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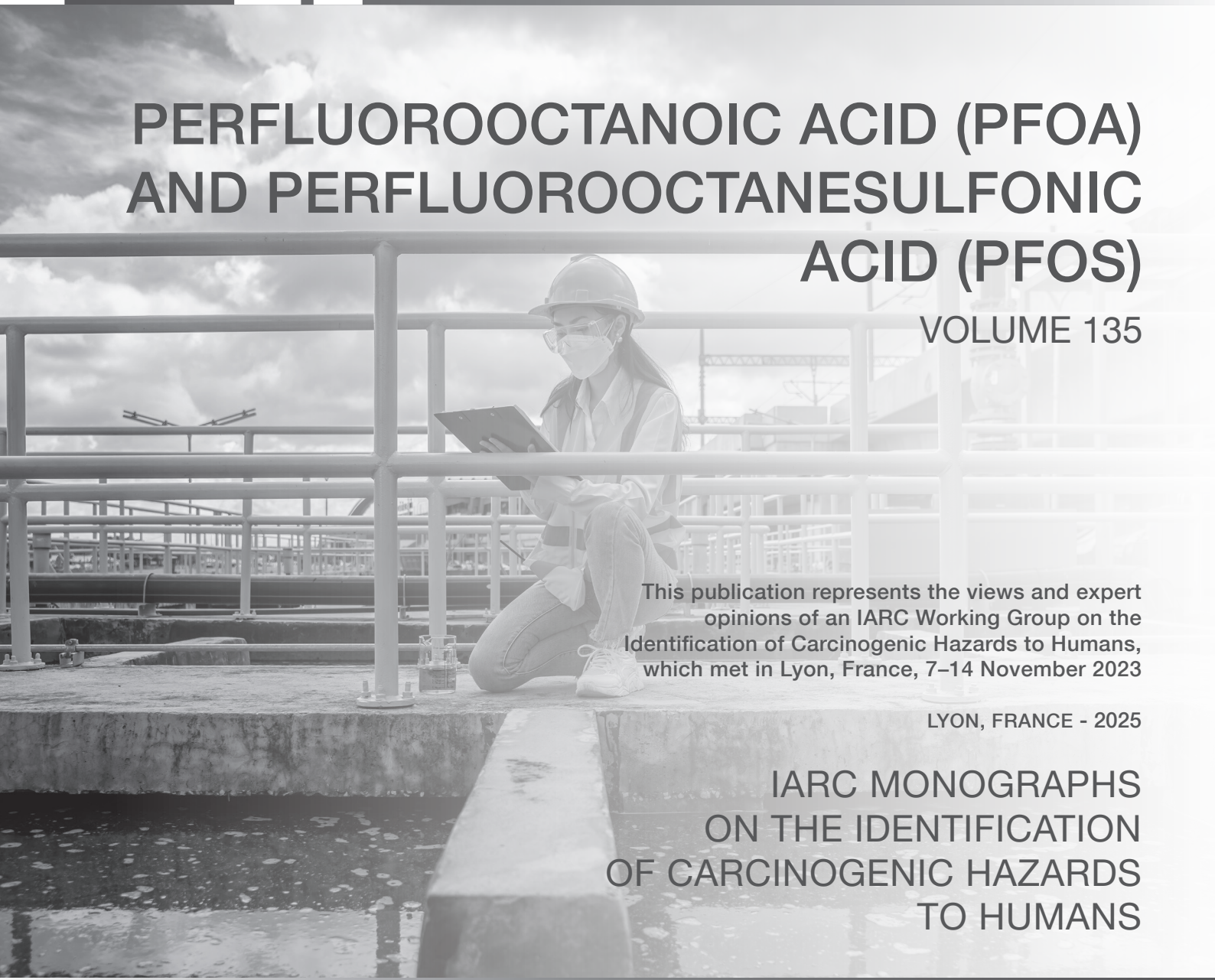

PERFLUOROOCTANOIC ACID (PFOA) AND PERFLUOROOCTANESULFONIC ACID (PFOS)

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3. CANCER IN EXPERIMENTAL ANIMALS

3.1 Perfluorooctanoic acid (PFOA)

Perfluorooctanoic acid (PFOA) was previously evaluated by the *IARC Monographs* programme in 2014 and the evaluation was published in Volume 110 (IARC, 2016). In its evaluation at that time, the Working Group concluded that there was *limited evidence* in experimental animals for the carcinogenicity of PFOA. Since the previous evaluation of PFOA by the *IARC Monographs* Programme, there have been new studies investigating the occurrence of cancer in experimental animals in relation to exposure to PFOA.

3.1.1 Mouse

See Table 3.1.

(a) Oral administration (drinking-water)

A cancer promotion study using the KC mouse model was conducted by Kamendulis et al. (2022). A mouse model was developed by selectively introducing a *Kras*^{G12D} mutation in pancreatic ductal cells using a Cre-lox technology, i.e. *PDX-1-Cre;LSL-Kras*^{G12D} transgenic mouse model (KC model) (Hingorani et al., 2003). This KC mouse model spontaneously develops pancreatic intraepithelial neoplasia (PanIN), mimicking human lesions as progression through four stages. At 9 months, 80% of mutant mice have PanIN lesions (considered to be a cancer precursor lesion), and eventually develop invasive and metastatic adenocarcinoma (Hingorani et al., 2003).

Groups of male and female *LSL-Kras*^{G12D}; *Pdx-1 Cre* (KC) transgenic mice (age, 8 weeks) were treated with PFOA (specifically, the ammonium salt; purity, 96%) at a concentration of 5 ppm in drinking-water for 4 or 7 months. The numbers of mice [number of each sex not reported] were 10 and 11 at 6 months, and 9 and 9 at 9 months, for the control and PFOA groups, respectively. [The Working Group noted that the administered dose of PFOA in milligrams per kilogram body weight (bw) was not reported.] Controls received tap water. PFOA exposure did not significantly alter body weight at either time point in treated mice compared with controls. No information on survival or food consumption was reported. At the end of the feeding period, the mice were killed (at age 6 or 9 months). PanIN grade, inflammation score and stroma evaluation were performed by pathologists blinded to the experimental groups on haematoxylin-and-eosin-stained slides using light microscopy to evaluate each section.

Administration of PFOA in drinking-water did not cause a significant increase in the incidence of any type of neoplasm in either males or females.

In the same study, there was a significant increase in both the PanIN lesion area (58%) and the number of PanIN lesions per mm^2 of pancreas

Table 3.1 Studies of carcinogenicity in mice exposed to PFOA

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence or multiplicity	Significance	Comments
Initiation–promotion (tested as promoter) Mouse (transgenic), *LSL-Kras^{G12D};Pdx-1 Cre* (KC) (M, F) (combined) 2 mo 6 mo Kamendulis et al. (2022)	Oral administration (drinking-water) PFOA (ammonium salt), 96% Tap water 0, 5 ppm, for 4 mo 10, 11 NR, NR	No significant increase in tumour incidence in treated animals		*Principal strengths*: end-points studied at two time points (age 6 and 9 mo, see below); PFOA measured in serum and pancreatic tissue at both time points; PanIN grade, inflammation score and stroma evaluation performed by pathologists blinded to the treatment. *Principal limitations*: intake of drinking-water was not measured, thus the PFOA dose was not known; data were combined for males and females; only one dose used; no survival data; short duration of exposure; limited number of animals per group. *Other comments*: the mean PanIN grade did not significantly differ between control and PFOA-treated mice at 6 mo; the composite histopathology severity score derived by incorporating PanIN lesion stage, inflammation and stromal density, was significantly increased at 6 mo; the lesion number per area was significantly increased.
Initiation–promotion (tested as promoter) Mouse (transgenic), *LSL-Kras^{G12D};Pdx-1 Cre* (KC) (M, F) (combined) 2 mo 9 mo Kamendulis et al. (2022)	Oral administration (drinking-water) PFOA (ammonium salt), 96% Tap water 0, 5 ppm for 7 mo 9, 9 NR, NR	No significant increase in tumour incidence in treated mice		*Principal strengths*: end-points studied at two time points (age 6 and 9 mo); PFOA measured in serum and pancreatic tissue at both time points; PanIN grade, inflammation score, and stroma evaluation performed by pathologists blinded to the treatment. *Principal limitations*: intake of drinking-water was not measured, thus the PFOA dose was not known; data were combined for males and females; only one dose used; no survival data; short duration of exposure; small number of animals per group. *Other comments*: the mean PanIN grade did not significantly differ between control and PFOA-treated mice at 9 mo; the composite histopathology severity score, derived by incorporating PanIN lesion stage, inflammation, and stromal density, was not significantly increased at 9 mo; the lesion number per area was not significantly increased.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence or multiplicity	Significance	Comments
Full carcinogenicity Mouse, C57BL/6J-*Apc*$^{Min/+}$ (M) Day 1 of gestation 11 wk Ngo et al. (2014)	Gavage PFOA (ammonium salt), ≥ 98% Water 0, 0.01, 0.1, 3.0 mg/kg bw per day 15, 3, 19, 0 15, 3, 19, 0	*Small intestine (duodenum, jejunum or ileum)* Tumour incidence: 15/15, 3/3, 19/19, 0/0 Tumour multiplicity: 146.7 ± 72.4, 128.0 ± 127.1, 82.2 ± 38.3, NR *Colon* Tumour incidence: 12/15 (80%), 3/3 (100%), 17/19 (89%), 0/0 Tumour multiplicity: 2.5 ± 2.2, 4.0 ± 3.5, 2.4 ± 2.2, NR	NS NS NS NS	*Principal strengths*: males and females studied; multiple doses used; analysed background levels of PFOA in feed and drinking-water; analysed internal doses of PFOA; tested stability of PFOA; blocks of PFOA administration were compared statistically. *Principal limitations*: no histopathological examination of the small intestinal tumours was performed; small number of mice per group. *Other comments*: study of transplacental exposure; increase in the incidence and multiplicity of spontaneous tumours was studied in this mouse model; small intestinal tumours were observed in all *Min*/+ mice in all experimental groups, including the vehicle group, demonstrating 100% incidence in this end-point, as is usual in this mouse model.
Full carcinogenicity Mouse, C57BL/6J-*Apc*$^{Min/+}$ (F) Day 1 of gestation 11 wk Ngo et al. (2014)	Gavage PFOA (ammonium salt), ≥ 98% Water 0, 0.01, 0.1, 3.0 mg/kg bw per day 23, 15, 26, 2 23, 15, 26, 2	*Small intestine (duodenum, jejunum or ileum)* Tumour incidence: 23/23, 15/15, 26/26, 2/2 Tumour multiplicity: 151.0 ± 102.3, 102.9 ± 40.7, 119.5 ± 73.0, 84.0 ± 36.8 *Colon* Tumour incidence: 9/23 (39%), 8/15 (53%), 17/27 (63%), 1/2 (50%) Tumour multiplicity: 0.6 ± 1.0, 0.8 ± 1.1, 1.1 ± 1.1, 1.0 ± 1.4	NS NS NS NS	*Principal strengths*: males and females studied; multiple doses used; analysed background levels of PFOA in feed and drinking-water, analysed internal doses of PFOA, tested stability of PFOA, blocks of PFOA administration were compared statistically. *Principal limitations*: no histopathological examination of the small intestinal tumours was performed; small number of mice per group; and short duration. *Other comments*: study of transplacental exposure; increase in incidence and multiplicity of spontaneous tumours was studied in this mouse model; small intestinal tumours were observed in all *Min*/+ mice in all experimental groups including the vehicle group, demonstrating 100% incidence in this end-point, as is usual in this mouse model.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence or multiplicity	Significance	Comments
Full carcinogenicity Mouse, CD-1 (F) Exposure on days 1–17 of gestation Killed at 18 mo Filgo et al. (2015)	Gavage PFOA (ammonium salt), > 98% (pure, linear product) Deionized water Control, 0.01, 0.1, 0.3, 1, 5 mg/kg bw, once per day 29, 29, 37, 26, 31, 21 29, 29, 37, 26, 31, 21	*Liver* Hepatocellular adenoma 0/29, 0/29, 1/37 (2.7%), 4/26* (15.38), 0/31, 1/21 (4.8%) Hepatocellular adenoma, multiple 0/29, 1/29 (3.4%), 0/37, 0/26, 0/31, 0/21 Hepatocellular carcinoma 0/29, 0/29, 0/37, 1/26 (3.8%), 0/31, 1/21 (4.8%) Haemangiosarcoma 0/29, 0/29, 0/37, 1/26 (3.8%), 0/31, 2/21 (9.5%) Histiocytic sarcoma 0/29, 0/29, 1/37 (2.7%), 0/26, 1/31 (3.2%), 1/21 (4.8%) Lymphoma 1/29 (3.4%), 0/29, 0/37, 1/26 (3.8%), 1/31 (3.2%), 1/21 (4.8%)	 *$P < 0.05$, Fisher exact test NS NS $P < 0.01$, Cochran–Armitage trend test NS NS	*Principal strengths*: three mouse strains used; multiple doses used. *Principal limitations*: only females were studied; number of tumours per animal was not reported; no statistical comparison between blocks of mice was reported; there was no statement that the dams were randomized to the treatment groups. *Other comments*: for mice killed before 18 mo when tumours were counted, only the percentage of mice born, which is unknown, was stated; thus, the numbers of mice reported at the start and surviving are both the numbers surviving at 18 mo and included in the study.
Full carcinogenicity Mouse, 129/Sv wildtype (F) Exposure on days 1–17 of gestation Killed at age 18 mo Filgo et al. (2015)	Gavage PFOA (ammonium salt), > 98% (pure, linear product) Deionized water Control, 0.1, 0.3, 0.6, 1 mg/kg bw, once per day 10, 10, 8, 6, 8 10, 10, 8, 6, 8	*Liver* Adenoma 0/10, 0/10, 0/8, 0/6, 0/8 Haemangiosarcoma 0/10, 0/10, 0/8, 0/6, 0/8 Histiocytic sarcoma 0/10, 1/10 (10%), 0/8, 0/6, 0/8 Ito cell tumour 0/10, 0/10, 0/8, 0/6, 0/8	 NS NS NS NS	*Principal strengths*: three mouse strains used; multiple doses used. *Principal limitations*: only females were studied; number of tumours per mouse was not reported; no statistical comparison between blocks of mice was reported; there was no statement that the dams were randomized to the treatment groups. *Other comments*: for mice killed before 18 mo when tumours were counted, only the percentage of mice born, which is unknown, was reported. Thus, the numbers of mice given at start and surviving are both the numbers surviving at 18 mo and included in the study.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence or multiplicity	Significance	Comments
Full carcinogenicity Mouse, 129/Sv PPARα knockout (F) Exposure on days 1–17 of gestation Killed at age 18 mo Filgo et al. (2015)	Gavage PFOA (ammonium salt), > 98% (pure, linear product) Deionized water Control, 0.1, 0.3, 1, 3 mg/kg bw, once per day 6, 10, 10, 9, 9 6, 10, 10, 9, 9	*Liver* Hepatocellular adenoma 0/6, 1/10 (10%), 1/10 (10%), 1/9 (11%), 2/9 (22%) Haemangiosarcoma 1/6 (16.7%), 0/10, 0/10, 0/9, 0/9 Histiocytic sarcoma 0/6, 0/10, 0/10, 0/9, 0/9 Ito cell tumour 0/6, 0/10, 1/10 (10%), 0/9, 0/9	NS NS NS NS	*Principal strengths*: three mouse strains used; multiple doses used. *Principal limitations*: only females were studied; number of tumours per mouse was not reported; no statistical comparison between blocks of mice was reported; there was no statement that the dams were randomized to the treatment groups. *Other comments*: for mice killed before 18 mo when tumours were counted, only the percentage of mice born, which is unknown, was reported. Thus, the numbers of mice given at start and surviving are both the numbers surviving at 18 mo and included in the study.

bw, body weight; F, female; M, male; mo, month(s); NR, not reported; NS, not significant; PanIN, pancreatic intraepithelial neoplasia; PFOA, perfluorooctanoic acid; PPARα, peroxisome proliferator-activated receptor alpha; ppm, parts per million; vs, versus; wk, week(s).

(twofold) in the group receiving PFOA versus the controls at 6 months, but not at 9 months. [The Working Group noted that PanIN progresses to pancreatic ductal adenocarcinoma through stages characterized by morphological changes and nuclear atypia (see Hezel et al., 2006).] [The Working Group noted that this model effectively examines the shortening of latency by the treatment. The Working Group also noted that this study included two time points and measured PFOA concentrations in the serum and tissue at both time points; however, there was a lack of information on the number of animals per sex (males and females were combined); a limited number of animals (for most end-points, the number of mice per group was 9–11); a lack of information on survival and on randomization to treatment groups; one dose only was used; and there was a lack of information on the exact dose of PFOA ingested, since the intake of drinking-water was not measured.]

