care and Use Committee and conformed to the "Guide for the Care and Use of Laboratory Animals" (ILAR, 1996).

2.2. Test substance

The potassium salt of PFBS (K^+PFBS, Lot 2, 97.90% pure) was provided by 3M Company (St. Paul, MN). The sample was 97.86% normal chain with 0.04% branched as determined by quantitative ^{19}F NMR. Solutions of K^+PFBS were prepared using 0.1% carboxymethyl cellulose in reverse osmosis processed deionized water.

2.3. Study design

The study was performed based on the following guidelines: Organisation for Economic Cooperation and Development (OECD) 416 and USEPA OPPTS 870.3800. The parental-generation (P) consisted of five treatment groups, 30 rats per sex per group. At least one first generation (F1) pup per sex per litter per group was selected for continued evaluation at weaning. K^+PFBS or vehicle (0.1% carboxymethyl cellulose) was administered by daily oral gavage to the P-generation rats, beginning at 6 weeks of age and at least 70 days before cohabitation, at daily dosages of 0, 30, 100, 300 and 1000 mg/kg using a constant volume of 10 mL/kg. The oral route was presumed to be most relevant, and doses were selected based on the results of a previous oral toxicity study in rats with K^+PFBS (Lieder et al., 2009). In addition, gavage dosing delivers a more accurate quantitative dose than other non-parenteral methods. The F1-generation rats were individually identified and given the same dosage level and volume of the test substance as their respective sires and dams beginning at weaning on lactation day (LD) 22. The P- and F1-generation rats were observed for clinical observations, abortions or premature deliveries, and deaths before and 60 min post-dose. Body weights and food consumption values of the male and female rats were recorded weekly and on gestation day (GD) 0, 7, 10, 14, 18, 21 and 25 (if females did not give birth), on LD 1, 5, 8, 11, 15 and 22 for the dams (terminal sacrifice), and for F1-generation rats on attainment of sexual maturation. Food consumption was recorded weekly but was not recorded during cohabitation and after LD 15, because pups begin to consume maternal food on or about this time.

F1-generation male and female rats were examined for age of vaginal patency or balanopreputial separation beginning on day 28 or 29 postpartum, respectively. Estrous cycling was evaluated by examination of vaginal cytology beginning 21 days before cohabitation and until mating. The cohabitation period was a maximum of 14 days. Evidence of mating was determined by spermatozoa observed in a vaginal smear or a copulatory plug observed *in situ*, and this was considered GD 0. On the day of sacrifice, stage of estrous was again determined to ascertain if there was a treatment effect and to aid the pathologist in the microscopic evaluation of uterine tissues.

The P- and F1-generation female rats were evaluated for duration of gestation, fertility and gestation indices, number and sex of offspring, number of implantation sites, condition of dam and litter, litter size, viability index, lactation index, percent survival and sex ratio. Second generation (F2) pup body weights were recorded on LD 1, 5, 8, 15 and 22.

At scheduled termination (after cohabitation for males and on LD 22 for females) P- and F1-generation rats were euthanized by carbon dioxide (CO_2) asphyxiation, necropsied and examined for gross lesions. The brain, kidneys, spleen, ovaries, testes, thymus, liver, adrenal glands, pituitary, uterus with oviducts and cervix, left epididymis, right epididymis, prostate and seminal vesicles with coagulating glands and fluid were individually weighed. The liver, kidneys, pituitary, adrenal glands, vagina, uterus with oviducts, cervix and ovaries, right testis (initially fixed in Bouin's solution), seminal vesicles (with coagulating glands), right epididymis and prostate were retained in formalin for histology.

All F2-generation pups culled on LD 22 were euthanized by CO_2 asphyxiation and three randomly selected pups per sex per litter were examined for gross lesions. The necropsy included a single cross-section of the head at the level of the frontal–parietal suture and examination for apparent hydrocephaly. The brain, spleen, liver, thymus, and kidneys and thymus from the first of the three randomly selected pups per litter were weighed.

Histological evaluation was performed by a Board Certified Veterinary Pathologist. While liver and kidneys collected from all of the control and treatment groups in P-, F1-, and F2-generation rats were examined microscopically, only the reproductive tissues from 10 randomly selected rats per sex from the control and high dosage groups in P- and F1-generations were assessed. Histopathological examination of the testis was conducted in order to identify retained spermatids, missing germ cell layers or types, multinucleated giant cells or sloughing of spermatogenic cells into the lumen. Examination of the intact epididymis (including the caput, corpus and cauda) was conducted in order to identify such lesions as sperm granulomas, leukocytic infiltration (inflammation), aberrant cell types within the lumen, or absence of clear cells in the cauda epididymal epithelium.

Sperm parameters were evaluated in male rats at sacrifice. The P-generation male rats were 106–110 days of age and the F1-generation male rats were 109–120 days of age. A portion of the left cauda epididymis was used for evaluation of cauda epididymal sperm concentration and motility using computer assisted sperm analysis (CASA). The motility was evaluated by the Hamilton Thorne IVOS. The remaining left cauda epididymis was used to manually evaluate sperm morphology. Sperm morphology evaluations included both the determination of the percentage of normal sperm (in a sample of at least 200) and qualitative evaluation of abnormal sperm. The left testis was used for evaluation of testicular spermatid concentration (10 fields) using the CASA (Hamilton Thorne Biosciences Inc., Beverly, MA). The left testis was weighed both before and after removal of the tunica albuginea and then homogenized; a sample from the homogenate was stained with an IDENT stain (Hamilton Thorne Biosciences Inc., Beverly, MA) before IVOS analysis (Hamilton Thorne Biosciences Inc., Beverly, MA).

Uteri of female rats were evaluated at sacrifice. The number and distribution of implantation sites were recorded. Uteri of apparently nonpregnant rats were examined while being pressed between glass plates to confirm the absence of implantation sites. A quantitative evaluation of ovarian follicles was conducted for F1 female rats.

2.4. Statistical analyses

Clinical observations and other proportional data were analyzed using the Variance Test for Homogeneity of the Binomial Distribution (Snedecor and Cochron, 1967). Continuous data (body weights, body weight changes, food consumption data, organ weights, duration of gestation, litter averages for pup body weights, percent male pups, pup viability and cumulative survival) were analyzed using Bartlett's Test of Homogeneity of Variances (Sokal and Rohlf, 1969) and the Analysis of Variance (Snedecor and Cochran, 1967), when Bartlett's Test was not significant ($p > 0.001$). If the Analysis of Variance was significant ($p < 0.05$), Dunnett's test (Dunnett, 1955) was used to identify the statistical significance of the individual groups as compared to the control. If Bartlett's Test was significant ($p \leq 0.001$), the Kruskal–Wallis Test (Sokol and Rohlf, 1969) was used, when 75% or fewer ties were present. When more than 75% ties were present, Fisher's Exact Test (Siegel, 1956) was used. In cases where the Kruskal–Wallis was statistically significant ($p < 0.05$), Dunn's Method of Multiple Comparisons (Dunn, 1964) was used to identify the statistical significance of the individual groups. All other natural delivery data involving discrete data were evaluated using the Kruskal–Wallis Test procedures.

3. Results

3.1. P-generation – reproductive outcome

Results of P-generation male sperm analyses are presented in Table 1. A statistically significant ($p \leq 0.05$) reduction in the number of spermatids per gram testis was observed only in the 1000 mg/kg/day dose group; however, this was not considered biologically meaningful, because there was not a similar statistically significant decrease in the F1-generation males (Table 1) at the same dose level when compared to concurrent controls and the effect was within the historical control of the testing facility (mean 131.3 million sperm per gram of tissue; ranging from 49.7 to 215.3 million for 104 studies conducted from 1998 to 2005). Therefore, there were no effects in K^+PFBS-treated groups relative to controls in sperm motility, sperm counts, and sperm morphology.

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EPA-HQ-2016-005679 06/14/2017

Table 1
Sperm analysis parameters for P- and F1-generation male rats. All numbers are mean ± S.D. (*N*).

	Generation	Dose group (mg/kg/day)				
		0	30	100	300	1000
Motility (%)	P	96 ± 4	95 ± 5	94 ± 12	95 ± 4	95 ± 5
	N	(30)	(30)	(30)	(30)	(28)
	F1	92 ± 10	92 ± 10	94 ± 6	95 ± 3	92 ± 10
	N	(29)	(26)	(29)	(27)	(27)
Morphology (%abnormal)	P	1.7 ± 1.5	2.0 ± 1.3	1.6 ± 1.0	1.5 ± 0.6	2.0 ± 0.6
	N	(30)	(30)	(30)	(30)	(29)
	F1	1.5 ± 0.7	1.3 ± 0.6	1.4 ± 0.5	1.5 ± 0.6	1.9 ± 0.5*
	N	(29)	(27)	(29)	(27)	(27)
Testicular sperm count (10^6/g)	P	148 ± 39	136 ± 34	146 ± 36	132 ± 21	122 ± 36*
	N	(30)	(30)	(30)	(30)	(29)
	F1	124 ± 33	109 ± 34	107 ± 29	110 ± 24	107 ± 32
	N	(29)	(27)	(29)	(27)	(27)
Epididymal sperm count (10^6/g)	P	1030 ± 209	1033 ± 226	957 ± 217	1019 ± 181	969 ± 275
	N	(30)	(30)	(30)	(30)	(29)
	F1	837 ± 237	770 ± 282	840 ± 262	857 ± 222	928 ± 273
	N	(29)	(27)	(29)	(27)	(27)

* Statistically significantly different from control value ($p \leq 0.05$).

Results for P-generation female estrous cycling are presented in Table 2. There were no statistically significant changes from control among K^+PFBS-treated females with respect to estrous cycling, including numbers of rats experiencing ≥6 continuous days of diestrus or ≥6 continuous days of estrus. A comparison of estrus stages at sacrifice showed no differences between K^+PFBS-treated groups and controls.

Absolute sex organ weight data are shown in Table 3. Sex organ weights and the ratios of their weight to body weight and brain weight (data not shown) were similar to control values.

Male and female fertility data are displayed in Tables 4 and 5, respectively. All mating and fertility parameters for the P-generation were unchanged relative to controls in K^+PFBS-treated groups. A slight, non-statistically-significant reduction in the fertility index and the number of pregnant rats per number of rats in cohabitation in the 1000 mg/kg/day dose group was considered unrelated to the test substance because it was within historical control range of the testing facility for the years 1998–2005 (in 2840 mating pairs mean 91.3%, range 50–100%).

P-generation delivery outcomes and the corresponding F1 litter observations are shown in Tables 6 and 7, respectively. There were no toxicologically meaningful changes in delivery outcomes and litter observations.

There were no K^+PFBS treatment-related microscopic changes in sex organs of males and females as compared to controls, including in those rats that were identified as having reduced fertility (data not shown).

Table 2
Estrous cycling of P- and F1-generation female rats.

	Generation	Dose group (mg/kg/day)				
		0	30	100	300	1000
Rats evaluated (*N*)	P	30	30	30	30	30
	F1	30	30	30	30	29
Estrous stages/21 days (mean ± S.D.)	P	4.8 ± 0.9	5.0 ± 0.8	4.9 ± 0.9	4.8 ± 0.7	4.7 ± 1.2
	F1	4.1 ± 1.4	4.1 ± 1.2	3.7 ± 1.5	4.4 ± 1.3	4.5 ± 0.9
≥6 Days of diestrus	P	1	0	0	3	1
	F1	7	10	15**	7	0*
≥6 Days of estrus	P	1	0	0	0	0
	F1	0	0	0	0	1
Stage at sacrifice (*N*)	P	28	28	30	30	30
	F1	30	30	30	29	29
Diestrus	P	24	24	27	28	26
	F1	24	24	28	25	23
Proestrus	P	0	0	0	0	0
	F1	1	1	0	1	0
Estrus	P	3	3	2	1	3
	F1	5	5	0	2	6
Metestrus	P	1	1	1	1	1
	F1	0	0	2	1	0

* Statistically significantly different from control value ($p \leq 0.05$).
** Statistically significantly different from control value ($p \leq 0.01$).

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EPA-HQ-2016-005679 06/14/2017

Table 3
Sex organ weights (mg) in P- and F1-generation male and female rats. All numbers are mean ± S.D. (*N*).

	Generation	Dose group (mg/kg/day)				
		0	30	100	300	1000
Left ovary	P	50 ± 11 (26)	47 ± 13 (29)	48 ± 13 (29)	47 ± 12 (28)	44 ± 10 (25)
	F1	57 ± 11 (23)	61 ± 14 (26)	56 ± 16 (28)	60 ± 12 (25)	56 ± 12 (25)
Right ovary	P	50 ± 9 (26)	47 ± 8 (29)	52 ± 24 (29)	47 ± 12 (28)	48 ± 9 (25)
	F1	57 ± 12 (23)	59 ± 13 (26)	63 ± 12 (28)	59 ± 12 (25)	54 ± 13 (25)
Left testis	P	1806 ± 148 (30)	1790 ± 148 (30)	1779 ± 163 (30)	1763 ± 234 (30)	1815 ± 274 (29)
	F1	1830 ± 163 (29)	1781 ± 236 (27)	1805 ± 146 (29)	1797 ± 156 (27)	1721 ± 229 (27)
Right testis	P	1786 ± 143 (30)	1795 ± 158 (30)	1827 ± 227 (30)	1800 ± 174 (30)	1839 ± 367 (29)
	F1	1819 ± 152 (29)	1770 ± 310 (27)	1804 ± 126 (29)	1819 ± 147 (27)	1785 ± 150 (27)
Left epididymis	P	766 ± 89 (30)	777 ± 96 (30)	737 ± 73 (30)	747 ± 111 (30)	729 ± 106 (29)
	F1	833 ± 101 (29)	800 ± 119 (27)	798 ± 88 (29)	770 ± 82 (27)	778 ± 89 (27)
Right epididymis	P	769 ± 113 (30)	779 ± 96 (30)	754 ± 66 (30)	760 ± 82 (30)	750 ± 110 (29)
	F1	835 ± 120 (29)	819 ± 117 (27)	821 ± 114 (29)	780 ± 100 (27)	808 ± 80 (27)
Left cauda epididymis	P	371 ± 42 (29)	374 ± 47 (30)	355 ± 42 (30)	351 ± 54 (29)	355 ± 62 (29)
	F1	378 ± 49 (29)	367 ± 52 (27)	369 ± 43 (29)	369 ± 50 (27)	365 ± 38 (27)
Seminal vesicles with fluid	P	2059 ± 395 (29)	2025 ± 295 (28)	2003 ± 356 (29)	2013 ± 296 (30)	1984 ± 280 (26)
	F1	2076 ± 400 (29)	2083 ± 441 (27)	2020 ± 429 (29)	2013 ± 396 (27)	1778 ± 396* (27)
Seminal vesicles w/o fluid	P	1094 ± 201 (29)	1087 ± 193 (30)	1076 ± 250 (30)	1099 ± 182 (30)	1069 ± 193 (29)
	F1	1214 ± 244 (29)	1189 ± 288 (27)	1138 ± 227 (29)	1181 ± 244 (27)	1016 ± 190** (27)
Prostate	P	1447 ± 274 (30)	1453 ± 274 (30)	1370 ± 243 (30)	1414 ± 219 (30)	1370 ± 176 (29)
	F1	1221 ± 287 (29)	1286 ± 242 (27)	1142 ± 317 (29)	1272 ± 309 (27)	1241 ± 243 (27)

* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group value ($p \leq 0.01$).

3.2. F1-generation – reproductive outcome

For F1 males, sperm motility and sperm counts were not statistically significantly different between the K^+PFBS-treated groups and the control group (Table 1). Although no treatment-related differences in head and/or tail abnormalities were observed, and the incidences of these abnormalities were low in all groups an incidental but statistically significant ($p < 0.05$) increase in the percent of abnormal sperm in the 1000 mg/kg/day dose group was observed. The extent of this increase was not considered biologically meaningful, because the mean percent of abnormal sperm in the P- and F1-generation rats was similar.

For F1 females, the average numbers of estrous stages per 21 days were not statistically significantly different among the five groups (Table 2). The number of rats with ≥6 consecutive days of diestrus was significantly increased ($p \leq 0.01$) in the 100 mg/kg/day dose group and significantly decreased ($p \leq 0.05$) in the 1000 mg/kg/day dose group. These statistically significant changes were not considered K^+PFBS treatment-related, because they were not dose-dependent; and they had no effect on fertility or mating.

Sex organ weights are presented in Table 3. The absolute weight of seminal vesicles with and without fluid was reduced in F1-generation males of the 1000 mg/kg/day group. However, this reduction was not apparent when compared on the basis of organ

Table 4
Male fertility of P- and F1-generation rats.

	Generation	Dose group (mg/kg/day)				
		0	30	100	300	1000
Number of cohabitating	P	30	30	30	30	29
	F1	30	28	30	28	29
Days in cohabitation (mean ± S.D.)	P	2.6 ± 1.1	2.5 ± 1.2	3.0 ± 2.4	2.7 ± 1.4	3.1 ± 2.4
	F1	3.0 ± 2.0	2.8 ± 1.0	2.4 ± 1.1	3.3 ± 3.9	2.8 ± 1.4
Percent mating	P	100	100	96.7	100	100
	F1	100	100	100	92.8	100
Rats with confirmed mating date	P	30	30	29	30	29
	F1	30	28	30	26	29
Rats mating days 1–7	P	30	30	29	29	28
	F1	29	28	30	25	29
Rats mating days 8–14	P	0	0	0	1	1
	F1	1	0	0	1	0
Number of pregnancies/number of cohab	P	27/30	29/30	29/30	29/30	24/29
	F1	24/30	24/28	28/30	24/28	27/29
Fertility index[a] (%)	P	90.0	96.7	100	96.7	82.8
	F1	80.0	85.7	93.3	92.3	93.1

[a] Fertility is the number pregnant divided by the number with evidence of mating (sperm in uterine lavage) and is restricted to rats with a confirmed mating date and rats that did not mate. (E.g, in the P-generation 100 mg/kg/day dose group, 30 rats were paired but only 29 had evidence of mating and all 29 were pregnant. Hence 29/29 = 100%).

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EPA-HQ-2016-005679 06/14/2017

Table 5
Female fertility of P- and F1-generation rats.

	Generation	Dose group (mg/kg/day)				
		0	30	100	300	1000
Number of cohabitating	P	30	30	30	30	30
	F1	30	30	30	30	29
Days in cohabitation (mean ± S.D.)	P	2.6 ± 1.1	2.5 ± 1.2	3.0 ± 2.4	2.7 ± 1.4	3.2 ± 2.4
	F1	3.0 ± 2.0	2.7 ± 1.0	2.4 ± 1.1	3.3 ± 3.8	2.8 ± 1.4
Rats with confirmed mating date	P	30	30	29	30	30
	F1	30	30	30	28	29
Rats mating days 1–7	P	30	30	29	29	29
	F1	29	30	30	27	29
Rats mating days 8–14	P	0	0	0	1	1
	F1	1	0	0	1	0
Number of pregnancies/number of cohab	P	27/30	29/30	29/30	29/30	25/30
	F1	24/30	26/30	28/30	25/30	27/29
Fertility index[a] (%)	P	90.0	96.7	100	96.7	83.3
	F1	80.0	86.7	93.3	89.3	93.1

[a] Fertility is the number pregnant divided by the number with evidence of mating (sperm in uterine lavage) and is restricted to rats with a confirmed mating date and rats that did not mate. (E.g., in the P-generation 100 mg/kg dose group, 30 rats were paired but only 29 had evidence of mating and all 29 were pregnant. Hence 29/29 = 100%).

weight to body weight ratios. Seminal vesicles with fluid were 0.351 ± 0.074% and 0.325 ± 0.073% of body weight in the control and high-dose males, respectively, and seminal vesicles without fluid were 0.204 ± 0.042% and 0.185 ± 0.038% of body weight in the control and 1000 mg/kg/day dose-group males, respectively. Because mean terminal body weights in the 1000 mg/kg/day dose-group males were reduced by approximately 9% from the control mean with statistical significance ($p \leq 0.01$), the effect on absolute seminal vesicle weight was likely the result of reduced body weight in the 1000 mg/kg/day dose-group.

Male and female mating and fertility data are shown in Tables 4 and 5, respectively. All F1-generation mating and fertility parameters measured were unaffected by K^+PFBS treatment. F1-generation delivery outcomes and the corresponding F1 litter observations are shown in Tables 6 and 7, respectively. There were no toxicologically meaningful changes in delivery outcomes and litter observations.

There were no K^+PFBS treatment-related microscopic changes in sex organs of F1 males and female rats as compared to controls, including those rats identified as having reduced fertility (data not shown). Ovarian follicle counts for females in the 1000 mg/kg/day dose group were not different than controls.

3.3. F1-generation – sexual maturation

Sexual maturation data for F1-generation male and female rats are presented in Table 8. In F1-generation males, the mean days to preputial separation was delayed with statistical significance in the 30 and 1000 mg/kg/day dose groups by 0.6 ($p \leq 0.05$) and 1.6 ($p \leq 0.01$) days, respectively. Mean (±S.D.) male body weights per group on the day of sexual maturation are also presented in Table 8. These data were used in analysis of co-variance for days to preputial separation versus body weight on day of preputial separation. When adjusted for body weight on day of preputial separation, there were no statistically significant differences across groups.

There were no advances or delays among K^+PFBS-treated females for the mean days to vaginal patency when compared to controls.

3.4. P-generation males – toxicology endpoints

3.4.1. Clinical observations

One male rat death occurred in the 1000 mg/kg/day dose group P-generation due to an intubation accident. The numbers of male rats in the 1000 mg/kg/day presenting with excessive salivation

Table 6
Delivery outcomes of P- and F1-generation dams.

	Dams	Dose group (mg/kg/day)				
		0	30	100	300	1000
Number of dams delivering	P	26	29	29	29	25
	F1	24	26	28	25	27
Length of gestation, days (mean ± S.D.)	P	22.7 ± 0.5	22.8 ± 0.4	22.8 ± 0.5	22.8 ± 0.4	22.7 ± 0.4
	F1	22.7 ± 0.5	22.8 ± 0.5	22.6 ± 0.5	22.6 ± 0.5	22.8 ± 0.4
Deliveries (%)	P	100	100	100	100	100
	F1	100	100	100	100	100
Percentage with liveborn pups[a]	P	100	100	100	100	100
	F1	100	100	100	100	100
Number with stillborn pups[b]	P	2	5	1	4	2
	F1	5	1	5	5	1
Mean implantation sites (mean ± S.D.)	P	15.2 ± 1.8	14.6 ± 3.0	14.3 ± 2.3	14.7 ± 1.7	14.0 ± 2.3
	F1	14.3 ± 2.3	14.6 ± 2.3	15.2 ± 2.0	15.2 ± 2.8	15.3 ± 2.0
Mean pups delivered (mean ± S.D.)	P	14.2 ± 2.2	13.9 ± 3.0	13.8 ± 3.0	14.0 ± 2.0	13.6 ± 2.3
	F1	13.2 ± 2.3	13.8 ± 2.2	14.1 ± 2.3	14.0 ± 2.1	14.0 ± 2.1

[a] Indicates the number of dams producing a litter containing at least one liveborn pup.
[b] Indicates the number of dams with at least one stillborn pup.

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EPA-HQ-2016-005679 06/14/2017

Table 7
Litter outcomes for F1- and F2-generation pups.

	Pups	Dose group (mg/kg/day)				
		0	30	100	300	1000
Number of litters delivered	F1	26	29	29	29	25
	F2	24	26	28	25	27
Mean (± S.D.) liveborn (*N*)	F1	14.2 ± 2.2	13.5 ± 2.8**	13.7 ± 3.0	13.8 ± 2.0	13.5 ± 2.3
	F2	13.0 ± 2.3	13.6 ± 2.3	13.9 ± 2.4	13.6 ± 2.6	14.0 ± 2.0
Mean (± S.D.) stillborn (*N*)	F1	0.1 ± 0.3	0.2 ± 0.6	0.1 ± 0.4	0.1 ± 0.4	0.1 ± 0.3
	F2	0.2 ± 0.4	0.1 ± 0.6	0.2 ± 0.5	0.4 ± 1.0	0.0 ± 0.2
Mean (± S.D.) pup weight at birth (g)	F1	6.4 ± 0.4	6.5 ± 0.5	6.6 ± 0.5	6.4 ± 0.4	6.3 ± 0.5
	F2	6.3 ± 0.6	6.4 ± 0.5	6.4 ± 0.4	6.2 ± 0.5	6.2 ± 0.8
Mean (± S.D.) pup weight at weaning (g)	F1	40.2 ± 5.6	40.2 ± 6.8	40.8 ± 7.8	39.2 ± 5.9[a]	39.3 ± 4.4
	F2	36.4 ± 7.7[b]	39.4 ± 7.3	37.1 ± 5.1	36.3 ± 6.6	35.0 ± 5.6[c]
Viability index[d] (%)	F1	98.1	96.7*	98.2	99.2	99.4*
	F2	95.5	96.9	97.2	96.8	95.8
Lactation index[e] (%)	F1	99.4	98.7	97.4**	97.7**	99.7
	F2	97.0	96.5	98.2	97.3	93.1**

[a] *N* = 28. Excludes values for litters wioth no surviving pups.
[b] *N* = 23. Excludes values for litters wioth no surviving pups.
[c] *N* = 25. Excludes values for litters wioth no surviving pups.
[d] (Number of pups alive on day 4/number of pups alive on day 1) × 100.
[e] (Number of pups alive on day 21/number of pups alive on day 4) × 100.
* Statistically significantly different from control value ($p \leq 0.05$).
** Statistically significantly different from control value ($p \leq 0.01$).

(144 instances among 27 rats), perioral substance (11 instances among 9 rats), and urine-stained abdominal fur (9 instance among 4 rats) were increased with statistical significance as compared to controls. No other K^+PFBS treatment-related clinical signs were observed in P-generation males.

3.4.2. Body weight and food consumption

Body weights on the initial day of dosing and on the day of terminal sacrifice as well as body weight change through terminal sacrifice are presented in Tables 9a for P-generation males. There were no statistically significant changes in mean absolute body weights at terminal sacrifice; therefore, treatment with K^+PFBS at doses up to 1000 mg/kg/day in P-generation male rats had no overall effect on body weight. In addition to the summary data provided in Table 9a, there were isolated periods of statistically significant differences in weight change in K^+PFBS-treated rats relative to control rats that were not considered treatment-related. Mean body weight gains were increased relative to controls between study days 36 and 43 in the 100 and 1000 mg/kg/day dose groups ($p \leq 0.05$ and $p \leq 0.01$, respectively) and between study days 64 and 70 in the 300 mg/kg/day dose group ($p \leq 0.01$). Decreased body weight gain relative to controls occurred between study days 43 and 57 ($p \leq 0.01$) in the 1000 mg/kg/day dose group, and a slight but significant ($p \leq 0.01$) negative body weight gain (representing body weight loss) occurred between study days 105 and 112 in the 1000 mg/kg/day dose group.

No K^+PFBS treatment-related changes in absolute or relative food consumption relative to controls occurred among P-generation male rats (data not shown).

3.4.3. Necropsy, histology and organ weight changes

There were no K^+PFBS treatment-related gross anatomical findings in P-generation male rats. As stated previously, no K^+PFBS treatment-related microscopic changes were present in reproductive organs of the 1000 mg/kg/day dose group P-generation male rats which were selected for histopathological evaluation. The microscopic incidence of mild enlargement of liver cells (hypertrophy) and minimal to mild proliferation of kidney medullary/papillary tubular and ductular epithelial cells (hyperplasia) was increased in the 300 and 1000 mg/kg/day dose groups (Table 10). Twenty-six of 30 (87%) 1000 mg/kg/day dose group P-generation male rats had hepatocellular hypertrophy with 25 minimal and 1 mild in severity. Three of 30 (10%) 300 mg/kg/day dose group males had minimal hepatocellular hypertrophy. In addition, six incidences of focal papillary edema (five minimal and one mild in severity) and one incident of moderate focal papillary necrosis were noted in the P-generation male rats in the 1000 mg/kg/day dose group. There were no other K^+PFBS

Table 8
Days to sexual maturation in F1-generation rats (mean ± S.D.). Mean body weight data (mean ± S.D.) at preputial separation for male rats are also included.

	Dose group (mg/kg/day)				
	0	30	100	300	1000
Males (*N*)	30	29	30	30	30
Days to preputial separation	47.7 ± 3.4	48.3 ± 1.8*	47.9 ± 2.3	48.2 ± 2.2	49.3 ± 1.8**
Weight (g) at preputial separation[a]	222.4 ± 20.1	223.2 ± 17.6	222. 4 ± 17.2	221.8 ± 17.7	219.6 ± 19.2
Females (*N*)	30	30	30	30	30
Days to vaginal patency	35.0 ± 2.5	35.6 ± 2.0	34.8 ± 1.6	35.9 ± 2.0	35.7 ± 1.8

[a] These data were used in analysis of co-variance for days to preputial separation versus body weight on day of preputial separation. When adjusted for body weight on day of preputial separation, there were no statistically significant differences across groups in days to preputial separation.
* Statistically significantly different from control value ($p \leq 0.05$).
** Statistically significantly different from control value ($p \leq 0.01$).

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EPA-HQ-2016-005679 06/14/2017

Table 9a
Body weight and body weight change in P-generation male and female rats (mean ± S.D.) (*N*).

Time point during study	Dose group (mg/kg/day)				
	0	30	100	300	1000
P-generation males					
Study day 1	181 ± 8 (30)	177 ± 17 (30)	179 ± 8 (30)	180 ± 11 (30)	178 ± 9 (30)
Termination	562 ± 56 (30)	569 ± 53 (30)	570 ± 45 (30)	572 ± 69 (30)	551 ± 46 (29)
Change	381 ± 54 (30)	392 ± 53 (30)	391 ± 44 (30)	393 ± 66 (30)	373 ± 44 (29)
P-generation females					
Study day 1	153 ± 7 (30)	153 ± 7 (30)	154 ± 8 (30)	152 ± 8 (30)	152 ± 7 (30)
Study day 70	285 ± 27 (30)	282 ± 29 (30)	289 ± 25 (30)	278 ± 28 (30)	276 ± 24 (30)
Change	132 ± 23 (30)	129 ± 25 (30)	135 ± 20 (30)	126 ± 23 (30)	124 ± 19 (30)
Gestation day 0	281 ± 26 (27)	284 ± 30 (29)	286 ± 25 (29)	277 ± 26 (29)	276 ± 22 (25)
Gestation day 21	426 ± 36 (22)	429 ± 50 (23)	430 ± 38 (22)	409 ± 33 (22)	409 ± 22 (19)
Change	143 ± 16 (22)	143 ± 24 (23)	143 ± 19 (22)	135 ± 21 (22)	133 ± 24 (19)
Lactation day 1	319 ± 28 (26)	317 ± 32 (29)	323 ± 29 (29)	313 ± 30 (29)	311 ± 18 (25)
Lactation day 22	346 ± 23 (26)	343 ± 26 (29)	351 ± 23 (29)	344 ± 30 (28)	336 ± 22 (25)
Change	27 ± 19 (26)	26 ± 16 (29)	29 ± 23 (29)	30 ± 18 (28)	26 ± 20 (25)

Table 9b
Body weight and body weight change in F1-generation male and female rats (mean ± SD) (*N*).

Time point during study	Dose group (mg/kg/day)				
	0	30	100	300	1000
F1-generation pups, pre-weaning (by litter)					
Postnatal day 1	6.4 ± 0.4 (26)	6.5 ± 0.5 (29)	6.6 ± 0.5 (29)	6.4 ± 0.4 (29)	6.3 ± 0.5 (25)
Postnatal day 22 (weaning)	40.2 ± 5.6 (26)	40.2 ± 6.8 (29)	40.8 ± 7.8 (29)	39.2 ± 5.9 (28)	39.3 ± 4.4 (25)
F1-generation males, post-weaning					
Post-weaning day 1	47 ± 5 (30)	46 ± 7 (30)	46 ± 8 (30)	45 ± 6 (30)	45 ± 5 (30)
Termination	594 ± 45 (29)	583 ± 61 (27)	593 ± 73 (29)	598 ± 65 (27)	549 ± 38** (27)
Change	546 ± 44 (29)	537 ± 59 (27)	547 ± 69 (29)	552 ± 63 (27)	504 ± 36** (27)
F1-generation females, post-weaning					
Post-weaning day 1	45 ± 7 (30)	45 ± 6 (30)	45 ± 9 (30)	44 ± 6 (30)	44 ± 4 (30)
Day of cohabitation[a]	277 ± 28 (30)	287 ± 28 (30)	288 ± 27 (30)	282 ± 24 (30)	274 ± 22 (29)
Change	232 ± 26 (30)	242 ± 27 (30)	243 ± 25 (30)	238 ± 22 (30)	231 ± 23 (29)
Gestation day 0	273 ± 26 (24)	287 ± 27 (26)	285 ± 25 (28)	279 ± 23 (25)	272 ± 22 (27)
Gestation day 21	407 ± 33 (22)	437 ± 32** (23)	435 ± 34** (20)	417 ± 30 (19)	412 ± 26 (21)
Change	132 ± 23 (22)	144 ± 27 (23)	146 ± 20 (20)	141 ± 18 (19)	141 ± 14 (21)
Lactation day 1	303 ± 27 (24)	319 ± 30 (26)	316 ± 27 (28)	311 ± 26 (25)	306 ± 26 (27)
Lactation day 22	331 ± 29 (23)	350 ± 33* (26)	353 ± 26** (28)	347 ± 16* (25)	348 ± 23* (25)
Change	29 ± 23 (23)	31 ± 26 (26)	37 ± 17 (28)	36 ± 20 (25)	38 ± 19 (25)

* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group value ($p \leq 0.01$).
[a] Rats were 102–110 days old.

treatment-related histopathological changes, including the spleen, thymus, and pituitary (data not shown).

P-generation male absolute and relative organ weight data for brain, liver, kidneys, and spleen are provided in Table 11. Liver weights (absolute and relative to body weight and brain weight) were statistically significantly increased in the 300 and 1000 mg/kg/day dose groups. Although mean liver weight relative to brain weight was increased with statistical significance in the 30 mg/kg/day dose-group males, this was likely due to the fact that mean absolute brain weight was slightly reduced but with statistical significance in the 30 mg/kg/day K^+PFBS dose group only; however, brain weight as a percent to body weight was not affected in this group. The relative left kidney weight to brain weight ratio was significantly increased in the 30 mg/kg/day K^+PFBS dose group, however, the absolute left kidney weight was not affected at this dose level and the finding was not dose-dependent. There were no other statistically significant changes relative to controls in absolute or relative organ weights.

3.5. P-generation females – toxicology endpoints

3.5.1. Clinical observations

There were no K^+PFBS treatment-related P-generation female rat deaths. One control rat died on GD 23, and one 300 mg/kg/day

Table 9c
Body weight and body weight change in F2-generation male and female rats (mean ± S.D.) (*N*).

Time point during study	Dose group (mg/kg/day)				
	0	30	100	300	1000
F2-Generation pups, pre-weaning (by litter)					
Postnatal day 1	6.3 ± 0.6 (24)	6.4 ± 0.5 (26)	6.4 ± 0.4 (28)	6.2 ± 0.5 (25)	6.2 ± 0.8 (27)
Postnatal day 22	36.4 ± 7.7 (23)	39.4 ± 7.3 (26)	37.1 ± 5.1 (28)	36.3 ± 6.6 (25)	35.0 ± 5.6 (25)

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EPA-HQ-2016-005679 06/14/2017

Table 10
Incidence and degree of severity of histomorphologic observations in livers and kidneys of P- and F1-generation rats.

Generation	Sex	Dose mg/kg	Liver	Kidney			
			Hepatocellular hypertrophy	Papillary epithelial tubular/ductal hyperplasia	Focal papillary edema	Focal necrosis of the papilla	Focal cortical tubular dilatation
			N[a]/30 (*N*[b]/deg.[c])	*N*/30 (*N*/deg.)	*N*/30 (*N*/deg.)	*N*/30 (*N*/deg.)	*N*/30 (*N*/deg.)
P	M	Cont[d]	0	0	1 (1+)	0	0
F1	M	Cont	0	3 (3+)	1 (1+)	0	0
P	M	300	3 (3+)	9 (7+; 2++)	2 (2+)	0	0
F1	M	300	3 (3+)	5 (4+; 1++)	0	2 (2+)	0
P	M	1000	26 (25+; 1++)	19 (9+; 9++; 1+++)	6 (5+; 1++)	1 (1++)	1 (1+)
F1	M	1000	14 (13+; 1++)	21 (8+; 13++)	9 (9+)	0	1 (1+)
P	F	Cont	0	3 (1+; 2++)	1 (1+)	0	0
F1	F	Cont	0	2 (2+)	0	0	1 (1++)
P	F	300	0	16 (7+; 8++; 1+++)	8 (7+; 1++)	3 (1+; 2++)	1 (1+++)
F1	F	300	0	13 (7+; 5++; 1+++)	7 (6+; 1++)	1 (1++)	1 (1+)
P	F	1000	0	21 (9+; 12++)	7 (7+)	0	1 (1++)
F1	F	1000	0	15 (7+; 7++; 1+++)	4 (3+; 1++)	1 (1++)	0

Key: M = males; F = females.
[a] Number of observations per 30 rats.
[b] Number of observations of a specific degree of severity are given in parentheses.
[c] Degree of severity (+ = minimal severity; ++ = mild severity; +++ = moderate severity).
[d] Control rats.

Table 11
P- and F1-generation male absolute oragn weights (g) and relative organ weights (%) (mean ± S.D.) (*N*).

	Gen[a]	Dose group (mg/kg/day)				
		0	30	100	300	1000
Body at term	P	562 ± 56 (30)	569 ± 53 (30)	570 ± 45 (30)	572 ± 69 (30)	551 ± 46 (29)
	F1	594 ± 45 (29)	583 ± 61 (27)	593 ± 73 (29)	598 ± 65 (27)	549 ± 38** (27)
Brain	P	2.14 ± 0.14 (30)	2.05 ± 0.12* (30)	2.17 ± 0.15 (30)	2.12 ± 0.14 (29)	2.14 ± 0.12 (29)
	F1	2.04 ± 0.19 (29)	2.03 ± 0.14 (25)	2.06 ± 0.12 (28)	2.00 ± 0.20 (27)	1.99 ± 0.12 (27)
Percentage to body[b]	P	0.38 ± 0.04 (30)	0.36 ± 0.04 (30)	0.38 ± 0.04 (30)	0.37 ± 0.05 (29)	0.39 ± 0.03 (29)
	F1	0.34 ± 0.04 (29)	0.35 ± 0.04 (25)	0.35 ± 0.05 (28)	0.34 ± 0.04 (27)	0.36 ± 0.03 (27)
Liver	P	19.2 ± 2.4 (30)	20.0 ± 2.9 (30)	20.5 ± 2.4 (30)	21.5 ± 2.9** (30)	2.7 ± 2.8** (29)
	F1	20.6 ± 2.5 (29)	20.1 ± 2.7 (27)	21.2 ± 4.4 (29)	21.7 ± 3.2 (27)	21.1 ± 2.2 (27)
Percentage to brain[c]	P	901 ± 113 (30)	982 ± 156* (30)	947 ± 118 (30)	1015 ± 125** (29)	1061 ± 121** (29)
	F1	1017 ± 148 (29)	988 ± 145 (25)	1038 ± 244 (28)	1097 ± 214 (27)	1062 ± 113 (27)
Percentage to body	P	3.4 ± 0.3 (30)	3.5 ± 0.4 (30)	3.6 ± 0.3 (30)	3.8 ± 0.3** (30)	4.1 ± 0.4** (29)
	F1	3.5 ± 0.4 (29)	3.4 ± 0.3 (27)	3.5 ± 0.4 (29)	3.6 ± 0.3 (27)	3.8 ± 0.3** (27)
Left kidney	P	2.10 ± 0.21 (30)	2.17 ± 0.20 (30)	2.18 ± 0.20 (30)	2.20 ± 0.28 (30)	2.20 ± 0.26 (29)
	F1	2.04 ± 0.21 (29)	2.06 ± 0.23 (27)	2.08 ± 0.20 (29)	2.14 ± 0.24 (27)	1.97 ± 0.16 (27)
Percentage to brain	P	98.2 ± 8.7 (30)	106.4 ± 112** (30)	100.7 ± 9.7 (30)	103.8 ± 11.1 (29)	102.9 ± 11.7 (29)
	F1	101.1 ± 12.2 (29)	101.7 ± 12.3 (25)	101.1 ± 10.8 (28)	108.3 ± 19.3 (27)	99.2 ± 8.0 (27)
Percentage to body	P	0.375 ± 0.034 (30)	0.382 ± 0.035 (30)	0.383 ± 0.032 (30)	0.385 ± 0.042 (30)	0.400 ± 0.047 (29)
	F1	0.344 ± 0.030 (29)	0.352 ± 0.021 (27)	0.353 ± 0.033 (29)	0.358 ± 0.038 (27)	0.357 ± 0.029 (27)
Right kidney	P	2.15 ± 0.22 (30)	2.18 ± 0.21 (30)	2.20 ± 0.21 (30)	2.21 ± 0.28 (30)	2.22 ± 0.25 (29)
	F1	2.05 ± 0.20 (29)	2.08 ± 0.22 (27)	2.09 ± 0.20 (28)	2.14 ± 0.25 (27)	2.00 ± 0.18 (27)
Percentage to brain	P	100.6 ± 8.8 (30)	106.7 ± 11.8 (30)	101.5 ± 11.1 (30)	104.5 ± 10.9 (29)	103.9 ± 10.4 (29)
	F1	101.5 ± 11.6 (29)	103.0 ± 12.0 (25)	101.3 ± 10.5 (27)	108.6 ± 20.6 (27)	100.4 ± 7.0 (27)
Percentage to body	P	0.383 ± 0.041 (30)	0.384 ± 0.034 (30)	0.385 ± 0.033 (30)	0.387 ± 0.042 (30)	0.403 ± 0.040 (29)
	F1	0.346 ± 0.030 (29)	0.356 ± 0.020 (27)	0.356 ± 0.029 (28)	0.359 ± 0.040 (27)	0.364 ± 0.032 (27)
Spleen	P	0.86 ± 0.13 (30)	0.92 ± 0.18 (30)	0.89 ± 0.11 (30)	0.88 ± 0.19 (30)	0.86 ± 0.09 (29)
	F1	0.90 ± 0.19 (29)	0.88 ± 0.08 (27)	0.87 ± 0.17 (29)	0.84 ± 0.14 (27)	0.79 ± 0.12 (27)
Percentage to brain	P	40.5 ± 6.1 (30)	44.9 ± 9.4 (30)	41.3 ± 5.3 (30)	41.9 ± 8.5 (29)	40.3 ± 4.7 (29)
	F1	44.7 ± 11.7 (29)	43.4 ± 5.3 (25)	42.8 ± 8.4 (28)	42.9 ± 9.8 (27)	39.8 ± 6.9 (27)
Percentage to body	P	0.153 ± 0.023 (30)	0.161 ± 0.030 (30)	0.156 ± 0.017 (30)	0.153 ± 0.025 (30)	0.157 ± 0.019 (29)
	F1	0.151 ± 0.033 (29)	0.151 ± 0.018 (27)	0.147 ± 0.025 (29)	0.141 ± 0.024 (27)	0.144 ± 0.023 (27)

[a] Generation.
[b] Percentage to body weight = (organ weight/terminal body weight) × 100.
[c] Percentage to terminal brain weight = (organ weight/brain weight) × 100.
* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group ($p \leq 0.01$).

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EPA-HQ-2016-005679 06/14/2017

dose group rat was moribund sacrificed on LD 15 for reasons believed not to be related to K^+PFBS treatment. During the precohabitation period, females in the 1000 mg/kg/day dose group experienced increased occurrences of dried or wet red perioral substance (7 of 30) and excessive salivation (5 of 30), and, during gestation, 1000 mg/kg/day dose group females continued to have red perioral substance (3 of 30).

3.5.2. Body weight and food consumption

Initial and terminal mean body weight and body weight change of the P-generation female rats during the precohabitation, gestation, and lactation periods are shown in Table 9a. There were no statistically significant findings in the presented data. In addition to the data provided in Table 9a, there were instances of statistically significant reduced body weight and weight gain in the 1000 mg/kg/day dose group compared to controls: (1) mean body weights were reduced on days 8 and 11 of lactation ($p \leq 0.05$ and 0.01, respectively) and (2) mean weight gain was reduced during the last week of dosing prior to mating (precohabitation days 64–70, $p \leq 0.01$) and during the 1st week of gestation ($p \leq 0.01$). Despite these isolated instances in the highest dose group, overall mean body weights and mean body weight changes at the end of each of these three periods were not significantly different from controls in all K^+PFBS-treated groups.

Statistically significant increases in mean relative food consumption compared to control P-generation female rats occurred in the 100 ($p \leq 0.05$), 300 ($p \leq 0.05$), and 1000 mg/kg/day ($p \leq 0.05$) dose groups between precohabitation days 29 and 36 and again in the 300 ($p \leq 0.05$) and 1000 mg/kg/day ($p \leq 0.05$) dose groups between precohabitation days 36 and 43. A decrease in absolute food consumption relative to control occurred between GD 10 and 14 in the 300 and 1000 mg/kg/day dose groups ($p \leq 0.05$). Through precohabitation, there was a significant ($p \leq 0.05$) decrease in overall relative food consumption in the 300 mg/kg/day dose group compared to controls. These were the only statistically significant changes from control noted for absolute and relative food consumption.

3.5.3. Necropsy, histology, and organ weight changes

There were no treatment-related gross anatomical findings in P-generation female rats.

Treatment-related microscopic findings were observed in the kidneys of female rats in the 300 and 1000 mg/kg/day dose groups (Table 10). These findings consisted of an increased incidence of minimal-to-moderate hyperplasia of the tubular and ductular epithelium of the inner medulla/papilla, and primarily minimal focal papillary edema in the 300 and 1000 mg/kg/day dose groups. In addition, three incidents of minimal-to-moderate focal papillary necrosis were observed in the 300 mg/kg/day dose group; although, none were observed in the 1000 mg/kg/day dose group. No liver hypertrophy was observed in the P-generation female rats.

Absolute and relative organ weight data for P-generation female rats are presented in Table 12 for brain, kidneys, and spleen. The mean absolute weight of the brain was reduced in the 1000 mg/kg/day dose group with statistical significance (2.04 ± 0.10 versus 2.13 ± 0.09 in controls). For brain weight rela-

Table 12
P- and F1-generation female absolute organ weights (g) and relative organ weights (%) (g) (mean ± S.D.) (*N*).

