In utero exposure to perfluorooctanoate (PFOA) or perfluorooctane sulfonate (PFOS) did not increase body weight or intestinal tumorigenesis in multiple intestinal neoplasia (Min/+) mice

by

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Abstract

We examined whether perfluorooctanoate (PFOA) or perfluorooctane sulfonate (PFOS) had obesogenic effects and if they increased spontaneous intestinal tumorigenesis in the mouse model C57BL/6J-Min+/+ (multiple intestinal neoplasia) after in utero exposure. The dams were exposed to PFOA or PFOS (0.01, 0.1 or 3.0 mg/kg bw/day) by po gavage on GD1-17. The Min+/+ and wild-type offspring were terminated at week 11 for examination of intestinal tumorigenesis or at week 20 for obesogenic effect, respectively. Body weights of the dams and pups were recorded throughout life. Food intake was determined at week 6 and 10. Blood glucose (non-fasted) was measured at week 6 and 11. No obesogenic effect of PFOA or PFOS was observed up to 20 weeks of age. PFOA or PFOS did not increase the incidence or number of tumors in the small intestine or colon of the Min+/+ mice or affect their location along the intestines. Feed intake was not affected. There were some indications of toxicity of PFOA, but not of PFOS. There was lower survival of pups after 3.0 mg/kg PFOA, lower body weight in pups after 3.0 and possibly 0.1 mg/kg PFOA, and increased relative liver weight after 0.01 and possibly 0.1 mg/kg PFOA. Plasma glucose was lower after 0.01 and 0.1 mg/kg PFOA. In conclusion, exposure to PFOA and PFOS in utero with the doses used did not have obesogenic effect on either Min/+ or wild-type mice, at least not up to 11 or 20 weeks of age, nor increased intestinal tumorigenesis in Min/+ mice.

Keywords: intestinal tumorigenesis; in utero exposure; Min/+ mouse; obesogen; perfluorooctane sulfonate; perfluorooctanoate.

Abbreviations: Apc, adenomatous polyposis coli; AUC, area under the curve; DIO, diet-induced obesity; DOHaD, developmental origins of health and disease; EDC, endocrine disrupting chemical; FAP, familial adenomatous polyposis; GD, gestational day; Min, multiple intestinal neoplasia; PFAA, perfluoroalkyl acid; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; PPAR, peroxisome proliferator-activated receptor; PND, postnatal day.
1. Introduction

Overweight and obesity have increased markedly over the past two-three decades, in parallel with increasing use of chemicals. Genetic variation contributes to an individual’s propensity to develop obesity, but genetic mutations cannot account for the rapid increase in obesity rates over such a short time period. Other factors in the environment, such as nutrition and chemicals, are being considered as contributing to the obesity epidemic (Heindel and vom Saal, 2009). The obesogen hypothesis (Grün and Blumberg, 2006) proposes that certain chemicals (natural, pharmaceutical or xenobiotic) are able to promote weight gain and obesity, especially when exposed during gestation (Heindel and vom Saal, 2009). Obesogens promote obesity by increasing the number of fat cells or the storage of fat into existing fat cells (Janesick and Blumberg, 2011). Obesogens can also act indirectly by altering the basal metabolic rate, by shifting the energy balance to favour storage of calories, alter lipid metabolism and alter hormonal control of appetite and satiety (Janesick and Blumberg, 2011; Schug et al., 2011). Many known obesogens are endocrine disrupting chemicals (EDCs) that may mimic or block hormones and disrupt the normal function of the body (De Coster and van Larebeke, 2012; Elobeid and Allison, 2008; Grün and Blumberg, 2009). EDCs may also be implicated in cancer (Soto and Sonnenschein, 2010).

The environmental EDCs studied in this work are the perfluoroalkyl acids (PFAAs) perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS). These substances are man-made fluorinated organic compounds used in consumer goods such as clothing, carpeting and food packaging materials, and as surfactants in industry, due to their grease and water-repellant properties (Buck et al., 2011; Post et al., 2012). PFAAs have carbon backbones of varying length, where hydrogen is substituted with fluorine and there is a functional group, which is a carboxylic acid and sulfonic acid for PFOA and PFOS, respectively (Buck et al., 2011). The
stability of the carbon-fluorine bond makes them wide-spread in the environment and in wildlife, and in humans (Lau et al., 2007; Post et al., 2012). PFAAs are readily absorbed, not known to be metabolized, and are poorly eliminated, with estimated half-lives in humans at 3.8 years for PFOA and 5.4 years for PFOS (Olsen et al., 2007; Post et al., 2012). The most common route of exposure is likely oral intake from diet and drinking water, and for infants via breast milk, while inhalation and dermal absorption are less important (EFSA, 2008).

In addition to their hormonal effects, animal studies have demonstrated developmental toxicity, reproductive toxicity, hepatotoxicity, neurotoxicity, immunotoxicity and tumorigenicity of the perfluorinated compounds (Johansson et al., 2008; Kennedy et al., 2004; Lau et al., 2004). Hepatocellular adenomas, testicular Leydig cell adenomas and pancreatic acinar cell adenomas have been reported in rats after exposure to PFOA in the diet for 2 years (Biegel et al., 2001; Butenhoff et al., 2012b). A 2-year study of PFOS given in the diet to rats reported hepatocellular and thyroid follicular cell adenomas and mammary fibroadenomas/adenomas (Butenhoff et al. 2012a). As far as we know, no tumorigenicity studies have been reported on mice, and no tumorigenic effects have been reported on the intestines of rats or mice, with PFOA or PFOS. PFOA increased body weight in female CD-1 mice after in utero exposure (Hines et al., 2009).

Earlier studies suggested that fetal nutrition plays an important role in development of diseases later in life (Barker and Osmond, 1986), which led to the “developmental origins of health and disease” (DOHaD) paradigm (Gluckman et al., 2007). Animal studies have documented that the in utero and neonatal developmental periods comprise “critical windows”, not just for nutrition factors, but also for environmental chemicals (Heindel and vom Saal, 2009).
Overweight and obesity are considered to be compelling risk factors for various cancers, including colorectal cancer (Calle and Kaaks, 2004). The C57BL/6J (B6) wild-type mouse strain is used as a diet-induced obesity (DIO) model. The C57BL/6J-Apc\textsuperscript{Min/+} (multiple intestinal neoplasia) mouse has a heterozygote mutation in the tumor suppressor gene adenomatous polyposis coli (Apc) (Moser et al., 1990; Su et al., 1992), and is therefore a model for both the inherited disorder familial adenomatous polyposis (FAP) and sporadic colorectal cancer in humans, having the same mutation in their APC gene. The Min/+ mouse is a sensitive model in which to test whether chemicals can affect intestinal tumorigenesis. In this study, we exposed C57BL/6J-Min/+ mice and their wild-type siblings \textit{in utero} to examine in the same model whether the environmental contaminants PFOA or PFOS had obesogenic effect, and if they increased spontaneous intestinal tumorigenesis.
2. Materials and methods

2.1. Environmental contaminants

The perfluoroalkyl acids (PFAAs) studied in this experiment were perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS). Perfluorooctanoic acid, ammonium salt, CAS no. 3825-26-1, and perfluorooctane sulfonic acid, potassium salt, CAS no. 2795-39-3, both with ≥98% purity, were purchased from Sigma-Aldrich Norway AS (Oslo, Norway). Water (Aqua B. Braun, B. Braun Melsungen AG, Melsungen, Germany) was used as vehicle. Both PFOA and PFOS were completely dissolved after agitation in an ultrasound water bath for a few minutes. When preparing the PFOA and PFOS solutions two days before start of the exposure, the concentrations were adjusted for conversion of salt to base, but not for <100% purity. The solutions were made separately for experimental blocks 1 and 2.

2.2. Determination of stability of PFOA and PFOS solutions

The stability of PFOA and PFOS in solution was tested by 9 repeated analyses during 9 weeks of concentrations of 2, 20, 200 and 600 ng/µl PFOA or PFOS. The solutions were analysed at the Norwegian Institute of Public Health by column-switching, isotope dilution LC-MS/MS methodology (Haug et al., 2009). PFOA showed hardly any change (<0.5%) in concentration, whereas PFOS showed a 24-32% decrease in concentration during this period in the four concentration levels tested (data not shown). During the first 3 weeks of this period, the concentrations of PFOS decreased 15-27%. During the 19 days from preparation to end of exposure in both experimental blocks 1 and 2, PFOA were stable, whereas PFOS may have decreased correspondingly.

2.3. Determination of background levels of PFOA and PFOS in water and feed
PFOA and PFOS were analyzed in various feed and drinking water given to the mice, as well as in the water used as vehicle for the PFOA and PFOS solutions. The analyses were done at Man-Technology-Environment (MTM) Research Centre, School of Science and Technology, Örebro University, Örebro, Sweden. Previously validated in-house methods were used for feed and ISO-25101 was used for water. All samples were analysed on an Acquity UPLC/Quattro Premier MS/MS, from Waters Corporation (Milford, MA, USA). The feed samples (approximately 40 g) were analysed using a modified version of the method previously described by Kärrman et al. (2009). Methods were controlled and the quality was assured by assessment of recoveries for each sample, and monitoring one or two qualifier ions. Additional quality assurance is the participation in international comparison studies, including the 2011 interlaboratory study on food and environmental samples (IVM, Free University of Amsterdam). Results above limit of detection (LOD) are reported (Table 1). Recoveries between 50 and 120% are considered acceptable and were achieved for all samples, except for PFOS in one feed sample, where it was 47%. LOD is based on the signal found in the procedural blank.