(b) Transplacental exposure

Ngo et al. (2014) examined the tumorigenic effects of gestational exposure to PFOA (specifically, the ammonium salt) in C57BL/6J-*Apc*$^{Min/+}$ mice, a mouse model that develops spontaneous intestinal tumours because of a heterozygous *Min* mutation in the tumour suppressor gene adenomatous polyposis coli (*Apc*). These mice are sensitive to chemicals that mutate or delete (parts of) the remaining wildtype *Apc* allele, and it is a model both for the inherited disorder, familial adenomatous polyposis, and for sporadic colorectal cancer. The wildtype C57BL/6J-*Apc*$^{+/+}$ dams were mated to heterozygous C57BL/6J-*Apc*$^{Min/+}$ males. Pregnant wildtype females were treated with PFOA (purity, ≥ 98%) by gavage at a dose of 0, 0.01, 0.1, or 3.0 mg/kg bw per day on days 1–17 of gestation. Insufficient rates of pregnancy and littering and low F_1 survival were observed in the first experimental block – block 1, 0 (vehicle, distilled water), 0.1, and 3.0 mg/kg per day; 104 exposed dams (age, 7–8 weeks) – thus, a second block was added for which the PFOA exposure was lower – block 2, 0, 0.01, and 0.1 mg/kg per day; 100 exposed dams (age, 9–10 weeks). The PFOA solutions were made separately for the two experimental blocks, and the gavage volumes for all doses were below 1 mL/100 g bw. The PFOA solutions were tested by chemical analysis and found to be stable during the experiment. Furthermore, the tap water (used as drinking-water for the mice) and both the breeding and maintenance diets, as well as the distilled water (used as the vehicle for PFOA), were analysed and showed very low background PFOA levels (picograms per litre and picograms per gram in water and feed, respectively). Internal exposure was quantified (1 or 2 mice per time point) in the dams on day 18 of gestation, postnatal day 23 (block 1) or postnatal days 26–28 (block 2), and F_1 pups on postnatal days 25–27 (depending on the block). The limit of quantification (LOQ) for PFOA was 0.05 ng/mL serum. Although minimal data were generated, they confirmed that the internal exposure within dams and pups increased with dose and decreased with time post-dosing (day 18 of gestation versus postnatal day 23 in dams). Serum concentrations of PFOA were significantly increased in mice exposed to PFOA, with mice in the control groups for both ages exhibiting an average PFOA concentration of 0.003 µg/mL, whereas serum concentrations in PFOA-treated KC mice aged 6 or 9 months were 41.96 ± 16.45 and 26.35 ± 17.53 µg/mL, respectively. PFOA concentrations in pancreatic tissue were also elevated (in the range of nanograms per milligram protein) in mice treated with PFOA. For *Min*/+ F_1 male offspring, the numbers of mice in each dose group were 15, 3, 19, and 0, in the groups exposed to PFOA at a dose of 0 (vehicle, water), 0.01, 0.1, or 3.0 mg/kg bw, respectively. The numbers of *Min*/+ F_1 female offspring obtained in each dose group (both blocks together) were 23, 15, 26, and 2 in the groups exposed in utero to PFOA at dose of 0 (vehicle, water), 0.01, 0.1, or 3.0 mg/kg bw, respectively. For the dams weighed

on days 1–18 of gestation, there were no differences in body weight as area under the curve (AUC, arbitrary units) between the experimental groups, in either experimental block 1 or 2, and there was no difference in body weight between the two experimental blocks. For the pups aged 3–18 days, including both *Min*/+ and wildtype (+/+) mice of both sexes, there were some significant differences in body weight between the treatment groups but in varying directions; thus, there were no consistent differences in pup body weight AUC for the pups between experimental blocks 1 and 2. Considering the individual pups in both experimental blocks, PFOA at doses of 3.0 and 0.1 mg/kg bw per day decreased the pup body weight compared with that of pups treated with the vehicle only (water). The offspring were weaned when aged 21 days and housed as a litter per cage, males and females separately. They were genotyped for the heterozygous *Min*/+ mutation using DNA collected from ear punches. The $Apc^{+/+}$ mice were not expected to develop intestinal tumours at age 11 weeks and were used for studies on non-cancer end-points. All C57BL/6J-$Apc^{Min/+}$ offspring mice were killed at age 11 weeks, before the onset of serious anaemia caused by their spontaneous tumours (based on experience with this model), and were used to study the effects of PFOA on intestinal tumorigenesis. The number, diameter, and localization of tumours in the small intestine and colon were measured by transillumination in an inverse light microscope. The reviewer scored lesions at 20× magnification and was blinded to mouse treatment. The diameter of the tumours was scored using an eyepiece graticule. Statistical analysis of incidence was performed on both an individual and litter basis; furthermore, the two experimental blocks were combined in the analysis if no statistical differences in incidence were found between them.

Neoplastic lesions (tumours of the small intestine) were observed in all *Min*/+ mice in all experimental groups including the vehicle group, demonstrating 100% incidence of this end-point, as is usual in this mouse model. PFOA, at any dose, did not cause a significant increase in the number of small intestine tumours, compared with the vehicle (control). In male *Min*/+ mice only, treated with PFOA at 0.01 mg/kg bw, the small intestine tumours were larger in size than those in mice that were treated with the vehicle. Most of the small intestine tumours were localized in the distal two thirds, i.e. in the middle and distal parts, of the small intestine, irrespective of treatment or sex, as seen in previous experiments with *Min*/+ mice (see Andreassen et al., 2002). [The Working Group noted that there was no clear linear dose–response relation in the number and size of small intestine tumours and that these results were found both when the data were analysed with individual mice or with the litter as the statistical unit.]

The incidence of colon tumours at the individual level showed no significant differences between experimental blocks 1 and 2. The only significant difference between the treatment groups was that the group treated with PFOA at 0.1 mg/kg bw had a higher incidence of colon tumours than did the group treated with the vehicle, for males and females together, in experimental block 1 ($P = 0.039$, Fisher exact test, two-tailed probability). However, when this result was tested with the litter as the statistical unit, it did not reach statistical significance. There were no statistically significant differences in the number or diameter of colon tumours between experimental blocks 1 and 2 on the individual level, and therefore the data from both experimental blocks were evaluated together. There were no significant differences in the number of tumours of the colon in mice from any of the groups treated with PFOA compared with that in mice in the vehicle group. The experimental design, such as duration of the study with termination at 11 weeks, was based on previous experience with this model. [The Working Group noted that, usually, when *Min*/+ mice are killed

at age 11 weeks, most intestinal tumours identified are adenomas (see Moser et al., 1990).] [The Working Group noted that this study used both sexes, multiple doses, tested PFOA stability, and analysed the internal dose and the background levels of PFOA in feed and drinking-water. However, no histological examination of tumours of the small intestine or colon was performed.]

Filgo et al. (2015) studied liver toxicity in CD-1 and 129/Sv strains of mice treated with PFOA (specifically, the ammonium salt; purity, > 98%) administered orally (by gavage) after gestational exposure. Both wildtype and peroxisome proliferator-activated receptor alpha (PPARα)-knockout transgenic 129/Sv mice were used. Two blocks of time-pregnant CD-1 mice (12, 12, 14, 13, 12, and 6 dams) were treated with distilled water (vehicle control), or PFOA at a dose of 0.01, 0.1, 0.3, 1, or 5 mg/kg bw, respectively, resulting in a final number of 29, 29 37, 26, 31, and 21 female offspring per group, respectively, surviving to age 18 months. Some mice died before 18 months (28%, 17%, 16%, 28%, 24%, and 22% of the numbers at the beginning of the experiment from the control group and groups treated with PFOA at 0.01, 0.1, 0.3, 1, and 5 mg/kg bw, respectively), because of sudden unknown causes (found on check; 28% of early deaths) or severe dermatitis (common to CD-1 mice; 32%) and other health problems (40%) that required pre-emptive euthanasia. [The Working Group noted that the percentages of early death in this study were reported to be in line with survival rates reported in several other studies of control CD-1 mice aged 18 months, with an average death rate before 18 months of 21.7% (see Giknis and Clifford, 2010).] For the 129/Sv mice, three blocks of animals were used, each separated by 2–3 weeks. 129/Sv wildtype mice were dosed with vehicle, or PFOA at 0.1, 0.3, 0.6, or 1 mg/kg bw, resulting in a final number of 10, 10, 8, 6, and 8 female offspring surviving to age 18 months (to be consistent with the CD-1 mice) and included in the necropsy (from 7, 7, 5, 3, and 5 pregnant dams, respectively). PPARα-knockout mice were dosed with vehicle, or PFOA at 0.1, 0.3, 1, or 3 mg/kg bw, resulting in a final number of 6, 10, 10, 9, and 9 offspring (from 5, 9, 8, 7, and 9 pregnant dams), respectively. Different dose ranges were used for the three strains because of differences in strain sensitivities to PFOA. The highest dose used per strain was selected to minimize developmental toxicities and litter loss (see Abbott et al., 2007). The lower doses were selected such that resulting adolescent mice would have PFOA blood serum concentrations comparable to those reported for highly exposed humans (see Macon et al., 2011). PFOA was administered to all mice by oral gavage on days 1–17 of gestation. To determine the dose amounts, the dams were weighed daily before dosing. At birth, the pups were individually weighed and sexed. Pups within a treatment group were pooled and randomly redistributed among the dams of their respective treatment groups, and litters were equalized to 10 male and female pups. [The Working Group noted that litter effects could not be evaluated because of the cross-fostering that only occurred in the CD-1 mice.] Among the CD-1 mice, small litters (fewer than 4 pups) were excluded from the study. Pups were weaned at age 21 days, and only female offspring were retained in this study and housed 3–5 mice per cage. At 18 months, all mice underwent full necropsy, and livers were collected from all surviving mice in the exposure groups. [The Working Group noted that the mice that died for various reasons before 18 months were not included in this study because of inconsistencies in age and quality of tissues that could be retrieved.] Liver sections underwent a pathology peer review by a team of board-certified veterinary pathologists (pathology working group) to determine the incidence of neoplastic and non-neoplastic lesions, and "INHAND" (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice) liver nomenclature was used when evaluating liver lesions (Thoolen et al., 2010). [The

Working Group noted that this study was not designed as a liver carcinogenesis study but was the consequence of finding liver tumours when investigating unscheduled deaths of PPARα-knockout mice in which no tumours were expected to be found. In addition, mice that died before termination of the study were not examined and, therefore, potential tumours in the liver were not included that could have affected the statistical analysis.]

Neoplastic lesions were present in female CD-1 mice treated with PFOA. The incidence of hepatocellular adenoma – 0/29, 0/29, 1/37 (2.7%), 4/26 (15.4%), 0/31, and 1/21 (4.8%) for the groups at 0 (control), 0.01, 0.1, 0.3, 1, and 5 mg/kg bw, respectively – was significantly increased ($P < 0.05$, Fisher exact test) at 0.3 mg/kg bw, and exceeded the upper bound of the range for historical controls – 3/897 (0.3%); range, 0–2% – reported by Giknis and Clifford (2010). There was one mouse with hepatocellular adenoma (multiple) in the group at the lowest PFOA dose. [The Working Group noted that there was a significant increase in the incidence of adenoma at the intermediate dose (0.3 mg/kg bw) compared with controls and that the upper bound of the range for historical controls was exceeded, but that there was no significant trend in the incidence of adenoma. Therefore, the Working Group was uncertain about the causal association between these tumours and PFOA exposure.] Hepatocellular carcinomas occurred in one mouse per group at 0.3 and 5.0 mg/kg bw, and histiocytic sarcomas developed in one mouse per group at 0.1, 1.0, and 5.0 mg/kg bw, but the incidence did not reach statistical significance for either tumour type. There was a significant positive trend ($P < 0.01$, Cochran–Armitage trend test) in the incidence of liver haemangiosarcoma – 0/29, 0/29, 0/37, 1/26 (3.8%), 0/31, 2/21 (9.5%) in the groups at 0 (control group), 0.01, 0.1, 0.3, 1, and 5 mg/kg bw, respectively – with the incidence exceeding the upper bound of the range observed in historical controls – 3/897 (0.3%), range, 0–2% – reported by Giknis and Clifford (2010). In the control group, the only tumour found was a single malignant lymphoma. [The Working Group noted that lymphoma was a background lesion in historical controls for CD-1 females – 112/900 (12.4%); range, 0–6% (see Giknis and Clifford, 2010).] The overall incidence of malignant lymphoma was 3/144 (2.1%) after PFOA exposure versus 1/29 (3.4%) in the controls.

In the vehicle-treated 129/Sv wildtype mice, no tumours were found. The only tumour found in PFOA-treated 129/Sv wildtype mice was a histiocytic sarcoma in the group at 0.1 mg/kg bw. Hepatocellular adenomas developed in five PFOA-treated PPARα-knockout mice – one mouse in each of the three groups at the lower doses and two mice in the group at the highest dose (3 mg/kg bw) – leading to an overall incidence of 5/38 (13.2%) in PFOA-treated mice. An Ito cell tumour developed in one PPARα-knockout mouse treated with PFOA at 0.3 mg/kg bw. [The Working Group noted that the power to detect an effect was low for this study because of the low number of animals and that the knockout control group contained only six mice.]

Regarding non-neoplastic lesions, basophilic or eosinophilic foci were found in one CD-1 mouse in each PFOA-treated group at 0.01 mg/kg bw, 0.1 mg/kg bw (basophilic foci), and 0.3 mg/kg bw (eosinophilic foci). A significant positive trend in the incidence of oval cell hyperplasia, Ito cell hypertrophy, and centrilobular hepatocyte hypertrophy was observed in CD-1 mice after PFOA exposure, with the incidence being significantly increased for Ito cell hypertrophy and centrilobular hepatocyte hypertrophy in the group at 5 mg/kg bw. Chronic inflammation was common in CD-1 mice, and there was a dose-related increase in severity scores in PFOA-exposed livers; mean severity in the two groups at the highest dose was significantly higher than in the controls. In PPARα-knockout mice, clear cell focus developed in one mouse in the group

treated with PFOA at 0.1 mg/kg bw, and eosinophilic foci developed in one mouse in each group at 0 (control) and 0.3 mg/kg bw. In 129/Sv wildtype mice, eosinophilic foci developed in one mouse in at each group at 0.3 and 0.6 mg/kg bw.

Non-neoplastic changes were also numerous in the 129/Sv strain after PFOA exposure. Significant positive trends were observed in the incidence of both bile duct hyperplasia and bile duct inclusion bodies (hyaline droplets) in 129/Sv PPARα-knockout mice, but there was no increase in the incidence of either bile duct hyperplasia (although there was a decreasing trend in severity with dose) or hyaline droplet accumulation (although there was a decreasing trend in incidence) in 129/Sv wildtype mice. The incidence of Ito cell hypertrophy decreased with increasing PFOA dose in PPARα-knockout mice. There was a significant positive trend in the incidence of haematopoietic cell proliferation with increasing PFOA dose in PPARα-knockout mice, but not in the 129/Sv wildtype mice. There was a significant increase in the incidence of centrilobular hepatocyte hypertrophy with increasing PFOA dose in the PPARα-knockout mice. Although the incidence of centrilobular hepatocyte hypertrophy in 129/Sv wildtype mice did not significantly change with PFOA dose, the severity increased significantly with PFOA dose. A similar increase in mean severity was noted in PPARα-knockout mice, but that effect did not reach statistical significance.

[The Working Group noted that only females were studied. For all three mouse strains, when tumours were counted for mice killed before 18 months, only percentages (%) of mice born were stated. The initial numbers of F_1 females for each study were not provided. Thus, in Table 3.1 the numbers of mice given at start and surviving are both the numbers surviving at 18 months and included in the study. The number of tumours per mouse was not reported, thus, multiplicity was not known. The numbers of mice in the wildtype and knockout studies were low. No statistical comparisons between experimental blocks of mice treated with PFOA were reported. There was no statement that the dams were randomized to the treatment groups. It was not reported whether histopathology was done without knowledge of treatment.]

3.1.2 Rat

See Table 3.2.