	Gen[a]	Dose group (mg/kg/day)				
		0	30	100	300	1000
Body at term	P	346 ± 23 (26)	343 ± 26 (29)	351 ± 23 (29)	344 ± 30 (28)	336 ± 22 (25)
	F1	331 ± 29 (23)	350 ± 33* (26)	353 ± 26** (28)	347 ± 16* (25)	348 ± 23* (25)
Brain	P	2.13 ± 0.09 (26)	2.11 ± 0.08 (29)	2.10 ± 0.15 (29)	2.12 ± 0.09 (28)	2.04 ± 0.10** (25)
	F1	2.11 ± 0.11 (23)	2.12 ± 0.11 (26)	2.18 ± 0.08 (28)	2.14 ± 0.10 (25)	2.10 ± 0.07 (25)
Percentage to body[b]	P	0.62 ± 0.05 (26)	0.62 ± 0.04 (29)	0.60 ± 0.05 (29)	0.62 ± 0.06 (28)	0.61 ± 0.05 (25)
	F1	0.64 ± 0.06 (23)	0.61 ± 0.07 (26)	0.62 ± 0.05 (28)	0.62 ± 0.03 (25)	0.61 ± 0.05 (25)
Left kidney	P	1.41 ± 0.12 (26)	1.42 ± 0.15 (29)	1.41 ± 0.15 (29)	1.48 ± 0.18 (28)	1.42 ± 0.17 (25)
	F1	1.51 ± 0.16 (23)	1.51 ± 0.13 (26)	1.52 ± 0.14 (28)	1.52 ± 0.18 (25)	1.48 ± 0.16 (25)
Percentage to brain[c]	P	66.1 ± 5.4 (26)	67.6 ± 6.9 (29)	67.6 ± 6.5 (29)	69.6 ± 7.8 (28)	69.8 ± 8.1 (25)
	F1	71.6 ± 8.0 (23)	71.3 ± 7.2 (26)	70.2 ± 6.9 (28)	70.7 ± 7.0 (25)	70.6 ± 7.1 (25)
Percentage to body	P	0.407 ± 0.032 (26)	0.416 ± 0.035 (29)	0.402 ± 0.034 (29)	0.430 ± 0.055 (28)	0.422 ± 0.051 (25)
	F1	0.462 ± 0.092 (23)	0.434 ± 0.039 (26)	0.433 ± 0.032 (28)	0.438 ± 0.046 (25)	0.428 ± 0.047 (25)
Right kidney	P	1.41 ± 0.13 (26)	1.39 ± 0.14 (29)	1.42 ± 0.16 (29)	1.44 ± 0.19 (28)	1.40 ± 0.15 (25)
	F1	1.55 ± 0.18 (23)	1.58 ± 0.13 (26)	1.57 ± 0.14 (28)	1.57 ± 0.18 (25)	1.56 ± 0.18 (25)
Percentage to brain	P	66.1 ± 5.4 (26)	66.1 ± 6.4 (29)	68.1 ± 6.8 (29)	67.8 ± 7.4 (28)	68.8 ± 6.8 (25)
	F1	73.4 ± 8.9 (23)	74.5 ± 6.7 (26)	72.4 ± 6.5 (28)	73.3 ± 6.7 (25)	74.1 ± 7.8 (25)
Percentage to body	P	0.406 ± 0.035 (26)	0.407 ± 0.036 (29)	0.406 ± 0.036 (29)	0.420 ± 0.053 (28)	0.418 ± 0.044 (25)
	F1	0.474 ± 0.101 (23)	0.454 ± 0.042 (26)	0.446 ± 0.024 (28)	0.454 ± 0.046 (25)	0.450 ± 0.048 (25)
Spleen	P	0.74 ± 0.15 (26)	0.70 ± 0.12 (29)	0.71 ± 0.16 (29)	0.74 ± 0.23 (28)	0.67 ± 0.12 (25)
	F1	0.62 ± 0.12 (23)	0.70 ± 0.11* (26)	0.70 ± 0.13* (28)	0.71 ± 0.11* (25)	0.67 ± 0.09 (25)
Percentage to brain	P	34.6 ± 6.9 (26)	33.5 ± 5.9 (29)	34.0 ± 6.8 (29)	34.8 ± 9.7 (28)	32.7 ± 5.8 (25)
	F1	29.3 ± 5.2 (23)	33.3 ± 5.3 (26)	32.3 ± 5.8 (28)	32.9 ± 5.4 (25)	31.9 ± 4.2 (25)
Percentage to body	P	0.213 ± 0.045 (26)	0.206 ± 0.033 (29)	0.202 ± 0.043 (29)	0.215 ± 0.061 (28)	0.198 ± 0.036 (25)
	F1	0.186 ± 0.034 (23)	0.201 ± 0.030 (26)	0.198 ± 0.035 (28)	0.204 ± 0.035 (25)	0.193 ± 0.027 (25)

[a] Generation.
[b] Percentage to body weight = (organ weight/terminal body weight) × 100.
[c] Percentage to brain weight = (liver weight/brain weight) × 100.
* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group value ($p \leq 0.01$).

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EPA-HQ-2016-005679 06/14/2017

tive to body weight, there was no difference between control and 1000 mg/kg/day dose group. There were no other statistically significant changes in organ weights.

3.6. F1-generation males – toxicology endpoints

3.6.1. Clinical observations

There were no treatment-related deaths in F1-generation male rats. Some excess salivation was observed in the 1000 mg/kg/day dose group (data not shown).

3.6.2. Body weight and food consumption

Mean body weight on the initial day of dosing and on the day of terminal sacrifice as well as body weight change through terminal sacrifice for the F1-generation male rats are presented in Table 9b. Terminal body weights and body weight change from weaning to termination, were significantly reduced for the 1000 mg/kg/day dose group. There were no statistically significant findings in the other presented data. In addition to the summary data provided in Table 9b, body weight gain was reduced with statistical significance ($p \leq 0.01$) during the 2nd week of dosing in the 1000 mg/kg/day dose group when compared to controls, with means of 46.7 g and 51.8g in 1000 mg/kg/day group and control, respectively. Although not statistically significant, mean body weight gains in the 1000 mg/kg/day dose group were generally less than controls during most of the period prior to termination. This reduced weight gain resulted in statistically significant reduced overall mean body weight gain through the day of terminal sacrifice ($p \leq 0.01$). As a result of reduced body weight gain, mean body weight in the 1000 mg/kg/day group was reduced with statistical significance beginning on post-weaning day 36 ($p < 0.05$) and, with the exception of post-weaning days 50 and 64, mean body weight was reduced with statistical significance ($p < 0.01$) through terminal sacrifice. Therefore, body weight was affected by K^+PFBS treatment in the 1000 mg/kg/day dose group F1-generation males, with this effect becoming statistically significant after about 5 weeks of dosing with K^+PFBS.

Absolute food consumption was unaffected in all dose groups (data not shown); although, due to lesser mean body weight, relative food consumption was typically higher in the 1000 mg/kg/day dose group, with statistical significance during the 6th ($p \leq 0.05$), 7th ($p \leq 0.01$), and 10th ($p \leq 0.05$) weeks of the precohabitation period. Relative food consumption was also slightly higher than control in the 300 mg/kg/day dose at all but one time interval and was significantly higher ($p \leq 0.01$) during the 4th week of dosing.

3.6.3. Necropsy, histology, and organ weight changes

There were no treatment-related gross anatomical findings in F1-generation male rats. As stated before, no K^+PFBS treatment-related microscopic changes were observed in the reproductive organs of any 1000 mg/kg/day dose group F1-generation male rats selected for histopathological evaluation. Microscopic examination of the liver and kidneys of the F1 generation males revealed treatment-related effects similar to those in the P-generations males (Table 10). The changes in the liver consisted of 3 and 14 incidences of minimal to mild severity of hepatocellular hypertrophy in the 300 and 1000 mg/kg/day dose groups, respectively. The liver cells were enlarged due to an increased amount of finely granular eosinophilic cytoplasm. The hypertrophy was generally present in all areas of the lobules but tended to be more prominent and prevalent in the centrilobular region. In the kidneys, the treatment-related microscopic change consisted primarily of an increased incidence and severity of hyperplasia of the tubular and ductular epithelium of the inner medulla/papilla area in the 1000 mg/kg/day dose group. There were also nine incidences of minimal focal papillary edema in the 1000 mg/kg/day dose group.

F1-generation male absolute and relative organ weight data are presented in Table 11 for brain, liver, kidneys, and spleen. Although absolute liver weights were not increased in the highest dose group, the ratio of the liver weight-to-body weight was significantly increased in the 1000 mg/kg/day dose group relative to control values. There were no statistically significant differences between controls and treated groups with respect to the ratios of organ weights to brain weights.

3.7. F1-generation females – toxicology endpoints

3.7.1. Clinical observations

No treatment-related clinical signs were observed in F1-generation female rats at any of the K^+PFBS dosages tested. Although one death occurred in a 1000 mg/kg/day dose group female on precohabitation day 35 within 28 min after dosing, this female had fed and gained weight normally and all tissues appeared normal on necropsy (data not shown); therefore, this death was considered not to be related to treatment.

3.7.2. Body weight and food consumption

Initial and terminal mean body weight and body weight change of the F1-generation female rats during the precohabitation, gestation, and lactation periods are shown in Table 9b. There were no statistically significant reductions in body weight change or body weights among K^+PFBS-treated female rats as compared to controls for the presented data. In addition to the summary data provided in Table 9b, occasional statistically significant increases in body weight change or body weight were observed in the K^+PFBS-treated groups when compared to controls. In the two lower K^+PFBS-treated dose groups (30 and 100 mg/kg/d), body weights were statistically significantly elevated relative to controls during the last half of gestation. Mean weight gain, although slightly greater in all K^+PFBS-treated groups relative to controls, was not statistically significantly different than the control weight gain. Therefore, the higher body weights in the two lowest K^+PFBS-treated groups may have been the result of somewhat higher body weights on GD 0 in these two groups (means of 287 and 285 g for the 30 and 100 mg/kg/day K^+PFBS dose groups, respectively, versus 279, 272 and 273 g in the 300 and 1000 mg/kg/day and control groups, respectively). At the beginning of lactation, the F1 control group females had the lowest mean body weight (303 g as compared to 319, 316, 311, and 306 g in the 30, 100, 300, and 1000 mg/kg/day K^+PFBS-treated groups, respectively). Although weight gains did not differ with statistical significance between K^+PFBS-treated and control groups, mean weights of the 30 and 100 mg/kg/day groups were higher than control with statistical significance ($p < 0.05$ or 0.01) on days 5 and 8 of lactation, and all K^+PFBS-treated groups had statistically significantly increased body weight on the last day of lactation.

3.7.3. Necropsy, histology, and organ weight changes

All necropsy observations were normal in all dose groups. There were no microscopic findings in the liver of the F1-generation female rats. Treatment-related microscopic changes similar to those observed in the P-generation female rats were observed in the kidneys of F1-generation female rats in the 300 and 1000 mg/kg/day dosage groups (Table 10). In the kidneys, the changes consisted of 13 and 15 incidences of minimal to moderate severity of hyperplasia of the tubular and ductular epithelium of the inner medulla/papilla area in the 300 and 1000 mg/kg/day dose groups, respectively. There were also seven and four incidences of minimal to mild focal papillary edema in the 300 and 1000 mg/kg/day dose groups, respectively.

F1-generation female absolute and relative organ weight data are presented in Table 12 for brain, kidneys, and spleen. With the

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EPA-HQ-2016-005679 06/14/2017

exception of absolute splenic weights, all organ weights and ratios of organ weight to body weight and to brain weight were similar to control values. Mean absolute splenic weights were elevated relative to controls with statistical significance ($p \leq 0.05$) in the 30, 100, and 300 mg/kg/day dose groups; however, ratios of splenic weight to body weight were not different than controls, suggesting that the elevations likely were due to statistically significant increased body weights in these same dose groups.

3.8. F2-generation pups

There were no abnormal clinical or necropsy observations for F2-generation pups from F1 maternal rats given K^+PFBS. At LD 1 and 22, mean F2 pup body weights from maternal K^+PFBS-treated groups were not statistically significantly different compared to control (Table 9c).

Several statistically significant changes in organ weights or ratios of organ weights to terminal body weight or brain weight were observed. In the 30 mg/kg/day maternal K^+PFBS-treatment group, the mean (±S.D.) absolute weights and weights as a percent to terminal body weight of the spleen on a litter basis (0.16 ± 0.06 g and 0.40 ± 0.10%) and in female pups (0.18 ± 0.07 g and 0.42 ± 0.13%) were significantly greater ($p < 0.01$) than controls (0.12 ± 0.04 g and 0.11 ± 0.04 g and 0.31 ± 0.06% and 0.31 ± 0.06% for litter and female pups, respectively). Spleen weights as a percent to brain weight were statistically significantly higher on a litter basis (11.28 ± 4.03%, $p < 0.01$) and for male (10.67 ± 3.48%, $p < 0.05$) and female pups (11.89 ± 4.83%) compared to control values (8.23 ± 2.61%, 8.47 ± 3.30%, and 7.99 ± 2.26%, respectively). These statistically significant changes in spleen weight parameters were not observed in the three higher dose groups. In addition to spleen, kidney weight was increased with statistical significance in female pups (but not on a litter basis or in males) from the 30 mg/kg/day dose group (0.56 ± 0.10g, $p < 0.05$) compared to control (0.50 ± 0.10g), and the kidney weight as a percentage to brain weight was increased on a litter basis (38.85 ± 5.08%, $p < 0.05$) and in female pups (39.61 ± 5.49%, $p < 0.01$) compared to controls (35.43 ± 6.02% and 35.26 ± 5.85%, respectively). In addition to these statistically significant observations, the thymus weight as a percent to brain weight in the 30 mg/kg/day maternal K^+PFBS dose group female pups (11.89 ± 2.63%, $p < 0.05$) was increased with statistical significance, and the absolute thymus weight of the 100 mg/kg/day maternal K^+PFBS dose group males (0.40 ± 0.08 g, $p < 0.01$) was increased with statistical significance compared to control (0.032 ± 0.11g). None of these statistically significant differences in organ weight or organ weight ratios for the F2-generation pups were considered treatment-related because they were not dosage-dependent.

4. Discussion

The objective of this study was to provide information on the potential reproductive and developmental effects of PFBS for safety evaluation and to compare findings from the study to the outcomes of two-generation studies conducted with PFOS and PFOA, both of which did affect postnatal developmental outcomes within the dose range administered.

In the PFBS study reported here, no adverse effects on functional aspects of reproduction were observed in either generation.

In both the P- and F1-generation males, minimal to mild hepatocellular hypertrophy was observed microscopically in the 300 and 1000 mg/kg/day K^+PFBS dose groups. In P-generation males, this was accompanied by increases in mean absolute and relative liver weights as compared to controls. For the F1-generation males from the 1000 mg/kg/day dose group, only liver weight as a percent of body weight was increased with statistical significance. The presence of increased eosinophilic granules suggests the presence of increased peroxisomes or lysosomes (David et al., 2000, 2001; Frame et al., 1992; Marsman, 1995; Toyosawa et al., 2001). Wolf et al., have recently demonstrated that PFBS is an agonist for mouse and human peroxisome proliferator activated receptor ffi (PPARffi) isoforms transiently transfected into COS-1 cells (Wolf et al., 2008). Therefore, the hepatocellular eosinophilia observed in the present study may have represented, in part, increased activation of PPARffi leading to increased peroxisomal bodies. Although endpoints reflecting liver changes other than weight and histology were not evaluated in the present study, in a 90-day oral gavage study with the same lot of K^+PFBS and in the same strain of rat, there were no indications of liver damage based on increases in serum concentrations of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, bilirubin, and albumin at K^+PFBS doses up to 600 mg/kg/day (Lieder et al., 2009). Based on the results of the 90-day study as well as the present study, there are no indications that daily K^+PFBS treatment for extended periods has caused liver injury in rats.

The F1-generation males appeared to have a lessened response to the liver hypertrophic effects of K^+PFBS compared to the P-generation males. Considering that the F1-generation males were potentially exposed *in utero* and during lactation, as well as during a 10-week period prior to mating and through mating, it is surprising that absolute and relative liver weights were not affected to the same extent. The reason for this is not understood. A possibility is that, because the age and maturation at the onset of dosing differed between the P- and F1-generation males, this difference may have influenced elimination and/or distribution kinetics of PFBS. The P-generation males were sexually mature at the onset of dosing with mean group body weights ranging from 177 ± 8 to 181 ± 8 g. Dosing for the F1-generation males was initiated on the day after weaning when the rats were 23 days old and mean group body weights ranged from 45 ± 6 to 47 ± 5 g. The elimination kinetics of PFOA have been shown to change during sexual maturation in male rats, such that the elimination rate after sexual maturation is significantly reduced (Hinderliter et al., 2006). This observation with PFOA may be the result of sexual hormone-mediated expression of renal tubular organic anion transporters in the rat (Buist et al., 2002, 2003), which has been shown to be affected by PFOA (Kudo et al., 2002). It is interesting to note that, in a developmental neurotoxicology study with perfluorohexanesulfonate (PFOS), F1 offspring developed an apparent difference in elimination rate of PFOS at some point between weaning and postnatal day 72, an observation that may have been influenced by sexual maturation (Butenhoff et al., 2009). In the latter case, male rats appeared to eliminate PFOS at a rate similar to females at weaning, but, by postnatal day 72, serum elimination in males appeared to be several times higher than in females. Liver organic anion transport processes could also be similarly affected by maturation (Buist and Klaassen, 2004). If the sex-determined expression of organic anion transport processes is a factor in determining PFBS pharmacokinetics kinetics in the rat, this could have resulted in differences between P- and F1-generation males in distribution of PFBS during the dosing periods, and could explain the apparent attenuated response in F1-generaton males. Another possibility is that early-life exposure may have conditioned the F1-generation males to be less responsive. Obviously, further study would be needed to fully understand the basis of the differences in liver weight response observed between the P- and F1-generations.

In the present study, increased incidence and severity of histological changes related to K^+PFBS treatment were noted in kidneys of males and females of both the P- and F1-generations in the 300 and 1000 mg/kg/day dose groups. These consisted of hyperplasia of the medullary/papillary tubular and ductular epithelium. Although there were low incidences of focal necrosis of the papilla observed

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EPA-HQ-2016-005679 06/14/2017

in K^+PFBS-treated males and females, these did not follow a clear dose–response pattern. Similar kidney effects were noted in the 90-day study cited above (Lieder et al., 2009), and, as discussed in that article, these changes likely were due to high concentrations of PFBS, a strong surface active compound, passing through the kidney. The recent confirmation of urine as the major excretory route for PFBS lends further support to the hypothesis that high concentrations of PFBS in kidney may have precipitated the histological changes in this organ observed in the present study and the 90-day study (Olsen et al., 2009).

The P-generation paternal no-observable-adverse effect dose level (NOAEL) was 100 mg/kg/day due to hepatocellular hypertrophy and liver weight increase and primarily minimal-to-mild hyperplasia of medullary/papillary tubular and ductular epithelium of the kidney which were observed at doses higher than 100 mg/kg/day. The P-generation maternal NOAEL was 100 mg/kg/day based on primarily minimal to mild hyperplasia of the medullary/papillary tubular and ductal epithelium in the kidney which was observed at doses above 100 mg/kg/day. All of these changes are believed to be due to a response to the high concentrations of surfactant in the kidney due to rapid elimination of high administered doses. No effects on reproductive function (estrous cycling, mating, fertility, and sperm parameters) were noted; therefore, the reproductive NOAEL in the male and female P-generation is >1000 mg/kg/day, the highest dose of the study, and the parental NOAEL for both sexes in the P-generation was 100 mg/kg/day.

The F1-generation paternal NOAEL was 100 mg/kg/day based on primarily minimal hypertrophy of liver cells observed which was observed at doses higher than 100 mg/kg/day. The F1-generation maternal NOAEL was 100 mg/kg/day based on increased minimal to mild hyperplasia of the tubular and ductular epithelium of the medulla and papilla of the kidney which was observed at doses higher than 100 mg/kg/day. The developmental NOAEL in the F1-generation is 300 mg/kg/day based on body weight effects. The slightly delayed sexual maturation likely was the result of reduced body weight at 1000 mg/kg/day (Lewis et al., 2002), because there were no statistically significant differences between groups when body weight on day of preputial separation was co-varied with days to preputial separation. As in the P-generation, no effects on reproductive function were noted; therefore, the reproductive NOAEL in the male and female F1-generation rats was >1000 mg/kg/day.

The F2-generation pups from parents treated with K^+PFBS were followed through lactation. Compared to F2-generation control peers, there were no differences in clinical observations; therefore, the F2-generation pup NOAEL is >1000 mg/kg/day.

This two-generation reproduction study with PFBS has demonstrated that PFBS does not adversely affect reproductive function in Sprague Dawley rats at doses as high as 1000 mg/kg/day or developmental outcomes at doses as high as 300 mg/kg/day. The ammonium salt of PFOA (APFO) and the potassium salt of PFOS (K^+PFOS) have previously been studied in two-generation reproduction studies in rats at the same laboratory (Butenhoff et al., 2004; Luebker et al., 2005a). The results obtained with APFO and K^+PFOS were qualitatively and quantitatively very different than the results obtained for K^+PFBS in this study. Neither K^+PFBS, K^+PFOS, nor APFO caused effects on functional reproduction (estrous cycling, mating, sperm parameters, and fertility). However, unlike K^+PFBS, K^+PFOS and APFO caused a number of postnatal developmental effects at doses that were 1–3 orders of magnitude less than the doses used for the K^+PFBS study.

The K^+PFOS study was conducted at doses of 0.1, 0.4, 1.6, and 3.2 mg/kg/day (Luebker et al., 2005a). The reproductive effects NOAEL for the study was 1.6 mg/kg/day; however, due to high incidences of deaths in the 1.6 and 3.2 mg/kg/day F1-generation neonates, the production of the F2-generation was limited to the 0.1 and 0.4 mg/kg/day F1-generation parental dose groups. In the P-generation 3.2 mg/kg/day group, several reproductive outcome parameters were adversely affected. These included: decreased gestation length; reduced implantation sites; and increased numbers of dams with stillborn pups or with all pups dying during LD 1–4. F1 neonatal toxicity was manifested in the 1.6 mg/kg/day dose group as decreased neonatal survival and reduced body weight gain through lactation. F1 pups also experienced slight developmental delays, including eye opening (0.4 and 1.6 mg/kg/day) and air righting, surface righting and pinna unfolding (1.6 mg/kg/day). Due to the severely-impaired survival of F1 pups in the 1.6 and 3.2 mg/kg/day dose groups, F2 pups were only obtained from the 0.1 and 0.4 mg/kg/day dose groups, and were normal as compared to controls. A cross-fostering study also reported by Luebker et al. (2005a) suggested that effects were largely due to exposure to PFOS *in utero*.

APFO was studied at dose levels of 1, 3, 10, and 30 mg/kg/day (Butenhoff et al., 2004). For the F1-generation pups at the highest dose of 30 mg/kg/day, APFO caused reductions in birth weight and survival, especially immediate post-weaning survival, as well as somewhat delayed sexual maturation in both sexes. The effects on survival and sexual maturation may have been the result of lower body weights. No developmental effects were noted in the F2-generation pups through study termination at the end of lactation. Both P-generation and F1-generation males experienced reduced body weights and increased kidney and liver weights at doses as low as 1 mg/kg/day. The reproductive toxicity NOAEL was >30 mg/kg/day, with a developmental NOAEL for the F1-generation of 10 mg/kg/day, and male parental NOAEL of < 1 mg/kg/day for the F1-generation.

The relative lack of developmental toxicity observed in rats with K^+PFBS, in contrast to K^+PFOS and APFO, may be due to differences in toxicokinetics and biochemical interactions, such as more rapid PFBS elimination and reduced binding affinity to critical receptors when compared to PFOS and PFOA. PFBS is eliminated rapidly in male and female rats (Olsen et al., 2009), with approximately 50% recovered in urine within 24 h and terminal serum elimination half-lives of 3 and 4 days. By contrast, unpublished PFOS elimination data in female rats from our laboratory indicate an elimination half-life of approximately 1 month. PFOA is eliminated rapidly in female rats, with elimination half-life of a few hours; however, male rats have an elimination half-life of several days (Kennedy et al., 2004; Kudo and Kawashima, 2003). In addition, PFBS is much more soluble in water compared to PFOS. Both PFOS and PFOA can transfer to the fetal compartment during gestation and, to a lesser extent, to the pups during lactation (Hinderliter et al., 2005; Luebker et al., 2005b). Although placental and lactational transfer of PFOS and PFOA to rat pups has been studied, similar experiments have not performed with PFBS. If these were to be done, additional insight into the differences between these compounds may become evident.

In conclusion, this two-generation reproduction study with PFBS in rats demonstrated that PFBS has a low potential to produce effects on reproduction or development, especially when compared to the eight-carbon analog, PFOA, and eight-carbon homolog, PFOS.

Conflict of interest statement

Paul H. Lieder, John L. Butenhoff, Shu-Ching Chang, and Daniel Hakes are employees of 3M Company, a manufacture of PFBS. Raymond York was an employee of Charles Rivers Laboratories in which the study was contracted. The funding for the study was provided by 3M Company.

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EPA-HQ-2016-005679 06/14/2017

the microscopic histological evaluation of tissues from the study.

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Evaluation of potential reproductive and developmental toxicity of potassium perfluorohexanesulfonate in Sprague Dawley rats

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This study evaluates the potential reproductive and developmental toxicity of perfluorohexanesulfonate (PFHxS), a surfactant found in sera of the general population. In a modified OECD 422 guideline-based design, 15 rats per sex and treatment group (control, 0.3, 1, 3, and 10 mg/kg-d) were dosed by gavage with potassium PFHxS (K^+PFHxS) or vehicle (0.5% carboxymethylcellulose) 14 days prior to cohabitation, during cohabitation, and until the day before sacrifice (21 days of lactation or presumed gestation day 25 (if not pregnant) for females and minimum of 42 days of treatment for males). Offspring were not dosed by gavage but were exposed by placental transfer *in utero* and potentially exposed via milk. Evaluations were made for reproductive success, clinical signs, body weight, food consumption, estrous cycling, neurobehavioral effects, gross and microscopic anatomy of selected organs, sperm, hematology, clinical pathology, and concentration of PFHxS in serum and liver. Additional three rats per sex per group were added to obtain sera and liver samples for PFHxS concentration determinations during the study. No reproductive or developmental effects were observed. There were no treatment-related effects in dams or offspring. K^+PFHxS-induced effects noted in parental males included: (1) at all doses, reductions in serum total cholesterol; (2) at 0.3, 3, and 10 mg/kg-d, decreased prothrombin time; (3) at 3 and 10 mg/kg-d, increased liver-to-body weight and liver-to-brain weight ratios, centrilobular hepatocellular hypertrophy, hyperplasia of thyroid follicular cells, and decreased hematocrit; (4) at 10 mg/kg-d, decreased triglycerides and increased albumin, BUN, ALP, Ca^{2+}, and A/G ratio. Serum and liver concentrations of PFHxS are reported for parents, fetuses, and pups. PFHxS was not a reproductive or developmental toxicant under study conditions.

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1. Introduction

Perfluorohexanesulfonate (PFHxS) is a perfluorinated surfactant in which all hydrogens in carbon–hydrogen bonds have been replaced with fluorine. As such, PFHxS is one of the number of functionalized, polyfluorinated compounds that have been produced for over half a century for use in specialized applications [1] as well as becoming the subject of increasing investigation with regard to environmental and health-related properties [2–5]. The unique properties of this and other perfluorinated surfactants, such as high surface activity, exceptional stability to degradation, density, solubility characteristics, and low intermolecular interactions, have been exploited in numerous industrial and consumer applications [6].

However, these same properties also create challenges for managing these materials in the environment. Exceptional stability to environmental and metabolic degradation together with poor elimination from the body in the case of several perfluorinated surfactants [7–9], including PFHxS, creates a potential for accumulation and biomagnification. The geometric mean serum PFHxS elimination half-life in 26 retired production workers was estimated to be approximately 7.3 years (95% CI = 5.8–9.2) [7]. Spliethoff et al. [10] estimated the serum elimination half-life for PFHxS to be 8.2 years (95% CI = 5.4–16.2) using PFHxS concentration data from neonatal screening blood spots. Although the reported half-life values from the latter two articles are imprecise estimates, they are consistent in suggesting poor elimination of PFHxS in humans. Due to these properties, it may not be surprising that Hansen et al. [11] found PFHxS in pooled serum from the United States general population, and numerous later biomonitoring studies have found PFHxS widely distributed at low ng/mL concentrations in individual samples from the general population [10,12–26].

The presence of PFHxS in umbilical cord blood at birth [19] and neonatal screening program blood spots [10] demonstrates that human exposures can begin *in utero*. The primary sources of exposure are not understood; however, exposure to PFHxS in human

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milk [20–22], house dust [27,28] or from contact with surfaces treated with PFHxS-containing carpet and upholstery protection formulations [14] may provide some contribution as well as potential dietary [24] and water sources [23,26,29]. These sources may contribute to PFHxS exposure to children after birth.

Between the years 2000 and 2002, due to persistence and evidence of widespread exposure of the general population, 3M Company discontinued production of PFHxS along with perfluorooctanoate (PFOA) and chemistries based on perfluorooctanesulfonyl fluoride, including perfluorooctanesulfonate (PFOS). Several recent biomonitoring studies have demonstrated that blood concentrations of PFHxS, PFOA, and PFOS in the general population have declined significantly since 3M Company ceased manufacturing of these materials [10,13,17]

Although its eight-carbon analog, PFOS, has been extensively studied for its potential heath effects [2,30,31], little information on the toxicity potential of PFHxS has been documented. The fact that exposure occurs *in utero* and is present at all life stages together with the known biological effects profile of PFOS from laboratory studies warrants a need to understand potential health risk associated with exposure. The objective of this study was to evaluate the potential reproductive, developmental, and neurological responses of rats to treatment with potassium PFHxS in an assessment based on OECD Method 422 guidelines [32]. Because internal dose metrics have become important in evaluating health risk [2,33–35], collection of serum and liver PFHxS concentration data was included in the study design. One of the earliest clinically measurable indicators of biological response to treatment of rats and non-human primates with PFOS has been reduction in serum cholesterol [36,37]. Accordingly, serum total cholesterol was included in the clinical chemistry profile. We believe that this is the first article to report on the biological response to treatment with PFHxS.

2. Materials and methods

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

K^+PFHxS (purity 99.98%), a white powder, was obtained from 3M Company (St. Paul, MN). The vehicle used in preparation of the test article formulations and for administration to the control group was 0.5% carboxymethylcellulose, medium viscosity (lot number 120K0252, Sigma–Aldrich, St. Louis, MO) in deionized water. Dosing formulations for four K^+PFHxS treatment levels of 0.3, 1, 3, and 10 mg/kg-d were prepared as K^+PFHxS concentrations in vehicle of 0.03, 0.1, 0.3, and 1 mg/mL, respectively, based on a dosing volume of 10 mL/kg and were not adjusted for purity. The purity and stability of the chemical were verified by analysis before the study. All samples were analyzed for PFHxS concentration using a high performance liquid chromatography/tandem mass spectrometry (LC–MS/MS) method.

2.2. Animal husbandry, assignment, and breeding

Crl:CD®(SD)IGS BR VAF/Plus® rats (n = 90/sex) supplied by Charles River Laboratories from St. Constant, Quebec, Canada (male rats) and Raleigh, NC, US (female rats) were used in this study. All rats were individually housed in stainless steel, wire-bottomed cages except during cohabitation and postpartum periods. During cohabitation, each pair of male and female rats was housed in the male rat's home cage. F_0 generation female rats were individually housed in nesting boxes (with Bed-o'cobs® bedding, The Andersons Industrial Products Group, Maumee, OH) until they either naturally delivered litters or were sacrificed. Each dam and her delivered litter were housed in a common nesting box during the postpartum period. All cage sizes and housing conditions were in compliance with the *Guide for the Care and Use of Laboratory Animals* [38].

Rats were given *ad libitum* access to Certified Rodent Diet #5002 (PMI Nutrition International, Inc., St. Louis, MO) except for the evening prior to sacrifice. Municipal water, processed through reverse osmosis membrane and fortified with chlorine, was given to the rats *ad libitum*. The study room was maintained under conditions of positive airflow with a minimum of ten changes per hour of air that passed through 99.97% HEPA filters. Room temperatures were maintained between 18 °C and 26 °C and relative humidity maintained at 30–70%. Lighting was maintained at 12-h light and 12-h dark cycles. The testing facility (Charles River Preclinical Services, Horsham, PA) is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. All procedures involving animals were approved by the testing facility's Institutional Animal Care and Use Committee.

2.3. Study design

This study was conducted according to OECD test guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test [32]). Male and female rats (n = 18/sex/dose) were allocated randomly into five oral gavage treatment groups consisting of the vehicle control, and 0.3, 1, 3, and 10 mg/kg-d K^+PFHxS. In each treatment group, rats were assigned to either the main study (15 rats/sex) or for collection of sera and liver samples for PFHxS concentration determination (3 rats/sex). The dosage volume (10 mL/kg) was adjusted daily on the basis of the individual body weights recorded before intubation. Rats were dosed at approximately the same time each day. Daily dosing of all rats began 14 days prior to cohabitation and continued until the day prior to scheduled sacrifice. F_1 generation pups were not dosed directly but were exposed to PFHxS *in utero* and were potentially exposed via the milk during the lactation period.

Male rats were sacrificed on study day (SD) 42 (3 males assigned for PFHxS concentration determinations) and SD 44 (main-study males). For main-study female rats, scheduled sacrifice was either through postnatal day (PND) 22 or presumed gestation day (GD) 25 for rats that did not deliver a litter. Female rats assigned for collection of sera and liver samples for PFHxS concentration determination were sacrificed on GD 21. F_1 pups were sacrificed on PND 22. Carbon dioxide asphyxiation was used for sacrifice.

2.4. Observations and evaluations (F_0 parental rats)

All main study rats were observed daily for body weight (except during cohabitation) and clinical signs of toxicity (before and approximately 60 min after dosing), and detailed clinical observations were conducted on a weekly basis. Feed consumption was evaluated weekly for males except during cohabitation. Feed consumption for females was evaluated up to cohabitation and on GD 0, 7, 10, 12, 15, 18, and 20 and PND 1, 5, 8 and 15. During parturition, female rats were evaluated for adverse clinical signs, duration of gestation, litter sizes (all pups delivered), and pup viability at birth. Maternal behavior was evaluated on PND 1, 5, 8, 15, and 22.

Estrous cycling was evaluated in all female rats (including rats assigned to sera and liver sample collection) by examination of vaginal cytology after the first dose and daily until spermatozoa were observed in a vaginal smear and/or a copulatory plug was observed *in situ* during the cohabitation period.

In each treatment group, functional observation battery (FOB) and motor activity assessments were conducted on 10 male rats (prior to scheduled sacrifice and before blood sample collection) and 10 female rats (prior to scheduled sacrifice during lactation period) [39–42]. The FOB evaluations were conducted in a blinded manner with the following parameters assessed: lacrimation, salivation, palpebral closure, prominence of the eye, pupillary reaction to light, piloerection, respiration, and urination and defecation (autonomic functions); sensorimotor responses to visual, auditory, tactile and painful stimuli (reactivity and sensitivity); reactions to handling and behavior in the open field (excitability); gait pattern in the open field, severity of gait abnormalities, air righting reaction and landing foot splay (gait and sensorimotor coordination); forelimb and hindlimb grip strength; and abnormal clinical signs including convulsions, tremors and other unusual behavior, hypotonia or hypertonia, emaciation, dehydration, unkempt appearance and deposits around the eyes, nose or mouth. Motor activity was evaluated by a passive infrared sensor. Each test session was 1.5 h in duration with the number of movements and time spent in movement tabulated at each 5-min interval.

Gross necropsy of all male and female rats included macroscopic evaluation of external surfaces and all orifices, as well as cranial, thoracic and abdominal cavities, and their contents for gross lesions. The reproductive organs were closely examined (see below).

Ten rats per sex from each treatment group that were assigned to FOB and motor activity testing were used for evaluation of organ weights, microscopic histological examination of tissues, detailed examination or sexual organs and related parameters, clinical chemistry, and hematology. Procedural descriptions follow.

Organ weights, including sexual organ weights, were determined, and microscopic histological samples were taken for potential examination. Liver, kidneys, adrenals, thymus, testes (left and right), epididymis (left and right), seminal vesicles (with and without fluid), prostate, spleen, brain, heart, ovaries (left and right) and uterus were weighed. Brain, small and large intestines, lungs, lymph nodes, peripheral nerve, stomach, kidneys, spleen, thymus, trachea, urinary bladder, spinal cord, liver, adrenals, heart, thyroid/parathyroid, bone marrow, testes, prostate, seminal vesicles, the remaining portion of the left epididymis, the right epididymis, ovaries, uterus, vagina, mammary gland and any gross lesions were retained in neutral buffered 10% formalin and evaluated histologically by Research Pathology Services, Inc. (New Britain, PA). The control and 10 mg/kg-d treatment groups were evaluated first. If differences were found between control and the high treatment group, the remaining three treatment groups were to be evaluated.

In addition to organ weights, potential toxicity to male and female reproductive systems was closely evaluated. For male rats, sperm concentration (per gram of tissue weight), sperm motility (by integrated visual optical system, or IVOS, Hamilton Thorne, Inc., Beverly, MA), and sperm morphology (by determining the percentage of normal and abnormal sperms) were assessed. Uteri of both pregnant and non-pregnant rats were examined for the presence/absence of and the number (if

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EPA-HQ-2016-005679 06/14/2017

present) of implantation sites. A quantitative evaluation of primordial follicles was also conducted for F_0 generation female rats.

Hematology and clinical chemistry samples were collected from the inferior vena cava of fasted rats assigned to the FOB assessment in the main study during the scheduled sacrifice. All samples were analyzed by Redfield Laboratories, Redfield, AK (a division of Charles River Laboratories).

Analyses of hematologic parameters included erythrocyte count (RBC), hematocrit (HCT), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), total leukocyte count (WBC), and differential leukocyte count, platelet count (PLAT), mean platelet volume (MPV), cell morphology, prothrombin time (PT), and activated partial thromboplastin time (APTT).

The evaluated clinical chemistry parameters were: total protein (TP), triglycerides (TRI), albumin (A), globulin (G), albumin/globulin ratio (A/G), glucose (GLU), cholesterol (CHOL), total bilirubin (TBILI), urea nitrogen (BUN), creatinine (CREAT), creatinine kinase (CK), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALK), calcium (CA), phosphorus (PHOS), sodium (NA), potassium (K), and chloride (CL).

Blood samples (processed to plasma) were collected from three male rats per treatment group on SD 14 and 42. Blood samples were also collected from the three female rats per treatment group on SD 14 and GD 21. Samples were collected from the orbital sinus on SD 14 and from the vena cava on SD 42. After the last blood collection on SD 42, all three male rats were euthanized and liver samples were harvested and weighed. Liver samples were also collected and weighed from the three female rats euthanized on GD 21 followed by the subsequent collection of fetal blood and liver (pooled by litter) samples. All samples were stored frozen until PFHxS concentration analysis (Exygen Research, State College, PA).

2.5. Observations and Evaluations (F_1 Generation)

The F_1 pups in each litter were counted once daily and clinical observations were recorded daily. Pup body weights were recorded on PND 1, 8, 15 and 22. No feed consumption or interim body-weight data were recorded prior to the scheduled sacrifice on PND 22. Pups were examined for gross lesions at sacrifice, and the necropsy also included a single cross-section of the head at the level of the frontal-parietal structure and examination of the cross-sectioned brain for apparent hydrocephaly.

At study termination, liver samples were excised and weighed and blood samples were collected and processed for serum from five pups per sex per litter from the 10 dams selected for FOB assessments. Serum samples were pooled by litter. The median liver lobe was stored frozen, and the remaining liver portion was retained in neutral buffered 10% formalin for possible histological evaluation. Samples remained frozen until PFHxS concentration analysis (Exygen Research, State College, PA).

2.6. Determination of PFHxS Concentrations in Serum and Liver

PFHxS concentrations were determined by LC–MS/MS. All samples underwent extraction procedures prior to chromatographic analyses. Blank serum and liver samples were purchased from Pel Freeze Biologicals (Rogers, AR) and these blank matrices were used to prepare the appropriate matrix-matched PFHxS standard curves with the addition of known amounts of PFHxS.

Serum (100 ffiL) and liver (100 mg) samples were diluted with deionized water so that the final volumes were 20 mL and 10 mL, respectively. After vortexing (serum and plasma) and homogenization (liver), 1 mL of the aliquot was mixed with 5 mL acetonitrile using a mechanical shaker (20 min, room temperature) and supernatant was obtained after centrifugation (3000 rpm, 5 min). After adding 35 mL of deionized water to the supernatant, the entire mixture was loaded onto Waters Sep-Pak® solid phase extraction (SPE) column (6 mL with 1 g C18 sorbent). The SPE columns were pre-conditioned with 10 mL methanol followed by 5 mL deionized water. Samples were eluted with 2 mL methanol.

The instrument used for PFHxS analysis was a Micromass Quattro Ultima mass spectrometer configured with electrospray ionization source (ESI) in negative ion mode. A Genesis® C8, 4 ffim, 50 mm × 2.1 mm i.d. HPLC column with a flow rate of 0.3 mL/min was used for PFHxS analysis. The gradient condition for the mobile phase started with 90% 2 mM ammonium acetate and 10% methanol to 100% methanol for 9.5 min. All source parameters were optimized under these conditions according to manufacturer's guidelines. Transition ions monitored for PFHxS were from 399 → 80 atomic mass units.

2.7. Statistical analysis

F_0 parental rat data were evaluated with the individual rat as the unit measured. Litter values were used in evaluation of pup data, as appropriate. Variables with interval or ratio scales of measurement, such as body weights, feed consumption values, latency and errors per trial scores in behavioral tests, and percent mortality per litter were analyzed as parametric data. Bartlett's Test of Homogeneity of Variances [43] was used to estimate the probability that the dosage groups have different variances. A non-significant result ($p > 0.001$) indicated that an assumption of homogeneity of variance was appropriate, and the data were compared using the Analysis of Variance (ANOVA) [44]. If ANOVA test was significant ($p \leq 0.05$), the groups given the test substance were compared with the control group using Dunnett's Test [45]. If Bartlett's Test was significant ($p \leq 0.001$), the ANOVA was not appropriate, and the data were analyzed as nonparametric data. When 75% or fewer of the scores in all the groups were tied, the Kruskal–Wallis Test [46] was used to analyze the data, and in the event of a significant result ($p \leq 0.05$), Dunn's Test [47] was used to compare the groups given the test substance with the control group. When more than 75% of the scores in any dosage group were tied, Fisher's Exact Test [48] was used to compare the proportion of ties in the groups.

Data from the motor activity test, with measurements recorded at intervals (blocks) throughout each test session, were analyzed using an ANOVA with repeated measures [49]. A significant result ($p \leq 0.05$) in that test could have appeared as effect of concentration (differences among dosage groups in the totals of all measurements in a session) or as an interaction between concentration and block (differences in the patterns of dosage group values across the measurement periods). If the concentration effect was significant, the totals for the control group and the groups given the test substance were compared using Dunnett's Test [45]. If the concentration × block interaction was significant, an ANOVA [44] was used to evaluate the data at each measurement period, and a significant result ($p \leq 0.05$) was followed by a comparison of the dosage groups using Dunnett's Test [45].

3. Results

All data reported in this study are presented as mean ± standard deviation (SD) unless specified otherwise.

3.1. Stability and homogeneity

Dosing solutions were found to be stable and homogenous. Mean PFHxS (and inferred K^+PFHxS) concentrations for seven sets of dosing solutions were within 70–125% of the target concentrations. The wide range of PFHxS concentrations obtained was likely due to the unavailability of appropriate internal standard at the time during which the analysis was conducted. (While the LC–MS/MS analytical coefficient of variation for PFHxS analysis typically ranges from 20 to 30% without employing an internal standard, it has been reduced significantly to less than 10% when an internal standard such as stable isotope ^{18}O-labeled PFHxS is used.)

3.2. F_0 parental rat results

All F_0 rats survived to the scheduled necropsy and no K^+PFHxS-treatment related mortalities occurred. No abnormal clinical findings were noted in any of the rats receiving either control or K^+PFHxS doses at the daily examinations or 1 h following dose administration.

Body weight and body-weight change data for F_0 males are summarized in Table 1. In males given K^+PFHxS, mean body weights by K^+PFHxS treatment level were not statistically significantly different than male control body weights at any time during the study. Statistically significant decreases in mean body-weight gains relative to mean control body-weight gains were noted in 0.3, 3, and 10 mg/kg-d K^+PFHxS-treated male groups between SD 29 (first value recorded after cohabitation) and 36, resulting in an overall decrease in body-weight gains through termination on SD 43, even though no differences from mean control values were noted between SD 36 and 43. Mean body-weight gain in the 10 mg/kg-d treatment group was decreased over the entire study period (SD 1–43) with statistical significance as compared to the mean for controls. Regardless of these variations in body-weight gain, mean terminal body weights were not statistically significantly different between K^+PFHxS-treated males and controls.

Body weight and body-weight change data for F_0 females also are summarized in Table 1. During pre-cohabitation and gestation, no statistically significant differences were noted in mean body weights or body-weight changes in K^+PFHxS-treated female groups when compared to the mean for controls for the same time periods. During lactation, there were no statistically significant differences between mean body-weight changes in K^+PFHxS-treated

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EPA-HQ-2016-005679 06/14/2017

Table 1
Body weights and body-weight changes summary for male and female parental rats during specified study periods (data in grams, $N = 15$ unless otherwise noted).

	Potassium perfluorohexanesulfonate dose (mg/kg-day)				
	0 (Control)	0.3	1	3	10
Male body-weight study day (SD) 1	342.7 ± 12.6	338.9 ± 13.1	341.9 ± 14.7	345.6 ± 11.2	343.3 ± 12.4
Male body-weight SD 43[a]	490.5 ± 37.2	475.1 ± 38.3	482.5 ± 53.1	490.5 ± 40.2	462.1 ± 22.0
Male body-weight SD 44[b]	472.5 ± 33.9	453.7 ± 34.3	463.1 ± 48.2	468.3 ± 37.1	441.5 ± 16.5
Male body-weight gain SDs 1–44[b,c]	129.8 ± 25.5	114.8 ± 27.5	121.2 ± 37.7	122.7 ± 30.0	98.2 ± 13.9**
Female body-weight SD 1	228.6 ± 8.0	224.7 ± 8.1	226.3 ± 7.7	226.8 ± 7.9	226.9 ± 8.0
Female body-weight SD 14	258.5 ± 16.1	250.7 ± 12.6	257.9 ± 13.6	253.0 ± 13.2	251.1 ± 18.0
Female body-weight gain SD 1–14[d]	29.9 ± 13.7	25.9 ± 7.2	31.6 ± 8.4	26.2 ± 8.3	24.2 ± 14.4
Maternal body-weight gestation day (GD) 0	270.1 ± 14.3	259.4 ± 12.0	270.9 ± 15.6[e]	265.6 ± 15.4[e]	260.9 ± 18.2
Maternal body-weight GD 20	412.0 ± 35.0	397.4 ± 27.8	412.1 ± 21.7[e]	398.8 ± 27.6[e]	395.1 ± 28.1
Maternal body-weight gain GD 0–20[d]	141.9 ± 29.0	138.0 ± 27.4	141.2 ± 14.1[e]	133.2 ± 21.9[e]	134.2 ± 16.9
Maternal body-weight postnatal day (PND) 1	313.1 ± 22.6	302.2 ± 16.8	309.5 ± 20.6[e]	304.7 ± 23.2[e]	296.3 ± 19.9
Maternal body-weight PND 8	342.3 ± 19.8	320.7 ± 12.6*	333.6 ± 19.6[e,f]	322.2 ± 21.5*	318.1 ± 26.0**
Maternal body-weight PND 15	355.3 ± 31.2	342.4 ± 10.4[g]	343.2 ± 17.2[e,f]	348.7 ± 21.4[e,f]	333.4 ± 33.6[g]
Maternal body-weight PND 21[a]	350.1 ± 26.9[g]	335.7 ± 18.7[g]	343.4 ± 19.8[e]	344.4 ± 25.8[e]	334.4 ± 35.9
Maternal body-weight PND 22[b]	306.3 ± 26.0	324.3 ± 27.1	311.2 ± 35.0[e]	319.6 ± 40.4[e]	303.5 ± 37.9
Maternal body-weight change (+/−) PND 1–22[h]	−6.9 ± 28.4	+22.1 ± 26.0	+1.7 ± 28.0[e]	+14.9 ± 31.8[e]	+7.3 ± 29.3

[a] Non-fasted body weight on last day before termination.
[b] Fasted terminal body weight.
[c] In males given potassium perfluorohexanesulfonate (K^+PFHxS), mean body weights by treatment level were not statistically significantly different than male control body weights at any time during the study. Statistically significant decreases in mean body-weight gains relative to mean control body-weight gains were noted in 0.3, 3, and 10 mg/kg-d K^+PFHxS-treated male groups between SD 29 (first value recorded after cohabitation) and 36 and from SD 29 through termination on SD 43; however, no differences from mean control values were noted between study days 36 and 43.
[d] During pre-cohabitation and gestation, no statistically significant differences were noted in mean body weights or body-weight changes in K^+PFHxS-treated female groups when compared to the mean for controls for the same time periods.
[e] $N = 13$ due to 2 nonpregnant females.
[f] $N = 12$ due to exclusion of 2 body-weight determinations that appeared to be incorrect as a result of interrupted water access.
[g] $N = 14$ due to exclusion of 1 body-weight determination that appeared to be incorrect as a result of interrupted water access.
[h] During lactation, there were no statistically significant differences between mean body-weight changes in K^+PFHxS-treated groups when compared to mean control values over the same time period. There were occasional periods of statistically significant differences in mean body weights among 0.3, 3, and 10 mg/kg-d K^+PFHxS-treated groups and controls from PND 4–14. Statistically significant reductions in mean body weight occurred in K^+PFHxS-treated females as compared to control mean values from PND 4 to PND 14 as follows: PND 4, 6–8, 11, and 13 in the 0.3 mg/kg-d group; PND 7 and 8 in the 3 mg/kg-d; and PND 4, 6–9, 11, 13, and 14 in the 10 mg/kg-d group. Mean body weights on PND 15 through term on PND 22 in K^+PFHxS-treated groups were not statistically significantly different than control means.
* Statistically significantly different from control value ($p \leq 0.05$).
** Statistically significantly different from control value ($p \leq 0.01$).

groups when compared to mean control values over the same time period. There were occasional periods of statistically significant differences in mean body weights among 0.3, 3, and 10 mg/kg-d K^+PFHxS-treated groups and controls from PND 4–14. Statistically significant reductions in mean body weight occurred in K^+PFHxS-treated females as compared to control mean values from PND 4 to PND 14 as follows: PND 4, 6–8, 11, and 13 in the 0.3 mg/kg-d group; PND 7 and 8 in the 3 mg/kg-d; and PND 4, 6–9, 11, 13, and 14 in the 10 mg/kg-d group. Mean body weights on PND 15 through term on PND 22 in K^+PFHxS-treated groups were not statistically significantly different than control means.