2.4. Mating and treatment of the mice

Wild-type C57BL/6J-Apc\(^{+/-}\) females (JAX™ Mice Stock Number 000664) (\(n=104\) in experimental block 1 and \(n=100\) in experimental block 2) were purchased from Charles River Laboratories International Inc., Sulzfeld, Germany, and housed in air flow IVC racks (Innovive Inc., San Diego, CA, USA) in 100% PET plastic disposable cages on Nestpak Aspen 4HK bedding (Datesand Ltd., Manchester, UK) in a room with 12-h light/dark cycle, and controlled humidity (55 ± 5%) and temperature (20 - 24°C). The room did not contain any male mice. Effort was made to synchronize the females in their estrous cycle by adding dirty bedding material from male cages to the female cages the last three days before mating.
C57BL/6J-Apc$^{Min/+}$ (multiple intestinal neoplasia) males were bred at The Norwegian Institute of Public Health, Oslo, Norway, by mating the C57BL/6J-Apc$^{+/+}$ females with C57BL/6J-Apc$^{Min/+}$ males originally purchased from The Jackson Laboratory (Bar Harbor, ME). Two types of diet were used in this study. The males were given a standard maintenance diet (SDS RM1 (E), from SDS Special Diet Services (Essex, UK) from weaning. In preparation for mating both female and male mice were given the breeding diet 2018 Teklad Global 18% Protein Rodent Diet, from Harlan Industries Inc. (Indianapolis, IN, USA), and the females were on this diet until weaning of the pups. After weaning, the pups were given the standard maintenance diet SDS RM1 (E). Water and diet were given to all mice ad libitum. When the females were 6-7 weeks of age, one female and one Min$^/+$/+ male with previous mating experience were housed together on gestational day 0 (GD0). The treatment of the dams with po gavage of PFOA and PFOS started the day after (GD1) and continued daily until GD17. The females were weighed daily to determine the dose since their body weight changed during this period because of the pregnancy. All the females in the experimental block were treated simultaneously. The females were checked for copulatory plugs twice a day during the presence of the males in the cages (one week), thereafter the dams were housed individually. However, this method was found to be very unreliable, since some dams with observed plugs were not pregnant and some dams where a plug was not observed were pregnant. Therefore, day of conception was later determined by counting 21 days backwards from day of delivery. Days to conception, number of pups born, time of death of litters etc. was recorded to see if PFOA or PFOS affected the reproduction and success of breeding (Tables 2 and 3).

The offspring were housed as a litter per cage after weaning, with females and males separated. All females had company (up to 5 mice per cage). Some wild-types males in all treatment groups were housed alone after termination of their Min$^/+$ littermates at week 11,
since severe fighting in this strain prohibits relocation of grown males together. All mice in each surviving litter were included in the experimental groups.

Genotyping of the offspring for the Apc gene was performed with allele-specific polymerase chain reaction (PCR) using DNA extracted from ~2 mm² samples obtained by ear puncture for identification of individual mice at weaning and kept on ice. The samples were suspended in 60 µl TE-buffer with SDS (10 mM Tris pH 7.4, 0.1 mM EDTA pH 8.0, 0.05% SDS) and incubated at 95°C for 10 min. Then aliquots of 6 µl of 10 mg/ml Proteinase K (Sigma-Aldrich Corp., St. Louis, MO, USA) were added and the samples incubated at 56°C overnight. Finally, the samples were incubated at 95°C for 10 min to inactivate the enzyme and stored at -20°C until PCR amplification. The PCR reactions for genotyping of Apc status were carried out with a BIO-RAD iCycler or a BIO-RAD S1000 Thermal cycler (BIO-RAD, Hercules, CA, USA) as follows. Genomic DNA (5 µl of 1:100 dilution of isolated DNA) was amplified in a 10 µl reaction volume per sample, which contained final primer concentration of 0.2 µM MAPC-9 (5’-GCC ATC CCT TCA CGT TAG-3’), 0.8 µM MAPC-MT (5’-TGA GAA AGA CAG AAG TTA-3’), and 0.4 µM MAPC-15 (5’-TTC CAC TTT GGC ATA AGG C-3’), purchased from Eurofins MWG Operon (Ebersberg, Germany), 0.2 mM each of dCTP, dGTP, dTTP and dATP (Promega Corp., Madison, WI, USA), 2.5 mM MgCl₂, 1x buffer II (10 mM Tris-HCl, pH 8.3, 50 mM KCl, both from Applied Biosystems (Foster City, CA, USA) and 0.017 U GoTaq polymerase (Promega Corp.). The amplification conditions were 3 min at 94°C, before 36 cycles at 94°C for 15 s, 54.5°C for 15 s and 72°C for 20 s, followed by a final extension at 72°C for 7 min. The PCR products were visualized by electrophoresis through a 2.2% agarose gel (Lonza FlashGel system, Lonza, Basel, Switzerland). The wild-type mice were identified as having a 600 bp PCR product and the heterozygous Apc<sup>Min/+</sup> mice as having
the 600 bp product and a 300 bp product. The reagents were purchased from Sigma-Aldrich Corp., Fluka (Buchs SG, Switzerland) or Promega Corp., if not stated otherwise.

All mice in the experimental groups with $Apc^{Min/+}$ genotype were terminated at 11 weeks of age, before onset of serious anemia caused by their tumors, and used for study of intestinal tumorigenesis and obesogenic effect. The mice with wild-type ($Apc^{+/+}$) genotype were terminated at 20 weeks, in order to see if the treatment with PFOA and PFOS had an obesogenic effect at older age. The mice were anesthetised by ZRF cocktail (containing 3.3 mg zolazepam, 3.3 mg tiletamine, 0.5 mg xylazine and 2,6 µg fentanyl per 1 ml 0.9% NaCl solution) and sacrificed by cervical dislocation.

The experiment reported in this paper was performed in conformity with the laws and regulations for animal experiments in Norway and were approved by the Norwegian Animal Research Authority in Norway.

2.5. Choice of doses of PFOA and PFOS

The doses of PFOA used in this study were 0.01, 0.1 or 3.0 mg/kg bw/day. The doses were reported in published literature to affect body weight without having major effects on pup development or survival. The doses of 0.01 and 0.1 mg/kg of PFOA given on GD 1-17 increased body weight observed from age 10-19 weeks and increased serum leptin and serum insulin at age 21-33 weeks in female CD-1 mice, whereas 0.1 mg/kg PFOA gave a nearly significant increase in blood glucose over control ($P=0.06$) at 20 min post-glucose challenge in 15-16 weeks old mice (Hines et al., 2009). The dose 3.0 mg/kg PFOA was reported to significantly affect the neonatal body weight (up to 25 days) and increased body weight of both genders at 48 weeks and older, without affecting pup survival on postnatal day (PND) 9.
although 3 mg/kg was reported to give some developmental effect (ossifications of limbs) (Lau et al., 2006). Higher doses than 3.0 mg/kg PFOA were not used in our experiment, since 5 mg/kg PFOA on GD 1-17 gave significantly increased number of dams with full litter resorptions in CD-1 mice (Lau et al., 2006).

The doses of PFOS used in this study were also 0.01, 0.1 or 3.0 mg/kg bw/day. Postnatal survival (up to 25 days) and neonatal body weight (up to 35 days) were not affected by 1 or 5 mg/kg PFOS after exposure daily on GD1-18 in CD-1 mice, whereas relative liver weight was increased by these doses (Lau et al., 2003). The % of live fetuses was not affected by 1 or 10 mg/kg PFOS, but was affected after 5 mg/kg exposure daily on GD1-17 in CD-1 mice (Thibodeux et al., 2003). Body weights of fetuses were not affected by 1 and 5 mg/kg, but decreased with 10 mg/kg and higher. Effects of the lower doses of PFOS (0.1 and 0.5 mg/kg) tested in these mice were not reported (Thibodeux et al., 2003).

2.6. Exposure to PFOA and PFOS

In experimental block 1, 104 females (age 7-8 weeks) were randomly divided into five groups given the vehicle distilled water, 0.1 mg/kg PFOA or PFOS or 3.0 mg/kg PFOA or PFOS by po gavage (<10 µl/g bw) from GD1-17 (n=20-21 in each group, see Table 3). Since this first experiment resulted in too few offspring for statistical analyses in some of the experimental groups when dividing them by gender and genotype, the experiment was repeated (experimental block 2) with lower doses of PFOA and PFOS. In experimental block 2, 100 females (aged 9-10 weeks) were randomly divided into five groups given the vehicle water, 0.01 mg/kg PFOA or PFOS or 0.1 mg/kg PFOA or PFOS. The po gavage was chosen as route of administration in order to mimic human exposure, which is mainly through food and drinking water. The po gavage was given at the same time each day, starting from approximately 9.00 a.m. The order of
treatment was first the vehicle water and then increasing doses of PFOA and PFOS. Different gavage tubes and syringes were used for each substance and dose.

2.7. Recording of feed intake

A toxic effect of PFOA or PFOS could lead to lower feed intake, or an obesogenic effect of these compounds could be caused by increasing the feed intake. Decreased or increased feed intake by the exposed mice could again affect the body weight of the mice. The amount of feed ingested for one week’s duration was recorded for each litter consisting of both Min/+ and wild-type mice at weeks 6-7 and weeks 10-11, representing phases of rapid and slower growth rates, respectively, in both experimental blocks 1 and 2, and for the wild-type mice at weeks 19-20 in experimental block 2. The feed consumed is given as g feed/g body weight per day for each gender separately.

