25/10/2022

Proposal for reporting data relevant for human health

Human Health Assessment of Perfluoroalkyl carboxylic Acids (PFCAs)

Current state of the assessment based on in vivo studies used in the EFSA Opinion (2020) and ATSDR Report (2018). The assessment is ongoing with more recent studies and epidemiological studies.

Content:

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When we have agreed on a format I believe we should combine all the PFASs in one doc?

Field Code Changed

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1. Toxicokinetics

Work in progress and to be added later.

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2. Repeated dose toxicity

Epidemiological studies

To be completed…

Animal studies (summarised in Table 2)

Oral exposure to **PFOA** by gavage induces strong adverse effects in rodent liver morphology, immune function, thyroid morphology/metabolism, and clinical chemistry, especially reduced cholesterol and triglycerides.

Strong increases in **liver weight** are observed after exposure to 0.625 mg/kg bw/d or 1 mg/kg bw/d and higher in male rats and mice, respectively (NTP, 2019b). In female rats liver weights increased at 25 mg/kg bw/d and higher.

Immunotoxic effects of PFOA include changes in weights of lymphoid organs (thymus, spleen), cellularity of white blood cells, and antibody responses to T-cell-dependent antigens. From immunological studies performed in male mice and rats, the ~~male~~ rat seems to be less sensitive (Loveless et al., 2008). In rats, thymic or splenic weight was reduced at 10 or 2.5 mg/kg bw/d or higher in males (NTP, 2019b); female rats have not been studied so far. Thymic weight was reduced in male or female mice at doses of 7.5 mg/kg bw/d or higher (DeWitt et al., 2016; Qazi et al., 2012); splenic weight was reduced at doses of 1 or 3.75 mg/kg bw/d or higher in male or female mice, respectively (DeWitt et al., 2008; Loveless et al., 2008). Antibody responses were reduced at doses of 10 or 1.88 mg/kg bw/d or higher in male or female mice, respectively (DeWitt et al., 2016; Loveless et al., 2008); no effects on antibody response were observed in male rats (Loveless et al., 2008). Numbers of white blood cells were altered doses of 0.49 and 3.75 mg/kg bw/d or higher in male and female mice, respectively (DeWitt et al., 2016; Son et al., 2008; Son et al., 2009).

PFOA induced an increase in **thyroid** weight at 1.25 mg/kg bw/d or higher and a reduction of serum thyroid hormones T3 and T4 at 0.625 or 100 mg/kg bw/d or higher in male and female rats (NTP, 2019b). Likewise, thyroid hormones were reduced in male in Cynomolgus monkeys at a dose of 20-30 mg/kg bw/d for T3, and at 3 or 10 mg/kg bw/d or higher for total or free T4 (Butenhoff et al., 2002).

With respect to **clinical chemistry**, reductions in cholesterol and triglycerides in blood serum are the most prominent findings for PFOA. Cholesterol was reduced at a LOAEL of 0.3 mg/kg bw/d in male rats (Loveless et al., 2006); female rats have not been studied. In male mice, PFOA reduced cholesterol at a LOAEL of 5 mg/kg bw/d (Wu et al., 2018); in female mice, 15 mg/kg bw/d is the lowest dose at which PFOA reduced cholesterol (DeWitt et al., 2009). Serum triglycerides were reduced at a LOAEL of 0.3 mg/kg bw/d in male rats and mice (Loveless et al., 2008); no data are available for female rats. Female mice showed reduced triglycerides at a LOAEL of 3.75 mg/kg bw/d (DeWitt et al., 2009).

PFNA, **PFDA**, **PFUnDA** and **PFDoDA** raise similar concerns in many of the effects described above in male and female rats and mice compared to repeated exposure to PFOA. Potencies are in comparable dose ranges as PFOA for the respective effect (Ding et al., 2009)Fang et al., 2008; Frawley et al., 2018; Harris and Birnbaum 1989; Kato et al., 2015; Kudo et al., 2006; NTP, 2019; Shi et al., 2009; Takahashi et al., 2014; Wang et al., 2015; Zhang et al., 2008).

Short chain PFCAs (C2: **TFA**, C4: **PFBA**, C6: **PFHxA**, C7: **PFHpA**) as well as long-chain (C14-C18) PFCAs (C14: **PFTeDA**, C16: **PFHxDA**, C18: **PFODA**) all induced increased liver weight, but LOAELs are (much) higher (10-1000-fold) when compared to C8-C12. Generally, at very short (TFA) or very long (PFODA) chain length, LOAELs are highest and only few effects have been reported (reduced body weight,

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Commented [A5]: Immunotoxicity should be a separate chapter perhaps- or should it be a subchapter under repeated dose? in order to avoid to much repetition its possible to refer to the different chapters and just describe it briefly here. Same goes for the endocrine part- perhaps we should have a separate chapter for endocrine disruptive effects as well.

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increased liver weight and for PFODA reduced cholesterol are the only significant effects). The most sensitive effects for short-chain (PFBA, PFHxA, PFHpA) and long-chain (PFTeDA, PFHxDA) PFCAs are those on the thyroid system (PFBA: Butenhoff et al., 2012a; PFHxA: NTP, 2019a; PFHpA: Anonymous, 2017; PFTeDA & PFHxDA: Hirata-Koizumi et al., 2015).

Research gaps

No data are available for **PFPA** (C3), **PFPeA** (C5), **PFTrDA** (C13), **PFPeDA** (C15) and **PFHpDA** (C17). Only one study is available for C2, C11, C14, C16, C18 each, and only for rats. Immunotoxicology in general is mainly studied in terms of lymphatic tissues (spleen and thymus) morphology and white blood cell count, but immunological effects of PFCAs can be much more complex as demonstrated in mice studies on PFOA (DeWitt et al., 2016; Loveless et al., 2008; Son et al., 2008, Son et al., 2009). Especially antibody responses have only been studied in mice after exposure to PFOA.

Dermal and inhalation application routes have been studied to a much more limited extent compared to the oral gavage route. Thus, there is a major research gap with respect to inhalation and dermal administration. The few available studies, however suggest that for these routes, affects are similar to those observed for the oral route (reduced body weight, increased liver weight, decreased weight of lymphatic tissues) after PFOA administration via these exposure routes (dermal: Kennedy et al., 1985; Fairley et al., 2007; O'Malley and Ebbins, 1981; inhalation: Kennedy et al., 1986).

In **conclusion**, there are overlaps in the type of adverse effects across all PFCAs but there are also marked differences in the potencies of PFCA to induce adverse effects on rodent physiology. Besides gradual variations in the magnitudes of identical effects, TFAA and PFODA lack some major health hazards that apply for PFOA and other PFCA.

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Commented [A9]: Even for PFOA the investigation is quite poor. Should be studied in a comparative way across PFCAs and PFSAs. To describe in a chapter on research gaps later

Commented [A10]: Need to say something on potency differences is dependent on study duration and tissue concentration (internal versus external dose), but perhaps that is planned in the TK part? And perhaps it belongs more in an overall consideration across PFCAs and PFSAs

3. Developmental and reproductive toxicity

Epidemiology

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Legacy PFAS, including, PFOA and PFOS have been associated with adverse effects on reproduction and development in epidemiological studies. Observed effects comprised decreased sperm counts, higher levels of luteinizing and follicle stimulation hormone (Vested et al., 2013; Song et al., 2018) and deteriorated semen parameters such as mobility and DNA fragmentation ratio (Pan et al., 2019). Exposure to PFOA was linked to reduced fecundity (Velez et al., 2015) or reduced odds for fecundability (Fei et al., 2009). Further, there is evidence for decreased infant weight in relation to PFOA exposure (Johnson et al., 2014), as well as delayed menarche (Kristensen et al., 2013). Although reported human and epidemiological data is not always consistent (Bach et al., 2016), systematic reviews of available studies indicated adverse effects of environmental exposure to legacy PFAS on both female and male reproduction (Fenton et al., 2019). This is further supported by assessments of human health hazards, based on in vivo studies in rodents of developmental and reproductive toxicity as listed in the attached table (Table 3).

Animal studies (summarised in Table 3)

Mice are regarded as the most sensitive species, showing severe developmental and reproductive effects after exposure to **PFOA** *in utero*. In particular, neonatal morbidity and reduced birth weights are observed after exposure to 5 mg/kg bw/d (Lau et al., 2006; Song et al., 2018; White et al., 2011; Yahia et al., 2010) or lower (starting at 0.6 mg/kg bw/d), depending on strain (Abbot et al., 2007; Tucker et al., 2015). Further, a substantial increase of full litter resorptions (FLR) was observed at a 5 mg/kg bw exposure levels. Concomitantly, placental lesions, impaired morphology and deteriorated functional scores of the mammary glands were also observed (Macon et al., 2011; Tucker et al., 2015).

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With respect to foetal and/or neonatal mortality, developmental toxicities of PFNA and PFDA were similar to that of PFOA, because these effects were observed at comparable exposure levels. For **PFNA**, neonatal survival was reduced down to 20% on PND 10 at 5 mg/kg bw/d (Das et al., 2015). Further, serum testosterone and other parameters of male fertility were affected by PFNA at a LOAEL as low as 0.5 mg/kg bw/d in mice (Singh and Singh, 2019a). **PFDA** exposure decreased the ratio of viable foetuses and increased litter resorption at a LOAEL level of 12.8 mg/kg bw/d in mice (Harris and Birnbaum, 1989). The LOAEL for reduced foetal weight was comparatively low (PFDA: 0.5 mg/kg bw/d GD10-13).

One study on **PFUnDA** demonstrated reduced birth weights in male (13.4%) and female (12.5%) rat pups, at an exposure level of 1 mg/kg bw/d (Takahashi et al., 2014). However, no other significant effects on reproduction or development were reported.

For **PFDoDA** parental body weight was reduced at LOAEL levels ranging between 0.5 – 3 mg/kg bw/d in rats (Chen et al., 2019; Shi et al., 2009a; Shi et al., 2009b). Further, mortality of pregnant female rats was increased at an LOAEL of only 2.5 mg/kg bw/d (Kato et al., 2015). PFDoDA did also affect expression of ovarian or testicular genes in rats (LOAEL of 0.5-3 mg/kg bw/d for females or 0.02-0.5 mg/kg bw/d for males), which led to decreased levels of reproductive hormones (LOAEL 5 mg/kg bw/d or below) and reduced weight of testes (LOAEL 10 mg/kg bw/d). This confirms substantial toxic effects on development and reproduction. However, comparison with PFOA is difficult as data on mice are lacking.

Exposure of up to 100 mg **PFTeDA** per kg bw/d did not affect oestrous cyclicity, fertility indices or other parameters of reproduction in rats (Hirata-Koizumi et al., 2015). Minor, but significantly

reduced maternal body weights have been observed during (LOAEL 3 mg/kg bw/d) and after lactation (10 mg/kg bw/d), as well as in mated males and pups (10 mg/kg bw/d).

Treatment of rats with **PFHxDA** resulted in reduced paternal body weights at a much higher LOAEL of 100 mg/kg bw/d. No other effects in relation to reproduction or development were observed. **PFODA** did affect both parental and pup body weights and fertility parameters, such as number of implantations or number of pups. The LOAEL for adversities on reproduction and development was 1000 mg/kg.

Compared to PFOA, PFHxA and PFBA are less potent in respect to effects on reproduction and development. For example, the NOAEL for pup mortality related to **PFHxA** exposure during pregnancy was 175 mg/kg bw/d (Iwai et al., 2019). Reduced birth weight or neonatal weight were observed at a LOAEL of 500 mg/kg bw/d. For **PFBA**, no increased foetal or pup mortality, reduced implantation or increased FLR was observed. Some mild developmental effects, such as slightly delayed eye opening (LOAEL 35 mg/kg bw/d), were reported.

In **conclusion**, there are overlaps in the type of adverse effects across all PFCAs but marked differences in the potencies of PFCA to induce adverse effects on development and reproduction. Besides gradual variations in the magnitudes of identical effects, PFBA, PFHxDA and PFODA lack some major health hazards that apply for PFOA and other C6-C14 chained PFCA. For both PFHxDA and PFODA, this could be related to an insufficient availability in potential target tissues or organs.

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4. Carcinogenicity

Current classifications

Several PFAS have previously been classified as possibly or probably carcinogenic to humans. The International Agency for Research on Cancer (IARC) classified PFOA as possibly carcinogenic (Group 2B; (IARC, 2017)) and tetrafluoroethylene as probably carcinogenic to humans (Group 2A; (IARC, 2017)), while other PFAS, such as 2-Chloro-1,1,1-trifluoroethane, are considered as not classifiable due to lack of data. The U.S. Environmental Protection Agency (EPA) found that there is suggestive evidence that PFOA (EPA US, 2016b), PFOS (EPA US, 2016a), and GenX (EPA US, 2018) may cause cancer. The EFSA Contam Panel concluded in 2018 that there is insufficient support for carcinogenicity of PFOS and PFOA in humans from epidemiological studies but that both compounds induced tumours in rats (EFSA, 2020b). According to the REACH regulation in the EU, 17 PFAS are currently harmonised classified as carcinogenic (Carc. 2 or Carc. 1B; e.g., PFOA and its ammonium salt, PFNA and its sodium and ammonium salts, PFDA and its sodium and ammonium salts, PFOS and it ammonium, lithium and potassium salts, trifluralin). Additionally, amongst 6790 PFAS registered in the EU, 76 PFAS are self-classified by registrants as Carc. 1A/B or Carc. 2.

Animal studies (summarised in Table 4)

Chronic repeated-dose toxicity studies performed in mammalian animals have been performed with PFHxA (Klaunig et al., 2015), PFOA (3M, 1983; Biegel et al., 2001; NTP, 2019a), PFOS (Butenhoff et al. 2012), GenX chemicals (EPA US, 2018; Temkin et al., 2020), and tetrafluoroethylene (IARC, 2017).
For PFHxA, there was no evidence for tumorigenic activity in SD rats (Klaunig et al., 2015).
For PFOA, 24 months repeated-dose toxicity studies with rats resulted in an increased incidence of Leydig cell adenoma (3M, 1983; Biegel et al., 2001), fibroadenoma in mammary glands (3M, 1983), liver adenoma (Biegel et al., 2001), and acinar cell adenoma (Biegel et al., 2001). A re-evaluation of the original slides of the 3M study by a Pathology Working Group resulted in no significant increase of fibroadenomas (Hardisty et al., 2010). A re-evaluation of male tissue samples of the 3M study by Caverly-Rae et al. (2014) resulted in an increase in acinar cell hyperplasia but not adenoma or carcinoma. In a more recent 2-year diet carcinogenicity study by the US-NTP (2019) with SD rats starting with prenatal exposure, the induction of pancreatic tumours (acinar cell adenomas and adenocarcinomas) as well as hepatocellular adenoma and carcinoma was confirmed at dose levels as low as 1.1 mg/kg bw/d. Additionally, PFOA was identified as tumour promoting in rat liver (Abdellatif et al., 1991) and trout liver (Benninghoff et al., 2012). Besides PFOA, also PFNA and PFDA showed tumour promoting activity in the trout study (Table 4).

Epidemiology

A variety of epidemiological studies, i.e., worker, high exposure community, and general population studies, assessed possible associations between PFAS exposure and cancer risk. These studies have been summarised in previous reports (ATSDR, 2018; EFSA, 2020a; EFSA, 2020b; IARC, 2017). In some studies PFOA exposure was associated with increased risks of testicular (Barry et al., 2013), kidney (Steenland and Woskie, 2012; Vieira et al., 2013) and prostate cancer (Gilliland and Mandel, 1993; Hardell et al., 2014; Lundin et al., 2009). In the study by Hardell et al. (2014), a statistically significant association between PFAS exposure and prostate cancer was also observed for PFDeA, PFUA , PFHxS, and PFOS. Inverse associations (i.e., decreased risk) were observed between PFOA exposure and bladder (Steenland et al., 2015), colorectal (Innes et al., 2014) and breast cancer (Barry et al., 2013). According to the EFSA CONTAM Panel (2018) these studies provide insufficient support for

Commented [A15]: Due to the limited data, we described in the text for carcinogenicity not only PFCAs but also other PFAS. For this endpoint it probably does not make much sense to discuss it per group?

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carcinogenicity of PFOS and PFOA in humans. The IARC assessment of PFOA (2017) concluded that the epidemiological evidence for increased testicular cancer risk through PFOA exposure is credible and unlikely to be explained by bias and confounding factors, however, it is based on a small number of testicular cancer cases (n=17; Barry et al., 2013). Also the increased kidney cancer risk through PFOA exposure is considered credible by IARC (IARC, 2017). IARCS overall conclusion was that there is limited evidence in humans for the carcinogenicity of PFOA despite the positive associations for cancers of the testis and kidney. It should be emphasized that the associations between testis cancer and PFOA exposure are in line with increased incidence of Leydig cell adenoma in animal studies (3M, 1983; Biegel et al., 2001).

Mode of Action

In a recent evaluation of the likely mechanisms of tumour formation by PFAS, Temkin and coworkes identified, that multiple PFAS induce oxidative stress, are immunosuppressive, and modulate receptor-mediated effects (Temkin et al., 2020). They also found suggestive evidence that some PFAS can induce epigenetic alterations and influence cell proliferation, while genotoxic mechanisms and metabolic activation is not supported by current scientific evidence.

For PFOA, the PPARα-agonistic mode of action is a plausible explanation for liver carcinogenicity as observed in rats (ATSDR, 2018). However, also interactions with hepatic estrogen receptors are discussed as potential PPARα-independent mechanisms for liver carcinogenicity (ATSDR, 2018). According to ATSDR, "the induction of Leydig cell tumors by PFOA may be mediated by effects on aromatase activity or testosterone biosynthesis, both of which may be related to PPARα activation". In the Background document to the Opinion on the Annex XV dossier proposing restrictions on PFOA, PFOA salts and PFOA-related substances, the Committee for Risk Assessment concluded that despite human PPARα does not seem to be involved in the induction of cell proliferation in the liver (Klaunig et al., 2012), PFOA-induced rat liver tumours cannot be regarded as irrelevant for humans (RAC 2015).

According to the EFSA Opinion on PFAS, the Leydig cell tumour induction by PFOA could be a result of "reduced serum testosterone levels and compensatory releases of luteotrophic hormone, which stimulates growth of Leydig cells and tumour formation." Also the induction of pancreatic acinar tumours by PFOA may be related to PPARα-agonistic activity. The Committee for Risk Assessment concluded that the data are insufficient to characterize the mode of action of PFOA induced Leydig cell adenomas and pancreatic acinar cell tumours and hence carcinogenic effects at these sites are presumed to be relevant for humans (RAC 2015).

In **conclusion**, for tetrafluoroethylene, PFOA, PFOS and GenX there is evidence for carcinogenic effects from animal studies but no clear evidence for carcinogenicity in humans. For the observed carcinogenicity, the available information is not sufficient to rule out human relevance of the underlying mode of action. According to the REACH regulation in the EU, 17 PFAS are currently harmonised classified as carcinogenic, partly based on read across to PFOA. For the vast majority of PFAS, long-term toxicity or carcinogenicity studies as well as epidemiological studies informative on potential carcinogenic effects are not available and thus, carcinogenicity of most PFAS is unclear.

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Of note, in the EFSA opinion the MoA was restricted to the most sensitive endpoints, so not everything is covered. Perhaps we can also here focus on the most prominent effects.
PPARa activation is relevant for human physiology and should not be discussed only in light of liver tumours.

Commented [A18]: Do we need a separate MoA search or is it likely to be covered by the main search for each PFAS-group?

5. References

3M (1983): TWO YEAR ORAL (DIET) TOXICITY / CARCINOGENICITY STUDY OF FLUOROCHEMICAL FC-143 IN RATS. RIKER Experiment No. 028]CR0012. Pathology and Toxicology, Riker Laboratories, Inc., St. Paul, MN, USA

Abbott B.D., Wolf C.J., Schmid J.E., Das K.P., Zehr R.D., Helfant L., Nakayama S., Lindstrom A.B., Strynar M.J., and Lau C. (2007): Perfluorooctanoic acid-induced developmental toxicity in the mouse is dependent on expression of peroxisome proliferator-activated receptor-alpha. Toxicological Sciences 98 (2), 571-581. DOI: 10.1093/toxsci/kfm110

Abdellatif A.G., Preat V., Taper H.S., and Roberfroid M. (1991): The modulation of rat liver carcinogenesis by perfluorooctanoic acid, a peroxisome proliferator. Toxicol Appl Pharmacol 111 (3), 530-537. DOI: 10.1016/0041-008x(91)90257-f

Abe T., Takahashi M., Kano M., Amaike Y., Ishii C., Maeda K., Kudoh Y., Morishita T., Hosaka T., Sasaki T., Kodama S., Matsuzawa A., Kojima H., and Yoshinari K. (2017): Activation of nuclear receptor CAR by an environmental pollutant perfluorooctanoic acid. Archives of Toxicology 91 (6), 2365-2374. DOI: 10.1007/s00204-016-1888-3

Ahmed D.Y. and Abd Ellah M.R. (2012): Effect of exposure to perfluorooctanoic acid on hepatic antioxidants in mice. Comparative Clinical Pathology 21 (6), 1643-1645. DOI: 10.1007/s00580-011-1341-1

Anonymous (2016): 90-Day Oral Toxicity in Wistar rats (OECD TG 408) with sodium trifluoroacetate. ECHA Dissemination website: https://echa.europa.eu/de/registration-dossier/-/registered-dossier/5203/7/6/2/?documentUUID=08bdbc2f-d202-478c-a6a9-eabc39479e69

Anonymous (2017): Combined 90-day repeated dose toxicity study with reproduction/developmental toxicity screening (OECD TG 408 and 422) of sodium perfluoroheptanoate

ATSDR (2018): Toxicological Profile for Perfluoroalkyls - Draft for Public Comment, date: 2018-06. Agency for Toxic Substances and Disease Registry. Registry A.f.T.S.a.D., Atlanta, GA, USA. https://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=1117&tid=237

Bach C.C., Vested A., Jørgensen K.T., Bonde J.P., Henriksen T.B., Toft G. (2016): Perfluoroalkyl and polyfluoroalkyl substances and measures of human fertility: a systematic review.

Crit Rev Toxicol. 46:735-55. DOI: 10.1080/10408444.2016.1182117.

Barry V., Winquist A., and Steenland K. (2013): Perfluorooctanoic acid (PFOA) exposures and incident cancers among adults living near a chemical plant. Environ Health Perspect 121 (11-12), 1313-1318. DOI: 10.1289/ehp.1306615

BayerCropScience (2014): Summary of the toxicological and metabolism studies for Flurtamone. https://www.cropscience.bayer.com/sites/cropscience/files/inline-files/M-482296-01-5.PDF

Benninghoff A.D., Orner G.A., Buchner C.H., Hendricks J.D., Duffy A.M., and Williams D.E. (2012): Promotion of Hepatocarcinogenesis by Perfluoroalkyl Acids in Rainbow Trout. Toxicological Sciences 125 (1), 69-78. DOI: 10.1093/toxsci/kfr267

Biegel L.B., Hurtt M.E., Frame S.R., O'Connor J.C., and Cook J.C. (2001): Mechanisms of extrahepatic tumor induction by peroxisome proliferators in male CD rats. Toxicological Sciences 60 (1), 44-55. DOI: 10.1093/toxsci/60.1.44

Biegel L.B., Liu R.C.M., Hurtt M.E., and Cook J.C. (1995): Effects of Ammonium Perfluorooctanoate on Leydig-Cell Function - in-Vitro, in-Vivo, and Ex-Vivo Studies. Toxicology and Applied Pharmacology 134 (1), 18-25. DOI: DOI 10.1006/taap.1995.1164

Blake B.E., Cope H.A., Hall S.M., Keys R.D., Mahler B.W., McCord J., Scott B., Stapleton H.M., Strynar M.J., Elmore S.A., and Fenton S.E. (2020): Evaluation of maternal, embryo, and placental effects in CD-1 mice following gestational exposure to perfluorooctanoic acid (PFOA) or hexafluoropropylene oxide dimer acid (HFPO-DA or GenX). Environmental Health Perspectives 128 (2). DOI: 10.1289/EHP6233

Butenhoff J., Costa G., Elcombe C., Farrar D., Hansen K., Iwai H., Jung R., Kennedy G., Jr., Lieder P., Olsen G., and Thomford P. (2002): Toxicity of ammonium perfluorooctanoate in male cynomolgus monkeys after oral dosing for 6 months. Toxicological Sciences 69 (1), 244-257. DOI: 10.1093/toxsci/69.1.244

Butenhoff J.L., Bjork J.A., Chang S.C., Ehresman D.J., Parker G.A., Das K., Lau C., Lieder P.H., van Otterdijk F.M., and Wallace K.B. (2012a): Toxicological evaluation of ammonium perfluorobutyrate in rats: Twenty-eight-day and ninety-day oral gavage studies. Reproductive Toxicology 33 (4), 513-530. DOI: 10.1016/j.reprotox.2011.08.004

Butenhoff J.L., Kennedy G.L., Chang S.C., and Olsen G.W. (2012b): 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

Butenhoff J.L., Kennedy G.L., Jr., Frame S.R., O'Connor J.C., and York R.G. (2004): The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat. Toxicology 196 (1-2), 95-116. DOI: 10.1016/j.tox.2003.11.005

Caverly Rae J.M., Frame S.R., Kennedy G.L., Butenhoff J.L., and 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

Chen Y., Li H., Mo J., Chen X., Wu K., Ge F., Ma L., Li X., Guo X., Zhao J., and Ge R.S. (2019): Perfluorododecanoic Acid Blocks Rat Leydig Cell Development during Prepuberty. Chem Res Toxicol 32 (1), 146-155. DOI: 10.1021/acs.chemrestox.8b00241

Chengelis C.P., Kirkpatrick J.B., Radovsky A., and Shinohara M. (2009): A 90-day repeated dose oral (gavage) toxicity study of perfluorohexanoic acid (PFHxA) in rats (with functional observational battery and motor activity determinations). Reproductive Toxicology 27 (3-4), 342-351. DOI: 10.1016/j.reprotox.2009.01.006

Christopher B. and Marias A. (1977): PFOA - Report to 3M Company - 28-day oral Toxicity study with FC-143 in Albino Mice. IBT NO. 8532-10655. Industrial BIO-TEST Laboratorial Inc. Company M.