(a) Oral administration (feed)

In a study by Biegel et al. (2001) that was designed to compare the carcinogenic effects of Wyeth-14,6431 (designated as WY group), a rodent peroxisome proliferator and carcinogen, with those of PFOA (specifically, ammonium salt; designated as the C8 group), an initial group of 156 male Sprague-Dawley rats [Crl:CD BR (CD)] (age, 6 weeks) were treated with feed containing PFOA (purity, 98–100%) at a dose of 300 ppm for 24 months. Two control groups (156 rats in each) were either fed ad libitum (designated as the control group) or received the same amount of feed as the PFOA-treated group (pair-fed control group, designated as the CP-C8 group), respectively. The average daily dose of PFOA was 13.6 mg/kg bw per day in the C8 group. There were initially 156 rats per group, and 6 rats per group were randomly selected and killed at eight interim time points (approximately 1, 3, 6, 9, 12, 15, 18, and 21 months) for histology evaluation (48 rats) and measurements of cell proliferation and peroxisome proliferation (48 rats), leaving 60 rats per group for the 2-year observation for carcinogenesis (Biegel et al., 2001). Hormone measurement was performed at the eight interim time points using 10 rats per group that were randomly selected and not killed. At study termination, survival rates were approximately 15%, 35%, 48%, and 16% for the control, CP-C8, C8, and WY groups, respectively. [The Working Group noted that, although not clearly documented, rats for hormone measurement were

Table 3.2 Studies of carcinogenicity in rats exposed to PFOA

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Crl:CD BR (M) 49 d [7 wk] 24 mo Biegel et al. (2001)	Oral administration (feed) PFOA (ammonium salt), 98–100% Feed 0 (controls fed ad libitum), 0 (pair-fed controls; CP-C8), 300 (C8) ppm (approximately 0, 0, and 13.6 mg/kg bw) 156, 156, 156 80, 79, 76	*Liver* Hepatocellular adenoma 2/80 (3%), 1/79 (1%), 10/76 (13%)* Hepatocellular carcinoma 0/80, 2/79 (3%), 0 /76 Hepatocellular adenoma or carcinoma (combined) 2/80 (3%), 3/79 (4%), 10/76 (13%)* *Testis* Leydig cell adenoma 0/80, 2/78 (3%), 8/76 (11%)* *Pancreas* Acinar cell adenoma 0/80, 1/79 (1%), 7/76 (9%)* Acinar cell carcinoma 0/80, 0/79, 1/76 (1%) Acinar cell adenoma or carcinoma (combined) 0/80, 1/79 (1%), 8/76 (11%)*	 *$P < 0.05$, Dunnett test; [$P = 0.0038$, Fisher exact test] NS *$P < 0.05$, Dunnett test; [$P = 0.0336$, Fisher exact test] *$P < 0.05$, Dunnett test; [$P = 0.0448$, Fisher exact test] *$P < 0.05$, Dunnett test; [$P = 0.0279$, Fisher exact test] NS *$P < 0.05$, Dunnett test; [$P = 0.0145$, Fisher exact test]	*Principal strengths*: long-term study; adequate number of rats per group; study covered most of the lifespan. *Principal limitations*: only one dose group; one sex used; age of rats when assessed for lesions (when killed) was not clearly documented; no results or data for trend test(s) were reported, despite large differences in survival rates among groups; scheduled and unscheduled deaths were not distinguishable and were shown as the denominator of the rat numbers in Table 2 of this paper. *Other comments*: only the liver, testes, epididymides, pancreas, and organs with gross lesions were examined microscopically; of 165 rats per group, 48 rats were designated for interim kill for measurement of cell proliferation, and another 48 rats for peroxisome proliferation (6 rats × 8 time points); hormone analysis was performed without killing; 60 rats were likely to be designated for pathological evaluation for the 2-yr time period; peer-review of the data on pancreatic lesions by a panel of pathologists (Caverly Rae et al., 2014) using the same diagnostic criteria as those applied in the study by Biegel et al. (2001) generated the following incidence data (which were statistically significant from those for pair-fed controls, *$P < 0.05$): pancreatic acinar hyperplasia: 14/80, 8/79, 30/76*

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley Crl: COBS (SD) BR (M) 39–41 d [6 wk] 104 wk Butenhoff et al. (2012a)	Oral administration (feed) PFOA (ammonium salt), 97.2% Feed 0, 30, 300 ppm (actual doses, 0, 1.3, and 14.2 mg/kg bw per day) 50, 50, 50 49, 50, 50	*Testis and epididymis* Leydig cell adenoma 0/49, 2/50 (4%), 7/50 (14%)*	[$P = 0.010$, Cochran–Armitage trend test] *$P \leq 0.05$, Fisher exact test (two-tailed) [*$P = 0.0067$, Fisher exact test (one-tailed)]	*Principal strengths*: adequate number of animals per group, males and females used, adequate duration; well-conducted study. *Other comments*: 65 rats in the control and higher-dose groups, 50 rats in the lower-dose group (15 rats from the control and higher-dose groups were killed at 1 yr); no neoplasms at 1-yr interim kill; peer review of the pancreatic lesion data by a panel of pathologists (Caverly Rae et al., 2014) using the same diagnostic criteria as those applied in the study by Biegel et al. (2001) generated the following incidence data: pancreatic acinar cell hyperplasia, 3/46, 1/46, 10/47* [*$P = 0.0382$, Fisher exact test (one-tailed)].
		Liver Hepatocellular carcinoma 3/49 (6%), 1/50 (2%), 5/50 (10%)	NS	
		Adrenal medulla Pheochromocytoma (benign) 2/49 (4%), 4/50 (8%), 4/50 (8%)	NS	
		Pheochromocytoma (malignant) 0/49, 1/50 (2%), 0/50	NS	
		Pituitary gland Adenoma 17/48 (35%), 17/47 (36%), 13/46 (28%)	NS	
		Thyroid gland, C-cell Adenoma 0/43, 2/47 (4%), 4/47 (9%)	NS	
		Carcinoma 2/43 (5%), 0/47, 0/47	NS	

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley Crl: COBS (SD) BR (F) 39–41 d [6 wk] 104 wk Butenhoff et al. (2012a)	Oral administration (feed) Ammonium salt, 97.2% Feed 0, 30, 300 ppm Actual doses: 0, 1.6, 16.1 mg/kg bw per day 50, 50, 50 46, 45, 44	*Mammary gland* Fibroadenoma 10/46 (22%), 19/45 (42%)*, 21/44 (48%)** Adenocarcinoma 7/46 (15%), 14/45 (31%), 5/44 (11%) Lymphangiosarcoma 0/46, 0/45, 1/44 (2%) *Adrenal medulla* Pheochromocytoma (malignant) 0/50, 0/50, 1/49 (2%) *Liver* Hepatocellular carcinoma 0/50, 0/50, 1/50 (2%) *Pituitary gland* Adenoma 33/46 (72%), 39/47 (83%), 36/50 (72%) Adenocarcinoma 9/50 (18%), 16/50 (32%), 5/50 (10%)	[$P = 0.024$, Cochran–Armitage trend test] [*$P = 0.0302$, Fisher exact test (one-tailed)] [**$P = 0.0086$, Fisher exact test (one-tailed)] NS NS NS NS NS	*Principal strengths*: adequate number of animals per group; males and females used; adequate duration. *Other comments*: 65 rats in the control and high-dose groups, 50 rats in the low-dose group (15 rats from the control and high-dose groups were killed at 1 yr); no neoplasms at 1-yr interim kill. Peer review of the mammary gland data by a panel of pathologists (Hardisty et al., 2010) using contemporary diagnostic criteria generated the following incidence data (with no statistical significance): adenocarcinoma of the mammary gland, 9/50 (18%), 16/50 (32%), 5/50 (10%); adenoma of the mammary gland: 1/50 (2%), 0/50, 0/50; fibroadenoma of the mammary gland: 16/50 (32%), 16/50 (32%), 20/50 (40%); fibroadenoma (multiple) of the mammary gland: 2/50 (4%), 6/50 (12%), 3/50 (6%) (not adjusted for survival).

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Hsd:Sprague-Dawley (M) Perinatal then PND 21–23 (study 2) 2 yr NTP (2020)	Oral administration (feed) PFOA, 98.8% NIH-07 (perinatal phase) and NTP-2000 (post-weaning phase) 0/0, 0/20, 0/40, 0/80, 300/0, 300/20, 300/40, 300/80 ppm Feed 50, 50, 50, 50, 50, 50, 50, 50 36, 42, 35, 37, 35, 38, 38, 39	*Liver* Hepatocellular adenoma (includes multiple) 0/50, 0/50, 7/50 (14%)*, 11/50 (22%)**, 0/50, 1/50 (2%), 5/50 (10%), 10/50 (20%)***	$P < 0.001$, Cochran–Armitage trend test *$P = 0.050$, Rao–Scott adjusted poly-3 test; [$P = 0.0062$, Fisher exact test] **$P = 0.010$, Rao–Scott adjusted poly-3 test; [$P = 0.0003$, Fisher exact test] ***$P = 0.006$, Rao–Scott adjusted poly-3 test; [$P = 0.0006$, Fisher exact test]	*Principal strengths*: adequate number of animals used; randomly allocated in groups; adequate duration; males and females used; multiple doses used; well-conducted GLP study. *Other comments*: historical controls: hepatocellular adenoma, all routes, 2/340 (0.67% ± 1.03%); range, 0–2%; hepatocellular carcinoma, 0/340; hepatocellular adenoma or carcinoma (combined), 2/340 (0.67% ± 1.03%); range, 0–2%; pancreatic acinar cell adenoma, all routes, 45/340 (12.33% ± 10.07%); range, 0–28%; pancreatic acinar cell adenocarcinoma, 2/340 (0.52% ± 0.85%); range, 0–2%; pancreatic acinar cell adenoma or adenocarcinoma (combined), 45/340 (12.33% ± 10.07%); range, 0–28%.
		Hepatocellular carcinoma 0/50, 0/50, 0/50, 0/50, 0/50, 0/50, 0/50, 4/50 (8%)	Positive trend for F_1 males with F_0 exposure only	
		Hepatocellular adenoma or carcinoma (combined) 0/50, 0/50, 7/50 (14%)*, 11/50 (22%)**, 0/50, 1/50 (2%), 5/50 (10)***, 12/50 (24%)****	$P < 0.001$, Cochran–Armitage trend test *$P = 0.050$, Rao–Scott adjusted poly-3 test; [$P = 0.0062$, Fisher exact test] **$P = 0.010$, Rao–Scott adjusted poly-3 test; [$P = 0.0003$, Fisher exact test] ***[$P = 0.0062$, Fisher exact test] ****$P = 0.003$, Rao–Scott adjusted poly-3 test; [$P = 0.0001$, Fisher exact test]	

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Hsd:Sprague-Dawley (M) Perinatal then PND 21–23 (study 2) 2 yr NTP (2020) (cont.)		*Pancreas* Acinar cell adenoma (includes multiple) 3/50 (6%), 28/50 (56%)**, 26/50 (52%)**, 32/50 (64%)**, 7/50 (14%), 18/50 (36%)*, 30/50 (60%)**, 30/50 (60%)**	$P < 0.001$, Cochran–Armitage trend test *$P = 0.016$, Rao–Scott adjusted poly-3 test; [$P = 0.0099$, Fisher exact test] **$P < 0.001$, Rao–Scott adjusted poly-3 test; [$P < 0.0001$, Fisher exact test]	
		Acinar cell adenocarcinoma 0/50, 3/50 (6), 1/50 (2%), 3/50 (6%), 0/50, 2/50 (4%), 1/50 (2%), 3/50 (6%)	NS	
		Acinar cell adenoma or adenocarcinoma (combined) 3/50 (6%), 29/50 (58%)**, 26/50 (52%)**, 32/50 (64%)**, 7/50 (14%), 20/50 (40%)*, 30/50 (60%)**, 30/50 (60%)**	$P < 0.001$, Cochran–Armitage trend test *$P = 0.006$, Rao–Scott adjusted poly-3 test; [$P < 0.0001$, Fisher exact test] **$P < 0.001$, Rao–Scott adjusted poly-3 test; [$P < 0.0001$, Fisher exact test]	

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Hsd:Sprague-Dawley (F) Perinatal then PND 21–23 (study 1) 2 yr NTP (2020)	Oral administration (feed) PFOA, 98.8% NIH-07 (perinatal phase) and NTP-2000 (post-weaning phase) 0/0, 0/300, 0/1000, 150/300, 300/1000 ppm Feed 50, 50, 49, 50, 50 23, 28, 23, 32, 23	*Liver* Hepatocellular adenoma 2/50 (4%), 0/50, 1/49 (2%), 0/50, 3/50 (6%) Hepatocellular carcinoma 1/50 (2%), 1/50 (2%), 3/49 (6%), 0/50, 4/50 (8%) Hepatocellular adenoma or carcinoma (combined) 3/50 (6%), 1/50 (2%), 4/49 (8%), 0/50, 6/50 (12%) *Pancreas* Acinar cell adenoma 0/50, 0/50, 1/49 (2%), 0/50, 3/50 (6%) Acinar cell adenocarcinoma 0/50, 0/50, 1/49 (2%), 0/50, 2/50 (4%) Acinar cell adenoma or adenocarcinoma (combined) 0/50, 0/50, 2/49 (4%), 0/50, 5/50 (10%) *Uterus* Adenoma (extended evaluation) 1/50 (2%), 0/49, 0/48, 0/50, 0/48 Acinar cell adenocarcinoma (extended evaluation) 1/50 (2%), 5/49 (10%), 7/48 (14%)*, 3/50 (6%), 5/48 (10%)	NS NS NS NS (*P* = 0.018, Rao–Scott adjusted trend poly-3 test) NS *P = 0.005, Rao–Scott adjusted poly-3 test; [*P* = 0.0258, Fisher exact test]	*Principal strengths*: adequate number of animals used, randomly allocated in groups; adequate duration; males and females used; multiple doses used; well-conducted GLP study. *Other comments*: historical controls: hepatocellular adenoma, all routes, 14/340 (3.63% ± 2.59%); range, 0–8%; hepatocellular carcinoma: 1/340 (0.33% ± 0.82%); range, 0–2%; hepatocellular adenoma or carcinoma (combined), 15/340 (3.96% ± 2.77%); range, 0–8%; pancreatic acinar cell adenoma, all routes, 0/340; pancreatic acinar cell adenocarcinoma, 0/340; pancreatic acinar cell adenoma or adenocarcinoma (combined), 0/340; adenoma of the uterus (standard evaluation), all routes, 1/150 (0.67% ± 1.15%); range, 0–2%; adenocarcinoma of the uterus (standard evaluation), 11/150 (7.33% ± 4.62%); range, 2–10%; adenoma or adenocarcinoma (combined) of the uterus, 12/150 (8% ± 3.46%); range (standard evaluation), 4–10%.

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Hsd:Sprague-Dawley (F) Perinatal then PND 21–23 (study 1) 2 yr NTP (2020) (cont.)		Acinar cell adenoma or adenocarcinoma (combined) (extended evaluation) 2/50 (4%), 5/49 (10%), 7/48 (15%), 3/50 (6%), 5/48 (10%) Acinar cell adenoma (standard or extended evaluation combined) 1/50 (2%), 1/49 (2%), 0/49, 0/50, 0/50 Acinar cell adenocarcinoma (standard or extended evaluation combined) 1/50 (2%), 5/49 (10%), 7/49 (14%)*, 3/50 (6%), 5/50 (10%) Acinar cell adenoma or adenocarcinoma (combined) (standard or extended evaluation combined) 2/50 (4%), 5/49 (10%), 7/49 (14%), 3/50 (6%), 5/50 (10%)	NS NS *P = 0.050, Rao–Scott adjusted poly-3 test; [P = 0.0277, Fisher exact test] NS	

bw, body weight; d, day(s); F, female; GLP, Good Laboratory Practice; M, male; mo, month(s); NIH, National Institutes of Health; NS, not significant; NTP, National Toxicology Program; PFOA, perfluorooctanoic acid; PND, postnatal day(s); ppm, parts per million; vs, versus; wk, week(s); yr, year(s).

not killed and were returned to the group for further treatment and observation. Accordingly, the Working Group estimated that the final number of rats for pathological evaluation at the 24-month time point was around 60 animals per group. Survival data were provided in graphic form only (the actual numbers were not reported) and therefore numbers were estimated by the Working Group from the graph presented in the original publication.] All surviving rats underwent complete necropsy with histopathological evaluation. The liver, testes, epididymis, pancreas, and organs with gross lesions were examined microscopically.

At 24 months, exposure to PFOA (C8 group) significantly increased the incidence of hepatocellular adenoma – 10/76 (13%) versus 1/79 (1%), ($P < 0.05$, Dunnett test; [$P = 0.0038$, Fisher exact test]) – and of hepatocellular adenoma or carcinoma (combined) – 10/76 (13%) versus 3/79 (4%), ($P < 0.05$, Dunnett test; [$P = 0.0336$, Fisher exact test]) – compared with the pair-fed control group CP-C8. There was a significant increase ($P < 0.05$, Dunnett test; [$P = 0.0448$, Fisher exact test]) in the incidence of testicular Leydig cell adenoma in the C8 group compared with the CP-C8 group – 8/76 (11%) versus 2/78 (3%). There were significant increases ($P < 0.05$, Dunnett test; [$P = 0.0279$, Fisher exact test]) in the incidence of pancreatic acinar cell adenoma in the C8 group compared with the CP-C8 group – 7/76 (9%) versus 1/79 (1%) – and in the incidence of pancreatic acinar cell adenoma or carcinoma (combined) in the C8 group ($P < 0.05$, Dunnett test; [$P = 0.0145$, Fisher exact test]) compared with the CP-C8 group – 8/76 (11%) versus 1/79 (1%). [The Working Group noted that the numbers of rats that were submitted for pathological diagnosis were indicated only in the footnote of Table 2 of the publication and are the sum of scheduled and unscheduled deaths.]

Regarding non-neoplastic lesions in the C8 group compared with the CP-C8 group, there was a significant increase in the incidence of pancreatic acinar cell hyperplasia.

[The Working Group noted that this was a long-term study using a large group size and that the duration was most of the lifespan, and that the stability of the test article was evaluated and the concentration in the diet measured. However, the study was limited by the use of a single dose; the use of one sex only; because the age of each animal at death was not clearly stated; and because no results or data for trend test(s) were reported, despite large differences in survival rates among groups.]