Treatment of male and female rats with K^+PFHxS at doses up to 10 mg/kg-d did not affect mean absolute and relative feed consumption when compared to controls.

Mating and fertility data are presented in Table 2. K^+PFHxS treatment did not affect any mating or fertility parameters investigated. Estrous cycling and all mating and fertility indices, including days in cohabitation, were unaffected by the K^+PFHxS treatments up to 10 mg/kg-d.

Pregnancy status, gestation length, and pregnancy outcome are summarized in Table 3. Pregnancy occurred in 88.7–100% of rats in all treatment groups and all pregnant F_0 dams delivered lit-

Table 2
Mating and fertility values (mean ± SD) of F_0 male and female rats.

	K^+PFHxS dose (mg/kg-d)				
	0	0.3	1.0	3.0	10.0
Male					
Number of animals	15	15	15	15	15
Cohabitation length (days)	3.2 ± 1.4	1.9 ± 1.2	2.8 ± 1.2	3.1 ± 2.0	2.5 ± 1.4
Mating index[a] (%)	14/15 (93.3%)	15/15 (100%)	13/15 (86.7%)	12/15 (80.0%)	15/15 (100%)
Fertility index[b] (%)	14/14 (100%)	15/15 (100%)	13/15 (86.7%)	12/13 (92.3%)	15/15 (100%)
Female					
Number of animals	15	15	15	15	15
Cohabitation length (days)	3.7 ± 3.0	1.9 ± 1.2	2.8 ± 1.2	4.0 ± 4.0	2.5 ± 1.4
Estrous cycle per 13-day	2.7 ± 0.9	2.8 ± 0.9	3.3 ± 0.6	2.9 ± 0.8	2.1 ± 1.2
Mating index (%)	15/15 (100%)	15/15 (100%)	13/15 (86.7%)	13/15 (86.7%)	15/15 (100%)
Fertility index (%)	15/15 (100%)	15/15 (100%)	13/15 (86.7%)	13/14 (92.8%)	15/15 (100%)

[a] Rats pregnant/number of rats in cohabitation.
[b] Pregnancies/number of rats that mated.

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EPA-HQ-2016-005679 06/14/2017

Table 3
Pregnancy outcome in F_0 female rats.

	K^+PFHxS dose (mg/kg-d)				
	0	0.3	1.0	3.0	10.0
Number of rats assigned for mating	15	15	15	15	15
Number of rats successfully mated	15	15	15	14	15
Number of rats became pregnant	15	15	13	13	15
Pregnancy rates[a] (%)	100	100	86.7	92.9	100
Duration of gestation (days)	22.5 ± 0.5	22.7 ± 0.4	22.7 ± 0.5	22.8 ± 0.4	22.6 ± 0.5
Total implants	251	230	206	192	231
Litters delivered	15	15	13	13	15
Implants/litter	16.7 ± 1.3	15.3 ± 2.9	15.8 ± 1.3	14.8 ± 2.8	15.4 ± 2.3
Gestation index (%)	15/15 (100%)	15/15 (100%)	13/13 (100%)	13/13 (100%)	15/15 (100%)
Total number of pups delivered	235	214	201	181	220
Liveborn	235	214	200	177	218
Stillborn	0	0	1	3	2
Unknown vital status	0	0	0	1	0

[a] Pregnancy rate = number of rats became pregnant relative to the total number assigned (for mating).

ters. Pregnancy status and outcomes were unaffected by K^+PFHxS treatments up to 10 mg/kg-d, the highest dose used in the study.

No test substance-related internal findings were observed for F_0 males and females at scheduled necropsies at any dose level. Macroscopic findings observed in the test article-treated groups occurred infrequently, at similar frequencies in the control group and/or in a manner that was not dose-related.

With the exception of liver weights in male rats, K^+PFHxS treatment did not affect the mean absolute or relative weights of organs when compared to control. K^+PFHxS treatment caused a statistically significant ($p < 0.05$) dose-related increase in mean absolute liver weight in male rats at doses of 3.0 and 10 mg/kg-d, which resulted in increases in mean liver weight of 20 and 56% over control, respectively. As a result, mean liver weight as a percent of body weight was also increased with statistical significance in male rats in the 3.0 and 10 mg/kg-d treatment groups (Fig. 1) as well as liver weight to brain weight ratio (data not shown). For female rats, K^+PFHxS did not result in treatment-related changes in absolute or relative liver weights.

With the exception of liver and thyroid tissues in male rats, there were no differences in tissue histology between control and K^+PFHxS-treatment groups. There were increased incidences of minimal to moderate hypertrophy seen in the liver and thyroid gland of male rats receiving 3.0 and 10 mg/kg-d K^+PFHxS doses (Table 4). The affected centrilobular hepatocytes were enlarged with an increased amount of dense eosinophilic granular cytoplasm. The changes in the thyroid gland consisted of hypertrophy and hyperplasia of the follicular epithelium cells. These microscopic changes in the liver and thyroid were consistent with the known effects of compounds that cause microsomal enzyme induction where the hepatocellular hypertrophy results in a compensatory hypertrophy and hyperplasia of the thyroid due to increased plasma turnover of thyroxine and associated stimulation of thyroid-stimulating hormone in rats [50]. No treatment-related microscopic changes were observed in any of the female rats or male rats administered 0.3 or 1.0 mg/kg-d K^+PFHxS doses.

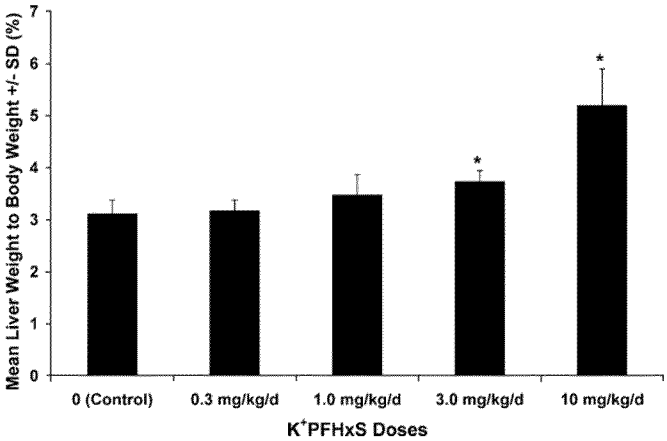

Fig. 1. Mean liver weight to body-weight percent data for F_0 male rats. Rats receiving daily K^+PFHxS treatments at 3.0 mg/kg-d and 10.0 mg/kg-d had statistically significant increased liver weight to body-weight percent than controls. Asterisk (*) denotes significant difference from control ($p < 0.05$).

Sperm motility, count, density, and morphology values for all F_0 male rats and primordial follicle counts for F_0 female rats (from control and 10 mg/kg-d K^+PFHxS treatment group only) were unaffected by treatment with K^+PFHxS.

Compared to controls, K^+PFHxS treatments did not affect any of the hematology or clinical chemistry in F_0 female rats.

For F_0 male rats, the hematology and clinical chemistry parameters that were statistically different than the values reported for controls are summarized in Table 5. While the hematocrit and RBC counts were significantly decreased in the 3 and 10 mg/kg-d treatment groups, hemoglobin concentrations were significantly decreased in the 1, 3 and 10 mg/kg-d groups. Prothrombin time was significantly increased in the 0.3, 3 and 10 mg/kg-d dosage groups but not the 1.0 mg/kg-d group. While albumin, albumin/globulin ratio, blood urea nitrogen, alkaline phosphatase, and calcium were significantly increased in the 10 mg/kg-d treatment group, cholesterol values were significantly decreased in all treatment groups and triglycerides was significantly decreased in the 10 mg/kg-d treatment group only.

Table 4
Histopathology of the F_0 generation male rats.

	K^+PFHxS dose (mg/kg-d)				
	0	0.3	1.0	3.0	10.0
Number of rats evaluated	10	10	10	10	10
Liver hypertrophy (hepatocellular, centrilobular)					
Minimal	0	0	0	8	4
Mild	0	0	0	1	5
Moderate	0	0	0	0	1
Total incidence	0	0	0	9	10
Thyroid hypertrophy/hyperplasia (follicular epithelium)					
Minimal	0	1	1	2	0
Mild	2	2	1	2	3
Moderate	0	0	0	0	4
Total incidence	2	3	2	4	7

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EPA-HQ-2016-005679 06/14/2017

Table 5
Hematological and clinical chemistry observations in male rats treated with K^+PFHxS for at least 42 days.

	K^+PFHxS dose (mg/kg-d)				
	0	0.3	1.0	3.0	10.0
Number of rats	10	10	10	10	10
Hematology					
Red blood cells (10^6/mm^3)	7.51 ± 0.40	7.33 ± 0.40	7.32 ± 0.44	6.93 ± 3.05*	6.99 ± 0.44*
Hemoglobin (g/dL)	16.5 ± 1.2	15.9 ± 0.4	15.7 ± 0.6*	15.4 ± 0.7**	15.6 ± 0.8*
Hematocrit (%)	43.5 ± 3.5	42.2 ± 1.6	42.0 ± 2.2	40.2 ± 2.3**	40.7 ± 1.8*
Prothrombin time (s)	13.4 ± 0.2	14.2 ± 0.3**	13.6 ± 0.2	13.8 ± 0.4*	14.0 ± 0.5**
Clinical chemistry					
Albumin (g/dL)	4.3 ± 0.2	4.1 ± 0.2	4.3 ± 0.2	4.2 ± 0.2	4.5 ± 0.2**
Albumin/globulin	2.1 ± 0.2	2.1 ± 0.2	2.2 ± 0.3	2.2 ± 0.2	2.5 ± 0.2**
BUN (mg/dL)	16.0 ± 1.5	16.0 ± 0.8	16.0 ± 1.8	17.0 ± 1.6	21.0 ± 2.4**
Creatinine (mg/dL)	0.30 ± 0.04	0.30 ± 0.05	0.30 ± 0.06	0.30 ± 0.03	0.30 ± 0.05
Calcium (mg/dL)	10.9 ± 0.5	10.7 ± 0.4	11.1 ± 0.4	11.1 ± 0.3	11.5 ± 0.4**
Sodium (mmol/L)	146.0 ± 1.4	146.0 ± 1.3	147.0 ± 1.6	147.0 ± 1.0	146.0 ± 2.2
Potassium (mmol/L)	6.6 ± 2.1	6.1 ± 0.9	6.5 ± 1.3	6.0 ± 0.6	6.9 ± 1.6
Chloride (mmol/L)	98.0 ± 2.7	99.0 ± 1.5	100.0 ± 2.3	100.0 ± 2.7	100.0 ± 1.2
Cholesterol (mg/dL)	57 ± 8	41 ± 11**	46 ± 12*	43 ± 13**	33 ± 7**
Triglycerides (mg/dL)	52 ± 21	47 ± 17	36 ± 14	36 ± 28	17 ± 8**
Alkaline phosphatase (U/L)	105 ± 14	111 ± 37	100 ± 12	115 ± 25	144 ± 38**
ALT (U/L)	42 ± 6	63 ± 70	60 ± 34	95 ± 124	45 ± 7
AST (U/L)	96 ± 22	121 ± 93	117 ± 41	198 ± 249	97 ± 16

* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group value ($p \leq 0.01$).

There were no statistically significant differences between control and K^+PFHxS treatments on the assessments of FOB parameters (autonomic functions, sensorimotor functions, excitability, gait and sensorimotor coordination and forelimb and hindlimb grip strength and abnormal clinical observations) and motor activity (data not shown).

3.3. F_1 Litter Results

In all K^+PFHxS-treated groups, there were no statistically significant differences in litter outcomes when compared to the control values (given in parentheses) for the numbers of F_1 pups delivered (235 per 15 litters), liveborn pups (235), stillborn pups (0), live pups on PND 1 (234), 8 (228), and 22 (228), and pups per litter on PND 22 (15.2 ± 1.8). The percent of pups that were male (45.2 ± 13.0), pup body weights (38.17 ± 4.72 and 36.67 ± 4.44 g for males and females, respectively), pup liver weights (1.48 ± 0.26 and 1.59 ± 0.24 g for males and females, respectively), liver-to-body-weight ratios (3.86 ± 0.27 and 4.33 ± 0.24 for males and females, respectively) on PND 22 also did not differ with statistical significance between control pups and those from K^+PFHxS-treated groups. The viability indices (number of live pups on PND 8 relative to number of live pups on PND 1 as a percent) and lactational indices (number of live pups on PND 22 relative to number of live pups on PND 8 as a percent) were not statistically significantly different between pups from K^+PFHxS-

Table 6
Mean serum/plasma and liver PFHxS concentration ± SD (ug/mL or ug/g) data summary.

		K^+PFHxS dose (mg/kg-d)				
		0	0.3	1.0	3.0	10.0
Serum PFHxS concentrations (ug/mL)						
SD 14	F_0 male[a]	0.14 ± 0.05	18.18 ± 2.42	80.97 ± 30.83	143.05 ± 82.09	182.67 ± 8.25
	F_0 female[b]	0.39[c]	2.78 ± 0.81	9.85 ± 3.91	20.67 ± 3.91	42.02 ± 6.47
SD 42	F_0 male	0.32 ± 0.09	44.22 ± 12.66	89.12 ± 0.80	128.67 ± 10.30	201.50 ± 20.02
GD 21	F_0 female	<LLOQ[d]	3.32 ± 0.71	10.65 ± 6.41	32.75 ± 7.83	59.80 ± 11.54
	F_1, pooled[e]	<LLOQ	5.32 ± 1.32	13.47 ± 2.06	37.10 ± 2.89	44.33 ± 6.50
PND 22	F_1, pooled	<LLOQ	8.57 ± 2.41	34.34 ± 10.86	32.35 ± 8.20	93.55 ± 55.79
Liver PFHxS concentrations (ug/g)						
SD 42	F_0 male	0.35 ± 0.23	43.80 ± 8.07	149.50 ± 26.06	338.67 ± 128.42	593.50 ± 81.41
GD 21	F_0 female	<LLOQ[f]	0.79 ± 0.19	2.61 ± 0.54	7.80 ± 1.58	16.53 ± 2.57
	F_1 fetus[g]	<LLOQ	1.37 ± 0.53	3.29 ± 1.17	7.19 ± 1.39	18.87 ± 4.28
PND 22	F_1 male[h]	<LLOQ	1.13 ± 0.31	3.86 ± 0.94	8.73 ± 1.65	16.22 ± 4.41
	F_1 female[i]	<LLOQ	1.04 ± 0.24	3.91 ± 1.05	9.96 ± 2.69	18.39 ± 2.32

[a] Parental male rats.
[b] Parental female rats (dams).
[c] Excludes data for two females that had serum measured below lower limit of quantification (LLOQ) of 0.1 ffig/mL.
[d] Serum LLOQ = 0.1 ffig/mL.
[e] GD 21 fetuses pooled by litter.
[f] Liver LLOQ = 0.ffig/g.
[g] Individual GD 21 fetuses.
[h] Male pups.
[i] Female pups.

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EPA-HQ-2016-005679 06/14/2017

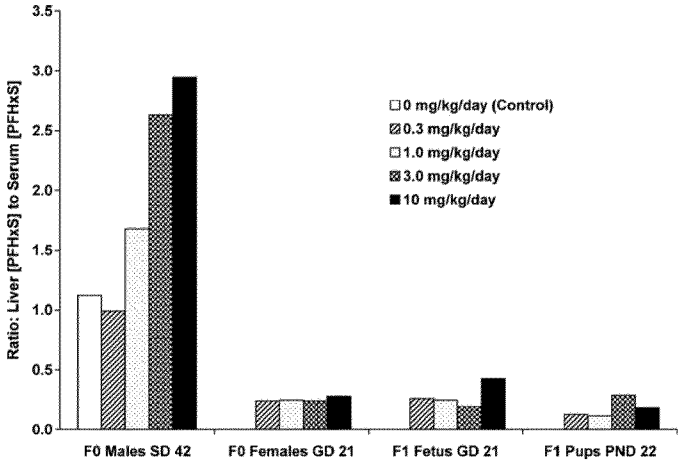

Fig. 2. Mean liver to serum PFHxS concentration ratio for F_0 males on study day (SD) 42, F_0 females on gestation day (GD) 21, F_1 fetus on GD 21, and F_1 pups on postnatal day 22. The F_1 generation rats did not directly receive K^+PFHxS treatment; therefore, presence of PFHxS in serum and liver was the result of *in utero* and lactational exposure.

treated and control groups (viability index = 97.4% and lactation index = 100%).

3.4. PFHxS concentrations in serum and liver

Mean PFHxS concentrations (±SD) in serum (or plasma) and livers are summarized in Table 6. The mean ratio between liver and serum PFHxS concentrations for each treatment groups are illustrated as Fig. 2.

4. Discussion

The purpose of this study was to provide information on the possible health hazards that may result from repeated exposure of Crl:CD®(SD)IGS BR VAF/Plus® male and female rats to K^+PFHxS beginning 14 days before cohabitation, through mating and continuing for at least 42 days (male rats) or through parturition until PND 21 (female rats). This repeated dose study incorporated a reproduction/developmental toxicity test that provided initial information on possible effects on male and female reproductive performance (e.g., gonadal function, mating behavior, conception, development of the conceptus and parturition). The study also included an evaluation of potential neurological effects in parental rats.

The overall body-weight gain from 10 mg/kg-d-dosed group F_0 males was statistically significantly lower than controls between SD 1 and SD 44, however, the terminal mean body weight was not statistically significantly different than control. The effect on body weight in 10 mg/kg-d dose-group males did not appear to be related to decreased feed consumption. In a two-generation reproduction and developmental study with the PFHxS congener, PFOS, with daily gavage dosing, F_0 male body weights were lower than controls at doses of 1.6 and 3.2 mg K^+PFOS/kg/d, becoming statistically significant by days 56 and 36 in these treatment groups, respectively [51]. Feed consumption and body-weight gain were also reduced with statistical significance by K^+PFOS treatment at doses ≥0.4 mg/kg-d. These data suggest that PFOS may be more effective in adversely affecting body weight in male SD rats than PFHxS at equivalent doses. This greater potency could be due to pharmacokinetic differences, pharmacodynamic differences, or both of these factors. Based on data presented by Luebker et al., the mean ffiM concentrations of PFOS in serum by dose were 91, 305, and 547 for the 0.4, 1.6, and 3.2 mg/kg-d dose F_0 male treatment groups, respectively. Mean liver PFOS concentrations in ffiM for the 0.4, 1.6, and 3.2 mg/kg-d dose F_0 male treatment groups from Luebker et al. were 353, 647, and 2719, respectively. Based on data presented in this article, for the 10 mg/kg-d K^+PFHxS-treated F_0 males, the mean ffiM PFHxS concentrations in serum and liver were 594 and 1487, respectively. Considering that the K^+PFOS-treated F_0 males in the Luebker et al. study had been dosed approximately three weeks longer than those from the study reported herein, it is apparent that PFOS may be more potent than PFHxS. At doses of 1.0 mg/kg-d or higher, K^+PFHxS-treated F_0 male rats appeared to have reached serum steady state by SD 14 as their serum PFHxS concentrations were not statistically different between SD 14 and SD 42. In contrast, serum PFHxS concentrations in F_0 females were 12–23% of the F_0 males on SD 14. This gender difference in body burden may have contributed to the absence in body-weight effect in F_0 females as compared to males in each K^+PFHxS treatment group. In addition, differences in pharmacodynamic response cannot be ruled out.

The only effect of treatment with K^+PFHxS on organ weights observed were 20 and 56% increases in mean liver weight in F_0 male rats in the 3 and 10 mg/kg-d treatment groups, respectively, when compared to F_0 male control mean liver weight. Liver weight relative to either body weight or brain weight was also increased to a similar extent in the 3 and 10 mg/kg-d dose-group males as compared to controls. The incidence and severity of centrilobular hepatocellular hypertrophy observed correlated well with liver weight observations.

In the study reported herein, the mean serum and liver concentrations in F_0 males associated with no effect on liver weight at the 1 mg/kg-d dose were 89 ffig/mL and 150 ffig/g, respectively, after six weeks of K^+PFHxS treatment. At the effective K^+PFHxS dose of 3 mg/kg-d, mean serum and liver PFHxS concentrations in males were 129 ffig/mL and 339 ffig/g, respectively. It is interesting to note that, in data from a currently unpublished 3M study, daily gavage dosing of male and female SD rats with potassium PFOS at a dose of 1 mg/kg-d for 28 days resulted in increased liver-weight-to-body-weight ratios in the absence of a statistically significant effect on body weight. This effect was noted at mean serum and liver PFOS concentrations of 46 ffig/mL and 107 ffig/g for males, respectively, and 74 ffig/mL and 126 ffig/g for females, respectively. This observation would suggest that PFOS may be more potent in inducing hepatic hypertrophy than PFHxS.

The observed increase in mild to moderate thyroid follicular epithelial hypertrophy/hyperplasia in the 10 mg/kg-d treatment group males was consistent with the increase in centrilobular hepatocellular hypertrophy [50]. Thyroid hormones were not measured, but prior work with PFOS has demonstrated that competition for binding resulting in increased displacement of thyroid hormones as well as potential induction of increased metabolism of thyroid hormones by liver results in hypothyroxinemia without a strong compensatory increase in pituitary thyrotropin (thyroid-stimulating hormone or TSH) [52]. This is not the result of decreased pituitary release of TSH or response to hypothalamic thyrotropin releasing hormone. Increased hepatocellular hypertrophy in rats is often accompanied by increased thyroid follicular epithelial hypertrophy/hyperplasia as a consequence of induction of thyroid hormone metabolism [50,53]. A two-year dietary study conducted with K^+PFOS in Sprague Dawley rats did not produce an increase in thyroid follicular cell tumors in rats given K^+PFOS continuously throughout the study. However, male rats at the high dose level (20 ppm in diet) that were given potassium PFOS for the first year of the study and control diet thereafter did have an increase in thyroid follicular cell adenoma [54]. Whether the latter observation was a chance occurrence or represents a true effect remains to be determined.

K^+PFHxS treatment resulted in mild but statistically significant decreases in RBC count, hematocrit, and hemoglobin in male rats in the 3 and 10 mg/kg-d treatment groups. Histological examination

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EPA-HQ-2016-005679 06/14/2017

of bone marrow in the 10 mg/kg-d dose-group males did not reveal any abnormalities as compared to controls. Taking the hematology data from the study as a whole, this pattern would suggest slight to mild plasma volume expansion; although other potential etiologies cannot be ruled out with the available data. A similar pattern has observed with the PFHxS homolog, perfluorobutanesulfonate (PFBS) [55]. The PFHxS homolog, PFOS, has been demonstrated to be both a PPARffl [56–59] and a PPAR[56] agonist; although Takacs and Abbott did not observe activation of PPAR with PFOS [60]. Plasma volume expansion is a known effect of thiazolidinediones mediated via PPAR stimulation of the epithelial sodium channel (ENaC) of the collecting duct in kidneys [61]. Typically, weight gain is a consequence of fluid retention with thiazolidinediones, and this was not observed with PFHxS. Indeed, K^+PFHxS treatment caused decreased weight gain at the highest dose in males. In addition, no increases in sodium levels were noted. Dual activation of PPAR and PPARffl could mask a weight gain effect due to the increased -oxidation associated with PPARffl activation. Additional investigation would be required to develop a better understanding of the etiology of the reduced RBC count, hematocrit, and hemoglobin occurring in male rats given PFHxS.

The statistically significant increase in serum urea nitrogen (BUN) in 10 mg/kg-d dose-group parental males without a change in serum creatinine is suggestive of mild dehydration. However, this would be inconsistent with the observed small but significant decreases in RBC count, hematocrit, and hemoglobin, which are suggestive of volume expansion. The mean control value for BUN was consistent with the reported control values for the sex and strain for fasted rats [62]. BUN could also be increased as a result of increased protein catabolism [63]. With the information available from the study, it is difficult to establish the cause of the apparent increase in BUN.

The statistically significant effects observed on prothrombin time in male rats given K^+PFHxS were slight and did not follow a specific trend. These may represent normal variation in this parameter, as the values were within the range of normal (mean 13.6 ± 2.7 s) for 10–20-week-old Sprague Dawley rats as reported by Matsuzawa et al. [62].

The mild hypercalcemia observed in male rats at the 10 mg/kg-d dose is likely due to the increase in serum albumin also occurring at this dose. The ratio of serum albumin to calcium concentration is relatively constant across doses for males. Serum calcium should be corrected for serum albumin in cases where serum albumin is known to be altered [63]. In 10 mg/kg-d dose-group parental males, serum albumin increased over controls by about 5%, with serum calcium increasing by about 6%. Less likely potential etiologies of the mild increase in serum calcium would be an effect on parathyroid function or slight hyperthyroid state. The 37% increase in mean serum alkaline phosphatase in 10 mg/kg-d dose-group males compared to controls, while of unknown origin, would support either of these scenarios. However, increased serum albumin is the simplest and most supportable explanation.

The effect of K^+PFHxS treatment on decreasing serum total cholesterol in male rats was the most pronounced effect observed, occurring at all dose levels. Although not reaching statistical significance until the 10 mg/kg-d dose, mean serum triglycerides were also lower in all K^+PFHxS-treated male groups compared to the male control group. This observation is consistent with cholesterol reduction as a sensitive clinical endpoint for response to the PFHxS congener, PFOS [36,37]. The mechanism resulting in the reduction in serum total cholesterol with PFOS remains to be elucidated. In Wistar rats fed PFOS, decreased activities of hydroxymethylglutaryl CoA reductase and acyl-CoA:cholesterol acyl transferase and increased fatty acid oxidation leading to decreased lipoprotein particle production due reduced synthesis and esterification of cholesterol have been suggested [64]. Stimulation of PPARffl is likely involved in the case of PFOS [56,59,60]. These modes of action are currently being further investigated with PFHxS. Wolf et al. have demonstrated that PFHxS may actually be a more potent activator of mouse and human PPARffl than either the four-carbon PFBS or eight-carbon PFOS in transiently transfected COS-1 cells [58].

The lack of effect of K^+PFHxS treatment on mating and fertility is consistent with prior observations with the congener, PFOS [51,65], and the perfluoroalkyl carboxylate, PFOA [66]. The dosing period did not allow for a complete cycle of spermatogenesis in the males.

In F_0 rats, the PFHxS concentrations in plasma and liver correlated well with the daily K^+PFHxS doses given. Prior to mating, the body burden (represented as plasma PFHxS concentration) in F_0 rats appeared to be sex-dependent: the plasma PFHxS concentrations in F_0 males were 4 to 8 times higher than that measured in F_0 females on SD 14.

Even though none of the F_1 rats received K^+PFHxS treatment directly, it was evident that neonates can receive PFHxS via *in utero* and lactational exposures. The same dose-dependent increases in serum and liver PFHxS concentrations were also observed in F_1 rats per daily K^+PFHxS doses given to the litter-matched F_0 female rats. The finding that serum and liver PFHxS concentrations in pups at PND 22 were higher than GD 21 indicated that exposure of F_1 progeny to PFHxS via the breast milk is as robust as that which occurs *in utero* across the placenta. Fetal serum PFHxS concentrations were slightly higher than maternal serum PFHxS concentrations on GD 21 in all treatment groups except for the highest treatment group. In both F_0 females and F_1 rats, liver PFHxS concentrations were lower than the respective serum PFHxS concentrations at all times.

While the ratio between F_0 male liver to serum PFHxS concentrations ranged from 1 to 3 on SD 42, the corresponding liver to serum PFHxS concentration ratios were approximately 0.5 or lower for all F_0 females on GD 21 and all F_1 pups on GD 21 and PND 22. Although there appeared to be a sex difference in serum and liver PFHxS concentrations in adult rats, there did not appear to be a sex difference between male and female F_1 rats throughout PND 22. The liver PFHxS concentrations remained similar between F_1 male and female rats in all treatment groups on PND 22. It is interesting that lack of a sex difference in PFOS serum and liver concentrations through PND 22 in rat pups from dams treated with K^+PFOS through gestation and lactation has also been reported [67], however, sex differences in serum PFOS concentrations became apparent after sexual maturation. Further study of the effect of sexual maturation on pharmacokinetic handling of PFHxS and PFOS would be of value.

The lack of significant postnatal effects in F_1 generation pups from K^+PFHxS-treated parents is quite different from the increased neonatal mortality and decreased postnatal growth observed for PFOS in Sprague Dawley rats and in mice (CD1 and SV129 PPARffl wild-type and null) [51,65,68,69] and in CD1 and SV129 PPARffl wild-type mice with PFOA [70–72]. The mean GD 21 maternal and fetal serum PFOS concentrations for the 10 mg/kg-d K^+PFHxS-treated dams (60 ffig/mL and 44 ffig/mL) approximated those of K^+PFOS-treated dams and their fetuses on GD 21 that were associated with significant reductions in postnatal pup survival (62 and 101 ffig/mL, respectively) [65]. This observation suggests that K^+PFHxS treatment in rats is either not capable of producing neonatal mortality or requires higher body burdens in the fetal compartment.

PFHxS was first reported to be present at low ng/mL (ppb) concentrations in human serum in 2001 by Hansen et al. [11]. A number of biomonitoring studies of human populations subsequently have confirmed the widespread presence of PFHxS in samples from non-occupationally exposed populations [10,12–14,19–23] These studies demonstrate that PFHxS can be found in umbilical cord blood serum collected in Canada [19], neonatal screening program blood spots from New York [10], and human milk samples from the

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EPA-HQ-2016-005679 06/14/2017

United States [20], China [21], and Sweden [22]. In addition, PFHxS has been found in plasma from children aged 5–6 from Bavaria [23], serum from children aged 2–12 from the United States [14], and serum from adolescents aged 12–19 from the United States [12,13] as well as adults [12,13,15–18]

The serum PFHxS concentrations in children from the United States are greater at the upper tails of the distribution than those of adults [14]. Olsen et al. [14] found that geometric mean serum PFHxS values among 598 children aged 2–12 from samples collected between 1995 and 1996 were not appreciably different than those of 645 American Red Cross blood donors taken between 2000 and 2001 [16] or 238 elderly blood donor serum samples from 1996 [15]. However, 67 of the 598 children (11%) had serum PFHxS concentrations above 30 ng/mL, as opposed to just one adult in each of the adult studies just cited. Similarly, Calafat et al., in reporting data from serum samples taken during 1999–2000 [12] and 2003–2004 [13] as part of the National Health and Nutrition Examination Survey (NHANES), demonstrated that, during both time periods, adolescents aged 12–19 years had the higher geometric mean serum PFHxS concentrations than the other three age groups (20–39 years, 40–59 years, and ≥60 years) and that the estimated 95th percentile serum PFHxS concentration of these adolescents was significantly greater than that of the three other age groups.

Exposure of children to PFHxS may begin very early in life. Monroy et al [19] studied PFHxS serum concentrations in 101 pregnant women and their matched umbilical cord serum at birth. They found that umbilical cord blood serum at birth had a slightly greater mean PFHxS concentration than the respective mean for maternal serum at delivery (5.05 ± 12.92 ng/mL for umbilical cord blood serum versus 4.05 ± 12.30 ng/mL for mothers' serum); however, PFHxS was detectable in 46% of mothers' serum at birth versus 21% of umbilical cord blood serum samples. Lactational exposure to PFHxS has also been demonstrated in human populations. Human milk concentrations have ranged from a median of 0.011 ng/mL in 19 samples from China [21], a mean of 0.0145 ng/mL in 45 samples from Massachusetts [20], and a mean of 0.085 ng/mL in 12 samples from Sweden [22]. In the Swedish study, the respective mean maternal serum concentration was 4.7 ng/mL.

Reflecting on the larger proportion of children with serum PFHxS concentrations greater than 30 ng/mL as compared to adults, Olsen et al. [14] suggested that differences in the exposure patterns of children as well as potential exposure to surfaces in the home treated with materials that may contain or release PFHxS may account for the greater PFHxS serum concentrations in the tails of the distribution. Kubwabo et al. [27] and Strynar and Lindstrom [28] demonstrated the presence of PFHxS in house dust. In the Kubwabo et al. study, PFHxS concentrations in house dust were positively associated with the presence and extent of carpeting in homes and negatively associated with the age of the homes, which was negatively associated with the presence of carpet. Because children may spend more time in the home and in contact with treated surfaces, and boys may be somewhat more prone to these types of exposures (boys were more likely to have higher serum PFHxS concentrations than girls in the studies reported by Olsen et al. [14] and Hölzer et al. [23]), these data combined with the biomonitoring data provide support for the suggestion made by Olsen et al. to explain the differences in the distributions of adults and children at the upper end.

Sources other than house dust or treated surfaces in the home may include exposure via water, as suggested by Hölzer et al. [23] and Ericson et al. [29]. In particular, Hölzer et al. found that maximum children's plasma PFHxS concentrations were greater than those of adults in two German towns, one with potential exposure via drinking water. PFHxS has been found, less frequently, in samples from various wildlife [73–77], and Falandysz et al. [24] found an association of PFHxS serum concentrations with consumption of fish in the Baltic. However, Ericson et al. [78] were not able to detect PFHxS in market food sources from the Catalan area of Spain.

Although a complete quantitative description of PFHxS exposure from various sources is not feasible at this time, it has become evident that PFHxS blood concentrations in the general public in the United States are declining since 2000–2002 when the principal manufacturer, 3M Company, discontinued production of PFHxS based on evidence of widespread distribution in non-occupationally-exposed populations [10,13,17] In analyzing data from the National Health and Nutrition Examination Survey (NHANES), Calafat et al. [13] reported a 10% decline in PFHxS serum concentrations between 1999–2000 and 2003–2004. Using newborn screening blood spots for the same time period, Spliethoff et al. [10] reported a 23% decline. Similarly, Olsen et al. [17] showed a 30% decline in measured serum PFHxS concentration with American Red Cross blood donors between 2000–2001 and 2006. These declines appear to be consistent with the reported geometric mean serum PFHxS elimination half-life for 26 retired fluorochemical workers of approximately 7 years [7]. However, in Germany, PFHxS serum concentrations have been shown to be rising between 1977 and 2004 [26].

5. Conclusion

Treatment of mature adult Sprague Dawley rats with K^+PFHxS by daily oral gavage at doses up to 10 mg/kg-d for two weeks prior to mating, and for females, through gestation and lactation, and, for males, for a minimum of 42 days, did not produce major detrimental effects on mating, fertility, birth outcome, and development of offspring. The most sensitive effect, observed in parental males, was a decrease in total serum cholesterol, observed at all treatment levels.

Conflict of interest statement

John L. Butenhoff, Shu-Ching Chang, and David J. Ehresman are employees of 3M Company, a former manufacturer of K^+PFHxS and the company supporting the work reported on in the article. Raymond G. York does not have competing interests other than employment in contract research facilities (Charles River Preclinical Services) conducting parts of the work.

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Toxicological evaluation of potassium perfluorobutanesulfonate in a 90-day oral gavage study with Sprague–Dawley rats

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abstract

Perfluorobutanesulfonate (PFBS) is a surfactant and degradation product of substances synthesized using perfluorobutanesulfonyl fluoride. A 90-day rat oral gavage study has been conducted with potassium PFBS (K^+PFBS). Rats were dosed with K^+PFBS at doses of 60, 200, and 600 mg/kg-day body weight. The following endpoints were evaluated: clinical observations, food consumption, body weight, gross and microscopic pathology, clinical chemistry, and hematology. In addition, functional observation battery and motor activity assessments were made. Histological examination included tissues in control and 600 mg/kg-day groups. Additional histological examinations were performed on nasal cavities and turbinates, stomachs, and kidneys in the 60 and 200 mg/kg-day groups. No treatment-related mortality, body weight, or neurological effects were noted. Chromorhinorrhea (perioral) and urine-stained abdominal fur were observed in males at 600 mg/kg-day. Red blood cell counts, hemoglobin, and hematocrit values were reduced in males receiving 200 and 600 mg/kg-day; however, there were no adverse histopathological findings in bone marrow. Total protein and albumin were lower in females at 600 mg/kg-day. There were no significant changes in clinical chemistry in either sex. All rats appeared normal at sacrifice. Microscopic changes were observed only at the highest dose in the stomach. These changes consisted of hyperplasia with some necrosis of the mucosa with some squamous metaplasia. These effects likely were due to a cumulative direct irritation effect resulting from oral dosing with K^+PFBS. Histopathological changes were also observed in the kidneys. The changes observed were minimal-to-mild hyperplasia of the epithelial cells of the medullary and papillary tubules and the ducts in the inner medullary region. There were no corresponding changes in kidney weights. Clinical chemistry parameters related to kidney function were unchanged. These kidney findings are likely due to a response to high concentration of K^+PFBS in tubules and ducts and represent a minimal-to-mild effect. Microscopic changes of an equivocal and uncertain nature were observed in the nasal mucosa and were likely attributable to the route of dosing (oral gavage). The NOAEL for the female rat in this study was 600 mg/kg-day (highest dose of study). The NOAEL for the male rat was 60 mg/kg-day based on hematological effects.

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1. Introduction

In the 1990s, industry (3M) began developing new technologies to augment and eventually replace a chemical class referred to "perfluorooctanyl" chemicals. This chemical class includes perfluorooctanesulfonyl fluoride (POSF), N-alkyl derivatives of POSF, perfluorooctanoate (PFOA), and perfluorooctanesulfonate (PFOS). In May 2000, 3M announced the voluntary manufacturing phase out of this chemical class due to its persistence and widespread distribution in the environment. Subsequently, the United States Environmental Protection Agency (USEPA) issued a Significant New Use Rule (USEPA, 2002), or SNUR, that more broadly regulates the production and import of many perfluorooctanyl-based chemicals. The USEPA subsequently proposed to amend the perfluoroalkyl substances SNUR (USEPA, 2006) to include additional perfluorooctanyl-based chemicals. Canada banned the use and importation of a number of long chain perfluorinated carboxylic acids because of concerns over potential adverse human and environmental effects (CGDE, 2008). In January 2006, the USEPA announced a voluntary 2010/2015 PFOA Stewardship Program to eliminate emissions and product content of PFOA and related chemicals (http://www.epa.gov/opptintr/pfoa/pubs/pfoastewardship). One of the new technologies devel-

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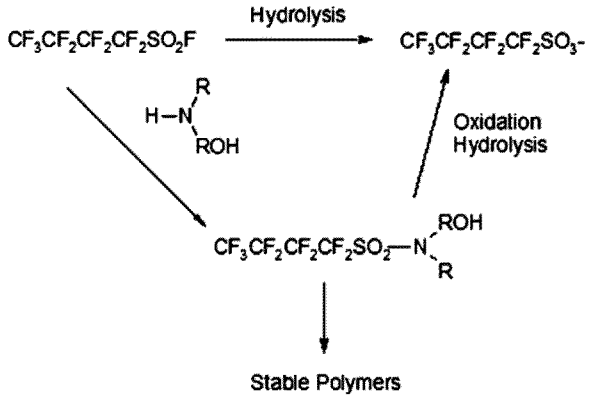

Fig. 1. Degradation pathways of N-alkyl-substituted perfluorobutanesulfonamide.

oped by 3M is based on products made from perfluorobutanesulfonyl fluoride (PBSF). The ultimate degradation and metabolic product from materials based on this chemistry is perfluorobutanesulfonate (PFBS). While PFBS can be formed from the hydrolysis of PBSF, it can also be formed from the metabolic and environmental degradation of certain derivatized perfluorobutanesulfonamides (Fig. 1). Derivatized perfluorobutanesulfonamides have been developed for use in numerous applications including fabric, carpet and upholstery protectants, and surfactants. Extensive testing has been performed on this material which, compared to the perfluorooctanyl-based chemicals, has a low potential to bioaccumulate (NICNAS, 2005; Olsen et al., 2008). We undertook this study to further develop the basic descriptive toxicological profile of PFBS.

2. Materials and methods

2.1. Animals

Crl:CD@(SD)IGS BR VAF/PlusTM rats were supplied by Charles Rivers Laboratory. Rats were assigned to individual housing in stainless steel, wire-bottomed cages. Animal care, cages, and housing conditions were in accordance with the Guide for the Care and Use of Laboratory Animals (ILAR, 1996). Rats were given *ad libitum* access to Certified Rodent Diet R #5002 (PMI Nutrition International Inc., St. Louis, MO) in individual feeders. Water (processed by reverses osmosis) was available to the rats *ad libitum* from individual bottles or an automatic watering system. Lighting was maintained at 12-h light and 12-h dark cycles. The study was conducted in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. All procedures involving laboratory animals were reviewed and approved by the Institutional Animal Care and Use Committee associated with the facility in which the laboratory animals were housed.

2.2. Test substance

The potassium salt of perfluorobutanesulfonate (K^+PFBS, Lot # 120K0252, 98.2% pure) was provided by 3M Company. Solutions of K^+PFBS were prepared in aqueous carboxymethyl cellulose (0.1% CMC, medium viscosity) using reverse osmosis membrane processed deionized water.

2.3. Study design

This study was performed according to Good Laboratory Practices (USEPA, 2000) and based on the test guidelines, OECD 408 and OPPTS 870.3100 (USEPA, 1998). Dose levels were selected based on the results of a 28-day toxicology study (unpublished) conducted with the test substance. In that study, 900 mg/kg-day produced significant increases in the liver weight of male rats and significant increases in kidney weights of female rats. The no-observable-adverse-effect level (NOAEL) in the 28-day study was 300 mg/kg-day. In this 90-day study, rats (10/(sexgroup)) received daily oral doses by gastric lavage of 0, 60, 200, and 600 mg/kg-day. The doses were prepared using a constant dosing volume of 10 mL/kg and were administered for approximately 90 days (90–93 days). Doses were adjusted for the most recently recorded body weight and administered at approximately the same time each day.

Rats were observed twice daily during the study. Observations for clinical signs were made daily before dosing and approximately 1 h after each dose administration and again on the day of sacrifice. Signs of toxicity were recorded as observed. Detailed clinical observations were conducted for all rats once before the first dose and at least once weekly thereafter. Signs noted included, but were not limited to changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions and autonomic activity (e.g., lacrymation, piloerection, pupil size, and unusual respiratory pattern). Changes in gait, posture, and response to handling, and other behavior patterns were recorded. Ophthalmologic examinations were performed by a veterinary ophthalmologist for all rats prior to the first dose and at termination of the study.

Body weights for male and female rats were recorded weekly during the acclimation and dose periods and at sacrifice. Feed consumption values were recorded weekly during the acclimation and dose periods. A functional observational battery (FOB) and motor activity assessment were conducted on five male and five female rats per group after the 11th week of dosing.

All rats were sacrificed by carbon dioxide asphyxiation on the day following the last administration of the test substance (days 91–94 of the study). Approximately 5 mL of blood was collected for hematological and clinical biochemical evaluations. One milliliter of blood was collected into EDTA-coated tubes and analyzed for the following hematological parameters: erythrocyte count (RBC); hematocrit (HCT); hemoglobin (HGB); mean corpuscular hemoglobin (MCH); mean corpuscular hemoglobin concentration (MCHC); mean corpuscular volume (MCV); total leukocyte count (WBC); differential leukocyte count; platelet count (PLAT); mean platelet volume (MPV); and cell morphology. Blood smears were prepared for each sample for measurements of differential leukocyte count. Another 1.8 mL of blood was added to a tube containing 0.2 mL of sodium citrate for determination of prothrombin time (PT) and activated partial thromboplastin time (APTT).

Two milliliters of blood were processed to obtain serum, immediately frozen on dry ice, and subsequently analyzed for the following parameters: total protein (TP); triglycerides (TRI); albumin (A); globulin (G); albumin/globulin ratio (A/G); glucose (GLU); cholesterol (CHOL); total bilirubin (TBILI); urea nitrogen (BUN); creatinine (CREAT); alanine aminotransferase (ALT); asparatate aminotransferase (AST); alkaline phosphatase (ALK); calcium (CA); phosphorus (PHOS); sodium (NA); potassium (K); and chloride (CL).

A gross necropsy of the thoracic, abdominal, and pelvic viscera was performed. In addition, the cranial, thoracic and abdominal cavities were examined. The lungs were perfused with neutral buffered 10% formalin. The following organs were excised, trimmed and weighed prior to fixation: adrenals; brain; kidneys; heart; thymus; spleen; liver; testes; epididymis; ovaries; and uterus. The following tissues were retained in neutral buffered formalin (10%): adrenals; aorta; bone marrow (sternum); brain; epididymis; esophagus; eyes; femur; heart; large intestines; small intestines; kidneys; larynx; pharynx and nose; liver; lungs; lymph nodes (mandibular and mesenteric); mammary gland; ovaries; pancreas; Peyer's patches; pituitary; prostate; salivary gland (mandibular); sciatic nerve; seminal vesicles; skeletal muscle; skin; spinal cord; spleen; stomach; testes; thymus; thyroid/parathyroid; trachea; uterus; urinary bladder; and vagina.

Tissues for histological examination were processed, embedded in paraffin, and sectioned at 5 ffim and stained with hematoxylin and eosin. Histological examination was performed on all tissues from the 0 (vehicle) and 600 mg/kg-day dose groups. In addition, the nasal cavities, nasal turbinates, stomachs and kidneys of the male and female rats in the 60 and 200 mg/kg-day dose groups were evaluated microscopically.

A 600-mg/kg-day male rat was found dead and examined for the cause of death on day 85 (prior to study termination). The rat was necropsied and examined to the extent possible as described for rats sacrificed according to schedule.

2.4. Statistical analysis

Data generated during the course of this study were recorded either by hand or by using the Argus Automated Data Collection and Management System, the Vivarium Temperature and Relative Humidity Monitoring System, the Coulbourn Instruments Passive Infrared Motor Activity System, the Coulbourn Instruments Auditory Startle System, the Coulbourn Instruments Spatial Delayed Alternation System, and/or the passive avoidance software. All data were tabulated, summarized and/or statistically analyzed using the Argus Automated Data Collection and Management System, the Vivarium Temperature and Relative Humidity Monitoring System, Microsoft Excel (part of Microsoft Office 97 (version SR-2)) and/or The SAS System (version 6.12).

Parameters in the FOB that used interval scales, such as the grip-strength tests and the landing foot splay test, as well as body weight data and feed consumption values, were analyzed initially as parametric data. Bartlett's test of homogeneity of variances (Sokol and Rohlf, 1969a) was used to estimate the probability that the groups had different variances. A non-statistically significant result ($p > 0.001$) indicated that an assumption of homogeneity of variance was appropriate, and the data were compared using the analysis of variance test (Snedecor and Cochron, 1967a,b). If that test was significant ($p < 0.05$), the groups exposed to the test substance were compared with the control group using Dunnett's test (Dunnett, 1955). If Bartlett's test was significant ($p < 0.001$), the analysis of variance test was not appropriate, and the data were analyzed using non-parametric methods. When 75% or fewer of the scores in all the groups were tied, the Kruskal–Wallis test (Sokol and Rohlf, 1969b) was used to analyze the data, and in the event of a significant result ($p < 0.05$), Dunn's test (Dunn, 1964) was used to compare the groups exposed to the test sub-

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EPA-HQ-2016-005679 06/14/2017

Table 1
Mean (±S.D.) body weights in grams by dose group in male and female rats.

Dosage (mg/kg-day)	Male				Female			
	Control	60	200	600	Control	60	200	600
N	10	10	10	10[a]	10	10	10	10
Day 1	192.1 ± 7.3	192.9 ± 6.8	190.0 ± 7.2	193.1 ± 7.2	161.5 ± 9.6	161.0 ± 7.7	157.9 ± 11.2	158.1 ± 10.9
Termination	510.9 ± 47.0	482.6 ± 60.4	479.6 ± 30.3	485.3 ± 49.4[a]	276.5 ± 24.0	286.0 ± 21.5	284.8 ± 30.1	264.6 ± 19.5

[a] Terminal body weights at this dose for males exclude the value for the rat which was found dead on day 85 of study.

stance with the control group. When more than 75% of the scores in any group were tied, Fisher's exact test (Siegel, 1956) was used to compare the proportion of ties in the groups.