Cook J.C., Murray S.M., Frame S.R., and Hurtt M.E. (1992): Induction of Leydig cell adenomas by ammonium perfluorooctanoate: a possible endocrine-related mechanism. Toxicol Appl Pharmacol 113 (2), 209-217. DOI: 10.1016/0041-008x(92)90116-a

Cui L., Zhou Q.F., Liao C.Y., Fu J.J., and Jiang G.B. (2009): Studies on the toxicological effects of PFOA and PFOS on rats using histological observation and chemical analysis. Arch Environ Contam Toxicol 56 (2), 338-349. DOI: 10.1007/s00244-008-9194-6

Das K.P., Grey B.E., Rosen M.B., Wood C.R., Tatum-Gibbs K.R., Zehr R.D., Strynar M.J., Lindstrom A.B., and Lau C. (2015): Developmental toxicity of perfluorononanoic acid in mice. Reproductive Toxicology 51, 133-144. DOI: 10.1016/j.reprotox.2014.12.012

Das K.P., Grey B.E., Zehr R.D., Wood C.R., Butenhoff J.L., Chang S.C., Ehresman D.J., Tan Y.M., and Lau C. (2008): Effects of perfluorobutyrate exposure during pregnancy in the mouse. Toxicological Sciences 105 (1), 173-181. DOI: 10.1093/toxsci/kfn099

Das K.P., Wood C.R., Lin M.T., Starkov A.A., Lau C., Wallace K.B., Corton J.C., and Abbott B.D. (2017): Perfluoroalkyl acids-induced liver steatosis: Effects on genes controlling lipid homeostasis. Toxicology 378, 37-52. DOI: 10.1016/j.tox.2016.12.007

DeWitt J.C., Copeland C.B., and Luebke R.W. (2009): Suppression of Humoral Immunity by Perfluorooctanoic Acid is Independent of Elevated Serum Corticosterone Concentration in Mice. Toxicological Sciences 109 (1), 106-112. DOI: 10.1093/toxsci/kfp040

DeWitt J.C., Copeland C.B., Strynar M.J., and Luebke R.W. (2008): Perfluorooctanoic acid-induced immunomodulation in adult C57BL/6J or C57BL/6N female mice. Environ Health Perspect 116 (5), 644-650. DOI: 10.1289/ehp.10896

DeWitt J.C., Williams W.C., Creech N.J., and Luebke R.W. (2016): Suppression of antigen-specific antibody responses in mice exposed to perfluorooctanoic acid: Role of PPARalpha and T- and B-cell targeting. J Immunotoxicol 13 (1), 38-45. DOI: 10.3109/1547691X.2014.996682

Ding L., Hao F., Shi Z., Wang Y., Zhang H., Tang H., and Dai J. (2009): Systems biological responses to chronic perfluorododecanoic acid exposure by integrated metabonomic and transcriptomic studies. J Proteome Res 8 (6), 2882-2891. DOI: 10.1021/pr9000256

EFSA (2020a): Public consultation on the draft scientific opinion on the risks to human health related to the presence of perfluoroalkyl substances in food. European Food Safety AuthorityDOI: 10.2903/j.efsa.20YY.NNNN

EFSA (2020b): Risk to human health related to the presence of perfluoroalkyl substances in food. EFSA Journal 18(9):6223

Elcombe C.R., Elcombe B.M., Foster J.R., Farrar D.G., Jung R., Chang S.C., Kennedy G.L., and Butenhoff J.L. (2010): Hepatocellular hypertrophy and cell proliferation in Sprague-Dawley rats following dietary exposure to ammonium perfluorooctanoate occurs through increased activation of the xenosensor nuclear receptors PPARalpha and CAR/PXR. Archives of Toxicology 84 (10), 787-798. DOI: 10.1007/s00204-010-0572-2

Eldasher L.M., Wen X., Little M.S., Bircsak K.M., Yacovino L.L., and Aleksunes L.M. (2013): Hepatic and renal Bcrp transporter expression in mice treated with perfluorooctanoic acid. Toxicology 306, 108-113. DOI: 10.1016/j.tox.2013.02.009

EPA US (2016a): Health Effects Support Document for Perfluorooctane Sulfonate (PFOS). U.S. Environmental Protection Agency, Office of Water (4304T) Health and Ecological Criteria Division, Washington, DC 20460 EPA Document Number: 822-R-16-002, May 2016

EPA US (2016b): Health Effects Support Document for Perfluorooctanoic Acid (PFOA). U.S. Environmental Protection Agency Office of Water (4304T), Health and Ecological Criteria Division, Washington, DC 20460 EPA Document Number: 822-R-16-003, May 2016

EPA US (2018): Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3), also known as "GenX Chemicals". U.S. Environmental Protection Agency, Office of Water (4304T) Health and Ecological Criteria Division, Washington, DC 20460 EPA Document Number: 823-P-18-001, NOVEMBER 2018

Fairley K.J., Purdy R., Kearns S., Anderson S.E., and Meade B.J. (2007): Exposure to the immunosuppresant, perfluorooctanoic acid, enhances the murine IgE and airway hyperreactivity response to ovalbumin. Toxicological Sciences 97 (2), 375-383. DOI: 10.1093/toxsci/kfm053

Fang X., Feng Y., Shi Z., and Dai J. (2009): Alterations of cytokines and MAPK signaling pathways are related to the immunotoxic effect of perfluorononanoic acid. Toxicological Sciences 108 (2), 367-376. DOI: 10.1093/toxsci/kfp019

Fang X., Feng Y., Wang J., and Dai J. (2010): Perfluorononanoic acid-induced apoptosis in rat spleen involves oxidative stress and the activation of caspase-independent death pathway. Toxicology 267 (1-3), 54-59. DOI: 10.1016/j.tox.2009.10.020

Fang X., Gao G., Xue H., Zhang X., and Wang H. (2012a): Exposure of perfluorononanoic acid suppresses the hepatic insulin signal pathway and increases serum glucose in rats. Toxicology 294 (2-3), 109-115. DOI: 10.1016/j.tox.2012.02.008

Fang X., Gao G., Xue H., Zhang X., and Wang H. (2012b): In vitro and in vivo studies of the toxic effects of perfluorononanoic acid on rat hepatocytes and Kupffer cells. Environ Toxicol Pharmacol 34 (2), 484-494. DOI: 10.1016/j.etap.2012.06.011

Fang X., Gao G., Zhang X., and Wang H. (2015): Perfluorononanoic acid disturbed the metabolism of lipid in the liver of streptozotocin-induced diabetic rats. Toxicol Mech Methods 25 (8), 622-627. DOI: 10.3109/15376516.2015.1053649

Fang X., Zhang L., Feng Y., Zhao Y., and Dai J. (2008): Immunotoxic effects of perfluorononanoic acid on BALB/c mice. Toxicological Sciences 105 (2), 312-321. DOI: 10.1093/toxsci/kfn127

Fei C., McLaughlin J.K., Lipworth L., Olsen J. (2009): Maternal levels of perfluorinated chemicals and subfecundity. Hum Reprod. 24:1200-5. DOI: 10.1093/humrep/den490.

Feng Y., Fang X., Shi Z., Xu M., and Dai J. (2010): Effects of PFNA exposure on expression of junction-associated molecules and secretory function in rat Sertoli cells. Reproductive Toxicology 30 (3), 429-437. DOI: 10.1016/j.reprotox.2010.05.010

Feng Y., Shi Z., Fang X., Xu M., and Dai J. (2009): Perfluorononanoic acid induces apoptosis involving the Fas death receptor signaling pathway in rat testis. Toxicol Lett 190 (2), 224-230. DOI: 10.1016/j.toxlet.2009.07.020

Fenton S.E., Ducatman A., Boobis A., DeWitt J.C., Lau C., Ng C., Smith J.S., Roberts S.M. (2020): Per- and Polyfluoroalkyl Substance Toxicity and Human Health Review: Current State of Knowledge and Strategies for Informing Future Research. Environ Toxicol Chem. doi: 10.1002/etc.4890. PMID: 33017053

Foreman J.E., Chang S.C., Ehresman D.J., Butenhoff J.L., Anderson C.R., Palkar P.S., Kang B.H., Gonzalez F.J., and Peters J.M. (2009): Differential Hepatic Effects of Perfluorobutyrate Mediated by Mouse and Human PPAR-alpha. Toxicological Sciences 110 (1), 204-211. DOI: 10.1093/toxsci/kfp077

Frawley R.P., Smith M., Cesta M.F., Hayes-Bouknight S., Blystone C., Kissling G.E., Harris S., and Germolec D. (2018): Immunotoxic and hepatotoxic effects of perfluoro-n-decanoic acid (PFDA) on female Harlan Sprague-Dawley rats and B6C3F1/N mice when administered by oral gavage for 28 days. J Immunotoxicol 15 (1), 41-52. DOI: 10.1080/1547691X.2018.1445145

Gilliland F.D. and Mandel J.S. (1993): Mortality among Employees of a Perfluorooctanoic Acid Production Plant. Journal of Occupational and Environmental Medicine 35 (9), 950-954. DOI: Doi 10.1097/00043764-199309000-00020

Goldenthal E.I. (1978a): Ninety day subacute rat toxicity study on Flurado®Fluorochemical FC-143. International Research and development corporation, Study No. 137-089, 3M Reference No. T-3141, November 6, 1978. US EPA AR226-0441

Goldenthal E.I. (1978b): Ninety day subacute rhesus monkey toxicity study International Research and Development Corporation, Study No. 137-090, November 10, 1978. US EPA Administrative Record, AR226-0447

Griffith F.D. and Long J.E. (1980): Animal toxicity studies with ammonium perfluorooctanoate. Am Ind Hyg Assoc J 41 (8), 576-583. DOI: 10.1080/15298668091425301

Hadrup N., Pedersen M., Skov K., Hansen N.L., Berthelsen L.O., Kongsbak K., Boberg J., Dybdahl M., Hass U., Frandsen H., and Vinggaard A.M. (2016): Perfluorononanoic acid in combination with 14 chemicals exerts low-dose mixture effects in rats. Archives of Toxicology 90 (3), 661-675. DOI: 10.1007/s00204-015-1452-6

Hardell E., Karrman A., van Bavel B., Bao J., Carlberg M., and Hardell L. (2014): Case-control study on perfluorinated alkyl acids (PFAAs) and the risk of prostate cancer. Environment International 63, 35-39. DOI: 10.1016/j.envint.2013.10.005

Hardisty J.F., Willson G.A., Brown W.R., McConnell E.E., Frame S.R., Gaylor D.W., Kennedy G.L., and Butenhoff J.L. (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), 131-137. DOI: 10.3109/01480541003667610

Harris M.W. and Birnbaum L.S. (1989): Developmental toxicity of perfluorodecanoic acid in C57BL/6N mice. Fundam Appl Toxicol 12 (3), 442-448. DOI: 10.1016/0272-0590(89)90018-3

Haughom B. and Spydevold O. (1992): The mechanism underlying the hypolipemic effect of perfluorooctanoic acid (PFOA), perfluorooctane sulphonic acid (PFOSA) and clofibric acid. Biochim Biophys Acta 1128 (1), 65-72. DOI: 10.1016/0005-2760(92)90258-w

Hirata-Koizumi M., Fujii S., Furukawa M., Ono A., and Hirose A. (2012): Repeated dose and reproductive/developmental toxicity of perfluorooctadecanoic acid in rats. J Toxicol Sci 37 (1), 63-79. DOI: 10.2131/jts.37.63

Hirata-Koizumi M., Fujii S., Hina K., Matsumoto M., Takahashi M., Ono A., and Hirose A. (2015): Repeated dose and reproductive/developmental toxicity of long-chain perfluoroalkyl carboxylic acids in rats: perfluorohexadecanoic acid and perfluorotetradecanoic acid. Fundamental Toxicological Sciences 2 (4), 177-190. DOI: 10.2131/fts.2.177

Hui Z.G., Li R.J., and Chen L. (2017): The impact of exposure to environmental contaminant on hepatocellular lipid metabolism. Gene 622, 67-71. DOI: 10.1016/j.gene.2017.04.024

IARC (2017): Some chemicals used as solvents and in polymer manufacture. International Agency for Research on Cancer. Available online: http://monographs.iarc.fr/ENG/Monographs/vol110/mono110-01.pdf

Ikeda T., Aiba K., Fukuda K., and Tanaka M. (1985): The induction of peroxisome proliferation in rat liver by perfluorinated fatty acids, metabolically inert derivatives of fatty acids. J Biochem 98 (2), 475-482. DOI: 10.1093/oxfordjournals.jbchem.a135302

Innes K.E., Wimsatt J.H., Frisbee S., and Ducatman A.M. (2014): Inverse association of colorectal cancer prevalence to serum levels of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in a large Appalachian population. BMC cancer 14, 45-45. DOI: 10.1186/1471-2407-14-45

Iwai H. and Hoberman A.M. (2014): Oral (Gavage) Combined Developmental and Perinatal/Postnatal Reproduction Toxicity Study of Ammonium Salt of Perfluorinated Hexanoic Acid in Mice. Int J Toxicol 33 (3), 219-237. DOI: 10.1177/1091581814529449

Iwai H., Hoberman A.M., Goodrum P.E., Mendelsohn E., and Anderson J.K. (2019): Addendum to Iwai and Hoberman (2014)-Reassessment of Developmental Toxicity of PFHxA in Mice. Int J Toxicol 38 (3), 183-191. DOI: 10.1177/1091581819837904

Iwai H. and Yamashita K. (2006): A fourteen-day repeated dose oral toxicity study of APFO in rats. Drug Chem Toxicol 29 (3), 323-332. DOI: 10.1080/01480540600653143

Johnson P.L., Sutton P., Atchley D.S., Koustas E., Lam J., Sen S., Robinson K.A., Axelrad D.A., Woodruff TJ. (2014): The Navigation Guide - evidence-based medicine meets environmental health: systematic review of human evidence for PFOA effects on fetal growth. Environ Health Perspect. 122:1028-39. DOI: 10.1289/ehp.1307893

Kato H., Fujii S., Takahashi M., Matsumoto M., Hirata-Koizumi M., Ono A., and Hirose A. (2015): Repeated dose and reproductive/developmental toxicity of perfluorododecanoic acid in rats. Environ Toxicol 30 (11), 1244-1263. DOI: 10.1002/tox.21996

Kawashima Y., Kobayashi H., Miura H., and Kozuka H. (1995): Characterization of hepatic responses of rat to administration of perfluorooctanoic and perfluorodecanoic acids at low levels. Toxicology 99 (3), 169-178. DOI: 10.1016/0300-483x(95)03027-d

Kennedy G.L., Jr. (1985): Dermal toxicity of ammonium perfluorooctanoate. Toxicol Appl Pharmacol 81 (2), 348-355. DOI: 10.1016/0041-008x(85)90172-3

Kennedy G.L., Jr. (1987): Increase in mouse liver weight following feeding of ammonium perfluorooctanoate and related fluorochemicals. Toxicol Lett 39 (2-3), 295-300. DOI: 10.1016/0378-4274(87)90245-1

Kennedy G.L., Jr., Hall G.T., Brittelli M.R., Barnes J.R., and Chen H.C. (1986): Inhalation toxicity of ammonium perfluorooctanoate. Food Chem Toxicol 24 (12), 1325-1329. DOI: 10.1016/0278-6915(86)90066-9

Kirkpatrick J.B. (2005): A combined 28-day repeated dose oral toxicity study with the reproduction/developmental toxicity screening test of perfluorhexanoic acid and 1H,1H,2H,2H-tridecafluoro-1-octanol in rats, with recovery. WIL-534001, date: 2005-09-02. WIL Research Laboratories. Chemical A., Asahi Glass Company L., 10 Goikaigan I.-s., and Chiba 290-8566 J.

Klaunig J.E., Shinohara M., Iwai H., Chengelis C.P., Kirkpatrick J.B., Wang Z.M., and Bruner R.H. (2015): Evaluation of the Chronic Toxicity and Carcinogenicity of Perfluorohexanoic Acid (PFHxA) in Sprague-Dawley Rats. Toxicologic Pathology 43 (2), 209-220. DOI: 10.1177/0192623314530532

Kristensen S.L., Ramlau-Hansen C.H., Ernst E., Olsen S.F., Bonde J.P., Vested A., Halldorsson T.I., Becher G., Haug L.S., Toft G. (2013): Long-term effects of prenatal exposure to perfluoroalkyl substances on female reproduction. Hum Reprod. 28:3337-48. DOI: 10.1093/humrep/det382.

Kudo N., Bandai N., Suzuki E., Katakura M., and Kawashima Y. (2000): Induction by perfluorinated fatty acids with different carbon chain length of peroxisomal beta-oxidation in the liver of rats. Chemico-Biological Interactions 124 (2), 119-132. DOI: 10.1016/s0009-2797(99)00150-7

Kudo N., Suzuki-Nakajima E., Mitsumoto A., and Kawashima Y. (2006): Responses of the liver to perfluorinated fatty acids with different carbon chain length in male and female mice: In relation to induction of hepatomegaly, peroxisomal beta-oxidation and microsomal 1-acylglycerophosphocholine acyltransferase. Biological & Pharmaceutical Bulletin 29 (9), 1952-1957. DOI: 10.1248/bpb.29.1952

Lau C., Thibodeaux J.R., Hanson R.G., Narotsky M.G., Rogers J.M., Lindstrom A.B., and Strynar M.J. (2006): Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. Toxicological Sciences 90 (2), 510-518. DOI: 10.1093/toxsci/kfj105

Li X.L., Wang Z.M., and Klaunig J.E. (2019): The effects of perfluorooctanoate on high fat diet induced non-alcoholic fatty liver disease in mice. Toxicology 416, 1-14. DOI: 10.1016/j.tox.2019.01.017

Liu H., Zhang H., Cui R., Guo X., Wang D., and Dai J. (2016): Activation of peroxisome proliferator-activated receptor alpha ameliorates perfluorododecanoic acid-induced production of reactive oxygen species in rat liver. Archives of Toxicology 90 (6), 1383-1397. DOI: 10.1007/s00204-015-1559-9

Liu R.C., Hurtt M.E., Cook J.C., and Biegel L.B. (1996): Effect of the peroxisome proliferator, ammonium perfluorooctanoate (C8), on hepatic aromatase activity in adult male Crl:CD BR (CD) rats. Fundam Appl Toxicol 30 (2), 220-228. DOI: 10.1006/faat.1996.0059

Loveless S.E., Finlay C., Everds N.E., Frame S.R., Gillies P.J., O'Connor J.C., Powley C.R., and Kennedy G.L. (2006): Comparative responses of rats and mice exposed to linear/branched, linear, or branched ammonium perfluorooctanoate (APFO). Toxicology 220 (2-3), 203-217. DOI: 10.1016/j.tox.2006.01.003

Loveless S.E., Hoban D., Sykes G., Frame S.R., and Everds N.E. (2008): Evaluation of the immune system in rats and mice administered linear ammonium perfluorooctanoate. Toxicological Sciences 105 (1), 86-96. DOI: 10.1093/toxsci/kfn113

Loveless S.E., Slezak B., Serex T., Lewis J., Mukerji P., O'Connor J.C., Donner E.M., Frame S.R., Korzeniowski S.H., and Buck R.C. (2009): Toxicological evaluation of sodium perfluorohexanoate. Toxicology 264 (1-2), 32-44. DOI: 10.1016/j.tox.2009.07.011

Lundin J.I., Alexander B.H., Olsen G.W., and Church T.R. (2009): Ammonium perfluorooctanoate production and occupational mortality. Epidemiology 20 (6), 921-928. DOI: 10.1097/EDE.0b013e3181b5f395

Macon M.B., Villanueva L.R., Tatum-Gibbs K., Zehr R.D., Strynar M.J., Stanko J.P., White S.S., Helfant L., and Fenton S.E. (2011): Prenatal perfluorooctanoic acid exposure in CD-1 mice: low-dose developmental effects and internal dosimetry. Toxicological Sciences 122 (1), 134-145. DOI: 10.1093/toxsci/kfr076

Metrick M. and Marisa A. (1977): 28-day oral toxicity study with FC-143 in albino Rats

Minata M., Harada K.H., Karrman A., Hitomi T., Hirosawa M., Murata M., Gonzalez F.J., and Koizumi A. (2010): Role of peroxisome proliferator-activated receptor-alpha in hepatobiliary injury induced by ammonium perfluorooctanoate in mouse liver. Ind Health 48 (1), 96-107. DOI: 10.2486/indhealth.48.96

NTP (2019a): NTP Draft Technical Report on the Toxicology and Carcinogenesis Studies of Perfluorooctanoic Acid (CAS No. 335-67-1) Administered in Feed to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats. National Toxicology Program, Public Health Service, U.S. Department of Health and Human Services, ISSN: 2473-4756 Technical Report 598

NTP (2019b): NTP Technical Report on the Toxicity Studies of Perfluoroalkyl Carboxylates (Perfluorohexanoic Acid, Perfluorooctanoic Acid, Perfluorononanoic Acid, and Perfluorodecanoic Acid) Administered by Gavage to Sprague Dawley (Hsd:Sprague Dawley SD) Rats. NTP TOX 97, date: August 2019. National Toxicology Program, Public Health Service, U.S. Department of Health and Human Services. Services U.S.D.o.H.a.H., Research Triangle Park, North Carolina, USA, Toxicity Report. https://ntp.niehs.nih.gov/ntp/htdocs/st_rpts/tox097_508.pdf?utm_source=direct&utm_medium=prod&utm_campaign=ntpgolinks&utm_term=tox097

O'Malley K.D. and Ebbins K.L. (1981): Repeat application 28 day percutaneous absorption study with T-2618 CoC in albino rabbits. USEPA Administrative Record 226-0446. Riker Laboratories, Inc., St. Paul, MN

Pan Y., Cui Q., Wang J., Sheng N., Jing J., Yao B., Dai J. (2019): Profiles of Emerging and Legacy Per-/Polyfluoroalkyl Substances in Matched Serum and Semen Samples: New Implications for Human Semen Quality. Environ Health Perspect. 127:127005. DOI: 10.1289/EHP4431.

Palazzolo M.J. (1993): Thirteen-week dietary toxicity study with T-5180, ammonium perfluorooctanoate (CAS No.3825-26-1) in male rats, date: 1993

Pastoor T.P., Lee K.P., Perri M.A., and Gillies P.J. (1987): Biochemical and Morphological-Studies of Ammonium Perfluorooctanoate-Induced Hepatomegaly and Peroxisome Proliferation. Experimental and Molecular Pathology 47 (1), 98-109. DOI: Doi 10.1016/0014-4800(87)90011-6

Perkins R.G., Butenhoff J.L., Kennedy G.L., Jr., and Palazzolo M.J. (2004): 13-week dietary toxicity study of ammonium perfluorooctanoate (APFO) in male rats. Drug Chem Toxicol 27 (4), 361-378. DOI: 10.1081/dct-200039773

Permadi H., Lundgren B., Andersson K., and Depierre J.W. (1992): Effects of Perfluoro Fatty-Acids on Xenobiotic-Metabolizing Enzymes, Enzymes Which Detoxify Reactive Forms of Oxygen and Lipid-Peroxidation in Mouse-Liver. Biochemical Pharmacology 44 (6), 1183-1191. DOI: Doi 10.1016/0006-2952(92)90383-T

Permadi H., Lundgren B., Andersson K., Sundberg C., and DePierre J.W. (1993): Effects of perfluoro fatty acids on peroxisome proliferation and mitochondrial size in mouse liver: dose and time factors and effect of chain length. Xenobiotica 23 (7), 761-770. DOI: 10.3109/00498259309166782

Qazi M.R., Abedi M.R., Nelson B.D., DePierre J.W., and Abedi-Valugerdi M. (2010): Dietary exposure to perfluorooctanoate or perfluorooctane sulfonate induces hypertrophy in centrilobular hepatocytes and alters the hepatic immune status in mice. Int Immunopharmacol 10 (11), 1420-1427. DOI: 10.1016/j.intimp.2010.08.009

Qazi M.R., Bogdanska J., Butenhoff J.L., Nelson B.D., DePierre J.W., and Abedi-Valugerdi M. (2009): High-dose, short-term exposure of mice to perfluorooctanesulfonate (PFOS) or perfluorooctanoate (PFOA) affects the number of circulating neutrophils differently, but enhances the inflammatory responses of macrophages to lipopolysaccharide (LPS) in a similar fashion. Toxicology 262 (3), 207-214. DOI: 10.1016/j.tox.2009.06.010

Qazi M.R., Nelson B.D., DePierre J.W., and Abedi-Valugerdi M. (2012): High-dose dietary exposure of mice to perfluorooctanoate or perfluorooctane sulfonate exerts toxic effects on myeloid and B-lymphoid cells in the bone marrow and these effects are partially dependent on reduced food consumption. Food Chem Toxicol 50 (9), 2955-2963. DOI: 10.1016/j.fct.2012.06.023

Rebholz S.L., Jones T., Herrick R.L., Xie C., Calafat A.M., Pinney S.M., and Woollett L.A. (2016): Hypercholesterolemia with consumption of PFOA-laced Western diets is dependent on strain and sex of mice. Toxicology Reports 3, 46-54. DOI: https://doi.org/10.1016/j.toxrep.2015.11.004

Rogers J.M., Ellis-Hutchings R.G., Grey B.E., Zucker R.M., Norwood J., Jr., Grace C.E., Gordon C.J., and Lau C. (2014): Elevated blood pressure in offspring of rats exposed to diverse chemicals during pregnancy. Toxicological Sciences 137 (2), 436-446. DOI: 10.1093/toxsci/kft248

Rosen M.B., Abbott B.D., Wolf D.C., Corton J.C., Wood C.R., Schmid J.E., Das K.P., Zehr R.D., Blair E.T., and Lau C. (2008): Gene Profiling in the Livers of Wild-type and PPAR alpha-Null Mice Exposed to Perfluorooctanoic Acid. Toxicologic Pathology 36 (4), 592-607. DOI: 10.1177/0192623308318208

Rosen M.B., Das K.P., Rooney J., Abbott B., Lau C., and Corton J.C. (2017): PPARalpha-independent transcriptional targets of perfluoroalkyl acids revealed by transcript profiling. Toxicology 387, 95-107. DOI: 10.1016/j.tox.2017.05.013

Shi Z., Ding L., Zhang H., Feng Y., Xu M., and Dai J. (2009): Chronic exposure to perfluorododecanoic acid disrupts testicular steroidogenesis and the expression of related genes in male rats. Toxicol Lett 188 (3), 192-200. DOI: 10.1016/j.toxlet.2009.04.014

Shi Z.M., Zhang H.X., Liu Y., Xu M., and Dai J.Y. (2007): Alterations in gene expression and testosterone synthesis in the testes of male rats exposed to perfluorododecanoic acid. Toxicological Sciences 98 (1), 206-215. DOI: 10.1093/toxsci/kfm070

Sibinski (1987): Final report of a two year oral (diet) toxicity and carcinogenicity study of fluorochemical FC-143 (perfluorooctane ammonium carboxylate) in rats. ol. 1-4, 3M Company/Riker exp. No. 0281CR0012; 8EHQ-1087-0394, October 16, 1987.