Butenhoff et al. (2012a) published a report of a well-conducted study carried out between 1981 and 1983. The original reports of this study (US EPA, 1987) were published in a peer-reviewed publication by Butenhoff et al. (2012a). In this study of chronic toxicity that complied with Good Laboratory Practice (GLP), groups of male and female Sprague-Dawley rats [Crl:COBS CD(SD)BR] (age, 39–41 days) were treated with feed containing PFOA (specifically, the ammonium salt; purity, 97.2%) at a concentration of 0 (control), 30, or 300 ppm, corresponding to an average daily dose of approximately 0, 1.3, and 14.2 mg/kg bw in males, and 0, 1.6, and 16.1 mg/kg bw in females. The control group and group at the higher dose contained 65 males and 65 females, and the group at the lower dose contained 50 males and 50 females. An interim kill at 1 year involved 15 males and 15 females from both the control group and the group at 300 ppm, and the remaining rats, 50 of each sex, were killed at 104 weeks (US EPA, 1987); also reported by Butenhoff et al., 2012a). At study termination at 104 weeks, survival was 70%, 72%, and 88% for male rats, and 50%, 48%, and 58% for female rats, for the groups at 0 (control), 30, and 300 ppm, respectively; the increased survival rate observed in males at the higher dose was statistically significant, compared with the males in the control group. For male rats at the higher dose, body-weight gains were decreased by more than 10% throughout the 66 weeks of the study, compared with controls. The largest

decrease was approximately 21% by week 6. In males at the lower dose, a slight but not significant (5%) decrease in body weight was observed at week 6, with little additional decrease subsequently. In female rats treated with PFOA, mean body weights did not change during the first 18 months of the study. After 18 months, there was a gradual decrease in mean body weight in females at 300 ppm; the maximum decrease was 11% lower than that of the controls at 22 months. Mean feed consumption relative to body weight was increased in all the PFOA-treated males throughout the study. In females, there was a trend towards lowered food consumption in both PFOA-treated groups.

In males, there was a significant positive trend [$P = 0.010$, Cochran–Armitage test] in the incidence of testicular Leydig cell adenoma, and the incidence – 0/49, 2/50 (4%), and 7/50 (14%) for the groups at 0 (control), 30, and 300 ppm, respectively – was significantly increased at 300 ppm ($P \leq 0.05$, Fisher exact test, two-tailed; [$P = 0.0067$, Fisher exact test, one-tailed]).

In females, there was a significant positive trend [$P = 0.024$, Cochran–Armitage test] in the incidence of fibroadenoma of the mammary gland, and the incidence – 10/46 (22%), 19/45 (42%), and 21/44 (48%) for the groups at 0 (control), 30, and 300 ppm, respectively – was significantly increased at 30 and 300 ppm ([$P = 0.0302$, $P = 0.0086$, Fisher exact test, respectively]).

In 2010, a pathology working group was convened to review the original slides of mammary glands from the study by US EPA (1987), to provide a consensus diagnosis for neoplasms of the mammary gland using contemporary diagnostic criteria (Hardisty et al., 2010). The pathology working group concluded that some lesions originally diagnosed as lobular hyperplasia had features consistent with fibroadenoma of the mammary gland (mainly in slides from the control group), and that, consequently, PFOA did not induce neoplasms of the mammary gland. Both the initial data on mammary pathology (US EPA, 1987) and the reviewed data (Hardisty et al., 2010) were reported by Butenhoff et al. (2012a).

There was an increase in the incidence of hepatocellular hypertrophy in males and females at the higher dose, and an increase in the incidence of liver cystoid degeneration, vascular mineralization of the testis, and portal mononuclear cell infiltrate in males at the higher dose (Butenhoff et al., 2012a). Increases in the incidence of pancreatic acinar cell hyperplasia in male rats – 0/46 (0%), 2/46 (4%), and 2/49 (4%) in the groups at 0 (control), 30, and 300 ppm, respectively – were not statistically significant. Pancreatic acinar hyperplasia was not reported in female rats in this study. [The Working Group noted that this study used an adequate number of rats per group, both sexes, and an adequate duration of exposure. Discrepancy between the original study pathology and the review pathology (Hardisty et al., 2010) regarding the diagnosis of mammary lesions was noted. The Working Group also noted that increases in the incidence of Leydig cell adenoma were the only positive finding when using contemporary diagnostic criteria. In addition, the Working Group noted that faster elimination occurs in female rats than in males, as outlined in Section 4.1 of the present monograph, which may explain why minimal effects were observed in females.]

In a review of the pancreatic lesions observed in male rats in US EPA (1987), also reported by Butenhoff et al. (2012a), using the same diagnostic criteria as those applied in the study by Biegel et al. (2001), a significant positive trend ($P < 0.05$, Cochran–Armitage trend test) in the incidence of pancreatic acinar cell hyperplasia was observed in males, and the incidence was significantly increased [$P = 0.0382$, Fisher exact test] at the higher dose – 3/46 (7%), 1/46 (2%), and 10/47 (21%) (Caverly Rae et al., 2014). There were no statistically significant or test-related increases in the incidence of acinar cell adenoma or in acinar cell carcinoma separately with either

PFOA dose, but there was a significant positive trend ($P < 0.05$, Cochran–Armitage trend test) in the incidence of all three lesions combined (hyperplasia, adenoma, and carcinoma) – 3/46 (7%), 1/46 (2%), and 11/47 (23%). [The Working Group noted that only one neoplasm was observed, which was a carcinoma in the group at the higher dose. Both pancreatic acinar cell hyperplasia and pancreatic acinar adenoma were considered to be proliferative lesions, and this review supported the conclusion that the pancreas is a target of PFOA in male rats. In addition, the Working Group noted that hyperplasia, adenoma, and carcinoma were combined under the assumption that they are sequential pathological lesions.]

In a well-conducted study of chronic toxicity that complied with GLP and in which early-life exposure to PFOA on carcinogenicity outcomes was investigated, PFOA (purity, 98.8%) was administered to groups of 36 Hsd:Sprague-Dawley pregnant rats from day 6 of gestation through lactation, and subsequently to their pups for 2 years (NTP, 2020, revised in 2023). The control group comprised 103 pregnant females. F_0 females received feed containing PFOA at a concentration of 0, 150, or 300 ppm and were housed individually during gestation and together with their respective litters during lactation. The pups (F_1) were weaned on postnatal days 21–23. All F_1 exposure groups comprised 50 males and 50 females and were treated with feed containing PFOA at a concentration of 150 or 300 ppm for males and 300 or 1000 ppm for females. The initial dose setting, i.e. 150 ppm and 300 ppm during the mating and preweaning period (F_0) combined with 300 ppm and 1000 ppm (females) or 150 ppm and 300 ppm (males) for 2-year dietary exposure to the offspring (F_1) was tolerated only by female offspring (designated as study 1 for females only). [The Working Group noted that elimination is faster in female rats than in males (as outlined in Section 4.1 in the present monograph), which was used to explain the higher post-weaning doses used in females.] Therefore, a second study was started that was focused entirely on males, and post-weaning concentrations were lowered (designated as study 2 for males only). A single perinatal exposure concentration was used, i.e. 300 ppm for the F_0 rats, and 20, 40 and 80 ppm for the F_1 rats. Total and live litter sizes and survival of the F_1 rats during lactation were not affected by exposure.

The treatment groups are indicated by the given doses in parts per million for the F_0 (gestation/lactation) and F_1 (post-weaning) as F_0/F_1, such as 0/1000.

At termination of study 2 (2 years, males only), group mean body weights for the groups at 0/20, 0/40, 0/80, 300/0, 300/20, and 300/40 ppm (males) were within 10% of those for the respective control groups (0/0 ppm or 300/0 ppm). The terminal mean body weight of the group at 300/80 ppm was 13% less than that of the control group at 0/0 ppm. Post-weaning consumption of PFOA in males was 1.1/1.0, 2.2/2.1 and 4.6/4.6 mg/kg per day for the groups at 20, 40, and 80 ppm, with or without perinatal exposure. At termination of study 1 (2 years, females only), group mean body weights for the groups at 0/1000 and 300/1000 ppm were lower (19% and 27%, respectively) than those in the 0/0 ppm control group (females). Group mean feed consumption in females over the course of the study averaged 93%, 99%, 96%, and 88% of that in the 0/0 ppm control group for the groups at 0/300, 150/300, 0/1000, and 300/1000 ppm, respectively. After weaning, PFOA consumption for females in the groups at 0/300 and 150/300 ppm averaged 18.2 and 18.4 mg/kg per day, respectively. PFOA consumption averaged 63.4 and 63.5 mg/kg per day for the groups at 0/1000 and 300/1000 ppm, respectively (NTP, 2020).

In F_1 male rats (2 years, study 2), there was a significant positive trend in the incidence of hepatocellular adenoma (includes multiple) ($P < 0.001$, Cochran–Armitage trend test) in

both the F_0 exposed and the F_0 unexposed groups, with the incidence being significantly increased (P = 0.050, Rao–Scott adjusted poly-3 test, [P = 0.0062, Fisher exact test]; P = 0.010, Rao–Scott adjusted poly-3 test, [P = 0.0003, Fisher exact test]; P = 0.006, Rao–Scott adjusted poly-3 test, [P = 0.0006, Fisher exact test]) at 0/40, 0/80, and 300/80 ppm, respectively) in both the F_0 exposed and the F_0 unexposed groups, i.e. 0/50, 0/50, 7/50 (14%), 11/50 (22%) at 0/0, 0/20, 0/40, 0/80 ppm, and 0/50, 1/50 (2%), 5/50 (10%), 10/50 (20%) at 300/0, 300/20, 300/40, and 300/80 ppm. In addition, the incidence in all treated groups, except the group at 300/20 ppm, exceeded the upper bound of the range observed in historical controls from this laboratory – 2/340 (0.067% ± 1.03%); range, 0–2%. There was a significant positive trend in the incidence of hepatocellular carcinoma (P = 0.049, Cochran–Armitage trend test) in male rats with perinatal exposure. No carcinomas were observed in the male rats with only post-weaning exposure. There was a significant positive trend in the incidence of hepatocellular adenoma or carcinoma (combined) (P < 0.001, Cochran–Armitage trend test) in both the F_0 exposed and the F_0 unexposed groups, with the incidence being significantly increased (P = 0.050, Rao–Scott adjusted poly-3 test, [P = 0.0062, Fisher exact test]; P = 0.010, Rao–Scott adjusted poly-3 test, [P = 0.0003, Fisher exact test]; P = 0.003, Rao–Scott adjusted poly-3 test, [P = 0.0001, Fisher exact test] at 0/40, 0/80, and 300/80 ppm, respectively, in both the F_0 exposed and the F_0 unexposed groups, i.e. 0/50, 0/50, 7/50 (14%), and 11/50 (22%) for the groups at 0/0, 0/20, 0/40, and 0/80 ppm, and 0/50, 1/50 (2%), 5/50 (10%), and 12/50 (24%) for the groups at 300/0, 300/20, 300/40, 300/80 ppm. In addition, the incidence in all treated groups, except in the group at 300/20 ppm, exceeded the upper bound of the range observed in historical controls from this laboratory – 2/340 (0.067% ± 1.03%); range, 0–2%.

There was a significant positive trend in the incidence of acinar cell adenoma of the pancreas (includes multiple) (P < 0.001, Cochran–Armitage trend test) in both the F_0 exposed and the F_0 unexposed groups, with the incidence being significantly increased (P < 0.0001, Rao–Scott adjusted poly-3 test, [P > 0.0001, Fisher exact test] at 0/20, 0/40, 0/80, and 300/40 ppm; and P = 0.016, Rao–Scott adjusted poly-3 test, [P = 0.0002, Fisher exact test] at 300/20 ppm) in both the F_0 exposed and the F_0 unexposed groups – 3/50 (6%), 28/50 (56%), 26/50 (52%), and 32/50 (64%) for the groups at 0/0, 0/20, 0/40, and 0/80 ppm, and 7/50 (14%), 18/50 (36%), 30/50 (60%), and 30/50 (60%) for the groups at 300/0, 300/20, 300/40, and 300/80 ppm, respectively). In addition, the incidence in all treated groups exceeded the upper bound of the range observed in historical controls from this laboratory – all routes, 45/340 (12.33% ± 10.07%); range, 0–28%. The incidence of pancreatic acinar cell adenocarcinoma was not statistically significant in any of the treated groups versus controls and exceeded the upper bound of the range observed in historical controls from this laboratory – all routes, 2/340 (0.52% ± 0.85%); range, 0–2% – for the groups at 0/20, 0/80, 300/20, and 300/80 ppm. There was a significant positive trend in the incidence of acinar cell adenoma or adenocarcinoma (combined) of the pancreas (P < 0.001, Cochran–Armitage trend test) in both the F_0 exposed and the F_0 unexposed groups, with the incidence being significantly increased (P < 0.0001, Rao–Scott adjusted poly-3 test, [P < 0.0001, Fisher exact test] for all treated groups) in both the F_0 exposed and the F_0 unexposed groups – 3/50 (6%), 29/50 (58%), 26/50 (52%), and 32/50 (64%) for the groups at 0/0, 0/20, 0/40, and 0/80 ppm, and 7/50 (14%), 20/50 (40%), 30/50 (60%), and 30/50 (60%) for the groups at 300/0, 300/20, 300/40, and 300/80 ppm, respectively). In addition, the incidence in all treated groups exceeded the upper bound of the range observed in historical

controls from this laboratory – all routes, 45/340 (12.33% ± 10.07%); range, 0–28%.

The effect of perinatal exposure (F_0) over the effect of postnatal exposure (F_1) was not apparent for hepatocellular adenoma and acinar cell adenoma of the pancreas. There was a suggestive but not statistically significant effect of perinatal exposure on the incidence of hepatocellular carcinoma in male rats – 0/50, 0/50, 0/50, and 0/50 versus 0/50, 0/50, 0/50, 4/50 (P = 0.049 by Rao–Scott adjusted poly-3 test). [The Working Group noted that hepatocellular carcinoma is a rare neoplasm (0/340 in historical controls).]

In female rats (2 years, study 1), there was a significant positive trend (P = 0.018, Rao–Scott adjusted poly-3 test) in the incidence of pancreatic acinar cell adenoma or adenocarcinoma (combined) with perinatal exposure – 0/50, 0/50, and 5/50 (10%) for the groups at 0/0, 150/300, and 300/1000 ppm, respectively). There was a significant increase in the incidence of adenocarcinoma of the uterus (standard or extended evaluation combined) (P = 0.050, Rao–Scott adjusted poly-3 test, [P = 0.0227, Fisher exact test] for the group at 0/1000 ppm) in F_0 exposed groups – 1/50 (2%), 5/49 (10%), 7/49 (14%), 3/50 (6%), and 5/48 (10%) for the groups at 0/0, 0/300, 0/1000, 150/300, and 300/1000 ppm, respectively). [The Working Group noted that the new and extended evaluation used a combination of two sectioning methods. Because of this change in methods, the historical controls were of limited utility for the results obtained by the new method. The Working Group also noted that the new data reflected the 2023 revision.]

In 2023, a revision was made due to the identification of an error in the combining process for the uterine adenocarcinomas: "One animal with a squamous cell carcinoma in the 0/1000 ppm group was inadvertently combined in the adenocarcinoma analysis of the extended evaluation. The number of animals examined during the standard, extended and standard or extended (combined) evaluations was also corrected in the 0/300, 0/1000, and 300/1000 ppm groups" (NTP, 2020; revised in 2023). [The Working Group noted that the squamous cell carcinoma is of the same origin as the endometrial epithelium and can be combined with the adenoma and carcinoma. The Working Group also noted that the significant difference in the incidence of adenocarcinoma of the uterus in the group with the highest exposure without perinatal exposure was still statistically significant.]

Regarding non-neoplastic lesions, exposure to PFOA resulted in increases in the incidence of non-neoplastic lesions in the liver (hepatocyte cytoplasmic alteration; hepatocyte hypertrophy; hepatocyte single cell death; necrosis; and pigment in males and females) in males; hepatocyte cytoplasmic alteration; hepatocyte hypertrophy; hepatocyte single cell death; necrosis; pigment; bile duct hyperplasia; hepatocyte increased mitoses, in females; pancreatic acinus hyperplasia in male rats; and follicular cell hypertrophy of the thyroid gland of female rats. [The Working Group noted that pancreatic acinus adenoma and adenocarcinoma are rare lesions in females of this rat species and that pancreatic acinus hyperplasia was also considered to be rare; although these effects were of low incidence, they were consistent with the increased incidence of pancreatic acinar cell lesions reported in male rats.]

[The Working Group noted that this was a well-conducted study that complied with GLP and that used an adequate number of rats per group, both sexes, and an adequate duration of exposure. The Working Group also noted that internal exposure was measured in male and female rats, and that the stability of the test article was tested.]

(b) Initiation–promotion studies

See Table 3.3.