Data from the motor activity test, with repeated measurements within a session, were analyzed using an analysis of variance with repeated measures (SAS Institute, 1988). A significant effect ($p < 0.05$) in that test can appear as effect of Concentration (a difference between groups in the total across all measurements in a session) or as an interaction between Concentration and Block (a difference between groups at specific measurement periods). If the Concentration effect was significant, the totals for the control group and the groups given the test substance were compared using Dunnett's test. If the Concentration × Block interaction was significant, an analysis of variance test was used to evaluate the data at each measurement period, and a significant result ($p < 0.05$) was followed by a comparison of the groups using Dunnett's test.

Parameters in the FOB that had graded or count scores were analyzed using non-parametric procedures.

Clinical observation incidence data, as well as the descriptive and quantal data from the FOB, were analyzed as contingency tables using the variance test for homogeneity of the binomial distribution (Snedecor and Cochron, 1967a,b).

3. Results

3.1. Mortality and clinical observations

3.1.1. Male rats

One male rat in the high dose group was found dead on study day 85. Clinical observations on day 84 included red perioral substance, urine-stained abdominal fur, decreased motor activity, cold to touch, ptosis, dehydration, brown substance around mouth, and ungroomed coat. This death was considered unrelated to administration of K^+PFBS. This was a single incident, and the sudden onset of adverse clinical observations indicates a possible injury. All other male rats survived until scheduled sacrifice.

In male rats in the 600 mg/kg-day dose group, the 9 incidences of a red perioral substance (slight to extreme in degree) occurring in 6 of 10 rats and the 27 incidences of urine-stained abdominal fur occurring in 3 of 10 rats were significantly different than control incidences ($p < 0.01$). Chromorhinorrhea occurred in 2 of 10 and 3 of 10 rats in the 200 and 600 mg/kg-day dose groups, respectively. All other clinical observations were considered unrelated to the test substance, because the incidences were not dose-dependent and/or the observations only occurred in one rat in a group. These clinical observations included localized alopecia of the limbs and neck, discolored fur, swollen ears or snout, excess salivation, red substance in cage pan, decreased motor activity, dehydration, ptosis, brown substance around mouth, ungroomed coat, cold to touch, soft or liquid feces, scab on the neck, head or forelimb, ulceration on neck, chromodacryorrhea, missing/broken incisors, and abrasion on neck or head. Ophthalmologic examination of the male rats at study termination revealed one male rat in the 200 mg/kg-day dose group with chromodacryorrhea of both eyes.

3.1.2. Female rats

All female rats survived to scheduled sacrifice. All clinical observations in the female rats were considered unrelated to K^+PFBS, because either the observations were not dose-dependent, and/or the observation only occurred in one or two rats in a group.

3.2. Effects on body weight

Body weights and body weight gains (data not shown) for both the male and female rats were similar in all four dose groups throughout the study period (Table 1). Body weight gain was significantly reduced ($p < 0.01$) in male rats on study days 15–22 in the 60 and 200 mg/kg-day dose groups and in the female rats on study days 43–50 in the 60 mg/kg-day dose group. These effects were not considered treatment related, because they were not dose-dependent and occurred only once.

3.2.1. Organ to body weight data

In male rats, the absolute weights of the spleen and the ratios of the weight of the spleen to terminal body weight and brain weight (data not shown) were reduced or significantly reduced in the 60, 200 and 600 mg/kg-day dose groups ($p < 0.05$ or $p < 0.01$) when compared to control values (Table 2). However, there was no trend in the reduction across the 10-fold increase in dose range (60–600 mg/kg/day), and reductions in spleen weights were not present among treated female rats when compared to controls. All other organ weights and organ weights to terminal body weights and brain weights for male rats were unaffected by doses of K^+PFBS up to 600 mg/kg-day.

In female rats, all organ weights and organ weights to terminal body weights and brain weights were unaffected in the female rats by dose of K^+PFBS up to 600 mg/kg-day (Table 3).

3.3. Feed consumption

There were no notable differences in feed consumption between K^+PFBS-treated male and female rats and their respective control rats.

3.4. Motor activity and functional observation battery

There were no statistically significant or biologically important differences between K^+PFBS-treated male and female rats and their respective control rats in any of the measured parameters. Parameters evaluated included: home cage behavior; autonomic functions (lacrymation, salivation, palpebral closure, prominence of the eye, papillary reaction to light, piloerection, respiration, defecation, and urination); sensorimotor functions; excitability; gait and sensorimotor coordination; forelimb and hindlimb grip strength; and abnormal clinical observations (including but not limited to: convulsions; tremors; unusual behavior; hypotonia; hypertonia; emaciation; dehydration; unkempt appearance; and deposits around the eyes nose or mouth).

3.5. Macroscopic observations at necropsy

All necropsy observations for animals sacrificed at the end of the study were normal.

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EPA-HQ-2016-005679 06/14/2017

Table 2
Mean (±S.D.) organ weights (g) and organ weight to terminal body weight ratios (%) in male rats.

Organ	Measure	Dose group (mg/kg-day)[a]			
		0 (*N*=10)	60 (*N*=10)	200 (*N*=10)	600 (*N*=9[b])
Epididymides	Weight (g)	1.65 ± 0.18	1.46 ± 0.10	1.58 ± 0.17	1.59 ± 0.16
	Ratio (%)	0.325 ± 0.043	0.306 ± 0.037	0.329 ± 0.037	0.332 ± 0.054
Testes	Weight (g)	3.54 ± 0.68	3.38 ± 0.22	3.56 ± 0.24	3.53 ± 0.57
	Ratio (%)	0.689 ± 0.118	0.708 ± 0.086	0.746 ± 0.074	0.733 ± 0.142
Brain	Weight (g)	2.28 ± 0.13	2.28 ± 0.11	2.25 ± 0.09	2.29 ± 0.10
	Ratio (%)	0.451 ± 0.046	0.479 ± 0.054	0.473 ± 0.042	0.474 ± 0.060
Liver	Weight (g)	14.48 ± 1.76	13.83 ± 2.67	13.50 ± 1.28	14.78 ± 1.78
	Ratio (%)	2.832 ± 0.198	2.846 ± 0.273	2.814 ± 0.175	3.049 ± 0.220
Kidneys	Weight (g)	4.18 ± 0.48	4.15 ± 0.55	3.93 ± 0.30	4.18 ± 0.33
	Ratio (%)	0.818 ± 0.058	0.859 ± 0.053	0.818 ± 0.053	0.864 ± 0.049
Adrenals	Weight (g)	0.066 ± 0.014	0.057 ± 0.016	0.068 ± 0.013	0.061 ± 0.010
	Ratio (%)	0.013 ± 0.005	0.012 ± 0.004	0.013 ± 0.005	0.011 ± 0.003
Spleen	Weight (g)	0.93 ± 0.13	0.77 ± 0.10**	0.83 ± 0.06*	0.80 ± 0.11**
	Ratio (%)	0.181 ± 0.018	0.158 ± 0.015**	0.172 ± 0.017	0.163 ± 0.020*
Thymus	Weight (g)	0.49 ± 0.19	0.48 ± 0.17	0.38 ± 0.10	0.38 ± 0.14
	Ratio (%)	0.096 ± 0.039	0.097 ± 0.033	0.078 ± 0.021	0.078 ± 0.023
Heart	Weight (g)	1.61 ± 0.19	1.54 ± 0.18	1.60 ± 0.23	1.59 ± 0.18
	Ratio (%)	0.317 ± 0.020	0.320 ± 0.022	0.334 ± 0.057	0.328 ± 0.037

[a] Dosage occurred on days 1 through 90, 91, 92, or 93 of study.
[b] Excludes values for a rat which was found dead on day 85 of study.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

3.6. Clinical chemistries

Results for clinical chemistry are shown in Table 4. The average value for chloride was significantly increased ($p < 0.01$) in male rats in the 600 mg/kg-day dose group. Average total protein and albumin values were significantly reduced ($p < 0.05$) in female rats in the 600 mg/kg-day group. Average values for glucose, cholesterol, total bilirubin, blood urea nitrogen, creatinine, alanine aminotransferase, asparatate aminotransferase, alkaline phosphate, calcium, inorganic phosphorus, triglycerides, sodium, potassium, globulin, and the albumin/globulin ratios were unaffected in either sex by doses of K^+PFBS as high as 600 mg/kg-day.

3.7. Hematology

Results for hematology are shown in Table 5. In male rats in the 200 and 600 mg/kg-day groups, statistically significant reductions in average values for red blood cells (600 mg/kg-day only), hemoglobin concentration, and hematocrit were noted. Average values for leukocytes, mean corpuscu-

Table 3
Mean (±S.D.) organ weights (g) and organ weight to terminal body weight ratios (%) in female rats.

Organ	Measure	Dose group (mg/kg-day)[a]			
		0 (*N*=10)	60 (*N*=10)	200 (*N*=10)	600 (*N*=10)
Ovaries	Weight (g)	0.136 ± 0.019	0.136 ± 0.020	0.139 ± 0.025	0.129 ± 0.018
	Ratio (%)	0.048 ± 0.008	0.046 ± 0.007	0.048 ± 0.009	0.047 ± 0.007
Uterus	Weight (g)	0.64 ± 0.16	0.76 ± 0.26	0.64 ± 0.14	0.80 ± 0.21
	Ratio (%)	0.234 ± 0.064	0.264 ± 0.087	0.228 ± 0.062	0.303 ± 0.077
Brain	Weight (g)	2.07 ± 0.08	2.08 ± 0.09	2.08 ± 0.08	2.06 ± 0.10
	Ratio (%)	0.752 ± 0.060	0.730 ± 0.052	0.736 ± 0.061	0.780 ± 0.080
Liver	Weight (g)	7.71 ± 0.78	8.30 ± 0.72	8.23 ± 0.91	7.79 ± 0.36
	Ratio (%)	2.788 ± 0.152	2.902 ± 0.154	2.890 ± 0.100	2.951 ± 0.204
Kidneys	Weight (g)	2.34 ± 0.22	2.40 ± 0.30	2.40 ± 0.18	2.39 ± 0.41
	Ratio (%)	0.847 ± 0.069	0.838 ± 0.071	0.846 ± 0.059	0.906 ± 0.150
Adrenals	Weight (g)	0.076 ± 0.014	0.078 ± 0.011	0.079 ± 0.013	0.074 ± 0.010
	Ratio (%)	0.026 ± 0.007	0.027 ± 0.005	0.028 ± 0.008	0.027 ± 0.007
Spleen	Weight (g)	0.58 ± 0.09	0.59 ± 0.11	0.57 ± 0.07	0.65 ± 0.11
	Ratio (%)	0.209 ± 0.033	0.208 ± 0.037	0.202 ± 0.030	0.248 ± 0.046
Thymus	Weight (g)	0.35 ± 0.07	0.34 ± 0.13	0.38 ± 0.10	0.36 ± 0.09
	Ratio (%)	0.128 ± 0.033	0.121 ± 0.047	0.131 ± 0.027	0.134 ± 0.031
Heart	Weight (g)	0.97 ± 0.08	1.02 ± 0.07	0.99 ± 0.10	0.98 ± 0.12
	Ratio (%)	0.356 ± 0.050	0.356 ± 0.031	0.348 ± 0.031	0.373 ± 0.046

[a] Dosage occurred on days 1 through 90, 91, 92, or 93 of study.

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EPA-HQ-2016-005679 06/14/2017

Table 4
Mean (±S.D.) clinical chemistry values in male and female rats.

Dosage (mg/kg-day)[a]	Male				Female			
	Control	60	200	600	Control	60	200	600
N	10	10	10	9[b]	10	10	10	9[c]
TP (g/dL)	6.6 ± 0.33	6.5 ± 0.16	6.4 ± 0.19	6.5 ± 0.50	7.2 ± 0.40	7.2 ± 0.34	7.1 ± 0.40	6.7 ± 0.23*
A (g/dL)	4.1 ± 0.17	4.1 ± 0.19	4.0 ± 0.11	4.0 ± 0.26	4.9 ± 0.38	4.8 ± 0.29	4.7 ± 0.31	4.4 ± 0.23*
GLU (mg/dL)	164 ± 29.2	157 ± 12.8	167 ± 21.6	166 ± 13.8	156 ± 16.7	160 ± 21.4	163 ± 30.1	161 ± 13.1
CHOL (mg/dL)	54 ± 12.9	51 ± 14.9	45 ± 8.4	53 ± 14.4	76 ± 11.8	69 ± 10.0	74 ± 18.7	68 ± 16.8
TBILI (mg/dL)	0.1 ± 0.03	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.03	0.1 ± 0.03	0.1 ± 0.03
BUN (mg/dL)	15 ± 1.4	14 ± 1.5	14 ± 2.2	15 ± 1.4	16 ± 1.9	16 ± 1.6	15 ± 1.8	16 ± 2.9
CREAT (mg/dL)	0.3 ± 0.05	0.2 ± 0.05	0.3 ± 0.06	0.3 ± 0.05	0.4 ± 0.05	0.4 ± 0.04	0.3 ± 0.05	0.4 ± 0.05
ALT (U/L)	42 ± 5.8	45 ± 7.1	41 ± 4.6	43 ± 7.8	44 ± 11.6	55 ± 23.6	49 ± 30.7	44 ± 13.3
AST (U/L)	84 ± 11.2	90 ± 10.9	88 ± 7.5	91 ± 12.7	85 ± 15.8	95 ± 25.8	94 ± 51.0	96 ± 19.6
ALK (U/L)	94 ± 15.0	90 ± 12.0	96 ± 12.1	107 ± 12.7	44 ± 8.0	46 ± 13.3	45 ± 12.1	59 ± 18.2
CA (mg/dL)	11.4± 0.37	11.2± 0.27	11.0± 0.24	11.1± 0.32	11.9± 0.50	11.6± 0.39	11.6± 0.53	11.4± 0.16
PHOS (mg/dL)	8.9 ± 0.68	8.7 ± 0.64	8.3 ± 0.71	8.3 ± 0.55	8.2 ± 1.35	8.0 ± 1.39	7.9 ± 1.17	8.4 ± 0.80
TRI (mg/dL)	60 ± 32.3	58 ± 21.0	50 ± 11.6	47 ± 13.7	36 ± 7.7	45 ± 19.0	41 ± 9.2	42 ± 13.5
NA (mmol/L)	148 ± 1.4	148 ± 0.7	149 ± 1.2	149 ± 0.9	146 ± 1.5	146 ± 0.9	146 ± 1.2	145 ± 1.1
K (mmol/L)	5.2 ± 0.59	5.3 ± 0.54	5.1 ± 0.29	5.1 ± 0.38	6.3 ± 0.76	5.9 ± 0.62	6.2 ± 0.60	6.2 ± 0.63
CL (mmol/L)	98 ± 2.0	100 ± 1.2	100 ± 1.4	101 ± 1.7**	101 ± 2.5	101 ± 2.3	102 ± 2.2	102 ± 1.1
G (g/dL)	2.5 ± 0.28	2.5 ± 0.14	2.4 ± 0.16	2.4 ± 0.37	2.4 ± 0.17	2.4 ± 0.22	2.4 ± 0.28	2.3 ± 0.26
A/G	1.7 ± 0.23	1.7 ± 0.14	1.7 ± 0.13	1.7 ± 0.23	2.1 ± 0.21	2.0 ± 0.23	2.0 ± 0.31	1.9 ± 0.26

[a] Dosage occurred on days 1 through 90, 91, 92, or 93 of study.
[b] Excludes data for a rat which was found dead on day 85 of study.
[c] Excludes a rat that did not have sufficient sample volume.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

lar volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, mean platelet volume, prothrombin time, activated partial thromboplastin time, nucleated red blood cell count, segmented neutrophils, bands, monocytes, eosinophils, basophils, abnormal lymphocytes, and other cells were similar to control values among the three K^+PFBS-treated groups of male and female rats. The mean corpuscular hemoglobin concentration was significantly increased ($p < 0.05$) in the 60 mg/kg-day dose-group female rats and was not considered treatment related because it was not dose-dependent.

3.8. Histopathology

Microscopic changes were observed in the kidneys and stomach of the male and female rats of the 600 mg/kg-day dose group (Table 6). In kidneys obtained from rats in the 600 mg/kg-day male and female dose groups, increased incidence of hyperplasia of the epithelial cells of the medullary and papillary tubules and ducts in the inner medullary regions was noted as compared to controls. These tubules had a dark tinctorial appearance with increased amounts of small interstitial cells with prominent dark nuclei. Other treatment-related changes included a lower incidence

Table 5
Mean (±S.D.) hematology values in male and female rats.

Dosage (mg/kg-day)[a]	Male				Female			
	Control	60	200	600	Control	60	200	600
N	10	10	10	9[b]	10	10	10	10
WBC (10^3/mm^3)	17.0 ± 3.66	15.4 ± 3.50	14.7 ± 2.31	15.2 ± 2.80	12.2 ± 3.95	11.5 ± 3.10	12.1 ± 3.77	13.5 ± 3.94
RBC (10^6/mm^3)	7.76 ± 0.469	7.62 ± 0.443	7.55 ± 0.282	7.19 ± 0.481*	7.17 ± 0.315	6.95 ± 0.226	7.16 ± 0.309	6.95 ± 0.297
HGB (g/dL)	16.4 ± 0.96	16.0 ± 0.41	15.6 ± 0.48*	15.5 ± 0.78*	15.9 ± 0.61	15.8 ± 0.57	15.6 ± 0.69	15.3 ± 0.82
HCT (%)	44.2 ± 2.32	42.7 ± 1.44	41.9 ± 1.50*	40.9 ± 2.24**	43.3 ± 1.85	42.1 ± 1.56	42.7 ± 2.14	41.2 ± 1.71
MCV (ffim^3)	57.0 ± 1.25	56.2 ± 2.12	55.6 ± 1.38	57.0 ± 2.08	60.5 ± 1.08	60.6 ± 1.47	59.5 ± 1.50	59.2 ± 1.21
MCH (pg)	21.2 ± 0.53	21.1 ± 1.18	20.7 ± 0.51	21.6 ± 1.01	22.2 ± 0.64	22.7 ± 0.65	21.8 ± 0.61	22.0 ± 0.75
MCHC (%)	37.2 ± 0.50	37.5 ± 1.11	37.2 ± 0.69	37.8 ± 0.96	36.7 ± 0.69	37.4 ± 0.54*	36.7 ± 0.51	37.1 ± 0.70
PLAT (10^3/mm^3)	1235 ± 241.3	1160 ± 175.6	1134 ± 149.4	1202 ± 165.0	1119 ± 88.2	1157 ± 195.8	1181 ± 181.6	1126 ± 283.3
MPV (ffim^3)	7.7 ± 0.35	8.0 ± 0.65	8.0 ± 0.64	7.6 ± 0.51	7.4 ± 0.41	7.2 ± 0.47	7.2 ± 0.45	7.2 ± 0.53
PT (s)	13.7 ± 0.41	14.2 ± 0.45	14.1 ± 0.68	14.2 ± 0.80	13.3 ± 0.29	13.5 ± 0.49	13.1 ± 0.40	13.4 ± 0.16[c]
APTT (s)	24.7 ± 2.80	25.7 ± 3.84	25.6 ± 2.94	25.5 ± 4.43	21.2 ± 2.41	24.3 ± 5.68	24.2 ± 3.27	24.5 ± 5.17[c]
NRBC (count)	0 ± 0.0	0 ± 0.3	0 ± 0.0	0 ± 0.7	0 ± 0.0	0 ± 0.3	0 ± 0.3	0 ± 0.0
Lymphocyte (10^3/mm^3)	13.6 ± 3.93	12.6 ± 3.66	12.2 ± 2.34	13.0 ± 2.62	10.3 ± 3.76	9.6 ± 2.56	9.6 ± 3.87	12.1 ± 3.94
Segmented (10^3/mm^3)	2.7 ± 1.48	2.2 ± 0.90	1.9 ± 0.57	1.6 ± 0.48	1.5 ± 0.59	1.6 ± 0.61	2.1 ± 1.45	1.2 ± 0.47
Bands (10^3/mm^3)	0.0 ± 0.00	0.0 ± 0.06	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Monocytes (10^3/mm^3)	0.5 ± 0.23	0.4 ± 0.26	0.5 ± 0.20	0.5 ± 0.26	0.2 ± 0.10	0.2 ± 0.20	0.2 ± 0.20	0.2 ± 0.18
Eosinophils (10^3/mm^3)	0.2 ± 0.29	0.2 ± 0.19	0.1 ± 0.18	0.1 ± 0.15	0.3 ± 0.15	0.2 ± 0.16	0.2 ± 0.17	0.1 ± 0.10
Basophils (10^3/mm^3)	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.03
Abnormal L (10^3/mm^3)	0.0 ± 0.06	0.0 ± 0.06	0.0 ± 0.07	0.0 ± 0.03	0.0 ± 0.08	0.0 ± 0.00	0.0 ± 0.03	0.0 ± 0.00

[a] Dosage occurred on days 1 through 90, 91, 92, or 93 of study.
[b] Excludes data for a rat which was found dead on day 85 of study.
[c] Excludes values for rats for which there was insufficient sample.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
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EPA-HQ-2016-005679 06/14/2017

Table 6
Microscopic incidence of histopathological findings by dose group in male and female rats.

Tissue	Observation	Dose group (mg/kg-day) and sex (M and F)							
		0		60		200		600	
		M	F	M	F	M	F	M	F
Kidney	Hyperplasia, tubular/ductular epithelium papilla	1/10	–[a]	–	–	1/10	1/10	8/10	6/10
	Edema, focal papillary	–	–	–	–	–	–	3/10	3/10
	Necrosis, papillary (both kidneys)	–	–	–	–	–	–	1/10	–
	Basophilia, tubular, multifocal	1/10	–	–	–	–	–	3/10	1/10
	Hyaline droplets, cortical tubules	2/10	–	–	–	–	–	1/10	1/10
	Mineralization, multifocal	–	5/10	–	5/10	–	2/10	–	2/10
	Pyelonephritis chronic	–	–	–	–	–	–	–	2/10
	Mononuclear cell infiltration, focal	1/10	–	2/10	3/10	4/10	–	–	2/10
Stomach	Dilation, mucosal glands	2/10	–	1/10	3/10	2/10	–	1/10	–
	Necrosis, individual cells in limiting ridge	–	1/10	2/10	–	2/10	1/10	8/10	9/10
	Hyperplasia/hyperkeratosis, limiting ridge	–	–	–	–	–	–	5/10	7/10
Liver	Inflammation, chronic, focal/multifocal	6/10	6/10	–	–	–	–	3/10	4/10
Lymph node, mandibular	Hyperplasia, lymphocytic/plasmacytic	5/10	7/10	–	–	–	–	6/10	6/10
Nasal cavity/turbinates	Necrosis, olfactory epithelium, multifocal	–	–	–	–	–	1/10	2/10	2/10
	Hypertosis, turbinate bone	–	–	–	–	–	–	2/10	2/10
	Inflammation, chronic, focal/multifocal	–	–	–	–	2/10	2/10	2/10	1/10

[a] No occurence found.

of focal papillary edema and a single incidence of papillary necrosis in both kidneys of one male rat in the 600 mg/kg-day dose group.

In stomachs obtained from rats in the 600 mg/kg-day male and female dose groups, increased incidence of necrosis of individual squamous epithelial cells in the limiting ridge of the forestomach was noted as compared to controls. This change was characterized by individual squamous epithelial cells with dark pyknotic nuclei surrounded by a clear cytoplasmic halo. This change was seen at a very low incidence in the rats of the other dose groups, including a control female rat, but the increased incidence of this change, along with minimal- or mild thickening of the mucosa of the limiting ridge due to hyperplasia and hyperkeratosis was considered to be treatment related in the 600 mg/kg-day dose group.

Microscopic examination of the nasal cavity and nasal turbinates revealed a few equivocal microscopic changes that occurred at low and sporadic incidences in rats in the 200 and 600 mg/kg-day dose groups. These changes occurred primarily in the posterior nasal cavity/turbinates. These histomorphologic changes included a single- or low incidence of multifocal necrosis or atrophy of the olfactory mucosa, focal acute/sub-acute or chronic inflammation, adhesions of the turbinate to either and adjacent turbinate or to the lateral nasal wall, focal hyperostosis of turbinate bone and/or foci of olfactory epithelia hyperplasia. Foci of inflammation may occur spontaneously in the nasal cavity of rats, but in several of the above-mentioned lesions, the inflammation was associated with these other changes. The lesions in the nasal cavity/turbinates are of uncertain significance and origin mainly because they occurred only in the 200 and 600 mg/kg-day dose groups at very low and sporadic incidence rates and were focal or multifocal in distribution. The nasal cavity/turbinates of most rats of all groups were histologically unremarkable. The varied and focal isomorphic characteristics of these lesions in the nasal cavity/turbinates are not typical or consistent with a systemic toxic effect. Although the mechanism is unknown, many of these lesions are more suggestive of a local irritating effect on the nasal mucosal membranes.

4. Discussion

On the basis of these data, the NOAEL for K^+PFBS in male rats was 60 mg/kg-day. Absolute and relative (to body weight and brain weight) spleen weights were lower than those of control males at all K^+PFBS treatment levels. However, there was no trend in this reduction across the 10-fold dose range and no adverse histopathological effects were noted. Furthermore, the spleen weight to body weight percent values obtained for K^+PFBS-treated males in this study were close to the central estimate of this parameter based on 16–24-week-old male Sprague–Dawley rats historical control data obtained from 19 studies conducted in the same laboratory between September 1994 and April 2008. In the study reported herein, mean spleen weight to body weight percents of 0.181, 0.158 ($p \leq 0.01$), 0.172, and 0.163 ± 0.020 ($p \leq 0.05$) were obtained for the control, 60, 200, and 600 mg/kg-day dose-group males, respectively. The mean (±S.D., range) for this parameter for controls from the 19 historical control studies was 0.159 (±0.015, 0.142–0.207). Therefore, the splenic weight effects were not considered to be of toxicological significance. The 200 and 600 mg/kg-day doses in the male rats were associated with increased adverse clinical observations and reductions in red blood cells, hemoglobin concentration, and hematocrit. Chloride was significantly increased at the 600 mg/kg-day dose.

PFBS treatment resulted in mild but statistically significant decreases in RBC count in male rats in the 600 mg/kg-day dose group and in hematocrit and hemoglobin in the 200 and 600 mg/kg-day dose groups. Histologic examination of bone marrow in the 600 mg/kg-day dose-group males did not reveal any abnormalities as compared to controls. Taking the hematology data from the study as a whole, this pattern would suggest slight to mild plasma volume expansion; although other potential etiologies cannot be ruled out with the available data. It is of interest to note that the same pattern was observed with the potassium salt of the PFBS congener, perfluorohexanesulfonate (PFHxS) in male rats given 3 and 10 mg/kg-day by gavage for at least 42 days (Butenhoff et al., submitted for publication). The PFBS and PFHxS congener, PFOS, has been demonstrated to be both a PPARffl (Shipley et al., 2004; Sohlenius et al., 1993; Vanden Heuvel et al., 2006; Wolf et al., 2008) and a PPAR] agonist (Vanden Heuvel et al., 2006); although Takacs and Abbott did not observe activation of PPAR] with PFOS (Takacs and Abbott, 2007). Plasma volume expansion is a known effect of thiazolidinediones mediated via PPAR] stimulation of the epithelial sodium channel (ENaC]) of the collecting duct in kidneys (Guan et al., 2007). Typically, weight gain is a consequence of fluid retention with thiazolidinediones, and

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EPA-HQ-2016-005679 06/14/2017

this was not observed with either PFBS or PFHxS. Indeed, PFHxS treatment caused decreased weight gain at the highest dose in males (Butenhoff et al., submitted for publication). In addition, no increase in sodium was noted. Dual activation of PPAR| and PPARffl could mask a weight gain effect due to the increased | -oxidation associated with PPARffl activation. Both PFBS and PFHxS have been shown to activate mouse and human PPARffl (Wolf et al., 2008). Additional investigation would be required to develop a better understanding of the etiology of the mild reduction in RBC count, hematocrit, and hemoglobin occurring in male rats given PFBS.

The NOAEL for K^+PFBS in female rats was 600 mg/kg-day. The 600 mg/kg-day dose was associated with significant reductions in average total protein and albumin values, but reductions were minor and were the only changes observed at the 600 mg/kg-day dose in the female rats.

The treatment-related histopathological findings observed in the study were likely related to the presence of high concentrations of the K^+PFBS surfactant, which is a strong surfactant (K^+PFBS is classified as a moderate eye irritant). The nasal and stomach effects may have been related to irritation from repeated exposure to K^+PFBS via gavage dosing. The microscopic kidney effects (mild tubular hyperplasia and papillary edema) were not associated with functional impairment or damage and may have been due to a response to high concentrations of PFBS passing through the kidney and into the urine. Because PFBS is not metabolized and is excreted rapidly in male and female urine with a serum elimination half-life of approximately 4 h in male rats (Olsen et al., 2008), almost 90% of each daily administered dose would be present in the daily urine output of these rats. For example, at the 600 mg/kg-day dose level, a 300-g rat would have received 200 mg of K^+PFBS per day, and excreted most of it in its daily urine output of about 15 mL; giving a urinary PFBS concentration of at least 1.2% or 12,000 ffig/mL. Although we do not understand the full biological consequences of relatively high concentrations of a strong surfactant in direct contact with the kidney tubular cells, it is likely that this was a factor contributing to the histopathological findings. Further work would be needed to differentiate between irritation and other modes of action in understanding the effects that occurred at the high dose of this study.

In recent years, there has been considerable attention on the potential toxicological properties of a higher molecular weight homolog of PFBS, PFOS (Lau et al., 2004, 2007; Organisation for Economic Cooperation and Development, 2002). When comparing the results of a 90-day dietary study with potassium PFOS in Sprague–Dawley rats (Seacat et al., 2003a, b) to the results reported herein for PFBS, it becomes evident that PFOS is more potent in its ability to elicit toxicological responses, both from the much lower doses required and the extent of responses elicited. In the study reported by Seacat et al. (2003a, b), PFOS was administered in the diet at doses of 0.5, 2, 5, and 20 ffig/g (ppm) for 90 days. Most effects were observed in the 20 ppm dose groups, equivalent to 1.33 ± 0.38 and 1.56 ± 0.35 mg/kg for males and females, respectively, based on diet consumption and feed analyses. Liver weight was increased in males, and relative liver weight was increased in males and females. These liver weight increases had histological correlates in increased centrilobular hepatocellular hypertrophy. This was accompanied with vacuolation and cytoplasmic eosinophilic granulation. The effects were also observed in the males given 5 ppm in the diet (equivalent to 0.34 ± 0.09 mg/kg daily dose). At the 20 ppm dose, average blood urea nitrogen concentrations were increased above control levels in males and females. In males, average alanine aminotransferase activity was increased, and serum total cholesterol was decreased when compared to control levels in males. The only hematological alteration was an increase in segmented neutrophiles in males in the 20-ppm dose group.

By contrast, on gavage dosing with K^+PFBS for 90 days at daily doses up to 600 mg/kg, no alterations in liver cell morphology were noted and observed effects were relatively minor. The decreased spleen weights at all treatment levels in males compared to male controls did not show a trend in reduction over a 10-fold dose range. The nasal and stomach effects may have been related to irritation from repeated exposure to K^+PFBS, and the microscopic kidney effects (mild tubular hyperplasia and papillary edema) were not associated with functional impairment or damage and may have been due to a response to high concentrations of PFBS in the urine. Average values for serum total protein and serum albumin were decreased at 600 mg/kg in females when compared to controls, and, at the 600 mg/kg dose level in males, average serum chloride was increased, and mean values for hematocrit, and hemoglobin were decreased at 200 and 600 mg/kg, with red blood cells reduced at 600 mg/kg. Thus, the pattern of effects elicited by treatment with PFBS for 90 days were different than that elicited by PFOS, and minimal doses to produce effects with PFBS were approximately 600 times higher on an administered dose basis (200 mg/kg versus 0.34 mg/kg, for PFBS and PFOS, respectively) and approximately a 1000 times higher on a molar dose basis. This may be due to the combination of more rapid elimination and reduced binding affinities of the PFBS surfactant ligand as compared to PFOS.

Conflict of interest

Paul H. Lieder, Shu-Ching Chang, and John L. Butenhoff are employed by 3M Company, a manufacturer of potassium perfluorobutanesulfonate. Raymond G. York is an employee of Charles River Preclinical Services which was contracted by 3M Company to perform the in-life portion of the study. The 3M Company funded all aspects of the work reported herein.

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A two-generation oral gavage reproduction study with potassium perfluorobutanesulfonate (K^+PFBS) in Sprague Dawley rats

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abstract

Perfluorobutanesulfonate (PFBS) is a surfactant and degradation product of substances based on perfluorobutanesulfonyl fluoride. A two-generation reproductive rat study has been conducted with potassium PFBS (K^+PFBS). Parental-generation (P) rats were dosed orally by gavage with 0, 30, 100, 300 and 1000 mg K^+PFBS/kg/day for 10 weeks prior to and through mating (males and females), as well as during gestation and lactation (females only). First generation (F1) pups were dosed similarly, beginning at weaning. Second generation (F2) pups were not directly dosed but potentially exposed to PFBS through placental transfer and nursing, and the study was terminated 3 weeks after their birth. Endpoints evaluated included body weight, food consumption, clinical signs, estrus cycling, sperm quality, pregnancy, natural delivery, litter outcomes, and developmental landmarks. The no-observable-adverse effect dose level (NOAEL) in the parental generations (P and F1) was 100 mg/kg/day. In the 300 and 1000 mg/kg/day dose group rats, there were (1) increased liver weight (absolute or relative) and corresponding increased incidence of adaptive hepatocellular hypertrophy (male only) and (2) increased incidence of minimal to mild microscopic findings in the medulla and papilla of the kidneys (male and female). There were no K^+PFBS treatment-related effects on fertility or reproduction among the P or the F1 rats. There were no microscopic changes in male or female reproductive organs, and no biologically relevant effects on sperm parameters, mating, estrous cycles, pregnancy, and natural delivery in the P- or F1-generations. There were no K^+PFBS treatment-related effects on survival of pups in the two-generation study. Litter size and average pup birth weight per litter were not statistically significantly different from controls in any dose group. In the F1-generation, terminal body weight was reduced in males at 1000 mg/kg/day. Preputial separation was slightly delayed (approximately 2 days) at this dose, a finding consistent with the body weight reduction. Essentially no effects were observed in the F1 females. F2 pups had normal body weights. The reproductive NOAEL was >1000 mg/kg/day in both generations.

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1. Introduction

In the 1990s, industry (3M) began developing new technologies to augment and eventually replace a chemical class referred to "perfluorooctanyl" compounds. This chemical class includes perfluorooctanoic acid (PFOA), perfluorooctanesulfonyl fluoride (POSF), and POSF-based chemistries, including N-alkyl-substituted perfluorooctanesulfonamides and perfluorooctanesulfonate (PFOS). In May 2000, 3M announced the voluntary manufacturing phase-out of this chemical class. Subsequently, the United States Environmental Protection Agency (USEPA) issued a Significant New Use Rule, or SNUR, that more broadly regulates the production and import of many perfluoroalkyl substances (PFAS) such as perfluorooctanyl-based chemicals (USEPA, 2002). The USEPA subsequently proposed to amend the PFAS SNUR to include additional perfluorooctanyl-based chemicals (USEPA, 2006). Canada banned the use and importation of a number of long-chain perfluorinated carboxylic acids because of concerns over potential adverse human and environmental effects (CanadaGazette, 2004, 2005). In January 2006, the USEPA announced a voluntary 2010/2015 PFOA Stewardship Program (http://www.epa.gov/opptintr/pfoa/pubs/pfoastewardship.htm) to eliminate emissions and product content of PFOA and related chemicals.

One of the new technologies developed by 3M is based on products made from perfluorobutanesulfonylfluoride (PBSF). Based on atmospheric degradation studies of N-methyl-N-(2-hydroxyethane)-perfluorobutanesulfonamide (D'Eon et al., 2006) and mammalian metabolism studies with the analogous materials made from N-ethoxy-N-(2-hydroxyethanol)-perfluorooctanesulfonamide (Xu et al., 2004, 2006), the degradation and metabolic

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product from materials based on this chemistry ultimately will be perfluorobutanesulfonate (PFBS). Consequently, extensive testing has been performed on this material, which, compared to the perfluorooctanyl-based chemicals, has a low potential to bioaccumulate (NICNAS, 2005; Olsen et al., 2009). This paper describes the reproductive effects of PFBS and briefly compares the effects to those of the perfluorooctanyl compounds, PFOA and PFOS, in rats (Butenhoff et al., 2004; Grasty et al., 2006; Lau et al., 2004, 2006, 2003; Luebker et al., 2005a,b; Thibodeaux et al., 2003).

2. Materials and methods

2.1. Animals

Crl:CD(SD)IGS BR VAF/Plus® (Sprague Dawley) rats were supplied by Charles River Laboratory (Kingston, NY – male rats; and Raleigh, NC – female rats). Rats were housed individually except during mating and lactation. Rats were maintained on a 12-h light/12-h dark cycle and fed Certified Rodent Diet® #5002 (PMI Nutrition International, St. Louis, MO) *ad libitum*. Water was provided *ad libitum* from individual bottles and/or from an automatic watering access system. Bed-o'cobs® bedding (Anderson's Industrial Products, Maumee, OH) was used as the nesting material.

The study was conducted in a facility accredited with by the Association for the Assessment and Accreditation of Laboratory Animal Care International. Procedures involving the use of laboratory rats were reviewed and approved by the facility Institutional Animal Care and Use Committee and conformed to the "Guide for the Care and Use of Laboratory Animals" (ILAR, 1996).

2.2. Test substance

The potassium salt of PFBS (K^+PFBS, Lot 2, 97.90% pure) was provided by 3M Company (St. Paul, MN). The sample was 97.86% normal chain with 0.04% branched as determined by quantitative ^{19}F NMR. Solutions of K^+PFBS were prepared using 0.1% carboxymethyl cellulose in reverse osmosis processed deionized water.

2.3. Study design

The study was performed based on the following guidelines: Organisation for Economic Cooperation and Development (OECD) 416 and USEPA OPPTS 870.3800. The parental-generation (P) consisted of five treatment groups, 30 rats per sex per group. At least one first generation (F1) pup per sex per litter per group was selected for continued evaluation at weaning. K^+PFBS or vehicle (0.1% carboxymethyl cellulose) was administered by daily oral gavage to the P-generation rats, beginning at 6 weeks of age and at least 70 days before cohabitation, at daily dosages of 0, 30, 100, 300 and 1000 mg/kg using a constant volume of 10 mL/kg. The oral route was presumed to be most relevant, and doses were selected based on the results of a previous oral toxicity study in rats with K^+PFBS (Lieder et al., 2009). In addition, gavage dosing delivers a more accurate quantitative dose than other non-parenteral methods. The F1-generation rats were individually identified and given the same dosage level and volume of the test substance as their respective sires and dams beginning at weaning on lactation day (LD) 22. The P- and F1-generation rats were observed for clinical observations, abortions or premature deliveries, and deaths before and 60 min post-dose. Body weights and food consumption values of the male and female rats were recorded weekly and on gestation day (GD) 0, 7, 10, 14, 18, 21 and 25 (if females did not give birth), on LD 1, 5, 8, 11, 15 and 22 for the dams (terminal sacrifice), and for F1-generation rats on attainment of sexual maturation. Food consumption was recorded weekly but was not recorded during cohabitation and after LD 15, because pups begin to consume maternal food on or about this time.

F1-generation male and female rats were examined for age of vaginal patency or balanopreputial separation beginning on day 28 or 29 postpartum, respectively. Estrous cycling was evaluated by examination of vaginal cytology beginning 21 days before cohabitation and until mating. The cohabitation period was a maximum of 14 days. Evidence of mating was determined by spermatozoa observed in a vaginal smear or a copulatory plug observed *in situ*, and this was considered GD 0. On the day of sacrifice, stage of estrous was again determined to ascertain if there was a treatment effect and to aid the pathologist in the microscopic evaluation of uterine tissues.

The P- and F1-generation female rats were evaluated for duration of gestation, fertility and gestation indices, number and sex of offspring, number of implantation sites, condition of dam and litter, litter size, viability index, lactation index, percent survival and sex ratio. Second generation (F2) pup body weights were recorded on LD 1, 5, 8, 15 and 22.

At scheduled termination (after cohabitation for males and on LD 22 for females) P- and F1-generation rats were euthanized by carbon dioxide (CO_2) asphyxiation, necropsied and examined for gross lesions. The brain, kidneys, spleen, ovaries, testes, thymus, liver, adrenal glands, pituitary, uterus with oviducts and cervix, left epididymis, right epididymis, prostate and seminal vesicles with coagulating glands and fluid were individually weighed. The liver, kidneys, pituitary, adrenal glands, vagina, uterus with oviducts, cervix and ovaries, right testis (initially fixed in Bouin's solution), seminal vesicles (with coagulating glands), right epididymis and prostate were retained in formalin for histology.

All F2-generation pups culled on LD 22 were euthanized by CO_2 asphyxiation and three randomly selected pups per sex per litter were examined for gross lesions. The necropsy included a single cross-section of the head at the level of the frontal–parietal suture and examination for apparent hydrocephaly. The brain, spleen, liver, thymus, and kidneys and thymus from the first of the three randomly selected pups per litter were weighed.

Histological evaluation was performed by a Board Certified Veterinary Pathologist. While liver and kidneys collected from all of the control and treatment groups in P-, F1-, and F2-generation rats were examined microscopically, only the reproductive tissues from 10 randomly selected rats per sex from the control and high dosage groups in P- and F1-generations were assessed. Histopathological examination of the testis was conducted in order to identify retained spermatids, missing germ cell layers or types, multinucleated giant cells or sloughing of spermatogenic cells into the lumen. Examination of the intact epididymis (including the caput, corpus and cauda) was conducted in order to identify such lesions as sperm granulomas, leukocytic infiltration (inflammation), aberrant cell types within the lumen, or absence of clear cells in the cauda epididymal epithelium.

Sperm parameters were evaluated in male rats at sacrifice. The P-generation male rats were 106–110 days of age and the F1-generation male rats were 109–120 days of age. A portion of the left cauda epididymis was used for evaluation of cauda epididymal sperm concentration and motility using computer assisted sperm analysis (CASA). The motility was evaluated by the Hamilton Thorne IVOS. The remaining left cauda epididymis was used to manually evaluate sperm morphology. Sperm morphology evaluations included both the determination of the percentage of normal sperm (in a sample of at least 200) and qualitative evaluation of abnormal sperm. The left testis was used for evaluation of testicular spermatid concentration (10 fields) using the CASA (Hamilton Thorne Biosciences Inc., Beverly, MA). The left testis was weighed both before and after removal of the tunica albuginea and then homogenized; a sample from the homogenate was stained with an IDENT stain (Hamilton Thorne Biosciences Inc., Beverly, MA) before IVOS analysis (Hamilton Thorne Biosciences Inc., Beverly, MA).

Uteri of female rats were evaluated at sacrifice. The number and distribution of implantation sites were recorded. Uteri of apparently nonpregnant rats were examined while being pressed between glass plates to confirm the absence of implantation sites. A quantitative evaluation of ovarian follicles was conducted for F1 female rats.

2.4. Statistical analyses

Clinical observations and other proportional data were analyzed using the Variance Test for Homogeneity of the Binomial Distribution (Snedecor and Cochron, 1967). Continuous data (body weights, body weight changes, food consumption data, organ weights, duration of gestation, litter averages for pup body weights, percent male pups, pup viability and cumulative survival) were analyzed using Bartlett's Test of Homogeneity of Variances (Sokal and Rohlf, 1969) and the Analysis of Variance (Snedecor and Cochran, 1967), when Bartlett's Test was not significant ($p > 0.001$). If the Analysis of Variance was significant ($p < 0.05$), Dunnett's test (Dunnett, 1955) was used to identify the statistical significance of the individual groups as compared to the control. If Bartlett's Test was significant ($p \leq 0.001$), the Kruskal–Wallis Test (Sokol and Rohlf, 1969) was used, when 75% or fewer ties were present. When more than 75% ties were present, Fisher's Exact Test (Siegel, 1956) was used. In cases where the Kruskal–Wallis was statistically significant ($p < 0.05$), Dunn's Method of Multiple Comparisons (Dunn, 1964) was used to identify the statistical significance of the individual groups. All other natural delivery data involving discrete data were evaluated using the Kruskal–Wallis Test procedures.

3. Results

3.1. P-generation – reproductive outcome

Results of P-generation male sperm analyses are presented in Table 1. A statistically significant ($p \leq 0.05$) reduction in the number of spermatids per gram testis was observed only in the 1000 mg/kg/day dose group; however, this was not considered biologically meaningful, because there was not a similar statistically significant decrease in the F1-generation males (Table 1) at the same dose level when compared to concurrent controls and the effect was within the historical control of the testing facility (mean 131.3 million sperm per gram of tissue; ranging from 49.7 to 215.3 million for 104 studies conducted from 1998 to 2005). Therefore, there were no effects in K^+PFBS-treated groups relative to controls in sperm motility, sperm counts, and sperm morphology.

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EPA-HQ-2016-005679 06/14/2017

Table 1
Sperm analysis parameters for P- and F1-generation male rats. All numbers are mean ± S.D. (*N*).

	Generation	Dose group (mg/kg/day)				
		0	30	100	300	1000
Motility (%)	P	96 ± 4	95 ± 5	94 ± 12	95 ± 4	95 ± 5
	N	(30)	(30)	(30)	(30)	(28)
	F1	92 ± 10	92 ± 10	94 ± 6	95 ± 3	92 ± 10
	N	(29)	(26)	(29)	(27)	(27)
Morphology (%abnormal)	P	1.7 ± 1.5	2.0 ± 1.3	1.6 ± 1.0	1.5 ± 0.6	2.0 ± 0.6
	N	(30)	(30)	(30)	(30)	(29)
	F1	1.5 ± 0.7	1.3 ± 0.6	1.4 ± 0.5	1.5 ± 0.6	1.9 ± 0.5*
	N	(29)	(27)	(29)	(27)	(27)
Testicular sperm count (10^6/g)	P	148 ± 39	136 ± 34	146 ± 36	132 ± 21	122 ± 36*
	N	(30)	(30)	(30)	(30)	(29)
	F1	124 ± 33	109 ± 34	107 ± 29	110 ± 24	107 ± 32
	N	(29)	(27)	(29)	(27)	(27)
Epididymal sperm count (10^6/g)	P	1030 ± 209	1033 ± 226	957 ± 217	1019 ± 181	969 ± 275
	N	(30)	(30)	(30)	(30)	(29)
	F1	837 ± 237	770 ± 282	840 ± 262	857 ± 222	928 ± 273
	N	(29)	(27)	(29)	(27)	(27)

* Statistically significantly different from control value ($p \leq 0.05$).

Results for P-generation female estrous cycling are presented in Table 2. There were no statistically significant changes from control among K^+PFBS-treated females with respect to estrous cycling, including numbers of rats experiencing ≥6 continuous days of diestrus or ≥6 continuous days of estrus. A comparison of estrus stages at sacrifice showed no differences between K^+PFBS-treated groups and controls.

Absolute sex organ weight data are shown in Table 3. Sex organ weights and the ratios of their weight to body weight and brain weight (data not shown) were similar to control values.

Male and female fertility data are displayed in Tables 4 and 5, respectively. All mating and fertility parameters for the P-generation were unchanged relative to controls in K^+PFBS-treated groups. A slight, non-statistically-significant reduction in the fertility index and the number of pregnant rats per number of rats in cohabitation in the 1000 mg/kg/day dose group was considered unrelated to the test substance because it was within historical control range of the testing facility for the years 1998–2005 (in 2840 mating pairs mean 91.3%, range 50–100%).

P-generation delivery outcomes and the corresponding F1 litter observations are shown in Tables 6 and 7, respectively. There were no toxicologically meaningful changes in delivery outcomes and litter observations.

There were no K^+PFBS treatment-related microscopic changes in sex organs of males and females as compared to controls, including in those rats that were identified as having reduced fertility (data not shown).

Table 2
Estrous cycling of P- and F1-generation female rats.

	Generation	Dose group (mg/kg/day)				
		0	30	100	300	1000
Rats evaluated (*N*)	P	30	30	30	30	30
	F1	30	30	30	30	29
Estrous stages/21 days (mean ± S.D.)	P	4.8 ± 0.9	5.0 ± 0.8	4.9 ± 0.9	4.8 ± 0.7	4.7 ± 1.2
	F1	4.1 ± 1.4	4.1 ± 1.2	3.7 ± 1.5	4.4 ± 1.3	4.5 ± 0.9
≥6 Days of diestrus	P	1	0	0	3	1
	F1	7	10	15**	7	0*
≥6 Days of estrus	P	1	0	0	0	0
	F1	0	0	0	0	1
Stage at sacrifice (*N*)	P	28	28	30	30	30
	F1	30	30	30	29	29
Diestrus	P	24	24	27	28	26
	F1	24	24	28	25	23
Proestrus	P	0	0	0	0	0
	F1	1	1	0	1	0
Estrus	P	3	3	2	1	3
	F1	5	5	0	2	6
Metestrus	P	1	1	1	1	1
	F1	0	0	2	1	0

* Statistically significantly different from control value ($p \leq 0.05$).
** Statistically significantly different from control value ($p \leq 0.01$).