Singh S. and Singh S.K. (2019a): Acute exposure to perfluorononanoic acid in prepubertal mice: Effect on germ cell dynamics and an insight into the possible mechanisms of its inhibitory action on testicular functions. Ecotoxicol Environ Saf 183, 109499. DOI: 10.1016/j.ecoenv.2019.109499

Singh S. and Singh S.K. (2019b): Chronic exposure to perfluorononanoic acid impairs spermatogenesis, steroidogenesis and fertility in male mice. J Appl Toxicol 39 (3), 420-431. DOI: 10.1002/jat.3733

Singh S. and Singh S.K. (2019c): Effect of gestational exposure to perfluorononanoic acid on neonatal mice testes. J Appl Toxicol 39 (12), 1663-1671. DOI: 10.1002/jat.3883

Singh S. and Singh S.K. (2019d): Prepubertal exposure to perfluorononanoic acid interferes with spermatogenesis and steroidogenesis in male mice. Ecotoxicol Environ Saf 170, 590-599. DOI: 10.1016/j.ecoenv.2018.12.034

Son H.Y., Kim S.H., Shin H.I., Bae H.I., and Yang J.H. (2008): Perfluorooctanoic acid-induced hepatic toxicity following 21-day oral exposure in mice. Archives of Toxicology 82 (4), 239-246. DOI: 10.1007/s00204-007-0246-x

Son H.Y., Lee S., Tak E.N., Cho H.S., Shin H.I., Kim S.H., and Yang J.H. (2009): Perfluorooctanoic acid alters T lymphocyte phenotypes and cytokine expression in mice. Environ Toxicol 24 (6), 580-588. DOI: 10.1002/tox.20459

Song P., Li D., Wang X., and Zhong X. (2018): Effects of perfluorooctanoic acid exposure during pregnancy on the reproduction and development of male offspring mice. Andrologia 50 (8), e13059. DOI: 10.1111/and.13059

Steenland K. and Woskie S. (2012): Cohort mortality study of workers exposed to perfluorooctanoic acid. Am J Epidemiol 176 (10), 909-917. DOI: 10.1093/aje/kws171

Steenland K., Zhao L., and Winquist A. (2015): A cohort incidence study of workers exposed to perfluorooctanoic acid (PFOA). Occup Environ Med 72 (5), 373-380. DOI: 10.1136/oemed-2014-102364

Takahashi M., Ishida S., Hirata-Koizumi M., Ono A., and Hirose A. (2014): Repeated dose and reproductive/developmental toxicity of perfluoroundecanoic acid in rats. J Toxicol Sci 39 (1), 97-108. DOI: 10.2131/jts.39.97

Tan X., Xie G., Sun X., Li Q., Zhong W., Qiao P., Sun X., Jia W., and Zhou Z. (2013): High fat diet feeding exaggerates perfluorooctanoic acid-induced liver injury in mice via modulating multiple metabolic pathways. PLoS One 8 (4), e61409. DOI: 10.1371/journal.pone.0061409

Temkin A.M., Hocevar B.A., Andrews D.Q., Naidenko O.V., and Kamendulis L.M. (2020): Application of the key characteristics of carcinogens to per and polyfluoroalkyl substances. International Journal of Environmental Research and Public Health 17 (5). DOI: 10.3390/ijerph17051668

Thomford P.J. (2001): 26-Week Capsule Toxicity Study with Ammonium Perfluorooctanoate (APFO) in Cynomolgus Monkeys. Covance 6329-231 / 3M T-6889.3. Covance Laboratories Inc.

Tucker D.K., Macon M.B., Strynar M.J., Dagnino S., Andersen E., and Fenton S.E. (2015): The mammary gland is a sensitive pubertal target in CD-1 and C57Bl/6 mice following perinatal perfluorooctanoic acid (PFOA) exposure. Reproductive Toxicology 54, 26-36. DOI: 10.1016/j.reprotox.2014.12.002

van Otterdijk F.M. (2007a): Project 470677: Repeated dose 28-day oral toxicity study with MTDID-8391 by daily gavage in the rat, followed by a 21-day recovery period. 3M, Maplewood, MN

van Otterdijk F.M. (2007b): Project 484492: Repeated dose 90-day oral toxicity study with MTDID 8391 by daily gavage in the rat followed by a 3-week recovery period. 3M, Maplewood, MN

Vélez M.P., Arbuckle T.E., Fraser W.D. (2015): Maternal exposure to perfluorinated chemicals and reduced fecundity: the MIREC study. Hum Reprod. 30:701-9. DOI: 10.1093/humrep/deu350.

Vested A., Ramlau-Hansen C.H., Olsen S.F., Bonde J.P., Kristensen S.L., Halldorsson T.I., Becher G., Haug L.S., Ernst E.H., Toft G. (2013): Associations of in utero exposure to perfluorinated alkyl acids with human semen quality and reproductive hormones in adult men. Environ Health Perspect. 453-8. DOI: 10.1289/ehp.1205118.

Vetvicka V. and Vetvickova J. (2013): Reversal of perfluorooctanesulfonate-induced immunotoxicity by a glucan-resveratrol-vitamin C combination. Oriental Pharmacy and Experimental Medicine 13 (1), 77-84. DOI: 10.1007/s13596-013-0105-7

Vieira V.M., Hoffman K., Shin H.M., Weinberg J.M., Webster T.F., and Fletcher T. (2013): Perfluorooctanoic acid exposure and cancer outcomes in a contaminated community: a geographic analysis. Environ Health Perspect 121 (3), 318-323. DOI: 10.1289/ehp.1205829

Wang J., Yan S., Zhang W., Zhang H., and Dai J. (2015): Integrated proteomic and miRNA transcriptional analysis reveals the hepatotoxicity mechanism of PFNA exposure in mice. J Proteome Res 14 (1), 330-341. DOI: 10.1021/pr500641b

Wang L., Zhao F., Kan M., Wen Z., Zhou Y., Yu L., and Liu H. (2017): [Effects of perfluorooctanoic acid on oxidative stress and PPARalpha and its related CYP4A1 gene expression in rat liver]. Wei Sheng Yan Jiu 46 (5), 802-806. https://www.ncbi.nlm.nih.gov/pubmed/29903312

White S.S., Stanko J.P., Kato K., Calafat A.M., Hines E.P., and Fenton S.E. (2011): Gestational and chronic low-dose PFOA exposures and mammary gland growth and differentiation in three generations of CD-1 mice. Environ Health Perspect 119 (8), 1070-1076. DOI: 10.1289/ehp.1002741

WIL R.L. (2005): A combined 28-day repeated dose oral toxicity study with the reproduction/developmental toxicity screening test of perfluorohexanoic acid and 1H, 1H, 2H, 2H tridecafluoro-1-octanol in rats, with recovery. Study number WIL-534001

Wolf C.J., Zehr R.D., Schmid J.E., Lau C., and Abbott B.D. (2010): Developmental effects of perfluorononanoic Acid in the mouse are dependent on peroxisome proliferator-activated receptor-alpha. PPAR research 2010, 282896. DOI: 10.1155/2010/282896

Wolf D.C., Moore T., Abbott B.D., Rosen M.B., Das K.P., Zehr R.D., Lindstrom A.B., Strynar M.J., and Lau C. (2008): Comparative Hepatic Effects of Perfluorooctanoic Acid and WY 14,643 in PPAR-α Knockout and Wild-type Mice. Toxicologic Pathology 36 (4), 632-639. DOI: 10.1177/0192623308318216

Wu X.M., Xie G.J., Xu X.X., Wu W., and Yang B. (2018): Adverse bioeffect of perfluorooctanoic acid on liver metabolic function in mice. Environmental Science and Pollution Research 25 (5), 4787-4793. DOI: 10.1007/s11356-017-0872-7

Xie Y., Yang Q., Nelson B.D., and DePierre J.W. (2003): The relationship between liver peroxisome proliferation and adipose tissue atrophy induced by peroxisome proliferator exposure and withdrawal in mice. Biochemical Pharmacology 66 (5), 749-756. DOI: 10.1016/S0006-2952(03)00386-1

Yahia D., El-Nasser M.A., Abedel-Latif M., Tsukuba C., Yoshida M., Sato I., and Tsuda S. (2010): Effects of perfluorooctanoic acid (PFOA) exposure to pregnant mice on reproduction. J Toxicol Sci 35 (4), 527-533. DOI: 10.2131/jts.35.527

Yang Q., Abedi-Valugerdi M., Xie Y., Zhao X.Y., Moller G., Nelson B.D., and DePierre J.W. (2002a): Potent suppression of the adaptive immune response in mice upon dietary exposure to the potent peroxisome proliferator, perfluorooctanoic acid. International Immunopharmacology 2 (2-3), 389-397. DOI: Doi 10.1016/S1567-5769(01)00164-3

Yang Q., Xie Y., Alexson S.E., Nelson B.D., and DePierre J.W. (2002b): Involvement of the peroxisome proliferator-activated receptor alpha in the immunomodulation caused by peroxisome proliferators in mice. Biochemical Pharmacology 63 (10), 1893-1900. DOI: 10.1016/s0006-2952(02)00923-1

Yang Q., Xie Y., and Depierre J.W. (2000): Effects of peroxisome proliferators on the thymus and spleen of mice. Clin Exp Immunol 122 (2), 219-226. DOI: 10.1046/j.1365-2249.2000.01367.x

Yang Q., Xie Y., Eriksson A.M., Nelson B.D., and DePierre J.W. (2001): Further evidence for the involvement of inhibition of cell proliferation and development in thymic and splenic atrophy induced by the peroxisome proliferator perfluoroctanoic acid in mice. Biochemical Pharmacology 62 (8), 1133-1140. DOI: Doi 10.1016/S0006-2952(01)00752-3

Zhang H., Ding L., Fang X., Shi Z., Zhang Y., Chen H., Yan X., and Dai J. (2011): Biological responses to perfluorododecanoic acid exposure in rat kidneys as determined by integrated proteomic and metabonomic studies. PLoS One 6 (6), e20862. DOI: 10.1371/journal.pone.0020862

Zhang H., Hou J., Cui R., Guo X., Shi Z., Yang F., and Dai J. (2013): Phosphoproteome analysis reveals an important role for glycogen synthase kinase-3 in perfluorododecanoic acid-induced rat liver toxicity. Toxicol Lett 218 (1), 61-69. DOI: 10.1016/j.toxlet.2013.01.012

Zhang H., Shi Z., Liu Y., Wei Y., and Dai J. (2008): Lipid homeostasis and oxidative stress in the liver of male rats exposed to perfluorododecanoic acid. Toxicol Appl Pharmacol 227 (1), 16-25. DOI: 10.1016/j.taap.2007.09.026

Zheng F., Sheng N., Zhang H.X., Yan S.M., Zhang J.H., and Wang J.S. (2017): Perfluorooctanoic acid exposure disturbs glucose metabolism in mouse liver. Toxicology and Applied Pharmacology 335, 41-48. DOI: 10.1016/j.taap.2017.09.019

6. Annex 1: Toxicokinetic studies

Work in progress and to be added later.

Table 1: Toxicokinetic studies

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentrate-ion of PFAS /metabolites (time of sampling)	Reference
"**PFCAs** In the past decades, a limited set of data was published on the toxicokinetics of PFCAs other than PFOA, such as PFBA, PFHxA, PFHpA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA and PFTeDA. The most extensive studies have been carried out in rodents. Oral exposure, mainly by gavage, of experimental animals to PFCAs having a perfluorinated carbon chain length of 3–11 was shown to result in an estimated absorption fraction greater than 95% of the administered dose (ATSDR, 2018). None of the experimental studies observed the formation of metabolites, suggesting, as previously reported for PFOA (EFSA CONTAM Panel, 2018), that the biotransformation of PFCAs is unlikely in mammals, irrespective of their chain length. Although distribution of PFCAs shows species and sex differences, which are attributed, at least in part, to differences in elimination kinetics, blood, liver and kidney are the tissues exhibiting the highest concentrations of absorbed PFCAs. In blood, PFCAs were found to bind to serum albumin, the affinity generally increasing with PFCAs hydrophobicity, but decreasing for perfluorinated carbon chain length beyond eight carbons. The primary route of elimination of PFCAs having a carbon chain length below 10 is via urine, whereas for PFDA, PFUnDA, PFDoDA, PFTrDA and PFTeDA, faecal excretion is predominant. In rodents, half-lives may vary from few hours (PFBA, PFHxA) to more than 1 month (PFNA, PFDA). Elimination of PFCAs exhibits pronounced sex differences in rats, with faster elimination in females than in males. It was shown that transport proteins such as serum albumin, liver fatty acid-binding proteins (L-FABP) and organic anion transporters play a key role in PFCA excretion and/or reabsorption (Appendix C)."						Summary from EFSA Opinion (2020)

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Potencies of different chain lengths might be due to longer time in the body or differences in take up of the body. This needs to be checked once the toxicokinetic analysis (for different chain length, but also differences across species or sexes) is finished.

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7. Annex 2: Repeated dose toxicity studies

Table 2: Repeated dose toxicity (RDT) animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
Perfluoroalkyl carboxylic acids (PFCAs)							
TFAA: Trifluoroacetic acid (Syn: Perfluoroethanoic acid, **CAS no:** 76-05-1, **EC no:** 200-929-3, **Mol. formula:** $C_2HF_3O_2$, **MW:** 114.023; full and intermediate registration under REACH: 1000-10,000 t/y, harmonised classification: Skin Corr. 1A, Acute Tox. 4 *, Aquatic Chronic 3)							
TFAA (99%)	92-day RDT study (OECD TG 408) Rat (Wistar) n/sex/group = 10 (m+f) Exposure: diet Doses: 0, 160, 1600, 16000 ppm (0, 9.9, 98, 1043 mg/kg bw/d for males; 0, 12.2, 123, 1216 mg/kg bw/d for females)	**Body weight** ↓ (≈ –17% in HD males) **Liver:** • Abs. + rel. liver weight ↑ (≈ up to 33% in HD) • Hepatocellular hypertrophy ↑ (in up to 100% of HD rats) **Haematopoietic system:** • Haemoglobin ↓ (females only, ≈ –8% in HD) • Mean corpuscular volume ↓ (females only, ≈ –6% in HD) **Clinical chemistry:** • Billirubin ↓ (≈ up to –81% in HD) • Glucose ↓ (≈ up to –29% in HD) • ALT, ALP ↑ (males only, (≈ up to + 95% and 38% in HD) **Other effects:** • Urinary ketones ↑ (in up to 100% of male rats)	98/1216 9.9/12.2 9.9/123 1043/12.2 1043/123 9.9/12.2 9.9/12.2 98/1216 9.9/123	1043/– 98/123 98/1216 –/123 –/1216 98/123 98/123 1043/– 98/1216	•		(Anonymous, 2016; BayerCropScience, 2014)
PFPA: Pentafluoropropionic acid (Syn: Perfluoroproprionic acid, **CAS no:** 422-64-0, **EC no:** 207-021-6, **Mol. formula:** $C_3HF_5O_2$, **MW:** 164.03; pre-registration process under REACH, self classification available**)**							
PFPA					•		
PFBA: Perfluorobutanoic acid (Syn: Heptafluorobutyric acid, **CAS no:** 375-22-4, **EC no:** 206-786-3, **Mol. formula:** $C_4HF_7O_2$, **MW:** 214,xx?; pre-registration process under REACH, self classification available**)**							
PFBA, perfluorobutyrate (purity not specified), $C_3F_7CO_2$	14-day RDT study, non-guideline study to assess liver toxicity Rat (SD) n/sex/group = 3 (m) Exposure: oral (diet, 0.02%) Doses: 0, 20 mg/kg bw/d	**Liver:** • Catalase activity ↑ (marker enzyme of peroxisomes, ≈ +42%) • Induction of 80K-protein (associated with peroxisome proliferation)	– –	20 20	• Body weight, kidney, nervous system, immune system, haematopoietic system, endocrine system, clinical chemistry		(Ikeda et al., 1985)
PFBA, perfluorobutyrate (98%), $C_3F_7CO_2$	10-day RDT study, non-guideline study to assess liver toxicity Mouse (C57BL/6N) n/sex/group = 4 (m)	**Body weight** ↑ (≈ +16%, but it was already significantly higher before treatment) **Body weight gain** ↑ (≈ +47% (+4.35% in Control, ≈ +6.38% in PFBA treatment, but no statistical analysis possible)) **Liver** • Abs.+rel. liver weight ↑ (≈ +63% (abs.) or +38% (rel.)) • Hepatic mitochondrial protein content ↑ (≈ +202%)			• Kidney, nervous system, immune system, haematopoietic system, endocrine system, clinical		(Permadi et al., 1992; Permadi et al., 1993)

Commented [A21]: We thought it was agreed to have one table for all studies, not to divide between repeat tox and developmental tox. Some studies are extended and cover both. We need to agree

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Commented [A23]: Too much information for this table for each substance

Table 2: Repeated dose toxicity (RDT) animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
	Exposure: oral (diet, 0.02%) Doses: 0, 24 (ATSDR: 78) mg/kg bw/d	• Hepatic microsomal protein content ↑ (≈ +79%) • Hepatic peroxisomal catalase specific/total activity ↓↑ (≈ –37%/+90%) • Hepatic lauroyl-CoA oxidase specific/total activity ↑ (≈ +112%/+543%) • Hepatic palmitoyl-CoA oxidation specific/total activity (↑) (≈ +112%/+539% (but ns)) • Microsomal cytochrome P450 reductase ↑ (≈ +101%) • Hepatic cytosolic SOD ↑ (≈ +74%) • Hepatic cytosolic epoxide hydrolase ↑ (≈ +112%) **Remark:** Perfluoroacetic acid was also tested and did not reveal any effects ("PFAA was inactive").			chemistry		
PFBA (ammonium salt, >98%)	18-day-ReproTox study (GD1-GD17) Mouse (CD-1) n/sex/group = 7-13 pregnant, 2-6 nonpregnant, 30-34 postweaning Exposure: oral (gavage) Doses: 0, 35, 175, 350 mg/kg bw/d	**Body weight (ns)** **Liver** • Abs. liver weight (pregnant/nonpregnant/postweaning) ↑ (≈ +24%/+33%/ns at LOEL) • Rel. liver weight (pregnant/nonpregnant/postweaning) ↑ (≈ +31%/+40%/ns at LOEL) **Remark:** Doses were calculated to match PFOA exposure of 1, 5, and 10 mg/kg (Lau et al. 2005). Elimination half-life of PFBA is about 3h (Chang et al.), PFOA 15-20 days (Lau et al. 2005)	35 35	175 175	•	Serum concentrations of PFBA in pregnant dams (µg/ml): Control: 0.002 ± 0.001 35 mg/kg: 3.78 ± 1.01 175 mg/kg: 4.44 ± 0.65 350 mg/kg: 2.49 ± 0.60 Serum concentrations of PFBA in nonpregnant females (µg/ml): Control: 0.006 ± 0.003 35 mg/kg: 1.96 ± 1.0 175 mg/kg: 2.41 ± 1.65 350 mg/kg: 2.67 ± 1.2 Liver concentrations of PFBA in pregnant dams (µg/g): Control: 0.003 ± 0.002 35 mg/kg: 1.41 ± 0.42 175 mg/kg: 1.60 ± 0.25 350 mg/kg: 0.96 ± 0.18 Liver concentrations of PFBA in nonpregnant females (µg/g): Control: 0.038 ± 0.017 35 mg/kg: 0.51 ± 0.20 175 mg/kg: 0.86 ± 0.55 350 mg/kg: 0.89 ± 0.38	(Das et al., 2008)

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Table 2: Repeated dose toxicity (RDT) animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
						(Data also available for postweaning stages PD1 and PD10)	
PFBA, perfluorobutyrate (purity not specified), $C_3F_7CO_2$	28-day RDT study, non-guideline study to address liver toxicity and its MoA Mouse (SV/129 wild-type, PPAR-α null, humanized PPAR- α transgenic mouse model) n/sex/group = 10 (m) Exposure: oral (gavage), vehicle: water Doses: 0, 35, 175, 350 mg/kg bw/d	**Liver:** • Rel. liver weight ↑ (≈ +50% in LD and ≈ +100% in HD of wild type mice) • Hepatocellular focal necrosis with inflammatory cell infiltrate ↑ (no statistical significance indicated but dose-dependent increase of incidences of mild cases) • Hepatocyte hypertrophy ↑ (no statistical significance indicated but 100% incidence in all dose groups and 0% in C) • Hepatic replicative DNA synthesis ↑ (only in LD) • mRNA of Cyp4A10/Aco ↑ **Remarks:** PFBA causes hepatomegaly and hepatocyte hypertrophy mediated via peroxisome proliferator-activated receptor-α (PPARα)	– 35 – – –	35 175 35 35 35	• Kidney, liver, nervous system, immune system, haematopoietic system, endocrine system, clinical chemistry	At 35 mg/kg bw per day (24h after final dosing) Serum: 80 µg/mL Liver: 27 µg/g	(Foreman et al., 2009)
PFBA, ammonium perfluorobutyrate (purity not specified), $NH_4C_3F_7CO_2$	28-day RDT study, similar to OECD TG 407 Rat (SD) n/sex/group = 10 (m+f) Exposure: oral (gavage) Doses: 0, 6, 30, 150 mg/kg bw/d	**Liver:** • Abs. liver weight (m) ↑ (≈ +27% in MD, no effect after recovery) • Hepatocyte hypertrophy ↑ (no significance indicated but 60% incidence in HD and 0% in C and other dose groups) • Hepatic Acox, Cyp4A1, Cyp2B2, Malic (all m only) ↑ **Thyroid:** • Follicular hypertrophy/hyperplasia ↑ (no significance indicated but 90% incidence in MD and 30% in C) **Endocrine system:** • Serum total T4 (m) ↓ (≈ −59% in LD) • Serum free T4 (m) ↓ (≈ −46% in LD) **Clinical chemistry:** • Serum cholesterol (m) ↓ (≈ −20% in MD) **Remarks:** During recovery, liver weight, histological, and cholesterol effects were resolved; mRNA transcript data for Acox and Cyp4A1 indicate activation of PPARα	6 30 6 6 – – 6	30 150 30 30 6 6 30	• Immune system	At 6 mg/kg bw/day in males Serum: 24.7 ± 17.6 µg/mL Liver: 7.5 ± 4.5 µg/g At 30 mg/kg bw/day in males Serum: 38.04 ± 23.2 µg/mL Liver: 17.4 ± 8.2 µg/g	(van Otterdijk, 2007a) (Butenhoff et al., 2012a)
PFBA (purity not specified), $NH_4C_3F_7CO_2$	90-day RDT study, similar to OECD TG 408 Rat (SD) n/sex/group = 10 (m+f)	**Liver:** • Abs. liver weight (m) ↑ (≈ +23% in HD) • Hepatocyte hypertrophy ↑ (no significance indicated but 90% incidence in HD and 0% in C and other dose groups) • Hepatic Acox, Cyp4A1, Cyp2B2, Malic (all m only) ↑ **Thyroid:** • Thyroid: Follicular hypertrophy/hyperplasia ↑ (no significance	6 6 6	30 30 30	• Immune system	At 6 mg/kg bw per day in males Serum: 13.6 ± 9.1 µg/mL Liver: 3.1 ± 2 µg/g At 30 mg/kg bw per day in males	(van Otterdijk, 2007b) (Butenhoff et al.,

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Table 2: Repeated dose toxicity (RDT) animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
	Exposure: oral (gavage) Doses: 0, 1.2, 6, 30 mg/kg bw/d	indicated but 90% incidence in HD and 40% in C) **Endocrine system:** • Serum total T4 (m) ↓ (≈ −39% in LD) **Remarks:** During recovery, liver weight, histological, and cholesterol effects were resolved; mRNA transcript data for Acox and Cyp4A1 indicate activation of PPARα	6 6	30 30		Serum: 52.2 ± 25 µg/mL Liver: 16.1 ± 9.1 µg/g	2012a)
PFBA	5-day RDT study Rat Dose: up to 184 mg/kg bw/d	**No effect on gross or microscopic morphology of brain or spinal cord** **Tbc**			•		3M 2007a
PFPeA: Perfluoropentanoic acid (Syn: Perfluorovaleric acid, **CAS no:** 2706-90-3, **EC no:** 220-300-7, **Mol. formula:** $C_5HF_9O_2$, **MW:** 264.05; pre-registration process under REACH, self classification available)							
					•		
PFHxA: Perfluorohexanoic acid (Syn: Undecafluorohexanoic acid, **CAS no:** 307-24-4, **EC no:** 206-196-6, **Mol. formula:** $C_6HF_{11}O_2$, **MW:** 264,xx?; under PBT assessment under REACH, self classification available)							
PFHxA (purity not specified)	5-day RDT study, non-guideline study Mouse (ddY) n/sex/group = 3-5 (m+f) Exposure: i.p. Doses: 0, 50, 100, 150 mg/kg bw/d	**Body weight (m/f) (ns)** **Liver:** • Rel. liver weight ↑ (m: ns; f, ≈ +17% at LOEL) • Peroxisomal β-oxidation ↑	 50 –	 100 50	• Kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Kudo et al., 2006)
PFHxA (>99%)	28-day RDT study, similar to OECD TG 407 Rat (SD) n/sex/group = 10 (m+f) Exposure: oral (gavage; in 2% Tween 80/ deionized water) Doses: 0, 62.5, 125, 250, 500, 1000 mg/kg bw/d	**Body weight** ↓ (m, ≈ −16% at LOEL; f ns) **Liver:** • Abs. liver weight ↑ (m/f, ≈ +27%/+14% at LOEL) • Rel. liver weight ↑ (m/f, ≈ +14%/+15% at LOEL) • Hepatocellular hypertrophy ↑ (m, 9 of 10 at 500 mg/kg bw/d) • Acyl-CoA oxidase ↑ (≈ +141% in males at 250 mg/kg bw/d) **Kidney:** • Rel. kidney weight ↑ (m/f, ≈ +12%/+12% at LOEL) **Immune system** • Abs. thymus weight ↓ (m, ≈ –27% at LOEL. Rel ns, f not measured) • Spleen: increased extramedullidary hematopoiesis **Thyroid weight** • Thyroid weight changes at all doses (m+f), but ns and not clearly dose related	500 250/250 125/250 250/500 250 250/500 500 250/500 –	1000 500/500 250/[redacted] [redacted] [redacted] [redacted] 1000 500/1000 62.5	• no histopathological description for Thyroid gland	Plasma conc. (ng/ml) at 62.6 mg/kg bw/d at end of study: 378 ± 178 (m) 129 ± 16 (f) Plasma conc. (ng/ml) at 250 mg/kg bw/d at end of study: 1297 ± 265 (m) Liver conc (ng/g) at 250 mg/kg bw/d at end of study: 655 ± 148 (m)	(NTP, 2019b) Exp.no. C20613

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Table 2: Repeated dose toxicity (RDT) animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
	twice daily at one-half dose (total): 31.3 (62.6), 62.5 (125), 125 (250), 250 (500), 500 (1000) mg/kg bw/d	**Haematopoietic system:** • Erythrocytes (≈ −5%/–7% at LOEL), haemoglobin (≈ −3%/–6% at LOEL), haematocrit (≈ −4%/–7% at LOEL) (m/f) ↓ **Endocrine system:** • T3 (≈ −18% in LD), fT4 (≈ −25% in LD), tT4 (≈ −20% in LD) (m) ↓ (f ns) **Clinical chemistry:** • ALT, ALP (m), AST (m+f) ↑ • Serum cholesterol ↓ (m, ≈ −20% at LOEL) **Other effects:** • Degeneration and hyperplasia of olfactory epithelium (m+f) **Reproductive tissues** (doses tested: 0, 250, 500, 1000 mg/kg bw/d) • Cauda epididymal sperm counts ↓ (m, ≈ −25%) • Epididymal weight ↓ (m, ≈ −5% at 1000 mg/kg bw/d) • Epididymal histopathology: Exfoliated germ cells (1/10 at LOEL) • Testis weight: in treated animals similar to controls • Spermatid counts: in treated animals to controls • Seminiferous tubule spermatid retention of the testis in 2/10 rats at 1000 mg/kg bw/d and 1/10 control rat (m) • Estrus cyclicity (f, ns)	−/125 250/250 – 125/125 500 500 500	62.5/250 500/500 62.5 [redacted] [redacted] 1000 1000			
PFHxA, Perfluorohexanoic acid (98.5%), CAS: 307-24-4/EC: 206-196-6, $C_6HF_{11}O_2$	28-day RDT study with reproduction/ developmental toxicity screening test, OECD TG 422 Rat (SD) n/sex/group = 10-15 (m) Exposure: oral (gavage), vehicle: deionized water Doses: 0, 50, 150, 300-450 mg/kg bw/d (reduced on day 4 from 450 to 300 mg/kg bw/d due to lethality in 4/15 males and 4/15 females within the first 4 days of dosing)	***Slash (/) indicates here the changed max. dose in HD** **Mortality** ↑ (death of 5/15 m and 6/15 f in HD) **Body weight** ↓ **Liver:** • Abs. + rel. liver weight (m/f) ↑ • Hepatocellular hypertrophy ↑ (minimal (MD) to mild (HD) **Kidney:** • Papillary necrosis↑ **Immune system:** • Lymphoid necrosis/depletion of T and B areas of lymph nodes, spleen, thymus ↑ • Abs. thymus weight (m) ↓ (considered spurious by authors) **Haematopoietic system:** • Mean corpuscular haemoglobin (m) ↓ • Haemoglobin (m)↓ • Globulin (m)↓ • No changes in total or differential leucocyte counts **Clinical chemistry:** • Serum cholesterol ↓ (m; f ns) • Triglycerides (NA)	150 50 50 50 150 150 150 150 150 150 150 150	300-450 150/300 150/300-450 150 300-450 300-450 300-450 300-450 300-450 300-450 300-450		Blood samples taken 1, 2, 4, 8, 24 h on study day 0 and 25 from three rats per group, Urine samples collected from all animals 0-6, 6-12, 12-24 h following last dosing Half-life in serum and for urinary elimination about 2-3 h Exposure in male rats 2-4-fold higher than in female rats (e.g., AUC (ng×h/mL): Day 25, HD males: 1637875; Days 25 HD females: 887031)	(Kirkpatrick, 2005) Proj. ID: WIL-534001) (WIL, 2005)