In an initiation–selection–promotion study, adult male Wistar rats [age not reported] were initiated with diethylnitrosamine (DEN)

Table 3.3 Initiation–promotion studies in rats and fish exposed to PFOA

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence or multiplicity	Significance	Comments
Initiation–promotion (tested as promoter) Rat, Wistar (ICO:WI IOPS AF:Han) (M) Age NR ("adult") 7 mo after initiation Abdellatif et al. (1990)	Oral administration (feed) PFOA, NR (analytical grade) Feed Basal feed (control), PB (positive control), PFOA (% feed) NDEA (single i.p. injection), 2-AAF (feed), carbon tetrachloride (gavage), PFOA and PB (feed) – Initiation: NDEA, 200 mg/kg bw (all three groups) – Selection: 2 wk after initiation, 0.03% 2-AAF for 2 wk; carbon tetrachloride, 2 mL/kg bw in 1:1 corn oil (after 1 wk of 2-AAF) – Promotion: 0% (control), 0.05% PB or 0.15% PFOA for 7 mo 7, 8, 12 7, 8, 12	*Liver* Total tumours Tumour incidence: 0/7, 6/8 (75%)**, 4/12 (33%)* Tumour multiplicity: 0, 3.4, 1.2	*$P < 0.05$, Student *t*-test **$P < 0.02$, Student *t*-test	*Principal strengths*: the only report on the promoting activity of PFOA identified in rats via an initiation–selection–promotion protocol; PFOA concentrations measured in serum; end-points were measure at two time points. *Principal limitations*: only a limited number of rats were used in the experiment; histopathological examination of the liver only; only one sex used; average daily dose of PFOA was not reported.
Initiation–promotion (tested as promoter) Rat, Wistar (ICO:WI IOPS AF:Han) (M) Age NR ("adult") 12 mo Abdellatif et al. (1991)	Oral administration (feed) PFOA, NR (analytical grade) Feed Basal feed (control), PB (positive control), PFOA (0.005%), PFOA (0.02%) % in feed NDEA (single i.p. injection), PB and PFOA (feed) – Initiation: NDEA, 200 mg/kg bw (all three groups) – Promotion: basal feed (control), 0.05% PB, or 0.005% or 0.02% PFOA for 12 mo 10, 10, 10, 10 7, 7, 7, 9	*Liver* Hepatocellular carcinoma Tumour incidence: 0/7, 2/7 (28%), 1/7 (14.3%), 5/9 (55.5%)*	*$P < 0.05$, Scheffé test NR	*Principal strengths*: the only report on promoting activity of PFOA identified in rats via an initiation–selection–promotion protocol; PFOA concentrations measured in serum; end-points were measured at two time points. *Principal limitations*: only a limited number of rats were included in the experiment; average daily dose of PFOA was not reported; histopathological examination of the liver only; only one sex used.

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence or multiplicity	Significance	Comments
Initiation–promotion (tested as promoter) Rat, Sprague-Dawley (F) 3 wk 7 mo Su et al. (2022)	Oral administration (gavage) PFOA, 95% Sesame oil 0, 0.01 mg/kg bw PFOA, zeranol, or PFOA + zeranol for 3 wk; at the age of 50 d, all rats received a single dose of DMBA 37, 37 37, 37	*Mammary gland* Papillary adenocarcinoma or cribriform carcinoma Tumour incidence: 35/37 (94.6%), 35/37 (94.6%) Tumour multiplicity: 3.5 ± 2.2, 3.7 ± 2.2 Total tumours: 121, 129	NS	*Principal limitations*: dose of the initiation agent DMBA was not optimal: extremely high tumour incidence in control (DMBA-treated group) did not allow investigation of potential enhancement of tumour incidence in the DMBA/PFOA-treated group; only one sex used; histological examination of the mammary gland only; only one dose. *Other comments*: the mixture of invasive papillary adenocarcinoma type 2 (prevalent) and invasive cribriform carcinoma was the most frequent mixed type for the PFOA group; histologically identified mammary tumours were also investigated by RNA-seq and qRT-PCR analyses; immunohistochemical analysis of selected receptors and effects on the endocrine system.
Initiation–promotion (tested as promoter) Rainbow trout (*Oncorhynchus mykiss*), Mount Shasta strain (M, F) (combined) 10 wk post-hatch 6 mo Tilton et al. (2008)	Oral administration (feed) PFOA, NR Feed 0, 200, 1800 ppm Initiation: aqueous exposure to AFB_1 or to vehicle (ethanol) for 30 min; after 3 mo, fed experimental diets containing lower or higher dose of PFOA 140, 140, 140 NR, NR, NR	*Liver* Mixed tumour, malignant 36%, 34%, 71%* Hepatocellular adenoma 3%, 0%, 8% Hepatocellular carcinoma 10%, 11%, 46%	*$P < 0.05$, logistic regression analysis (compared with AFB_1/control) NR NR	*Principal strengths*: adequate number of fish; two doses used. *Principal limitations*: data for males and females combined; no survival data; histopathological examination of the liver only. *Other comments*: no tumours were observed in non-initiated fish treated with feed containing PFOA; liver tumour enhancement after AFB_1/PFOA treatment might be related to induced estrogen-like signalling; the historical incidence of spontaneous liver tumours in trout (age 9 mo) fed control feed was 0.1%.

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence or multiplicity	Significance	Comments
Initiation–promotion (tested as promoter) Rainbow trout (*Oncorhynchus mykiss*), Mount Shasta strain (M, F) (combined) 10 wk post-spawn 12 mo Benninghoff et al. (2012)	Oral administration (feed) PFOA, "analytical grade" aqueous exposure Sham/control, Sham/PFOA, AFB_1/control, AFB_1/PFOA ppm Initiation: aqueous exposure to AFB_1 (10 ppb [0.01 ppm]) for 30 min; promotion: after 1 mo, fed experimental diets containing PFOA (2000 ppm) 5 d/wk for 6 mo 250, 250, 250, 250 NR, NR, NR, NR	*Liver* Total tumours 0, 0, 13%, 62%* Hepatocellular adenoma 0, 0, 26%, 10% Hepatocellular carcinoma 0, 0, 0, 27% Mixed carcinoma 0, 0, 47%, 54%	*$P < 0.01$, logistic regression analysis (compared with AFB_1/control) NS NS NS	*Principal strengths*: the experiment was supplemented with hepatic gene expression analysis, adequate number of fish per group. *Principal limitations*: a short-term exposure was used in the global gene-expression experiment; males and females combined; only one dose used; no survival data. *Other comments*: no liver tumours were observed in non-initiated fish treated with PFOA; increased multiplicity and size of liver tumours, but statistical analysis not provided.
Initiation–promotion (tested as promoter) Rainbow trout, Mount Shasta strain (M, F) (combined) 10 wk post-spawn 12 mo Benninghoff et al. (2012)	Oral administration (feed) PFOA, "analytical grade" Aqueous exposure Sham/control, sham/PFOA, MNNG/control, MNNG/PFOA Initiation: aqueous exposure to MNNG (35 ppm) for 30 min; promotion: after 1 mo, fed experimental diets containing PFOA (2000 ppm) 5 d/wk for 6 mo 250, 250, 250, 250 NR, NR, NR, NR	*Liver* Total tumours 0, 0, 51%, 81%* Hepatocellular adenoma 0, 0, 25%, 26% Hepatocellular carcinoma 0, 0, 28%, 11% Hepatocellular carcinoma [mixed] 0, 0, 39%, 55% *Stomach, kidney, swim bladder* No significant increase in the incidence of tumours	*$P < 0.0001$, logistic regression analysis (compared with MNNG/control) NS NS NS	*Principal strengths*: the use of MNNG as the initiation agent allowed investigation of whether tumorigenesis in other organs (kidney and swim bladder) was affected by PFOA treatment; adequate number of fish per group. *Principal limitations*: the MNNG dose was too high for estimation of effects of PFOA on stomach carcinogenesis (stomach tumour incidence in control fish was 99%); males and females combined; only one dose used; no survival data. *Other comments*: significant increase in liver tumour multiplicity ($P < 0.005$, Kruskal–Wallis test with Dunnett post hoc test for multiple comparisons).

2-AAF, 2-acetylaminofluorene; AFB_1, aflatoxin B_1; bw, body weight; d, day(s); DMBA, 7,12-dimethylbenz[*a*]anthracene; F, female; i.p., intraperitoneal; M, male; min, minute(s); MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; mo, month(s); NDEA, *N*-nitrosodiethylamine; NR, not reported; NS, not significant; PB, phenobarbital; PFOA, perfluorooctanoic acid; ppb, parts per billion; ppm, parts per million; RNA-seq, RNA sequencing; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; vs, versus; wk, week(s); yr, year(s).

(200 mg/kg bw, intraperitoneally), followed 2 weeks later by a selection procedure – feed containing 0.03% 2-acetylaminofluorene (2-AAF) for 2 weeks and in the middle of this treatment, after week 1, a treatment with a single dose of carbon tetrachloride at 2 mL/kg bw by gavage, 1:1 in corn oil (Abdellatif et al., 1990, also reported by Abdellatif et al., 1991 and Nilsson et al., 1991). One week after the selection procedure, the rats were divided into three groups receiving a basal diet (control), or a diet supplemented with either 0.05% phenobarbital (positive control) or 0.015% PFOA (analytical grade) for 23 weeks. [The average daily doses of 2-AAF, phenobarbital, and PFOA were not reported.] There were 7 rats in the control group, 8 rats in the phenobarbital-treated group, and 12 rats in the PFOA-treated group. Body weight was slightly but non-significantly decreased in the PFOA-treated group compared with the control group. [No data on survival at study termination were reported.] Liver samples were collected, and histological and histochemical evaluations were performed.

There was a significant increase ($P < 0.05$, Student *t*-test) in the incidence of total liver tumours in the phenobarbital-treated and PFOA-treated groups compared with the control group: 0/7, 6/8 (75%), and 4/12 (33%) for the control group, and groups receiving phenobarbital and PFOA, respectively. In the PFOA-treated group, 25% of the liver tumours were hepatocellular carcinomas (type I) and 8% were other tumours. In the phenobarbital-treated group, 63% were hepatocellular carcinomas (type I), and 12% were hepatocellular carcinomas (type IV). The tumour multiplicity was 3.4 and 1.2 for the phenobarbital- and PFOA-treated rats, respectively (Abdellatif et al., 1990).

Regarding pre-neoplastic lesions, some eosinophilic, basophilic, or mixed cell type foci and a few nodules were detected (Abdellatif et al., 1991). According to Nilsson et al. (1991), there were 8 nodules in the PFOA-treated group.

In the initiation–promotion study performed by the same research team (Abdellatif et al., 1991), groups of 15 adult male Wistar rats [age not reported] received a single intraperitoneal dose of DEN at 200 mg/kg bw for initiation. Control groups did not receive initiation treatment with DEN. After 2 weeks, the rats were fed basal feed (control), or feed containing 0.05% phenobarbital (positive control), or 0.005% or 0.02% PFOA (analytical grade) until termination at 12 months. The average daily doses of phenobarbital and PFOA were not reported. From each group, 5 rats were killed at the interim time of 3 months, and 10 rats were killed 12 months after the start of the experiment. Survival in the initiated groups was 7/10 (total in the control, phenobarbital-treated, and 0.005% PFOA-treated groups) and 9/10 in 0.02% PFOA-treated rats. Liver samples were collected, and histological and histochemical evaluations were performed.

There was a significant increase ($P < 0.05$, Sheffe test) in the incidence of hepatocellular carcinoma in the group at the higher dose of PFOA (DEN-initiated) – 0/7, 1/7 (14.3%), and 5/9 (55.5%), for the control group and the groups at 0.005% PFOA and 0.02% PFOA, respectively – at 12 months. For the positive control, phenobarbital, the result was 2/7 (28.6%) (Abdellatif et al., 1991). All the malignant tumours were well-differentiated type I hepatocellular carcinoma in rats treated with 0.005% PFOA. In the rats treated with 0.02% PFOA, four out of nine rats had moderately differentiated type II hepatocellular carcinoma, and one rat had a poorly differentiated type III hepatocellular carcinoma. No tumours were identified in rats that died at an early stage of the experiment, all within the first 8 months of the study, with the cause of death reported to be pneumonia in all cases. Tumour multiplicity was not reported for any treatment groups.

Regarding pre-neoplastic lesions, the rats with or without malignant tumours had foci and nodules containing a mainly eosinophilic, but

also basophilic, clear cell population or a mixed cell pattern (Abdellatif et al., 1991). No foci, nodules or malignant tumours were observed in non-initiated control rats either after 3 or 12 months, or in the initiated rats killed after 3 months. [The Working Group noted that PFOA concentrations were measured in the serum, and end-points were measured at two time points. However, both studies (Abdellatif et al., 1990, 1991) used only one sex; the purity of PFOA was not reported for either of these two protocols, only that it was of the purest available analytical grade; the average daily doses of PFOA, phenobarbital, and 2-AAF, and survival at study termination were not reported; and the histopathological examination was limited to the liver.]

In a study by Su et al. (2022), the effect of pubertal exposure to an environmentally relevant dose of PFOA was investigated, using a model of 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced tumorigenesis in the rat mammary gland. The aim of the study was to investigate whether exposure to PFOA during puberty might alter susceptibility to breast cancer. Female Sprague-Dawley rats (age, 21 days) were randomized into 36 or 37 rats per group and exposed via gavage to sesame oil (controls), or to PFOA (purity, 95%) at a dose of 0.01 mg/kg bw, or to a combination of PFOA and zeranol (a metabolite of the mycotoxin zearalenone) (0.01 mg/kg bw), 5 days per week from age 21 to 42 days. At age 50 days, all rats were challenged with a single dose of DMBA (30 mg/kg bw) via gavage. There was no significant difference in body weight between treated and control groups. Survival was not significantly affected by PFOA treatment. The rats were monitored for the development of mammary gland tumours for 7 months.

There were no significant differences in tumour incidence or the number of tumours per rat in the groups treated with PFOA or with PFOA plus zeranol compared with the DMBA control group. Overall, tumour latency, based on tumour-free survival, was not significantly affected with PFOA alone.

Regarding pre-neoplastic lesions, none were reported. [The Working Group noted that this study used only one sex and one dose, and histological examination was performed on the mammary glands only. The Working Group also noted that tumour incidence in both the control group (DMBA-treated) and in groups treated with PFOA and DMBA was extremely high – 35/37 (94.6%) – therefore, it may have been very difficult to detect any promotion effects.]

In an initiation–promotion study in rainbow trout (*Oncorhynchus mykiss*), approximately 1000 fry were initiated at 10 weeks post-hatch with aqueous exposure to aflatoxin B_1 (AFB_1) at 0.01 ppm for 30 minutes. The non-initiated controls were sham-exposed trout, exposed to vehicle alone (0.01% ethanol). After 3 months, initiated trout were randomly divided among experimental treatment groups (140 animals per group) and fed a semi-purified casein-based diet containing PFOA [purity not reported] at a concentration of 200 or 1800 ppm (equivalent to doses of 5 and 50 mg/kg per day, respectively) for 5 days per week. [The Working Group noted that the concentration of PFOA in the water tank was not reported.] At 9 months post-initiation, juvenile fish were killed and sampled for liver tumour histological identification and examination using haematoxylin and eosin (Tilton et al., 2008).

No tumours were observed in non-initiated fish fed with control or PFOA diets, indicating lack of carcinogenic potential by themselves in this model. There was a significant increase ($P < 0.05$, logistic regression analysis) in the incidence of total liver tumours (cholangiocellular carcinoma, hepatocellular adenoma, hepatocellular carcinoma, and mixed adenoma and mixed carcinoma) – 36%, 34%, and 71% for the AFB_1/0 (control group), and the groups treated with AFB_1/200 ppm PFOA, and AFB_1/1800 ppm PFOA, respectively. [The Working Group noted

that mixed adenoma and carcinoma comprised both cholangiocellular and hepatocellular cell types that are considered to be originated from a common progenitor cell of bile duct cells and liver cells.] Specifically, there was a significant increase in overall tumour incidence (71%) in the group treated with AFB_1/1800 ppm PFOA compared with the control group ($P < 0.05$, logistic regression analysis). There was also an increase in the incidence of hepatocellular carcinoma – 10%, 11%, and 46% for the groups treated with AFB_1/0 (control), AFB_1/200, and AFB_1/1800 ppm, respectively – and in the incidence expressed as a percentage of hepatocellular adenoma – 3%, 0%, and 8% for the groups treated with AFB_1/0 (control), AFB_1/200, and AFB_1/1800 ppm, respectively – although no statistical testing for the individual tumour types was reported. In addition, the multiplicity of the induced tumours per animal was also increased.

Regarding non-neoplastic lesions, PFOA exposure produced hepatomegaly and basophilic foci (Tilton et al., 2008). [The Working Group noted that this study used an adequate number of animals per group and tested two doses of PFOA. However, data were combined for males and females, the purity of PFOA was not reported, no information on survival was provided, and histopathological examination was performed on the liver only.]