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EPA-HQ-2016-005679 06/14/2017

Table 3
Sex organ weights (mg) in P- and F1-generation male and female rats. All numbers are mean ± S.D. (*N*).

	Generation	Dose group (mg/kg/day)				
		0	30	100	300	1000
Left ovary	P	50 ± 11 (26)	47 ± 13 (29)	48 ± 13 (29)	47 ± 12 (28)	44 ± 10 (25)
	F1	57 ± 11 (23)	61 ± 14 (26)	56 ± 16 (28)	60 ± 12 (25)	56 ± 12 (25)
Right ovary	P	50 ± 9 (26)	47 ± 8 (29)	52 ± 24 (29)	47 ± 12 (28)	48 ± 9 (25)
	F1	57 ± 12 (23)	59 ± 13 (26)	63 ± 12 (28)	59 ± 12 (25)	54 ± 13 (25)
Left testis	P	1806 ± 148 (30)	1790 ± 148 (30)	1779 ± 163 (30)	1763 ± 234 (30)	1815 ± 274 (29)
	F1	1830 ± 163 (29)	1781 ± 236 (27)	1805 ± 146 (29)	1797 ± 156 (27)	1721 ± 229 (27)
Right testis	P	1786 ± 143 (30)	1795 ± 158 (30)	1827 ± 227 (30)	1800 ± 174 (30)	1839 ± 367 (29)
	F1	1819 ± 152 (29)	1770 ± 310 (27)	1804 ± 126 (29)	1819 ± 147 (27)	1785 ± 150 (27)
Left epididymis	P	766 ± 89 (30)	777 ± 96 (30)	737 ± 73 (30)	747 ± 111 (30)	729 ± 106 (29)
	F1	833 ± 101 (29)	800 ± 119 (27)	798 ± 88 (29)	770 ± 82 (27)	778 ± 89 (27)
Right epididymis	P	769 ± 113 (30)	779 ± 96 (30)	754 ± 66 (30)	760 ± 82 (30)	750 ± 110 (29)
	F1	835 ± 120 (29)	819 ± 117 (27)	821 ± 114 (29)	780 ± 100 (27)	808 ± 80 (27)
Left cauda epididymis	P	371 ± 42 (29)	374 ± 47 (30)	355 ± 42 (30)	351 ± 54 (29)	355 ± 62 (29)
	F1	378 ± 49 (29)	367 ± 52 (27)	369 ± 43 (29)	369 ± 50 (27)	365 ± 38 (27)
Seminal vesicles with fluid	P	2059 ± 395 (29)	2025 ± 295 (28)	2003 ± 356 (29)	2013 ± 296 (30)	1984 ± 280 (26)
	F1	2076 ± 400 (29)	2083 ± 441 (27)	2020 ± 429 (29)	2013 ± 396 (27)	1778 ± 396* (27)
Seminal vesicles w/o fluid	P	1094 ± 201 (29)	1087 ± 193 (30)	1076 ± 250 (30)	1099 ± 182 (30)	1069 ± 193 (29)
	F1	1214 ± 244 (29)	1189 ± 288 (27)	1138 ± 227 (29)	1181 ± 244 (27)	1016 ± 190** (27)
Prostate	P	1447 ± 274 (30)	1453 ± 274 (30)	1370 ± 243 (30)	1414 ± 219 (30)	1370 ± 176 (29)
	F1	1221 ± 287 (29)	1286 ± 242 (27)	1142 ± 317 (29)	1272 ± 309 (27)	1241 ± 243 (27)

* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group value ($p \leq 0.01$).

3.2. F1-generation – reproductive outcome

For F1 males, sperm motility and sperm counts were not statistically significantly different between the K^+PFBS-treated groups and the control group (Table 1). Although no treatment-related differences in head and/or tail abnormalities were observed, and the incidences of these abnormalities were low in all groups an incidental but statistically significant ($p < 0.05$) increase in the percent of abnormal sperm in the 1000 mg/kg/day dose group was observed. The extent of this increase was not considered biologically meaningful, because the mean percent of abnormal sperm in the P- and F1-generation rats was similar.

For F1 females, the average numbers of estrous stages per 21 days were not statistically significantly different among the five groups (Table 2). The number of rats with ≥6 consecutive days of diestrus was significantly increased ($p \leq 0.01$) in the 100 mg/kg/day dose group and significantly decreased ($p \leq 0.05$) in the 1000 mg/kg/day dose group. These statistically significant changes were not considered K^+PFBS treatment-related, because they were not dose-dependent; and they had no effect on fertility or mating.

Sex organ weights are presented in Table 3. The absolute weight of seminal vesicles with and without fluid was reduced in F1-generation males of the 1000 mg/kg/day group. However, this reduction was not apparent when compared on the basis of organ

Table 4
Male fertility of P- and F1-generation rats.

	Generation	Dose group (mg/kg/day)				
		0	30	100	300	1000
Number of cohabitating	P	30	30	30	30	29
	F1	30	28	30	28	29
Days in cohabitation (mean ± S.D.)	P	2.6 ± 1.1	2.5 ± 1.2	3.0 ± 2.4	2.7 ± 1.4	3.1 ± 2.4
	F1	3.0 ± 2.0	2.8 ± 1.0	2.4 ± 1.1	3.3 ± 3.9	2.8 ± 1.4
Percent mating	P	100	100	96.7	100	100
	F1	100	100	100	92.8	100
Rats with confirmed mating date	P	30	30	29	30	29
	F1	30	28	30	26	29
Rats mating days 1–7	P	30	30	29	29	28
	F1	29	28	30	25	29
Rats mating days 8–14	P	0	0	0	1	1
	F1	1	0	0	1	0
Number of pregnancies/number of cohab	P	27/30	29/30	29/30	29/30	24/29
	F1	24/30	24/28	28/30	24/28	27/29
Fertility index[a] (%)	P	90.0	96.7	100	96.7	82.8
	F1	80.0	85.7	93.3	92.3	93.1

[a] Fertility is the number pregnant divided by the number with evidence of mating (sperm in uterine lavage) and is restricted to rats with a confirmed mating date and rats that did not mate. (E.g, in the P-generation 100 mg/kg/day dose group, 30 rats were paired but only 29 had evidence of mating and all 29 were pregnant. Hence 29/29 = 100%).

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EPA-HQ-2016-005679 06/14/2017

Table 5
Female fertility of P- and F1-generation rats.

	Generation	Dose group (mg/kg/day)				
		0	30	100	300	1000
Number of cohabitating	P	30	30	30	30	30
	F1	30	30	30	30	29
Days in cohabitation (mean ± S.D.)	P	2.6 ± 1.1	2.5 ± 1.2	3.0 ± 2.4	2.7 ± 1.4	3.2 ± 2.4
	F1	3.0 ± 2.0	2.7 ± 1.0	2.4 ± 1.1	3.3 ± 3.8	2.8 ± 1.4
Rats with confirmed mating date	P	30	30	29	30	30
	F1	30	30	30	28	29
Rats mating days 1–7	P	30	30	29	29	29
	F1	29	30	30	27	29
Rats mating days 8–14	P	0	0	0	1	1
	F1	1	0	0	1	0
Number of pregnancies/number of cohab	P	27/30	29/30	29/30	29/30	25/30
	F1	24/30	26/30	28/30	25/30	27/29
Fertility index[a] (%)	P	90.0	96.7	100	96.7	83.3
	F1	80.0	86.7	93.3	89.3	93.1

[a] Fertility is the number pregnant divided by the number with evidence of mating (sperm in uterine lavage) and is restricted to rats with a confirmed mating date and rats that did not mate. (E.g., in the P-generation 100 mg/kg dose group, 30 rats were paired but only 29 had evidence of mating and all 29 were pregnant. Hence 29/29 = 100%).

weight to body weight ratios. Seminal vesicles with fluid were 0.351 ± 0.074% and 0.325 ± 0.073% of body weight in the control and high-dose males, respectively, and seminal vesicles without fluid were 0.204 ± 0.042% and 0.185 ± 0.038% of body weight in the control and 1000 mg/kg/day dose-group males, respectively. Because mean terminal body weights in the 1000 mg/kg/day dose-group males were reduced by approximately 9% from the control mean with statistical significance ($p \leq 0.01$), the effect on absolute seminal vesicle weight was likely the result of reduced body weight in the 1000 mg/kg/day dose-group.

Male and female mating and fertility data are shown in Tables 4 and 5, respectively. All F1-generation mating and fertility parameters measured were unaffected by K^+PFBS treatment. F1-generation delivery outcomes and the corresponding F1 litter observations are shown in Tables 6 and 7, respectively. There were no toxicologically meaningful changes in delivery outcomes and litter observations.

There were no K^+PFBS treatment-related microscopic changes in sex organs of F1 males and female rats as compared to controls, including those rats identified as having reduced fertility (data not shown). Ovarian follicle counts for females in the 1000 mg/kg/day dose group were not different than controls.

3.3. F1-generation – sexual maturation

Sexual maturation data for F1-generation male and female rats are presented in Table 8. In F1-generation males, the mean days to preputial separation was delayed with statistical significance in the 30 and 1000 mg/kg/day dose groups by 0.6 ($p \leq 0.05$) and 1.6 ($p \leq 0.01$) days, respectively. Mean (±S.D.) male body weights per group on the day of sexual maturation are also presented in Table 8. These data were used in analysis of co-variance for days to preputial separation versus body weight on day of preputial separation. When adjusted for body weight on day of preputial separation, there were no statistically significant differences across groups.

There were no advances or delays among K^+PFBS-treated females for the mean days to vaginal patency when compared to controls.

3.4. P-generation males – toxicology endpoints

3.4.1. Clinical observations

One male rat death occurred in the 1000 mg/kg/day dose group P-generation due to an intubation accident. The numbers of male rats in the 1000 mg/kg/day presenting with excessive salivation

Table 6
Delivery outcomes of P- and F1-generation dams.

	Dams	Dose group (mg/kg/day)				
		0	30	100	300	1000
Number of dams delivering	P	26	29	29	29	25
	F1	24	26	28	25	27
Length of gestation, days (mean ± S.D.)	P	22.7 ± 0.5	22.8 ± 0.4	22.8 ± 0.5	22.8 ± 0.4	22.7 ± 0.4
	F1	22.7 ± 0.5	22.8 ± 0.5	22.6 ± 0.5	22.6 ± 0.5	22.8 ± 0.4
Deliveries (%)	P	100	100	100	100	100
	F1	100	100	100	100	100
Percentage with liveborn pups[a]	P	100	100	100	100	100
	F1	100	100	100	100	100
Number with stillborn pups[b]	P	2	5	1	4	2
	F1	5	1	5	5	1
Mean implantation sites (mean ± S.D.)	P	15.2 ± 1.8	14.6 ± 3.0	14.3 ± 2.3	14.7 ± 1.7	14.0 ± 2.3
	F1	14.3 ± 2.3	14.6 ± 2.3	15.2 ± 2.0	15.2 ± 2.8	15.3 ± 2.0
Mean pups delivered (mean ± S.D.)	P	14.2 ± 2.2	13.9 ± 3.0	13.8 ± 3.0	14.0 ± 2.0	13.6 ± 2.3
	F1	13.2 ± 2.3	13.8 ± 2.2	14.1 ± 2.3	14.0 ± 2.1	14.0 ± 2.1

[a] Indicates the number of dams producing a litter containing at least one liveborn pup.
[b] Indicates the number of dams with at least one stillborn pup.

ED_000954(915)_Processed_PSTs-2_DD_00000890-00005

EPA-HQ-2016-005679 06/14/2017

Table 7
Litter outcomes for F1- and F2-generation pups.

	Pups	Dose group (mg/kg/day)				
		0	30	100	300	1000
Number of litters delivered	F1	26	29	29	29	25
	F2	24	26	28	25	27
Mean (± S.D.) liveborn (*N*)	F1	14.2 ± 2.2	13.5 ± 2.8**	13.7 ± 3.0	13.8 ± 2.0	13.5 ± 2.3
	F2	13.0 ± 2.3	13.6 ± 2.3	13.9 ± 2.4	13.6 ± 2.6	14.0 ± 2.0
Mean (± S.D.) stillborn (*N*)	F1	0.1 ± 0.3	0.2 ± 0.6	0.1 ± 0.4	0.1 ± 0.4	0.1 ± 0.3
	F2	0.2 ± 0.4	0.1 ± 0.6	0.2 ± 0.5	0.4 ± 1.0	0.0 ± 0.2
Mean (± S.D.) pup weight at birth (g)	F1	6.4 ± 0.4	6.5 ± 0.5	6.6 ± 0.5	6.4 ± 0.4	6.3 ± 0.5
	F2	6.3 ± 0.6	6.4 ± 0.5	6.4 ± 0.4	6.2 ± 0.5	6.2 ± 0.8
Mean (± S.D.) pup weight at weaning (g)	F1	40.2 ± 5.6	40.2 ± 6.8	40.8 ± 7.8	39.2 ± 5.9[a]	39.3 ± 4.4
	F2	36.4 ± 7.7[b]	39.4 ± 7.3	37.1 ± 5.1	36.3 ± 6.6	35.0 ± 5.6[c]
Viability index[d] (%)	F1	98.1	96.7*	98.2	99.2	99.4*
	F2	95.5	96.9	97.2	96.8	95.8
Lactation index[e] (%)	F1	99.4	98.7	97.4**	97.7**	99.7
	F2	97.0	96.5	98.2	97.3	93.1**

[a] *N* = 28. Excludes values for litters wioth no surviving pups.
[b] *N* = 23. Excludes values for litters wioth no surviving pups.
[c] *N* = 25. Excludes values for litters wioth no surviving pups.
[d] (Number of pups alive on day 4/number of pups alive on day 1) × 100.
[e] (Number of pups alive on day 21/number of pups alive on day 4) × 100.
* Statistically significantly different from control value ($p \leq 0.05$).
** Statistically significantly different from control value ($p \leq 0.01$).

(144 instances among 27 rats), perioral substance (11 instances among 9 rats), and urine-stained abdominal fur (9 instance among 4 rats) were increased with statistical significance as compared to controls. No other K^+PFBS treatment-related clinical signs were observed in P-generation males.

3.4.2. Body weight and food consumption

Body weights on the initial day of dosing and on the day of terminal sacrifice as well as body weight change through terminal sacrifice are presented in Tables 9a for P-generation males. There were no statistically significant changes in mean absolute body weights at terminal sacrifice; therefore, treatment with K^+PFBS at doses up to 1000 mg/kg/day in P-generation male rats had no overall effect on body weight. In addition to the summary data provided in Table 9a, there were isolated periods of statistically significant differences in weight change in K^+PFBS-treated rats relative to control rats that were not considered treatment-related. Mean body weight gains were increased relative to controls between study days 36 and 43 in the 100 and 1000 mg/kg/day dose groups ($p \leq 0.05$ and $p \leq 0.01$, respectively) and between study days 64 and 70 in the 300 mg/kg/day dose group ($p \leq 0.01$). Decreased body weight gain relative to controls occurred between study days 43 and 57 ($p \leq 0.01$) in the 1000 mg/kg/day dose group, and a slight but significant ($p \leq 0.01$) negative body weight gain (representing body weight loss) occurred between study days 105 and 112 in the 1000 mg/kg/day dose group.

No K^+PFBS treatment-related changes in absolute or relative food consumption relative to controls occurred among P-generation male rats (data not shown).

3.4.3. Necropsy, histology and organ weight changes

There were no K^+PFBS treatment-related gross anatomical findings in P-generation male rats. As stated previously, no K^+PFBS treatment-related microscopic changes were present in reproductive organs of the 1000 mg/kg/day dose group P-generation male rats which were selected for histopathological evaluation. The microscopic incidence of mild enlargement of liver cells (hypertrophy) and minimal to mild proliferation of kidney medullary/papillary tubular and ductular epithelial cells (hyperplasia) was increased in the 300 and 1000 mg/kg/day dose groups (Table 10). Twenty-six of 30 (87%) 1000 mg/kg/day dose group P-generation male rats had hepatocellular hypertrophy with 25 minimal and 1 mild in severity. Three of 30 (10%) 300 mg/kg/day dose group males had minimal hepatocellular hypertrophy. In addition, six incidences of focal papillary edema (five minimal and one mild in severity) and one incident of moderate focal papillary necrosis were noted in the P-generation male rats in the 1000 mg/kg/day dose group. There were no other K^+PFBS

Table 8
Days to sexual maturation in F1-generation rats (mean ± S.D.). Mean body weight data (mean ± S.D.) at preputial separation for male rats are also included.

	Dose group (mg/kg/day)				
	0	30	100	300	1000
Males (*N*)	30	29	30	30	30
Days to preputial separation	47.7 ± 3.4	48.3 ± 1.8*	47.9 ± 2.3	48.2 ± 2.2	49.3 ± 1.8**
Weight (g) at preputial separation[a]	222.4 ± 20.1	223.2 ± 17.6	222. 4 ± 17.2	221.8 ± 17.7	219.6 ± 19.2
Females (*N*)	30	30	30	30	30
Days to vaginal patency	35.0 ± 2.5	35.6 ± 2.0	34.8 ± 1.6	35.9 ± 2.0	35.7 ± 1.8

[a] These data were used in analysis of co-variance for days to preputial separation versus body weight on day of preputial separation. When adjusted for body weight on day of preputial separation, there were no statistically significant differences across groups in days to preputial separation.
* Statistically significantly different from control value ($p \leq 0.05$).
** Statistically significantly different from control value ($p \leq 0.01$).

ED_000954(915)_Processed_PSTs-2_DD_00000890-00006

EPA-HQ-2016-005679 06/14/2017

Table 9a
Body weight and body weight change in P-generation male and female rats (mean ± S.D.) (*N*).

Time point during study	Dose group (mg/kg/day)				
	0	30	100	300	1000
P-generation males					
Study day 1	181 ± 8 (30)	177 ± 17 (30)	179 ± 8 (30)	180 ± 11 (30)	178 ± 9 (30)
Termination	562 ± 56 (30)	569 ± 53 (30)	570 ± 45 (30)	572 ± 69 (30)	551 ± 46 (29)
Change	381 ± 54 (30)	392 ± 53 (30)	391 ± 44 (30)	393 ± 66 (30)	373 ± 44 (29)
P-generation females					
Study day 1	153 ± 7 (30)	153 ± 7 (30)	154 ± 8 (30)	152 ± 8 (30)	152 ± 7 (30)
Study day 70	285 ± 27 (30)	282 ± 29 (30)	289 ± 25 (30)	278 ± 28 (30)	276 ± 24 (30)
Change	132 ± 23 (30)	129 ± 25 (30)	135 ± 20 (30)	126 ± 23 (30)	124 ± 19 (30)
Gestation day 0	281 ± 26 (27)	284 ± 30 (29)	286 ± 25 (29)	277 ± 26 (29)	276 ± 22 (25)
Gestation day 21	426 ± 36 (22)	429 ± 50 (23)	430 ± 38 (22)	409 ± 33 (22)	409 ± 22 (19)
Change	143 ± 16 (22)	143 ± 24 (23)	143 ± 19 (22)	135 ± 21 (22)	133 ± 24 (19)
Lactation day 1	319 ± 28 (26)	317 ± 32 (29)	323 ± 29 (29)	313 ± 30 (29)	311 ± 18 (25)
Lactation day 22	346 ± 23 (26)	343 ± 26 (29)	351 ± 23 (29)	344 ± 30 (28)	336 ± 22 (25)
Change	27 ± 19 (26)	26 ± 16 (29)	29 ± 23 (29)	30 ± 18 (28)	26 ± 20 (25)

Table 9b
Body weight and body weight change in F1-generation male and female rats (mean ± SD) (*N*).

Time point during study	Dose group (mg/kg/day)				
	0	30	100	300	1000
F1-generation pups, pre-weaning (by litter)					
Postnatal day 1	6.4 ± 0.4 (26)	6.5 ± 0.5 (29)	6.6 ± 0.5 (29)	6.4 ± 0.4 (29)	6.3 ± 0.5 (25)
Postnatal day 22 (weaning)	40.2 ± 5.6 (26)	40.2 ± 6.8 (29)	40.8 ± 7.8 (29)	39.2 ± 5.9 (28)	39.3 ± 4.4 (25)
F1-generation males, post-weaning					
Post-weaning day 1	47 ± 5 (30)	46 ± 7 (30)	46 ± 8 (30)	45 ± 6 (30)	45 ± 5 (30)
Termination	594 ± 45 (29)	583 ± 61 (27)	593 ± 73 (29)	598 ± 65 (27)	549 ± 38** (27)
Change	546 ± 44 (29)	537 ± 59 (27)	547 ± 69 (29)	552 ± 63 (27)	504 ± 36** (27)
F1-generation females, post-weaning					
Post-weaning day 1	45 ± 7 (30)	45 ± 6 (30)	45 ± 9 (30)	44 ± 6 (30)	44 ± 4 (30)
Day of cohabitation[a]	277 ± 28 (30)	287 ± 28 (30)	288 ± 27 (30)	282 ± 24 (30)	274 ± 22 (29)
Change	232 ± 26 (30)	242 ± 27 (30)	243 ± 25 (30)	238 ± 22 (30)	231 ± 23 (29)
Gestation day 0	273 ± 26 (24)	287 ± 27 (26)	285 ± 25 (28)	279 ± 23 (25)	272 ± 22 (27)
Gestation day 21	407 ± 33 (22)	437 ± 32** (23)	435 ± 34** (20)	417 ± 30 (19)	412 ± 26 (21)
Change	132 ± 23 (22)	144 ± 27 (23)	146 ± 20 (20)	141 ± 18 (19)	141 ± 14 (21)
Lactation day 1	303 ± 27 (24)	319 ± 30 (26)	316 ± 27 (28)	311 ± 26 (25)	306 ± 26 (27)
Lactation day 22	331 ± 29 (23)	350 ± 33* (26)	353 ± 26** (28)	347 ± 16* (25)	348 ± 23* (25)
Change	29 ± 23 (23)	31 ± 26 (26)	37 ± 17 (28)	36 ± 20 (25)	38 ± 19 (25)

* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group value ($p \leq 0.01$).
[a] Rats were 102–110 days old.

treatment-related histopathological changes, including the spleen, thymus, and pituitary (data not shown).

P-generation male absolute and relative organ weight data for brain, liver, kidneys, and spleen are provided in Table 11. Liver weights (absolute and relative to body weight and brain weight) were statistically significantly increased in the 300 and 1000 mg/kg/day dose groups. Although mean liver weight relative to brain weight was increased with statistical significance in the 30 mg/kg/day dose-group males, this was likely due to the fact that mean absolute brain weight was slightly reduced but with statistical significance in the 30 mg/kg/day K^+PFBS dose group only; however, brain weight as a percent to body weight was not affected in this group. The relative left kidney weight to brain weight ratio was significantly increased in the 30 mg/kg/day K^+PFBS dose group, however, the absolute left kidney weight was not affected at this dose level and the finding was not dose-dependent. There were no other statistically significant changes relative to controls in absolute or relative organ weights.

3.5. P-generation females – toxicology endpoints

3.5.1. Clinical observations

There were no K^+PFBS treatment-related P-generation female rat deaths. One control rat died on GD 23, and one 300 mg/kg/day

Table 9c
Body weight and body weight change in F2-generation male and female rats (mean ± S.D.) (*N*).

Time point during study	Dose group (mg/kg/day)				
	0	30	100	300	1000
F2-Generation pups, pre-weaning (by litter)					
Postnatal day 1	6.3 ± 0.6 (24)	6.4 ± 0.5 (26)	6.4 ± 0.4 (28)	6.2 ± 0.5 (25)	6.2 ± 0.8 (27)
Postnatal day 22	36.4 ± 7.7 (23)	39.4 ± 7.3 (26)	37.1 ± 5.1 (28)	36.3 ± 6.6 (25)	35.0 ± 5.6 (25)

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EPA-HQ-2016-005679 06/14/2017

Table 10
Incidence and degree of severity of histomorphologic observations in livers and kidneys of P- and F1-generation rats.

Generation	Sex	Dose mg/kg	Liver	Kidney			
			Hepatocellular hypertrophy	Papillary epithelial tubular/ductal hyperplasia	Focal papillary edema	Focal necrosis of the papilla	Focal cortical tubular dilatation
			N[a]/30 (*N*[b]/deg.[c])	*N*/30 (*N*/deg.)	*N*/30 (*N*/deg.)	*N*/30 (*N*/deg.)	*N*/30 (*N*/deg.)
P	M	Cont[d]	0	0	1 (1+)	0	0
F1	M	Cont	0	3 (3+)	1 (1+)	0	0
P	M	300	3 (3+)	9 (7+; 2++)	2 (2+)	0	0
F1	M	300	3 (3+)	5 (4+; 1++)	0	2 (2+)	0
P	M	1000	26 (25+; 1++)	19 (9+; 9++; 1+++)	6 (5+; 1++)	1 (1++)	1 (1+)
F1	M	1000	14 (13+; 1++)	21 (8+; 13++)	9 (9+)	0	1 (1+)
P	F	Cont	0	3 (1+; 2++)	1 (1+)	0	0
F1	F	Cont	0	2 (2+)	0	0	1 (1++)
P	F	300	0	16 (7+; 8++; 1+++)	8 (7+; 1++)	3 (1+; 2++)	1 (1+++)
F1	F	300	0	13 (7+; 5++; 1+++)	7 (6+; 1++)	1 (1++)	1 (1+)
P	F	1000	0	21 (9+; 12++)	7 (7+)	0	1 (1++)
F1	F	1000	0	15 (7+; 7++; 1+++)	4 (3+; 1++)	1 (1++)	0

Key: M = males; F = females.
[a] Number of observations per 30 rats.
[b] Number of observations of a specific degree of severity are given in parentheses.
[c] Degree of severity (+ = minimal severity; ++ = mild severity; +++ = moderate severity).
[d] Control rats.

Table 11
P- and F1-generation male absolute oragn weights (g) and relative organ weights (%) (mean ± S.D.) (*N*).

	Gen[a]	Dose group (mg/kg/day)				
		0	30	100	300	1000
Body at term	P	562 ± 56 (30)	569 ± 53 (30)	570 ± 45 (30)	572 ± 69 (30)	551 ± 46 (29)
	F1	594 ± 45 (29)	583 ± 61 (27)	593 ± 73 (29)	598 ± 65 (27)	549 ± 38** (27)
Brain	P	2.14 ± 0.14 (30)	2.05 ± 0.12* (30)	2.17 ± 0.15 (30)	2.12 ± 0.14 (29)	2.14 ± 0.12 (29)
	F1	2.04 ± 0.19 (29)	2.03 ± 0.14 (25)	2.06 ± 0.12 (28)	2.00 ± 0.20 (27)	1.99 ± 0.12 (27)
Percentage to body[b]	P	0.38 ± 0.04 (30)	0.36 ± 0.04 (30)	0.38 ± 0.04 (30)	0.37 ± 0.05 (29)	0.39 ± 0.03 (29)
	F1	0.34 ± 0.04 (29)	0.35 ± 0.04 (25)	0.35 ± 0.05 (28)	0.34 ± 0.04 (27)	0.36 ± 0.03 (27)
Liver	P	19.2 ± 2.4 (30)	20.0 ± 2.9 (30)	20.5 ± 2.4 (30)	21.5 ± 2.9** (30)	2.7 ± 2.8** (29)
	F1	20.6 ± 2.5 (29)	20.1 ± 2.7 (27)	21.2 ± 4.4 (29)	21.7 ± 3.2 (27)	21.1 ± 2.2 (27)
Percentage to brain[c]	P	901 ± 113 (30)	982 ± 156* (30)	947 ± 118 (30)	1015 ± 125** (29)	1061 ± 121** (29)
	F1	1017 ± 148 (29)	988 ± 145 (25)	1038 ± 244 (28)	1097 ± 214 (27)	1062 ± 113 (27)
Percentage to body	P	3.4 ± 0.3 (30)	3.5 ± 0.4 (30)	3.6 ± 0.3 (30)	3.8 ± 0.3** (30)	4.1 ± 0.4** (29)
	F1	3.5 ± 0.4 (29)	3.4 ± 0.3 (27)	3.5 ± 0.4 (29)	3.6 ± 0.3 (27)	3.8 ± 0.3** (27)
Left kidney	P	2.10 ± 0.21 (30)	2.17 ± 0.20 (30)	2.18 ± 0.20 (30)	2.20 ± 0.28 (30)	2.20 ± 0.26 (29)
	F1	2.04 ± 0.21 (29)	2.06 ± 0.23 (27)	2.08 ± 0.20 (29)	2.14 ± 0.24 (27)	1.97 ± 0.16 (27)
Percentage to brain	P	98.2 ± 8.7 (30)	106.4 ± 112** (30)	100.7 ± 9.7 (30)	103.8 ± 11.1 (29)	102.9 ± 11.7 (29)
	F1	101.1 ± 12.2 (29)	101.7 ± 12.3 (25)	101.1 ± 10.8 (28)	108.3 ± 19.3 (27)	99.2 ± 8.0 (27)
Percentage to body	P	0.375 ± 0.034 (30)	0.382 ± 0.035 (30)	0.383 ± 0.032 (30)	0.385 ± 0.042 (30)	0.400 ± 0.047 (29)
	F1	0.344 ± 0.030 (29)	0.352 ± 0.021 (27)	0.353 ± 0.033 (29)	0.358 ± 0.038 (27)	0.357 ± 0.029 (27)
Right kidney	P	2.15 ± 0.22 (30)	2.18 ± 0.21 (30)	2.20 ± 0.21 (30)	2.21 ± 0.28 (30)	2.22 ± 0.25 (29)
	F1	2.05 ± 0.20 (29)	2.08 ± 0.22 (27)	2.09 ± 0.20 (28)	2.14 ± 0.25 (27)	2.00 ± 0.18 (27)
Percentage to brain	P	100.6 ± 8.8 (30)	106.7 ± 11.8 (30)	101.5 ± 11.1 (30)	104.5 ± 10.9 (29)	103.9 ± 10.4 (29)
	F1	101.5 ± 11.6 (29)	103.0 ± 12.0 (25)	101.3 ± 10.5 (27)	108.6 ± 20.6 (27)	100.4 ± 7.0 (27)
Percentage to body	P	0.383 ± 0.041 (30)	0.384 ± 0.034 (30)	0.385 ± 0.033 (30)	0.387 ± 0.042 (30)	0.403 ± 0.040 (29)
	F1	0.346 ± 0.030 (29)	0.356 ± 0.020 (27)	0.356 ± 0.029 (28)	0.359 ± 0.040 (27)	0.364 ± 0.032 (27)
Spleen	P	0.86 ± 0.13 (30)	0.92 ± 0.18 (30)	0.89 ± 0.11 (30)	0.88 ± 0.19 (30)	0.86 ± 0.09 (29)
	F1	0.90 ± 0.19 (29)	0.88 ± 0.08 (27)	0.87 ± 0.17 (29)	0.84 ± 0.14 (27)	0.79 ± 0.12 (27)
Percentage to brain	P	40.5 ± 6.1 (30)	44.9 ± 9.4 (30)	41.3 ± 5.3 (30)	41.9 ± 8.5 (29)	40.3 ± 4.7 (29)
	F1	44.7 ± 11.7 (29)	43.4 ± 5.3 (25)	42.8 ± 8.4 (28)	42.9 ± 9.8 (27)	39.8 ± 6.9 (27)
Percentage to body	P	0.153 ± 0.023 (30)	0.161 ± 0.030 (30)	0.156 ± 0.017 (30)	0.153 ± 0.025 (30)	0.157 ± 0.019 (29)
	F1	0.151 ± 0.033 (29)	0.151 ± 0.018 (27)	0.147 ± 0.025 (29)	0.141 ± 0.024 (27)	0.144 ± 0.023 (27)

[a] Generation.
[b] Percentage to body weight = (organ weight/terminal body weight) × 100.
[c] Percentage to terminal brain weight = (organ weight/brain weight) × 100.
* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group ($p \leq 0.01$).

ED_000954(915)_Processed_PSTs-2_DD_00000890-00008

EPA-HQ-2016-005679 06/14/2017

dose group rat was moribund sacrificed on LD 15 for reasons believed not to be related to K^+PFBS treatment. During the precohabitation period, females in the 1000 mg/kg/day dose group experienced increased occurrences of dried or wet red perioral substance (7 of 30) and excessive salivation (5 of 30), and, during gestation, 1000 mg/kg/day dose group females continued to have red perioral substance (3 of 30).

3.5.2. Body weight and food consumption

Initial and terminal mean body weight and body weight change of the P-generation female rats during the precohabitation, gestation, and lactation periods are shown in Table 9a. There were no statistically significant findings in the presented data. In addition to the data provided in Table 9a, there were instances of statistically significant reduced body weight and weight gain in the 1000 mg/kg/day dose group compared to controls: (1) mean body weights were reduced on days 8 and 11 of lactation ($p \leq 0.05$ and 0.01, respectively) and (2) mean weight gain was reduced during the last week of dosing prior to mating (precohabitation days 64–70, $p \leq 0.01$) and during the 1st week of gestation ($p \leq 0.01$). Despite these isolated instances in the highest dose group, overall mean body weights and mean body weight changes at the end of each of these three periods were not significantly different from controls in all K^+PFBS-treated groups.

Statistically significant increases in mean relative food consumption compared to control P-generation female rats occurred in the 100 ($p \leq 0.05$), 300 ($p \leq 0.05$), and 1000 mg/kg/day ($p \leq 0.05$) dose groups between precohabitation days 29 and 36 and again in the 300 ($p \leq 0.05$) and 1000 mg/kg/day ($p \leq 0.05$) dose groups between precohabitation days 36 and 43. A decrease in absolute food consumption relative to control occurred between GD 10 and 14 in the 300 and 1000 mg/kg/day dose groups ($p \leq 0.05$). Through precohabitation, there was a significant ($p \leq 0.05$) decrease in overall relative food consumption in the 300 mg/kg/day dose group compared to controls. These were the only statistically significant changes from control noted for absolute and relative food consumption.

3.5.3. Necropsy, histology, and organ weight changes

There were no treatment-related gross anatomical findings in P-generation female rats.

Treatment-related microscopic findings were observed in the kidneys of female rats in the 300 and 1000 mg/kg/day dose groups (Table 10). These findings consisted of an increased incidence of minimal-to-moderate hyperplasia of the tubular and ductular epithelium of the inner medulla/papilla, and primarily minimal focal papillary edema in the 300 and 1000 mg/kg/day dose groups. In addition, three incidents of minimal-to-moderate focal papillary necrosis were observed in the 300 mg/kg/day dose group; although, none were observed in the 1000 mg/kg/day dose group. No liver hypertrophy was observed in the P-generation female rats.

Absolute and relative organ weight data for P-generation female rats are presented in Table 12 for brain, kidneys, and spleen. The mean absolute weight of the brain was reduced in the 1000 mg/kg/day dose group with statistical significance (2.04 ± 0.10 versus 2.13 ± 0.09 in controls). For brain weight rela-

Table 12
P- and F1-generation female absolute organ weights (g) and relative organ weights (%) (g) (mean ± S.D.) (*N*).

	Gen[a]	Dose group (mg/kg/day)				
		0	30	100	300	1000
Body at term	P	346 ± 23 (26)	343 ± 26 (29)	351 ± 23 (29)	344 ± 30 (28)	336 ± 22 (25)
	F1	331 ± 29 (23)	350 ± 33* (26)	353 ± 26** (28)	347 ± 16* (25)	348 ± 23* (25)
Brain	P	2.13 ± 0.09 (26)	2.11 ± 0.08 (29)	2.10 ± 0.15 (29)	2.12 ± 0.09 (28)	2.04 ± 0.10** (25)
	F1	2.11 ± 0.11 (23)	2.12 ± 0.11 (26)	2.18 ± 0.08 (28)	2.14 ± 0.10 (25)	2.10 ± 0.07 (25)
Percentage to body[b]	P	0.62 ± 0.05 (26)	0.62 ± 0.04 (29)	0.60 ± 0.05 (29)	0.62 ± 0.06 (28)	0.61 ± 0.05 (25)
	F1	0.64 ± 0.06 (23)	0.61 ± 0.07 (26)	0.62 ± 0.05 (28)	0.62 ± 0.03 (25)	0.61 ± 0.05 (25)
Left kidney	P	1.41 ± 0.12 (26)	1.42 ± 0.15 (29)	1.41 ± 0.15 (29)	1.48 ± 0.18 (28)	1.42 ± 0.17 (25)
	F1	1.51 ± 0.16 (23)	1.51 ± 0.13 (26)	1.52 ± 0.14 (28)	1.52 ± 0.18 (25)	1.48 ± 0.16 (25)
Percentage to brain[c]	P	66.1 ± 5.4 (26)	67.6 ± 6.9 (29)	67.6 ± 6.5 (29)	69.6 ± 7.8 (28)	69.8 ± 8.1 (25)
	F1	71.6 ± 8.0 (23)	71.3 ± 7.2 (26)	70.2 ± 6.9 (28)	70.7 ± 7.0 (25)	70.6 ± 7.1 (25)
Percentage to body	P	0.407 ± 0.032 (26)	0.416 ± 0.035 (29)	0.402 ± 0.034 (29)	0.430 ± 0.055 (28)	0.422 ± 0.051 (25)
	F1	0.462 ± 0.092 (23)	0.434 ± 0.039 (26)	0.433 ± 0.032 (28)	0.438 ± 0.046 (25)	0.428 ± 0.047 (25)
Right kidney	P	1.41 ± 0.13 (26)	1.39 ± 0.14 (29)	1.42 ± 0.16 (29)	1.44 ± 0.19 (28)	1.40 ± 0.15 (25)
	F1	1.55 ± 0.18 (23)	1.58 ± 0.13 (26)	1.57 ± 0.14 (28)	1.57 ± 0.18 (25)	1.56 ± 0.18 (25)
Percentage to brain	P	66.1 ± 5.4 (26)	66.1 ± 6.4 (29)	68.1 ± 6.8 (29)	67.8 ± 7.4 (28)	68.8 ± 6.8 (25)
	F1	73.4 ± 8.9 (23)	74.5 ± 6.7 (26)	72.4 ± 6.5 (28)	73.3 ± 6.7 (25)	74.1 ± 7.8 (25)
Percentage to body	P	0.406 ± 0.035 (26)	0.407 ± 0.036 (29)	0.406 ± 0.036 (29)	0.420 ± 0.053 (28)	0.418 ± 0.044 (25)
	F1	0.474 ± 0.101 (23)	0.454 ± 0.042 (26)	0.446 ± 0.024 (28)	0.454 ± 0.046 (25)	0.450 ± 0.048 (25)
Spleen	P	0.74 ± 0.15 (26)	0.70 ± 0.12 (29)	0.71 ± 0.16 (29)	0.74 ± 0.23 (28)	0.67 ± 0.12 (25)
	F1	0.62 ± 0.12 (23)	0.70 ± 0.11* (26)	0.70 ± 0.13* (28)	0.71 ± 0.11* (25)	0.67 ± 0.09 (25)
Percentage to brain	P	34.6 ± 6.9 (26)	33.5 ± 5.9 (29)	34.0 ± 6.8 (29)	34.8 ± 9.7 (28)	32.7 ± 5.8 (25)
	F1	29.3 ± 5.2 (23)	33.3 ± 5.3 (26)	32.3 ± 5.8 (28)	32.9 ± 5.4 (25)	31.9 ± 4.2 (25)
Percentage to body	P	0.213 ± 0.045 (26)	0.206 ± 0.033 (29)	0.202 ± 0.043 (29)	0.215 ± 0.061 (28)	0.198 ± 0.036 (25)
	F1	0.186 ± 0.034 (23)	0.201 ± 0.030 (26)	0.198 ± 0.035 (28)	0.204 ± 0.035 (25)	0.193 ± 0.027 (25)

[a] Generation.
[b] Percentage to body weight = (organ weight/terminal body weight) × 100.
[c] Percentage to brain weight = (liver weight/brain weight) × 100.
* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group value ($p \leq 0.01$).

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EPA-HQ-2016-005679 06/14/2017

tive to body weight, there was no difference between control and 1000 mg/kg/day dose group. There were no other statistically significant changes in organ weights.

3.6. F1-generation males – toxicology endpoints

3.6.1. Clinical observations

There were no treatment-related deaths in F1-generation male rats. Some excess salivation was observed in the 1000 mg/kg/day dose group (data not shown).

3.6.2. Body weight and food consumption

Mean body weight on the initial day of dosing and on the day of terminal sacrifice as well as body weight change through terminal sacrifice for the F1-generation male rats are presented in Table 9b. Terminal body weights and body weight change from weaning to termination, were significantly reduced for the 1000 mg/kg/day dose group. There were no statistically significant findings in the other presented data. In addition to the summary data provided in Table 9b, body weight gain was reduced with statistical significance ($p \leq 0.01$) during the 2nd week of dosing in the 1000 mg/kg/day dose group when compared to controls, with means of 46.7 g and 51.8g in 1000 mg/kg/day group and control, respectively. Although not statistically significant, mean body weight gains in the 1000 mg/kg/day dose group were generally less than controls during most of the period prior to termination. This reduced weight gain resulted in statistically significant reduced overall mean body weight gain through the day of terminal sacrifice ($p \leq 0.01$). As a result of reduced body weight gain, mean body weight in the 1000 mg/kg/day group was reduced with statistical significance beginning on post-weaning day 36 ($p < 0.05$) and, with the exception of post-weaning days 50 and 64, mean body weight was reduced with statistical significance ($p < 0.01$) through terminal sacrifice. Therefore, body weight was affected by K^+PFBS treatment in the 1000 mg/kg/day dose group F1-generation males, with this effect becoming statistically significant after about 5 weeks of dosing with K^+PFBS.

Absolute food consumption was unaffected in all dose groups (data not shown); although, due to lesser mean body weight, relative food consumption was typically higher in the 1000 mg/kg/day dose group, with statistical significance during the 6th ($p \leq 0.05$), 7th ($p \leq 0.01$), and 10th ($p \leq 0.05$) weeks of the precohabitation period. Relative food consumption was also slightly higher than control in the 300 mg/kg/day dose at all but one time interval and was significantly higher ($p \leq 0.01$) during the 4th week of dosing.

3.6.3. Necropsy, histology, and organ weight changes

There were no treatment-related gross anatomical findings in F1-generation male rats. As stated before, no K^+PFBS treatment-related microscopic changes were observed in the reproductive organs of any 1000 mg/kg/day dose group F1-generation male rats selected for histopathological evaluation. Microscopic examination of the liver and kidneys of the F1 generation males revealed treatment-related effects similar to those in the P-generations males (Table 10). The changes in the liver consisted of 3 and 14 incidences of minimal to mild severity of hepatocellular hypertrophy in the 300 and 1000 mg/kg/day dose groups, respectively. The liver cells were enlarged due to an increased amount of finely granular eosinophilic cytoplasm. The hypertrophy was generally present in all areas of the lobules but tended to be more prominent and prevalent in the centrilobular region. In the kidneys, the treatment-related microscopic change consisted primarily of an increased incidence and severity of hyperplasia of the tubular and ductular epithelium of the inner medulla/papilla area in the 1000 mg/kg/day dose group. There were also nine incidences of minimal focal papillary edema in the 1000 mg/kg/day dose group.

F1-generation male absolute and relative organ weight data are presented in Table 11 for brain, liver, kidneys, and spleen. Although absolute liver weights were not increased in the highest dose group, the ratio of the liver weight-to-body weight was significantly increased in the 1000 mg/kg/day dose group relative to control values. There were no statistically significant differences between controls and treated groups with respect to the ratios of organ weights to brain weights.

3.7. F1-generation females – toxicology endpoints

3.7.1. Clinical observations

No treatment-related clinical signs were observed in F1-generation female rats at any of the K^+PFBS dosages tested. Although one death occurred in a 1000 mg/kg/day dose group female on precohabitation day 35 within 28 min after dosing, this female had fed and gained weight normally and all tissues appeared normal on necropsy (data not shown); therefore, this death was considered not to be related to treatment.

3.7.2. Body weight and food consumption

Initial and terminal mean body weight and body weight change of the F1-generation female rats during the precohabitation, gestation, and lactation periods are shown in Table 9b. There were no statistically significant reductions in body weight change or body weights among K^+PFBS-treated female rats as compared to controls for the presented data. In addition to the summary data provided in Table 9b, occasional statistically significant increases in body weight change or body weight were observed in the K^+PFBS-treated groups when compared to controls. In the two lower K^+PFBS-treated dose groups (30 and 100 mg/kg/d), body weights were statistically significantly elevated relative to controls during the last half of gestation. Mean weight gain, although slightly greater in all K^+PFBS-treated groups relative to controls, was not statistically significantly different than the control weight gain. Therefore, the higher body weights in the two lowest K^+PFBS-treated groups may have been the result of somewhat higher body weights on GD 0 in these two groups (means of 287 and 285 g for the 30 and 100 mg/kg/day K^+PFBS dose groups, respectively, versus 279, 272 and 273 g in the 300 and 1000 mg/kg/day and control groups, respectively). At the beginning of lactation, the F1 control group females had the lowest mean body weight (303 g as compared to 319, 316, 311, and 306 g in the 30, 100, 300, and 1000 mg/kg/day K^+PFBS-treated groups, respectively). Although weight gains did not differ with statistical significance between K^+PFBS-treated and control groups, mean weights of the 30 and 100 mg/kg/day groups were higher than control with statistical significance ($p < 0.05$ or 0.01) on days 5 and 8 of lactation, and all K^+PFBS-treated groups had statistically significantly increased body weight on the last day of lactation.

3.7.3. Necropsy, histology, and organ weight changes

All necropsy observations were normal in all dose groups. There were no microscopic findings in the liver of the F1-generation female rats. Treatment-related microscopic changes similar to those observed in the P-generation female rats were observed in the kidneys of F1-generation female rats in the 300 and 1000 mg/kg/day dosage groups (Table 10). In the kidneys, the changes consisted of 13 and 15 incidences of minimal to moderate severity of hyperplasia of the tubular and ductular epithelium of the inner medulla/papilla area in the 300 and 1000 mg/kg/day dose groups, respectively. There were also seven and four incidences of minimal to mild focal papillary edema in the 300 and 1000 mg/kg/day dose groups, respectively.

F1-generation female absolute and relative organ weight data are presented in Table 12 for brain, kidneys, and spleen. With the

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exception of absolute splenic weights, all organ weights and ratios of organ weight to body weight and to brain weight were similar to control values. Mean absolute splenic weights were elevated relative to controls with statistical significance ($p \leq 0.05$) in the 30, 100, and 300 mg/kg/day dose groups; however, ratios of splenic weight to body weight were not different than controls, suggesting that the elevations likely were due to statistically significant increased body weights in these same dose groups.

3.8. F2-generation pups

There were no abnormal clinical or necropsy observations for F2-generation pups from F1 maternal rats given K^+PFBS. At LD 1 and 22, mean F2 pup body weights from maternal K^+PFBS-treated groups were not statistically significantly different compared to control (Table 9c).

Several statistically significant changes in organ weights or ratios of organ weights to terminal body weight or brain weight were observed. In the 30 mg/kg/day maternal K^+PFBS-treatment group, the mean (±S.D.) absolute weights and weights as a percent to terminal body weight of the spleen on a litter basis (0.16 ± 0.06 g and 0.40 ± 0.10%) and in female pups (0.18 ± 0.07 g and 0.42 ± 0.13%) were significantly greater ($p < 0.01$) than controls (0.12 ± 0.04 g and 0.11 ± 0.04 g and 0.31 ± 0.06% and 0.31 ± 0.06% for litter and female pups, respectively). Spleen weights as a percent to brain weight were statistically significantly higher on a litter basis (11.28 ± 4.03%, $p < 0.01$) and for male (10.67 ± 3.48%, $p < 0.05$) and female pups (11.89 ± 4.83%) compared to control values (8.23 ± 2.61%, 8.47 ± 3.30%, and 7.99 ± 2.26%, respectively). These statistically significant changes in spleen weight parameters were not observed in the three higher dose groups. In addition to spleen, kidney weight was increased with statistical significance in female pups (but not on a litter basis or in males) from the 30 mg/kg/day dose group (0.56 ± 0.10g, $p < 0.05$) compared to control (0.50 ± 0.10g), and the kidney weight as a percentage to brain weight was increased on a litter basis (38.85 ± 5.08%, $p < 0.05$) and in female pups (39.61 ± 5.49%, $p < 0.01$) compared to controls (35.43 ± 6.02% and 35.26 ± 5.85%, respectively). In addition to these statistically significant observations, the thymus weight as a percent to brain weight in the 30 mg/kg/day maternal K^+PFBS dose group female pups (11.89 ± 2.63%, $p < 0.05$) was increased with statistical significance, and the absolute thymus weight of the 100 mg/kg/day maternal K^+PFBS dose group males (0.40 ± 0.08 g, $p < 0.01$) was increased with statistical significance compared to control (0.032 ± 0.11g). None of these statistically significant differences in organ weight or organ weight ratios for the F2-generation pups were considered treatment-related because they were not dosage-dependent.