2.8. Recording of body weight and body mass index (BMI)

Body weight of the dams was recorded daily from GD1 to GD18, while the pups were weighed every third day from day 3 after birth until weaning on day 21 in experimental block 1 and on day 25 in experimental block 2. After weaning, the mice were weighed weekly until termination at week 11 (Min/+ mice) or week 20 (wild-type mice). The body weight data were analysed as area under the curve (AUC) for the various periods of life. At termination, the nasoanal lengths of the mice were recorded. The BMI was calculated as body weight divided by the nasoanal length squared (g/cm²).

2.9. Blood glucose measurements

Blood glucose levels (non-fasted) were measured with the glucometer FreeStyle Freedom Lite (Abbott Diabetes Care Inc., Alameda, CA, USA) by puncture of the saphenous vein in the
hind leg in Min/+ and wild-type mice at 6 and 11 weeks of age, and in wild-type mice at termination at 20 weeks of age.

2.10. Absolute and relative organ weights

The liver and spleen were dissected and weighed at termination, and the data are presented as absolute weight (in g), or as relative weight (in %) calculated as absolute weight/BW x 100.

2.11. Scoring of small intestinal and colonic tumors

Colon and small intestine were removed separately, rinsed in ice-cold phosphate-buffered saline (PBS) and slit open along the longitudinal axis. Intestinal tissues were then spread flat between sheets of filter paper, and fixed for at least 48 h in 10% neutral buffered formalin prior to staining with 0.2% methylene blue purchased from Sigma-Aldrich Norway AS. Number, diameter and localization of tumors in the small intestine and colon were scored by transillumination in an inverse light microscope at a magnification of x20. The scoring was done in order of consecutive mouse numbers unaware of their treatment. Diameters of tumors were scored with an eyepiece graticule. Tumor position along the intestines was registered in cm from the stomach. For each experimental group, incidence of tumors (number of mice with tumors/number of mice in the group), tumor number (mean number of tumors/mouse ± SD) and tumor diameter in mm (mean of all tumors in all mice in the group ± SD) were calculated, for small intestine and colon separately.

2.12. Determination of PFOA and PFOS concentrations in serum of exposed mice

Serum samples were obtained from two pregnant dams from each experimental group on GD18, 24 h after the last gavage, in experimental block 1 (Table 4). Since the number of surviving litters was rather low also in experimental block 2, samples from one pregnant dam
were obtained at the same time point only from the new dose groups given 0.01 mg/kg of PFOA or PFOS. Serum samples were obtained from two dams from each treatment group, two days after weaning of the pups on day 21 after birth (on PND23) in experimental block 1, and 1-3 days after weaning on day 25 after birth (on PND26-28) in experimental block 2. Serum samples from two pups were obtained from the vehicle and the 0.1 mg/kg PFOA and PFOS groups 4-6 days after weaning on day 21 (on PND25-27) in experimental block 1, and from each of the 0.01 mg/kg PFOA and PFOS groups one day after weaning on day 25 (on PND26) in experimental block 2.

Concentrations of PFOA and PFOS in serum from the mice were determined by column-switching, isotope dilution LC-MS/MS methodology at the Norwegian Institute of Public Health as previously described (Haug et al., 2009), except that only 10 µl serum was used due to expected high concentrations. The limit of quantification (LOQ) for PFOA and PFOS was 0.05 ng/ml serum. The quality of the analytical procedure was monitored by analyzing in-house quality control samples (n=2). The laboratory also regularly participates in an interlaboratory comparison study organized by Institute national de santé publique du Québec (Canada) for the Arctic Monitoring and Assessment Programme (AMAP). The PFOA and PFOS results in serum samples from AMAP interlaboratory ring test for persistent organic pollutants in human serum, round 1, 2013, had z-score values lower than 1.10 for all three samples. Procedure blanks analysed along with the samples did not contain PFOA and PFOS above LOQ.

2.13. Statistical analyses

In experiments where the exposure is via the dams, using litter instead of individual mice as the statistical unit is often regarded as the most appropriate way of analysing the data.
However, we have experienced through numerous experiments for many years with the Min/+
mouse model, that there are great variations in number of spontaneous or chemically
induced intestinal tumors also among siblings from the same litter (up to 320% variation in
number of small intestinal tumors between littermates with the same gender in this
experiment). Obviously, unknown factors can give rise to inter-sibling variation in utero
and/or other factors than only the in utero conditions affect this as counted at termination 11
weeks later. Therefore, both approaches were used in the present work. When statistically
significant effects were found on the individual level, i.e. as mean of all mice in the treatment
groups, the data was also re-analyzed with mean of the litter as the statistical unit, and both
results were reported.

When no consistent differences were found for an end point between the data from
experimental blocks 1 and 2, the data from both blocks were analysed together, unless stated
otherwise.

The data are presented as mean ± SD and were analysed using SigmaPlot 12.3 (Systat
Software Inc., San Jose, CA, USA). For data on number and diameter of small intestinal and
colic tumors, AUC for body weight development, terminal BMI, blood glucose levels,
organ weights and feed intake, analysis of variance (ANOVA) was used with an appropriate
multiple comparison procedure (as suggested by the programme). When testing the influence
of a single factor, one-way ANOVA with the Holm-Sidak test for multiple comparisons was
used for parametric data or the Kruskal-Wallis ANOVA on ranks with Dunn’s test for
multiple comparisons was used for non-parametric data. When testing the influence of two or
three factors together the data were analysed by two- or three-way ANOVA, respectively,
with the Holm-Sidak test for multiple comparisons. AUC for body weight development was
calculated using the macro in SigmaPlot 12.3, which integrates the area under the curve using the trapezoidal rule. The incidence of pregnant dams and the incidence of colonic tumors were analysed by Fischer exact test (two-tailed probability). A $P$-value of $<0.05$ was considered statistically significant.
3. Results

3.1. Analyses of PFOA and PFOS in drinking water, feed and vehicle

The tap water used as drinking water for the mice and both the breeding and maintenance diets used in these experiments, as well as the commercial water used as vehicle for the PFOA and PFOS solutions, were analysed for background levels of PFOA and PFOS (Table 1). The levels were in the pg/l and pg/g range for the water and feed, respectively, demonstrating very low background levels compared with the doses intentionally administered to the mice (0.01, 0.1 and 3.0 mg/kg bw/day).

3.2. Reproduction and breeding

No overt toxicity was observed in the dams by the gavage of PFOA or PFOS during GD1-17. Reproduction, as evaluated as mean number of days to conception, was not statistically different among any of the treatment groups either in experimental blocks 1 or 2, or both blocks together (Table 2). The incidence of pregnancy was not significantly different among any treatment groups, either in experimental blocks 1 or 2 or both blocks together (Table 3). There were no differences in days to pregnancy or incidence of pregnancy among the three common treatment groups in experimental block 1 versus experimental block 2. However, the survival of the pups after birth and therefore the final numbers of litters and individual pups varied between the experimental groups (Table 3). In experimental block 1, all dosing with PFOA or PFOS led to fewer surviving mice compared with the vehicle group. After the 3.0 mg/kg dose of PFOA, only one litter with three pups survived, whereas both the 0.1 and the 3.0 mg/kg doses of PFOS gave four surviving litters. In experimental block 2, the 0.1 mg/kg dose was repeated to obtain more mice per group, and a lower dose of 0.01 mg/kg of both substances was included. In this experimental block, the survival of the vehicle-treated mice was lower than in the previous experiment, whereas the number of litters after both the PFOA
and PFOS doses was fairly similar. No overt toxicity was observed for the rest of their lifetime for the pups who survived weaning.

3.3. Feed intake

The feed intake calculated as g feed/g body weight per day was similar between females and males at age 6 weeks (Fig. 1A), but at both age 10 weeks (Fig. 1B) and 20 weeks (Fig. 1C) the feed intake was higher in females than in males ($P<0.001$, for both comparisons). The feed intake was higher for all mice at week 6 compared with week 10 ($P<0.001$), also both in females ($P=0.029$) and males ($P<0.001$) separately, and at week 6 compared with week 20 for all mice ($P<0.001$), and in males separately ($P<0.001$). The feed intake was higher for all mice at week 10 compared with week 20 ($P<0.001$), and in males separately ($P<0.001$). There were no significant differences in feed intake between any of the treatment groups either at week 6 (Fig. 1A), week 10 (Fig. 1B), or week 20 (Fig. 1C). Since litter siblings of both Min/+ and wild-type genotypes were mixed in the cages, the influence on Apc genotype on feed intake could not be investigated.

3.4. Body weight

Body weight of the dams was recorded daily from GD1-18, while the pups were weighed every third day from day 3 after birth until weaning on day 21 in experimental block 1 and on day 25 in experimental block 2. After weaning, the pups were weighed weekly until termination at week 11 (Min/+ mice) or week 20 (wild-type mice). The body weight development is analysed as area under the curve (AUC) for the various life periods.