In another initiation–promotion study in Mount Shasta rainbow trout (*Oncorhynchus mykiss*), PFOA was evaluated by initiating about 3500 fry (age, 10 weeks) with AFB_1 at 10 ppb [0.01 ppm] or about 1000 fry (age, 10 weeks) with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) at 35 ppm for 30 minutes (Benninghoff et al., 2012). Since MNNG is a multiorgan carcinogen, MNNG initiation was used to determine whether the tumour-promoting effects of dietary PFOA were specific to hepatocarcinogenesis or dependent on the initiating carcinogen. Non-initiated sham controls in the two experiments were treated with the vehicle (0.01% ethanol or 0.01% dimethyl sulfoxide, DMSO, respectively). After initiation, the fry were given untreated feed (a semi-purified casein-based diet) for 1 month. Then, within each initiation cohort, trout were randomly divided into the treatment groups. In the first (AFB_1) cohort, fish were fed experimental diets containing PFOA at 2000 ppm (equivalent to 50 mg/kg per day, analytical grade), ad libitum, 5 days per week for 6 months. Untreated feed was used for controls. There were four exposure groups, each containing 250 fish: sham/control, sham/+PFOA, AFB_1/control, and AFB_1/+PFOA. The MNNG-initiated trout were exposed to the vehicle or PFOA at 2000 ppm. There were four exposure groups, each containing 250 fish: sham/–PFOA, sham/+PFOA, MNNG/–PFOA, and MNNG/+PFOA. The diet was prepared on a monthly basis and kept frozen at –20°C before use. The fish were terminated at 12.5 months post-spawn and examination of tumours was performed. Neoplasms were classified according to the criteria described in Hendricks et al. (1984).

A total of cholangiocellular adenomas or cholangiocellular carcinomas, hepatocellular adenomas or hepatocellular carcinomas, and mixed adenomas, and mixed carcinomas that consist of both cholangiocellular and hepatocellular cell types were counted as liver tumours. Initiation with AFB_1 at 10 ppb resulted in a moderate increase in liver tumour incidence (13%) compared with the control group. PFOA exposure significantly enhanced the incidence of liver tumours (62%) ($P < 0.01$, logistic regression analysis), and increased liver tumour multiplicity and size (both $P < 0.05$, Kruskal–Wallis test with Dunnett post hoc test for multiple comparisons). There was a significant increase ($P < 0.0001$, logistic regression analysis) in liver tumour incidence in the MNNG/PFOA group (86%) compared with the MNNG/control group (51%). Tumour multiplicity and size were also significantly increased by PFOA treatment (both $P < 0.001$, Kruskal–Wallis test with Dunnett post

hoc test for multiple comparisons). After MNNG initiation, kidney and stomach carcinogenesis was not significantly affected by PFOA exposure. Mixed carcinoma followed by hepatocellular adenoma and hepatocellular carcinoma were the most prevalent liver tumour types in both experiments, with the prevalence being lower than that seen in groups treated with AFB_1 alone and MNNG alone (individual tumour types were not statistically analysed). [The Working Group noted that this study used an adequate number of animals per group; however, data were combined for males and females, only one dose of PFOA was tested, and the purity for PFOA was reported only as "analytical grade". It was noted that the results of these studies indicated that PFOA can act as a promoter in this fish model. Furthermore, the data reflect chemical-specific responses in the liver with both AFB_1 (liver-specific) and MNNG (multiorgan) initiators.]

3.2 Perfluorooctanesulfonic acid (PFOS)

See Table 3.4.

3.2.1 Mouse

Transplacental exposure

The tumorigenic effects of gestational exposure to PFOS were evaluated in C57BL/6J-*Apc*$^{Min/+}$ mice, a mouse model that develops intestinal tumours because of a mutation in the tumour suppressor gene, adenomatous polyposis cell (*Apc*) (Ngo et al., 2014). The wildtype (*Apc*$^{+/+}$) females were mated to heterozygotic males (*Apc*$^{Min/+}$). Wildtype dams were then treated by gavage with PFOS (purity, ≥ 98%) at a dose of 0 (water vehicle), 0.01, 0.1, or 3.0 mg/kg per day on days 1–17 of gestation. Insufficient rates of pregnancy and littering rates and low F_1 survival were observed in the first experimental block – block 1, 0 (water vehicle), PFOS at 0.1 and 3.0 mg/kg bw per day; 104 mice (age, 7–8 weeks) – thus a second block was added that had a lower PFOS exposure (block 2, 0 (water vehicle), PFOS at 0.01 and 0.1 mg/kg bw per day; 100 dams (age, 9–10 weeks). The numbers of mice (*Min*/+) obtained in each dose group (both blocks combined) were 15, 10, 12, and 7 for males and 23, 6, 13, and 5 for females exposed in utero to vehicle (water), 0.01, 0.1, and 3.0 mg/kg bw of PFOS, respectively. Because of difficulty in ascertaining pregnancy status, exposure varied on days 14 to 17 of gestation. F_1 offspring were genotyped via polymerase chain reaction (PCR) using DNA collected from ear punches. Offspring were genotyped as wildtype (*Apc*$^{+/+}$) and heterozygous (*Apc*$^{Min/+}$), and only the *Min*/+ mice were used for the carcinogenesis study. Efforts were made to verify dosing level by measuring PFOS stability in the dosing solution. Furthermore, PFOS concentrations in tap water and feed used for the study were quantified, and levels of PFOS in the serum of mice in the vehicle control group were below the LOQ. Internal exposure was quantified in the serum (2 mice per time point) in dams on day 18 of gestation, and postnatal day 23 (block 1) or postnatal days 26–28 (block 2), and F_1 pups on postnatal days 25–27 (depending on block). The LOQ in serum was 0.05 ng/mL. Although minimal data were generated, they confirmed that there was internal exposure within dams and pups and that it increased with dose and decreased with time after dosing (day 18 of gestation versus postnatal day 23 in dams). Evaluation of intestinal tumorigenesis occurred at age 11 weeks in the F_1 *Min*/+ mice. The number, diameter, and localization of tumours in the small intestine and colon were measured by transillumination in an inverse light microscope. The reviewer scored lesions at 20× magnification and was blinded to treatment. Statistical analysis of incidence was conducted on both an individual and litter basis; furthermore, blocks were combined in the analysis if no consistent differences were found between blocks 1 and 2. None of the PFOS doses

Table 3.4 Studies of carcinogenicity in experimental animals exposed to PFOS

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Results	Significance	Comments
Full carcinogenicity Mouse, C57BL/6J-*Apc*$^{Min/+}$ (M) Day 1 of gestation 11 wk Ngo et al. (2014)	Oral administration (gavage) PFOS, ≥ 98% Water 0, 0.01, 0.1, 3.0 mg/kg bw per day 15, 10, 12, 7 15, 10, 12, 7	*Small intestine (duodenum, jejunum or ileum)* No significant increase in tumour incidence 15/15, 10/10, 12/12, 7/7 *Colon* No significant increase in tumour incidence 15/15, 3/3, 19/19, 0	NS NS	*Principal strengths*: males and females studied; multiple doses; analysed background levels of PFOS in feed and drinking-water; analysed internal doses of PFOS; tested stability of PFOS; blocks of PFOS administration were compared statistically. *Principal limitations*: small number of mice per group; short duration of study; histopathological examination not conducted; batch number of PFOS was not stated; varied exposure (14–17 d). *Other comments*: study of transplacental exposure; increase in the incidence of spontaneous tumours was studied in this mouse model; tumours of the small intestine were observed in all experimental groups of *Min*/+ mice, demonstrating 100% incidence for this end-point, as is usual in this mouse model; multiplicity was not increased by PFOS exposure.

Table 3.4 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Results	Significance	Comments
Full carcinogenicity Mouse, C57BL/6J-*Apc*$^{Min/+}$ (F) Day 1 of gestation 11 wk Ngo et al. (2014)	Oral administration (gavage) PFOS, ≥ 98% Water 0, 0.01, 0.1, 3.0 mg/kg bw per day 23, 6, 13, 5 23, 6, 13, 5	*Small intestine (duodenum, jejunum or ileum)* No significant increase in tumour incidence 23/23, 6/6, 13/13, 5/5 *Colon* No significant increase in tumour incidence	NS	*Principal strengths*: males and females studied; multiple doses; analysed background concentrations of PFOS in feed and drinking-water; analysed internal doses of PFOS; tested stability of PFOS; blocks of PFOS administration were compared statistically. *Principal limitations*: small and unbalanced number of mice per group; short duration of study; histopathological examination not conducted; batch number of PFOS was not reported. *Other comments*: study of transplacental exposure; increase in incidence of spontaneous tumours was studied in this mouse model; small intestinal tumours were observed in all experimental groups of *Min*/+ mice, demonstrating 100% incidence for this end-point, as is usual in this mouse model; multiplicity was not increased by PFOS exposure.
Full carcinogenicity Rat, Crl:CD (SD)IGS BR (M) Approx. 41 d 104 wk Butenhoff et al. (2012b)	Oral administration (feed) PFOS (potassium salt), 86.9% Acetone 0, 0.5, 2, 5, 20 ppm 7 d/wk 50, 50, 50, 50, 51 11, 11, 17, 25, 23	*Liver* Hepatocellular adenoma 0/60, 3/50, 3/50, 1/50, 7/60* *Thyroid gland* Follicular cell adenoma 3/60, 5/49, 4/50, 4/49, 4/59 Follicular cell carcinoma 3/60, 1/49, 1/50, 2/49, 1/59 Follicular cell adenoma or carcinoma (combined) 6/60, 6/49, 5/50, 5/49, 5/59	$P = 0.0276$, Cochran–Armitage trend test *$P = 0.046$, Dunnett t-test NS NS NS	*Principal strengths*: adequate number of rats used; randomly allocated in groups; males and females used; adequate duration. *Other comments*: "N at Start" removed rats from 4, 14, and 52 wk interim necropsy; note: Laboratory Report and Butenhoff include 52-wk interim animals in "*n*" for tumour incidence in the 0 and 20 ppm groups (e.g. 20 ppm $n = 7/60$ including 52 interim weeks vs $n = 7/50$ using only ≥ 53 wk); survival data in laboratory report were calculated using $n = 50$.

Table 3.4 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Results	Significance	Comments
Full carcinogenicity Rat, Crl:CD (SD)IGS BR (M) Approx. 41 d 104 wk Butenhoff et al. (2012b) (cont.)		*Pancreas* Islet cell adenoma 4/60, 3/49, 4/50, 4/50, 4/60 Islet cell carcinoma 1/60, 2/49, 2/50, 5/50, 5/60 Islet cell adenoma or carcinoma (combined) 5/60, 5/49, 6/50, 8/50, 9/60	 NS [$P = 0.02$, Cochran–Armitage trend test (not survival adjusted)] [$P = 0.13$, poly-3 trend test (survival adjusted, with interim animals)] [$P = 0.117$, poly-3 trend test (survival adjusted, without interim animals)] NS	
Full carcinogenicity Rat, Crl:CD (SD)IGS (F) Approx. 41 d 104 wk Butenhoff et al. (2012b)	Oral administration (feed) PFOS (potassium salt), 86.9% Acetone 0, 0.5, 2, 5, 20 ppm 7 d/wk 50, 50, 50, 50, 50 25, 15, 10, 17, 26	*Liver* Hepatocellular adenoma 0/60, 1/50, 1/49, 1/50, 5/60* Hepatocellular carcinoma 0/60, 0/50, 0/49, 0/50, 1/60 Hepatocellular adenoma or carcinoma (combined) 0/60, 1/50, 1/49, 1/50, 6/60* *Thyroid gland* Follicular cell adenoma 0/60, 0/50, 0/49, 2/50, 1/60	 $P = 0.0153$, Cochran–Armitage trend test *$P = 0.0386$, Dunnett *t*-test NS $P = 0.0057$, Cochran–Armitage trend test *$P = 0.0204$, Dunnett *t*-test NS	*Principal strengths*: adequate number of rats used; randomly allocated in groups; males and females used; adequate duration. *Other comments*: inclusion of animals killed at the 52-wk interim time point in statistical analysis; experiment terminated at 103 wk for the group at 2 ppm; "N at Start" removed rats from 4, 14, and 52 wk interim necropsy; note: US EPA (2002) and Butenhoff included 52-wk interim animals in "*n*" for tumour incidence in the groups at 0 and 20 ppm (e.g. 20 ppm, $n = 7/60$ including 52 interim weeks vs $n = 7/50$ using only ≥ 53 wk); survival data in this laboratory report were calculated using $n = 50$.

Table 3.4 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Results	Significance	Comments
Full carcinogenicity Rat, Crl:CD (SD)IGS (F) Approx. 41 d 104 wk Butenhoff et al. (2012b) (cont.)		Follicular cell carcinoma 0/60, 0/50, 0/49, 1/50, 0/60	NS	
		Follicular cell adenoma or carcinoma (combined) 0/60, 0/50, 0/49, 3/50*, 1/60	*P = 0.047, Dunnett t-test	
		Mammary gland Fibroadenoma 20/60, 27/50*, 19/48, 24/50, 11/60	*P = 0.0337, Dunnett t-test	
		Adenoma 7/60, 6/50, 5/48, 7/50, 4/60	NS	
		Carcinoma 11/60, 12/50, 15/48, 11/50, 14/60	NS	
		Fibroadenoma or adenoma (combined) 23/60, 30/50*, 22/48, 26/50, 15/60	*P = 0.318, Dunnett t-test	
		Fibroadenoma, adenoma, or carcinoma (combined) 29/60, 36/50*, 31/48**, 29/50, 24/60	P = 0.0482, Cochran–Armitage trend test *P = 0.0474, Dunnett t-test **P = 0.0066, Dunnett t-test	

Table 3.4 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Results	Significance	Comments
Full carcinogenicity Rat, Crl:CD (SD)IGS BR rats (M) Approx. 41 d 52 wk Butenhoff et al. (2012b)	Oral administration (feed) PFOS (potassium salt), 86.9% Acetone 0, 20 (recovery) ppm 0, 20 ppm, 7 d/wk for 52 wk followed by basal feed with acetone control for 52 wk 50, 40 11, 11	*Liver* Hepatocellular adenoma 0/60, 0/40 *Thyroid gland* Follicular cell adenoma 3/60, 9/39* Follicular cell carcinoma 3/60, 1/39 Follicular cell adenoma or carcinoma (combined) 6/60, 10/39 *Pancreas* Islet cell adenoma 4/60, 11/40 Islet cell carcinoma 1/60, 3/40 Islet cell adenoma or carcinoma (combined) 5/60, 4/40	 *P = 0.0280, Dunnett t-test NS NS NS NS NS	*Principal strengths*: adequate number of rats used; randomly allocated in groups; males and females used; adequate duration. *Other comments*: "N at Start" removed rats from the interim necropsy at 4, 14, and 52 wk; note: US EPA (2002) and Butenhoff included 52-wk interim rats in "n" for tumour incidence in the groups at 0 and 20 ppm (e.g. 20 ppm n = 7/60 including 52 interim weeks vs n = 7/50 using only ≥ 53 wk); survival data in this laboratory report were calculated using n = 50.
Full carcinogenicity Rat, Crl:CD (SD)IGS (F) Approx. 41 d 52 wk Butenhoff et al. (2012b)	Oral administration (feed) PFOS (potassium salt), 86.9% Acetone 0, 20 (recovery) ppm 0, 20 ppm, 7 d/wk for 52 wk followed by basal feed with acetone control for 52 wk 50, 40 25, 19	*Liver* Hepatocellular adenoma 0/60, 2/40 Hepatocellular carcinoma 0/60, 0/40 Hepatocellular adenoma or carcinoma (combined) 0/60, 2/40 *Thyroid gland* Follicular cell adenoma 0/60, 1/40 Follicular cell carcinoma 0/60, 0/40 Follicular cell adenoma or carcinoma (combined) 0/60, 1/40	 NS NS NS NS NS NS	*Principal strengths*: adequate number of rats used, randomly allocated in groups, males and females used, adequate duration; well-conducted GLP study. *Other comments*: inclusion of 52-wk interim rats in statistical analysis; experiment terminated at 103 wk for the group at 2 ppm; "N at Start" removed rats from interim necropsy at 4, 14, and 52 wk; note: US EPA (2002) and Butenhoff included 52-wk interim rats in "n" for tumour incidence in the groups at 0 and 20 ppm (e.g. 20 ppm, n = 7/60 including 52 interim weeks vs n = 7/50 using only ≥ 53 wk); survival data in this laboratory report were calculated using n = 50.