4. Discussion

The objective of this study was to provide information on the potential reproductive and developmental effects of PFBS for safety evaluation and to compare findings from the study to the outcomes of two-generation studies conducted with PFOS and PFOA, both of which did affect postnatal developmental outcomes within the dose range administered.

In the PFBS study reported here, no adverse effects on functional aspects of reproduction were observed in either generation.

In both the P- and F1-generation males, minimal to mild hepatocellular hypertrophy was observed microscopically in the 300 and 1000 mg/kg/day K^+PFBS dose groups. In P-generation males, this was accompanied by increases in mean absolute and relative liver weights as compared to controls. For the F1-generation males from the 1000 mg/kg/day dose group, only liver weight as a percent of body weight was increased with statistical significance. The presence of increased eosinophilic granules suggests the presence of increased peroxisomes or lysosomes (David et al., 2000, 2001; Frame et al., 1992; Marsman, 1995; Toyosawa et al., 2001). Wolf et al., have recently demonstrated that PFBS is an agonist for mouse and human peroxisome proliferator activated receptor ffi (PPARffi) isoforms transiently transfected into COS-1 cells (Wolf et al., 2008). Therefore, the hepatocellular eosinophilia observed in the present study may have represented, in part, increased activation of PPARffi leading to increased peroxisomal bodies. Although endpoints reflecting liver changes other than weight and histology were not evaluated in the present study, in a 90-day oral gavage study with the same lot of K^+PFBS and in the same strain of rat, there were no indications of liver damage based on increases in serum concentrations of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, bilirubin, and albumin at K^+PFBS doses up to 600 mg/kg/day (Lieder et al., 2009). Based on the results of the 90-day study as well as the present study, there are no indications that daily K^+PFBS treatment for extended periods has caused liver injury in rats.

The F1-generation males appeared to have a lessened response to the liver hypertrophic effects of K^+PFBS compared to the P-generation males. Considering that the F1-generation males were potentially exposed *in utero* and during lactation, as well as during a 10-week period prior to mating and through mating, it is surprising that absolute and relative liver weights were not affected to the same extent. The reason for this is not understood. A possibility is that, because the age and maturation at the onset of dosing differed between the P- and F1-generation males, this difference may have influenced elimination and/or distribution kinetics of PFBS. The P-generation males were sexually mature at the onset of dosing with mean group body weights ranging from 177 ± 8 to 181 ± 8 g. Dosing for the F1-generation males was initiated on the day after weaning when the rats were 23 days old and mean group body weights ranged from 45 ± 6 to 47 ± 5 g. The elimination kinetics of PFOA have been shown to change during sexual maturation in male rats, such that the elimination rate after sexual maturation is significantly reduced (Hinderliter et al., 2006). This observation with PFOA may be the result of sexual hormone-mediated expression of renal tubular organic anion transporters in the rat (Buist et al., 2002, 2003), which has been shown to be affected by PFOA (Kudo et al., 2002). It is interesting to note that, in a developmental neurotoxicology study with perfluorohexanesulfonate (PFOS), F1 offspring developed an apparent difference in elimination rate of PFOS at some point between weaning and postnatal day 72, an observation that may have been influenced by sexual maturation (Butenhoff et al., 2009). In the latter case, male rats appeared to eliminate PFOS at a rate similar to females at weaning, but, by postnatal day 72, serum elimination in males appeared to be several times higher than in females. Liver organic anion transport processes could also be similarly affected by maturation (Buist and Klaassen, 2004). If the sex-determined expression of organic anion transport processes is a factor in determining PFBS pharmacokinetics kinetics in the rat, this could have resulted in differences between P- and F1-generation males in distribution of PFBS during the dosing periods, and could explain the apparent attenuated response in F1-generaton males. Another possibility is that early-life exposure may have conditioned the F1-generation males to be less responsive. Obviously, further study would be needed to fully understand the basis of the differences in liver weight response observed between the P- and F1-generations.

In the present study, increased incidence and severity of histological changes related to K^+PFBS treatment were noted in kidneys of males and females of both the P- and F1-generations in the 300 and 1000 mg/kg/day dose groups. These consisted of hyperplasia of the medullary/papillary tubular and ductular epithelium. Although there were low incidences of focal necrosis of the papilla observed

EPA-HQ-2016-005679 06/14/2017

in K^+PFBS-treated males and females, these did not follow a clear dose–response pattern. Similar kidney effects were noted in the 90-day study cited above (Lieder et al., 2009), and, as discussed in that article, these changes likely were due to high concentrations of PFBS, a strong surface active compound, passing through the kidney. The recent confirmation of urine as the major excretory route for PFBS lends further support to the hypothesis that high concentrations of PFBS in kidney may have precipitated the histological changes in this organ observed in the present study and the 90-day study (Olsen et al., 2009).

The P-generation paternal no-observable-adverse effect dose level (NOAEL) was 100 mg/kg/day due to hepatocellular hypertrophy and liver weight increase and primarily minimal-to-mild hyperplasia of medullary/papillary tubular and ductular epithelium of the kidney which were observed at doses higher than 100 mg/kg/day. The P-generation maternal NOAEL was 100 mg/kg/day based on primarily minimal to mild hyperplasia of the medullary/papillary tubular and ductal epithelium in the kidney which was observed at doses above 100 mg/kg/day. All of these changes are believed to be due to a response to the high concentrations of surfactant in the kidney due to rapid elimination of high administered doses. No effects on reproductive function (estrous cycling, mating, fertility, and sperm parameters) were noted; therefore, the reproductive NOAEL in the male and female P-generation is >1000 mg/kg/day, the highest dose of the study, and the parental NOAEL for both sexes in the P-generation was 100 mg/kg/day.

The F1-generation paternal NOAEL was 100 mg/kg/day based on primarily minimal hypertrophy of liver cells observed which was observed at doses higher than 100 mg/kg/day. The F1-generation maternal NOAEL was 100 mg/kg/day based on increased minimal to mild hyperplasia of the tubular and ductular epithelium of the medulla and papilla of the kidney which was observed at doses higher than 100 mg/kg/day. The developmental NOAEL in the F1-generation is 300 mg/kg/day based on body weight effects. The slightly delayed sexual maturation likely was the result of reduced body weight at 1000 mg/kg/day (Lewis et al., 2002), because there were no statistically significant differences between groups when body weight on day of preputial separation was co-varied with days to preputial separation. As in the P-generation, no effects on reproductive function were noted; therefore, the reproductive NOAEL in the male and female F1-generation rats was >1000 mg/kg/day.

The F2-generation pups from parents treated with K^+PFBS were followed through lactation. Compared to F2-generation control peers, there were no differences in clinical observations; therefore, the F2-generation pup NOAEL is >1000 mg/kg/day.

This two-generation reproduction study with PFBS has demonstrated that PFBS does not adversely affect reproductive function in Sprague Dawley rats at doses as high as 1000 mg/kg/day or developmental outcomes at doses as high as 300 mg/kg/day. The ammonium salt of PFOA (APFO) and the potassium salt of PFOS (K^+PFOS) have previously been studied in two-generation reproduction studies in rats at the same laboratory (Butenhoff et al., 2004; Luebker et al., 2005a). The results obtained with APFO and K^+PFOS were qualitatively and quantitatively very different than the results obtained for K^+PFBS in this study. Neither K^+PFBS, K^+PFOS, nor APFO caused effects on functional reproduction (estrous cycling, mating, sperm parameters, and fertility). However, unlike K^+PFBS, K^+PFOS and APFO caused a number of postnatal developmental effects at doses that were 1–3 orders of magnitude less than the doses used for the K^+PFBS study.

The K^+PFOS study was conducted at doses of 0.1, 0.4, 1.6, and 3.2 mg/kg/day (Luebker et al., 2005a). The reproductive effects NOAEL for the study was 1.6 mg/kg/day; however, due to high incidences of deaths in the 1.6 and 3.2 mg/kg/day F1-generation neonates, the production of the F2-generation was limited to the 0.1 and 0.4 mg/kg/day F1-generation parental dose groups. In the P-generation 3.2 mg/kg/day group, several reproductive outcome parameters were adversely affected. These included: decreased gestation length; reduced implantation sites; and increased numbers of dams with stillborn pups or with all pups dying during LD 1–4. F1 neonatal toxicity was manifested in the 1.6 mg/kg/day dose group as decreased neonatal survival and reduced body weight gain through lactation. F1 pups also experienced slight developmental delays, including eye opening (0.4 and 1.6 mg/kg/day) and air righting, surface righting and pinna unfolding (1.6 mg/kg/day). Due to the severely-impaired survival of F1 pups in the 1.6 and 3.2 mg/kg/day dose groups, F2 pups were only obtained from the 0.1 and 0.4 mg/kg/day dose groups, and were normal as compared to controls. A cross-fostering study also reported by Luebker et al. (2005a) suggested that effects were largely due to exposure to PFOS *in utero*.

APFO was studied at dose levels of 1, 3, 10, and 30 mg/kg/day (Butenhoff et al., 2004). For the F1-generation pups at the highest dose of 30 mg/kg/day, APFO caused reductions in birth weight and survival, especially immediate post-weaning survival, as well as somewhat delayed sexual maturation in both sexes. The effects on survival and sexual maturation may have been the result of lower body weights. No developmental effects were noted in the F2-generation pups through study termination at the end of lactation. Both P-generation and F1-generation males experienced reduced body weights and increased kidney and liver weights at doses as low as 1 mg/kg/day. The reproductive toxicity NOAEL was >30 mg/kg/day, with a developmental NOAEL for the F1-generation of 10 mg/kg/day, and male parental NOAEL of < 1 mg/kg/day for the F1-generation.

The relative lack of developmental toxicity observed in rats with K^+PFBS, in contrast to K^+PFOS and APFO, may be due to differences in toxicokinetics and biochemical interactions, such as more rapid PFBS elimination and reduced binding affinity to critical receptors when compared to PFOS and PFOA. PFBS is eliminated rapidly in male and female rats (Olsen et al., 2009), with approximately 50% recovered in urine within 24 h and terminal serum elimination half-lives of 3 and 4 days. By contrast, unpublished PFOS elimination data in female rats from our laboratory indicate an elimination half-life of approximately 1 month. PFOA is eliminated rapidly in female rats, with elimination half-life of a few hours; however, male rats have an elimination half-life of several days (Kennedy et al., 2004; Kudo and Kawashima, 2003). In addition, PFBS is much more soluble in water compared to PFOS. Both PFOS and PFOA can transfer to the fetal compartment during gestation and, to a lesser extent, to the pups during lactation (Hinderliter et al., 2005; Luebker et al., 2005b). Although placental and lactational transfer of PFOS and PFOA to rat pups has been studied, similar experiments have not performed with PFBS. If these were to be done, additional insight into the differences between these compounds may become evident.

In conclusion, this two-generation reproduction study with PFBS in rats demonstrated that PFBS has a low potential to produce effects on reproduction or development, especially when compared to the eight-carbon analog, PFOA, and eight-carbon homolog, PFOS.

Conflict of interest statement

Paul H. Lieder, John L. Butenhoff, Shu-Ching Chang, and Daniel Hakes are employees of 3M Company, a manufacture of PFBS. Raymond York was an employee of Charles Rivers Laboratories in which the study was contracted. The funding for the study was provided by 3M Company.

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EPA-HQ-2016-005679 06/14/2017

the microscopic histological evaluation of tissues from the study.

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To: Donohue, Joyce[Donohue.Joyce@epa.gov]
From: Strong, Jamie
Sent: Wed 6/1/2016 4:13:33 PM
Subject: R1 written response on PFOA PFOS
R1 Call Responses 06 01 2016.docx

joyce take a look at the two yellow highlighted comments and send back to me please.

Jamie Strong, Chief Human Health Risk Assessment Branch

Health and Ecological Criteria Division, 4304-T

Office of Science and Technology, Office of Water

United States Environmental Protection Agency

1200 Pennsylvania Avenue, NW

Washington DC 20460

phone: 202.566.0056

fax: 202.566.1140

To: Donohue, Joyce[Donohue.Joyce@epa.gov]
From: Wood, Carol S.
Sent: Fri 5/27/2016 4:20:59 PM
Subject: RE: media clips for PFOA PFOS

Goodness, that created quite a flurry of news articles!

Carol S. Wood, Ph.D., D.A.B.T.

Oak Ridge National Laboratory

P.O. Box 2008

Bldg. 1507; MS-6407

1 Bethel Valley Road

Oak Ridge, TN 37831-6407

ph: 865-574-0596; fax: 865-574-5353

woodcs@ornl.gov

From: Donohue, Joyce [mailto:Donohue.Joyce@epa.gov]
Sent: Friday, May 27, 2016 11:52 AM
To: Wood, Carol S.
Subject: FW: media clips for PFOA PFOS

From: Southerland, Elizabeth
Sent: Friday, May 20, 2016 10:38 AM
To: Donohue, Joyce <Donohue.Joyce@epa.gov>; Strong, Jamie <Strong.Jamie@epa.gov>; Flaherty, Colleen <Flaherty.Colleen@epa.gov>
Cc: Behl, Betsy <Behl.Betsy@epa.gov>
Subject: Fwd: media clips for PFOA PFOS

What a difference you are making! My beloved WV has already switched 3 communities to new

sources (Martinsburg, Parkersburg, Vienna). Also I am so glad you finally released it in response to Senator Schumer...

Sent from my iPhone

Begin forwarded message:

From: "Loop, Travis" <Loop.Travis@epa.gov>
Date: May 20, 2016 at 9:09:22 AM EDT
To: "Beauvais, Joel" <Beauvais.Joel@epa.gov>, "Gilinsky, Ellen" <Gilinsky.Ellen@epa.gov>, "Campbell, Ann" <Campbell.Ann@epa.gov>, "Gude, Karen" <Gude.Karen@epa.gov>, "Dennis, Allison" <Dennis.Allison@epa.gov>, "Fuld, John" <Fuld.John@epa.gov>, "Altieri, Sonia" <Altieri.Sonia@epa.gov>, "Duke, Nia" <Duke.Nia@epa.gov>, "Grevatt, Peter" <Grevatt.Peter@epa.gov>, "Burneson, Eric" <Burneson.Eric@epa.gov>, "Wadlington, Christina" <Wadlington.Christina@epa.gov>, "Southerland, Elizabeth" <Southerland.Elizabeth@epa.gov>, "Behl, Betsy" <Behl.Betsy@epa.gov>, "Harper, Ashley" <harper.ashley@epa.gov>
Subject: media clips for PFOA PFOS

Here is a compilation of media clips on PFOA PFOS. The biggest common error is referring to it as a standard.

Because they asked and had done some digging through data, we provided the list of 63 communities Joel did interviews with USA Today/Gannett and BuzzFeed. I have not seen those stories yet.

EPA sets health advisory level for lifetime exposure to **PFOA**

NEWS10 ABC - 19 hours ago

WASHINGTON (NEWS10) – The U.S. Environmental Protection Agency (**EPA**) announced a lifetime drinking water health advisory of 70 parts per trillion for human exposure to **PFOA**. The **EPA**said the advisory is to provide Americans, including the most ...

EPA sets new level for chemical **PFOA** in drinking water

Albany Times Union - 12 hours ago

The **EPA's** long-awaited action comes as health and environmental officials in New York, Vermont

and New Hampshire are grappling with the discovery of elevated levels of **PFOA** in public water systems and private wells. The contamination has been linked ...

EPA suggests tighter limits for **PFOA**, PFOS in drinking water

PhillyVoice.com - 3 hours ago

The agency said the new limits were prompted by recent scientific studies linking **PFOA** and PFOS to testicular and kidney cancers, as well as birth defects and liver damage. "**EPA** will continue sharing the latest science and information so that state and **...**

With New **EPA** Advisory, Dozens of Communities Suddenly Have Dangerous Drinking Water

The Intercept - 17 hours ago

The **EPA** announced new drinking water health advisory levels today for the industrial chemicals**PFOA** and PFOS. The new levels — .07 parts per billion (ppb) for both chemicals — are significantly lower than standards the agency issued in 2009, which **...**

EPA issues health advisory for long-term **PFOA** exposure

The Union Leader - 16 hours ago

“I am pleased that the **EPA** has answered my repeated requests and released the new health advisory standards for **PFOA** and (perfluorooctyl sulfonate) levels,” U.S. Sen. Kelly Ayotte said in a statement on Thursday. “New Hampshire residents deserve to ...

EPA issues **PFOA** health advisory

Nashua Telegraph - 8 hours ago

"The **EPA** has set the health advisory for both perfluorooctanoic acid (**PFOA**) and perfluorooctane sulfonate (PFOS) at 70 parts per trillion and has recommended that when these two chemicals co-occur in the drinking water source, the sum of the ...

EPA Sets New Guidelines For **PFOA**, PFOS; Superfund Declared For Petersburgh

WAMC - 15 hours ago

In 2009, **EPA** set a short-term exposure guideline of 100 ppt for **PFOA** and PFOS. Perhaps due to a lack of long-term exposure guidlelines, state governments have not had consistent recommended levels. Vermont has acted under a 20 ppt threshold while ...

In Significant Reduction, **EPA** Sets Health Advisory For **PFOA** At 70 Parts Per Trillion

Vermont Public Radio - 18 hours ago

The **EPA** announced last year that it was looking at its health advisory level of 400 parts per trillion for **PFOA**, which was established in 2009. After the chemical was detected in North Bennington, the state of Vermont set a more conservative level **...**

EPA Releases New Health Guidelines for **PFOA**, PFOS Found in Drinking Water

TWC News - 18 hours ago

The **EPA** has released new health guidelines for **PFOA** and PFOS found in drinking water. **PFOA**was the chemical found in the water supply in Hoosick Falls and Petersburgh. The new health advisory levels have been set to 70 parts per trillion. When first ...

EPA Announces Lifetime **PFOA** and PFOS Advisory Level

New Hampshire Public Radio - 13 hours ago

The **EPA** has announced a lifetime health advisory level for two chemicals that have contaminated water in Southern NH and at the former Pease Air Force Base. **PFOA** and PFOS are slippery, stable chemicals used since the 1940s in products like nonstick ...

EPA issues new federal standards for **PFOA** exposure

WCAX - 14 hours ago

There are new federal standards for exposure to **PFOA** and it's three times higher than the level Vermont is using.**PFOA** has been found in drinking water supplies in Bennington and Pownal. Officials believe the chemical came from old Teflon factories.

EPA Releases New 'Lifetime' **PFOA** Exposure Level Data

Patch.com - 14 hours ago

CONCORD, NH - The U.S. Environmental Protection Agency has issued new “lifetime drinking water health advisory levels” for both perfluorooctanoic acid (**PFOA**) and perfluorooctane sulfonate (PFOS) at 70 parts per trillion (ppt), according to Jim Martin **...**

EPA releases drinking water health advisories for **PFOA** and PFOS

WaterWorld - 18 hours ago

WASHINGTON, May 19, 2016 -- Based on the latest science on two chemical contaminants called**PFOA** and PFOS, **EPA** has released drinking water health advisories to provide the most up-to-date information on the health risks of these chemicals.

EPA Releases New **PFOA**, PFOS Guidelines

WAMC - 19 hours ago

“I am pleased that the **EPA** has heeded my call to release updated drinking water health advisories for the highly-toxic chemicals **PFOA** and PFOS," New York U.S. Senator Charles Schumer said in a statement. "Communities like Hoosick Falls, Petersburgh ...

EPA declares official standard for **PFOA**, PFOS contamination for lifetime exposure

WRGB - 19 hours ago

'EPA's assessment indicates that drinking water with individual or combined concentrations of**PFOA** and PFOS below 70 parts per trillion is not expected to result in adverse health effects over a lifetime of exposure. These levels reflect a margin of **...**

3 Arizona water systems afoul of new **EPA** water guidelines

azcentral.com - 9 hours ago

The agency's latest advisory drops the recommended limit of **PFOA** and PFOS in drinking water from 400 parts per trillion to 70 parts per trillion. The **EPA** cannot enforce that limit, as it is just a recommendation intended to advise water systems on how **...**

EPA Issues New Health Advisories for Chemical Found Near Some Plastics Plants

Wall Street Journal - 17 hours ago

On Thursday, the **EPA** declared that drinking water with **PFOA** concentrations of 70 parts per trillion or more were not healthy for human consumption. In 2009, the agency issued a provisional guideline of 400 parts per trillion. The **EPA** has been reviewing ...

EPA sets new standards for contaminants

Eagle-Tribune - 1 hour ago

The state has been waiting for some time to hear from the federal **EPA** about its recommendations about acceptable levels of **PFOA** in drinking water, said DES spokesman Jim Martin on Thursday. The new data recommends no more than 70 parts per trillion, ...

New **EPA** Guidelines on PFCs Welcome – but Still Fall Short

Environmental Working Group (blog) - 15 hours ago

Today the Environmental Protection Agency issued a long-awaited drinking water health advisory for the perfluorinated chemicals **PFOA** and PFOS. But **EPA's** advisory falls far short of what's needed to fully protect public health, and it is not a legally ...

Air Force applies new **EPA** guidance

Air Force Link - 15 hours ago

The Air Force has been following a 2009 **EPA** issued short-term provisional health advisory level for **PFOA** at 400 parts per trillion and 200 ppt for PFOS. On May 19, the **EPA** released new lifetime exposure health advisories for PFOS at 70 ppt and for **PFOA ...**

Martinsburg shuts down water plant after **EPA** advisory

Herald-Mail Media - 11 hours ago

The **EPA** issued the stricter guidelines for the chemicals after years of pressure from public-health experts and advocacy groups. The agency said the new limits were prompted by recent scientific studies linking **PFOA** and PFOS to testicular and kidney ...

EPA releases new safety advisories for chemicals

Burlington County Times - 14 hours ago

Up until Thursday, the military had been gauging its response using short-term, provisional health advisories for the chemicals set by the **EPA** in 2009: 0.4 parts per billion for **PFOA** in drinking water, and 0.2 ppb for PFOS. Thursday's announcement **...**

EPA sets new level for contaminant found in drinking water

WMUR Manchester - 19 hours ago

H. "I will continue to monitor the increasing instances of potentially dangerous **PFOA** and PFOS levels in water resources in several communities across our state and ensure that the **EPA** is working with local officials and communities to take every **...**

'Do not drink' ordered for Vienna water after **EPA** issues C8 guidance

Charleston Gazette-Mail (subscription) - 15 hours ago

“Although it's a long overdue step in the right direction, the guideline is still too high as it allows unacceptable accumulation of **PFOA** to build up in the blood of people drinking it,” said Robert Bilott, a longtime lawyer for residents who had their **...**

EPA Sets New Health Advisory for Toxic Chemicals in Drinking Water

NBC Bay Area - 15 hours ago

The agency set the advisory at 70 parts per trillion of human exposure to two perfluoronated compounds called PFOS and **PFOA**. That means people should not drink water with detections of the chemicals above that level. The new level is nearly six times ...

Amherst water tested for **PFOA**

Cabinet.com - May 19, 2016

After the presence of **PFOA** was detected in private wells in those towns, the DES declared a provisional safe exposure limit of 100 parts per trillion, which is the standard used by Maine. The **EPA** recommends avoiding short-term exposure to more than 400 ...

Federal **EPA** warns of high chemical levels in Martinsburg, Parkersburg and Vienna water sources

West Virginia MetroNews - 14 hours ago

WASHINGTON, D.C. — The federal **EPA** announced Thursday that water sources in Martinsburg, Parkersburg and Vienna contain the chemicals **PFOA** and PFOS at levels higher than previously recommended for public water systems. A ' do not drink' ...

Vermont Department of Health setting **PFOA** advisory level at 20 parts per trillion

NEWS10 ABC - 17 hours ago

Now that **EPA** has set a new advisory level of 70 parts per trillion for **PFOA** and PFOS combined, the department says it will evaluate the hundreds of pages of supporting documents just released by**EPA** to assess how this differs from their current **...**

EPA Issues Tighter Limits for Chemical In Water

WILX-TV - 13 hours ago

The cancer-causing chemical **PFOA** has been found in the tap water of dozens of factory towns near industrial sites where it was manufactured. DuPont, 3M and other U.S. chemical companies voluntarily phased out the use of**PFOA** in recent years.

EPA issues advisory on chemicals found in West Morgan-East Lawrence drinking water

The Decatur Daily - 17 hours ago

The **EPA** advisory says some studies indicate long-term exposure to higher levels of **PFOA** and PFOS may result in adverse health effects, including developmental effects to fetuses during pregnancy or to breast-fed infants, cancer, liver damage, immune ...

Do not drink advisory issued for citizens of Vienna

WSAZ-TV - 14 hours ago

WOOD COUNTY, W.Va. (WSAZ) -- The Bureau for Public health has reviewed new information regarding **PFOA** and PFOS that the U.S. **EPA** released Thursday, and have decided to issue a Do Not Drink advisory for citizens of Vienna until additional testing ...

Amherst addresses **PFOA** contamination

WMUR Manchester - May 18, 2016

In Amherst, state environmental officials recently identified the former site of Textiles Coated International on Route 101-A as a likely source of **PFOA** contamination in nearby wells. “Why did this happen, and how is it allowed that we have industry **...**

West Morgan-East Lawrence water customers to receive health warning

The Decatur Daily - 7 hours ago

After the **EPA** on Thursday issued a health advisory, the West Morgan-East Lawrence Water and Sewer Authority said it will notify its 25,000 direct and indirect customers that their drinking water contains excessive amounts of two chemicals.

Newburgh water pollutant now more than twice level of new **EPA** guidelines

Times Herald-Record - 18 hours ago

Sean Maloney had been urging the **EPA** to expedite release of its updated guidelines. “I am pleased that the **EPA**has heeded my call to release updated drinking water health advisories for the highly-toxic chemicals **PFOA** and PFOS,” Schumer said in a ...

La Crosse releases drinking water advisory

WXOW.com - 13 hours ago

The contaminants are called Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoic Acid (**PFOA**). The Environmental Protection Agency or **EPA** now wants municipal water supplies across the county to be tested for unregulated contaminants to be proactive ...

West Virginia communities altering their water sources after new

WHAG - 8 hours ago

... specifically in Martinsburg, Parkersburg and the town of Vienna, are altering their water sources after new **EPA**thresholds were released on Thursday. This comes after officials found raised levels of industrial chemicals of**PFOA** and PFOS in the water.

Doylestown Township shuts down contaminated well

The Intelligencer - 14 hours ago

It (**PFOA**) had not been on any chemical list that governs our permit," the director said. **PFOA** was first found in the Cross Keys well in 2014, the first year the **EPA** tested for the chemical, John said. "Had the **EPA** not looked for it, we wouldn't have **...**

New Water Research Foundation Study Addresses Most Effective Methods for Removing PFOS/**PFOA** from Water and **...**

Broadway World - 17 hours ago

The **EPA** issued two public health advisories today for perfluorooctanoic acid (**PFOA**) and perfluorooctane sulfonate (PFOS), each at 70 parts per trillion. **EPA's** assessment indicates that drinking water with individual or combined concentrations of **PFOA ...**

Newest clips below: PoliticoPro; (Manchester) Union Leader; (all previously clips forwarded below that).

Politico Pro Energy

EPA SETS PFOA LIMIT: EPA today set its long-awaited PFOA limit at 70 parts per trillion. Scott Waldman reports the limit "applies to both long- and short-term exposure and could have a significant effect on water testing and lawsuits against industry. It is also lower than the current advisory of 100 parts per trillion that the EPA issued in many upstate communities facing exposure to the toxic chemical, which is used in the manufacturing of nonstick products." Communities from New England to West Virginia have been shown to have high levels of the chemical in their drinking water. The New York Times magazine did a deep dive into the chemical back in January.

Union Leader (New Hampshire)

http://www.unionleader.com/environment/epa-issues-health-advisory-for-long-term-pfoa-exposure--20160519

EPA issues health advisory for long-term PFOA exposure

By Kimberly Houghton on 5/19/16 at 4:51pm

Federal officials on Thursday issued a lifetime health advisory for the chemical perfluorooctanoic acid, which is being detected in some water sources in southern New Hampshire.

The U.S. Environmental Protection Agency has now identified 70 parts per trillion of PFOA as a new lifetime drinking water health advisory. Previously, the EPA had a 400 ppt PFOA advisory for short-term exposure.

The new advisory level is less than some levels of PFOA detected in the municipal public water supply wells operated by Merrimack Village District and provided to about 25,000 customers in the area.

At least one Village District well was found to have a PFOA level of 90 ppt, though that well is currently off-line. Ron Miner, superintendent of the district, was not immediately available for comment Thursday. Town Manager Eileen Cabanel directed questions to MVD.

Recently, the New Hampshire Department of Environmental Services began providing bottled water to about 200 properties throughout Litchfield, Merrimack and Amherst that have private wells detecting PFOA at 100 ppt or more. It will now begin offering bottled water to homeowners with PFOA at 70 ppt in light of the newest recommendations from EPA, according to a release.

The federal advisory level of 70 ppt was derived from a developmental toxicity study in mice, according to the EPA's advisory released on Thursday.

"This lifetime health advisory is based on the latest health effects information for noncancer and cancer effects for PFOA - the lifetime health advisory is therefore protective of the population at large," states the advisory.

Tests on monkeys, rats and mice discovered developmental effects, liver toxicity, kidney toxicity, immune effects and cancers such as liver cancer, testicular cancer and pancreatic cancer as a result of long-term PFOA exposure, according to the advisory.

"Water resources contaminated by PFOA have been associated with releases from manufacturing sites, industrial sites, fire/crash training areas and industrial or municipal waste sites where products are disposed of or applied," it states.

Saint-Gobain Performance Plastics has been identified as the likely source of water contamination in Merrimack and surrounding areas, according to officials with DES. PFOA has also been discovered at the old Merrimack landfill and the former Textiles Coated International plant in Amherst.

To date, 73 private wells within Merrimack, Litchfield and Amherst have detected elevated levels of PFOA above 100 ppt.

"I am pleased that the EPA has answered my repeated requests and released the new health advisory standards for PFOA and (perfluorooctyl sulfonate) levels," U.S. Sen. Kelly Ayotte said in a statement on Thursday. "New Hampshire residents deserve to know whether or not their water resources are safe.

"I will continue to monitor the increasing instances of potentially dangerous PFOA and PFOS levels in water resources in several communities across our state and ensure that the EPA is working with local officials and communities to take every action possible for the safety of all Granite State residents," she said.

4:23 PM

Associated Press

http://hosted.ap.org/dynamic/stories/U/US_DRINKING_WATER_CHEMICALS?SITE=AP&SECTION

EPA ISSUES TIGHTER LIMITS FOR INDUSTRIAL CHEMICAL IN WATER

By Michael Biesecker on 5/19/16 at 4:12pm

Federal regulators are tightening limits for human exposure to an industrial chemical used for decades in such consumer products as non-stick pans, stain-resistant carpets and microwave popcorn bags.

The cancer-causing chemical PFOA has been found in the tap water of dozens of factory towns near industrial sites where it was manufactured. DuPont, 3M and other U.S. chemical companies voluntarily phased out the use of PFOA in recent years.

Also at issue is the related chemical PFOS used in firefighting foam.

The Environmental Protection Agency issued the stricter standards for the chemicals on Thursday, following years of public pressure by advocacy groups. The agency said the new limits we prompted by recent scientific studies linking PFOA and PFOS to testicular and kidney cancers, as well as birth defects and liver damage.

4:12 PM

Wall Street Journal

http://www.wsj.com/articles/epa-issues-new-health-advisories-for-chemical-from-some-plastics-plants-1463687484

EPA Issues New Health Advisories for Chemical Found Near Some Plastics Plants

By Cameron McWhirter on 5/19/16 at 3:51pm

The Environmental Protection Agency announced new health advisories Thursday for perfluorooctanoic acid, a potentially toxic chemical, calling for water utilities across the nation to adhere to stricter guidelines than the agency had previously recommended.

In recent months, state investigators in New York, Vermont and New Hampshire have found high levels of the chemical, known as PFOA, in drinking-water wells and groundwater near former and current chemical plants, alarming residents and raising concern about PFOA contamination in other parts of the country.

The Environmental Working Group, a Washington-based advocacy group that assesses chemicals in consumer products and the environment, sent a letter in late April to EPA Administrator Gina McCarthy urging the agency to set an enforceable drinking-water standard for the chemical and to force former manufacturers to disclose all sites in the U.S. where they used, made or dumped PFOA.

On Thursday, the EPA declared that drinking water with PFOA concentrations of 70 parts per trillion or more were not healthy for human consumption. In 2009, the agency issued a provisional guideline of 400 parts per trillion. The EPA has been reviewing PFOA guidelines for months, and state environmental agencies have been setting their own guidelines. New York and New Hampshire both set limits of 100 parts per trillion, while Vermont had a stricter standard of 20 parts per trillion.

PFOA can be harmful to animals in high doses, causing tumors in the liver and other parts of the body, according to several scientific studies. A multiyear medical study in the 2000s of 70,000 people near a plant in West Virginia that made PFOA found “some suggestions” of “probable links” between high exposure to the chemical and illnesses, including cancer.

The agency Thursday also set a 70 parts per trillion guideline for perfluorooctane sulfonate, or PFOS, a chemical similar to PFOA that was used to make products stain resistant. PFOS has been found in studies to be harmful to the immune systems of animals.

For both chemicals, the guidelines are lifetime-exposure advisories.

The provisional health advisory for PFOS had been 200 parts per trillion. The EPA stated that if both chemicals were found in drinking water, the 70 parts per trillion limit applies for the two chemicals combined. The advisories are not regulations that can be enforced by the EPA, but health guidelines issued for water utilities, and state environmental and health agencies to consider.

"If these chemicals are found in drinking-water systems above these levels, system operators should quickly conduct additional sampling to assess the level, scope and source of contamination," according to an EPA statement. "They should also promptly notify consumers and consult with their state drinking-water agency to discuss appropriate next steps. Public notification is especially important for pregnant or nursing women because of the impact these chemicals can have on the development of fetuses and breast-fed or formula-fed infants."

Some state officials were quick to applaud the new guidelines. The New Hampshire Department of Environmental Services spokesman Jim Martin said his department would work to provide bottled water to any communities where drinking-water tests exceeded the 70 parts per trillion limit.

The Environmental Working Group said the new levels aren't strict enough and urged the agency to impose a legally enforceable limit.

Factories for decades used PFOA as a plastic coating and to make consumer products such as Teflon nonstick pans, waterproof jackets and pizza boxes. Former large manufacturers or users of PFOA, including 3M Co. and DuPont Co., agreed in 2006 to phase out PFOA

production and use by December 2015. Public concern over PFOA has spread through upstate New York and New England since August 2014, when a resident of Hoosick Falls, N.Y., near the Vermont border, tested his drinking water and found high levels of the acid. The man was concerned because his father, a former employee of the town's plastics plant that used PFOA, died of cancer.

Saint-Gobain Performance Plastics, a subsidiary of Saint-Gobain SA of France, operates plants in Merrimack, N.H., and Hoosick Falls, N.Y., and used to operate a plant in North Bennington, Vt. Since last year, state investigators have found PFOA contamination near all three sites, and they cited the plants as potential sources.

A company spokeswoman said in a statement that, according to the EPA, the new advisory is "not a legally enforceable federal standard and is subject to change as new information becomes available." The company, however, believes the new guidelines will help "state and local governments to make consistent decisions concerning the levels of PFOA in drinking water," she said.

Shawn Dalton, 65, a retired communications manager who lives not far from the Merrimack plant, thought until Thursday that water from his well, which tested 73 parts per trillion for PFOA, was safe. Now he knows it is three parts above the new guideline. He was glad for the new guidelines, however, because it means he now can hold government officials and companies accountable, he said.

"At least now the federal government has put a stake in the ground," he said.

News 10 (ABC)

http://news10.com/2016/05/19/vermont-department-of-health-setting-pfoa-advisory-level-at-20-parts-per-trillion/

Vermont Department of Health setting PFOA advisory level at 20 parts per trillion

By Ali Stewart on 5/19/16 at 3:24pm

The Vermont Health Department says they evaluated all of the scientific information available at the time when setting Vermont's advisory level of 20 parts per trillion for PFOA in drinking water.

Now that EPA has set a new advisory level of 70 parts per trillion for PFOA and PFOS combined, the department says it will evaluate the hundreds of pages of supporting documents just released by EPA to assess how this differs from their current advisory level, and consider if any changes are warranted.

The department says they remain committed to protecting the health of Vermonters from these harmful chemicals.

3:29pm

Decatur Daily

http://www.decaturdaily.com/news/lawrence_county/epa-issues-advisory-on-chemicals-found-in-west-morgan-east/article_2d66630b-b85c-5f14-9c45-e11f422b8457.html

EPA issues advisory on chemicals found in West Morgan-East Lawrence drinking water

By Eric Fleischauer on 5/19/16 at 3:22pm

The Environmental Protection Agency today issued a health advisory for two chemicals that are found in the drinking water of West Morgan-East Lawrence Water Authority.

The lifetime advisory warns of adverse health effects for people exposed to drinking water

with more than 70 parts per trillion of two chemicals, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). According to court documents, West Morgan-East Lawrence drinking water exceeds the EPA advisory limit for both chemicals.

The EPA advisory says some studies indicate long-term exposure to higher levels of PFOA and PFOS may result in adverse health effects, including developmental effects to fetuses during pregnancy or to breast-fed infants, cancer, liver damage, immune effects, thyroid effects and cholesterol changes.

The authority sells water to water departments in Vinemont, Falkville, Trinity, Town Creek and the West Lawrence Water Cooperative. About 25,000 people use water supplied by the authority, it has said in court documents.

West Morgan-East Lawrence in October filed a federal lawsuit alleging 3M Co. and its subsidiary, Dyneon LLC, and Daikin America Inc. released the chemicals into the Tennessee River. The lawsuit alleges the chemical entered the authorities water intake downstream of the companies.

Virtual-Strategy Magazine (Reprint from Business Wire)

http://www.virtual-strategy.com/2016/05/19/new-water-research-foundation-study-addresses-most-effective-methods-removing-pfospfoa-wa#axzz498AKvTCX

New Water Research Foundation Study Addresses Most Effective Methods for Removing PFOS/PFOA from Water and Wastewater

(See Business Wire story below)

3:02pm

Times Hearld-Record

http://www.recordonline.com/news/20160519/newburgh-water-pollutant-now-more-than-twice-level-of-new-epa-guidelines

Newburgh water pollutant now more than twice level of new EPA guidelines

By Leonard Sparks on 5/19/16 at 2:49pm

New federal health guidelines issued Thursday for the chemical that has shut down Newburgh's primary water supply are less than half the levels recorded in the city.

The Environmental Protection Agency is now urging action if levels of perfluorooctanoic acid or perfluorooctane sulfonate are above 70 parts per trillion, a significantly stricter standard than previously issued advisory guidelines of 100 parts per trillion for PFOA and 200 parts per trillion for PFOS.

Since its water was first tested in December 2013, Newburgh's Washington Lake has had levels of PFOS ranging from 140 to 170 parts per trillion.

While below the initial EPA guidelines, those levels spurred the closure of the lake on May 2, and Newburgh has been drawing water from its backup supply at Brown's Pond.

U.S. Sen. Charles Schumer and U.S. Rep. Sean Maloney had been urging the EPA to expedite release of its updated guidelines.

"I am pleased that the EPA has heeded my call to release updated drinking water health advisories for the highly-toxic chemicals PFOA and PFOS," Schumer said in a statement. "Communities like Hoosick Falls, Petersburgh and Newburgh understand all too well the uncertainty and anxiety that contamination from these chemicals can cause."

The Intercept

https://theintercept.com/2016/05/19/with-new-pfoa-drinking-water-advisory-dozens-of-communities-suddenly-have-dangerous-water/

WITH NEW DRINKING WATER ADVISORY, DOZENS OF COMMUNITIES SUDDENLY HAVE DANGEROUS WATER

By Sharon Lerner on 5/19/16 at 2:36pm

The EPA announced new drinking water health advisory levels today for the industrial chemicals PFOA and PFOS. The new levels — .07 parts per billion (ppb) for both chemicals — are significantly lower than standards the agency issued in 2009, which were .4 ppb for PFOA and .2 ppb for PFOS. In areas where both PFOA and PFOS are present, the advisory suggests a maximum combined level of .07 ppb. While the old levels were calculated based on the assumption that people were drinking the contaminants only for weeks or months, the new standards assume lifetime exposure and reflect more recent research.

The new federal standards may unify what has been an inconsistent official response to the presence of these perfluorinated chemicals, or PFCs, in drinking water. They will also instantaneously create official water contamination crises in dozens of cities and towns across the country.

According to the EPA's most recent data on unregulated drinking water contaminants, released in January, 14 drinking water systems around the country reported levels of PFOA that exceed the new federal threshold, while 40 reported PFOS above the new cutoff. In all, water systems in 18 states, as well as in Guam, are contaminated.

Some of these water systems have already begun to quietly address the problem. In Suffolk County, New York, where public drinking water wells show PFOS levels of .33 and .53 ppb, the contaminated water "has either been blended with other wells to reduce the level of the compound to non-detection or their use has been limited to the greatest extent possible," according to Kevin Durk, director of water quality and laboratory services for the Suffolk County Water Authority. Though he does not know the level of PFOS in the water that comes out of local taps, Durk wrote in an email that "it is a virtual certainty that levels of any detected chemical would have been reduced."

Similarly, the Security Water and Sanitation District in Colorado Springs has been struggling to clean up its contaminated water since 142 tests detected PFCs. The district has shut down seven out of 26 wells and is blending water to lower levels, according to Roy Heald, the district's general manager.

But other water company operators have yet to lower their PFC levels. Steve Anderson, owner of the Oatman Water Company in Scottsdale, Arizona, where PFOS measured .2 and .23 in the most recent EPA testing, learned that the chemical was in his water only recently, after he received a call from the Arizona Department of Environmental Quality. Anderson, who suspects the PFOS originated from firefighting foam used by the nearby Oatman Fire Department, said he is "trying to come up with a solution."

Until today, there was a wide range of official opinion on the level of contamination that presented a health danger. The military, which is in the throes of a massive cleanup of 664 contaminated fire- and crash-training sites, has been using the EPA's older standards for PFOA and PFOS to guide its efforts and help determine who receives clean drinking water and remediation of contaminated private wells. (The Department of Defense did not responded to inquiries about how the new advisory levels would alter its cleanup plan.)

Others have set more stringent standards. On January 28, the EPA advised residents of Hoosick Falls, New York, not to use water with PFOA levels above .1 ppb. And a panel of scientists who spent years researching some 70,000 people whose water contained PFOA levels of at least .05 ppb, found probable links between that level of exposure and testicular cancer, kidney cancer, thyroid disease, preeclampsia, ulcerative colitis and high cholesterol. In 2010, New Jersey's Drinking Water Quality Institute calculated a safety limit of .04 for PFOA. Vermont currently has the lowest state drinking water limit for PFOA, .02 ppb.

The levels released today are based on numerous studies connecting the chemicals with health effects. For PFOS, the report notes, studies of lab animals exposed to the chemical reported "developmental effects (decreased body weight, survival, and increased serum glucose levels and insulin resistance in adult offspring), reproductive (mating behavior), liver toxicity (liver weight co-occurring with decreased cholesterol, hepatic steatosis), developmental neurotoxicity (altered spatial learning and memory), immune effects, and cancer (thyroid and liver)."

The report also acknowledged research on human populations that has found associations between PFOS and immune suppression, thyroid disease, high cholesterol, and reduced fertility. It also acknowledged a possible connection between PFOS and bladder, colon, and prostate cancer.

For PFOA, the research included studies on monkeys, rats, and mice showing "developmental effects (survival, body weight changes, reduced ossification, delays in eye opening, altered puberty, and retarded mammary gland development), liver toxicity (hypertrophy, necrosis, and effects on the metabolism and deposition of dietary lipids), kidney toxicity (weight), immune effects, and cancer (liver, testicular, and pancreatic)."

The new health advisory for PFOA was also based on human studies, which showed "associations between PFOA exposure and high cholesterol, increased liver enzymes, decreased vaccination response, thyroid disorders, pregnancy-induced hypertension and preeclampsia, and cancer (testicular and kidney)." The EPA report noted that in humans "the developing fetus and newborn is particularly sensitive to PFOA-induced toxicity."

"Taken together," the report notes, "the weight of evidence for human studies supports the conclusion that PFOA exposure is a human health hazard." The exact phrasing was used in the PFOS report as well.

While calling the new level "a very long-overdue step in the right direction," Robert Bilott, an attorney overseeing a class-action suit over PFOA contamination near a DuPont plant in West Virginia, cautioned that "the new guideline is still too high, as exposures at even the new guideline level would allow PFOA to continue to build up to ever-increasing, unacceptable levels in human blood."

Bilott also noted that the new levels are informal guidelines, as opposed to enforceable regulatory limits. "If it was enforceable," he said, "the EPA could issue unilateral orders requiring the responsible party to clean it up."

WaterWorld

http://www.waterworld.com/articles/2016/05/epa-releases-drinking-water-health-advisories-for-pfoa-and-pfos.html

EPA RELEASES DRINKING WATER HEALTH ADVISORIES FOR PFOA AND PFOS

By (No author) on 5/19/16 at 2:42pm

Based on the latest science on two chemical contaminants called PFOA and PFOS, EPA has released drinking water health advisories to provide the most up-to-date information on the health risks of these chemicals. These advisories will help local water systems and state, tribal and local officials take the appropriate steps to address PFOA and PFOS if needed.

EPA's assessment indicates that drinking water with individual or combined concentrations of PFOA and PFOS below 70 parts per trillion is not expected to result in adverse health effects over a lifetime of exposure. These levels reflect a margin of protection, including for the most sensitive populations.

For many years, PFOA and PFOS were widely used in carpets, clothing, furniture fabrics, food packaging, and other materials to make them more resistant to water, grease, and stains. PFOA and PFOS were also used for firefighting at airfields and in a number of industrial processes. Between 2000 and 2002, PFOS was voluntarily phased out of production in the U.S. by its primary manufacturer. And EPA asked eight major companies to commit to eliminate their production and use of PFOA by the end of 2015 and they have indicated that they have met their commitments. While there are some limited ongoing uses of these chemicals, in recent years, blood testing data has shown that exposures are declining across the country.

For most people, their source of exposure to PFOA and PFOS has come through food and consumer products. But drinking water can be an additional source of exposure in the small percentage of communities where these chemicals have contaminated water supplies. This is typically a localized issue associated with a specific facility -- for example, in communities where a manufacturing plant or airfield made or used these chemicals.

If these chemicals are found in drinking water systems above these levels, system operators should quickly conduct additional sampling to assess the level, scope, and source of contamination. They should also promptly notify consumers and consult with their state drinking water agency to discuss appropriate next steps. Public notification is especially important for pregnant or nursing women because of the impact these chemicals can have on the development of fetuses and breastfed or formula-fed infants. There are a number of options available to water systems to lower concentrations of these chemicals in the drinking water supply.

EPA will continue sharing the latest science and information so that state and local officials can make informed decisions and take actions to protect public health. This is an important part of our broader effort to support states and public water systems as we work together to strengthen the safety of America's drinking water.

Business Wire

http://www.businesswire.com/news/home/20160519006484/en/Water-Research-Foundation-Study-Addresses-Effective-Methods

New Water Research Foundation Study Addresses Most Effective Methods for Removing PFOS/PFOA from Water and Wastewater

By (No author) on 5/19/16 at 2:44pm

The Water Research Foundation (WRF), a leading sponsor of research supporting the water community, has released findings of a study addressing effective methods for removing poly- and perfluoroalkyl substances (PFASs) from water and wastewater. The research report, Treatment Mitigation Strategies for Poly- and Perfluorinated Chemicals (WRF project #4322), contains results of an in-depth treatment study conducted on waters from 13 water and wastewater treatment plants in the United States. Additionally, WRF will be hosting a Webcast on June 2 addressing the project's results and has posted a State of the Science document on PFASs.

The research from project #4322 demonstrated that conventional treatment at wastewater treatment plants and most drinking water treatment plants is ineffective at removing PFASs.

Activated carbon and anion exchange can remove many PFASs but are less effective at removing shorter chain PFASs. The most effective treatment technologies are nanofiltration and reverse osmosis, which work even for the smallest PFASs studied.