Despite the effort to synchronize the female estrus cycle and using males with previous mating experience, the females got pregnant on various days after being housed together with the male
(Table 2). Since the exposure and also the weighing were done simultaneously for all females in each experimental block, the exposure and weighing stopped on various days after the dams got pregnant (Table 2). However, the pregnancy had not started to affect the dam’s body weight this early (GD1-3), and hence, the body weights are comparable and are shown as AUC for all dams giving birth to litters being included in the experimental groups (Fig. 2A). For the dams weighed on GD1-18, there were no differences in AUC between the experimental groups either in experimental blocks 1 or 2, and there was no difference between the two experimental blocks (Fig. 2A).

Determination of the pups’ gender was done at weaning, and genotyping of the pups was done just after weaning. Therefore, the body weight data for pups aged 3-18 days include both Min/+ and wild-type mice, and both genders (Fig. 2B). There was no consistent difference in AUC for the pups between experimental blocks 1 and 2. Based on all individual pups in both experimental blocks, pups given 0.1 mg/kg PFOA were significantly lighter than pups given water ($P=0.002$), and pups given 0.01 mg/kg PFOA ($P=0.003$). The few surviving pups ($n=5$) after exposure to 3.0 mg/kg PFOA were lighter than pups given water ($P<0.001$), 0.01 mg/kg PFOA ($P<0.001$) or 0.1 mg/kg PFOA ($P=0.002$). Also in experimental block 1 separately, pups given 0.1 mg/kg PFOA were borderline significantly lighter than pups given water ($P=0.046$). For PFOS, the pups were lighter after exposure to 3.0 mg/kg than after 0.01 mg/kg ($P=0.023$). However, after none of the PFOS doses were the body weight significantly different from after exposure to water. If litter was used as statistical unit instead of individual mice, pups given 3.0 mg/kg PFOA were significantly lighter than pups given water, 0.01 mg/kg PFOA or 0.1 mg/kg PFOA ($P=0.049$, $P=0.005$ and $P=0.039$, respectively), but the differences between 0.1 mg/kg PFOA and water or 0.01 mg/kg PFOA were not significant.
The body weight data for the mice from weaning at 3 weeks until 11 weeks of age, when the Min/+ mice were terminated for examination of intestinal tumorigenesis, were analysed for Min/+ (Fig. 2C) and wild-type (Fig. 2D) mice separately, and for each gender separately. There was no consistent difference in AUC for the mice between experimental blocks 1 and 2. Based on mice in both experimental blocks, males were heavier than females, both in the Min/+ (P<0.001) and wild-type genotypes (P<0.001), and wild-type mice (Fig. 2D) were heavier than Min/+ mice (P<0.001) (Fig. 2C), as is commonly seen in this mouse model. Mice exposed to 0.1 mg/kg PFOA were significantly lighter that mice given water (P=0.003), based on all mice. In Min/+ mice separately, mice given 0.1 mg/kg PFOA were lighter than mice given water (P=0.001) or 0.01 mg/kg PFOA (P=0.013). When evaluated for each gender separately, these effects were found in males (P=0.010 and P=0.015, for comparison of 0.1 mg/kg PFOA with water and 0.01 mg/kg PFOA, respectively), but not in females. For wild-type mice separately, no effects of treatment on AUC were found. Body weight (in g) throughout life for mice exposed to water and 0.1 mg/kg PFOA is illustrated in Fig. 3. When the data were analysed with litter instead of individual mice as statistical unit, the above-mentioned results of heavier males than females (P<0.001), and heavier wild-type than Min/+ mice (P<0.001) were still obtained, but in this case no differences between the treatment groups were seen.

The body weight data for the wild-type mice were also analysed as AUC from week 12 until termination at 20 weeks of age (Fig. 2E). There was no consistent difference in AUC for the mice between experimental blocks 1 and 2. Based on mice in both experimental blocks, males were heavier than females (P<0.001). This was also the case within each treatment group separately (P<0.001, for all comparisons). There were no significant differences in AUC among the treatment groups.
3.5. Terminal BMI

The males had higher BMI at termination than the females, both among the Min/+ mice terminated at 11 weeks (P<0.001) and the wild-type mice terminated at 20 weeks (P<0.001) (data not shown). There were no differences in terminal BMI between the treatment groups in either Min/+ or wild-type mice (data not shown).

3.6. Blood glucose levels

The blood glucose levels were measured at weeks 6 and 11 for Min/+ mice (Fig. 4A and B), and at weeks 6, 11 and 20 for the wild-type mice (Fig. 4C and D). There was no consistent difference in blood glucose levels between experimental blocks 1 and 2. Based on mice in both experimental blocks, and using individual mice as the statistical unit, the male mice had higher blood glucose levels than the females, both in the Min/+ and wild-type genotypes, in all treatment groups, and at week 6, 11 and 20 (P<0.001, for all comparisons). The Min/+ mice had higher blood glucose levels than the wild-type mice, based on all mice (P=0.029) and in the subgroups males (P<0.001) and in mice given water (P<0.001). Based on all mice, the blood glucose levels were significantly higher at 6 weeks compared with both 11 weeks and 20 weeks (P<0.001, both comparisons), and at 11 weeks compared with 20 weeks (P=0.004), indicating decreased blood glucose levels with age. When the data were analysed using litter as the statistical unit, the same results were reached, except that the glucose levels were not significantly higher in males compared with females in the treatment group water, and that the difference between 11 weeks and 20 weeks was no longer statistically significant.

When analysing the data using individual mice as the statistical unit, the mice given 0.01 mg/kg PFOA had significantly lower blood glucose levels compared with the control group who received water (P=0.002), based on all mice. This was significant also in the subgroups Min/+
mice ($P=0.004$), and at 6 weeks ($P<0.001$), but not in the subgroups wild-type mice and at weeks 11 and 20. The blood glucose levels after 0.1 mg/kg PFOA were not significantly different from exposure to water based on all mice, but significantly lower in Min/+ mice separately ($P=0.028$). However, if tested only on mice exposed to water or PFOA (not PFOS) glucose levels after 0.1 mg/kg PFOA were also significantly lower than from exposure to water based on all mice ($P=0.014$). The doses of PFOA, 0.01 and 0.1 mg/kg, were not significantly different based on all mice, but the glucose levels were higher after 0.1 mg/kg than 0.01 mg/kg PFOA at 6 weeks ($P<0.001$). Also mice exposed to 0.01 mg/kg PFOS had higher blood glucose levels than mice given 0.1 mg/kg PFOS, based on all mice ($P=0.016$), and in the subgroup males ($P=0.033$) and at 20 weeks ($P=0.029$). However, neither of the PFOS doses, 0.01 or 0.1 mg/kg, affected the glucose levels differently from water. When the data were analysed using litter as the statistical unit, the same results were reached, with only details different; the blood glucose levels after 0.1 mg/kg PFOA were not significantly different from exposure to water based on all mice, but significantly lower in female mice separately ($P=0.010$).

The levels of blood glucose were within the normal range (>3.3–<13.3 mmol/l) except for one value of 3.0 in a male Min/+ mice at week 6 exposed to 0.1 mg/kg PFOA and one value of 13.6 in a male Min/+ mice at week 6 exposed to 0.01 mg/kg PFOS, both in experimental block 2.

3.7. Absolute and relative liver and spleen weights

As indicators of toxicity of PFOA and PFOS, absolute and relative weights of liver and spleen were determined both for Min/+ mice terminated at 11 weeks of age, and for wild-type mice terminated at 20 weeks of age (Tables 5 and 6). When analysing the data using individual mice
as the statistical unit, the absolute weight of liver was larger in males than in females, both in Min/+ (P<0.001) and wild-type (P<0.001) mice. There were no statistically significant differences between the treatment groups in absolute liver weights either in Min/+ or wild-type mice. Based on all mice, the relative liver weight in the wild-type mice given 0.01 mg/kg PFOA was higher than in mice given water, but also higher than in mice given 0.1 mg/kg PFOA (P<0.001, both comparisons) (Table 6). When using litter as statistical unit, these differences in relative liver weight between treatment groups in wild-type mice were no longer statistically significant. In experimental block 2 separately, in individual Min/+ mice given 0.1 mg/kg PFOA, the relative liver weight was higher than in mice given water (P=0.027). When using litter as statistical unit, this difference in relative liver weight in Min/+ mice between 0.1 mg/kg PFOA and water was no longer statistically significant, whereas the difference between 0.01 mg/kg PFOA and water was significant (P=0.007). Based on all individual mice, the female wild-type mice, but not the female Min/+ mice, had higher relative spleen weights than the males in the treatment groups given water, 0.1 mg/kg PFOA and 0.1 mg/kg PFOS (P<0.05, for all comparisons) (Table 6). Using litter as the statistical unit, this gender difference was statistically significant for mice in experimental block 2. There was a larger variation in the spleen weights than in the liver weights. There were no statistically significant differences among the treatment groups in absolute spleen weights or in relative spleen weights, neither in Min/+ nor wild-type mice, either based on individual mice or litter.

3.8. Intestinal tumorigenesis

All Min/+ mice in all experimental groups had small intestinal tumors, demonstrating 100% incidence in this end point, as is usual in this mouse model. There were no statistically significant differences in small intestinal tumor number between female (Fig. 5A) and male
(Fig. 5B) Min/+ mice. None of the doses, 0.01, 0.1 or 3.0 mg/kg per day, of either PFOA or PFOS, increased the number of small intestinal tumors above the level found in the vehicle control group treated with water (Fig. 5). There was no linear dose response in this end point neither for PFOA nor PFOS (Fig. 5). These results were found both when the data were analysed with individual mice or with litter as the statistical unit (data not shown). At least in this mouse model, neither PFOA nor PFOS increased the number of small intestinal tumors.