Table 3.4 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Results	Significance	Comments
Full carcinogenicity Rat, Crl:CD (SD)IGS (F) Approx. 41 d 52 wk Butenhoff et al. (2012b) (cont.)		*Mammary gland* Fibroadenoma 20/60, 15/40 Adenoma 7/60, 4/40 Carcinoma 11/60, 4/40 Fibroadenoma or adenoma (combined) 23/60, 16/40 Fibroadenoma, adenoma, or carcinoma (combined) 29/60, 17/40	 NS NS NS NS	
Initiation–promotion (tested as promoter) Rainbow trout, (*Oncorhynchus mykiss*) (M, F) (combined) 15 wks (at initiation) 6 mo Benninghoff et al. (2012)	Oral administration (feed) PFOS, unspecified 0.01% EtOH Sham/control, Sham/PFOS, AFB_1/control, AFB_1/PFOS ppm 5d/wk 250, 250, 250, 250 NR, NR, NR, NR	*Liver* Total tumours 0, 0, 1%, 13%*	*$P < 0.01$, logistic regression analysis (compared with AFB_1/control)	*Principal strengths*: adequate number of animals used; randomly allocated in groups. *Principal limitations*: males and females combined; only one dose; purity not reported. *Other comments*: survival and incidence number not reported, just the percentage incidence in each group; appeared that multiplicity increased somewhat, but numbers not provided; liver tumour diameter not increased.
Initiation–promotion (tested as promoter) Zebrafish (*Danio rerio*), *Kras*^{V12} transgenic (M, F) (combined) 90 d post fertilization 10 d Zhu et al. (2021)	Aqueous exposure PFOS (potassium salt), > 98% 0.1% DMSO (v/v) DMSO, DOX, PFOS, DOX + PFOS µg/L 7 d/wk 24, 24, 24, 24 NR, NR, NR, NR	*Liver* Hepatocellular adenoma 0/6, 3/6, 0/6, 1/6 Hepatocellular carcinoma 0/6, 2/6, 0/6, 5/6*	 [NS] [*$P = 0.0076$, Fisher exact test]	*Principal strengths*: liver histology conducted. *Principal limitations*: neoplasm incidences not reported; short duration; small number of animals per group; data combined for males and females; limited histopathological description. *Other comments*: incidence derived from a graph; histology conducted on 6 fish per treatment.

AFB_1, aflatoxin B_1; bw, body weight; d, day(s); DMSO, dimethyl sulfoxide; DOX, doxycycline; EtOH, ethanol; F, female; M, male; mo, month(s); NR, not reported; NS, not significant; PFOS, perfluorooctanesulfonic acid; ppm, part per million; v/v, volume per volume; wk, week(s).

affected body weight, compared with dams and F_1 offspring in the vehicle control group.

There was a 100% incidence of tumours of the small intestine in heterozygous *Min*/+ mice, as is usual in this mouse model. Exposure to PFOS did not cause a significant increase in the number or multiplicity of small intestine tumours among male and female heterozygous mice. Tumour diameters were not significantly increased with increasing PFOS exposure within male mice, but there was an increase in tumour size in females in the groups treated with PFOS at 0.01 and 3.0 mg/kg bw compared with the control group (mice at the intermediate dose of 0.1 mg/kg bw were unaffected). Fewer tumours were observed in the colon than in the small intestine, as usual in this model, and no statistical differences were observed between PFOS-exposed groups and the control group with regard to incidence, number per mouse (multiplicity), or size of colon tumours. The localization of tumours along the small intestine and colon was not affected by treatment with PFOS. [The Working Group noted that this study used both sexes and multiple doses; PFOS stability was tested and the internal dose was analysed, as were background levels of PFOS in feed and drinking-water. However, no histological examination was performed.]

3.2.2 Rat

Oral administration (feed)

There was only one study on the carcinogenicity of PFOS in rodents. For this well-conducted study that complied with GLP, the data were available in the original laboratory report (US EPA, 2002) and as a manuscript published at a later date (Butenhoff et al., 2012b). In this study, PFOS (T-6295; purity, 86.9%) was administered in the feed at a concentration of 0 (control), 0.5, 2, 5, or 20 ppm to initial groups of 70, 60, 60, 60, 70, and 40 (20 ppm recovery group) Sprague-Dawley (Crl:CD(SD)IGS BR) male and female rats, respectively. Of these rats, 50 rats per group received control feed or PFOS for the full 2-year exposure (groups at 0–20 ppm). A recovery group of 40 males and 40 females was also included; rats in this group were treated with feed containing PFOS at 20 ppm for 52 weeks, and then allowed a recovery of 52 weeks. Controls received the control feed (basal feed with acetone as vehicle). Interim necropsies were carried out at 4, 14, and 53 weeks, when clinical chemistry, PFOS concentrations, and liver end-points (palmitoyl-CoA oxidase activity for PPARα activity, cell proliferation) were evaluated. PFOS consumption was 0.024, 0.098, 0.242, and 0.984 mg/kg bw per day for males and 0.029, 0.120, 0.299, and 1.251 mg/kg bw per day for females over the 104-week period, for the groups at 0.5, 2, 5, and 20 ppm, respectively. PFOS consumption for the recovery groups at 20 ppm was 1.144 and 1.385 mg/kg bw per day for males and females, respectively. Body weight was lower in the recovery groups of males and females at 20 ppm compared with that in the control group at the end of the dosing period, but body weights in the recovery group post-exposure tended back towards control values. At 104 weeks, survival of rats selected for the 2-year evaluation was: 11/50, 11/50, 17/50, 25/50, 23/51, and 11/40 in males and 25/50, 15/50, 10/50 (at 102 weeks), 17/50, 26/50, and 19/40 in females, for the groups at 0 (control), 0.5, 2, 5, 20 ppm, and 20 ppm (recovery), respectively. There was some indication of longer survival among male rats at 5 and 20 ppm compared with controls, possibly because of lower survival in the controls. [The Working Group noted that survival was lower in the male control groups compared with the treated groups, and the Peto statistical test was performed, taking differences in the survival into account.] One group of treated females (2 ppm) had decreased survival compared with controls (US EPA, 2002; also reported by Butenhoff et al., 2012b).

Rats from the 53-week evaluation of the control group and the group at 20 ppm (highest dose) and from the 104-week terminal necropsy were

included to determine tumour incidence within the groups. For the evaluation of the incidence of non-neoplastic lesions, rats were included from the 14-, 53-, and 104-week necropsies.

In males, there was a significant positive trend (P = 0.0276, Cochran–Armitage trend test) in the incidence of hepatocellular adenoma, and the incidence – 0/60, 3/50, 3/50, 1/50, and 7/60 for the groups at 0 (control), 0.5, 2, 5, and 20 ppm, respectively – was significantly increased at 20 ppm (P = 0.0456, Dunnett t-test), compared with controls. Incidence in the recovery group at the highest dose (20 ppm) was similar to that in controls (0/60 versus 0/40). There was no significant increase in the incidence of thyroid follicular cell adenoma in continuously exposed male rats – 3/60, 5/49, 4/50, 4/49, and 4/59 for the groups at 0 (control), 0.5, 2, 5, and 20 ppm, respectively – however, there was a significant increase (P = 0.0280, Dunnett t-test) in the incidence of thyroid follicular cell adenoma in the recovery group at 20 ppm (9/39) compared with the control group (3/60).

In females, there was a significant positive trend (P = 0.0153, Cochran–Armitage trend test) in the incidence of hepatocellular adenoma – 0/60, 1/50, 1/49, 1/50, and 5/60, for the groups at 0 (control), 0.5, 2, 5, and 20 ppm, respectively – with the incidence being significantly increased (P = 0.0386, Dunnett t-test) in the group at 20 ppm compared with controls. There was a significant positive trend (P = 0.0057, Cochran–Armitage trend test) in the incidence of hepatocellular adenoma or carcinoma (combined), with the incidence – 0/60, 1/50, 1/49, 1/50, and 6/60 for the groups at 0 (control), 0.5, 2, 5, and 20 ppm, respectively – being significantly increased (P = 0.0204, Dunnett t-test) in the groups at 20 ppm compared with controls. In the group at 20 ppm, the incidence of hepatocellular carcinoma was 2% (1/60). For females, there was no difference between the recovery group (20 ppm) and the controls. There was a significant increase (P = 0.047, Dunnett t-test) in the incidence of thyroid follicular cell adenoma or carcinoma (combined) in females in the group at 5 ppm – 0/60, 0/50, 0/49, 3/50, and 1/60 for the groups at 0 (control), 0.5, 2, 5, and 20 ppm, respectively. The incidence of mammary gland fibroadenoma – 20/60, 27/50, 19/48, 24/50, and 11/60 for the groups at 0 (control), 0.5, 2, 5, and 20 ppm, respectively – was significantly increased in the group at 0.5 ppm compared with controls (P = 0.0337, Dunnett t-test). The incidence of mammary gland fibroadenoma or adenoma (combined) was increased in the group at 0.5 ppm (P = 0.0318, Dunnett t-test). Not reported in Butenhoff et al. (2012b), but present in the laboratory report (US EPA, 2002), the incidence of mammary gland fibroadenoma, adenoma, or carcinoma (combined) – 29/60, 36/50, 31/48, 29/50, and 24/60 for the groups at 0 (control), 0.5, 2, 5, and 20 ppm, respectively – was significantly increased in the groups at 0.5 and 2.0 ppm (P = 0.0474 and P = 0.0066, Dunnett t-test, respectively). The increases in the incidence of combined mammary neoplasms within these groups were mostly because of increases in the incidence of fibroadenoma. There was no increase in the incidence of tumours in females in the recovery group (20 ppm) compared with the controls. [The Working Group noted that there was a tumour response for cancer of the thyroid gland in male (recovery group at 20 ppm) and female (continuous exposure at 5 ppm) rats compared with controls. However, the response was not dose-dependent in females, and no increase was observed in males at the highest dose and continuous exposure. Furthermore, mammary gland tumours were only observed at the lowest exposures and the association with PFOS was uncertain. The positive findings from this study were liver tumours in males and females.]

Regarding non-neoplastic lesions, these were observed in the liver in both males and females, primarily in the group at 20 ppm, and included hepatocellular hypertrophy, eosinophilic granular cytoplasm, hepatocellular pigmentation,

individual hepatocyte necrosis, hepatocellular vacuolation, and cystic degeneration (males only). In females, there was an increase in the incidence of lymphohistiocytic infiltrate and pigmented macrophage infiltrate within the liver. Increased liver weight (absolute and relative) in males and hepatocyte hypertrophy in both males and females in the group at 20 ppm were observed at weeks 14 and 53. However, for both sexes, no significant increases in the incidence of liver cell proliferation were observed at weeks 4 and 14 (proliferating cell nuclear antigen, PCNA) or at week 53 (bromodeoxyuridine).

[The Working Group noted that this study used an adequate number of animals per group, both sexes, and an adequate duration of exposure. The reason for the inclusion of interim animals (control group and 20 ppm) from week 52 with animals from week 104 in the report by US EPA (2002) and Butenhoff et al. (2012b) was unclear, as the exposure was significantly different. A review of the histopathology results for males and females from week 52 showed that many of the rats had no neoplasms (26/39) or only had pituitary adenomas (11/39). The Working Group also noted the nearly significant positive trend in the incidence of pancreatic islet carcinoma, but there were no significant changes by pairwise comparison. The Working Group was uncertain of this finding and the association with PFOS exposure because hyperplasia, adenomas, and the combination of adenoma or carcinoma were also not significant in males, and no pancreatic islet cell effect in female rats was observed. The Working Group also noted that the pancreatic islet cell tumours were not reported in Butenhoff et al. (2012b) but were reported in US EPA (2002). The Working Group conducted survival-adjusted statistical analyses on the data for pancreatic islet cell carcinoma, because it was noted that survival in controls was low, using the poly-3 test method. There was no significant difference in the trend test results in analyses including the 53-week interim animals ($P = 0.130$) or excluding the 53-week interim animals ($P = 0.117$), and, similarly to in the report by US EPA (2002), no significant pairwise comparisons with incidence in the controls were found.]

3.2.3 Fish

Initiation–promotion studies

Benninghoff et al. (2012) used Mount Shasta rainbow trout (*Oncorhynchus mykiss*) to evaluate PFOS promoter activity by initiating 1000 fry (age, 15 weeks) for 30 minutes with either aflatoxin B_1 (AFB_1) at a concentration of 10 ppb or a sham control of 0.01% ethanol. These initiated fry were then treated with a diet containing PFOS (purity not reported; analytical grade) at a concentration of 100 ppm (equivalent to 2.5 mg/kg bw per day). In all, there were four exposure groups, each containing 250 fish: sham/control, sham/PFOS, AFB_1/control, and AFB_1/PFOS. The dietary concentration of PFOS was selected on the basis of a pilot study in which the concentration used, 2000 ppm, resulted in high mortality. The PFOS diet was provided for 6 months; during this time, the diet was prepared on a monthly basis and kept frozen at –20 °C before use. Body weight was decreased after AFB_1/PFOS exposure compared with sham/control ($P < 0.05$, logistic regression analysis), whereas body weight in sham/control and sham/PFOS exposure groups was not significantly different. Liver weights (absolute and relative) were increased after treatment with PFOS, with and without initiation. The fish were killed at 12.5 months post-spawn, and examination of tumours was performed.

After initiation with AFB_1 at 10 ppb and in the absence of subsequent PFOS exposure, there was a 1% induction of liver tumours. In the sham controls with and without PFOS exposure, there was no induction of liver tumours. After initiation followed by PFOS exposure, there was a significant increase in the incidence of liver tumours, compared with controls ($P < 0.01$, logistic regression analysis; 13% compared with

1%). Tumour multiplicity and tumour size after PFOS exposure were not different from those for the controls. Mixed carcinoma was the most prevalent tumour type after AFB_1/PFOS exposure and AFB_1/control. [The Working Group noted that an adequate number of fish per group was used in this study; however, data were combined for males and females, only one dose of PFOS was tested, and PFOS purity was not reported.]

In a study by Zhu et al. (2021), male *Kras*^{V12} transgenic zebrafish (*Danio rerio*) (age, 90 days post-fertilization), in which hepatocellular carcinomas can be initiated via doxycycline (DOX)-induced expression of the *Kras* G12V oncogene in the liver (see Chew et al., 2014), were used to determine whether PFOS alone or DOX + PFOS could initiate or promote hepatocellular carcinoma, respectively. Adult transgenic zebrafish were immersed in PFOS (purity, > 98%) with or without DOX, in the dark for 10 days, to avoid photodegradation of the DOX. Four exposure groups were evaluated: 0.01% DMSO (control), DOX (20 mg/L), PFOS (500 µg/L), and DOX + PFOS (20 mg/L plus 500 µg/L, respectively). The selection of PFOS exposures was made on the basis of a short-term (4 day) study in zebrafish larvae exposed to PFOS at concentrations of 50, 100, 200, 500, and 1000 µg/L. After the 10-day exposure, the fish were killed, and livers were evaluated. Three exposure replicates were conducted, with 8 fish per replicate. From these 24 fish, livers from 6 fish were used for histological analysis and livers from 3 fish were used for transcriptomics analysis. [The Working Group noted that PFOS concentrations were not verified either in the aqueous exposure or in the internal dose.]

The hepatosomatic index (liver weight relative to body weight) in adult zebrafish was increased after treatment with DOX or DOX + PFOS, compared with DMSO (both $P < 0.05$), but not with PFOS alone, and was increased after treatment with DOX + PFOS versus DOX ($P < 0.05$). Liver size (mm^2), and fluorescence intensity (resulting from expression of a liver-specific enhanced green fluorescent protein) were increased in the group exposed to DOX + PFOS compared with the group exposed to DOX alone (both $P < 0.05$). The incidence of hepatocellular carcinoma was higher in the group exposed to DOX + PFOS (5/6) than in the group exposed to DOX alone (2/6), and no hepatocellular carcinomas were observed in the groups exposed to PFOS alone (0/6) or DMSO (0/6). [The Working Group noted that a small number of fish were used ($n = 6$) for histopathological evaluation of liver tumours. Statistical analysis by the Working Group showed no significant difference between the DOX group (2/6; 40%) and the DOX + PFOS group (5/6; 83%) [$P = 0.1212$, Fisher exact test]. The duration of the study appeared to be based on a 3-month study in which the establishing of this transgenic line was reported, and in which increasing mortality was observed shortly after induction or initiation in a 3-month study.]

3.3 Evidence synthesis for cancer in experimental animals

3.3.1 PFOA

The carcinogenicity of PFOA has been assessed in two well-conducted GLP studies, one in male and female Sprague-Dawley rats treated by oral administration (in the feed) in a combination of F_0 (in utero and lactation) and F_1 (post-weaning) exposure (NTP, 2020) and the other in male and female Sprague-Dawley rats treated by oral administration (in the feed) (US EPA, 1987); also reported by Butenhoff et al., 2012a) [histological re-analysis by Hardisty et al. (2010) (mammary gland) and Caverly Rae et al., 2014 (pancreas)]. The carcinogenicity of PFOA was also evaluated in studies that did not comply with GLP. Specifically, these were studies of oral administration (feed) in male Sprague-Dawley rats (Biegel et al., 2001); oral administration (gavage) in male and female C57BL/6J-*Apc*$^{Min/+}$

mice (Ngo et al., 2014); and studies in female CD-1 mice, female 129/Sv wildtype, and 129/Sv PPARα-knockout mice (Filgo et al., 2015). In addition, there were six initiation–promotion studies of oral administration (feed) in male Wistar rats (Abdellatif et al., 1990, 1991; also reported by Nilsson et al., 1991), in male and female rainbow trout (Tilton et al., 2008; Benninghoff et al., 2012); of oral administration (drinking-water) in male and female *LSL-Kras^{G12D};Pdx-1 Cre* (KC) transgenic mice (Kamendulis et al., 2022); and of oral administration (gavage) in female Sprague-Dawley rats (Su et al., 2022).