The EPA issued two public health advisories today for perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), each at 70 parts per trillion. EPA's assessment indicates that drinking water with individual or combined concentrations of PFOA and PFOS below 70 parts per trillion is not expected to result in adverse health effects over a lifetime of exposure.

"The research results from project #4322 will help the water community understand the best options for removing PFASs from water and wastewater, said Rob Renner, CEO of the Water Research Foundation. "This knowledge is especially important as the EPA continues the process of regulating these chemicals in water."

Poly- and perfluoroalkyl substances (PFASs) are a group of manmade chemicals with past and current uses in industrial processes and consumer products. PFASs are also commonly referred to as perfluorinated chemicals or PFCs. The most notable PFASs are PFOA and PFOS, but there are many others. PFASs are used in firefighting foams, coating for food packaging, ScotchGardTM and TeflonTM, among other products.

Exposure to PFASs can occur through use of products or consumption of food or water containing PFASs. PFASs do not break down easily and therefore persist in the environment. They are also soluble in water and can enter source waters through industrial releases, discharges from wastewater treatment plants, storm water runoff, release of firefighting foams, and land application of contaminated biosolids.

PFASs are a concern because they have been shown to have health effects in animal studies. Data from some human studies suggest that PFASs also affect human health. Additional research is ongoing in order to gain a better understanding of human health effects.

2:45pm

WRGB Albany (CBS)

http://cbs6albany.com/news/hoosick-falls-crisis/epa-declares-official-standard-for-pfoa-pfoh-contamination

EPA declares official standard for PFOA, PFOS contamination for lifetime exposure

By WRGB Staff on 5/19/16 at 2:19pm

The EPA has released official long- term exposure guidelines for PFOA, PFOS contamination.

Released documents state that concentrations of PFOA and PFOS below 70 parts per trillion is not expected to result in adverse health effects over a lifetime of exposure.

Vermont lists safe exposure levels at 20 parts per trillion, including babies and children.

Regional EPA originally set short term exposure as 100 ppt in Hoosick Falls.

'EPA's assessment indicates that drinking water with individual or combined concentrations of PFOA and PFOS below 70 parts per trillion is not expected to result in adverse health effects over a lifetime of exposure. These levels reflect a margin of protection, including for the most sensitive populations. " , says the EPA in a released document.

2:27 PM

Vermont Public Radio

http://digital.vpr.net/post/significant-reduction-epa-sets-health-advisory-pfoa-70-parts-trillion#stream/0

In Significant Reduction, EPA Sets Health Advisory For PFOA At 70 Parts Per Trillion

By Howard Weiss-Tisman on 5/19/16 at 2:11pm

The Environmental Protection Agency on Thursday issued an updated health advisory for the suspected carcinogen PFOA.

The EPA says water that contains PFOA in levels at 70 parts per trillion should be considered unsafe to drink.

The EPA previously set its level at 400 ppt. The new, lifetime health advisory replaces that standard.

PFOA, a chemical that was used in a variety of manufacturing applications, has been found in water in North Bennington, Bennington and Pownal.

The contaminant has also been detected in New Hampshire and New York, and in other states around the country.

Vermont set its level at 20 parts per trillion.

(Reprint of Bucks County Courier Times story)

http://www.theintell.com/news/local/epa-releases-new-safety-advisories-for-chemicals/article_a404ad5d-a246-5ec2-9d1b-a9af8b6e70d5.html

EPA releases new safety advisories for chemicals

2:20 PM

Time Warner Cable News - Capital Region

http://www.twcnews.com/nys/capital-region/news/2016/05/19/epa-releases-new-pfoa-and-pfos-health-guidelines.html

EPA Releases New Health Guidelines for PFOA, PFOS Found in Drinking Water

By TWC News Web Staff on 5/19/16 at 1:46pm

The EPA has released new health guidelines for PFOA and PFOS found in drinking water.

PFOA was the chemical found in the water supply in Hoosick Falls and Petersburgh.

The new health advisory levels have been set to 70 parts per trillion.

When first tested at the St. Gobain facility in Hoosick Falls, the EPA measured the levels at 18,000 parts per trillion.

A filtration system has since been installed, eliminating traces of the chemical, making the water safe to use again in Hoosick Falls.

In Petersburgh, multiple homes have tested above the new acceptable levels.

Taconic Plastics is believed to be the source of contamination. Residents in the town have grown impatient with leaders as they await to learn when they can get blood tested.

The town even ran out of bottled water to give to residents over the weekend. That supply has since been replenished.

Also, PFOS levels in Washington Lake and Silver Stream are in the range of 145 to 150 parts per trillion.

Washington Lake used to supply Newburgh's drinking water, until the city found out about the continuing levels of PFOS.

The city switched to Brown's Pond as a drinking source, since almost no levels of PFOS were found in that water.

Newburgh is still working to determine where the PFOS contamination is coming from.

Senator Schumer on New Standards:

"I am pleased that the EPA has heeded my call to release updated drinking water health advisories for the highly-toxic chemicals PFOA and PFOS. Communities like Hoosick Falls, Petersburgh and Newburgh understand all too well the uncertainty and anxiety that contamination from these chemicals can cause. The EPA's new guidance will go a long way in protecting public health and arming local officials with the most up-to-date information to keep our drinking water safe from harmful contaminates."

Bucks County Courier Times (also ran in the Intelligencer)

http://www.buckscountycouriertimes.com/news/local/epa-releases-new-safety-advisories-for-chemicals/article_97946008-1de8-11e6-83d3-334d7ea7ce37.html

EPA Releases New Safety Advisories for Chemicals

By Kyle Bagenstose on 5/19/16 at 2:00pm

The Environmental Protection Agency on Thursday released a long-awaited update to its drinking water advisories for unregulated chemicals perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS).

According to prior testing data of public and private water supplies in Bucks and Montgomery counties, it appears the updated advisories could mean some drinking water in the area is contaminated with the chemicals at a level above what the EPA deems is safe to consume.

The chemicals already had contaminated about 100 public and private water wells near a trio of current and former military bases in the region: The former Naval Air Station Joint Reserve Base Willow Grove, Horsham Air Guard Station, and former Naval Air Warfare Center in Warminster.

The chemicals are suspected to have originated in firefighting foams used at the bases, and the military already has agreed to spend approximately $19 million to provide replacement water and install filtration systems for public water wells affected by the chemicals, and bottled water and hook-ups to public systems for homes with affected private wells.

But the number of wells now likely will increase.

Up until Thursday, the military had been gauging its response using short-term, provisional health advisories for the chemicals set by the EPA in 2009: .4 parts per billion for PFOA in drinking water, and .2 ppb for PFOS.

Thursday's announcement, however, set an advised limit of .07 ppb for PFOS and PFOA combined, in order to protect against health effects from a lifetime of exposure. In a worst case scenario, that means drinking water with .58 ppb of the chemicals (.39 ppb PFOA and .19 ppb PFOS) previously considered safe, now would be more than eight times the recommended limit.

After the chemicals first were discovered in some local public water supplies by a nationwide EPA testing program in 2013 and 2014, multiple public wells in the area were taken offline.

The Horsham Water and Sewer Authority removed two drinking water wells from its system in the summer of 2014 after PFOS was found at 1 and .7 ppb. Data from the testing program shows that three more wells also were contaminated with the chemicals at levels above the EPA's new advisory, with a combined .071 ppb, .123 ppb, and .14 ppb.

In a brief email Thursday, authority manager Tina O'Rourke said only two additional wells are above the .07 ppb level as of the most recent testing.

She added that the authority intends to take the wells offline and that existing agreements with the military allow for the authority to take action immediately.

The Warminster Municipal Authority, which previously removed three wells from its supply, also appears to have two more wells contaminated above the new level, with a combined .091 ppb and .09 ppb.

The Warrington Township Water Department previously removed a trio of wells that combined to form a single water source. Based on the EPA's data showing a combined .082 ppb, another well could also be above the new health advisory.

This news organization reached out to all three utilities to obtain the most recent testing information and will update this story as it receives responses.

Although unregulated, a growing body of science has established associations between PFOA and PFOS and a range of health effects, including a variety of cancers.

Joel Beauvais, deputy assistant administrator for the EPA's Office of Water, said in a phone interview Thursday that the updated advisories reflect a review of available science and are intended to be protective for lifetime exposure to the chemicals for even vulnerable populations, such as nursing mothers and their children.

"These health advisories are scientific documents … the point is to provide the best available information to state and local authorities and drinking water system operators," Beauvais said. "They're calculated to reflect a margin or protection to the most sensitive populations."

Beauvais added that the next step for the chemicals is to be considered for a federal drinking water standard that can be enforced, as is the case with more well-known contaminants such as lead or arsenic. However, he was unable to say how long it might take for that decision to be made.

1:59 PM

Albany Times Union

http://www.timesunion.com/local/article/EPA-sets-new-level-for-chemical-in-local-water-7716825.php

EPA Sets New Level for Chemical in Drinking Water

By Brendan J. Lyons on 5/19/16 at 1:17pm

The U.S. Environmental Protection Agency on Thursday issued a lifetime drinking water health advisory of 70 parts per trillion for human exposure to the man-made chemical, perfluorooctanoic acid, or PFOA.

The EPA's long-awaited action comes as health and environmental officials in New York, Vermont and New Hampshire are grappling with the discovery of elevated levels of PFOA in public water systems and private wells. The contamination has been linked to factories that used PFOA in their manufacturing processes, in some cases dating back decades.

"Where these chemicals are detected above the advisory levels, we're recommending the water system operators go back and do confirmatory testing," said Joel Beauvais, deputy assistant administrator for the EPA's Office of Water. "We're also recommending that systems promptly provide notice to residents and users of water systems."

Beauvais said the EPA's recommendation is based on toxicity studies in mice.

The EPA's advisory level is well below the levels of PFOA that were detected in public water supplies over the past two years in Hoosick Falls, Petersburgh, North Bennington, Vt., and Merrimack, N.H.

In addition, health and environmental officials said tests have also revealed levels of PFOA in dozens of private wells in Rensselaer County that are much higher than the EPA's guideline.

Officials with the state Health Department and Department of Environmental Conservation could not immediately provide information Thursday about how many water systems in New York may contain PFOA, which has been used since the 1940s to make products such as nonstick coatings, such as Teflon, and heat-resistant wiring and other specialty products.

In 2009, the EPA established a guideline that a level of 400 parts per trillion is a safe level for "short-term" consumption of the water. Earlier this year, the EPA issued a provisional health advisory of 100 ppt. Meanwhile, other states have set far lower acceptable levels of

PFOA in public water systems, including New Jersey, which has set a level of 40 ppt.

State Health Commissioner Dr. Howard A. Zucker in January said the state Health Department was reviewing its position on acceptable levels of PFOA in drinking water and would set a new safety limit within weeks. But the state did not issue a new advisory.

Robert A. Bilott, an Ohio attorney who is helping represent an estimated 3,500 people in a class-action lawsuit against DuPont, which manufactured and used perfluorinated chemicals such as PFOA in its products, has been urging the EPA for years to adopt a permanent health advisory for long-term exposure to PFOA in drinking water. Bilott has represented "tens of thousands of individuals in various communities across the country who have been injured because of the contamination of their drinking water with PFOA."

"The lower number and acknowledgement of the need to consider the combined amount of PFCs in water is a long-overdue step in the right direction, but the guideline is still too high, as it will allow ever-increasing, unacceptable levels of PFOA to continue to build up in the blood of those exposed," Bilott said.

Three years ago, a science panel released the results of a multiyear study that examined PFOA exposure and health risks in several Ohio Valley communities, where PFOA, also known as C8, was emitted since the 1950s from the Washington Works plant in Parkersburg, W.Va. The panel found probable links between PFOA exposure and high cholesterol, ulcerative colitis, thyroid disease, testicular cancer, kidney cancer and pregnancy-induced hypertension.

Bilott said the science panel's peer-reviewed report was paid for by DuPont and, as a result of the panel's findings, the company has to concede in the personal-injury lawsuits that PFOA causes cancer.

In Albany, numerous lawsuits were filed this year in U.S. District Court seeking class-action status on behalf of current and former Hoosick Falls residents, including some who allege they suffered serious health effects that could be linked to PFOA exposure.

According to the EPA, PFOA was detected in blood serum in 99 percent of the U.S. population between 1999 and 2012. However, the EPA said the levels of PFOA in human blood have been decreasing since U.S. companies began to phase out production of the chemical more than 10 years ago. Still, water resources contaminated by PFOA have been associated with releases from manufacturing sites, industrial sites, fire/crash training areas, and industrial or municipal waste sites where products are disposed of or applied, the agency said.

Thousands of people whose blood was tested in that area of the Ohio Valley had elevated levels of PFOA, including at levels as high as 40 parts per billion.

In Hoosick Falls, where hundreds of people signed up for state-sponsored blood tests in February, the results of those tests are expected to be released beginning next week.

In February, the state Department of Environmental Conservation asked two corporations, Saint-Gobain Performance Plastics and Honeywell International, to enter consent orders that would require them to clean up the remnants of the toxic chemical that polluted water supplies in and around Hoosick Falls.

The DEC said its preliminary investigation determined the two corporations, and possibly others, are the "parties responsible" for the presence of high levels of PFOA that were discovered two years ago in the village's public water supply. The chemical has since been detected in private wells in and around the village and at other locations in Rensselaer County, including the town of Petersburgh.

Saint-Gobain operates two plants in Hoosick Falls, including a McCaffrey Street facility the company has owned since 1999. That plant is a few hundred yards from the village's water-treatment plant and has been a focus of the contamination.

PFOA, a man-made chemical, was discovered in Hoosick Falls' water system by a resident, Michael Hickey, who began researching the issue because of what he believed was a high rate of cancer in the village where he grew up. His father, John, who worked at the Saint-Gobain plant for decades, died of kidney cancer in 2013. Hickey paid to have samples of

village water tested for PFOA and notified village officials in 2014 that the tests showed the levels of the chemical found in the system exceeded federal health advisories.

Saint-Gobain, which said it learned of the pollution in December 2014, has voluntarily funded the distribution of bottled water and said it will pay for the installation of a long-term water filtration system at the village's water treatment plant. The state has been installing individual filter systems at residences and businesses that have private wells with PFOA contamination.

In 2006, the EPA reached an agreement with DuPont and other manufacturers to stop producing or using PFOA, although DuPont continued producing PFOA because the agreement did not call for the end of production of the chemical until 2015. The EPA settlement with DuPont came less than a year after DuPont agreed to pay $10.25 million in civil penalties to settle the complaint brought by the EPA regarding the company's PFOA pollution in the Midwest. At the time, it was the largest civil administrative penalty ever obtained by the EPA under federal environmental statutes.

WAMC, Northeast Public Radio

http://wamc.org/post/epa-releases-new-pfoa-pfos-guidelines

EPA Releases New PFOA, PFOS Guidelines

By (No author) on 5/19/16 at 1:44pm

The U.S. Environmental Protection Agency has released new health advisories for PFOA and PFOS, the manufacturing chemicals at the center of water contamination crises in several communities in the region including Hoosick Falls, Petersburgh, and North Bennington. The guidelines released today lower the lifetime exposure from drinking water level to 70 parts per trillion. The level of PFOA in affected areas has been much higher than the new benchmark, leading communities to establish alternate water supplies. High exposure can result in cancer, birth defects and other diseases.

WMUR Manchester (ABC)

http://www.wmur.com/health/epa-sets-new-level-for-contaminant-found-in-drinking-water/39629266

EPA sets new level for contaminant found in drinking water

By Kirk Enstrom on 5/19/16 at 1:41pm

The Environmental Protection Agency has set new advisory levels for a contaminant that has been found in drinking water supplies in parts of New Hampshire.

The state Department of Environmental Services said Thursday that the EPA set a lifetime drinking water health advisory level for perfluorooctanoic acid at 70 parts per trillion.

The same level was set for a related chemical, perfluorooctane sulfonate. The EPA said that when both chemicals are present, the combined concentration shouldn't exceed 70 parts per trillion.

State environmental officials had been advising well water users whose water tested above 100 parts per trillion to drink bottled water instead.

PFOA was found earlier this year in groundwater near the Saint-Gobain Performance Plastics facility in Merrimack after that company conducted tests. The contaminant has since been found in private wells in Litchfield, Manchester, Bedford and other areas.

It was most recently found in Amherst near a former industrial site.

State environmental officials said they are working to review the scientific basis for the new levels. DES said it's also taking steps to provide bottled water to all known private well users who have detected PFOA or PFOS levels greater than 70 parts per trillion.

"The state of New Hampshire will take immediate action to expand distribution of bottled water in affected areas to those whose water supply falls under the new advisory," Gov. Maggie Hassan said.

DES said it plans to move quickly to develop emergency rules for groundwater cleanup and drinking water standards. There will be a public comment process before permanent rules are established.

DES has set up a website to discuss the PFOA investigation.

NEWS10 ABC

http://news10.com/2016/05/19/epa-issues-health-advisory-level-for-lifetime-exposure-to-pfoa/

EPA Sets Health Advisory Level for Lifetime Exposure to PFOA

By Joe Gullo on 5/19/16 at 1:21pm

The U.S. Environmental Protection Agency (EPA) announced a lifetime drinking water health advisory of 70 parts per trillion for human exposure to PFOA.

The EPA says the advisory is to provide Americans, including the most sensitive populations, with a margin of protection from a lifetime exposure to PFOA and PFOS from drinking water.

PFOA has been linked to serious health problems such as cancer.

Some local communities, including Petersburgh, Hoosick Falls, Bennington, and Pownal have had wells test positive for the chemical.

PFOA and PFOS have been used to make carpets, clothing, fabrics for furniture, paper packaging for food, and other materials that are resistant to water, grease, or stains. In 2006, the EPA says they asked eight companies using PFOA to phase out use and chemicals used to break down PFOA. Those companies have stated at the end of 2015, they phased out the use of PFOA.

According to the EPA, the establishment of the advisories follows an assessment from the latest peer-reviewed science to provide water system operators, and state, tribal, and local officials who have the primary responsibility of overseeing these systems.

Advisories issued by the EPA are designed to provide information on contaminants that can cause human health effects and are known or anticipated to occur in water.

Travis Loop

Director of Communications
Office of Water

U.S. Environmental Protection Agency

202-870-6922

loop.travis@epa.gov

To: Stern, Alan[Alan.Stern@dep.nj.gov]
Cc: Pachkowski, Brian[Brian.Pachkowski@dep.nj.gov]; Donohue, Joyce[Donohue.Joyce@epa.gov]
From: Strong, Jamie
Sent: Fri 5/27/2016 12:57:50 PM
Subject: RE: discussion of PFOS Health Advisory

Alan,
Thank you for your voicemail. I forwarded your email to Joyce Donohue, the chemical manager for PFOA and PFOS. She will reach out to Brian.

Thanks,

Jamie

From: Stern, Alan [mailto:Alan.Stern@dep.nj.gov]
Sent: Thursday, May 26, 2016 3:25 PM
To: Strong, Jamie <Strong.Jamie@epa.gov>
Cc: Pachkowski, Brian <Brian.Pachkowski@dep.nj.gov>
Subject: discussion of PFOS Health Advisory

Jamie,

Not having heard from you since leaving our voice mail for you this morning, I wanted to let you know that I will be out of the office tomorrow (Friday), but Brian (Dr. Pachkowski) will be in. If you can, please contact him at Personal Phone / Ex. 6 If you are, however, still able to call us today, I will be in the office until about 4:45 PM (Personal Phone / Ex. 6 Thank you.

Alan H. Stern, Dr.P.H., D.A.B.T.

Bureau Chief – Risk Analysis

Division of Science, Research and Environmental Health

To: Donohue, Joyce[Donohue.Joyce@epa.gov]
From: Strong, Jamie
Sent: Thur 5/26/2016 7:25:20 PM
Subject: FW: discussion of PFOS Health Advisory

can you call him?

From: Stern, Alan [mailto:Alan.Stern@dep.nj.gov]
Sent: Thursday, May 26, 2016 3:25 PM
To: Strong, Jamie <Strong.Jamie@epa.gov>
Cc: Pachkowski, Brian <Brian.Pachkowski@dep.nj.gov>
Subject: discussion of PFOS Health Advisory

Jamie,

Not having heard from you since leaving our voice mail for you this morning, I wanted to let you know that I will be out of the office tomorrow (Friday), but Brian (Dr. Pachkowski) will be in. If you can, please contact him at (Personal Phone / Ex. 6 . If you are, however, still able to call us today, I will be in the office until about 4:45 PM (Personal Phone / Ex. 6 Thank you.

Alan H. Stern, Dr.P.H., D.A.B.T.

Bureau Chief – Risk Analysis

Division of Science, Research and Environmental Health

To: Glassmeyer, Susan[glassmeyer.susan@epa.gov]; Benson, Bob[Benson.Bob@epa.gov]; Conerly, Octavia[Conerly.Octavia@epa.gov]; sander_william@bah.com[sander_william@bah.com]; Furlong, Edward[efurlong@usgs.gov]; Dana Kolpin[dwkolpin@usgs.gov]; Boone, Scott[sboone@mscl.msstate.edu]; Donohue, Joyce[Donohue.Joyce@epa.gov]
Cc: Wilson, Vickie[Wilson.Vickie@epa.gov]
From: Simmons, Jane
Sent: Thur 5/26/2016 3:05:01 PM
Subject: RE: Human Health Manuscript - now that the PFOA and PFOS chronic health values are published by EPA, how do we want to proceed?

Susan

Deeply in favor of deep thoughts, but you deserve a holiday weekend!!

Please enjoy yourself at least a little bit!!

BEST

JES

From: Glassmeyer, Susan
Sent: Thursday, May 26, 2016 10:58 AM
To: Benson, Bob <Benson.Bob@epa.gov>; Simmons, Jane <Simmons.Jane@epa.gov>; Conerly, Octavia <Conerly.Octavia@epa.gov>; sander_william@bah.com; Furlong, Edward <efurlong@usgs.gov>; Dana Kolpin <dwkolpin@usgs.gov>; Boone, Scott <sboone@mscl.msstate.edu>; Donohue, Joyce <Donohue.Joyce@epa.gov>
Cc: Wilson, Vickie <Wilson.Vickie@epa.gov>
Subject: RE: Human Health Manuscript - now that the PFOA and PFOS chronic health values are published by EPA, how do we want to proceed?

Looks like PFASs are in the air. I've added Joyce to this conversation.

Bob laid out the issues with the non PFOS and PFOA detections, and how to deal with the other health effects below.

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

From: Benson, Bob
Sent: Thursday, May 26, 2016 10:38 AM
To: Simmons, Jane <Simmons.Jane@epa.gov>; Glassmeyer, Susan <glassmeyer.susan@epa.gov>; Conerly, Octavia <Conerly.Octavia@epa.gov>; sander_william@bah.com; Furlong, Edward <efurlong@usgs.gov>; Dana Kolpin <dwkolpin@usgs.gov>; Boone, Scott <sboone@mscl.msstate.edu>
Cc: Wilson, Vickie <Wilson.Vickie@epa.gov>
Subject: RE: Human Health Manuscript - now that the PFOA and PFOS chronic health values are published by EPA, how do we want to proceed?

Deliberative Process / Ex. 5

Some choices will need to be made on how to conduct and present the data analysis.

Here is my recommendation. Comments are welcome.

Deliberative Process / Ex. 5

From: Simmons, Jane
Sent: Tuesday, May 24, 2016 6:23 AM
To: Glassmeyer, Susan <glassmeyer.susan@epa.gov>; Benson, Bob <Benson.Bob@epa.gov>; Conerly, Octavia <Conerly.Octavia@epa.gov>; sander_william@bah.com; Furlong, Edward

<efurlong@usgs.gov>; Dana Kolpin <dwkolpin@usgs.gov>; Boone, Scott <sboone@mscl.msstate.edu>
Cc: Wilson, Vickie <Wilson.Vickie@epa.gov>
Subject: RE: Human Health Manuscript - now that the PFOA and PFOS chronic health values are published by EPA, how do we want to proceed?

Susan,

Thanks for the update. I think that the Project Lead needs to be involved, so I am cc'ing Vickie Wilson.

JES

From: Glassmeyer, Susan
Sent: Monday, May 23, 2016 6:09 PM
To: Simmons, Jane <Simmons.Jane@epa.gov>; Benson, Bob <Benson.Bob@epa.gov>; Conerly, Octavia <Conerly.Octavia@epa.gov>; sander_william@bah.com; Furlong, Edward <efurlong@usgs.gov>; Dana Kolpin <dwkolpin@usgs.gov>; Boone, Scott <sboone@mscl.msstate.edu>
Subject: RE: Human Health Manuscript - now that the PFOA and PFOS chronic health values are published by EPA, how do we want to proceed?

Deliberative Process / Ex. 5

From: Simmons, Jane
Sent: Friday, May 20, 2016 1:21 PM
To: Benson, Bob <Benson.Bob@epa.gov>; Conerly, Octavia <Conerly.Octavia@epa.gov>; sander_william@bah.com; Glassmeyer, Susan <glassmeyer.susan@epa.gov>; Furlong, Edward

<efurlong@usgs.gov>; Dana Kolpin <dwkolpin@usgs.gov>; Boone, Scott <sboone@mscl.msstate.edu>
Subject: RE: Human Health Manuscript - now that the PFOA and PFOS chronic health values are published by EPA, how do we want to proceed?
Importance: High

YEA,

The new PFOA and PFOS health advisory is published.

How should we proceed with the PFOA and PFOS paper? I think that Bob and I can be especially useful in placing the exposure from water into context with other exposure sources and other exposure levels, but will work on any section where help is needed (but please not the method itself ☺

I know that we are not trying to include it in the series, but getting it written and out there as soon as possible will be great!

Also, any news on when the embargo will end? I had to answer another question about it today…. – in that I had to provide the accepted date and then explain why it was not available online. I recall the embargo would only last through May – is that the latest?

JES

From: Benson, Bob
Sent: Monday, April 11, 2016 4:54 PM
To: Conerly, Octavia <Conerly.Octavia@epa.gov>; sander_william@bah.com; Glassmeyer, Susan <glassmeyer.susan@epa.gov>; Furlong, Edward <efurlong@usgs.gov>; Dana Kolpin <dwkolpin@usgs.gov>; Simmons, Jane <Simmons.Jane@epa.gov>
Subject: Human Health Manuscript

I submitted the corrections to the page proofs to STOTEN today.

To: Benson, Bob[Benson.Bob@epa.gov]; Simmons, Jane[Simmons.Jane@epa.gov]; Conerly, Octavia[Conerly.Octavia@epa.gov]; sander_william@bah.com[sander_william@bah.com]; Furlong, Edward[efurlong@usgs.gov]; Dana Kolpin[dwkolpin@usgs.gov]; Boone, Scott[sboone@mscl.msstate.edu]; Donohue, Joyce[Donohue.Joyce@epa.gov]
Cc: Wilson, Vickie[Wilson.Vickie@epa.gov]
From: Glassmeyer, Susan
Sent: Thur 5/26/2016 2:57:31 PM
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Deliberative Process / Ex. 5

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Deliberative Process / Ex. 5

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Deliberative Process / Ex. 5

From: Simmons, Jane
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Subject: Human Health Manuscript

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To: Benson, Bob[Benson.Bob@epa.gov]
Cc: Strong, Jamie[Strong.Jamie@epa.gov]; Donohue, Joyce[Donohue.Joyce@epa.gov]; Bahrman, Sarah[Bahrman.Sarah@epa.gov]; Garcia, Bert[Garcia.Bert@epa.gov]; O'Connor, Darcy[oconnor.darcy@epa.gov]; Southerland, Elizabeth[Southerland.Elizabeth@epa.gov]; Hisel-Mccoy, Sara[Hisel-McCoy.Sara@epa.gov]
From: Behl, Betsy
Sent: Thur 5/26/2016 11:22:07 AM
Subject: FW: PFASs in Colorado
Memo to CDPHE.docx

Bob, We published the health advisories for PFOA and PFOS last week after a full panel peer review and significant internal and external review. Here is a link.

https://www.epa.gov/ground-water-and-drinking-water/drinking-water-health-advisories-pfoa-and-pfos

Best, Betsy

From: Benson, Bob
Sent: Wednesday, May 25, 2016 3:02 PM
To: Donohue, Joyce <Donohue.Joyce@epa.gov>; Strong, Jamie <Strong.Jamie@epa.gov>; Behl, Betsy <Behl.Betsy@epa.gov>
Cc: Bahrman, Sarah <Bahrman.Sarah@epa.gov>; Garcia, Bert <Garcia.Bert@epa.gov>; O'Connor, Darcy <oconnor.darcy@epa.gov>
Subject: PFASs in Colorado

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

The memo I drafted is based on my best profession judgment. I would welcome any comments if your professional judgment differs significantly from mine.

To: Behl, Betsy[Behl.Betsy@epa.gov]
Cc: Donohue, Joyce[Donohue.Joyce@epa.gov]
From: Strong, Jamie
Sent: Wed 5/25/2016 7:21:12 PM
Subject: FW: PFASs in Colorado
Memo to CDPHE.docx

Shouldn't this go through OGWDW too?

From: Benson, Bob
Sent: Wednesday, May 25, 2016 3:02 PM
To: Donohue, Joyce <Donohue.Joyce@epa.gov>; Strong, Jamie <Strong.Jamie@epa.gov>; Behl, Betsy <Behl.Betsy@epa.gov>
Cc: Bahrman, Sarah <Bahrman.Sarah@epa.gov>; Garcia, Bert <Garcia.Bert@epa.gov>; O'Connor, Darcy <oconnor.darcy@epa.gov>
Subject: PFASs in Colorado

Deliberative Process / Ex. 5

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To: Donohue, Joyce[Donohue.Joyce@epa.gov]; Strong, Jamie[Strong.Jamie@epa.gov]; Behl, Betsy[Behl.Betsy@epa.gov]
Cc: Bahrman, Sarah[Bahrman.Sarah@epa.gov]; Garcia, Bert[Garcia.Bert@epa.gov]; O'Connor, Darcy[oconnor.darcy@epa.gov]
From: Benson, Bob
Sent: Wed 5/25/2016 7:02:23 PM
Subject: PFASs in Colorado
Memo to CDPHE.docx

Deliberative Process / Ex. 5

The memo I drafted is based on my best profession judgment. I would welcome any comments if your professional judgment differs significantly from mine.

To: Donohue, Joyce[Donohue.Joyce@epa.gov]
From: Strong, Jamie
Sent: Mon 3/21/2016 7:39:59 PM
Subject: PFOA HA
PFOA HA Draft 03 21 2016 for JMD.docx

Joyce,

I am sending you the PFOA HA. I understand you are working on the synthesis and the susceptible population sections, but I wanted to get this to you today as there are few things I need you to look at in addition to these pieces. You can dump the synth and susceptible pop discussion into this version or send to me separately if that's easier and I will add. I have several comment bubbles that I need you to address. I have highlighted these in yellow. It's not too many. This is the final push on this HA.

Jamie

Jamie Strong, Chief Human Health Risk Assessment Branch

Health and Ecological Criteria Division, 4304-T

Office of Science and Technology, Office of Water

United States Environmental Protection Agency

1200 Pennsylvania Avenue, NW

Washington DC 20460

phone: 202.566.0056

fax: 202.566.1140

To: Donohue, Joyce[Donohue.Joyce@epa.gov]
From: Strong, Jamie
Sent: Mon 3/21/2016 11:55:09 AM
Subject: DR PFOA HA
DR PFOA HA W COMMENTS.docx

Can you read this again and edit per comments.

Jamie Strong, Chief Human Health Risk Assessment Branch

Health and Ecological Criteria Division, 4304-T

Office of Science and Technology, Office of Water

United States Environmental Protection Agency

1200 Pennsylvania Avenue, NW

Washington DC 20460

phone: 202.566.0056

fax: 202.566.1140

To: Southerland, Elizabeth[Southerland.Elizabeth@epa.gov]
Cc: Strong, Jamie[Strong.Jamie@epa.gov]; Donohue, Joyce[Donohue.Joyce@epa.gov]; Flaherty, Colleen[Flaherty.Colleen@epa.gov]
From: Behl, Betsy
Sent: Fri 3/18/2016 2:40:35 PM
Subject: FW: Meeting Re: Health Advisories for Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoic Acid (PFOA)
PFOA-PFOS-OW HAs ADMIN BRIEF 03 18 16 AA draft.ppt

Betsy: This is the final draft for Joel of the briefing for the administrator. It includes all comments from other offices with the exception of Travis (final slide on the Roadmap).

Deliberative Process / Ex. 5

I think it is very good and I hope he likes it!

Betsy

From: Conerly, Octavia
Sent: Thursday, March 17, 2016 1:12 PM
To: Behl, Betsy <Behl.Betsy@epa.gov>; Strong, Jamie <Strong.Jamie@epa.gov>
Cc: Southerland, Elizabeth <Southerland.Elizabeth@epa.gov>
Subject: Meeting Re: Health Advisories for Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoic Acid (PFOA)

Does Joel have the slides for this briefing? I have not seen any email. They are due to the Administrator tomorrow.

Subject:	Meeting Re: Health Advisories for Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoic Acid (PFOA)
Location:	Alm Conference Room
Start:	Wed 3/23/2016 1:30 PM
End:	Wed 3/23/2016 2:30 PM
Recurrence:	(none)
Meeting Status:	Accepted
Organizer:	**Personal Address / Ex. 6**
Required Attendees:	Southerland, Elizabeth; Behl, Betsy; Flaherty, Colleen; Strong, Jamie; Donohue, Joyce; Grevatt, Peter; Clark, Becki; Burneson, Eric; Henry, Tala; Cleland-Hamnett, Wendy; Conerly, Octavia; Miller, Gregory; Etzel, Ruth; Scozzafava, MichaelE; Cooke, Maryt; Vaught, Laura; Gilinsky, Ellen; Fritz, Matthew; Klasen, Matthew; Burke, Thomas; Thomas, Russell; Wambaugh, John; Slotkin, Ron; Rodgers, Ryan;

Carter, Donnell; Sampson, Jamaal; Vitalien, Christal; EPAVTC; MCClain, Mike; Meiburg, Stan; Purchia, Liz; Nancy Grantham (Grantham.Nancy@epa.gov); Distefano, Nichole; Rupp, Mark; Beauvais, Joel; Stanislaus, Mathy; Barry Breen (Breen.Barry@epa.gov); Rennert, Kevin

******Please DO NOT forward this calendar invitation. You may "Accept" or "Decline" the invitation but DO NOT respond with questions or concerns. If you do have any questions or concerns about this invitation please contact the Scheduling Office at scheduling@epa.gov . ******

SCt: Liel Azoolin
Ct: Crystal Penman, 202-564-3318

Staff:
Deputy Meiburg, Matt Fritz (OA)
Liz Purchia, Nancy Grantham (OPA)
Nichole Distefano, Mark Rupp (OCIR)
Joel Beauvais, Ellen Gilinsky, Elizabeth Southerland, Betsy Behl, Colleen Flaherty, Jamie Strong, Joyce Donohue, Peter Grevatt, Becki Clark, Eric Burneson (OW)
Tom Burke, Russell Thomas, John Wambaugh (ORD)
Mathy Stanislaus, Barry Breen (OLEM)
Laura Vaught, Kevin Rennert (OP)

Optional:
Octavia Conerly (OW)
Ruth Etzel, Greg Miller (OCHP)
Michael Scozzafava, Mary Cooke (OLEM)
Tala Henry, Wendy Cleland-Hamnett (OCSPP)

Conference Code/ Ex. 6

To: Behl, Betsy[Behl.Betsy@epa.gov]; Strong, Jamie[Strong.Jamie@epa.gov]; Donohue, Joyce[Donohue.Joyce@epa.gov]
Cc: Flowers, Lynn[Flowers.Lynn@epa.gov]; Glenn, Barbara[Glenn.Barbara@epa.gov]
From: Hines, Erin
Sent: Thur 3/17/2016 8:35:37 PM
Subject: PFOS thyroid questions
PFOS EPI COMMENTS AND QUESTIONS eh.docx

Dear Joyce and OW-

I hope I was able to clarify some of the thyroid questions you posed. My comments are included in the attached document.

Deliberative Process / Ex. 5

Erin

Erin Pias Hines, Ph.D., DABT

Biologist

National Center for Environmental Assessment

U.S. Environmental Protection Agency, B243-01

Research Triangle Park, NC 27711
Phone: 919-541-4204 | Fax: 919-541-2985

Email: hines.erin@epa.gov

To: Donohue, Joyce[Donohue.Joyce@epa.gov]
From: Strong, Jamie
Sent: Thur 3/17/2016 3:50:36 PM
Subject: PFOS HA
PFOS HA 03 17 2016.docx

See remaining comments that are highlighted yellow.

Jamie Strong, Chief Human Health Risk Assessment Branch

Health and Ecological Criteria Division, 4304-T

Office of Science and Technology, Office of Water

United States Environmental Protection Agency

1200 Pennsylvania Avenue, NW

Washington DC 20460

phone: 202.566.0056

fax: 202.566.1140

To: Behl, Betsy[Behl.Betsy@epa.gov]
Cc: Strong, Jamie[Strong.Jamie@epa.gov]; Donohue, Joyce[Donohue.Joyce@epa.gov]
From: Flaherty, Colleen
Sent: Tue 3/15/2016 7:29:53 PM
Subject: Health advisory slides
PFOA-PFOS-OW HAs ADMIN BRIEF 03.14.16.ppt

Betsy, here's the latest draft of the slides for you to send to OGWDW, OPPT, OCHP, and OLEM. Please ask them for comments by tomorrow morning, if possible, so we can send to Joel later tomorrow. Thanks to Jamie and Joyce for their helpful reviews.

Thanks,

Colleen

To: Donohue, Joyce[Donohue.Joyce@epa.gov]
Cc: Strong, Jamie[Strong.Jamie@epa.gov]
From: Flaherty, Colleen
Sent: Tue 3/15/2016 7:26:32 PM
Subject: FW: slides
PFOA-PFOS-OW HAs ADMIN BRIEF 031416 cf-JMD.ppt

Thanks, Joyce! Great suggestions!

From: Strong, Jamie
Sent: Tuesday, March 15, 2016 1:36 PM
To: Flaherty, Colleen <Flaherty.Colleen@epa.gov>
Subject: RE: slides

Here are Joyce's changes.

From: Flaherty, Colleen
Sent: Tuesday, March 15, 2016 12:06 PM
To: Strong, Jamie <Strong.Jamie@epa.gov>
Subject: slides

Colleen M. Flaherty | Deputy Director

Health and Ecological Criteria Division | Office of Water

U.S. Environmental Protection Agency

1200 Pennsylvania Avenue, NW, Washington, DC 20460

☎ Phone: (202) 564-5939 | ✉ flaherty.colleen@epa.gov

To: Donohue, Joyce[Donohue.Joyce@epa.gov]
From: Strong, Jamie
Sent: Tue 3/15/2016 4:06:47 PM
Subject: Admin briefing slides for review
PFOA-PFOS-OW HAs ADMIN BRIEF 031416 cf.ppt

Take a look and send me any comments.

Thanks,

Jamie

From: Flaherty, Colleen
Sent: Tuesday, March 15, 2016 12:06 PM
To: Strong, Jamie <Strong.Jamie@epa.gov>
Subject: slides

Colleen M. Flaherty | Deputy Director

Health and Ecological Criteria Division | Office of Water

U.S. Environmental Protection Agency

1200 Pennsylvania Avenue, NW, Washington, DC 20460

☎ Phone: (202) 564-5939 | ✉ flaherty.colleen@epa.gov

To: Donohue, Joyce[Donohue.Joyce@epa.gov]
From: Strong, Jamie
Sent: Mon 3/14/2016 12:47:49 PM
Subject: PFOS HA FOR REVIEW
PFOS HA 03 14 2016.docx

Joyce,

Here is the PFOS HA for your review. I have added in the ORD edits for the epi. I have also added in comments from the internal review that I either cant address or need help with addressing. I have also done a side by side with the PFOA HA and made those universal changes here. I need you to go through this draft and review and respond to comments as appropriate.

Thanks,
Jamie

Jamie Strong, Chief Human Health Risk Assessment Branch

Health and Ecological Criteria Division, 4304-T

Office of Science and Technology, Office of Water

United States Environmental Protection Agency

1200 Pennsylvania Avenue, NW

Washington DC 20460

phone: 202.566.0056

fax: 202.566.1140

To: Donohue, Joyce[Donohue.Joyce@epa.gov]; Wood, Carol S.[woodcs@ornl.gov]
From: Strong, Jamie
Sent: Mon 3/14/2016 11:48:45 AM
Subject: FW: PFOS HESD and HA
PFOS HESD Epi edits 11Mar2016.docx
ATT00001.htm
PFOS HA Epi edits_11Mar2016.docx
ATT00002.htm

From: Flowers, Lynn
Sent: Saturday, March 12, 2016 6:46 AM
To: Behl, Betsy <Behl.Betsy@epa.gov>; Strong, Jamie <Strong.Jamie@epa.gov>
Cc: Cantilli, Robert <Cantilli.Robert@epa.gov>; Fegley, Robert <Fegley.Robert@epa.gov>
Subject: Fwd: PFOS HESD and HA

Betsy and Jamie: Barbara's email is self explanatory. Again, if you have any questions, just email or call.

Lynn

Sent from my iPhone

Begin forwarded message:

From: "Glenn, Barbara" <Glenn.Barbara@epa.gov>
To: "Flowers, Lynn" <Flowers.Lynn@epa.gov>
Cc: "Wright, Michael" <Wright.Michael@epa.gov>, "Hines, Erin" <Hines.Erin@epa.gov>
Subject: PFOS HESD and HA

Hi Lynn,
I am sending the HESD and HA files with our edits to the epidemiology discussions which are found throughout these documents. It is mostly in track changes but we did not retain the track changes when moving sections around.

Deliberative Process / Ex. 5

I want to give credit to Erin and Mike who put in a tremendous effort over the last week and half to assure that the epidemiology literature on lipids, thyroid and reproductive effects is characterized properly. I think we hit the outcomes with the most literature â€“ lipids, thyroid disease and hormones, infectious disease and antibody titers, reproductive and developmental outcomes, and cancer.

Have a great weekend. -Barbara

To: Strong, Jamie[Strong.Jamie@epa.gov]; Donohue, Joyce[Donohue.Joyce@epa.gov]; Albert, Ryan[Albert.Ryan@epa.gov]; Burneson, Eric[Burneson.Eric@epa.gov]; Rodgers-Jenkins, Crystal[Rodgers-Jenkins.Crystal@epa.gov]
Cc: Moraff, Kenneth[Moraff.Ken@epa.gov]; Hamjian, Lynne[Hamjian.Lynne@epa.gov]
From: Downing, Jane
Sent: Thur 3/10/2016 9:03:23 PM
Subject: FW: RELEASE: Governor Hassan, NY Governor Cuomo and VT Governor Shumlin Urge U.S. Environmental Protection Agency to Review and Issue New PFOA Guidelines for Safe Drinking Water
removed.txt

Afternoon All

FYI on PFOA letters from Governors – I am sure you have or will see this soon.

Take Care

Jane

From: Pillsbury, Sarah [mailto:Sarah.Pillsbury@des.nh.gov]
Sent: Thursday, March 10, 2016 3:16 PM
To: Downing, Jane <Downing.Jane@epa.gov>
Subject: FW: RELEASE: Governor Hassan, NY Governor Cuomo and VT Governor Shumlin Urge U.S. Environmental Protection Agency to Review and Issue New PFOA Guidelines for Safe Drinking Water

Sarah Pillsbury, Administrator

Drinking Water & Groundwater Bureau, NHDES

29 Hazen Drive, PO Box 95, Concord, NH 03302

Tel: (603) 271-1168 | Fax: (603) 271-5171

NHDES Be Well Informed Private Well App - An interactive web application for private well owners to interpret water quality test results and identify appropriate water treatment options

From: Martin, James
Sent: Thursday, March 10, 2016 2:17 PM
To: DES-Everyone
Subject: FW: RELEASE: Governor Hassan, NY Governor Cuomo and VT Governor Shumlin Urge U.S. Environmental Protection Agency to Review and Issue New PFOA Guidelines for Safe Drinking Water

From: Hinkle, William
Sent: Thursday, March 10, 2016 2:14 PM
To: Hinkle, William
Subject: RELEASE: Governor Hassan, NY Governor Cuomo and VT Governor Shumlin Urge U.S. Environmental Protection Agency to Review and Issue New PFOA Guidelines for Safe Drinking Water

Office of New Hampshire Governor Maggie Hassan

For Immediate Release

Thursday, March 10, 2016

Contact: William Hinkle

Office: (603) 271-2121; Cell: (336) 682-8799

Governor Hassan, NY Governor Cuomo and VT Governor Shumlin Urge U.S. Environmental Protection Agency to Review and Issue New PFOA Guidelines for Safe Drinking Water

Governors also Call for Full Federal Funding of Programs to Support State Investments in Modern and Safe Drinking Water Upgrades

Governors to EPA Administrator McCarthy: "PFOA Contamination is not a State Problem or a Regional Problem – It's a National Problem that Requires Federal Guidelines and a Consistent, Science-Based Approach."

CONCORD – Today, Governor Maggie Hassan, Governor Andrew M. Cuomo of New York and Governor Peter Shumlin of Vermont officially requested that the U.S. Environmental Protection Agency conduct a review of the best available science regarding perfluorooctanoic acid (PFOA), in order to assess and ensure the safety of drinking water exposed to the contaminant. Additionally, the Governors called for full federal funding of the Drinking Water State Revolving Fund and the Clean Water Revolving Fund, which are critical tools for supporting state efforts to upgrade drinking water infrastructure.

These requests, which were made in a letter to EPA Administrator Gina McCarthy, can be viewed here. The text of that letter is also available below:

March 10, 2016

Gina McCarthy

Administrator

U.S. Environmental Protection Agency

1200 Pennsylvania Avenue, NW

Washington, DC 20460

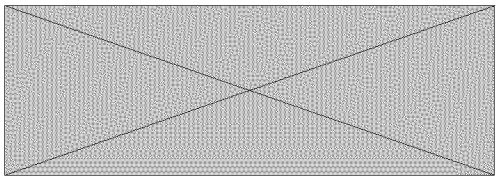
Dear Administrator McCarthy,

We write as Governors whose states are all in the midst of addressing local drinking water contamination involving the federally unregulated chemical perfluorooctanoic acid (PFOA). We are deeply concerned for the health and well-being of our communities grappling with this contaminant. In New York and New Hampshire, tests have indicated the presence of this chemical in public drinking water systems, and in New York and Vermont the chemical has been detected in several private wells. It is clear that PFOA contamination is not a state problem or a regional problem – it's a national problem that requires federal guidelines and a consistent, science-based approach.

The EPA's PFOA health advisory was recently lowered in one village in New York by the EPA's Regional Office, though the higher advisory remains in the rest of the country. We urge the EPA, under your leadership, to expeditiously review the best available science on this contaminant, and provide uniform guidance to states that our health and environmental officials can use in assessing the safety of our drinking water. In addition, we seek your help and support for additional drinking water testing and analysis in communities exposed to PFOA.

We also are all strong supporters of full federal funding for water infrastructure. The Drinking Water State Revolving Fund is a critical tool for states to invest in modern and safe drinking water upgrades. Unfortunately, over the last six years funding for this program, and its companion Clean Water Revolving Fund, have been flat or declining. This comes even as the American Society of Civil Engineers points out massive gaps between our water infrastructure needs and our investment. We should invest more in both the Drinking Water State Revolving Fund and the Clean Water Revolving Fund, and we look forward to working with you to make that happen.

We respectfully request your personal attention to the challenges created by PFOA contamination in our states. Consistency, accuracy, and uniformity are paramount. We look forward to your response and assistance as we work to identify the polluters responsible for this contamination, and hold them accountable for their actions. It is unacceptable to us that any community should have to be concerned about the safety of their drinking water. Families in our states are worried about potentially tragic short and long-term health impacts, not to mention the potential loss in property values for homes in affected areas. It has been our priority to ensure that residents are being provided clean and safe water immediately, and that our infrastructure be modernized to eliminate these concerns in the future.



Sincerely,

Margaret Wood Hassan

Governor of New Hampshire

Andrew M. Cuomo

Governor of New York

Peter Shumlin

Governor of Vermont

###

To: Behl, Betsy[Behl.Betsy@epa.gov]; Donohue, Joyce[Donohue.Joyce@epa.gov]; Flaherty, Colleen[Flaherty.Colleen@epa.gov]
From: Strong, Jamie
Sent: Wed 3/9/2016 8:34:19 PM
Subject: NJ presentation for tomorrow
PFOA final presentation for EPA OW draft deliberative 3_9.pptx

Did you look at this?