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Table 2: Repeated dose toxicity (RDT) animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentrate-ion of PFAS /metabolites (time of sampling)	Reference
		Thyroid • No changes in thyroid weights **Endocrine system:** • Hyperplasia of adrenal cortex ↑ **Other effects:** • Litter size ↓ • Pup number ↓ • Implantation size (f) ↓ • Food consumption (f) ↓ during gestation and lactation • Hemorragic ring around iris ↑	 150 150 150 150 150 -	 300-450 300 300 300 300 50			
PFHxA (98.5%)	90-day RDT study, similar to OECD TG 408 Rat (SD) n/sex/group = 10 (m+f) Exposure: oral (gavage) Doses: 0, 10, 50, 200 mg/kg bw/d	**Body weight gain** (m) ↓ (≈ −7% in LD&HD, –10% in MD at day 90) **Body weight gain** (f) (≈ −5% in LD&HD at day 90, but ns) **Liver:** • Rel. liver weight (m) ↑ (≈ +22% in HD) (f ns) • Centrilobular hepatocellular hypertrophy (m) • Hepatic peroxisomal β-oxidation (m) ↑ **Kidney:** • Rel. kidney weight (m) ↑ (≈ +8% in LD) **Immune system** • White blood cell count (ns) • Thymus and spleen weight (m+f) (ns) **Haematopoietic system:** • Erythrocytes, haemoglobin ↓ (≈ −8% in HD) • Reticulocyte ↑ (≈ +59% in HD) • Globulin (m)↓ (≈ −8% in HD) **Clinical chemistry:** • Serum cholesterol (m) ↓ (≈ −26% in MD) (f ns • Triglycerides not measured • Calcium (m) ↓ (≈ −3% in MD) • Serum ALT (≈ +237% in HD) and ALP (m) ↑ (≈ +109% in HD) **Remark:** "Based on liver histopathology and liver weight changes, the no-observed-adverse-effect level (NOAEL) for oral administration was 50 mg/kg bw/day for males and 200 mg/kg bw/day for females." (200 mg/kg bw/d is an unbounded NOAEL,i.e. the NOAEL is the highest measured dose and the "real" NOAEL could be even higher)	10 50 50 50 – 50 50 50 10 10 50	50 200 200 200 10 200 200 200 50 50 200			(Chengelis et al., 2009)
PFHxA (sodium salt, 100%)	90-day RDT study, OECD TG 408, plus 30 day recovery group Rat (Crl:CD(SD))	**Body weight** (m) ↓ (≈–10% at day 90 at LOEL) **Liver:** • Abs./rel. liver weight ↑ (≈ +63% in HD) • Hepatocellular hypertrophy ↑ (no significance indicated but for males 40% incidence in MD, 100% in HD and 0% in C and LD)	100 100 20/100	500 500 100/500	•		(Loveless et al., 2009)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
	n/sex/group = 10 (m+f) Exposure: oral (gavage) Doses: 0, 20, 100, 500 mg/kg bw/d	• Hepatic peroxisomal β-oxidation (m/f) ↑ (≈ +85% in MD, ≈ +330% in HD) **Kidney:** • Rel. kidney weight ↑ (≈ +16% in HD) • Urine volume ↑ (≈ +207% in HD) **Immune system** • Abs. thymus weight (m) ↓ (≈ –31% at LOEL, rel. weight ns) • Abs. spleen weight (m) ↓ (≈ –16% at LOEL rel. weight ns) **Haematopoietic system:** • Erythrocytes (≈ –31% in HD) , haemoglobin (≈ –36% in HD) ↓ • Reticulocyte ↑ (≈ +210% in HD) **Thyroid:** • Thyroid hypertrophy ↑ (no significance indicated but for females 40% incidence in HD, 0% in other dose groups) • Thyroid weight ↑ (only in f after 30 day recovery) **Clinical chemistry:** • Serum ALT (m) ↑ (≈ +133% in LD) • Cholesterol (m) ↓ (≈ –35% at 100 mg/kg bw/d, at HD only significant after 3 months of treatment, effect not considered adverse) • Triglycerides (ns) **Other effects:** • Degeneration/atrophy in nasal cavity (no significance indicated but for males 40% incidence in MD, 70% in HD and 0% in C and LD) **Remark:** "subchronic toxicity no observed adverse effect level (NOAEL)was 20 mg/(kg day), based on nasal lesions observed at 100 and 500 mg/(kg day)"	20/100 100 100 100 100 100 100 100 100 – 20	100/500 500 500 500 500 500 500 500 500 20 100			
PFHxA sodium salt (100%)	110-day RDT study, as part of a one-generation reproduction study, OECD TG 415 Rat (Crl:CD(SD)) n/sex/group = 10 (m+f) Exposure: oral (gavage) Doses: 0, 20, 100, 500 mg/kg bw/d	**Body weight** (m) ↓ (≈ –12% at LOEL) **Body weight** (f, GD0-7)) ↓ (≈ –31% at LOEL)	20 100	100 500	• Liver, kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Loveless et al., 2009)

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Table 2: Repeated dose toxicity (RDT) animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
PFHxA ammonium salt (93.4%)	Combined Developmental and perinatal/postnatal reproduction toxicity study ICH Harmonised Tripartite Guideline - S5(R2), stage C-F Mouse Crl:CD1(ICR), 69-76 d.o. at mating F0: 20 F/dose GDs 6-18(Males only used for breeding, no treatment) F1: 20/sex/dose (no direct treatment; observed until weaning to sexual maturity) Exposure: oral (gavage; in deionized water) Phase I: Doses: 0, 100, 350, 500 mg/kg bw/d Phase II Doses: 0, 7, 35, 175 mg/kg bw/d	**Phase I** **Body weight (ns)** **Liver:** • Abs/rel liver weight (ns) **Phase II** **Body weight (slight reduction at mid and high dose on GD 18, lowest dose slightly higher than control)** **Liver:** • Abs/rel liver weight (ns) **Remark:** Iwai and Hoberman 2014 abstract: "Based on these data, the maternal and reproductive no observable adverse effect level of PFHxA Ammonium Salt is 100 mg/kg bw/d."			• Postnatal functional parameters are not evaluated in this type of study		(Iwai and Hoberman, 2014; Iwai et al., 2019) Study reports: Hoberman, 2011a (Phase I) Hoberman, 2011b (Phase II)
PFHxA (98.1%)	104-weeks Carcinogenic study Combined chronic tox/carc study Rat Crl:CD(SD) n/sex/group = 60-70 Male Dosage: 0, 2.5, 15, 100 mg/kg bw/d Female Dosage: 0, 5, 30, 200 mg/kg bw/d	**Mortality** ↑ (f only: 22% survival at 200 mg/kg bw/d vs 36% survival in control, i.e. +100% mortality at 200 mg/kg bw/d, but no statistical testing) **Body weight (no effect)** **Liver:** • Hepatocellular necrosis (weak increase at highest dose in m+f) **Kidney:** • Necrosis ↑ (f only, minimal to severe papillary necrosis in 17 of 70 test animals and/or minimal to moderate renal tubular degeneration in 7 of 70 test animals) • Urinary parameters: mean urine volume ↑ (f); slightly lower specific gravity at 200 mg/kg bw/d ↓ (f, 26. Week) **Haematopoietic system:** • White blood cell count (ns) • Red blood cell count ↓ (–8% after 51 weeks only in females) • Heamoglobin ↓ (–5% after 51 weeks only in females) **Clinical chemistry:**	30 30 30 30	200 200 200 200			(Klaunig et al., 2015)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentrate-ion of PFAS /metabolites (time of sampling)	Reference
		• Serum triglycerides ↓ (m: –43% at2.5 and 100, but not at 15 mg/kg bw/d after 52 weeks, authors conclude no effect) • Serum LDL (f: –44% at LOEL after 26 weeks, but not after 52 weeks) • Serum HDL (ns) **Remark:** not tumorgenic (f+m) all dosages, authors in abstract: "heamatology and serum chemistry unaffected", no statistically sign. Alterations in hormones LH, Testosterone, Estradiol (Jung et al. 2016 stated: there were no histological changes in ED-related tissues)	– 30	2.5 200			
PFHpA: Perfluoroheptanoic acid (Syn: Tridecafluoroheptanoic Acid, **CAS no:** 375-85-9, **EC no:** 206-798-9, **Mol. formula:** $C_7HF_{13}O_2$, **MW:** 364,xx?; pre-registration process under REACH, self classification available)							
PFHpA, Perfluoroheptanoic acid (purity not specified), CAS: 375-85-9 / EC: 206-798-9, $C_7HF_{13}O_2$	5-day RDT study, non-guideline study, to assess hepatic peroxisomal β-oxidation through PFCAs Rat (Wistar) n/sex/group = 4 (m+f) Exposure: i.p. Doses: 0, 30, 160 mg/kg bw/d	**Liver:** • hepatic peroxisomal β-oxidation ↑ (m/f) **Remarks:** potency of the induction of peroxisomal β-oxidation was compared between PFCAs; highly significant correlation between the induction and hepatic concentrations of PFCAs in the liver regardless of their carbon chain lengths	30/160	160/–	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system, clinical chemistry	concentrations of PFHA in the liver were below detection limit <3 g:g liver	(Kudo et al., 2000)
PFHpA (purity not specified)	5-day RDT study, non-guideline study, Mouse (ddY) n/sex/group = 3-5 Exposure: i.p. Doses: 0, 20, 50, 100 mg/kg bw/d	**Liver:** • Abs. and rel. liver weight ↑ (m/f, ≈ +68% in MD) • Peroxisomal β-oxidation ↑ • Activity of hepatic microsomal 1-Acyl-GPC acyltransferase (m, only HD tested) ↑ **Remarks:** the longer the perfluoroalkyl chain, the more PFCA accumulates in the liver; accumulated PFCAs induce hepatomegaly, peroxisomal β-oxidation and microsomal 1-acyl-GPC acyltransferase	20/50 –/20 –	50/100 20/50 100	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system, clinical chemistry		(Kudo et al., 2006)
PFHpA (purity not specified)	3-day RDT study, non-guideline study Mouse (C57BL/6) n/sex/group: 4 Exposure: i.p.	**Liver:** • Hepatic mRNA levels of Cyp4a10 ↑ **Remarks:** Mechanistic study to assess the MoA of PFCAs	–	20	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system, clinical chemistry		(Abe et al., 2017)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
	Doses: 0 , 20 mg/kg bw/d						
PFHpA as Sodium perfluoroheptanoate (>99.3%)	combined 90-day RDT study with reproduction/developmental toxicity screening (similar to OECD TG 408 and 422) Mouse (CD1) exposure time F0, m: 90d prior to mating (total 109-113 d); F0, f: 90d prior to pairing and until lactation (total exposure time: 130-142 d) exposure time F1: during PND 22 to 42 (total of 21 days) n/sex/group: in F0 20 (except 25 f for controls and highest dose and 15 for clinical pathology phase); in F1 n=16-20 Exposure: oral (gavage), vehicle: deionised water Doses: 0, 0.5, 10, 50 mg/kg bw/d	**Liver:** • Centrilobular hypertrophy of hepatocytes ↑ • Abs. and rel. liver weight ↑ **Clinical chemistry:** • ALT levels in lactating females (D21) ↑ • ALP, ALT and Trig. in m and in ALP and Trig. in non-mated f ↑ **Endocrine system:** • Thyroid T4 levels in serum ↓ (m only) **Developmental effects in F1:** • Centrilobular hypertrophy of hepatocytes ↑ • Hepatocellular necrosis (single cell to coalescing) ↑ • Abs. and rel. liver weight ↑ • T4 serum levels in f ↑ • Cleft palate in 6 pups from 1 litter at LD • Cleft palates in 3 pups from 2 litters at HD • % of males per litter ↓ in LD • Postnatal survival ↓ in HD • Mean body weight ↓ in HD • Vaginal patency ↑ in HD • Adrenal rel. and abs. weights in f ↑ in HD	–/– 0.5/0.5 0.5 10/10 0.5 –/– 0.5/0.5 0.5/10 –	0.5/0.5 10/10 10 50/50 10 0.5/0.5 10/10 10/50 0.5	•		(Anonymous, 2017)
PFOA: Perfluorooctanoic acid (Syn: Pentadecafluorooctanoic acid, **CAS no:** 335-67-1, **EC no:** 206-397-9, **Mol. formula:** $C_8HF_{15}O_2$, **MW:** 414,xx?; registered under REACH, self classification available)							
PFOA, Perfluorooctanoic acid (analytical grade), EC: 206-397-9, CAS: 335-67-1, $C_8HF_{15}O_2$	5-day RDT study, non-guideline study Rat (Wistar) n/sex/group = 4 Exposure: i.p. injection (vehicle: propyleneglycol:water (1:1, v:v) Doses: 0, 2.5, 5, 10, 15, 20 mg/kg bw/d PFCAs assessed: PFHpA, PFOA, PFNA,	**Liver:** • Peroxisomal β-oxidation ↑ (m/f) **Remarks:** Mechanistic study to assess the MoA and to investigate influence of chain length; peroxisomal β-oxidation is statistically highly correlated with PFCA concentration in the liver (r=0.850, p<0.001) à internal dose in liver decisive for effect, not carbon chain length or sex	5/20	10/–	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system, clinical chemistry		(Kudo et al., 2000)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
	PFDA						
PFOA (purity not specified)	5-day RDT study, non-guideline study Mouse (ddY) n/sex/group = 3-5 Exposure: i.p. Doses: 0, 2.5, 5, 10, 20 mg/kg bw/d	**Liver:** • Abs. and rel. liver weight (m/f) ↑ (≈ +39% in LD) • Peroxisomal β-oxidation ↑ • Activity of hepatic microsomal 1-Acly-GPC acyltransferase (m, only 20 mg/kg bw/d tested) ↑ **Remarks:** "(…) the longer the perfluoroalkyl chain, the more PFCA accumulates in the liver (… and) accumulated PFCAs induce hepatomegaly, peroxisomal β-oxidation and microsomal 1-acyl-GPC acyltransferase (…)"(from abstract; refers to C6-C9)	–/– –/– –	2.5/2.5 2.5/2.5 20	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system, clinical chemistry	Chain length (C6-C9 investigated) and sex dependent accumulation of PFCAs in the liver (higher concentrations in males and with higher C length)	(Kudo et al., 2006)
PFOA (purity not specified)	3-day RDT study, non-guideline study Mouse (C57BL/6) n/sex/group = 4 Exposure: i.p. Doses: 0 , 20 mg/kg bw/d	**Liver:** • Rel. liver weight ↑ (≈ +100%) • Hepatic mRNA levels of Cyp4a10, Acox1, Cyp2b10, Aldh1 ↑ **Remarks:** Mechanistic study to assess the MoA of PFCAs	– –	20 20	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system, clinical chemistry		(Abe et al., 2017)
PFOA (>96%)	7-days RDT study, non-guideline study Mouse (Balb/c) n/sex/group = not reported Exposure: oral (vi distilled water) Doses: 0, 1, 5 mg/kg bw/d	**Body weight** ↓ **Liver:** • Abs. liver weight ↑ (≈ +60% at LOAEL) • Necrosis and vacuolation of hepatocytes ↑ • Hepatic triglycerides ↑ • mRNA levels of fatty acid translocase and lipoprotein lipase ↑ **Clinical chemistry:** • ALT ↑ (≈ +80% at LOAEL) • Serum fatty acids ↓ (≈ -62% at LOAEL) • Serum triglycerides ↓ (≈ -58% at LOAEL) **Remarks:** Mechanistic study to assess the MoA of PFCAs	– 1 – – – – 1 –	1 5 1 1 1 1 5 1	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Hui et al., 2017)

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Table 2: Repeated dose toxicity (RDT) animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
PFOA (purity not specified)	14-days RDT study, non-guideline study Rat (SD) n/sex/group = 7 (m only) Exposure: oral (gavage) Doses: 0, 1, 5, 25 mg/kg bw/d	**Body weight** ↓ **Liver:** • Abs. + rel. liver weight ↑ • Activity of hepatic superoxide dismutase and glutathione peroxidase ↑ • MDA content in liver ↑ • Expression of PPARα mRNA ↑ • Expression of CYP4A1 mRNA ↑ • PPARα protein ↑ **Remark:** CYP4A1 is a PPARα-related gene	5 1 – 1 – – –	25 5 1 5 1 1 1	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Wang et al., 2017) Article in Chinese, only abstract in English
PFOA (96%)	21-days RDT study, non-guideline study Mouse (Balb/c) n/sex/group = 3-10 (m only) Exposure: oral (gavage) Doses: 0, 1.25 mg/kg bw/d	**Liver:** • Rel. liver weight ↑ (≈ +120%) • Glycogen (≈ −80%) and glucose (≈ −33%) content in the liver ↓ • Pyruvate ↑ (≈ +80%) **Clinical chemistry:** • Fasting blood glucose levels ↑ (≈ +50%) • Blood glucagon ↑ (≈ +50%) **Remarks:** Mechanistic study to assess the MoA: indicates that subacute exposure to PFOA might enhance glycogenolysis and gluconeogenesis and promote carbohydrate consumption	 – – – – –	 1.25 1.25 1.25 1.25 1.25	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system	PFOA level in liver: 125.9 ± 10.0 µg/g PFOA level in serum: 55.5 ± 0.50 µg/mL	(Zheng et al., 2017)
PFOA (ammonium salt, <98%)	21-days RDT study, non-guideline study Mouse (Kunming) n/sex/group: 8 (m only) Exposure: oral (gavage, in peanut oil + 0.5% DMSO) Doses: 0, 1, 5 mg/kg bw/d	**Liver:** • Abs. and rel. liver weight ↑ • Hepatic functional enzymes (GPT, GOT) ↑ • Hepatic triglycerides ↑ (≈ +150% in HD) • Visible vacuoles around liver portal area ↑ • Hepatic FGF21 protein ↑ **Clinical chemistry:** • Serum cholesterol ↓ (≈ −26% in MD) • Serum glucose ↓ (≈ −25% in HD) • Serum insulin ↓ (≈ −45% in HD) • Serum triglyceride ↓ (≈ −57% in HD) • Serum L-LDL ↑ (≈ +140% in HD) • Serum H-LDL ↓ (≈ −50% in HD) **Other effects:** • Insulin-positive cells in pancreatic islets ↑	 1 1 1 1 1 1 1 1 1 1 1 1	 5 5 5 5 5 5 5 5 5 5 5 5	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Wu et al., 2018)
PFOA (ammonium salt, >98%)	2, 8 or 16 weeks RDT study, non-guideline study Mouse (57BlL/6)	**Body weight** ↓ (≈ −22 – −37%) **Liver:** • Abs./rel. liver weight ↑ (≈ +50%)	– –	1 1	• kidney, nervous system, immune system,		(Li et al., 2019)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
	n/sex/group = 5 (m only) Exposure: oral (gavage, in distilled water) Doses: 0, 1 mg/kg bw/d	• Replication of hepatocytes (week 2+8) ↑ (4.3-fold increase of the hepatocytes DNA synthesis • Hepatic peroxisomal ß-oxidation activity (week 2-16) • Activation of PPARα • Expression of fatty acid metabolism genes ↑ **Remarks:** Mechanistic study to assess the MoA	– – – –	1 1 1 1	haematopoietic system, thyroid, endocrine system, clinical chemistry		
PFOA (ammonium salt, purity not specified)	28-day RDT study Rat (ChR-CD albino) n/sex/group = 5 Exposure: oral (dietary) Doses: 0, 30, 100, 300, 1000, 3000, 10000, 30000 ppm (equivalent to 0, 3.5, 12, 37, 115, 340, "1600", "4800" mg/kg bw/d, last two values hypothetical as animals in these groups died, female doses slightly higher than males)	**Mortality 100% within 7 days** **Body weight** ↓ (m, ≈ –21/–26.5/–29/–33%; f, -22.5/-23/-18/-20% after 1/2/3/4 week at LOEL) **Liver:** • Abs. liver weight ↑ (m, ≈ +38% at 30 ppm, ≈ +60% at 300 ppm; f: ≈ +25% at LOEL) • Rel. liver weight ↑ (m, ≈ +104% at LOEL; f: ns) **Remarks:** Acute oral toxicity (LD_{50} 540 mg/kg), primary skin irritation (no effects), eye irrigation (moderate) and one-hour inhalation data (no death from inhalation) as well as in vitro mutagenicity tests (all negative) are also available 3M's effort	340 37/115 –/37 115/–	1600 115/340 –/115 340/–	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system, clinical chemistry		(Griffith and Long, 1980; Study report by Metrick and Marisa, 1977)
PFOA ("FC-143" (3M), ammonium salt, purity not specified)	28-day RDT study Mouse (albino) n/sex/group = 5 Exposure: oral (dietary) Doses: 0, 30, 100, 300, 1000, 3000, 10000, 30000 ppm (of diet) (equivalent to 0, 13, 51, 240, "760", "1900", "6200", "19000" mg/kg bw/d, last four values hypothetical as animals in these groups died, female doses	**Mortality 100% after 9 days** **Mortality 90% after 26 days** **Body weight** (week 1) ↓ (m/f, –18%/-29% for m/f at LOEL) **Body weight** (week 2) ↓ (-32%/-28% for m/f at LOEL) **Body weight** (week 3) ↓ (-34%/-33% for m/f at LOEL) **Body weight** (week 4) ↓ (-37.5%/-25% for m/f at LOEL) **Liver:** • Abs. liver weight ↑ (m/f, ≈ +171%/+202% at LOEL) • Rel. liver weight ↑ (m/f, ≈ +252%/+294% at LOEL) • Hepatic hypertrophy ↑ (mild to moderate) • Hepatic lipid vacuolation ↑ (minimal to mild) • Hepatocellular degeneration or necrosis ↑ (minimal to mild)	760 240 13/51 13 13 13/– – 13/– – – –	1900 760 51/240 51 51 51/13 13 240/13 13 13 13	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system, clinical chemistry		(Study report by Christopher and Marias, 1977; Griffith and Long, 1980)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
	slightly higher than males)						
PFOA (ammonium salt)	90-day RDT study Rat (ChR-CD albino), n/sex/group = 5 Exposure: oral (dietary) Doses: 0, 10, 30, 100, 300, 1000 ppm (equivalent to 0.7, 2.2, 7.2, 22, 75 mg/kg bw/d, female doses slightly higher than males)	**Body weight** ↓ (m, ≈ –12% at LOAEL) **Liver:** • Abs. liver weight ↑ (m, ≈ +51% at LOAEL) • Rel. liver weight ↑ (m/f, ≈ +62/+28% at LOEL) **Kidney:** • Abs. kidney weight ↑ (m, ≈ +24% only at 30 ppm) • Rel. kidney weight ↑ (m, ≈ +22% at LOEL; f, +30% only at 10 ppm)	22 7.2/– 7.2/22 7.2/–	75 22– 22/75 7.2/–	• nervous system, immune system, haematopoietic system, thyroid, endocrine system, clinical chemistry	Fluorine in pooled (n=4-5) serum (ppm) (m/f) 0 ppm: ND/ND 10 ppm: 21/NM 30 ppm: 34/0.15 100 ppm: 36/NM 300 ppm: 38/0.25 1000 ppm: 49/0.65 ND = None detected NM = Not measured	(Study report by Goldenthal, 1978a; Griffith and Long, 1980)
PFOA (ammonium salt)	90-day RDT study Rhesus monkey, n/sex/group = 2 Exposure: oral (gavage) Doses: 0, 3, 10, 30, 100 mg/kg bw/d	**Mortality:** • 30 mg/kg bw/d: 2 f and 1 m died in weeks 7-12 • 100 mg/kg bw/d: All died in weeks 2-5	10	30	• body weight, liver, kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system, clinical chemistry	Ammonium PFOA in serum (ppm) (m/f) 0 mg/kg bw/d: NM/1 3 mg/kg bw/d: 48.53/50.65 10 mg/kg bw/d: 45.71/71.79 30 mg/kg bw/d: 145/NM 100 mg/kg bw/d: NM/NM Ammonium PFOA in liver (ppm) (m/f): 0 mg/kg bw/d: 0.05/0.07 3 mg/kg bw/d: 3/7 10 mg/kg bw/d: 9/10 30 mg/kg bw/d: 60.125/80.125 100 mg/kg bw/d: 100/325	(Study report by Goldenthal, 1978b; Griffith and Long, 1980)
PFOA (ammonium salt)	13-week (91 days)+8week recovery Rat (Crl:CD®BR) n/sex/group = 10-25 (males only) Exposure: oral (dietary)	**Body weight (ns)** **Liver:** • Abs. liver weight ↑ (≈ +36%% at LOEL in week 8) • Rel. liver weight ↑ (≈ +37% at LOEL in week 8) • Hepatocellular hypertrophy ↑ • Hepatic palmitoyl CoA oxidase activity ↑ (≈ +133% at LOEL after in week 14)	 0.06 0.06 0.06 0.65	 0.65 0.65 0.65 1.94	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system, clinical chemistry		(Palazzolo, 1993)