In the dietary study that complied with GLP in F_1 male and female Sprague-Dawley rats (NTP, 2020), a significant positive trend in the incidence of hepatocellular adenoma (includes multiple) was observed in males, and the incidence was significantly increased in both the F_0 exposed and F_0 unexposed groups. There was a positive trend in the incidence of hepatocellular carcinoma only in F_1 males with F_0 exposure. There was a significant positive trend in the incidence of hepatocellular adenoma or carcinoma (combined) in F_1 males in both the F_0 exposed and F_0 unexposed groups, and the incidence was significantly increased in both groups. There was a significant positive trend in the incidence of acinar cell adenoma of the pancreas (includes multiple) in F_1 males in both the F_0 exposed and F_0 unexposed groups, and the incidence was significantly increased in both groups. There was a significant positive trend in the incidence of acinar cell adenoma or adenocarcinoma (combined) of the pancreas in F_1 males in both the F_0 exposed and F_0 unexposed groups, and the incidence was significantly increased in both groups. In female rats, there was a significant increase in the incidence of adenocarcinoma of the uterus in the group with F_1 exposure at the highest dose without F_0 exposure. In female rats, there was a significant positive trend in the incidence of pancreatic acinar cell adenoma or adenocarcinoma (combined) in F_1 rats with F_0 exposure. [The Working Group noted that a low incidence of pancreatic acinar cell adenoma or carcinoma (combined) and of pancreatic acinus hyperplasia was observed in females; these rare lesions in female rats were considered to be associated with PFOA exposure.]

In another dietary study that complied with GLP in male and female Sprague-Dawley rats (Butenhoff et al., 2012a), there was a significant positive trend in the incidence of testicular Leydig cell adenoma, and the incidence was significantly increased at the highest dose in males. In females, there was a significant positive trend in the incidence of fibroadenoma of the mammary gland, and the incidence was significantly increased at both doses. A pathology working group was convened by the study sponsor(s) to review the original slides of the mammary glands from the study by US EPA (1987), a study that was also reported by Butenhoff et al. (2012a), and concluded that PFOA did not induce neoplasms of the mammary gland in those studies (Hardisty et al., 2010; Butenhoff et al., 2012a). [The Working Group agreed with the conclusion of Hardisty et al. (2010) that PFOA did not induce neoplasms of the mammary gland.]

In the single-dose dietary study in male Sprague-Dawley rats (Biegel et al., 2001), there was a significant increase in the incidence of hepatocellular adenoma, and of hepatocellular adenoma or carcinoma (combined). There was a significant increase in the incidence of testicular Leydig cell adenoma. There were significant increases in the incidence of pancreatic acinar cell adenoma, and in the incidence of pancreatic acinar cell adenoma or carcinoma (combined).

In a study of oral administration (gavage) in female CD-1 mice, there was a significant increase in the incidence of hepatocellular adenoma at the intermediate dose only, and a significant positive trend in the incidence of liver haemangiosarcoma (Filgo et al., 2015). No increase in the incidence of hepatic neoplasms was observed in treated female 129/Sv wildtype and PPARα-knockout

mice. [The Working Group considered the liver haemangiosarcomas to be possibly associated with PFOA exposure; however, the Working Group acknowledged that 16–28% of unscheduled deaths in all groups were not examined. The Working Group was uncertain regarding the biological significance of the hepatocellular adenoma results.]

In studies of oral administration (feed) and initiation–promotion in rats (Abdellatif et al., 1990, 1991; also reported by Nilsson et al., 1991) and fish (Tilton et al., 2008; Benninghoff et al., 2012), the promoting activity of PFOA was investigated. There was a significant increase in the incidence of total tumours of the liver at the intermediate and higher doses (Abdellatif et al., 1990) and a significant increase in the incidence of hepatocellular carcinoma at the highest dose (Abdellatif et al., 1991) in male Wistar rats. There was a significant increase in the incidence of total tumours of the liver (malignant and benign) (Tilton et al., 2008) and of total tumours of the liver Benninghoff et al. (2012) in male and female rainbow trout. [The Working Group noted that the proportion of malignant tumours (as a percentage of the total liver tumours) was higher than that of benign tumours. The Working Group also noted that PFOA acted as a cancer promoter in these studies.]

In a study of oral administration (gavage) in male and female C57BL/6J-*Apc*$^{Min/+}$ mice (Ngo et al., 2014), and a promotion study of oral administration (drinking-water) in male and female *LSL-Kras*^{G12D}*;Pdx-1 Cre* (KC) transgenic mice (Kamendulis et al., 2022) there was no significant increase in the incidence of tumours. In a promotion study of oral administration (gavage) in female Sprague-Dawley rats, no significant increase in tumour incidence was found (Su et al., 2022). [The Working Group noted that this negative result may have been due to the high initiating dose.] [The Working Group noted that an effect in the liver and pancreas in male rats was observed consistently throughout the studies, while an effect in the pancreas in female rats was only observed in the NTP study. This effect in the female pancreas is possibly a result of the higher exposure to PFOA in females than in males, compensating for the faster elimination in females than in males.]

3.3.2 PFOS

The carcinogenicity of PFOS has been assessed in one well-conducted study that complied with GLP in male and female Sprague-Dawley rats treated by oral administration (feed) (US EPA, 2002; also reported by Butenhoff et al., 2012b). The carcinogenicity of PFOS was also evaluated in three studies that did not comply with GLP. One study was of oral administration (gavage) in male and female C57BL/6J-*Apc*$^{Min/+}$ mice (Ngo et al., 2014). Two initiation–promotion studies were of oral administration (feed) in male and female rainbow trout (*Oncorhynchus mykiss*) (Benninghoff et al., 2012) and of aqueous exposure in male and female *Kras*^{V12} transgenic zebrafish (Zhu et al., 2021).

In a dietary study in male and female Sprague-Dawley rats (Butenhoff et al., 2012b), there was a significant positive trend in the incidence of hepatocellular adenoma in males, and the incidence was significantly increased at the highest dose. In females, there was a significant positive trend in the incidence of hepatocellular adenoma, and hepatocellular adenoma or carcinoma (combined), with the incidence being significantly increased at the highest dose. There was a significant increase in the incidence of thyroid follicular cell adenoma or carcinoma (combined) at the higher intermediate dose in females. There was a significant increase in the incidence of fibroadenoma of the mammary gland and of fibroadenoma or adenoma (combined) at the lowest dose. There was significant positive trend in the incidence of fibroadenoma, adenoma or carcinoma (combined) of the mammary gland, with the incidence being significantly increased

at the two lower doses. In the recovery group of males, there was a significant increase in the incidence of thyroid follicular cell adenoma. [The Working Group noted that the liver was a target organ for PFOS in both male and female rats. The Working Group also noted that the association between PFOS exposure and the incidence of thyroid follicular cell tumours and mammary gland tumours was uncertain.]

In an initiation–promotion study of oral administration (in the feed) of PFOS, there was a significant increase in the incidence of total liver tumours in male and female rainbow trout (*Oncorhynchus mykiss*) at the highest dose (Benninghoff et al., 2012). [The Working Group noted that this study provided evidence that PFOS can be a cancer promoter in a rainbow trout model.]

In a study of oral administration (gavage) in male and female C57BL/6J-*Apc*$^{Min/+}$ mice (Ngo et al., 2014) and of aqueous exposure in male and female *Kras*^{V12} transgenic zebrafish (Zhu et al., 2021), there was no significant increase in the incidence of tumours.

References

Abbott BD, Wolf CJ, Schmid JE, Das KP, Zehr RD, Helfant L, et al. (2007). Perfluorooctanoic acid induced developmental toxicity in the mouse is dependent on expression of peroxisome proliferator activated receptor-alpha. *Toxicol Sci*. 98(2):571–81. doi:10.1093/toxsci/kfm110 PMID:17488742

Abdellatif AG, Préat V, Taper HS, Roberfroid M (1991). The modulation of rat liver carcinogenesis by perfluorooctanoic acid, a peroxisome proliferator. *Toxicol Appl Pharmacol*. 111(3):530–7. doi:10.1016/0041-008X(91)90257-F PMID:1684073

Abdellatif AG, Préat V, Vamecq J, Nilsson R, Roberfroid M (1990). Peroxisome proliferation and modulation of rat liver carcinogenesis by 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, perfluorooctanoic acid and nafenopin. *Carcinogenesis*. 11(11):1899–902. doi:10.1093/carcin/11.11.1899 PMID:2225320

Andreassen A, Møllersen L, Vikse R, Steffensen I-L, Mikalsen A, Paulsen JE, et al. (2002). One dose of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) or 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) induces tumours in Min/+ mice by truncation mutations or LOH in the Apc gene. *Mutat Res*. 517(1–2):157–66. doi:10.1016/S1383-5718(02)00065-7 PMID:12034317

Benninghoff AD, Orner GA, Buchner CH, Hendricks JD, Duffy AM, Williams DE (2012). Promotion of hepatocarcinogenesis by perfluoroalkyl acids in rainbow trout. *Toxicol Sci*. 125(1):69–78. doi:10.1093/toxsci/kfr267 PMID:21984479

Biegel LB, Hurtt ME, Frame SR, O'Connor JC, Cook JC (2001). Mechanisms of extrahepatic tumor induction by peroxisome proliferators in male CD rats. *Toxicol Sci*. 60(1):44–55. doi:10.1093/toxsci/60.1.44 PMID:11222872

Butenhoff JL, Chang S-C, Olsen GW, Thomford PJ (2012b). Chronic dietary toxicity and carcinogenicity study with potassium perfluorooctanesulfonate in Sprague-Dawley rats. *Toxicology*. 293(1–3):1–15. doi:10.1016/j.tox.2012.01.003 PMID:22266392

Butenhoff JL, Kennedy GL Jr, Chang S-C, Olsen GW (2012a). Chronic dietary toxicity and carcinogenicity study with ammonium perfluorooctanoate in Sprague-Dawley rats. *Toxicology*. 298(1–3):1–13. doi:10.1016/j.tox.2012.04.001 PMID:22531602

Caverly Rae JM, Frame SR, Kennedy GL, Butenhoff JL, Chang S-C (2014). Pathology review of proliferative lesions of the exocrine pancreas in two chronic feeding studies in rats with ammonium perfluorooctanoate. *Toxicol Rep*. 1:85–91. doi:10.1016/j.toxrep.2014.04.005 PMID:28962229

Chew TW, Liu XJ, Liu L, Spitsbergen JM, Gong Z, Low BC (2014). Crosstalk of Ras and Rho: activation of RhoA abates Kras-induced liver tumorigenesis in transgenic zebrafish models. *Oncogene*. 33(21):2717–27. doi:10.1038/onc.2013.240 PMID:23812423

Filgo AJ, Quist EM, Hoenerhoff MJ, Brix AE, Kissling GE, Fenton SE (2015). Perfluorooctanoic acid (PFOA)-induced liver lesions in two strains of mice following developmental exposures: PPARα is not required. *Toxicol Pathol*. 43(4):558–68. doi:10.1177/0192623314558463 PMID:25398757

Giknis MLA, Clifford CB (2010). Spontaneous neoplastic lesions in the Crl:CD-1(ICR) mouse in control groups from 18 month to 2 year studies – March 2010. Charles River Laboratories. Available from: https://www.criver.com/sites/default/files/resources/doc_a/SpontaneousNeoplasticLesionsintheCrlCD-1ICRMouseinControlGroupsfrom18Monthto2YearStudies%E2%80%94March2010.pdf.

Hardisty JF, Willson GA, Brown WR, McConnell EE, Frame SR, Gaylor DW, et al. (2010). Pathology Working Group review and evaluation of proliferative lesions of mammary gland tissues in female rats fed ammonium perfluorooctanoate (APFO) in

the diet for 2 years. *Drug Chem Toxicol*. 33(2):131–7. doi:10.3109/01480541003667610 PMID:20307141

Hendricks JD, Meyers TR, Shelton DW (1984). Histological progression of hepatic neoplasia in rainbow trout (*Salmo gairdneri*). *Natl Cancer Inst Monogr*. 65:321–36. PMID:6087143

Hezel AF, Kimmelman AC, Stanger BZ, Bardeesy N, Depinho RA (2006). Genetics and biology of pancreatic ductal adenocarcinoma. *Genes Dev*. 20(10):1218–49. doi:10.1101/gad.1415606 PMID:16702400

Hingorani SR, Petricoin EF 3rd, Maitra A, Rajapakse V, King C, Jacobetz MA, et al. (2003). Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. *Cancer Cell*. 4(6):437–50. doi:10.1016/S1535-6108(03)00309-X PMID:14706336

IARC (2016). Some chemicals used as solvents and in polymer manufacture. *IARC Monogr Eval Carcinog Risks Hum*. 110:1–276. Available from: https://publications.iarc.who.int/547 PMID:31829531

Kamendulis LM, Hocevar JM, Stephens M, Sandusky GE, Hocevar BA (2022). Exposure to perfluorooctanoic acid leads to promotion of pancreatic cancer. *Carcinogenesis*. 43(5):469–78. doi:10.1093/carcin/bgac005 PMID:35022659

Macon MB, Villanueva LR, Tatum-Gibbs K, Zehr RD, Strynar MJ, Stanko JP, et al. (2011). Prenatal perfluorooctanoic acid exposure in CD-1 mice: low-dose developmental effects and internal dosimetry. *Toxicol Sci*. 122(1):134–45. doi:10.1093/toxsci/kfr076 PMID:21482639

Moser AR, Pitot HC, Dove WF (1990). A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. *Science*. 247(4940):322–4. doi:10.1126/science.2296722 PMID:2296722

Ngo HT, Hetland RB, Sabaredzovic A, Haug LS, Steffensen I-L (2014). In utero exposure to perfluorooctanoate (PFOA) or perfluorooctane sulfonate (PFOS) did not increase body weight or intestinal tumorigenesis in multiple intestinal neoplasia (*Min*/+) mice. *Environ Res*. 132:251–63. doi:10.1016/j.envres.2014.03.033 PMID:24834819

Nilsson R, Beije B, Préat V, Erixon K, Ramel C (1991). On the mechanism of the hepatocarcinogenicity of peroxisome proliferators. *Chem Biol Interact*. 78(2):235–50. doi:10.1016/0009-2797(91)90017-2 PMID:2040027

NTP (2020). NTP Technical Report on the toxicology and carcinogenesis studies of perfluorooctanoic acid (CASRN 335-67-1) administered in feed to Sprague Dawley (Hsd:Sprague Dawley® SD®) rats (revised). *Natl Toxicol Program Tech Rep Ser*. (598) Revised February 2023.

Su Y, Santucci-Pereira J, Dang NM, Kanefsky J, Rahulkannan V, Hillegass M, et al. (2022). Effects of pubertal exposure to butyl benzyl phthalate, perfluorooctanoic acid, and zeranol on mammary gland development and tumorigenesis in rats. *Int J Mol Sci*. 23(3):1398. doi:10.3390/ijms23031398 PMID:35163327

Thoolen B, Maronpot RR, Harada T, Nyska A, Rousseaux C, Nolte T, et al. (2010). Proliferative and nonproliferative lesions of the rat and mouse hepatobiliary system. *Toxicol Pathol*. 38(7 Suppl):5S–81S. doi:10.1177/0192623310386499 PMID:21191096

Tilton SC, Orner GA, Benninghoff AD, Carpenter HM, Hendricks JD, Pereira CB, et al. (2008). Genomic profiling reveals an alternate mechanism for hepatic tumor promotion by perfluorooctanoic acid in rainbow trout. *Environ Health Perspect*. 116(8):1047–55. doi:10.1289/ehp.11190 PMID:18709148

US EPA (1987). Two-year oral (diet) toxicity/oncogenicity study of fluorochemical FC-143 in rats. Prepared by Sibinski LJ. Riker Experiment No. 0281CR0012, Riker Laboratories Inc/3M Company. Washington (DC), USA: United States Environmental Toxicology Program. Available from: https://hero.epa.gov/hero/index.cfm/reference/details/reference_id/5432403.

US EPA (2002). 104-Week dietary chronic toxicity and carcinogenicity study with perfluorooctane sulfonic acid potassium salt (PFOS; T-6295) in rats. Technical report. Prepared by Thomford PJ. Prepared by Thomford PJ for Covance Laboratories Inc. Study No. 6329-183. Washington (DC), USA: United States Environmental Toxicology Program. Available from: https://hero.epa.gov/hero/index.cfm/reference/details/reference_id/5029075.

Zhu Y, Yang D, Duan X, Zhang Y, Chen D, Gong Z, et al. (2021). Perfluorooctane sulfonate promotes doxycycline-induced liver tumor progression in male *Kras*^{v12} transgenic zebrafish. *Environ Res*. 196:110962. doi:10.1016/j.envres.2021.110962 PMID:33675800