The Min/+ mice in the vehicle group had small intestinal tumors with diameters of 0.2-3.0 mm and 0.2-3.2 in females and males, respectively. In the PFOA groups, the females had tumor diameters of 0.2-3.6 mm and the males had tumors diameters of 0.2-3.2 mm. In the PFOS groups, the tumor diameters were 0.2-2.7 mm in females and 0.2-3.6 mm in males. Based on all mice, the males had significantly larger tumors than the females (P<0.001) (Fig. 6). In females, the small intestinal tumors in mice treated with 0.01 mg/kg PFOS or 3.0 mg/kg PFOS, but not with 0.1 mg/kg PFOS, were larger than the tumors in mice treated with water (P<0.05, both comparisons), and the tumors were larger after 3.0 mg/kg PFOS compared with after 0.1 mg/kg PFOS (P<0.05) (Fig. 6). There were no significant effects in females with PFOA. In males, the small intestinal tumors in mice treated with 0.01 mg/kg PFOA were larger than the tumors in mice treated with water (P<0.05), and the tumors were larger after 0.01 mg/kg PFOA compared with after 0.1 mg/kg PFOA (P<0.05) (Fig. 6). There were no significant effects in males with PFOS. Thus, the results were essentially contrary between female and male mice.

In the colon, much fewer tumors are found than in the small intestine in Min/+ mice on C57BL/6J background (Andreassen et al., 2002). When evaluating the incidence of colonic tumors on the individual level, there were no significant differences between experimental
blocks 1 and 2. There was a higher incidence of colonic tumors in males compared with females in the vehicle group in experimental block 1 \((P=0.029)\) and in experimental blocks 1 and 2 together \((P=0.020)\) (data not shown). The only significant difference between the treatment groups was that 0.1 mg/kg PFOA had a higher incidence than the vehicle group for females and males together in experimental block 1 \((P=0.039)\) (data not shown). However, when these three comparisons were tested with litter as statistical unit, none of them reached significance.

There were no statistically significant differences in number of colonic tumors between experimental blocks 1 and 2 on the individual level, and therefore the data were evaluated from both experimental blocks together. The number of colonic tumors was significantly higher in males compared with females \((P<0.001)\) (data not shown). This was also the case within the separate groups given vehicle \((P=0.002)\), after treatment with 0.01 mg/kg PFOA \((P=0.002)\), 0.1 mg/kg PFOA \((P=0.008)\) and 0.01 mg/kg PFOS \((P=0.007)\). There were no significant differences in number of colonic tumors between mice from any of the PFOA and PFOS groups compared with the vehicle group.

The Min+/− mice in the vehicle group had colonic tumor diameters of 1.0-4.0 and 1.1-5.0 mm in females and males, respectively. In the PFOA groups, the colonic tumor diameters were 1.1-4.5 and 1.0-4.7 mm in the females and males, respectively. In the PFOS groups, the tumor diameters were 1.1-4.3 mm in females and 0.7-5.2 mm in males. There was no significant difference in colonic tumor diameter when evaluated on the individual level between the experimental blocks, the genders or between any of the treatment groups (data not shown).
The majority of the small intestinal tumors were localized in the distal two-thirds, i.e. in the middle and distal parts, of the small intestine, irrespective of treatment or gender, and in the middle to distal parts of the colon (Fig. 7), as seen in our previous experiments with Min/+ mice (Andreassen et al., 2002).

3.9. Determination of internal doses of PFOA and PFOS

PFOA and PFOS were analysed in serum of exposed mice in order to determine the internal dose after the exposure. Serum was collected from exposed dams on GD18 (24 h after last po gavage), from dams 1-3 days after weaning of the pups, and from the pups 1-6 days after being weaned from the dams (Table 4). There was a dose-response in the internal serum concentrations of both PFOA and PFOS (in ng/ml) corresponding to the administered doses (Table 4). For all doses, the levels had decreased 2-4 times in the dams from GD18 to after weaning of the pups. The levels in the pups were approximately 1.3–4 times lower than the levels in the dams shortly after weaning. It has been shown in CD-1 mice that substantial amounts of these substances were transferred from the dams to their pups during lactation (Fenton et al., 2009). Therefore, the exposure of the pups continued via the milk after the in utero exposure through po gavage of the dams had finished on GD17.

Low levels of PFOA were found as contamination in some of the PFOS samples, shown as numbers in parentheses in Table 4. The PFOA (23 ng/ml) determined in one dam after weaning in the 0.01 mg/kg PFOS group in experimental block 2 is due to one po gavage of 0.1 mg/kg PFOA given on GD3 by a mistake. The reason for the low level PFOA contamination in some of the mice in experimental block 1 is not known, since great care was taken to avoid cross-contamination of the mice, the solutions and the equipment used. However, when checking the results of tumor numbers, body weight, organ weights etc. of the
individually affected mice, the results did not deviate from the results from the mice without PFOA contamination. Also, the few treatment effects observed in this study were mainly from PFOA, not PFOS, and should therefore not have been influenced by this contamination of PFOA in the PFOS-exposed mice.
4. Discussion

PFOA or PFOS did not increase body weight in any of the doses tested (0.01, 0.1 or 3.0 mg/kg bw/day) up to 20 weeks. Exposure of CD-1 mice on GD1-17 to 0.01, 0.1 and 3.0 mg/kg PFOA significantly increased body weight, and 0.01 and 0.1 mg/kg affected serum insulin and leptin levels in female offspring (Hines et al., 2009). Associations between maternal PFOA, but not PFOS, serum concentration and overweight/obesity and waist circumference in their daughters, and with serum insulin and leptin levels, were also reported in a prospective cohort study (Halldorsson et al., 2012). Thus both animal and human data indicate that PFOA may be obesogenic. Comparable doses were used in our study and by Hines et al. (2009). However, we used C57BL/6J mice, whereas Hines et al. used CD-1 mice. Difference in sensitivity for PFOA on body weight between mouse strains is possible, since it was shown that C57BL/6 and Balb/c reacted differently to the effects of PFOA on mammary gland development (Yang et al., 2009).

Regarding obesogenic effect of PFOS, no effect on body weight was reported on PND0-35 after exposure of CD-1 mice on GD1-18 with 1 or 5 mg/kg PFOS (Lau et al., 2003).

We examined whether PFOA and PFOS affected intestinal tumorigenesis in the sensitive Min/+ mouse model, since adenomas in liver, testicular Leydig cells and pancreatic acinar cells were reported in male Sprague-Dawley and CD (only highest dose) rats after 2 year exposure to 0, 30 or 300 mg PFOA/kg diet, equal to mean daily doses of approximately 0, 1.5 and 15 mg/kg (Biegel et al., 2001; Butenhoff et al., 2012b). Originally reported significant increase in mammary lesions was comparable with controls and non-significant upon pathological reevaluation (Butenhoff et al., 2012b). Negative outcome in many in vitro and in vivo tests at gene and/or chromosome level indicated that PFOA is not genotoxic (EFSA, 2008). Thus, cancers detected after exposure to PFOA in liver, Leydig cells in testis and pancreas appears to be induced by non-genotoxic mechanisms, probably involving
peroxisome proliferation and activation of the peroxisome proliferator-activated receptor alpha (PPARα) and disturbance of the endocrine system (Biegel et al., 2001; EFSA, 2008; Klaunig et al., 2012).

PFOS was given to Crl:CD(SD)IGS BR rats for 2 years in concentrations of 0.5, 2, 5 or 20 mg/kg diet, corresponding to mean daily doses of 0.029-1.385 and 0.024-1.144 mg/kg in females and males, respectively (Butenhoff et al., 2012a). The significant neoplastic findings were hepatocellular and thyroid follicular cell adenomas and mammary fibroadenomas/adenomas. The European Food Safety Authority (EFSA) concluded that PFOS was carcinogenic in the liver, but that the evidence for induction of thyroid and mammary tumors was limited (EFSA, 2008). Negative results in many in vitro and/or in vivo tests at gene and/or chromosome or DNA repair levels indicate that PFOS is not genotoxic (EFSA, 2008).

PFOA or PFOS did not increase the incidence or number of small intestinal or colonic tumors in Min/+ mice, nor affect the location of tumors. Since we measured these substances in serum (Table 4), they are definitely bioavailable, and the reason for lack of tumorigenic effect cannot be because they did not reach the target organ, i.e. the intestines. However, since this model is highly sensitive to substances that disrupt the remaining wild-type allele of Apc by loss of heterozygocity (LOH) or mutations (Andreassen et al., 2002), the lack of tumorigenic effect in the intestines of PFOA and PFOS could be explained by their apparent lack of genotoxicity. Also, if the tumorigenic effect of PFOA and PFOS involves peroxisome proliferation with activation of PPARα and disturbance of the endocrine system, the intestines are less likely to be affected compared with hormone-sensitive organs such as testis, thyroid and mammary glands. PPARα mRNA and protein levels were not increased in the intestines.
after PFOA exposure of mice, although mRNA expression of genes involved in lipid and glucose metabolism regulated by this receptor was (Abbott et al., 2012). We did not observe any tumors in other organs, however, a pathological examination was not performed. *Min/+* mice were terminated at 11 weeks, and wild-type mice at 20 weeks. This observation time was probably not sufficient for tumors to become manifest in the wild-type mice. Also, the exposure time (17 days *in utero* and via milk during the three weeks nursing period) may have been too short, although in putative sensitive periods. The two highest doses (0.1 and 3.0 mg/kg) were comparable with doses of PFOA and PFOS used in the 104 weeks chronic and carcinogenicity studies.