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Table 2: Repeated dose toxicity (RDT) animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
	Doses: 0, 0 (pair-fed to 100 ppm), 1, 10, 30, 100 ppm equivalent to 0, 0.06, 0.65, 1.94, 6.5 mg/kg bw/day (calculated in study report)	**Remark:** Effects seemingly reversible. Overall, NOAEL is 100 ppm (6.5 mg/kg bw/d), NOEL is 1 ppm (0.06 mg/kg bw/d)					
PFOA (ammonium salt, 95.2% purity)	26 weeks + recovery (2 control and 2 mid-dose monkeys) 13 weeks more Cynomolgus monkey n/sex/group = (males only) Exposure: oral (gelatine capsules) Doses: 0, 3, 10, 20-30 mg/kg bw/d (30 mg/kg bw/d was reduced to 20 mg/kg bw/d at day 22 after no dosing on days 12-21)	**Body weight** • 30 mg/kg bw/d (in first 11 days) -3 to -7.5% (no food consumption), then 10 days of no dose, then continued with 20 mg/kg bw/d with reduced body weight and body weight gain until week 14, then steep increase in body weight but overall weight still reduced. • Recovery animals from 10 mg/kg bw/d group much lower body weight gain in 14 weeks after treatment **Liver:** • Abs. liver weight ↑ (≈ +35% at LOEL) • Rel. liver weight ↑ (≈ +57% at LOEL) **Endocrine system:** • Total T4 ↓ (≈ –37.5/–35/–31% at MD after 5/10/14 weeks; ≈ –33% at LOEL after 27 weeks) • Free T4 ↓ (≈ –32/–27/–38% after 5/10/27 weeks at LOEL) • Free T3 ↓ (≈ –31/–47/–40% after 5/10/27 weeks at LOEL) **Clinical chemistry:** • Triglycerides ↑ (≈ +145/+109% after 5/27 weeks at HD; ≈ +120% after 14 weeks at MD) • Total bilirubin ↓ (≈ –60% only after 10 weeks, only at MD) **Remarks:** Effects are mainly in the 30/20 mg/kg bw/d group, which was an inconsistent treatment due to toxic effects (no food uptake) in the first 10 days with the 30 mg/kg bw/d dosage. Data on some hepatic marker enzymes also available but no methods described… Liver PFOA content returned to initial levels after 14 weeks of recovery	10 - 0 10 - 3 10 10 3	20-30 10 3 20-30 3 10 20-30 10	• kidney, nervous system, immune system, haematopoietic system, thyroid	Liver tissue PFOA concentration (µg/g) (values of individuals, mean ± sd in bold)) Control: 0.09, 0.23, <LOQ (2x) 3 mg/kg bw/d: 15.2, 18.5, 11.3, 18.3 **(15.8 ± 3.4)** 10 mg/kg bw/d: 21.9, 6.3, 8.9, 18.8 **(14.0 ± 7.6)** 30/20 mg/kg bw/d: 16, 83.3 Control recovery: <LOQ (2x) 10 mg/kg bw/d recovery: 0.15, 0.08	(Butenhoff et al., 2002; Study report by Thomford, 2001)
PFOA (ammonium salt, 97.99%)	70-days (parental-generation, before cohabilitation), 2-generation guideline study EPA OPPTS 8703800 Rat (Sprague-Dawley) n/sex/group = 30 (males only)	**Body weight ↓ (≈ –7%)** **Liver:** • Abs.+rel. liver weight ↑ (≈ +20% at LOEL) **Kidney:** • Rel. kidney weight ↑ (≈ +16.5% at LOEL)	1 - -	3 1 1	• Nervous system, immune system, haematopoietic system, thyroid, endocrine system, clinical chemistry		(Butenhoff et al., 2004)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
	Exposure: oral (gavage) Doses: 0, 1, 3, 10 mg/kg bw/d						
PFOA (ammonium salt, purity)	7 day-study Mouse (PPARα-null mice (129S4/SvJae-*Ppara*^{tm1Gonz}/J) and wildtype (WT)(129S1/SvlmJ - SV/129 and CD-1)) n/sex/group = (m only) Exposure: oral (gavage), PFOA in deionized water Doses: 0, 1, 3 mg/kg bw/d	**Liver:** • Abs. liver weight ↑ (≈ +30% at LOEL) • Rel. liver weight ↑ (≈ +32% at LOEL) • Hepatocyte hypertrophy (lesion score) ↑ (≈ +73% at LOEL) • Labelling index (LI) ↑ (≈ +3750% at LOEL) • • Peroxisome proliferation • Increased cell proliferation (only in wild-type) • Primary effects of PFOA on WT mice was on genes associated with fatty acid metabolism, consistent with activation of PPARα • Other alterations on genes related to inflammation (complement/coagulation cascades), and xenobiotic metabolism • Altered expression of genes associated with cell cycle control, peroxisome biogenesis and proteasome structure/organization • Cyp2b and Cyp2c genes upregulated **Remark:** "In wild-type mice, PFOA (…) induced changes consistent with activation of PPARα. (…)In PFOA-treated null mice, changes were observed in transcripts related to fatty acid metabolism, inflammation, xenobiotic metabolism, and cell cycle regulation. Hence, a component of the PFOA response was found to be independent of PPARα." (from abstract of Rosen et al. 2008)	0 0 0 3	1 1 1 10	• Body weight, kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system, clinical chemistry	Serum concentrations of PFOA in WT/CD-1/KO mice (µg/ml): Control: 0.012 ± 0.006/ 0.032 ± 0.023/ 0.010 ± 0.004 1 mg/kg: 14.1 ± 4.3/17.2 ± 7.3/ 17.7 ± 4.4 3 mg/kg: 33.3 ± 15.0/ - / 47.9 ± 18.9 10 mg/kg: 99.0 ± 33.5/ 112.7 ± 20.4/ 85.6 ± 31.1 Liver concentrations of PFOA in WT/CD-1/KO mice (µg/ml): Control: 0.042 ± 0.020/ 0.046 ± 0.056/ 0.053 ± 0.059 1 mg/kg: 58.2 ± 6.7/ 56.9 ± 7.7/ 37.1 ± 7.9 3 mg/kg: 111.1 ± 23.4/ - / 90.7 ± 9.2 10 mg/kg: 296.3 ± 57.0/ 310.0 ± 59.2/ 209.5 ± 29.1	(Wolf et al., 2008) (histopathology) (Rosen et al., 2008) (Gene expression)
PFOA (ammonium salt, linear/branched (80:20) isoforms)	14-day-study Rat (Crl:CD®(SD)IGS BR) n/sex/group = 10 (m only) Exposure: oral (gavage, in water) Doses: 0, 0.3, 1, 3, 10, or 30 mg/kg bw/d	**Body weight** (day 7) ↓ (≈ –17% at LOEL) **Body weight** (day 13) ↓ (≈ –19% at LOEL) **Body weight gain** (0-13) ↓ (≈ –45% at LOEL) **Liver:** • Abs. liver weight ↑ (≈ +60% at LOEL) • Rel. liver weight ↑ (≈ +15% at LOEL) • Hepatic peroxisomal ß-oxidation activity ↑ (≈ +48% at LOEL) **Kidney:** • Abs. kidney weight (≈ +11% at 3 mg/kg bw/d, ≈ –12% at 30 mg/kg bw/d) • Rel. kidney weight ↑ (+9% at LOEL) **Haematopoietic system:** • Haemolysis in vena cava • Haemolysis in orbital sinus	10 10 3 1 0.3 0.3 - 1 1 0	30 30 10 3 1 1 - 3 3 0.3	• nervous system, immune system, thyroid, endocrine system	Serum PFOA concentrations (µg/ml): 0 mg/kg: 0.37 ± 0.14 0.3 mg/kg: 19 ± 2.5 1 mg/kg: 51 ± 10 3 mg/kg: 106 ± 10 10 mg/kg: 183 ± 46 30 mg/kg: 208 ± 51	(Loveless et al., 2006)

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Table 2: Repeated dose toxicity (RDT) animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
		Clinical chemistry: • Serum cholesterol ↓ (≈ –55% at LOEL) • Serum HDL ↓ (≈ –48% at LOEL) • Serum non-HDL ↓ (≈ –43% at LOEL) • Serum triglycerides ↓ (≈ –34% at LOEL)	 1 1 0.3 0.3	 3 3 1 1			
PFOA (ammonium salt, linear isoforms)	14-day-study Rat (Crl:CD®(SD)IGS BR) n/sex/group = 10 (m only) Exposure: oral (gavage, in water) Doses: 0, 0.3, 1, 3, 10, or 30 mg/kg bw/d	**Body weight** (day 7) ↓ (≈ –31% at LOEL) **Body weight** (day 13) ↓ (≈ –19% at LOEL) **Body weight gain** (0-13) ↓ (≈ –26% at LOEL) **Liver:** • Abs. liver weight ↑ (≈ +28% at LOEL) • Rel. liver weight ↑ (≈ +14% at LOEL) • Hepatic peroxisomal ß-oxidation activity ↑ (≈ +90% at LOEL) **Kidney:** • Abs. kidney weight ↓ (≈ –16% at LOEL) **Haematopoietic system:** • Haemolysis in vena cava • Haemolysis in orbital sinus **Clinical chemistry:** • Serum cholesterol ↓ (≈ –27% at LOEL) • Serum HDL ↓ (≈ –42% at LOEL) • Serum non-HDL (≈ –32% at LOEL) • Serum triglycerides (≈ –21% at LOEL) **Remark**: Conclusion of the study: Linear PFOA is similarly toxic as linear/branched-mix (80:20), branched isoforms have weaker toxicity	10 10 1 1 0.3 0.3 10 1 0.3 0 1 0 0	30 30 3 3 1 1 30 3 1 0.3 3 0.3 0.3	• nervous system, immune system, haematopoietic system, thyroid, endocrine system	Serum PFOA concentrations (µg/ml): 0 mg/kg: 0.39 ± 0.51 µg/ml 0.3 mg/kg: 20 ± 3.2 1 mg/kg: 65 ± 11 3 mg/kg: 137 ± 18 10 mg/kg: 206 ± 65 30 mg/kg: 223 ± 77	(Loveless et al., 2006)
PFOA (ammonium salt, branched isoforms)	14-day-study Rat (Crl:CD®(SD)IGS BR) n/sex/group=10 (m only) Exposure: oral (gavage, in water) Doses: 0, 0.3, 1, 3, 10, or 30 mg/kg bw/d	**Body weight gain** (0-13) ↓ (≈ –24% at LOEL) **Liver:** • Abs. liver weight ↑ (≈ +19% at LOEL) • Rel. liver weight ↑ (≈ +16% at LOEL) • Hepatic peroxisomal ß-oxidation activity ↑ (≈ +34% at LOEL) **Kidney:** • Rel. kidney weight ↑ (≈ +10% at LOEL) **Haematopoietic system:** • Haemolysis in vena cava • Haemolysis in orbital sinus **Clinical chemistry:** • Serum cholesterol ↓ (≈ –22% at LOEL) • Serum HDL ↓ (≈ –30% at LOEL) • Serum non-HDL (≈ –50% at LOEL) • Serum triglycerides (≈ –44% at LOEL)	10 0.3 0.3 1 3 3 1 0.3 1 1 3	30 1 1 3 10 10 3 1 3 3 10	• nervous system, immune system, haematopoietic system, thyroid, endocrine system	Serum PFOA concentrations (µg/ml): 0 mg/kg: 0.11 ± 0.24 0.3 mg/kg: 16 ± 2.5 1 mg/kg: 48 ± 12 3 mg/kg: 73 ± 25 10 mg/kg: 92 ± 20 30 mg/kg: 124 ± 33	(Loveless et al., 2006)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
PFOA (ammonium salt, linear/branched (80:20) isoforms)	14-day-study Mouse (Crl:DC®-1(ICR)BR) n/sex/group = 10 (m only) Exposure: oral (gavage, in water) Doses: 0, 0.3, 1, 3, 10, or 30 mg/kg bw/d	**Body weight** (day 7) ↓ (≈ –16% at LOEL) **Body weight** (day 13) ↓ (≈ –12% at LOEL) **Body weight gain** (0-13) ↓ (≈ –200% at LOEL) **Liver:** • Abs. liver weight ↑ (≈ +112% at LOEL) • Rel. liver weight ↑ (≈ +109% at LOEL) • Hepatic peroxisomal ß-oxidation activity ↑ (≈ +92% at LOEL) **Kidney:** • Abs. kidney weight ↓ (≈ –20% at LOEL) **Clinical chemistry:** • Serum cholesterol ↓ (≈ –33% at LOEL) • Serum HDL ↓ (≈ –43% at LOEL) • Serum triglycerides ↑ (≈ +37% at LOEL)	10 3 3 1 1 0 10 1 1 0	30 10 10 3 3 0.3 30 3 3 0.3	• nervous system, immune system, haematopoietic system, thyroid, endocrine system	Serum PFOA concentrations (µg/ml): 0 mg/kg: 0.04 ± 0.02 0.3 mg/kg: 10 ± 1.4 1 mg/kg: 27 ± 5 3 mg/kg: 66 ± 8.6 10 mg/kg: 190 ± 29 30 mg/kg: 241 ± 28	(Loveless et al., 2006)
PFOA (ammonium salt, linear isoforms)	14-day-study Mouse (Crl:DC®-1(ICR)BR) n/sex/group = 10 (m only) Exposure: oral (gavage, in water) Doses: 0, 0.3, 1, 3, 10, or 30 mg/kg bw/d	**Body weight** (day 7) ↓ (≈ –16%) **Body weight** (day 13) ↓ (≈ –18%) **Body weight gain** (0-13) ↓ (≈ –500%) **Liver:** • Abs. liver weight ↑ (≈ +61% at LOEL) • Rel. liver weight ↑ (≈ +17% at LOEL) • Hepatic peroxisomal ß-oxidation activity ↑ (≈ +143% at LOEL) **Kidney:** • Abs. kidney weight ↓ (≈ –18% at LOEL) • Rel. kidney weight ↓ (≈ –11% at LOEL) **Clinical chemistry:** • Serum cholesterol ↓ (≈ –35% at LOEL) • Serum HDL ↓ (≈ –46% at LOEL)	10 10 10 0.3 0 0 3 3 1 1	30 30 30 1 0.3 0.3 10 10 3 3	• nervous system, immune system, haematopoietic system, thyroid, endocrine system	Serum PFOA concentrations (µg/ml): 0 mg/kg: 0.07 ± 0.06 0.3 mg/kg: 13 ± 2.4 1 mg/kg: 32 ± 5.2 3 mg/kg: 69 ± 10 10 mg/kg: 225 ± 68 30 mg/kg: 259 ± 34	(Loveless et al., 2006)
PFOA (ammonium salt, branched isoforms)	14-day-study Mouse (Crl:DC®-1(ICR)BR) n/sex/group = 10 (m only) Exposure: oral (gavage, in water) Doses: 0, 0.3, 1, 3, 10, or 30 mg/kg bw/d	**Body weight** (day 13) ↑ (≈ +6%) **Body weight gain** (0-13) ↑ (≈ +200%) **Liver:** • Abs. liver weight ↑ (≈ +149% at LOEL) • Rel. liver weight ↑ (≈ +19% at LOEL) • Hepatic peroxisomal ß-oxidation activity ↑ (≈ +103% at LOEL) **Clinical chemistry:** • Serum cholesterol ↓ (≈ –34% at LOEL) • Serum HDL ↓ (≈ –34% at LOEL) • Serum non-HDL (≈ –35% only at 3 mg/kg) **Remarks:** Body weight changes only significant at 3 and 10 mg/kg, at highest dose of 30 mg/kg no significant changes.	1 1 1 0 0.3 1 1 -	3 3 3 0.3 1 3 3 -	• nervous system, immune system, haematopoietic system, thyroid, endocrine system	Serum PFOA concentrations (µg/ml): 0 mg/kg: 0.02 ± 0.03 0.3 mg/kg: 14 ± 3.5 1 mg/kg: 34 ± 10 3 mg/kg: 82 ± 10 10 mg/kg: 172 ± 29 30 mg/kg: 244 ± 50	(Loveless et al., 2006)
PFOA (ammonium	28-day-study, USEPA, OPPTS 870.7800: Immunotoxicity, Health Effects Test	**Body weight** ↓ (≈ –11% at LOEL after 28 days) **Body weight gain over 28 days** ↓ (≈ –26% at LOEL)	1 1	10 10	• Kidney, nervous system,		(Loveless et al.,

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Table 2: Repeated dose toxicity (RDT) animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
salt, linear) 19.5% aqueous solution (in NANOpure water)	Guidelines (1998) Rat (Crl:CD(SD)IGS BR) n/sex/group = 10 (m only) Exposure: oral (gavage) Doses: 0, 0.3, 1, 10, 30, 30/0 mg/kg bw/d	**Liver:** • Abs. liver weight ↑ (≈ +30%% at LOEL) • Rel. liver weight ↑ (≈ +83% at LOEL) **Immune system:** • Rel. thymus weight ↑ (≈ +44% at LOEL) • Abs./rel. spleen weight (ns) • Anti-sRBC IgM antibody (ns) **Endocrine system:** Serum corticosterone (ns) **Clinical chemistry:** • Cholesterol ↓ (≈ –36/–31% at 0.3/1 mg/kg bw/d) • HDL ↓ (≈ –25% at LOEL) • Non-HDL ↓ (≈ –42%/–37% at 0.3/1 mg/kg bw/d) • Triglycerides ↓ (≈ –31% at LOEL)	0.3 1 10 - -	1 10 30 (30/0) 0.3 0.3	haematopoietic system, thyroid		2008)
PFOA (ammonium salt, linear) 19.5% aqueous solution (in NANOpure water)	28-day-study, USEPA, OPPTS 870.7800: Immunotoxicity, Health Effects Test Guidelines (1998) Mouse (Crl:CD-1(ICR)BR) n/sex/group=20 (m only) Exposure: oral (gavage) Doses: 0, 0.3, 1, 10, 30, 30/0 mg/kg bw/d	**Body weight ↓ (≈ –14% at LOEL after 28 days)** **Liver:** • Abs.+ rel. liver weight ↑ (≈ +80% at LOEL) **Immune system:** • Abs. spleen weight ↓ (≈ –44% at LOEL) • Rel. spleen weight ↓ (≈ –14% at LOEL) • Abs. + rel. thymus weight ↓ (≈ –45% at LOEL) • Anti-sRBC IgM antibody ↓ (≈ –22% at LOEL) **Endocrine system:** Serum corticosterone ↑ (≈ +120% at 10 mg/k/d) **Clinical chemistry:** • Cholesterol ↓ (≈ –31% at LOEL) • HDL ↓ (≈ –29% at LOEL) • Non-HDL (ns) • Triglycerides ↓ (≈ –53% at LOEL)	1 0.3 1 0.3 1 1 1 0.3 1	10 1 10 1 10 10 10 1 10	• Kidney, nervous system, haematopoietic system, thyroid		(Loveless et al., 2008)
PFOA (90%)	3-week study, non-guideline Mouse (no strain given) n/sex/group = 5 (f only) exposure: oral (gavage, PFOA in water) doses: 0, 1, 5, 10 mg/kg bw/d	**Liver:** • Abs. liver weight ↑ (≈ +77% at LOEL) • Total glutathione ↑ (≈ +101% at LOEL) • Hepatic catalase ↑ (≈ +482% at LOEL) • Hepatic SOD (ns) • Hepatic Glutathione reductase ↑ (≈ +156% at LOEL)	1 1 1 10 5	5 5 5 10	• Body weight, kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Ahmed and Abd Ellah, 2012)
PFOA (99.9%)	14-days, rat (CD) n/sex/group = 15 (m only)	**Body weight ↓ (≈ –9% at LOEL)** **Body weight gain ↓ (≈ –68% at LOEL)** **Liver:** • Abs. liver weight ↑ (≈ +70% at LOEL)	1 1 1	10 10 10	• kidney, nervous system, immune system, haematopoietic		(Cook et al., 1992)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
	Exposure: oral (gavage) Doses: 0, 1, 10, 25, 50 mg/kg bw/d	• Rel. liver weight ↑ (≈ +181% at LOEL) • Hepatic β-oxidation activity ↑ (≈ +246% at LOEL) **Endocrine system:** • Serum estradiol ↑ (≈ +64% at LOEL) **Reproductive tissue:** • Abs./rel. testes weight ↑ (≈ +20%/+21% at LOEL) • Abs. accessory sex organ weight ↓ (≈ –16% at LOEL) • Abs. ventral prostate weight ↓ (≈ –12% at LOEL)	1 1 1 10 10 25	10 10 10 25 25 50	system, thyroid,		
PFOA (99.9%)	14-days Rat (Crl:CD BR(CD)) n/sex/group = 15 (m only) Exposure: oral (gavage) Doses: 0, 25 mg/kg bw/d	**Body weight ↓ (≈ –16%)** **Liver:** • Rel. liver weight ↑ (≈ +79%) • Hepatic β-oxidation ↑ (≈ +760%) • Hepatic aromatase ↑ (≈ +354%) **Endocrine system:** • Serum estradiol ↑ (≈ +184%) **Reproductive tissue:** • Testicular interstitial fluid testosterone ↑ (≈ +93%) • Testicular interstitial fluid estradiol ↑ (≈ +115%)			• kidney, nervous system, immune system, haematopoietic system, thyroid,		(Biegel et al., 1995)
PFOA (98-100%)	24-months-study Rat (Crl:CD BR (CD)) n/sex/group = 156 (m only) n/group (hormonal analysis) = 10 n/group (peroxisome prolif.) = 6 Exposure: oral (dietary) Doses: 0, 300 ppm (equivalent to 13.6 mg/kg bw/d)	**Body weight gain (ns)** **Liver:** • Liver adenoma ↑ (≈ +1200% (13/1)) **Endocrine system:** • Estradiol ↑ (≈ +271/+110/+70/+71/+78% after 1/3/6/9/12 month(s) • Prolactin ↓ (≈ –40/–29% after 3/6 months) **Reproductive tissue:** • Testis weight ↑ (≈ +8% after 24 months) • Leydig cell adenoma ↑ (≈ +267%) **Other effects:** • Pancreatic cell proliferation ↑ (≈ +175/+125% after 15/18 months) • Pancreatic acinar cell hyperplasia ↑ (≈ +290%) • Pancreatic acinar cell adenoma ↑ (≈ +800%) **Remark:** Mechanistic study on tumor induction			•		(Biegel et al., 2001)
PFOA (96%)	28-day RDT-study, non-guideline Rat (SD) n/sex/group = 10 (m only) Exposure: oral (gavage, in water)	**Body weight ↓** (≈ –6% at LOEL after 27 days) **Liver:** • Rel. liver weight ↑ (≈ +75% at LOEL) • Cytoplasmic vacuolation • Focal or flakelike necrosis • Hepatocellular hypertrophy **Kidney:**	5 – – – –	20 5 5 5 5	• , nervous system, immune system, haematopoietic system, thyroid	Blood PFOA concentrations (µg/ml): 0 mg/kg: 0 5 mg/kg: 39.2 ± 14.4 20 mg/kg: 58.8 ± 17.6 Liver PFOA concentrations	(Cui et al., 2009)

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Table 2: Repeated dose toxicity (RDT) animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentrate-ion of PFAS /metabolites (time of sampling)	Reference
	Doses: 0, 5, 20 mg/kg bw/d	• Rel. kidney weight ↑ (≈ +15% at LOEL) **Reproductive tissue:** • Rel. testis weight ↑ (≈ +20% at LOEL) **Other effects:** • Pulmonary congestion • Focal or diffuse thickened epithelial walls of respiratory system	– – – –	5 5 5 5		<u>(µg/g):</u> 0 mg/kg: 0 5 mg/kg: 218 ± 21 20 mg/kg: 196 ± 10 <u>Kidney PFOA concentrations (µg/g):</u> 0 mg/kg: 0 5 mg/kg: 228 ± 37 20 mg/kg: 209 ± 74 <u>Lung PFOA concentrations (µg/g):</u> 0 mg/kg: 0 5 mg/kg: 63.0 ± 11.3 20 mg/kg: 64.3 ± 15.9 <u>Heart PFOA concentrations (µg/g):</u> 0 mg/kg: 0 5 mg/kg: 35.5 ± 17.6 20 mg/kg: 34.6 ± 18.0 <u>Spleen PFOA concentrations (µg/g):</u> 0 mg/kg: 0 5 mg/kg: 13.6 ± 2.4 20 mg/kg: 6.92 ± 9.31 <u>Testicle PFOA concentrations (µg/g):</u> 0 mg/kg: 0 5 mg/kg: 16.7 ± 16.9 20 mg/kg: 16.8 ± 19.2 <u>Brain PFOA concentrations (µg/g):</u> 0 mg/kg: 0	