Jamie Strong, Chief Human Health Risk Assessment Branch

Health and Ecological Criteria Division, 4304-T

Office of Science and Technology, Office of Water

United States Environmental Protection Agency

1200 Pennsylvania Avenue, NW

Washington DC 20460

phone: 202.566.0056

fax: 202.566.1140

NJDEP Technical Comments on: USEPA OW Draft PFOA Health Effects Document & PFOA Drinking Water Level of 0.1 ppb (100 ppt)

USEPA & NJDEP Meeting on PFOA
Washington, DC
March 10, 2016

Executive Summary

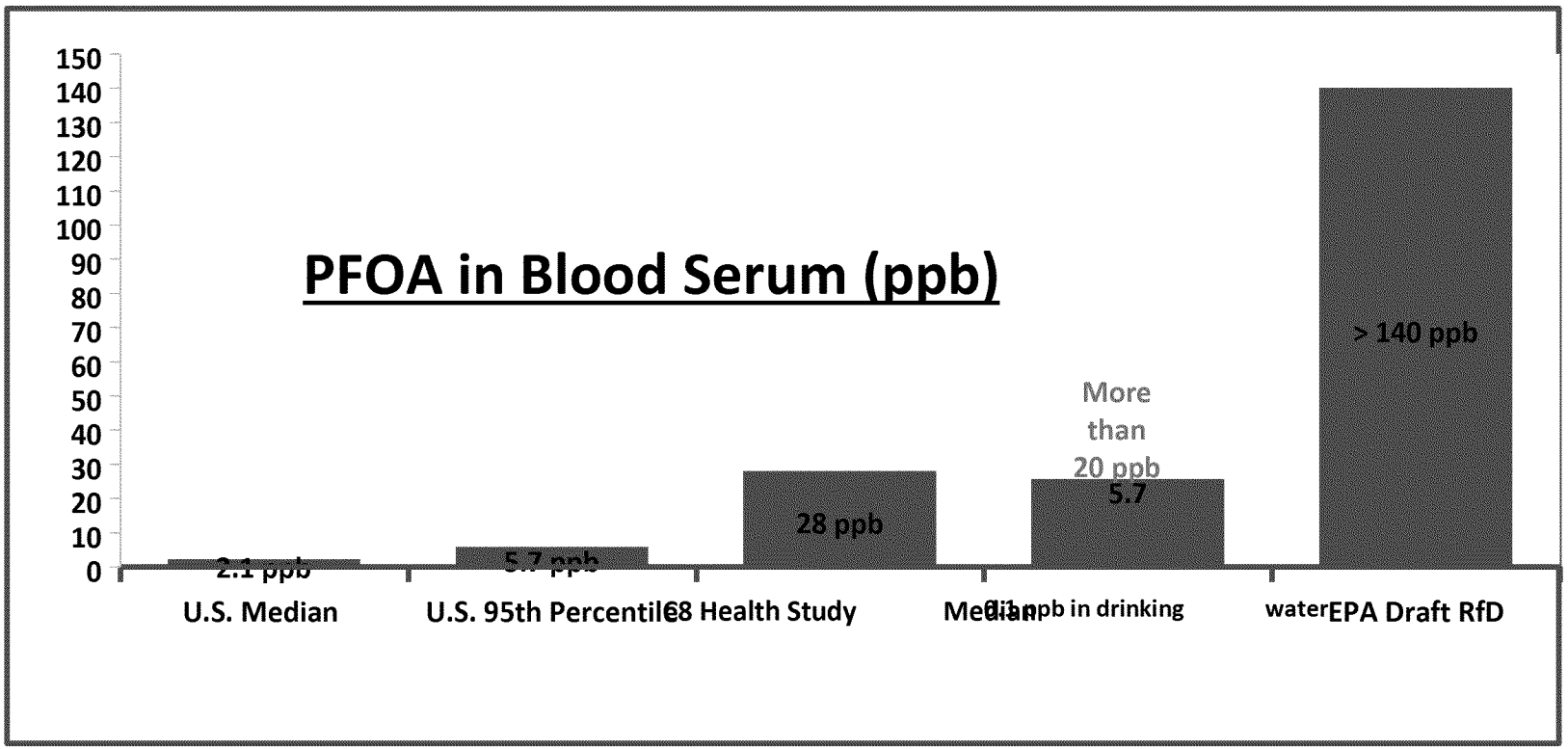
- NJDEP believes that the draft PFOA Reference Dose (RfD; 0.0002 mg/kg/day) and a Lifetime Health Advisory (0.1 ppb) are not sufficiently protective of public health.

- Major points include:

1. Human data should be considered, since EPA's formula to convert animal serum levels to external human doses can also be used to calculate the external human dose from human serum levels.

Executive Summary

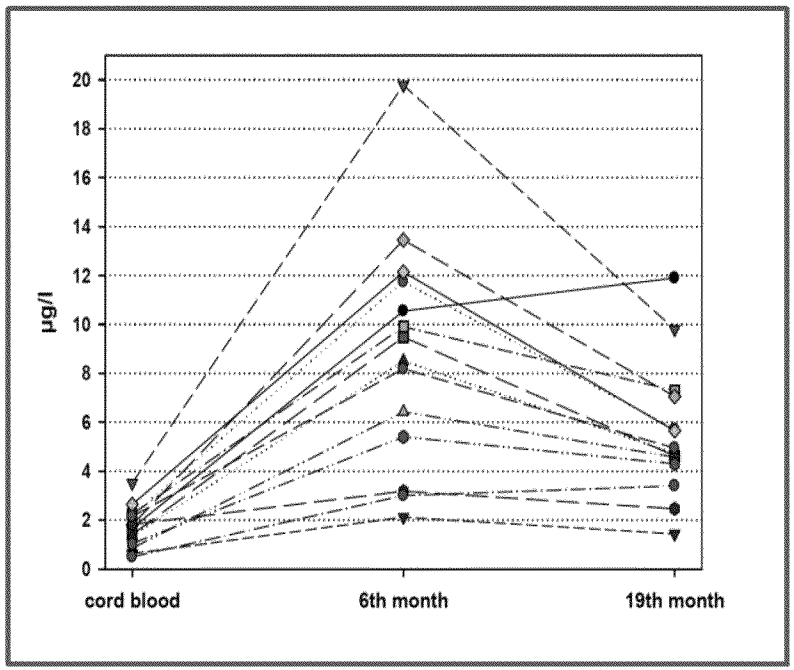
2. Request that EPA consider the elevated human serum levels expected to result from exposure to the proposed Reference Dose (RfD) and drinking water concentration of 0.1 ppb.



Executive Summary

3. Infants are a sensitive subpopulation for PFOA's potential developmental effects.
   - They have higher exposures and blood serum levels than older individuals and should be considered.

PFOA in Infants' Blood Serum



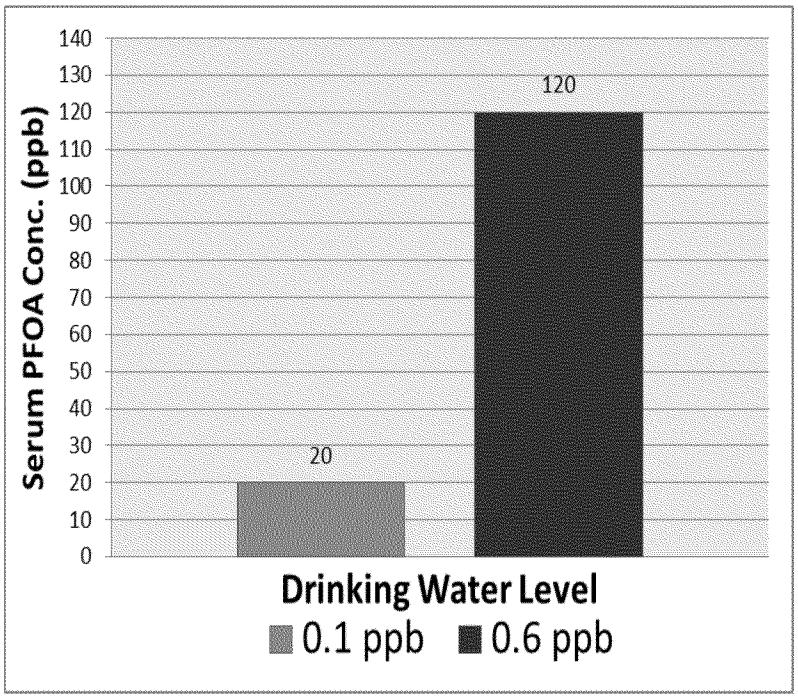
4. There are more sensitive and relevant toxicological endpoints that should be considered as the basis for the risk assessment.

Executive Summary

5. **RELATIVE SOURCE CONTRIBUTION (RSC):**

- Available data supports use of a RSC of 80% rather then the default RSC of 20% to account for non-drinking water exposures.
- However, the RSC of 80% and the draft RfD would result in a drinking water level of **0.6 ppb**.
- This 0.6 ppb value predicts an increased PFOA serum level of **120 ppb.**
- This is four (4) times the median in communities (C8 Health Studies) where health effects are associated with drinking water exposure.

Lifetime Health Advisories from Draft RfD & Default (20%) or Chemical-Specific (80%) RSC Values



Context for NJDEP Review & Overview of Presentation

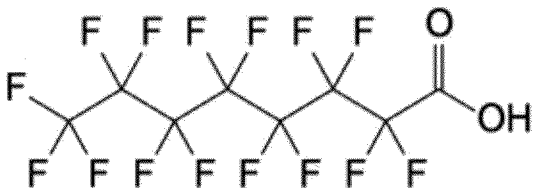
- General context for NJDEP review:
  - *Reference Dose (RfD)*: An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.
  - *Lifetime Health Advisory*: The concentration of a chemical in drinking water that is not expected to cause any adverse non-carcinogenic effects for a lifetime of exposure.

- Content of presentation:
  - Main points from NJDEP public comments on 2014 EPA OW draft document.
    - *Note*: EPA OW draft document developed **Reference Dose (RfD)** of 0.00002 mg/kg/day but did not propose a drinking water level.
  - Comments on a potential **Lifetime Health Advisory of 0.1 ppb.**
  - Title of slides presenting **summaries of points listed in the Agenda are in blue.**

- **Based on its review, NJDEP is concerned that the EPA OW draft RfD and a Lifetime Health Advisory of 0.1 ppb for PFOA are not sufficiently protective of public health, for the reasons to be discussed here.**

Overview of Perfluorooctanoic Acid (PFOA) as a Drinking Water Contaminant

- Manmade chemical with totally fluorinated carbon chain & carboxyl group.
- Does not break down in the environment.
- Water soluble – occurs in drinking water.
- Accumulates in blood serum after exposure.



Perfluorooctanoic acid (PFOA)

- Ongoing exposure to low drinking water levels results in much greater increases in blood serum levels.
  - EPA model predicts serum levels that are 200 times higher than levels in drinking water with chronic exposure.
- Slowly excreted from the body after exposure ceases - human half-life is several years.
- Causes a variety of toxic effects in animal studies.
- Associated with numerous health effects in humans.
  - Including in the general population and/or communities with drinking water exposure.

Units for PFOA Drinking Water and Serum Levels

- Unit used in this presentation for PFOA drinking water and serum levels is **ppb (***parts per billion; equivalent to ng/ml and µg/L)*

- *Note:* Units for PFOA drinking water & serum levels differ among documents and studies, including:
  - *µg/ml* – equivalent to parts per million (ppm)
  - *µg/L, ng/ml* – equivalent to parts per billion (ppb)
  - *ng/L* – equivalent to parts per trillion (ppt)

Rationale for Discounting Human Data-

- EPA OW states (p. 5-1; 5-19) that human studies cannot be used for quantitative exposure-response because they provide only PFOA serum levels, and that **external exposure (e.g. administered dose) to PFOA is not known**.
- However, EPA OW used a **species–independent factor** (clearance) to convert animal serum levels from toxicology studies to human administered doses.
- This factor can also be used in the reverse direction to convert human serum levels to human administered doses.
- **Therefore, external exposures (administered doses) that result in the serum levels reported in the human studies can be determined.**
  - This information can be considered in the risk assessment.

Rationale for Discounting Human Data (cont.)

- Human-to-animal comparisons for PFOA are based on serum levels (internal doses), not administered doses.
  - A given serum level results from a much lower administered dose in humans than in animals, due to much longer human half-life.
- The following equation was used by EPA OW to determine human equivalent doses from animal serum data:

Human Dose (µg/kg/day) =
Average Serum Concentration (µg/L) x Clearance

Clearance = 1.4 x 10^{-4} L/kg body wt/day (p. 5-17)

- This relationship can also be used to determine the external human dose expected to result in a given human serum level.

Consideration of Human Serum Levels from Exposure to Draft RfD & 0.1 ppb in Drinking Water

- EPA OW should consider the increases in PFOA serum levels expected from the draft RfD and 0.1 ppb in drinking water.
  - Draft Reference Dose (RfD) – predicted increase is 140 ppb.
  - 0.1 ppb in drinking water – predicted increase is 20 ppb.*
- Numerous human health effects are associated with serum levels well below those predicted from the draft RfD and 0.1 in drinking water.
- For comparison, U.S. general population serum PFOA levels (NHANES, 2011-12) are 2.1 ppb (median) and 5.7 ppb (95th percentile).
- Although causality cannot be definitively proven for the associations of PFOA with health effects, due to the nature of observational epidemiology in general, **caution is needed about exposures that are expected to increase serum levels to this degree.**
- This is particularly true for PFOA because **elevated serum levels persist for years after exposure ends**, due to its long human half-life of several years.

** Based on default EPA chronic drinking water exposure assumptions: (2 L/day, 70 kg) or (2.4 L/day, 80 kg) and 20% Relative Source Contribution factor.*

Study of Communities with Drinking Water Exposure to PFOA

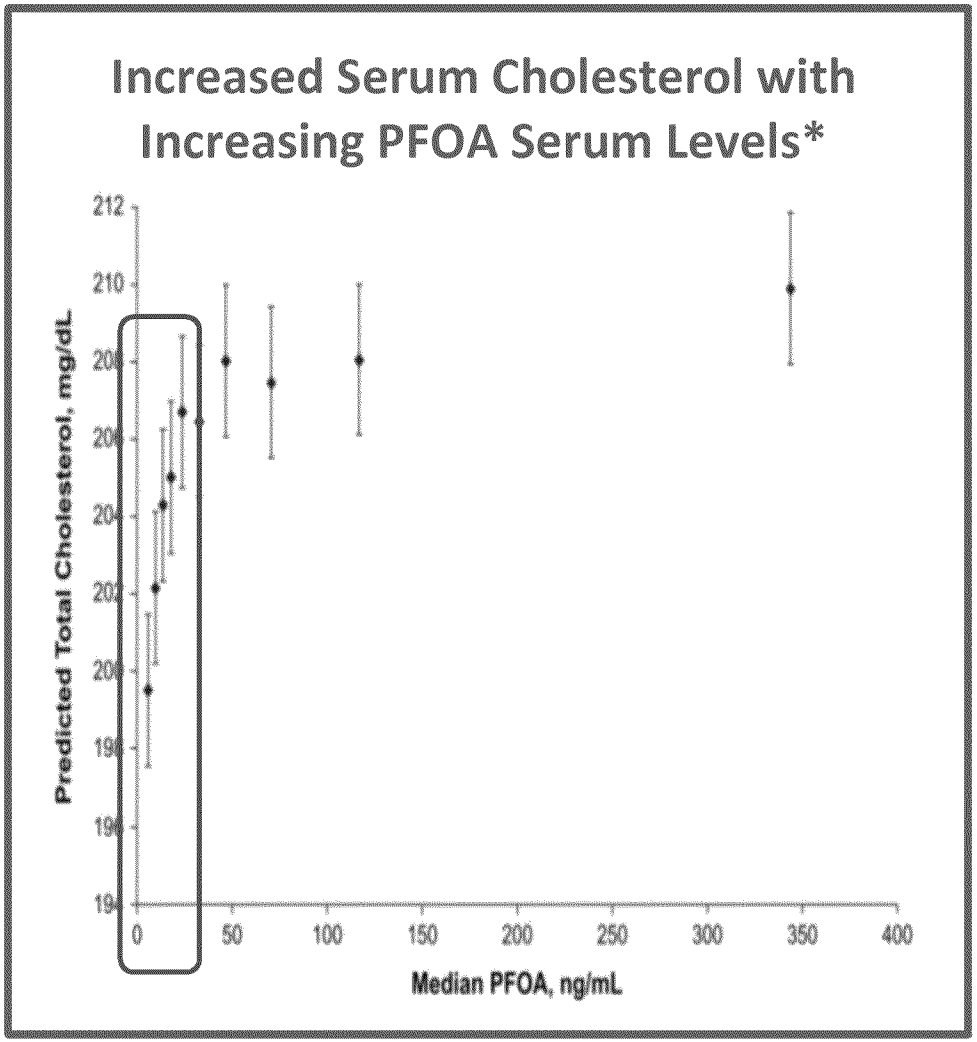
- Study of ~70,000 people in six OH and WV water districts exposed to PFOA in drinking water at **0.05 ppb** or higher for at least one year.
  - Drinking water levels in 3 of 6 districts, serving more than 40% of subjects, were **below or close to 0.1 ppb**.
  - Median serum PFOA was 28 ppb**, close to 20 ppb predicted from 0.1 ppb in drinking water.**
- Independent panel of epidemiologists found "probable links" with PFOA in drinking water and six diseases:
  - High cholesterol, thyroid disease, ulcerative colitis, pregnancy induced hypertension, testicular cancer, and kidney cancer.
- Associations also found with health endpoints not classified as diseases, including increased serum liver enzymes and others.
- Results for some endpoints generally consistent with numerous general population studies (for example – increased cholesterol, liver enzymes).
- For several effects, steepest dose-response at **lower serum levels, including levels below those expected from exposure to 0.1 ppb guidance.**

Data from Community Drinking Water Study

Water District	Drinking Water Levels *(ppb)**
Little Hocking	1.7-4.3
Lubeck	0.4-3.9
Tuppers	0.25-0.37
Belpre	**0.08-0.13**
Mason	**0.06-0.1**
Village of Pomeroy	**0.06-0.07**

From: Post et al., 2009

***Median serum level in study was 28 ppb.**



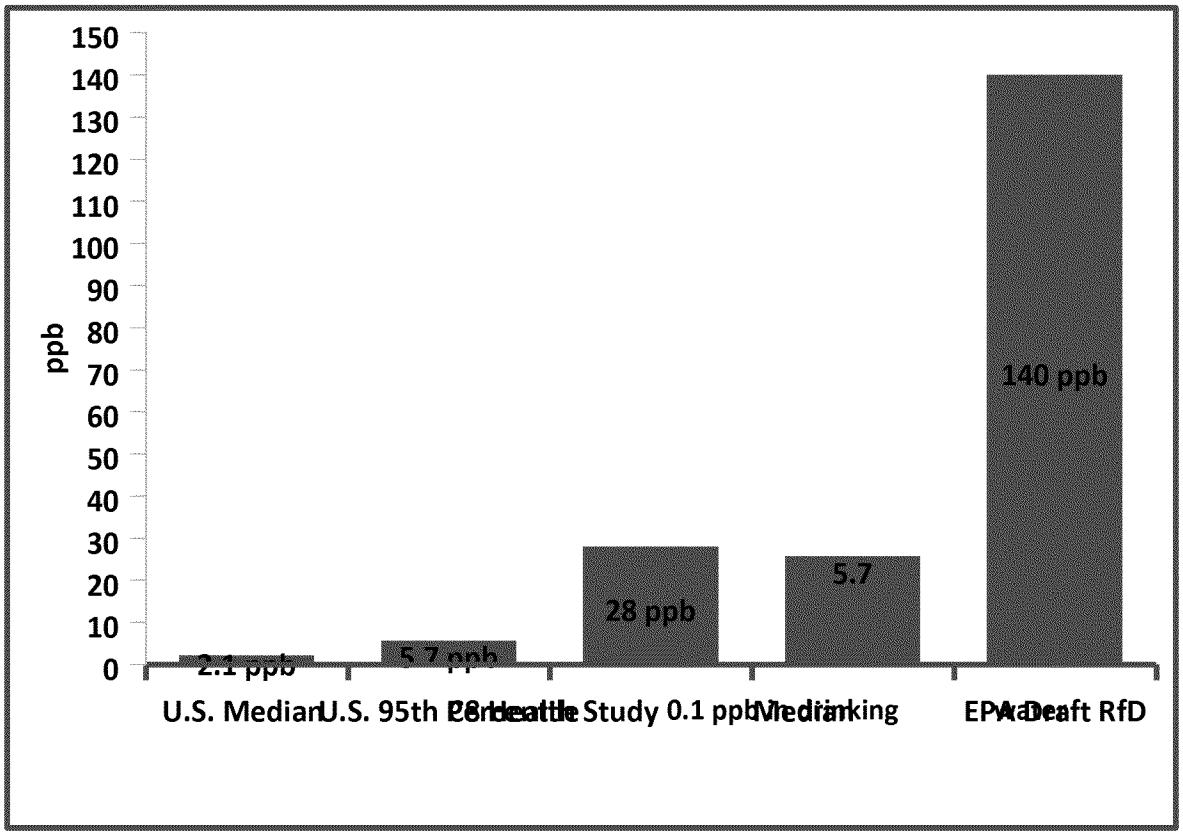
From: Steenland et al., 2009

Some Additional Epidemiological Findings from the General Population

- Recent systematic review by the UCSF Navigation Guide group concluded that PFOA exposure in the general population range is **associated with decreased birth weight** (Johnson et al., 2014).

- At least 5 studies have associated PFOA and other perfluorinated compounds (PFCs) with **decreased vaccine response** at general population exposure levels, although the specific vaccines with decreased response differed among studies and it was not possible to distinguish the impact of individual PFCs.

PFOA Serum Levels in U.S. Populations Compared to Serum Levels Predicted from Draft RfD and 0.1 ppb in Drinking Water



U.S. Median & 95th percentiles:
NHANES (2011-12).

C8 Health Study median:
Study of OH & WV communities with PFOA in drinking water.

0.1 ppb in drinking water:
Based on EPA OW clearance factor, EPA OW draft RfD, and EPA OW default drinking water exposure assumptions.

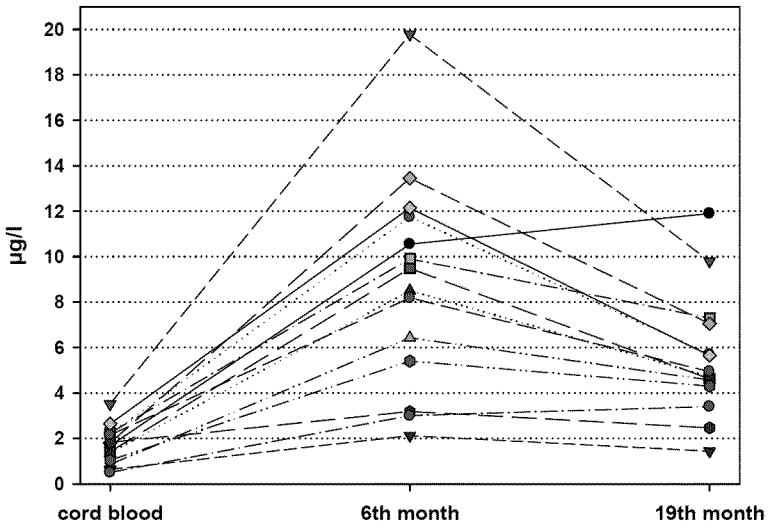
EPA draft RfD:
Based on EPA OW clearance factor.

Need to Consider Greater Exposures of Infants

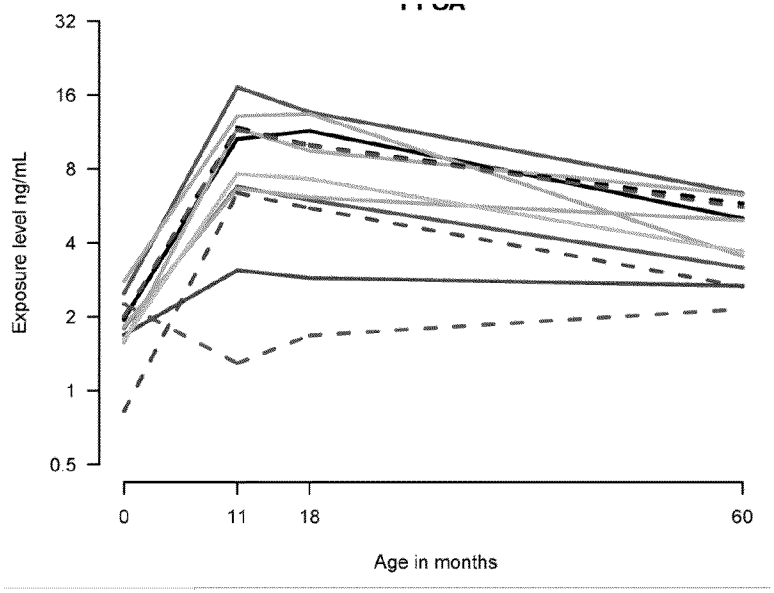
- Infants receive higher PFOA exposures on a body weight basis than do their mothers or other older individuals using the same drinking water.
  - Infants consume more fluid (breast milk and/or formula) than older individuals on a body weight basis.
  - PFOA concentrations in breast milk are similar or higher than in the mother's drinking water source.
- PFOA serum levels in infants (consuming breast milk or formula prepared with contaminated water) increase rapidly by several-fold after birth to levels much higher than would occur in older individual.
  - Peak serum levels in infants exposed to 0.1 ppb in drinking water (through breastmilk from exposed mother or prepared formula) are expected to be much higher than the serum level of 20 ppb predicted by the EPA OW clearance factor.
- Infants are considered a susceptible subpopulation for PFOA because of its potential to cause developmental effects.
- EPA OW should consider the higher peak serum levels in infants.

Greater Exposures of Infants

- At birth, serum PFOA levels in infants are similar to maternal serum levels.
- PFOA serum levels in infants (consuming breast milk or prepared formula) increase rapidly by several-fold after birth to levels that are much higher than in older individuals using the same drinking water.



Fromme et al., 2010



Mogensen et al., 2015

(Each line on the graphs represents data from a different subject.)

Need to Consider More Sensitive Toxicological Endpoints

- EPA OW states that an RfD based on increased liver weight is **protective for co-occurring toxicological effects** (p. 1-2, 5-23, 5-28).
- However, delayed mammary gland development from perinatal exposure, a clearly established effect of PFOA in mice, is a **more sensitive endpoint** than increased liver weight. Other more sensitive endpoints have also been reported.
- Structural changes in the mammary gland persist until adulthood and are considered to be permanent.
- EPA OW's rationale was that "more studies are needed to elucidate the mode of action" (p. 4-121).
  - This rationale does not appear to be consistent with EPA risk assessment guidance.
  - Default EPA risk assessment assumption is that **effects in animals are assumed relevant to humans unless shown otherwise**.
  - The rodent is considered to be a good model for human mammary gland development, and there is no information indicating that PFOA's effects on this endpoint are not relevant to humans.
- **Even if delayed mammary gland development is not used as the basis for the RfD, an uncertainty factor for an incomplete database appears warranted.**

Need to Consider More Sensitive Toxicological Endpoints (cont.)

- Developmental exposures to PFOA caused delayed mammary gland development in pregnant and lactating dams and/or female offspring in two strains of mice.
  - Reported in 9 separate studies in 5 publications.
  - Only one study (Albrecht et al., 2013) did not find this effect. NJDEP's review concluded that this study has several general problematic issues (details can be provided).
- When both endpoints were assessed in the same animals, delayed mammary gland development occurred at lower doses than increased liver weight.
- Delayed mammary gland development is dose-related, and a Benchmark Dose was developed for this effect (Post et al., 2012).
- IRIS preamble states: "*If an incomplete database raises concern that further studies might identify* ***a more sensitive effect****, organ system, or lifestage, the assessment may apply a* ***database uncertainty factor****.*"
  - As above, a large body of data shows that delayed MG development is a more sensitive endpoint than increased liver weight.

Uncertainty Factor (UF) for Subchronic-to-Chronic Exposure for Subchronic Studies Used in Draft RfD

- EPA OW states that the UF for subchronic-to-chronic exposure is not needed for the subchronic studies because Human Equivalent Doses (HEDs) used as the basis for the RfD "reflect steady state serum values" (p. 5-21).
- Recommend that EPA review this decision in conjunction with EPA risk assessment guidance.
  - The EPA IRIS Preamble states: *If a point of departure is based on subchronic studies, the assessment considers* ***whether lifetime exposure could have effects at lower levels of exposure****. A factor of 10 is applied to account for the uncertainty in using subchronic studies to make inferences about lifetime exposure.*
  - This UF is intended to protect from additional or more severe effects that can occur at lower doses with longer exposures, **not because steady state is not reached during the dosing period.**
- The subchronic-to-chronic UF is routinely used when subchronic studies are used as the basis for chronic RfDs.
  - This includes many chemicals which reach steady state well before the end of the subchronic study.

Additional Information on Time to Reach Steady State Serum Levels

- EPA OW states (p. 3-39) that the model indicates that a 30 year exposure is needed to reach steady state at the very high (several ppb) PFOA drinking water levels in Little Hocking, Ohio.
- However, at lower drinking water concentrations (0.06 - 0.13 ppb) more relevant to chronic drinking water guidance, serum levels close to steady state (70-80%) were reached after only one year of exposure (from data presented graphically by Seals et al., 2011).

*Animal-to-Human Dose Extrapolation for Cancer Slope Factor**

- EPA OW used the default animal-to-human dose extrapolation (body weight$^{3/4}$) to derive the cancer slope factor based on testicular tumors in a chronic rat study.
- Recommend that this default assumption be revisited in terms of EPA (2005) Guidelines for Carcinogen Risk Assessment.
- Animal-to-human extrapolation should be done by applying the rat-to-human pharmacokinetic adjustment factor of 73 (p. 5-21) used by EPA OW for non-cancer endpoints from rat studies.
  - This approach accounts for the higher internal dose (serum level) in humans than in rats from the same external dose.
- The resulting slope factor is **1.48 (mg/kg/day)$^{-1}$** which is **21-fold higher** than the slope factor of 0.07 (mg/kg/day)$^{-1}$ developed by EPA OW.

** Comments on cancer risk assessment were not included in NJDEP comments submitted on 2014 EPA OW Draft document.*

Animal-to-Human Dose Extrapolation for Cancer Slope Factor

EPA (2005) Guidelines for Carcinogen Risk Assessment state:

> *"As a default for oral exposure, a human equivalent dose for adults is estimated from data on another species by an adjustment of animal applied oral dose by a scaling factor based on body weight to the 3/4 power. ….. **Because the factor adjusts for a parameter that can be improved on and brought into more sophisticated toxicokinetic modeling when such data become available, they are usually preferable to the default option.**"*

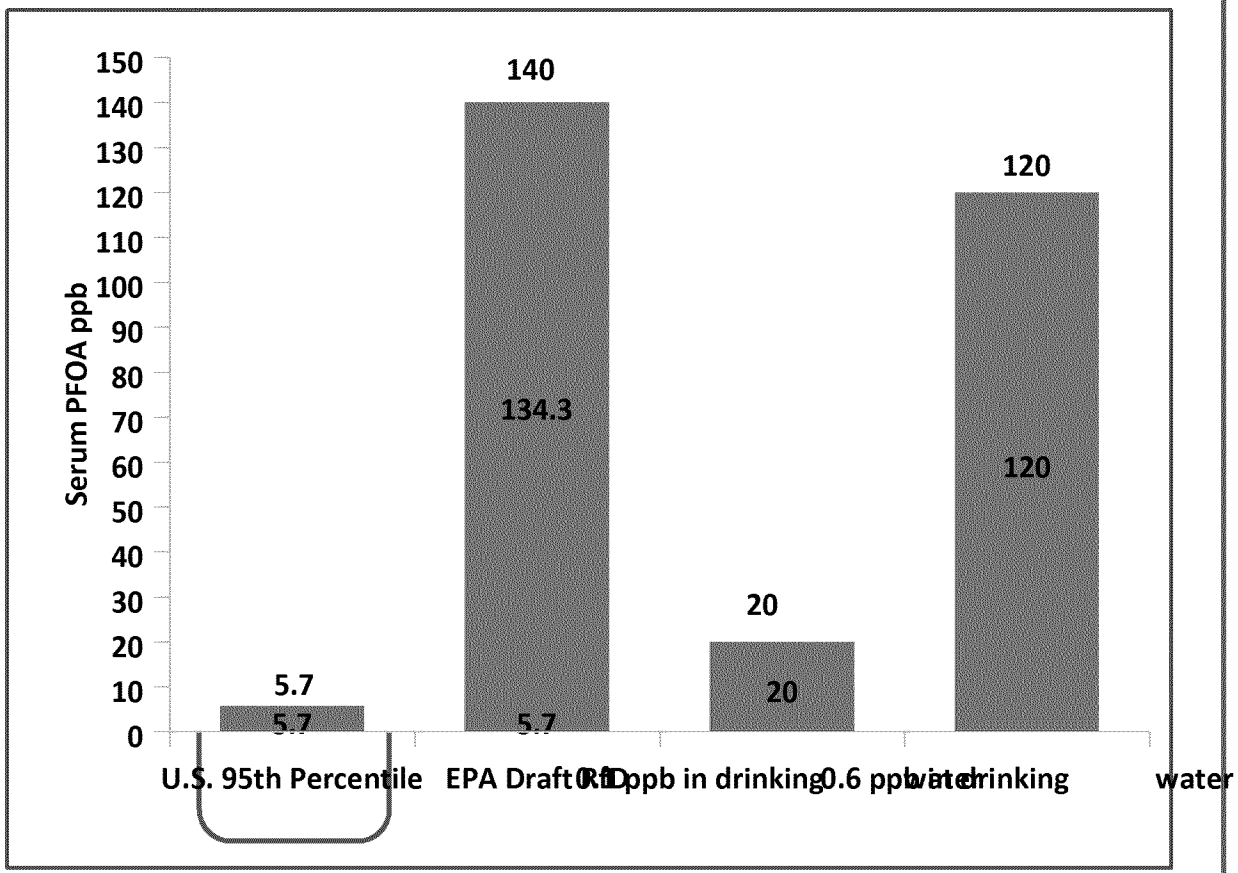
Use of Default Relative Source Contribution (RSC) Factor

- RSC is used in drinking water risk assessment to account for exposures from **non-drinking water sources** (e.g. food, air) such that total exposure will not exceed the RfD.
  - The EPA default RSC is **20%**. This assumes that 20% of the RfD comes from drinking water and 80% from non-drinking water sources.
  - EPA OW (2000) states that a chemical-specific RSC from 20% to 80% is used when chemical-specific exposure data are available.
- EPA OW used the default RSC of 20% to develop the 0.1 ppb guidance value.
  - Suggest this be reviewed because chemical-specific data are available.
- The chemical-specific RSC for PFOA (using the draft RfD) is **80%**.
  - This RSC is based on the "subtraction" method recommended by EPA OW (2000); this method is appropriate for PFOA.
  - Serum PFOA levels in the U.S. general population from NHANES reflect non-drinking water exposures and are used to derive the RSC.
- The drinking water level based on the draft RfD and the chemical specific RSC of 80% is 0.56 ppb (rounds to **0.6 ppb**).
  - An increased PFOA serum level of **120 ppb** is predicted from this exposure.
- This issue arises because the draft RfD results in a high serum level (140 ppb). Drinking water exposure to a large portion (80%) of this RfD also results in a high serum level.

Development of Chemical-Specific RSC

- A chemical-specific RSC based on the "subtraction" approach (EPA OW 2000 "Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health“) is appropriate for PFOA.
  - The 95th % PFOA serum level from NHANES (6 ppb) represents the upper bound for non-drinking water exposures in the U.S. general population.
  - The portion of the RfD allotted to exposure from drinking water is derived by subtracting the 95th percentile NHANES serum level (6 ppb) from the serum level predicted from the draft Reference Dose (140 ppb).
- Subtracting the current 95th % NHANES from the draft RfD (140 ppb) leaves **134 ppb** in serum allotted to drinking water exposure (**96% of the RfD, i.e. an RSC of 96%**).
  - *Note*: RSCs based on the subtraction method are dependent on the RfD value and will vary if the RfD is changed.
- An **RSC of 80%** is used, since 80% is the upper RSC value by policy (EPA OW, 2000).
- This subtraction approach was used in chronic drinking water risk assessments for PFOA by Maine Dept. of Health & Human Services and for PFNA by NJDEP.

Drinking Water Level Based on Draft RfD and Chemical-Specific RSC is 0.6 ppb



***U.S. 95th percentile*:**
Represents upper bound of non-drinking water exposure.

EPA Draft RfD:
Represents dose without appreciable risk from lifetime exposure. Only 4% of RfD can be assumed to come from **non-drinking water exposure**. Chemical-specific RSC is 96%.

0.1 ppb in drinking water:
Based on default RSC of 20% (assumes 80% of total exposure comes from non-drinking water sources).

0.6 ppb in drinking water
Based on chemical-specific RSC of 80%. Data indicate RSC of 96%, but upper value for RSC is 80% by policy.

Conclusions

- For the reasons discussed above, NJDEP is concerned that the draft RfD of 0.0002 mg/kg/day and a Lifetime Health Advisory of 0.1 ppb for PFOA are not sufficiently protective of public health.
- NJDEP hopes that its comments are helpful to EPA OW's efforts to develop an RfD and Lifetime Health Advisory that are scientifically sound and are protective of health risks from lifetime exposure to PFOA.
- Please feel free to contact us if you have any questions about our comments.

Thank you!

Additional NJDEP Comments

Additional NJDEP comments on the 2014 EPA OW draft PFOA document can be found in the NJDEP submission posted on *regulations.gov*

To: Burneson, Eric[Burneson.Eric@epa.gov]; Rodgers-Jenkins, Crystal[Rodgers-Jenkins.Crystal@epa.gov]; Donohue, Joyce[Donohue.Joyce@epa.gov]; Strong, Jamie[Strong.Jamie@epa.gov]
From: Downing, Jane
Sent: Wed 3/9/2016 2:31:24 PM
Subject: FW: PFOA - New Hampshire
NH PFOA Letter.pdf

Hi All

Consider this just FYI as the cases on PFOA plays out, including in VT and NH.

Let me know if any questions

Thanks

Jane

From: Szaro, Deb
Sent: Wednesday, March 09, 2016 8:07 AM
To: Olson, Bryan <Olson.Bryan@epa.gov>; Moraff, Kenneth <Moraff.Ken@epa.gov>; Lovely, William <Lovely.William@epa.gov>; Downing, Jane <Downing.Jane@epa.gov>
Subject: FW: PFOA - New Hampshire

fyi

From: Enck, Judith
Sent: Tuesday, March 08, 2016 5:12 PM
To: Szaro, Deb <Szaro.Deb@epa.gov>; Spalding, Curt <Spalding.Curt@epa.gov>
Subject: FW: PFOA - New Hampshire

Taft /

Robert A. Bilott / Partner
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From: Bilott, Robert A.
Sent: Tuesday, March 08, 2016 2:32 PM
To: 'thomas.burack@des.nh.gov'; 'ron.miner@mvdwater.org'
Cc: Enck.judith@Epa.gov
Subject: PFOA - New Hampshire

Please see attached. Thank you.

Taft/

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ROBERTA. BILOTT
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March 8, 2016

BY E-MAIL AND REGULAR U.S. MAIL

Tom Burak, Commissioner
New Hampshire Department of Environmental Services
29 Hazen Drive
P.O. Box 95
Concord, NH 03302-0095

Ron Miner, Superintendent
Merrimack Village District Water Works
2 Green Ponds Road
Merrimack, NH 03054

Re: Accuracy of New Hampshire/Merrimack Water PFOA Information

Commissioner Burak and Superintendent Miner:

Over the last 15 years, I have represented tens of thousands of individuals in various communities across the country who have been injured because of the contamination of their drinking water with PFOA. As a result of a 2005 settlement of the claims of approximately 70,000 such individuals exposed to PFOA-contaminated drinking water in Ohio and West Virginia, in which I served as class counsel for the residents, extensive investigation and study into the actual human health effects of community-level exposure to PFOA in drinking water was undertaken by an independent panel of scientists known as the "C8 Science Panel." Under the terms of that class action settlement, the C8 Science Panel spent over seven years and over $30 Million analyzing data from approximately 69,000 residents (including actual PFOA blood results from tens of thousands of such residents and decades of actual medical records and actual drinking water exposure histories) to determine which diseases

actually were linked to community resident exposures to PFOA in drinking water for as little as one year and at PFOA concentrations as low as 0.05 ppb.[1]

By the end of 2012, the independent C8 Science Panel had determined, after reviewing all of the data from the tens of thousands of community residents and all available scientific data (epidemiological and toxicological), that human exposure to even low levels (as low as 0.05 ppb) of PFOA in that community drinking water had a probable link to six serious diseases: 1) kidney cancer; 2) testicular cancer; 3) ulcerative colitis; 4) thyroid disease; 5) preeclampsia/pregnancy-induced hypertension; and 6) medically-diagnosed high cholesterol (hypercholesterolemia). The C8 Science Panel's final reports on each of these diseases (and links to the peer-reviewed, published papers confirming all of their findings and the scientific standards they used) are publicly-available through the C8 Science Panel's website: www.c8sciencepanel.org.

Thus, the New Hampshire Department of Environmental Services' ("DES'") representation to the public in its March 4, 2016, press release, (copy attached at Ex. A), that "[p]otential health effects from exposures to low levels of PFCs are not well understood" and that the available studies are "inconclusive," requiring "[f]urther research ... to determine whether PFCs can cause health changes in humans" is inaccurate, and ignores the vast wealth of published, peer-reviewed, and independent C8 Science Panel research. Likewise, DES' representations that EPA believes "that existing evidence is too limited to support a strong link between PFCs and cancer in people" and that there is a "lack of health effects studies" are not accurate. EPA's own Science Advisory Board recommended that PFOA be characterized as a "likely" human carcinogen *almost a decade ago* (even before the independent Science Panel cancer findings), and recent public presentations by EPA in connection with PFOA drinking water contamination in nearby Hoosick Falls, New York, reflect that EPA is well-aware of the published, peer-reviewed, independent Science panel work that confirmed probable links between PFOA drinking water exposures and both kidney cancer and testicular cancer. (*See* EPA Region 2 public presentation materials attached at Ex. B.) DES's public statements in this regard should, therefore, be modified accordingly.

DES and the Merrimack Village District Water Works (the "Village") also should correct their public representations[2] regarding the meaning and significance of the 0.4 ppb Provisional Health Advisory ("PHA") released by the United States Environmental Protection Agency ("EPA") in 2009. Comparing levels found in a public drinking water supply that may have been used by residents *for years* to the PHA is inappropriate. EPA has made clear, repeatedly, that the PHA was developed for use only in situations of ***acute, short term*** (only a few days or few weeks) of exposures to PFOA in drinking

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[1] More information on that settlement is available at: www.C8claim.com.
[2] The Village's most recent public statement on these issues, printed from its website, is attached at Ex. C.

water. Although we have been asking EPA to do so for more than fifteen years, to date, EPA has never released or adopted any standard or guideline to apply in situations where there may have been ***long term*** (more than a few days or weeks) exposures to PFOA in human drinking water. Moreover, both DES and the Village failed to point out in their public statements that EPA, as recently as January 28, 2016, advised the community near Hoosick Falls, New York, not to drink water containing more than 0.1 ppb PFOA. (*See* attached Ex. D.) Thus, at a minimum, EPA has dropped its 0.4 ppb guideline for short-term exposures down to 0.1 ppb while it continues to work on a long-term exposure guideline. In the meantime, other states have adopted even more protective PFOA drinking water guidelines. For example, in Vermont, the most recent State to evaluate PFOA drinking water guidelines, the State has advised residents not to drink water containing more than 0.02 ppb PFOA. (*See* attached Ex. E.) Based on the sampling date referenced in DES's and the Village's public statements, it appears that the level found in the Village's water – 0.03 ppb – exceeds that latest drinking water guideline.

Given the biopersistent nature of PFOA – meaning that even the tiniest, barely detectable amounts in drinking water will build up in the human body over time – and the finding of adverse health effects in laboratory studies at lower and lower dose levels, many scientists are questioning how a "safe" level can even be set for PFOA in drinking water. (*See e.g.,* attached Ex. F.) Regardless, while such research and work is underway, the public should not be led to believe that it is perfectly "safe" to drink water containing as much as 0.4 ppb PFOA or that there is no evidence of adverse health effects among people exposed to such levels of PFOA (or lower) in their drinking water.

We would be happy to discuss any of this publicly-available data with you in further detail. Thank you.

Sincerely yours,

Robert A. Bilott

Attachs.
cc: Judith A. Enck (US EPA Region 2) (w/attachs.)

EXHIBIT A

Media Center

FOR IMMEDIATE RELEASE:
DATE: March 4, 2016
CONTACT: Jim Martin, 603 271-3710

NHDES, Merrimack Village Water District, and Saint-Gobain Performance Plastics Investigating Perfluorochemical Found in Drinking Water

Concord, NH - The New Hampshire Department of Environmental Services (NHDES) is today announcing that it is working with the Merrimack Village District Water System (MVDWS) and Saint-Gobain Performance Plastics to investigate the potential presence of perfluorochemicals (PFCs) in drinking water in Merrimack. Late last week, Saint-Gobain notified NHDES that perfluorooctanoic acid (PFOA) was detected at low levels [0.03 micrograms per liter (µg/L)] in samples taken from four water faucets within their Merrimack facility, which is served by the Merrimack Village District Water System. Due in part to the lack of health effect studies, the United States Environmental Protection Agency (EPA) has not set an enforceable drinking water standard for PFOA. EPA's Office of Water has, however, established a Provisional Health Advisory of 0.4 micrograms per liter (µg/L) for PFOA. Sampling to date in Merrimack has not identified PFOA at levels above the Provisional Health Advisory level. Provisional Health Advisories reflect reasonable, health-based hazard concentrations above which action should be taken to reduce exposure to unregulated contaminants in drinking water. Currently, this Provisional Health Advisory is under review and is expected to be revised this spring.

PFOA has been detected in the Hoosick Falls, NY, water supply and in some private wells in North Bennington, VT, near other Saint-Gobain facilities. Because materials containing PFOA have been used at the plant in Merrimack and out of an abundance of caution, Saint-Gobain voluntarily tested the water at its Merrimack facility and intends to test the groundwater at its facility. NHDES will oversee this investigation and is also working closely with MVDWS and the Town of Merrimack to determine if PFOA is present in any of the MVDWS wells that cumulatively serve 25,000 customers in Merrimack. NHDES will also investigate private wells in the vicinity of the Saint-Gobain facility and the MVDWS wells to determine whether they have been affected.

PFCs are a family of manmade chemicals that have been used for decades as an ingredient to make products that resist heat, oil, stains, grease, and water, such as non-stick cookware, weather resistant outdoor clothing and gear, and stain resistant carpeting. Many chemicals in this group, including PFOA, are commonly present in the environment and do not break down easily. According to the company, polytetrafluoroethylene (PTFE), which contains PFOA, has been used at the plant in Merrimack, by Saint-Gobain and previous facility owners.

Studies have shown that nearly all people have some level of PFCs in their blood. Potential health effects from exposure to low levels of PFCs are not well understood. To date studies have been inconclusive as to whether PFCs can affect growth and development, hormone levels including thyroid hormone, liver enzyme levels, cholesterol levels, immune function or occurrence of certain types of cancer. Further research is needed to determine whether PFCs can cause health changes in humans. The EPA states that existing evidence is too limited to support a strong link between PFCs and cancer in people.

The EPA has also done nation-wide sampling for PFCs as a preliminary step toward determining the need for national drinking water standards that would be enforceable under the Safe Drinking Water Act. In 2014, the wells in Merrimack that provide water to Saint-Gobain were tested and PFOA was detected once at 0.042 micrograms per liter (µg/L)(which is below the Provisional Health Advisory level), but was not detected in a subsequent sample. MVDWS is resampling those and their other wells in response to the detection of PFOA in water samples taken at Saint-Gobain. This testing is in addition to the routine water quality monitoring performed by MVDWS.

NHDES will work closely with MVDWS and the Town of Merrimack to ensure that residents are promptly informed of the results of the MVDWS well tests and any additional information resulting from the planned groundwater investigations being conducted by Saint-Gobain and NHDES. For additional information please contact Jim Martin at (603)-271-3710.

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NH Department of Environmental Services | 29 Hazen Drive | PO Box 95 | Concord, NH 03302-0095
(603) 271-3503 | TDD Access: Relay NH 1-800-735-2964 | Hours: M-F, 8am-4pm

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EXHIBIT B

EPA-HQ-2016-005679 06/14/2017

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

Judith Enck

EPA Region 2 Administrator

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