Regarding effects of PFOA and PFOS on size of the small intestinal tumors, the results were essentially contrary between females and males. Since these apparent effects were not consistent across genders or doses, and were found using all tumors present in all mice in the treatment groups (up to 3227 tumors/group), it is likely that they reached statistical significance merely by chance. Similar results are often seen for this end point in the *Min/+* mice.

We observed some indications of toxicity of PFOA, but not of PFOS. There was lower survival of pups after 3.0 mg/kg PFOA. There was indication of lower body weight as AUC in pups after 0.1 mg/kg PFOA (significant for individuals, but not for litter as statistical unit), and also the few surviving pups (*n*=5) after exposure to 3.0 mg/kg PFOA were lighter than pups given 0.1 or 0.01 mg/kg PFOA or water (still significant with litter as statistical unit). In a similar experiment with CD-1 mice given PFOA on GD1-17, 1 and 3 mg/kg did not affect pup survival after birth (on PND9), whereas 5 mg/kg did (Lau et al., 2006), indicating that C57BL/6J mice are slightly more sensitive to PFOA than CD-1 mice. In this study by Lau et
al. (2006), 1 mg/kg PFOA did not affect the neonatal body weight (up to 25 days), whereas ≥3 mg/kg doses did. At 48-60 weeks, 1 and 3 mg/kg PFOA (and 5 mg/kg in males) slightly increased body weight (Lau et al., 2006). The differences in body weight we observed were not caused by variation in food intake, which was not affected by PFOA or PFOS. There were also indications of increased relative liver weight after 0.01 and 0.1 mg/kg PFOA, varying with statistical unit, in the Min/+ mice. Increased relative liver weight and hepatocellular hypertrophy were reported in C57BL/6 and CD-1 mice after ≥1 mg/kg PFOA (Lau et al., 2003; Yang et al., 2009).

The mice tolerated PFOS better than PFOA, judged by higher survival after the 3.0 mg/kg dose (Table 3). None of the PFOS doses affected the body weight (Fig. 2) or the blood glucose levels (Fig. 4) compared with water. There were no significant differences in absolute or relative liver or spleen weight with PFOS (Tables 5 and 6). However, we showed that the concentrations of PFOS decreased approximately 20% during the administration period, so the total dose was lower than for PFOA. In humans, it has been shown that PFOA crosses the placental barrier approximately twice as efficiently as PFOS (Midasch et al., 2007).

Non-fasted blood glucose levels were lower after 0.01 and 0.1 mg/kg PFOA compared with water in all mice or in subgroups of the mice, but not after PFOS. Since the treatments with PFOA did not affect feed intake, this could indicate a direct effect of PFOA on glucose regulation. Pancreas is a target organ for PFOA, as shown by the pancreatic acinar cell adenomas in rats (Butenhoff et al., 2012b). Fasted serum glucose and levels after challenge in glucose tolerance test were not affected by 0.1, 1 or 5 mg/kg PFOA in mice, although serum insulin and leptin were increased in female offspring after 0.01 and 0.1 mg/kg PFOA (Hines et al., 2009). In rats, fasted serum glucose was not affected by 0.5 or 1.5 mg/kg PFOS, although
insulin and leptin were increased (Lv et al., 2013). In a human study, PFOA and PFOS concentrations were negatively associated with serum glucose levels both in adolescents and adults, but not statistically significant (Lin et al., 2009). However, associations between PFOA or PFOS and various parameters linked to metabolic syndrome were significant.

Min/+ mice had higher blood glucose than wild-type mice. This is unlikely caused by higher feed intake since Min/+ mice had lower body weight than wild-types. A possible explanation is that Apc is involved in regulation of epithelial glucose transport in the intestines, since Min/+ mice have increased activity of the electrogenic glucose carrier (SGLT1) compared with wild-types (Rexhepaj et al., 2011).

Mean serum PFOA concentrations in background-exposed European populations are 0.5-40 ng/ml (Fromme et al., 2009), which are somewhat lower than the concentrations we determined after 0.01 mg/kg PFOA (Table 4). However, in industrially contaminated areas median serum concentration of 354 ng/ml PFOA was reported (Emmett et al., 2006), corresponding to serum concentrations after 0.1 mg/kg PFOA (Table 4). The concentrations determined after 0.01 mg/kg PFOS (Table 4) are comparable to serum concentrations of 1-116 ng/ml in general European populations (Fromme et al., 2009).

In conclusion, exposure of C57BL/6J-Min/+ mice and their wild-type siblings in utero to PFOA and PFOS did not have an obesogenic effect in either Min/+ or wild-type mice, nor increased the number of intestinal tumors in Min/+ mice.
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**Figure captions**

**Fig. 1.** Feed intake (g feed/g body weight (bw)/day, mean ± SD of litters) after exposure to the vehicle water, 0.01, 0.1 or 3.0 mg/kg bw/day PFOA or PFOS recorded during one week at (A) weeks 6-7 and (B) weeks 10-11 for litters consisting of both Min/+ and wild-type mice, and (C) at weeks 19-20 for litters of wild-type mice, for each gender separately; (□) Females, (■) males.

**Fig. 2.** Body weight development (mean ± SD of individual dams or pups) as area under the curve (AUC) after exposure to the vehicle water, 0.01, 0.1 or 3.0 mg/kg bw/day PFOA or PFOS for (A) dams on GD0-18, (B) pups (pooled Min/+ and wild-type, females and males) on days 3-18, (C) Min/+ females and males separately, weeks 3-11, (D) wild-type females and males separately, weeks 3-11, (E) wild-type females and males separately, weeks 12-20. (□) Females, (■) males. a-f Experimental groups with similar letters were significantly different, when individual data for both experimental blocks were used.

**Fig. 3.** Body weight (g) throughout life for Min/+ (females (□), males (■) and wild-type mice (females (○), males (●) exposed to the vehicle water (solid line) or 0.1 mg/kg bw/day PFOA (dotted line) (mean of individual mice). The vertical lines indicate weaning at three weeks and termination of Min/+ mice at 11 weeks.

**Fig. 4.** Blood glucose levels (non-fasted) after exposure to the vehicle water, 0.01, 0.1 or 3.0 mg/kg bw/day PFOA or PFOS in (A) Min/+ females and (B) Min/+ males measured at 6 (■) and 11 (□) weeks of age, (C) wild-type females and (D) wild-type males measured at 6 (■), 11 (□) or 20 (○) weeks of age (mean ± SD of individual mice).
Fig. 5. Number of small intestinal tumors in Min/+ mice after exposure to the vehicle water, 0.01, 0.1 or 3.0 mg/kg bw/day PFOA or PFOS (mean ± SD of individual mice). (A) Females, (B) males.

Fig. 6. Diameter (mm) of the small intestinal tumors in Min/+ mice after exposure to the vehicle water, 0.01, 0.1 or 3.0 mg/kg bw/day PFOA or PFOS (mean ± SD of individual mice). (A) Females, (B) males. a-c Experimental groups with similar letters were significantly different, when individual data for both experimental blocks were used.

Fig. 7. Localization of tumors along the small intestine and colon of pooled female and male Min/+ mice after exposure to the vehicle water (●), 0.01 (◇), 0.1 (□) or 3.0 (Δ) mg/kg bw/day PFOA (grey) or PFOS (white) (mean of individual mice).
## Table 1
Analyses of background levels of PFOA and PFOS in drinking water, vehicle and feed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Use</th>
<th>Level of PFOA</th>
<th>Level of PFOS</th>
<th>% recovery of PFOA</th>
<th>% recovery of PFOS</th>
</tr>
</thead>
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<tr>
<td>Water</td>
<td>Tap water</td>
<td>439 pg/l</td>
<td>&lt;350 pg/l</td>
<td>106</td>
<td>85</td>
</tr>
<tr>
<td>Water</td>
<td>Water (vehicle)</td>
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<td>&lt;350 pg/l</td>
<td>106</td>
<td>84</td>
</tr>
<tr>
<td>Feed</td>
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<td>23 pg/g</td>
<td>59</td>
<td>47</td>
</tr>
<tr>
<td>Feed</td>
<td>Harlan Teklad 2018X</td>
<td>&lt;200 pg/g</td>
<td>&lt;10 pg/g</td>
<td>54</td>
<td>80</td>
</tr>
</tbody>
</table>

Tap water is the drinking water for the mice. Water (Aqua B. Braun, B. Braun Melsungen AG, Melsungen, Germany) was used as vehicle for the PFOA and PFOS solutions. SDS RM1(E) from SDS Special Diets Services (Essex, UK) was used as maintenance diet. 2018X Teklad Global 18% Protein Rodent Diet from Harlan Industries Inc. (Indianapolis, IN, USA) was used as breeding diet.
Table 2
Duration of exposure to PFOA and PFOS during GD1-17 and mean numbers of days to conception for the dams in each experimental group.