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
						5 mg/kg: 10.5 ± 9.8 20 mg/kg: 7.20 ± 6.03	
PFOA (97%)	7 days Rat (SV129 WT and PPARα-null) n/sex/group = 4 (m only) Exposure: oral (gavage) Doses: 0, 10 mg/kg bw/d	**Body weight** (ns) **Liver:** • Abs. liver weight (WT/PPARAα-null) ↑ (≈ +170%/+175%) • Rel. liver weight (WT/PPARAα-null) ↑ (≈ +143%/+171%) • Cell area (WT/PPARAα-null) ↑ (≈ +105%/+96%) • Hepatic DNA content (WT/PPARAα-null) ↓ (≈ –43%/–35%) • Hepatic lipid accumulation (WT) ↑ (≈ +900%) • Hepatic triglycerides (WT) ↑ (≈ +225%)			• kidney, nervous system, immune system, haematopoietic system, thyroid		(Das et al., 2017)
PFOA (≥98%)	15 days, non-guideline study Mouse (C57BL/6N) n/sex/group = 8 (f only) Exposure: oral (drinking water) Doses: 0, 0.94, 1.88, 3.75, 7.5, 15, 30 mg/kg bw/d	**Body weight** ↓ (1 day postdosing) (≈ –4% at LOEL) **Immune system:** • Abs. + rel. spleen weight ↓ (≈ –16% at LOEL at 1 day PD) • Abs. + rel. thymus weight ↓ (≈ –30% at LOEL at 1 day PD) • SRBC IgM antibody titers ↓ (≈ –10% at LOEL) • SRBC IgG antibody titers ↑ (≈ +15% at LOEL)	7.5 1.88 7.5 1.88 1.88	15 3.75 15 3.75 3.75	• Liver, kidney, nervous system, haematopoietic system, thyroid	<u>Serum PFOA concentrations (ng/mL) at 1 day postdosing:</u> 0 mg/kg: 54.3 ± 4.9 3.75 mg/kg: 74.9 ± 2.7 7.5 mg/kg: 87.2 ± 3.3 15 mg/kg: 128.1 ± 6.8 30 mg/kg: 162.6 ± 8.4 <u>Serum PFOA concentrations (ng/mL) at 15 day postdosing:</u> 0 mg/kg: **156.4** ± 14.9 3.75 mg/kg: 35.9 ± 1.6 7.5 mg/kg: 42.8 ± 1.7 15 mg/kg: 50.0 ± 1.5 30 mg/kg: 52.7 ± 3.2	(DeWitt et al., 2008)
PFOA (≥98%)	10-day-study Mouse (C57BL/6N; adx/sham-adrenolectomized) n/sex/group = 5-7 (f only) Exposure: oral (drinking water) Doses: 0, 3.75, 7.5, 15 mg/kg bw/d	**Body weight (sham)** ↓ (≈ –9.8% at LOEL at 2 days postdosing) **Body weight (adx)** ↓ (≈ –10.3% at LOEL, at 2 days postdosing) **Immune system:** • SRBC IgM titer (sham) ↓ (≈ –18% at LOEL) • SRBC IgM titer (adx) ↓ (≈ –14% at LOEL) **Endocrine system:** • Serum corticosterone ↑ (≈ +168% at LOEL) **Clinical Chemistry** (after 5 days of dosing, no effects after 10 days) • ALT (adx) ↑ (≈ +250% at LOEL) • AST (adx) ↑ (≈ +72% at LOEL) • SDH (adx) ↑ (≈ +135% at LOEL) • Cholesterol (sham) ↓ (≈ –19% at LOEL) • Triglycerides (sham) (≈ –45% at LOEL)	3.75 7.5 7.5 3.75 7.5 7.5 7.5 7.5 7.5 –	7.5 15 15 7.5 15 15 15 15 15 3.75	• Liver, kidney, nervous system, haematopoietic system, thyroid		(DeWitt et al., 2009)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
		• Triglycerides (adx) (≈ –60% at LOEL) • LDH (adx) ↑ (≈ +36% at LOEL)	3.75 7.5	7.5 15			
PFOA (ammonium salt, ≥98%)	15-day-study (immunization on day 11) Mouse (WT (C57BL/6-Tac)) and PPARα KO (B6.129S4-Ppar^{tm1Gonz}N12)) n/sex/group = 6 (f only) Exposure: oral (drinking water) Doses: 0, 7.5, 30 mg/kg bw/d	**Body weight ↓ (≈ –14% at LOEL from day 12 onwards, only in WT)** **Immune system:** • Rel. spleen weight (only WT) ↓ (≈ –32% at LOEL) • Rel. thymus weight (only WT) ↓ (≈ –58% at LD, no effect at HD) • T-cell dependent IgM antibody response (WT) ↓ (≈ –17%) • T-cell dependent IgM antibody response (PPARα-KO) ↓ (≈ –14%) **Remark:** Suppression of T-cell dependent antibody response seemed to be PPARα-independent. "…effects on humoral immunity are likely mediated by disruption of B-cell/plasma cell function."	7.5 7.5 7.5 7.5	30 30 30 30	• Liver, kidney, nervous system, haematopoietic system, thyroid, endocrine system, clinical chemistry		(DeWitt et al., 2016)
PFOA (ammonium salt, ≥98%)	15-day-study (immunization on day 11), mouse (WT (C57BL/6N)) n/sex/group = 8 (f only) Exposure: oral (drinking water) Doses: 0, 0.94, 1.88, 3.75, 7.5 mg/kg bw/d	**Body weight (ns)** **Immune system:** • Rel. spleen weight ↓ (≈ –17% at LOEL) • Rel. thymus weight ↓ (≈ –14% at LOEL) • T-cell-independent IgM antibody response ↓ (≈ –10% at LOEL)	3.75 3.75 0.94	7.5 7.5 1.88	• Liver, kidney, nervous system, haematopoietic system, thyroid, endocrine system, clinical chemistry		(DeWitt et al., 2016)
PFOA (ammonium salt, ≥98%)	15-day-study (immunization on days 11) Mouse (WT (C57BL/6N)) n/sex/group = 4 (f only) Exposure: oral (drinking water) Doses: 0, 3.75, 7.5 mg/kg bw/d	**Immune system: (**Splenic lymphocyte phenotypes) • CD4-/CD8+ ↑ (≈ +14% at HD after 13 days (i.e. 2 days after immunization)) ↓ (≈ –10% only at LD after 15 days (i.e 5 days after immunization)) • CD4-/CD8- ↓ (≈ –30% at LOEL after 13 days, but no effect after 15 days) • CD4+/CD8- ↑ (≈ +7% only at LD after 15 days) • NK cells ↓ (≈ –21% only at LD after 15 days) • **Remark:** there were no PFOA effects before immunization on day 10	-	3.75	• body weight, liver, kidney, nervous system, haematopoietic system, thyroid, clinical chemistry		(DeWitt et al., 2016)
PFOA	7-day study Rat (Wistar) n/sex/group = 4 (m only) Exposure: oral (dietary)	**Body weight ↓ (≈ –6%)** **Liver:** • Abs. liver weight ↑ (≈ +66%) • Liver non-esterified cholesterol ↑ (≈ +48%) • Cholesterol synthesis in hepatocytes ↓ (≈ –75% from acetate/-55% from pyruvate/-80% from hydroxyl-methylglutarate) • Fatty acid synthesis from acetate in hepatocytes ↓ (≈ –64%) • Enzymatic activity in hepatocytes (≈ –60% for acetate thiokinase, ≈ +272% for malate dehydrogenase(NADP)(decarboxylating), ≈ –			• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Haughom and Spydevold, 1992)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
	Doses: 0, 0.02% (equivalent to 17 mg/kg bw/d)	54% for Glucose-6-phosphate dehydrogenase) • Acetyl-CoA Cholesterol acyltransferase (ACAT) activity) ↓ (≈ –51%) **Clinical chemistry:** • Serum cholesterol ↓ (≈ –56%) • Serum triglycerols ↓ (≈ –39%) **Remark:** Also PFOSA data available					
PFOA (ammonium salt, 98%)	28-day-studies (study 1 and 2) Rat (Sprague-Dawley) n/sex/group = 10 (m only) Exposure: oral (dietary) Doses: 0, 300 ppm (equivalent to 20 mg/kg bw/d)	**Body weight ↓ (≈ –12%/–11% on day 8; ≈ –23%/–13% on day 29)** **Liver:** • Abs. liver weight ↑ (≈ +25%/+37% on day 8 in study 1/2; ≈ +55% on day 29 but only in study 2, not in study 1) • Rel. liver weight ↑ (≈ +42%/+53% on day 8; ≈ +47%/+66% on day 29) • Hepatic cell proliferation ↑ (≈ +236%/+114% on day 2; ≈ +318%/+413% on day 8; ≈ +164% on day 29 (only study 2 data available) • Relative liver DNA concentration ↓ (≈ –13% on day 2 (only study 1 significant effect); ≈ –22%/-26% on day 8; ≈ –13%/–32% on day 29) • Peroxisome proliferation (PCO) ↑ (≈ +120%/+166% on day 2; ≈ +558%/+677% on day 8; ≈ +888%/+656% on day 29) **Clinical chemistry (study 1 only):** • Plasma AST ↓ (≈ –25%/-19% on day 2/29) • Plasma cholesterol ↓ (≈ –61%/–40% on day 8/29) • Plasma glucose ↓ (≈ –38%/–38% on day 8/29) • Plasma triglycerides ↑/↓ (≈ +37% on day 2; ≈ –75%/–73% on day 8/29)			• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system	Plasma levels of APFO (µg/mL): Control: < quantification limit Day2: 259 ± 39 Day 8: 234 ± 33 Day 29: 252 ± 45	(Elcombe et al., 2010)
PFOA (ammonium salt)	7-day RDT study Mouse (C57BL/6) n/sex/group = 6 (m only) Exposure: oral (gavage) Doses: 0, 1, 3 mg/kg bw/d	**Liver:** • Abs. + rel. liver weight ↑ (≈ +35% at LOEL) • Hepatic mRNA/protein expression of Cyp4a14 ↑ (≈ +11900%/+1150% at LOEL) • Hepatic mRNA/protein expression of Bcrp ↑ (≈ +90%/+70% at LOEL) **Kidney:** • Renal mRNA/protein expression of Cyp4a14 ↑ (≈ +90%/ns at LOEL)	- - - 1	1 1 1 3	• Body weight, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Eldasher et al., 2013)
PFOA (ammonium salt, >96%)	10-day RDT study (hypersensitivity study) Mouse (BALB/c)	**Liver:** • Abs. liver weight ↑ (≈ +53% at LOEL) **Immune system:** • Thymus weight ↓ (≈ –41% at LOEL)	2.5 25	6.25 50	• Body weight, kidney, nervous system, haematopoietic		(Fairley et al., 2007)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
	n/sex/group= 5 (f only) Exposure: **dermal** (PFOA in acetone, exposure on ear), 4 days exposure, sacrifice 6 days after final exposure Doses: 0, 0.25, 2.5, 6.25, 12.5, 25, 50 mg/kg bw/d	• Spleen weight ↓ (≈ –28% at LOEL) **Remark**: Hypersensitivity study results not listed here	12.5	25	system, thyroid, endocrine system		
PFOA	14-day RDT study Rat (SD) n/sex/group = 3 (m only) Exposure: oral (dietary) Doses: 0, 0.02% (equivalent to 20 mg/kg bw/d)	**Liver:** • Rel. liver weight ↑ (≈ +45%) • CAT activity ↑ (≈ +57%) • Induction of 80K-protein **Remark:** Acute toxicity dose response for PFOA also available (i.p. injection, 5-150 mg/kg)			• Body weight, kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Ikeda et al., 1985)
PFOA (ammonium salt, 10% aqueous solution)	14-day RDT study Rat (Crj:CD(SD)IGS) n/sex/group = 4 (m only) Exposure: oral (gavage) Doses: 0, 0.5, 5, 50 mg/kg bw/d	**Body weight (increase but ns)** **Liver:** • Rel. liver weight ↑ (≈ +102% at LOEL) • Cyanide-insensitive palmitoyl CoA β-oxidation activity ↑ (≈ +598% at LOEL) • Carnitine acetyltransferase activity ↑ (≈ +1960% at LOEL)	 5 5 5	 50 50 50	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Iwai and Yamashita, 2006)
PFOA	1-week RDT-study Rat (Wistar) n/sex/group= (males only) Exposure: oral (dietary) Doses: 0, 0.0025, 0.005, 0.01, 0.02,	**Body weight (ns)** **Liver:** • Abs. + rel. liver weight ↑ (≈ +33% at LOEL) • Peroxisomal β-oxidation ↑ (≈ +300% at LOEL) • Hepatic acyltransferase ↑ (≈ +30% at LOEL) • Hepatic hydrolase ↑ (≈ +700% at LOEL) • Hepatic GSH S-transferase ↓ (≈ –22% at LOEL) • Hepatic triacylglycerol ↑ (≈ +160% at LOEL) • Hepatic cholesterol ↑ (≈ +50% at LOEL) • Hepatic phospholipid ↑ (≈ +33% at LOEL)	 2.5 2.5 – 2.5 – – 5 5	 5 5 2.5 5 2.5 2.5 10 10	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Kawashima et al., 1995)

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	0.04 % (equivalent to 2.5, 5, 10, 21, and 43 mg/kg bw/d)						
PFOA (ammonium salt, 99%)	21-day RDT studies Mouse (Crl:CD-1) n/sex/group=5 Exposure: oral (dietary) Doses: 0, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, 300, 3000 ppm (300 and 3000 ppm only in 14 day study) (equivalent to 0, 0.0013, 0.0038, 0.013, 0.038, 0.13, 0.38, 1.3, 3.8, 38, 380 mg/kg bw/d)	**Mortality** ↑ (100% mortality at 3000 ppm within 14 days) **Body weight** ↓ (data not shown) **Liver:** • Abs. liver weight ↑ (m/f, ≈ +35/+32% at LOEL) • Rel. liver weight ↑ (m/f, ≈ +31/+29% at LOEL)	38 3.8 0.13/0.13 0.13/0.13	380 38 0.38/0.38 0.38/0.38	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Kennedy, 1987)
PFOA (>99.9%)	14-day RDT study Rat (Crl:CD BR(CD)) n/sex/group=15 (males only) Exposure: oral (gavage) Doses: 0, 0, 0.02, 2, 20, 40 mg/kg bw/d (controls ad libitum and pair-fed to 40 mg control)	**Body weight ↓ (-14% at LOEL)** **Liver:** • Abs. + rel. liver weight ↑ (≈ +34% at LOEL) • Protein of hepatic microsomes ↑ (≈ +7% at LOEL) • Hepatic aromatase activity ↑ (≈ +300% at LOEL) • Total hepatic aromatase ↑ (≈ +560% at LOEL) • Hepatic β-oxidation activity ↑ (≈ +190% at LOEL) • Total hepatic cytochrome P450 ↑ (≈ +70% at LOEL) **Endocrine system:** Serum estradiol ↑ (≈ +56% at LOEL) **Reproductive tissue:** • Rel. testes weight ↑ (≈ +12% at LOEL) (compared to ad libitum control); ↓ (≈ –11% at 40 mg compared to pair-fed control)	2 0.2 - 0.2 0.2 0.2 2 0.2 2	20 2 0.2 2 2 2 20 2 20	• kidney, nervous system, immune system, haematopoietic system, thyroid		(Liu et al., 1996)
PFOA (ammonium salt, >98%)	4-week RDT study Mouse (Wild-type mice (129S4/SvlmJ) and Pparα-null mice) (129S4/SvJae-Pparα^{tm1Gonz}/J) n/sex/group = 10 (males only) Exposure: oral (gavage)	**Body weight (WT)** ↓ (≈ –14% at LOEL) **Body weight gain (WT)** ↓ (≈ -167% at LOEL) **Body weight (PPARα KO)** ↑ (≈ +11% at LOEL, ns at highest dose) **Liver:** • Abs. + rel. liver weight (WT) ↑ (≈ +240% at LOEL) • Abs./rel. liver weight (PPARα KO) ↑ (≈ +180% at LOEL) **Clinical Chemistry:** • Plasma AST (WT) ↑ (≈ +83% at LOEL) • Plasma ALT (WT) ↑ (≈ +577% at LOEL) • Plasma total bilirubin (WT)(≈ –44% at 12.5 µmol/kg; ≈ +67% at 50	10.8 5.4 – – – 5.4 –	21.6 10.8 5.4 5.4 5.4 10.8 5.4	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system	PFOA concentrations in whole blood (WT) (µg/ml): 12.5 µmol/kg: 20.6 ± 2.4 25 µmol/kg: 46.9 ± 3.2 50µmol/kg:64.2 ± 6.5 PFOA concentration in bile (WT) (µg/ml): 12.5 µmol/kg: 56.8 ± 26.9 25 µmol/kg: 784.0 ± 137.6	(Minata et al., 2010)

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	Doses: 0, 12.5, 25, 50 µmol/kg/d (0, 5.4, 10.8, 21.6 mg/kg bw/d)	µmol/kg) • Plasma total cholesterol (WT) ↓ (≈ –17% at LOEL) • Plasma triglycerides (WT) ↑ (≈ +47% at LOEL, ns at highest dose) • Plasma AST (PPARα KO) ↑ (≈ +535% at LOEL) • Plasma ALT (PPARα KO) ↑ (≈ +491% at LOEL) • Plasma total bilirubin (PPARα KO)(≈ +683% at 50 µmol/kg) • Plasma total bile acid (PPARα KO) (≈ +1350% at 50 µmol/kg) • Plasma total cholesterol (PPARα KO) (≈ –38%/-36% at 12.5/25 µmol/kg; ≈ +66% at 50 µmol/kg) • Plasma triglycerides (PPARα KO) ↑ (≈ +102% at LOEL)	5.4 – 10.8 – –	10.8 5.4 21.6 5.4 5.4		50µmol/kg: 2174.0 ± 332.4 PFOA concentration in liver (WT) (µg/ml): 12.5 µmol/kg: 181.2 ± 6.3 25 µmol/kg: 198.8 ± 15.4 50µmol/kg: 211.6 ± 13.3 PFOA concentrations in whole blood (*Pparα*$^{(-/-)}$) (µg/ml): 12.5 µmol/kg: 19.3 ± 2.2 25 µmol/kg: 36.4 ± 2.7 50µmol/kg: 71.2 ± 8.0 PFOA concentrations in bile (*Pparα*$^{(-/-)}$) (µg/ml): 12.5 µmol/kg: 19.6 ± 2.2 25 µmol/kg: 62.9 ± 16.7 50µmol/kg: 383.0 ± 109.9 PFOA concentrations in liver (*Pparα*$^{(-/-)}$) (µg/ml): 12.5 µmol/kg: 172.3 ± 8.9 25 µmol/kg: 218.3 ± 14.5 50µmol/kg: 239.7 ± 25.0	
PFOA (ammonium salt, 70:30 linear:branched)	7-day RDT study Rat (Crl:CD(SD)BR) n/sex/group = 6 (males only) Exposure: oral (gavage) Dose: 0 (pair-fed), 50 mg/kg bw/d	**Body weight** (no difference compared to control; decrease in body weight in both control and treatment over 7 days) **Liver:** • Abs./rel. liver weight ↑ (≈ +119/+91% after 7 days) • Hepatic DNA ↓ (≈ –57% after 7 days) • Hepatic cytochrome P-450 activity ↑ (≈ +120% after 1 day, +220% after 3 days) • Hepatic benzphetamine N-demethylase activity ↑ (≈ +100% after 3 days) • Hepatic ethyresorufin O-deethylase activity ↓ (≈ –47% after 1 day; -51% after 3 days) • Hepatic carnitine acetyltransferase activity ↑ (≈ +1117% after 3 days) • Hepatic carnitine palmitoyltransferase activity ↑ (≈ +94% after 3 days) • Incorporation of [^{14}C] acetate into hepatic neutral lipids ↑ (≈ +2763% for triacylglycerols, ≈ +1200% for cholesterylesters, all			• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Pastoor et al., 1987)

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		after 7 days) • Incorporation of [^{14}C] acetate into hepatic neutral lipids ↑ (≈ +764% for phosphatidyl ethanolamine, ≈ +2433% for phosphatidyl serine, ≈ +1514% for phosphatidyl inositol, ≈ +937% for phosphatidyl choline, ≈ +543% for sphingomyelin, all after 7 days) **Clinical chemistry** • Plasma cholesterol and triacylglycerol (both ns after 7 days) **Remark:** The increased ^{14}C incorporation is interpreted as increased lipogenesis, which contrasts hypolipidemic effects of other peroxisome proliferators, e.g. decreased lipogenesis with DHEP					
PFOA (ammonium salt, 98% purity)	13-week-RDT study,+8-week recovery Rat (Crl:CDBR) n/sex/group = 5-15 (m only) Exposure: oral (dietary) Doses: 0, 1, 10, 30, 100 ppm (ad libitum control and pair-fed control with 100 ppm) equivalent to 0, 0.06, 0.64, 1.94, 6.5 mg/kg bw/d	**Body weight** ↓ after 1-13 weeks, but not in recovery (≈ –9% after 13 weeks compared to ad libitum, ns compared to pair-fed control) **Body weight gain** ↓ over weeks 1-13 (≈ –14% compared to ad libitum control, ns compared to pair-fed control) **Liver:** • Rel. liver weight ↑ (≈ +13% at LOEL after 4 weeks, after 7 and 13 weeks, LOEL at 30 ppm!, after 13 weeks significant reduction of -8% at 1 ppm…) • Hepatic palmitoyl CoA oxidase activity ↑ (≈ +75% at LOEL compared to ad libitum control after 4 weeks, after 7 and 13 weeks LOEL at 30 ppm (≈ +75% compared to pair-fed control after 13 weeks)) • Hepatocyte hypertrophy (minimal at LOEL, mild at 30 and 100 ppm) **Endocrine system:** • Estradiol, LH and testosterone (ns) **Remark:** Effects are reversible in recovery (Serum levels of PFOA decline rapidly in recovery). Overall, "study no effect level was 1 ppm (0.06 µg/mg) with doses of 10 ppm (0.64 µg/mg) and higher producing adaptive and reversible liver changes."	1.94 1.94 0.06 0.06 0.06	6.5 6.5 0.64 0.64 0.64	• kidney, nervous system, immune system, haematopoietic system, thyroid, clinical chemistry	Serum PFOA concentrations after 4 weeks: 0 ppm: - 0 (pair fed): - 1 ppm: 6.5 ± 1 10 ppm: 55.81 ± 8.1 30 ppm: 104 ± 14 100 ppm: 159+ ± 30 Serum PFOA concentrations after 7 weeks: 0 ppm: - 0 (pair fed): - 1 ppm: 7.5 ± 1.3 10 ppm: 46 ± 16 30 ppm: 87 ± 28 100 ppm: 149 ± 35 Serum PFOA concentrations after 13 weeks: 0 ppm: - 0 (pair fed): - 1 ppm: 7.1 ± 1.2 10 ppm: 41 ± 13 30 ppm: 70 ± 16 100 ppm: 138 ± 34 Serum PFOA concentrations after 21 weeks (13 weeks RDT + 8 weeks recovery): 0 ppm: - 0 (pair fed): not applicable	(Perkins et al., 2004)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
						1 ppm: 1.2 10 ppm: 1.1 ± 1.3 30 ppm: 1.6 ± 0.9 100 ppm: 2.5 ± 0.9	
PFOA (98% purity)	2/5/10 days Mouse (C57B1/6) n/sex/group = 3 (m only) Exposure: oral (dietary) Doses: 0, 0.02, 0.1% (data from all treatments only for 5 days) (equivalent to 0, 24, 120 mg/kg bw/d (calculations by ATSDR 3x higher)) (0.001, 0.003, 0.01% only for hepatic enzymes)	**Body weight ↓ (≈ –6% at LOEL after 5 days)** **Liver:** • Abs./rel. liver weight ↑ (≈ +75%/+112% at LOEL after 5 days) • Hepatic protein content ↑ (≈ +36% at LOEL after 5 days) • Hepatic mitochondrial protein content ↑ (≈ +767% at LOEL after 5 days) • Hepatic microsomal protein content ↑ (≈ +55% at LOEL after 5 days) • Hepatic peroxisomal catalase specific activity↑ (≈ +154% at LOEL after 5 days) • Hepatic lauroyl-CoA oxidase specific activity ↑ (≈ +171% at LOEL after 5 days) • Hepatic palmitoyl-CoA oxidation specific activity ↑ (+244% at LOEL after 5 days) • Hepatic cyanide-insensitive palmitoyl-CoA oxidation specific activity ↑ (≈ +312% at LOEL after 10 days)	– – – – 24 – – – –	24 24 24 24 120 24 24 24 1.2	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Permadi et al., 1993)
PFOA (98% purity)	10-day study Mouse (C57B1/6) n/sex/group = 3 (m only) Exposure: oral (dietary) Doses: 0, 0.02, (0.1)% (control only for 10 days, 0.1% only 2 and 5 days) (equivalent to 24, 120 mg/kg bw/d)	**Body weight and Liver mass and liver enzymes see Permadi et al. 1993 (same study)** **Liver:** • Microsomal cytochrome P450 ↑ (≈ +293% in Table5, +200% in Table 6) • Microsomal cytochrome P450 reductase ↑ (≈ +100% in Table5, +285% in Table 6) • Microsomal Cytochrome B5 (↑) (ns in Table 5, +70% in Table 6) • Microsomal epoxide hydrolase ↑ (≈ +239% in Table5, +191% in Table 6) • Hepatic cytosolic DT-diaphorase ↑ (≈ +391% in Table7, +438% in Table 8) • Hepatic cytosolic glutathione peroxidase (↓) (ns in Table7, –29% in Table 8) • Hepatic cytosolic SOD (↑) (ns in Table7, ≈ +53% in Table 8) • Hepatic cytosolic epoxide hydrolase ↑ (≈ +105% in Table7, +107% in Table 8) • Lipid peroxidation in mitochondrial fraction from liver (TBARS) ↓ (≈ –34%/–58% in Exp.1/Exp.2) • Lipid peroxidation in mitochondrial fraction from liver (O2			• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Permadi et al., 1992)

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Table 2: Repeated dose toxicity (RDT) animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
		consumption in Fe presence) ↓ (≈ –54%/–55% in Exp.1/Exp.2) **Remark:** Also data for 2 days (0.1%) or 5 days (0.02% and 0.1%) available, but in this publication only control levels for 10 days are reported. It is not clear, why data in Table 5 and 6/ table 7 and 8 are different…					
PFOA (96% purity)	10-day study Mouse (C57BL/6 (H-2^b)) n/sex/group = 4 (m only) Exposure: oral (dietary) Doses: 0, 0.001, 0.02 % (w/w) (equivalent to 0, 0.34, 4.3 mg/kg bw/d)	**Body weight** ↓ (data not shown) **Liver weight** ↑ (data not shown) **Immune system:** • Number of total white blood cells ↓ (≈ –72%) • Number of lymphocytes ↓ (≈ –73%) • Number of neutrophils ↓ (≈ –52%) • LPS-induced TNF-α production (*Ex vivo*, *in vitro* LPS-stimulation) ↑ (≈ +112% in peritoneal cavity, +103% in bone marrow, +14% (ns?) in spleen) • LPS-induced IL-6 levels (*Ex vivo*, *in vitro* LPS-stimulation) ↑ (≈ +33% in peritoneal cavity, +50% in bone marrow, +152% in spleen) • LPS-induced TNF-α production (*Ex vivo*, *in vivo* LPS-stimulation) ↑ (+126% in peritoneal cavity, +150% in bone marrow); ↓ (-73% in spleen) • LPS-induced IL-6 levels (*Ex vivo*, *in vivo* LPS-stimulation) ↑ (≈ +40% in peritoneal cavity, +150% in bone marrow); ↓ (≈ –60% in spleen) • Serum TNF-α levels after LPS injection ↑ (≈ +473%) • Serum IL-6 levels without LPS injection ↑ (≈ +150%) • Serum IL-6 levels with LPS injection ↑ (≈ +92%)	0.34 0.34 0.34 0.34 0.34 0.34 0.34 0.34 0.34	4.3 4.3 4.3 4.3 4.3 4.3 4.3 4.3 4.3	• kidney, nervous system, haematopoietic system, thyroid, endocrine system, clinical chemistry	Serum PFOA concentration after 10 days: 0.02%: 152 ± 8.6 µg/ml (357 ± 20.8 µM)	(Qazi et al., 2009)
PFOA (96% purity)	10-day-study Mouse (C57BL/6 (H-2^b)) n/sex/group = 4-7 (m only) Exposure: oral (dietary) Dose: 0, 0.002% (w/w) (equivalent to 0.65 mg/kg bw/d)	**Body weight gain (ns)** **Liver:** • Rel. liver weight ↑ (≈ +67%) • Histological alterations in structure of parenchymal cells • Hypertrophy of hepatocytes around central vein • Larger cell surface in centrilobular hepatocytes • Total number of intrahepatic immune cells ↑ (≈ +100%) • Total number of intrahepatic myeloid-, lymphoid-, and erythroid-related cells ↑ (e.g. +64% for granulocytes, +118% for macrophages, +267% for myeloid suppressor cells, +283% for erythroid progenitor TER119, +85% for NK cells, +70% for B cells, +78% for Helper T cells) **Clinical chemistry:** • Serum ALP ↑ (≈ +45%) • Serum total cholesterol ↓ (≈ –23%) • Serum triglycerides (≈ –36%)			• kidney, nervous system, haematopoietic system, thyroid, endocrine system	Serum PFOA concentration after 10 days: 0.002%: 87.6 ± 2.1 µg/ml (211.6 ± 5.1 µM)	(Qazi et al., 2010)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
PFOA (96% purity)	10-day study (+10 days recovery) Mouse (C57BL/6 (H-2^b)) n/sex/group = 8 (m only) Exposure: oral (dietary) Doses: 0, 0.001, 0.002%, 0.02 % (w/w) (control ad libitum or restricted/pair-fed to 0.02%) (equivalent to 0, 0.4, 0.75, 7.5 mg/kg bw/d)	**Body weight ↓ (≈ –27% at LOEL)** **Liver:** • Rel. liver weight ↑ (≈ +73% at LOEL) **Immune system:** • Rel. thymus weight ↓ (≈ –79% at LOEL) • Rel. spleen weight ↓ (≈ –44% at LOEL) • Rel. epididymal fat weight ↓ (≈ –93% at LOEL) • Number of myeloid cells in bone marrow ↓ (≈ –35% at LOEL) • Number of bone marrow B-lymphoid cells ↓ (≈ –27% at LOEL) **Remarks:** Most effects not recovered after 10 additional days. Pair-fed controls indicate that effects at 0.02% are likely due to reduced food uptake	0.75 – 0.75 0.75 0.75 0.75 0.4	7.5 0.4 7.5 7.5 7.5 7.5 0.75	• kidney, nervous system, haematopoietic system, thyroid, endocrine system, clinical chemistry		(Qazi et al., 2012)
PFOA (no purity specified, but supplier was Sigma Aldrich which today sells 95% purity PFOA)	5-week Mouse (C57BL/6 and BALB/c, premature) n/sex/group = 2x3 Exposure: oral (dietary) Doses: 0, 3.5 mg/kg diet (equivalent to 0.55 mg/kd bw/d at 4 weeks of age (males and females); at 10 weeks of age, male mice consumed ca. 0.33 and female mice ca. 0.44 mg PFOA/kg bw/d)	**Body weight (ns)** **Liver:** • Rel. liver weight (C57) ↑ (m/f, ≈ +67%/+66%) • Rel. liver weight (BALB/c) ↑ (m/f, ≈ +55%/+65%) • Liver cholesterol (BALB/c) ↓ (m/f, ≈ –80/60% in BALB/c mice) • PPARα gene ex (BALB/c) ↑ (m, ≈ +33%; f: ns) **Clinical chemistry** • Plasma cholesterol (C57) ↑ (m/f, ≈ +27/+62%) • Plasma cholesterol (BALB/c) ↑ (m, ≈ +24%; f: ns) **Reproductive tissue** • Cholesterol in mammary (C57) ↑ (f, ≈ +10%) • Cholesterol in ovary (C57) ↑ (f, ≈ +70%) **Remark:** This study concludes that PFOA effects may depend on diet composition (PFOA leads to higher cholesterol in blood)			• kidney, nervous system, haematopoietic system, thyroid	PFOA levels in plasma in C57 male/female mice: Control: 2/28 **ng**/mL PFOA exposed: 26.9/44.3 **µg**/mL PFOA levels in plasma in BALB/c mice: Control: 5/86 **ng**/mL PFOA-exposed: 28.2/35.6 **µg**/mL	(Rebholz et al., 2016)
PFOA (ammonium salt, 98% purity)	21-day study Mouse (ICR) n/sex/group = 10 (m only) Exposure: oral (drinking water) Doses: 0, 2, 10, 50, 250 ppm (mg/l drinking water) (equivalent to 0.49,	**Body weight gain ↓** (≈ –192% at LOEL) **Body weight ↓** (≈ –33% at LOEL, unclear at which day or whether averaged over all days) **Liver:** • Rel. liver weight ↑ (≈ +27%) • Liver ALT activity ↑ (≈ +189% at LOEL) • Liver AST activity ↑ (≈ +230% at LOEL) • Hepatic TNF-α gene ex ↓ (≈ –50% at LOEL) • Hepatic IL-1β gene ex ↓ (≈ –60% at LOEL) • Hepatic TGF-β gene ex ↑ (≈ +40% at LOEL) **Immune system:**	2.64 2.64 - 0.49 2.64 2.64 17.63 2.64	17.63 17.63 0.49 2.64 17.63 17.63 47.21 17.63	• nervous system, haematopoietic system, thyroid, endocrine system, clinical chemistry		(Son et al., 2008; Son et al., 2009)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
	2.64, 17.63, 47.21 mg/kg bw/d)	• Alterations of splenic T-lymphocyte populations (↓ in CD4-CD8-, CD4-CD8+, and CD4+CD8+; ↑ in CD4+CD8-) • Splenic CD4-CD8+ Lymphocytes ↓ (≈ –65% at LOEL) • Alterations of thymic T-lymphocyte populations (↑ in CD4-CD8-, CD4-CD8+; ↓ in CD4+CD8+) • Splenic TNF-α gene ex ↑ (≈ +83% at LOEL) • Splenic IL-1β gene ex ↑ (≈ +100% at LOEL) • Splenic TGF-β gene ex ↑ (≈ +100% at LOEL) • Splenic c-myc gene ex ↑ (≈ +150% at LOEL) • Thymic c-myc gene ex ↑ (≈ +90% at LOEL) **Remark:** increased ALT and AST suggest hepatotoxicity	– 17.63 2.64 17.63 17.63 2.64	0.49 47.21 17.63 47.21 47.21 17.63			
PFOA	3-week study Mouse (C57BL/6N) n/sex/group = 7-8 (m) Exposure: oral (dietary, high fat diet (HFD)) Doses: 0 (pair-fed to PFOA group), 5 mg/kg bw/d	**Body weight** ↓ (≈ –11%/–19% with normal/HF diet) **Liver:** • Abs. liver weight ↑ (≈ +136%/+254% with normal/HF diet) • Rel. liver weight ↑ (≈ +197%/+213% with normal/HF diet) • Liver triglyceride ↑ (≈ +70%/+80% with normal/HF diet) **Clinical chemistry:** • Plasma ALT ↑ (≈ +608%/+1173% with normal/HF diet) • Plasma AST ↑ (≈ +61%/+112% with normal/HF diet) • Plasma ALP ↑ (≈ +467%/+400% with normal/HF diet) • Plasma FFAs ↑ (≈ +24% only under HFD) **Other effects:** • Abs. epididymal white adipose tissue ↓ (≈ –78%/–63% with normal/high fat diet) • Rel. epididymal white adipose tissue ↓ (≈ –72%/–55% with normal/high fat diet) • Abs. subcutaneous white adipose tissue ↓ (≈ –72%/–66% with normal/high fat diet) • Rel. subcutaneous white adipose tissue ↓ (≈ –65%/–59% with normal/high fat diet) **Remark:** more gene expression and metabolomics data available (not listed here). PFOA effects stronger than HFD effects. PFOA and HFD effects add up in liver metabolism. "(…)HFD increases the risk of PFOA exposure (…)"			• Kidney, nervous system, thyroid, endocrine system		(Tan et al., 2013)
PFOA (96%)	7-day-study Mouse (Balb/c) n/sex/group = 3x5 (f)	**Body weight after 7 days** ↓ (≈ –23%) **Body weight gain** ↓ (≈ –12% in PFOA, +14% in controlà –186% in body weight gain, data in graph do not fit data from table) **Liver:** • Rel. liver weight ↑ (≈ +32/+10% without/with RVB 300)			• Kidney, nervous system, haematopoietic system, thyroid, endocrine		(Vetvicka and Vetvickova, 2013)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
	Exposure: oral (gavage, in PBS) Doses: 0, 20 mg/kg bw/d	**Immune system:** • Rel. spleen and thymus weight (ns) • Thymic cellularity ↓ (≈ –47/–21% without/with RVB 300) • T cell lymphocyte proliferation ↓ (≈ –22/–14% without/with RVB 300) • B cell lymphocyte proliferation ↓ (≈ –14% only without RVB 300) • Phagocytosis of peripheral neutrophils ↓ (≈ –57/–8% without/with RVB 300) • Splenic NK cell activity ↓ (≈ –64/–16% without/with RVB 300) **Remark:** PFOS data available. Focus of study on combined treatment of PFAS and RVB (Glucan-resveratrol-vitamin C), which basically counteracts PFOA effects.			system, clinical chemistry		
PFOA (purity was not specified, but Sigma Aldrich delivers in 95% purity)	7-day-study+2, 5, or 10 days recovery Mouse (C57BL/6) n/sex/group = 4 (m) Exposure: oral (dietary) Doses:0, 0.02% (w/w) (equivalent to 24 mg/kg bw/d)	**Body weight** ↓ (≈ –20%, recovery after 5 days) **Liver** • Abs liver weight ↑ (≈ +90%, further increase during recovery: +110% after 2 days, +130% after 5 days recovery, slow recovery after 10 days (+80%)) • Hepatic CPT activity ↑ (≈ +300%, slow recovery (down to +160% after 10 days)) • Hepatic COT activity ↑ (≈ +900%, slow recovery (down to +400% after 10 days) **Fat tissue** • Epididymal adipose tissue ↓ (≈ –80%, recovery after 10 days) • Retroperitoneal adipose tissue ↓ (≈ –90%, recovery after 5 days) • LPL activity of epididymal adipose tissue ↓ (≈ –61%, recovery after 5 days) **Clinical chemistry** • Serum triglycerides ↓ (≈ –29%, even further reduction after 2 days recovery (-57%), after 5 and 10 days recovery level comparable to levels of treatment day 7 • Serum cholesterol ↑ (≈ +100% after 2 days recovery, then recovery after 10 days)			• Kidney, nervous system, haematopoietic system, thyroid, endocrine system,		(Xie et al., 2003)
PFOA	10-day-study, mouse (C57BL/6) n/sex/group = 4 (m) Exposure: oral (dietary)	**Body weight** ↓ (≈ –17%) **Liver** • Abs./rel. liver weight ↑ (≈ +96%/+136) **Immune system:** • Abs. + rel. thymus weight ↓ (≈ –85%) • Abs.+ rel. thymic DNA contet ↓ (≈ –39% for rel) • Abs. + rel. spleen weight ↓ (≈ –30%) • Abs. splenic DNA content ↓ (≈ –49%)			• Kidney, nervous system, haematopoietic system, thyroid, endocrine system, clinical chemistry		(Yang et al., 2000)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
	Doses: 0, 0.02% (w/w) (equivalent to 30 mg/kg bw/d)	• Thymocyte cellularity ↓ (≈ –56% for CD4-CD8-, -95% for CD4+CD8+, -64% for CD4+, -72% for CD8+) • Splenocyte cellularity ↓ (≈ –75% for CD3+, -78% for CD4+, -74% for CD8+, -86% for CD19+) **Remark:** most effects already present after 5 days					
PFOA	10-day-study, 2-10 days Mouse (C57BL/6) n/sex/group = 4 (m) Exposure: oral (dietary) Doses: 0, 0.001%, 0.003%, 0.01%, 0.02%, 0.05% (w/w) (equivalent to 0, 1, 3.5, 11.5, 23, 57.5 mg/kg bw/d)	**Body weight** ↓ (≈ –15% at 0.02% after 5 days, data not shown for other doses) **Liver:** • Rel. liver weight ↑ (≈ +60% at LOEL) **Immune system:** • Rel. thymus weight ↓ (≈ –50% at LOEL) • Rel. spleen weight ↓ (≈ –35% at LOEL) • Hepatic acyl-CoA oxidase activity ↑ (≈ +300% with palmitoyl-CoA as substrate, +1000% with lauroyl-CoA as substrate, increase starts already on day 1) • Total number of thymocytes ↓ (≈ –90% at 0.02% after 7 days) • Thymocyte cellularity ↓ (≈ –96% for CD4+CD8+, –62% for CD4+, –77% for CD8+, –67% for CD4-CD8-; recovery complete after 10 days) • Splenocyte cellularity ↓ (≈ –73% for CD3+, –68% for CD4+, –63% for CD8+, –75% for CD19+; recovery complete after 10 days)	11.5 3.5 3.5 –	23 11.5 11.5 1	• Kidney, nervous system, haematopoietic system, thyroid, endocrine system, clinical chemistry		(Yang et al., 2001)
PFOA	7-day-study Mouse (C57BL/6 (WT) or PPARα-null) n/sex/group = 4 (m) Exposure: oral (dietary) Doses:0, 0.02% (w/w) (equivalent to 33.3 mg/kg bw/d (WT) or 29.8 mg/kg bw/d (PPARα))	**Body weight ↓ (≈ –14% in WT)** **Liver:** • Abs. liver weight ↑ (≈ +86%/+119% in WT/PPARα-null) **Immune system:** • Abs. spleen weight ↓ (≈ –39% in WT) • Splenocyte number ↓ (≈ –78% in WT) • Abs. thymus weight ↓ (≈ –79%/–39% in WT/ PPARα-null) • Thymocyte number ↓ (≈ –84%/–39% in WT/ PPARα-null) • Hepatic Palmitoyl-CoA oxidase activity ↑ (≈ +1900% in WT) • Thymocyte cellularity ↓ (≈ –93% for CD4+CD8+, –67% for CD4+CD8-, –68% for CD4-CD8+, –55% for CD4-CD8-) • Splenocyte cellularity ↓ (≈ –42% for CD3+, -85% for CD19+)			• kidney, nervous system, haematopoietic system, immune system, endocrine system, clinical chemistry		(Yang et al., 2002b)
PFOA	10-day-study, 2-10 days Mouse (C57BL/6) n/sex/group = 4 (m) Exposure: oral (dietary)	**Immune system:** • IgM ↓ (≈ –73%/-72% without/with HRBC- immunisation) • IgG1 ↓ (≈ –29%/–99.95% without/with HRBC-immunisation) • IgG2b ↓ (≈ –50%/–95% without/with HRBC-immunisation) • IgG3 ↓ (≈ –33%/–91% without/with HRBC-immunisation) **Remark:** IgM only antibody almost recovered after 6 days, other			• Body weight, liver, kidney, nervous system, haematopoietic system, endocrine system, clinical		(Yang et al., 2002a)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
	Doses: 0, 0.02% (w/w) (equivalent to 30 mg/kg bw/d)	antibodies: not fully recovered			chemistry		
PFOA (ammonium salt)	14 days-study + 14 days recovery Rabbit (albino) n/sex/group = 6-10 Exposure: **dermal (percutaneous, 40% of body surface area covered, 6h/day** (10 applications: 5 application days, 2 rest days, 5 application days, 2 days rest, then 14 days recovery**)** Dose: 100 mg/kg (no control group, only compared to initial values…)	**Body weight** decreased in 14 days of treatment (compared to intial values) and increased again after 14 recovery days (males and females; no control group)			•		(O'Malley and Ebbins, 1981)
PFOA (100%)	12-days Rat (Crl:CD) n/sex/group = 15 (m) Exposure: **Dermal** (skin patch on back, 15% of body surface), 6h/day (10 applications: 5 application days, 2 rest days, 5 application days) Doses: 0, 20, 200, 2000 mg/kg	**Body weight (day 5)** ↓ (≈ –13%) **Body weight (day 10)** ↓ (≈ –15%) **Body weight (recovery day 14)** ↓ (≈ –8%) **Liver** • Abs./rel. liver weight directly after last application ↑ (≈ +53%/+55% at LOEL) • Abs./rel. liver weight after 14 days of recovery ↑ (≈ +33%/+54% at LOEL) • Abs./rel. liver weight after 42 days of recovery ↑ (≈ +30%/+31% at LOEL) **Haematopoietic system:** • Red blood cell directly after last application ↑ (≈ +53%/+55% at LOEL) **Remarks:** some treatment related toxicity resolved during a 42-day recovery period (but not liver weight); dermal LD50 (7000 mg/kg for males, 7500 mg/kg for females)	20 20 20 0 20 200	200 200 200 20 200 2000	•	Organofluorine concentrations in blood (ppm) after last application: 0 mg/kg: 10.2 ± 5.5 20 mg/kg: 52.4 ± 3.7 200 mg/kg: 79.2 ± 29.9 2000 mg/kg: 117.8 ± 21.1 Organofluorine concentrations in blood (ppm) after 14 days recovery: 0 mg/kg: 2.7 ± 0.9 20 mg/kg: 9.5 ± 0.5 200 mg/kg: 26.2 ± 4.8 2000 mg/kg:44.8 ± 13.1 Organofluorine concentrations in blood (ppm) after 42 days recovery: 0 mg/kg: 0.5 ± 0.2 20 mg/kg: 1.2 ± 0.5 200 mg/kg: 3.7 ± 2.0	(Kennedy, 1985)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
						2000 mg/kg:8.2 ± 4.1	
PFOA (ammonium salt, 100%)	Repeated-dose-inhalation Rat (Crl:CD) n/sex/group = 24 (m) Exposure: **inhalation**, 6h/day (10 applications: 5 application days, 2 rest days, 5 application days) Doses: 0, 1, 8, 84 mg/m³	**Body weight (day 5) ↓ (≈ −7%)** **Body weight (recovery day 8) ↑ (≈ +5% at MD)** **Liver:** • Abs./rel. liver weight directly after last application ↑ (≈ +52%/+45% at LOEL) • Abs./rel. liver weight after 14 days of recovery ↑ (≈ +19%/+23% at LOEL) • Abs./rel. liver weight after 28 days of recovery ↑ (≈ +16%/+23% at LOEL) **Clinical chemistry:** • Serum ALP activity (after last application) ↑ (≈ +36% at LOEL) • Serum ALP activity (after 14 days recovery) ↑ (≈ +38% at LOEL)	8 1 1/8 1/8 1 8	84 8 8/84 8/84 8 84	•	APFO concentrations in blood (ppm) after last application: 0 mg/m³: 1.4 1 mg/m³: 13 8 mg/m³: 47 84 mg/m³: 108 Organofluorine concentrations in blood (ppm) after 14 days recovery: 0 mg/m³: 0.28 1 mg/m³: NA 8 mg/m³: NA 84 mg/m³: 10 Organofluorine concentrations in blood (ppm) after 28 days recovery: 0 mg/m³: 0.10 1 mg/m³: 1.2 8 mg/m³: 3.8 84 mg/m³: 7.1 Organofluorine concentrations in blood (ppm) after 42 days recovery: 0 mg/m³: 0.032 1 mg/m³: NA 8 mg/m³: NA 84 mg/m³: 1.8 Organofluorine concentrations in blood (ppm) after 84 days recovery: 0 mg/m³: 0.015 1 mg/m³: NA 8 mg/m³: NA 84 mg/m³: 0.84	(Kennedy et al., 1986)
PFOA (>98%)	28-day RDT study, NTP study, similar to OECD TG 407	**Body weight** ↓ (m, 5 mg/kg bw/d: ≈ −12%, 10 mg/kg bw/d: −19%) **Liver:** • Abs.+ rel. liver weight ↑ (m/f, ≈ +16/14% at LOAEL)	1.25/100 −/12.5	2.5/− 0.625/25	•	Although females were administered a 10-fold higher dose of PFOA, males had a	(NTP, 2019b)

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Table 2: Repeated dose toxicity (RDT) animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
	Rat (SD) n/sex/group = 10 (m+f) Exposure: oral (gavage, in distilled water) Doses (m): 0, 0.625, 1.25, 2.5, 5, 10 mg/kg bw/d Doses (f): 0, 6.25, 12.5, 25, 50, 100 mg/kg bw/d	• Hepatocyte hypertrophy ↑ • Acetyl-CoA activity↑ (m, ≈ +222% at LOAEL) • Gene expression of *Acox1, Cyp4a1, Cyp2b1, Cyp2b2* ↑ (≈ +246-1971% at LOAEL) **Kidney:** • Abs./rel. kidney weight ↑ (m/f, ≈ +11/+7% at LOAEL) **Immune system:** • Abs. spleen weight ↓ (m, ≈ −12% at LOAEL; f: ns) • Abs. thymus weight ↓ (m, ≈ −12% at LOAEL; f: ns) **Thyroid:** • Rel. thyroid weight ↑ (m, ≈ +17% at LOAEL; f: ns)) • Thyroid gland follicular cell hypertrophy **Haematopoietic system:** • Erythrocytes , haemoglobin, Haematocrit ↓ (m, ≈ −4-6% at LOAEL) • Reticulocyte ↓ (m, ≈−15% at LOAEL) **Clinical chemistry:** • ALT ↑ (m, ≈ +19-47%) • ALP ↑ (m, ≈ +13-89%) • ALP ↑ (m, ≈ +22-38%) • Cholesterol ↓ (m, ≈ -37%) **Endocrine system (m):** • T3 (≈ −40% at LOAEL), free T4 (≈ −79% at LOAEL), total T4 ↓ (≈ −91% at LOAEL) **Reproductive Tissue (m only):** • L. Cauda Epididymis weight ↓ (m, ≈ -11% at LOAEL) • L. Epididymis weight ↓ (m, ≈ -9% at LOAEL) • Sperm (10^6/g cauda epididymis) ↓ (m, ≈ -24% at LOAEL) **Other effects:** • Hyperplasia/inflammation of respiratory epithelium	−/50 − − −/25 1.25 5 0.625 5/50 0.625 − − − 1,25 − −/50 2.5 5 5 −/6.25	0.625/100 0.625 0.625 0.625/50 2.5 10 1.25 10/100 1.25 0.625 0.625 0.625 2.5 0.625 0.625/100 5 10 10 0.625/12.5		higher plasma concentration compared to females across the dose groups (up to 1,000-fold higher levels)	
PFNA: Perfluorononanoic acid (Syn: Heptadecafluorononanoic Acid, **CAS no:** 375-95-1, **EC no:** 206-801-3, **Mol. formula:** $C_9HF_{17}O_2$, **MW:** 464,xx?; registered under REACH, self classification available)							
PFNA, Perfluorononanoic acid (analytical grade), EC: 206-801-3, CAS: 375-95-1, $C_9HF_{17}O_2$	5-day RDT study, non-guideline study Rat (Wistar) n/sex/group = 4 Exposure: i.p. injection (vehicle: propyleneglycol:water (1:1, v:v)	**Liver:** • Peroxisomal β-oxidation ↑ (m/f) **Remarks:** Mechanistic study to assess the MoA; peroxisomal β-oxidation is statistically highly correlated with PFCA concentration in the liver (r=0.850, p<0.001) à internal dose in liver decisive of effect not carbon chain length or sex	−/5	2.5/10	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system	PFNA conc. in the liver at highest dose: m: 358 ± 19 µg/g liver f: 102 ± 11 µg/g liver	(Kudo et al., 2000)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
	Doses: 0, 2.5, 5, 10, 15, 20 mg/kg bw/d PFCAs assessed: PFHpA, PFOA, PFNA, PFDA						
PFNA	5-day RDT study, non-guideline study Mouse (ddy) n/sex/group = 3-5 Exposure: i.p. injection (vehicle: propyleneglycol:water (1:1, v:v) Doses: 0, 2.5, 5, 10, 15, 20 mg/kg bw/d PFCAs assessed: PFHxA, PFHpA, PFOA, PFNA	**Liver:** • Abs./rel. liver weight ↑ • Peroxisomal β-oxidation ↑ (m/f) • Activity of hepatic microsomal 1-Acly-GPC acyltransferase (m, only 20 mg/kg bw/d tested) ↑ **Remarks:** Mechanistic study to assess the MoA; hepatic peroxisomal β-oxidation is statistically highly significantly correlated with PFCA concentration in the liver (m: r=0.9201, p<0.001; f: r=0.9254, p<0.001) à internal dose in liver decisive for effect	 –/– –/– –	 2.5/2.5 2.5/2.5 20	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system	C-length and sex dependent accumulation of PFCAs in the liver (higher concentrations in males and with higher C length)	(Kudo et al., 2006)
PFNA (97%)	14-days RDT study, non-guideline Rat (SD) n/sex/group = 6 (m) Exposure: oral (gavage, in distilled water) Doses: 0, 0.2, 1, 5 mg/kg bw/d	**Liver:** • Hepatocytes with focal vacuolations ↑ • mRNA levels of CYP4A1 and ACOX ↑ • Hepatic TNFα level ↑ • Lipid accumulation in liver ↑ **Remarks:** Mechanistic study to assess the MoA	 1 – – 1	 5 0.2 0.2 5	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Fang et al., 2012b)
PFNA (97%)	14-days RDT study, non-guideline Rat (SD) n/sex/group = 6 (m) Exposure: oral (gavage, in distilled water) Doses: 0, 0.2, 1, 5 mg/kg bw/d	**Liver:** • MDA in liver ↑ • Glycogen in liver ↑ • mRNA of G6PC/GLUT2 ↑ **Clinical chemistry:** • Serum glucose ↑ (≈ +9% at LOEL) • Serum HDL ↓ (≈ -18-55%) • Serum LDL ↑ (≈ +100% at LOEL) **Remarks:** Mechanistic study to assess the MoA: PFNA caused oxidative stress in liver and inhibited hepatic insulin signal pathway, accelerating the output of glucose and increasing glycogen synthesis	 1 1 1 0.2 – 1	 5 5 5 1 0.2 5	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Fang et al., 2012a)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentrate-ion of PFAS /metabolites (time of sampling)	Reference
		in the rat liver					
PFNA (97%)	7-days RDT study, non-guideline Rat (SD, diabetes induced via streptozotocin) n/sex/group = 10 (m) Exposure: oral (gavage, in distilled water) Doses: 0, 0.2, 1, 5 mg/kg bw/d	**Liver:** • Hepatic cholesterol ↑ (≈ +91% at LOEL) • Hepatic triglycerides ↑ (≈ +180% at LOEL) • G6P-dehydrogenase activity ↑ (≈ +19% at LOEL) • Hepatic lipase ↓ (≈ -68% at LOEL) **Clinical chemistry:** • Serum ALT ↑ (≈ +60% at LOEL) **Remarks:** Mechanistic study to assess the MoA	0.2 1 0.2 1 0.2	1 5 1 5 1	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Fang et al., 2015)
PFNA (97%)	14-days RDT study, non-guideline Mouse (BALB/c (SD)) n/sex/group = 6 (m) Exposure: oral (gavage) Doses: 0, 1, 3, 5 mg/kg bw/d	**Body weight**↓ (≈ –15% at LOEL) • Body weight gain ↓ (≈ –517%) **Immune system:** • Abs. spleen weight ↓ (≈ –9.6% (text), ≈ –34% (graph) at LOEL) • Rel. spleen weight ↓ (≈ –32%) • Splenocyte apoptosis ↑ (≈ +90%) • Abs./rel. thymus weight ↓ (≈ –33.3%/–39% at LOEL) • Thymocyte apoptosis ↑ (≈ –+67%) • IL-4 in spleen homogenates ↓ (≈ –17%) • IFN-γ in spleen homogenates ↓ (≈ –50%) • Thymic gene expression of PPAR-α ↑ (≈ +510%/6.1-fold at 1 mg/kg) • Thymic gene expression of PPAR-γ ↑ (≈ +160%/2.6-fold at 1 mg/kg) • Thymic gene expression of IL-1-γ ↑ (≈ +200%/3-fold at 1 mg/kg) **Endocrine system:** • ACTH ↑ (≈ +72% at LOEL) • Cortisol ↑ (≈ +17% at LOEL)	1 1 3 3 1 - 3 3 1	3 3 5 5 3 5 1 5 5 3	• Liver, kidney, nervous system, Clinical chemistry		(Fang et al., 2008)
PFNA (97%)	14-day-study Rat (SD) n/sex/group =6 (m) Exposure: Oral (gavage) Doses: 0, 1, 3, 5 mg/kg bw/d	**Body weight** ↓ (≈ –18% at LOEL) **Immune system:** • Abs. thymus weight ↓ (≈ –20% at LOEL) • Rel. thymus weight ↓ (≈ –58% at LOEL) • Thymocyte apoptosis ↑ • Serum IL-1 ↑ (≈ +215% at LOEL) • Serum IL-2 ↓ (≈ –29% at LOEL) • Serum IL-4 ↑ (≈ +106% at LOEL) • Thymic PPARα gene ex. ↑ (≈ +120% at LOEL) • Thymic PPARγ gene ex. ↑ (≈ +190% at LOEL) **Endocrine system:**	1 1 3 1 1 3 - 1	3 3 5 3 3 5 1 3	• Liver, kidney, nervous system, Clinical chemistry		(Fang et al., 2009)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
		• Cortisol ↑ (≈ +67% at LOEL) **Remark:** More data (mainly related to MAPK and NF-κB signalling pathways) available Feng 2009, 2010 and Fang 2009, 2010 probably all one experiment and different endpoints published separately	3	5			
PFNA (97%) 14-day-study Rat (SD) n/sex/group = 6 (m) Exposure: oral (gavage) Doses: 0, 1, 3, 5 mg/kg bw/d		**Immune system** • Abs. spleen weight ↓ (≈ –22% at LOEL) • Rel. spleen weight ↓ (≈ –8% at LOEL) • Lymphoid cell apoptosis in spleen ↑ • Splenic IL-1 ↑ (≈ +49% at LOEL) • Splenic IL-6 ↑ (≈ +51% at LOEL) • Splenic TNF-α ↑ (≈ +30% at LOEL) • Splenic IFNγ ↓ (≈ –35% at LOEL) • Splenic IL-10 ↓ (≈ –57% at LOEL) • Splenic H_2O_2 conc. ↑ (≈ +31% at LOEL) • Splenic SOD activity ↓ (≈ –42% at LOEL) • Splenic PPARα gene ex. ↑ (≈ +160% at LOEL) • Splenic PPARγ gene ex. ↑ (≈ +130% at LOEL) **Remark:** IL-1, IL-6, IFNγ, and TNF-α are pro-inflammatory, IL-10 is anti-inflammatory. More protein expression data (by Western blotting) available	- 3 1 3 3 3 3 3 3 1 1 1	1 5 3 5 5 5 5 5 5 3 3 3	• Body weight, liver, kidney, nervous system, endocrine system, clinical chemistry		(Fang et al., 2010)
PFNA (97%) 14-day-study Rat (SD) n/sex/group =6 (m) Exposure: oral (gavage) Doses: 0, 1, 3, 5 mg/kg bw/d		**Endocrine system** • Serum testosterone ↓ (≈ –85% at LOEL) • Serum estradiol ↑ (≈ +104% at LOEL) • Apoptotic cells in testes ↑ (≈ +367% at LOEL) • Fas gene ex ↑ (≈ +90% at LOEL) • Bax gene ex ↑ (≈ +36% at LOEL) • Bcl-2 gene ex ↓ (≈ –27% at LOEL)	3 3 1 3 3 1	5 5 3 5 5 3	•		(Feng et al., 2009)
PFNA (97%) 14-day-study Rat (SD) n/sex/group =6 (m) Exposure: oral (gavage)		**Reproductive tissue** • Vacuolization between Sertoli cells and spermatogonia ↑ • Testicular WT1 protein levels ↑ (≈ +59% at LOEL) • Testicular transferrin protein levels ↓ (≈ –33% at LOEL) • Serum MIS ↑ (≈ +22% at LOEL) • Serum inhibin B ↓ (≈ –10% at LOEL)	1 - - 3 -	3 1 1 5 1	•		(Feng et al., 2010)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentrate-ion of PFAS /metabolites (time of sampling)	Reference
	Doses: 0, 1, 3, 5 mg/kg bw/d	**Remark:** in vitro study with primary sertoli cells also available. Overall, the study shows that PFNA treatment led to the damage of specific secretory functions of Sertoli cells					
PFNA (ammonium salt, 99%)	14-day RDT studies Mouse (Crl:CD-1) n/sex/group = 5 Exposure: oral (dietary) Doses: 0, 3, 10, 30, 300, 3000 ppm (equivalent to 0, 0.38, 1.3, 3.8, 38, 380 mg/kg bw/d)	**Mortality** ↑ (all mice at 300 and 3000 ppm died before end of study) **Body weight** ↓ (data not shown) **Liver** • Abs. liver weight ↑ (m/f, ≈ +58/+56% at LOEL) • Rel. liver weight ↑ (m/f, ≈ +48/+35% at LOEL)	3.8 1.3 -/- -/-	38 3.8 0.38/0.38 0.38/0.38	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Kennedy, 1987)
PFNA (97%)	14-days RDT study, non-guideline Mouse (Balb/c) n/sex/group = 8 (m) Exposure: oral (gavage) Doses: 0, 0.2, 1, 5 mg/kg bw/d	**Body weight** ↓ (≈ -29% at LOAEL) **Liver:** • Rel. liver weight ↑ (≈ +9–140%) • Hepatic triglycerides ↑ (≈ +67% at LOEL) • Hepatic cholesterol ↑ (≈ +18% at LOEL) • mRNA of Cyp4A10/ACOX1 ↑ (≈ +% at LOEL) **Clinical chemistry:** • Serum ALT ↑ (≈ +275% at LOEL) • Serum AST ↑ (≈ +185% at LOEL) • Serum triglyceride ↓ (≈ -71% at LOEL) • Serum cholesterol ↓ (≈ -33% at LOEL) **Remarks** Mechanistic study to assess the MoA	1 – – – – 1 1 1 1	5 0.2 0.2 0.2 0.2 5 5 5 5	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Wang et al., 2015)
PFNA (purity not specified)	14-days RDT study, non-guideline study Rat (Wistar) n/sex/group = 10 (m) Exposure: oral (gavage) Doses: 0, 0.0125, 0.25, 5 mg/kg bw/d	**Body weight** ↓ (≈ -31% at LOAEL) **Liver:** • Hepatic OATP4C1 protein ↓ **Clinical chemistry:** • Serum corticosterone ↑	0.25 – –	5 0.0125 0.0125	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system	Serum levels (µg/ml): at 0.0125 mg/kg bw/d: 0.396 at 0.25 mg/kg bw/d: 30 at 5 mg/kg bw/d: 602	(Hadrup et al., 2016)