<table>
<thead>
<tr>
<th>Duration of exposure&lt;sup&gt;a&lt;/sup&gt;</th>
<th>17 days</th>
<th>16 days</th>
<th>15 days</th>
<th>14 days</th>
<th>Total no. of litters</th>
<th>Mean no. of days to conception (mean ± SD)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experimental block 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water (vehicle)</td>
<td>7</td>
<td>2</td>
<td>3</td>
<td></td>
<td>12</td>
<td>0.67 ± 0.89</td>
</tr>
<tr>
<td>0.1 mg/kg PFOA</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td></td>
<td>8</td>
<td>0.62 ± 0.96</td>
</tr>
<tr>
<td>3.0 mg/kg PFOA</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>1.30 ± 1.42</td>
</tr>
<tr>
<td>0.1 mg/kg PFOS</td>
<td>2</td>
<td></td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>1.00 ± 1.18</td>
</tr>
<tr>
<td>3.0 mg/kg PFOS</td>
<td>2</td>
<td></td>
<td></td>
<td>2</td>
<td>4</td>
<td>0.33 ± 0.49</td>
</tr>
<tr>
<td><strong>Experimental block 2</strong></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Water (vehicle)</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
<td>4</td>
<td>1.57 ± 1.27</td>
</tr>
<tr>
<td>0.01 mg/kg PFOA</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>10</td>
<td>1.69 ± 1.96</td>
</tr>
<tr>
<td>0.1 mg/kg PFOA</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>9</td>
<td>0.75 ± 1.06</td>
</tr>
<tr>
<td>0.01 mg/kg PFOS</td>
<td>5</td>
<td>3</td>
<td></td>
<td></td>
<td>8</td>
<td>0.46 ± 0.88</td>
</tr>
<tr>
<td>0.1 mg/kg PFOS</td>
<td>4</td>
<td>2</td>
<td></td>
<td>3</td>
<td>9</td>
<td>1.00 ± 1.07</td>
</tr>
</tbody>
</table>

Females and males were housed together on GD0. All dams were given water (vehicle), PFOA and PFOS by po gavage daily for 17 days (GD1-17).

<sup>a</sup>If conception occurred immediately, the exposure was on GD1-17 of pregnancy. However, not all females conceived immediately, as judged by counting 21 days backwards from giving birth. Therefore, the number of dams with a given duration of exposure to PFOA and PFOS during pregnancy is given (only including dams providing living litters included in the experimental groups).

<sup>b</sup>The mean number of days (counting full days) to conception is also given, and this numbers include all dams giving birth even if their litters later died and were not included in the experimental groups.
Table 3
Incidence of pregnant females and the resulting numbers of litters and pups in the experimental groups after exposure to PFOA or PFOS on GD1-17.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>No. of dams exposed</th>
<th>No. of dams pregnant (%)</th>
<th>No. of successful births</th>
<th>No. of litters that died perinatally</th>
<th>No. of litters that died around weaning</th>
<th>No. of litters in exp. groups</th>
<th>No. of pups in exp. groups</th>
<th>Mean no. of pups/litter in exp. groups</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experimental block 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water (vehicle)</td>
<td>20</td>
<td>15 (75)</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1</td>
<td>0</td>
<td>12</td>
<td>70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.0</td>
</tr>
<tr>
<td>0.1 mg/kg PFOA</td>
<td>21</td>
<td>15 (71)</td>
<td>10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3</td>
<td>2</td>
<td>8</td>
<td>40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.3</td>
</tr>
<tr>
<td>3.0 mg/kg PFOA</td>
<td>21</td>
<td>12 (57)</td>
<td>2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3.0</td>
</tr>
<tr>
<td>0.1 mg/kg PFOS</td>
<td>21</td>
<td>13 (62)</td>
<td>7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.0</td>
</tr>
<tr>
<td>3.0 mg/kg PFOS</td>
<td>21</td>
<td>14 (67)</td>
<td>5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7</td>
<td>1</td>
<td>4</td>
<td>20</td>
<td>5.0</td>
</tr>
<tr>
<td>104</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Experimental block 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water (vehicle)</td>
<td>10</td>
<td>7 (70)</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>4</td>
<td>15</td>
<td>3.8</td>
</tr>
<tr>
<td>0.01 mg/kg PFOA</td>
<td>23</td>
<td>17 (74)</td>
<td>10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6</td>
<td>0</td>
<td>10</td>
<td>45&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>4.8</td>
</tr>
<tr>
<td>0.1 mg/kg PFOA</td>
<td>20</td>
<td>12 (60)</td>
<td>9</td>
<td>3</td>
<td>0</td>
<td>9</td>
<td>54&lt;sup&gt;e&lt;/sup&gt;</td>
<td>6.1</td>
</tr>
<tr>
<td>0.01 mg/kg PFOS</td>
<td>23</td>
<td>16 (70)</td>
<td>9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6</td>
<td>1</td>
<td>8</td>
<td>40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.3</td>
</tr>
<tr>
<td>0.1 mg/kg PFOS</td>
<td>24</td>
<td>15 (63)</td>
<td>9</td>
<td>6</td>
<td>0</td>
<td>9</td>
<td>41</td>
<td>4.6</td>
</tr>
<tr>
<td>100</td>
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<td></td>
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<tr>
<td><strong>Experimental blocks 1 + 2</strong></td>
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<td></td>
</tr>
<tr>
<td>Water (vehicle)</td>
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<td></td>
<td></td>
<td></td>
<td>85&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.01 mg/kg PFOA</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>45&lt;sup&gt;b,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.1 mg/kg PFOA</td>
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<td></td>
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<td></td>
<td></td>
<td>94&lt;sup&gt;b,e&lt;/sup&gt;</td>
</tr>
<tr>
<td>3.0 mg/kg PFOA</td>
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<td></td>
<td></td>
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<td></td>
<td>3</td>
</tr>
<tr>
<td>0.01 mg/kg PFOS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>40&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.1 mg/kg PFOS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>59&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3.0 mg/kg PFOS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Not all females conceived or had successful births, and some litters died at varying times after birth.</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>
<sup>a</sup>Two dams/experimental group were terminated on GD18 for analyses of PFOA and PFOS (see Table 4).
<sup>b</sup>Two pups/experimental group were terminated after weaning for analyses of PFOA and PFOS (see Table 4).
<sup>c</sup>One dam/experimental group was terminated on GD18 for analyses of PFOA and PFOS (see Table 4).
<sup>d</sup>One wild-type male mouse was found dead after fighting week 9.
<sup>e</sup>One Min/+ female was terminated because of rectal prolapse week 9.
### Table 4
Analyses of PFOA and PFOS in serum from exposed dams and pups (ng/ml).

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Dams GD18</th>
<th>Dams after weaning</th>
<th>Pups after weaning</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experimental block 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water (vehicle control)</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
</tr>
<tr>
<td>0.1 mg/kg PFOA</td>
<td>2 176/ 2 680</td>
<td>866/ 799</td>
<td>213/ 236</td>
</tr>
<tr>
<td>0.1 mg/kg PFOS</td>
<td>1 334/ 1 237 (23/ 25)</td>
<td>476/ 544 (7.7/ 7.2)</td>
<td>377/ 298 (3.1)</td>
</tr>
<tr>
<td>3.0 mg/kg PFOA</td>
<td>35 321/ 49 717</td>
<td>14 498/ 12 663</td>
<td>n.a.</td>
</tr>
<tr>
<td>3.0 mg/kg PFOS</td>
<td>36 646/ 44 634</td>
<td>17 227/ 22 249</td>
<td>n.a.</td>
</tr>
<tr>
<td><strong>Experimental block 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water (vehicle control)</td>
<td>n.a.</td>
<td>0/0</td>
<td>n.a.</td>
</tr>
<tr>
<td>0.01 mg/kg PFOA</td>
<td>194</td>
<td>90/ 67</td>
<td>26/ 12</td>
</tr>
<tr>
<td>0.01 mg/kg PFOS</td>
<td>131</td>
<td>66/ 37 (23)</td>
<td>20/ 39</td>
</tr>
<tr>
<td>0.1 mg/kg PFOA</td>
<td>n.a.</td>
<td>593/ 599</td>
<td>n.a.</td>
</tr>
<tr>
<td>0.1 mg/kg PFOS</td>
<td>n.a.</td>
<td>710/ 496</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

Pregnant dams were terminated at GD18, 24 hr after last po gavage, or 2 days after weaning of the pups on day 21 after birth (on PND23) in experimental block 1, and 1-3 days after weaning on day 25 after birth (on PND 26-28) in experimental block 2. The pups were terminated 4-6 days after weaning in experimental block 1 (PND25-27), or 1 day after weaning in experimental block 2 (PND26). Serum samples were taken from one or two mice at each time point (sample 1/sample 2). Numbers in parentheses are contamination of PFOA in the mice administered PFOS. n.a. = not analysed.
Table 5
Effects of PFOA and PFOS on body weight (BW), absolute liver weight (ALW), relative liver weight (RLW), absolute spleen weight (ASW) and relative spleen weight (RSW) in female and male Min/+ mice terminated at 11 weeks of age (mean of individual mice in the group ± SD).

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>n</th>
<th>BW (g)</th>
<th>ALW (g)</th>
<th>RLW (%)</th>
<th>ASW (g)</th>
<th>RSW (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Min/+, females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water (vehicle)</td>
<td>23</td>
<td>19.8 ± 0.9</td>
<td>0.93 ± 0.12</td>
<td>4.67 ± 0.48</td>
<td>0.12 ± 0.03</td>
<td>0.61 ± 0.14</td>
</tr>
<tr>
<td>0.01 mg/kg PFOA</td>
<td>15</td>
<td>19.3 ± 0.9</td>
<td>0.91 ± 0.12</td>
<td>4.69 ± 0.46</td>
<td>0.12 ± 0.04</td>
<td>0.62 ± 0.23</td>
</tr>
<tr>
<td>0.1 mg/kg PFOA</td>
<td>26</td>
<td>18.9 ± 1.1</td>
<td>0.89 ± 0.11</td>
<td>4.71 ± 0.48</td>
<td>0.11 ± 0.05</td>
<td>0.60 ± 0.23</td>
</tr>
<tr>
<td>3.0 mg/kg PFOA</td>
<td>2</td>
<td>19.7 ± 0.4</td>
<td>0.82 ± 0.03</td>
<td>4.19 ± 0.24</td>
<td>0.11 ± 0.01</td>
<td>0.55 ± 0.05</td>
</tr>
<tr>
<td>0.01 mg/kg PFOS</td>
<td>6</td>
<td>19.2 ± 0.5</td>
<td>0.79 ± 0.08</td>
<td>4.11 ± 0.43</td>
<td>0.12 ± 0.07</td>
<td>0.65 ± 0.38</td>
</tr>
<tr>
<td>0.1 mg/kg PFOS</td>
<td>13</td>
<td>19.1 ± 1.0</td>
<td>0.88 ± 0.12</td>
<td>4.60 ± 0.43</td>
<td>0.10 ± 0.03</td>
<td>0.53 ± 0.18</td>
</tr>
<tr>
<td>3.0 mg/kg PFOS</td>
<td>5</td>
<td>19.4 ± 1.5</td>
<td>0.88 ± 0.15</td>
<td>4.52 ± 0.55</td>
<td>0.11 ± 0.02</td>
<td>0.58 ± 0.06</td>
</tr>
<tr>
<td><strong>Min/+, males</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water (vehicle)</td>
<td>15</td>
<td>24.7 ± 1.8</td>
<td>1.16 ± 0.15</td>
<td>4.70 ± 0.40</td>
<td>0.15 ± 0.05</td>
<td>0.60 ± 0.21</td>
</tr>
<tr>
<td>0.01 mg/kg PFOA</td>
<td>3</td>
<td>24.6 ± 0.6</td>
<td>1.26 ± 0.14</td>
<td>5.10 ± 0.45</td>
<td>0.16 ± 0.13</td>
<td>0.66 ± 0.52</td>
</tr>
<tr>
<td>0.1 mg/kg PFOA</td>
<td>19</td>
<td>23.7 ± 2.9</td>
<td>1.11 ± 0.19</td>
<td>4.69 ± 0.53</td>
<td>0.20 ± 0.26</td>
<td>0.99 ± 1.67</td>
</tr>
<tr>
<td>3.0 mg/kg PFOA</td>
<td>0</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>0.01 mg/kg PFOS</td>
<td>10</td>
<td>24.8 ± 1.2</td>
<td>1.12 ± 0.07</td>
<td>4.51 ± 0.25</td>
<td>0.15 ± 0.08</td>
<td>0.61 ± 0.33</td>
</tr>
<tr>
<td>0.1 mg/kg PFOS</td>
<td>12</td>
<td>24.5 ± 1.8</td>
<td>1.08 ± 0.26</td>
<td>4.39 ± 0.80</td>
<td>0.10 ± 0.03</td>
<td>0.42 ± 0.12</td>
</tr>
<tr>
<td>3.0 mg/kg PFOS</td>
<td>7</td>
<td>22.7 ± 1.5</td>
<td>1.08 ± 0.13</td>
<td>4.74 ± 0.41</td>
<td>0.23 ± 0.37</td>
<td>1.04 ± 1.73</td>
</tr>
</tbody>
</table>

Relative liver weight (RLW) (%) = absolute liver weight (ALW)/BW x 100, relative spleen weight (RSW) (%) = absolute spleen weight (ASW)/BW x 100. The numbers for water (vehicle), 0.1 mg/kg PFOA and PFOS are the mean of experimental blocks 1 and 2, the rest of the treatment groups are either from experimental block 1 (3.0 mg/kg PFOA and PFOS) or from experimental block 2 (0.01 mg/kg PFOA and PFOS).
Table 6
Effects of PFOA and PFOS on body weight (BW), absolute liver weight (ALW), relative liver weight (RLW), absolute spleen weight (ASW) and relative spleen weight (RSW) in female and male wild-type mice terminated at 20 weeks of age (mean of individual mice in the group ± SD).

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>n</th>
<th>BW (g)</th>
<th>ALW (g)</th>
<th>RLW (%)</th>
<th>ASW (g)</th>
<th>RSW (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>+/+, females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water (vehicle)</td>
<td>20</td>
<td>22.4 ± 1.1</td>
<td>0.98 ± 0.12</td>
<td>4.38 ± 0.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.12 ± 0.16</td>
<td>0.55 ± 0.73&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.01 mg/kg PFOA</td>
<td>17</td>
<td>21.2 ± 1.2</td>
<td>0.97 ± 0.10</td>
<td>4.96 ± 0.36&lt;sup&gt;a&lt;/sup&gt;&lt;sup,b&lt;/sup&gt;</td>
<td>0.15 ± 0.25</td>
<td>0.70 ± 1.15</td>
</tr>
<tr>
<td>0.1 mg/kg PFOA</td>
<td>25</td>
<td>21.9 ± 1.3</td>
<td>0.97 ± 0.10</td>
<td>4.42 ± 0.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.09 ± 0.01</td>
<td>0.40 ± 0.05&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>3.0 mg/kg PFOA</td>
<td>0</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>0.01 mg/kg PFOS</td>
<td>15</td>
<td>21.8 ± 1.6</td>
<td>1.01 ± 0.06</td>
<td>4.66 ± 0.34</td>
<td>0.20 ± 0.28</td>
<td>0.93 ± 1.31</td>
</tr>
<tr>
<td>0.1 mg/kg PFOS</td>
<td>14</td>
<td>22.1 ± 1.8</td>
<td>1.01 ± 0.12</td>
<td>4.57 ± 0.56</td>
<td>0.09 ± 0.01</td>
<td>0.43 ± 0.05&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>3.0 mg/kg PFOS</td>
<td>5</td>
<td>21.0 ± 1.4</td>
<td>0.94 ± 0.06</td>
<td>4.45 ± 0.10</td>
<td>0.08 ± 0.01</td>
<td>0.37 ± 0.02</td>
</tr>
<tr>
<td><strong>+/+, males</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water (vehicle)</td>
<td>27</td>
<td>30.0 ± 1.8</td>
<td>1.33 ± 0.13</td>
<td>4.42 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.09 ± 0.02</td>
<td>0.29 ± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.01 mg/kg PFOA</td>
<td>10</td>
<td>29.2 ± 3.0</td>
<td>1.29 ± 0.17</td>
<td>4.64 ± 0.29&lt;sup&gt;a&lt;/sup&gt;&lt;sup,b&lt;/sup&gt;</td>
<td>0.13 ± 0.19</td>
<td>0.48 ± 0.74</td>
</tr>
<tr>
<td>0.1 mg/kg PFOA</td>
<td>24</td>
<td>29.1 ± 2.4</td>
<td>1.29 ± 0.17</td>
<td>4.42 ± 0.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.08 ± 0.03</td>
<td>0.29 ± 0.15&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>3.0 mg/kg PFOA</td>
<td>1</td>
<td>25.0</td>
<td>1.04</td>
<td>4.15</td>
<td>0.06</td>
<td>0.24</td>
</tr>
<tr>
<td>0.01 mg/kg PFOS</td>
<td>9</td>
<td>29.5 ± 1.8</td>
<td>1.31 ± 0.14</td>
<td>4.43 ± 0.38</td>
<td>0.08 ± 0.02</td>
<td>0.29 ± 0.07</td>
</tr>
<tr>
<td>0.1 mg/kg PFOS</td>
<td>20</td>
<td>29.0 ± 2.4</td>
<td>1.26 ± 0.12</td>
<td>4.35 ± 0.38</td>
<td>0.10 ± 0.13</td>
<td>0.35 ± 0.43&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>3.0 mg/kg PFOS</td>
<td>3</td>
<td>29.2 ± 4.4</td>
<td>1.36 ± 0.24</td>
<td>4.65 ± 0.16</td>
<td>0.08 ± 0.00</td>
<td>0.27 ± 0.04</td>
</tr>
</tbody>
</table>

Relative liver weight (RLW) (%) = absolute liver weight (ALW)/BW x 100, relative spleen weight (RSW) (%) = absolute spleen weight (ASW)/BW x 100. The numbers for water (vehicle), 0.1 mg/kg PFOA and PFOS are the mean of experimental blocks 1 and 2, the rest of the treatment groups are either from experimental block 1 (3.0 mg/kg PFOA and PFOS) or from experimental block 2 (0.01 mg/kg PFOA and PFOS). Experimental groups with similar letters were significantly different.

<sup>a,b,c</sup>The relative liver weight in the wild-type mice given 0.01 mg/kg PFOA was higher than in mice given water, but also higher than in mice given 0.1 mg/kg PFOA (<i>P</i>&lt;0.001, based on all wild-type mice; females and males, experimental blocks 1 and 2, using individual data).

<sup>c,d,e</sup>Females had higher relative spleen weight than males (<i>P</i>&lt;0.005, based on all wild-type mice, experimental blocks 1 and 2, using individual data).
Figure 1

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Figure 3
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