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PFNA (purity not specified)	3-day RDT study, non-guideline study Mouse (C57BL/6) n/sex/group = 4 Exposure: i.p. (vehicle: corn oil) Doses: 0 , 20 mg/kg bw/d	**Liver:** • Rel. liver weight ↑ (≈ +88%) • mRNA of Cyp4A10 and Cyp2b10 ↑ **Remarks** Mechanistic study to assess the MoA	– –	20 20	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Abe et al., 2017)
PFNA	7-day RDT study, non-guideline study Mouse (SV129) n/sex/group = 4 (m) Exposure: oral (gavage) Doses: 0 , 10 mg/kg bw/d	**Liver:** • Abs. + rel. liver weight ↑ (≈ +120%) • Hepatic triglyceride ↑ (≈ +260%) • Hepatic lipid content ↑ (≈ +540%) **Remarks** Mechanistic study to assess the MoA of PFOA, PFNA, PFHxS	– – –	10 10 10	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Das et al., 2017)
PFNA	7-day RDT study, non-guideline study Mouse (129S1/SvlmJ) n/sex/group = 4 (m) Exposure: oral (gavage) Doses: 0 , 1, 3 mg/kg bw/d	**Liver:** • Abs. + rel. liver weight ↑ (≈ +56%) **Remarks** Mechanistic study to assess the MoA of PFOA, PFNA, PFHxS, PFOS: gene expression profiles significantly similar to profiles from mouse tissues exposed to agonists of the constitutive activated receptor (CAR), estrogen receptor α (ERα), and PPARγ; PPARγ and ERα were activated by all four PFCAs in trans-activation assays from the ToxCast screening program	–	1	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Rosen et al., 2017)
PFNA (>98%)	28-day RDT study, NTP study, similar to OECD TG 407 Rat (SD) n/sex/group = 10 Exposure: oral (gavage, in distilled water)	**Mortality** ↑ (m/f, ≈80-90% at LOAEL) **Body weight** ↓ (m/f, ≈ −17/−10% at LOAEL) **Liver:** • Abs. + rel. liver weight ↑ (m/f, ≈ +23/+21% at LOAEL) • Hepatocyte, hypertrophy ↑ (m, 70% incidence at LOAEL) • Hepatocyte, cytoplasmic alteration ↑ (m, 100% incidence at LOAEL) • Hepatocyte necrosis ↑ (m, 50% incidence at LOAEL) • Hepatocyte, cytoplasmic vacuolization (m, 60% incidence at LOAEL) • Acetyl-CoA activity ↑ (m, ≈ +391% at LOAEL) • Gene expression of *Acox1, Cyp4a1, Cyp2b1, Cyp2b2* ↑ (≈ +216-	2.5/6.25 0.625/ 1.56 – –/3.12 –/– 1.25/6.25 0.625 – –	5/12.5 1.25/3.12 0.625/1.56 0.625/6.25 0.625/1.56 2.5/12.5 1.25 0.625/1.56 0.625/1.56	•	Plasma concentrations normalized to dose generally increased with dose in males and were similar across the available doses in females; Normalized plasma concentrations were generally five- to nine-fold higher in males compared to females	(NTP, 2019b)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
		1889% at LOAEL)					
	Doses (m): 0, 0.625, 1.25, 2.5, 5, 10 mg/kg bw/d Doses (f): 0, 1.56, 3.12, 6.25, 12.5, 25 mg/kg bw/d	**Kidney:**				Plasma concentrations after 28-d in m (µg/mL): 0.625 mg/kg bw/d: 57 1.25 mg/kg bw/d: 161 2.5 mg/kg bw/d: 380 5 mg/kg bw/d: 358 Liver concentrations after 28-d in m (µg/mL): 0.625 mg/kg bw/d: 146 1.25 mg/kg bw/d: 249 2.5 mg/kg bw/d: 311 5 mg/kg bw/d: 313 Plasma concentrations after 28-d in f (µg/mL): 1.56 mg/kg bw/d: 26.4 3.12 mg/kg bw/d: 54.4 6.26 mg/kg bw/d: 112.2 12.5 mg/kg bw/d: too less survivor	
		• Abs.+ rel. kidney weight ↑ (m/f, ≈ +15/+7% at LOAEL)	–/–	0.625/1.56			
		Immune system:					
		• Abs. spleen weight ↓ (m/f, ≈ –26/–11% at LOAEL)	0.625/1.56	1.25/3.12			
		• Rel. Spleen weight ↓ (m/f, ≈ –12/–11% at LOAEL)	0.625/3.12	1.25/6.25			
		• Abs. thymus weight ↓ (m, ≈ –31% at LOAEL; f: ns)	0.625	1.25			
		• Rel. thymus weight↓ (m, ≈ –18% at LOAEL; f: ns)	0.625	1.25			
		• Bone marrow hypocellularity	1.25/6.25	2.5/12.5			
		• Spleen atrophy	2.5/6.25	5/12.5			
		• Thymus atrophy	1.25/6.25	2.5/12.5			
		• Lymph node mandibular atrophy	2.5/6.25	5/12.5			
		• Lymph node mesenteric atrophy	2.5/6.25	5/12.5			
		Thyroid:					
		• Abs./rel. Thyroid weight ↑ (only m; f: NA)	1.25	2.5			
		Haematopoietic system (only m):					
		• Leukocytes , lymphocytes ↓ (m, ≈ –42/–51% at LOAEL)	1.25	2.5			
		• Reticulocyte ↓ (m, ≈–19% at LOAEL)	–	0.625			
		Endocrine system (m):					
		• Testosterone in m ↓ (≈ –81% at LOEL)	1.25	2.5			
		• Testosterone in f ↑ (≈ +30% at LOEL)	–	1.56			
		• Free T4 (≈ –75% at LOAEL), total T4 ↓ (≈ –91% at LOAEL)	–/1.56	0.625/3.12			
		• Thyroid stimulating hormone ↓ (≈ –46% at LOAEL)	0.625	1.25			
		Clinical chemistry:					
		• Total protein, globulin, albumin ↓	–/–	0.625/1.56			
		• Urea nitrogen ↑	–/1.56	0.625/3.12			
		• ALT ↑ (m, ≈ +48% at LOAEL)	0.625/1.56	1.25/3.12			
		• ALP ↑ (m, ≈ +92% at LOAEL)	0.625/1.56	1.25/3.12			
		• AST ↑ (m, ≈ +13% at LOAEL)	0.625	1.25			
		• Cholesterol ↓ (m, ≈ -26% at LOAEL)	–	0.625			
		• Triglycerides ↓ (m, ≈ -51% at LOAEL)	–	0.625			
		• Bile salts ↑ (m, ≈ +111% at LOAEL)	–	0.625			
		Reproductive Tissue (m only):					
		• L. Cauda Epididymis weight ↓ (m, ≈ -11% at LOAEL)	0.625	1.25			
		• L. Epididymis weight ↓ (m, ≈ -7% at LOAEL)	–	0.625			
		• Testis weight ↓ (m, ≈ -7% at LOAEL)	0.625	1.25			
		• Sperm (10^6/g cauda epididymis) ↓ (m, ≈ -18% at LOAEL)	0.625	1.25			
		• Germinal epithelium degeneration ↑	1.25	2.5			
		• Interstitial cell atrophy in testes ↑	1.25	2.5			
		• Seminiferous tubule spermatid retention ↑	1.25	2.5			
		Other effects:					

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
		• Olfactory Epithelium Degeneration in f ↑ Forestomach: Epithelium hyperplasia, chronic inflammation ↑	12.5 1.25/6.25	25 2.5/12.5			
PFDA: Perfluorodecanoic acid (Syn: Nonadecafluorodecanoic acid, **CAS no:** 335-76-2, **EC no:** 206-400-3, **Mol. formula:** $C_{10}HF_{19}O_2$, **MW:** 514,xx?; registered under REACH, self classification available)							
PFDA, Perfluorodecanoic acid (purity not specified), EC: 206-400-3, CAS: 335-76-2, $C_{10}HF_{19}O_2$	7-day RDT study, non-guideline study Rat (Wistar) n/sex/group = 4 (m) Exposure: oral (diet) Doses: 0, 0.00125, 0.0025, 0.005 or 0.01% (equivalent to 0 , 1.5, 3, 6, 12 mg/kg bw/d)	**Body weight** ↓ **Liver:** • Abs./rel. liver weight ↑ • Peroxisomal β-oxidation ↑ • Microsomal I-acyl-GPC acyltransferase ↑ • Cytosolic long-chain acyl-CoA hydrolase ↑ • GSH S-transferase ↓ • Hepatic Triacylglycerol ↑ • Hepatic Cholesterol ↑	6 – 1.5 – 1.5 3 – 6	12 1.5 3 1.5 3 6 1.5 12	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Kawashima et al., 1995)
PFDA (analytical grade)	5-day RDT study, non-guideline study Rat (Wistar) n/sex/group = 4 Exposure: i.p. injection (vehicle: propyleneglycol:water (1:1, v:v) Doses: 0, 2.5, 5, 10, 15, 20 mg/kg bw/d PFCAs assessed: PFHpA, PFOA, PFNA, PFDA	**Liver:** • Peroxisomal β-oxidation ↑ (m/f) **Remarks** Mechanistic study to assess the MoA; peroxisomal β-oxidation is statistically highly correlated with PFCA concentration in the liver (r=0.850, p<0.001) à internal dose in liver decisive of effect not carbon chain length or sex	2.5/2.5	5/5	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system	PFDA conc. in the liver at highest dose: m: 453 ± 19 µg/g liver f: 412 ± 33 µg/g liver	(Kudo et al., 2000)
PFDA (purity not specified)	3-day RDT study, non-guideline study Mouse (C57BL/6) n/sex/group = 4 (sex not reported) Exposure: i.p. (vehicle: corn oil) Doses: 0, 20 mg/kg bw/d	**Liver:** • Rel. liver weight ↑ (≈ +55%) • mRNA of Cyp4A10 ↑ **Remarks** Mechanistic study to assess the MoA	– –	20 20	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Abe et al., 2017)
PFDA (97.3%)	28-day RDT study, NTP study to assess	**Body weight** ↓ (≈ -22%)	1	2	• nervous system,		(Frawley et

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Table 2: Repeated dose toxicity (RDT) animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
	immunotoxicity and hepatotoxicity Rat (SD) n/sex/group = 8 (f) Exposure: oral (gavage) Doses: 0, 0.125, 0.25, 0.5, 1, 2 mg/kg bw/d	**Liver:** • Abs. liver weight ↑ • Rel. liver weight ↑ • Single cell hepatocyte necrosis (minimal severity) ↑ **Kidney:** • Abs. kidney weight ↑ • Rel. kidney weight ↑ **Haematopoietic system:** • Mean corpuscular haemoglobin ↓ (≈ −6% at LOAEL)	 0.125 – 0.25 0.25 0.125 0.125	 0.25 0.125 0.5 0.5 0.25 0.25	thyroid, endocrine system		al., 2018)
PFDA	28-day RDT study, NTP study to assess immunotoxicity and hepatotoxicity Mouse (B6C3F1/N) n/sex/group = 8 (f) Exposure: oral (gavage) Doses: 0, 0.31, 0.625, 1.25, 2.5, 5 mg/kg bw/**week**	**LOAEL/NOAELs given in <u>mg/kg bw/week</u>** **Liver:** • Abs./rel. liver weight ↑ **Immune system:** • Rel. spleen weight ↑ • Total spleen cell numbers ↓ (≈ –24% at LOAEL) • Number of B-cells ↓ (≈ –27% at LOAEL) • Number of T-cells ↓ (≈ –22% at LOAEL) • Number of T-helper cells ↓ (≈ –19% at LOAEL) • Number of cytotoxic T-lymphocytes ↓ (≈ –19% at LOAEL) • Number of NK cells ↓ (≈ –18% at LOAEL) • Number of macrophages ↓ (≈ –21% at LOAEL)	 0.31 0.625 2.5 2.5 0.625 0.625 0.31 0.625 0.31	 0.625 1.25 5 5 1.25 1.25 0.625 1.25 0.625	• nervous system, thyroid, endocrine system		(Frawley et al., 2018)
PFDA (98%)	10-day-study Mouse (C57B1/6) n/Sex/group = 4 (m) Exposure: oral (dietary) Doses: 0, 0.02% (w/w) (equivalent to 24 mg/kg bw/d)	**Body weight ↓ (≈ –32%)** **Liver:** • Abs./rel. liver weight ↑ (≈ +36%/+100%) • Hepatic protein content ↑ (≈ +24%) • Hepatic mitochondrial protein content ↑ (≈ +298%) • Hepatic cytosolic protein content ↓ (≈ –29%) • Hepatic peroxisomal catalase specific/total activity ↓↑ (≈ –58%/+66%) • Hepatic lauroyl-CoA oxidase specific/total activity ↑ (≈ +257%/+1325%) • Hepatic palmitoyl-CoA oxidation specific/total activity ↑ (≈ +442%/+1908%) • Microsomal cytochrome P450 reductase ↑ (≈ +251%) • Microsomal epoxide hydrolase ↑ (≈ +125%) • Hepatic cytosolic DT-diaphorase ↑ (≈ +149%) • Hepatic cytosolic glutathione peroxidase ↓ (≈ –36%) • Hepatic cytosolic SOD ↑ (≈ +59%)			• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Permadi et al., 1992; Permadi et al., 1993)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
		• Hepatic cytosolic epoxide hydrolase ↑ (≈ +144%)					
PFDA 96%	10 day, non-guideline ReproTox study Mouse (C57BL/6N) n/sex/group = 10-14 Exposure: oral (gavage, in corn oil) Doses: 0, 0.25, 0.5, 1, 2, 4, 8, 16, 32 mg/kg bw/day on gestation days (GD) 10-13 (4 consecutive days) or 0, 0.03, 0.1, 0.3, 1, 3, 6.4, or 12.8 mg/kg bw/day, on GD 6-15 (10 consecutive days)	**Gd 10-13:** • Maternal body weight gain ↓ (≈ –61% at LOEL) • Abs. liver weight ↑ (≈ +10% at LOEL) • Rel. liver weight ↑ (≈ +3% at LOEL) • **Gd 6-15:** • Maternal body weight gain ↓ (≈ –92% at LOEL) • Abs./rel. liver weight ↑ (≈ +61%/+127% at LOEL)	 8 0.5 0.25 3 0.3	 16 1 0.5 6.4 1	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Harris and Birnbaum, 1989)
PFDA (>98%)	28-day RDT study, NTP study, similar to OECD TG 407 Rat (SD) n/sex/group = 10 Exposure: oral (gavage, in distilled water) Doses: males: 0, 0.156, 0.312, 0.625, 1.25, or 2.5 mg/kg bw/d	**Body weight** ↓ (m: ≈ –21–38%, f: ≈ –12–36% at LOAEL) **Liver:** • Abs./rel. liver weight ↑ (≈ +11/12% in m/f at LOAEL) • Hepatocyte, hypertrophy ↑ (≈ 80-100% incidence at LOAEL) • Hepatocyte, cytoplasmic alteration ↑ (80-100% incidence at LOAEL) • Hepatocyte necrosis ↑ (f only, 40% incidence at LOAEL) • Hepatocyte, cytoplasmic vacuolization (90-100% incidence at LOAEL) • Acetyl-CoA activity ↑ (m only, ≈ +22-404%) • Gene expression of *Acox1, Cyp4a1, Cyp2b1, Cyp2b2* ↑ **Kidney:** • Abs./rel. kidney weight ↑ (≈ +8–15% at LOAEL) **Immune system:** • Abs. spleen weight ↓ (m/f, ≈ –26/–36% at LOAEL) • Rel. spleen weight ↓ (m/f, ≈ –19/–9% at LOAEL) • Abs.thymus weight ↓ (m/f, ≈ –44/–65% at LOAEL) • Rel. thymus weight ↓ (m/f, ≈ –29/–46% at LOAEL) • Bone marrow hypocellularity (100% incidence at LOAEL) • Thymus atrophy (80-90% incidence at LOAEL) **Thyroid:** • Abs.+rel. thyroid weight ↑ (m/f, ≈ +27–45%) **Haematopoietic system (only in m determined):** • Reticulocytes ↓ (≈–54–62% at LOAEL)	0.625/0.625 –/3.12 0.625/0.625 0.325/0.325 1.25 0.625/1.25 – –/– 0.312/0.156 0.625 1.25/0.312 0.625/1.25 0.625/1.25 1.25/1.25 1.25/1.25 0.625/0.156 0.625/0.625	1.25/1.25 0.125/0.125 1.25/1.25 0.625/0.625 2.5 1.25/2.5 0.156 0.156/0.156 0.625/0.312 1.25 2.5/0.625 1.25/2.5 1.25/2.5 2.5/2.5 2.5/2.5 1.25/0.312 1.25/1.25	•	Plasma concentrations were higher in females (30% or less); normalized to dose plasma concentrations increased with dose in males and females; Normalized plasma concentrations increased with dose, 1.9-fold increase in males and a 1.4-fold increase in females from lowest to highest dose; Liver concentrations in males increased with dose, but when normalized to dose (µM/mol/kg), values decreased with increasing dose <u>Plasma concentrations after 28-d in m (µg/mL):</u> 0.156 mg/kg bw/d: 8.5 0.312 mg/kg bw/d: 23 0.625 mg/kg bw/d: 47.7	(NTP, 2019b)

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Table 2: Repeated dose toxicity (RDT) animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
		• Erythrocytes ↑ (≈ +13–23% at LOAEL)	1.25/0.625	2.5/1.25		1.25 mg/kg bw/d: 101.6	
		• Mean cell haemoglobin ↑	0.312/1.25	0.625/2.5		2.5 mg/kg bw/d: 259.4	
		Endocrine system (m):				Liver concentrations after 28-d in m (µg/mL):	
		• Adrenal gland weight abs. ↓ (≈ −15% (m) to ≈ −36% (f) at LOAEL)	−/1.25	0.156/2.5		0.156 mg/kg bw/d: 44.7	
		• Testosterone in m ↓ (≈ −64% at LOAEL)	0.625	1.25		0.312 mg/kg bw/d: 87.2	
		• Testosterone in f ↑ (≈ +32-355%)	0.156	0.312		0.625 mg/kg bw/d: 163.9	
		• free T4 ↓ (≈ −39-42% at LOAEL)	−/1.56	0.625/3.12		1.25 mg/kg bw/d: 308.8	
		• T3, female only ↑ (≈ +24% at LOAEL)	0.156/0.625	0.312/1.25		2.5 mg/kg bw/d: 403.6	
		Clinical chemistry:					
		• Globulin (g/dL) ↓ (≈−14–22% at LOAEL)	−/0.156	0.156/0.312		Plasma concentrations after 28-d in f (µg/mL):	
		• Urea nitrogen ↑ (≈ +25–38% at LOAEL)	0.625/0.625	1.25/1.25		0.156 mg/kg bw/d: 11.2	
		• Glucose ↓ (≈−31% at LOAEL)	0.625/0.625	1.25/1.25		0.312 mg/kg bw/d: 25.7	
		• ALT ↑ (≈ +44–45% at LOAEL)	0.156/0.625	0.312/1.25		0.625 mg/kg bw/d: 50.3	
		• ALP ↑ (≈ +22–35% at LOAEL)	0.156/0.156	0.312/0.312		1.25 mg/kg bw/d: 117.2	
		• AST ↑ (m, ≈ +14–30% at LOAEL)	−/0.625	0.156/1.25		2.5 mg/kg bw/d: 246.9	
		• Cholesterol ↓ (≈−25–34% at LOAEL)	−/1.25	0.156/2.5			
		• Triglycerides ↓ (m only, ≈ -36% at LOAEL)	0.625	1.25			
		• Bile salts ↑ (m, ≈ +205–440% at LOAEL)	0.625/0.625	1.25/1.25			
		Reproductive Tissue (m only):					
		• L. Cauda Epididymis weight ↓ (m, ≈ -11% at LOAEL)	0.625	1.25			
		• L. Epididymis weight ↓ (m, ≈ -10% at LOAEL)	0.625	1.25			
		• Testis weight ↓ (m, ≈ -11% at LOAEL)	1.25	2.5			
		• Sperm (10^6/g cauda epididymis) ↓ (m, ≈ -30% at LOAEL)	1.25	2.5			
		• Interstitial cell atrophy in testes ↑ (80% incidence at LOAEL)	0.625	1.25			
PFUnDA: Perfluoroundecanoic acid (Syn: Henicosafluoroundecanoic acid, **CAS no:** 2058-94-8, **EC no:** 218-165-4, **Mol. formula:** $C_{11}HF_{21}O_2$, **MW:** 564,xx?; SVHC, candidate for authorisation under REACH, self classification available)							
PFUnDA (98.5%), Perfluoroundecanoic acid, EC: 218-165-4, CAS: 2058-94-8, $C_{11}HF_{21}O_2$	42-day RDT study with the Reproduction/Developmental Toxicity Screening Test, according to OECD TG 422 Rat Crl:CD (SD) n/sex/group = 12 Exposure: oral (gavage, vehicle: corn oil)	**Liver:**			• nervous system, endocrine system		(Takahashi et al., 2014)
		• Abs. liver weight ↑	0.3/0.3	1/1			
		• Rel. liver weight ↑	0.1/0.3	0.3/1			
		• Hepatocyte hypertrophy ↑ (≈58-52% incidence at LOAEL)	0.3/0.3	1/1			
		Immune system:					
		• Abs./rel. spleen weight ↓ (m only, ≈ −19–23% at LOAEL)	0.3/0.3	1/1			
		Clinical chemistry:					
		• Serum ALP, ALT ↑ (m only, ≈ +26% for ALT and +139% for ALP)	0.3	1			
		• Serum urea nitrogen ↑ (≈ +46-61% at LOAEL)	0.3/0.3	1/1			
		• Serum albumin ↓ (m only, ≈ −7% at LOAEL)	0.3	1			
		• Total protein ↓ (≈ −10-11% at LOAEL)	0.3/0.3	1/1			

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
	Doses: 0, 0.1, 0.3, 1.0 mg/kg bw/d						
PFDoDA: Perfluorododecanoic acid **(Syn:** Tricosafluorododecanoic acid, **CAS no:** 307-55-1, **EC no:** 206-203-2, **Mol. formula:** $C_{12}HF_{23}O_2$, **MW:** 614.10; SVHC substance, candidate list for authorisation, self class.available)							
PFDoDA, Perfluorododecanoic acid (>99%), EC: 206-203-2, CAS: 307-55-1, $C_{12}HF_{23}O_2$	14-day RDT study Rat (SD) n/sex/group = 6 (m) Exposure: oral (gavage), vehicle: 0.5% Tween-20) Doses: 0, 1, 5, 10 mg/kg bw/d	**Liver:** • Abs. liver weight ↓ (≈ −19-26%) • Rel. liver weight ↑ (≈ +25-30% at LOAEL) • Hepatic triglyceride ↑ (≈ +27% at LOAEL) • Hepatic SOD/CAT activity ↑ • Hepatic mRNA of PPARα/γ, ACOX, CPT1, Cyp4A1, LDLR ↑ • Hepatic content of cholesterol ↑ (≈ +39% at LOAEL) **Clinical chemistry:** • Serum triglyceride ↑ (≈ +96% at LOAEL)	 1 1 5 – – 5 5	 5 5 10 1 1 10 10	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Zhang et al., 2008)
PFDoDA (95%)	110-day RDT study Rat (SD) n/sex/group = 10 (m) Exposure: oral (gavage), vehicle: 0.2% Tween-20) Doses: 0, 0.02, 0.05, 0.2, 0.5 mg/kg bw/d	**Body weight** ↓ (≈ −7% at LOAEL) **Liver:** • Abs./rel. liver weight ↑ (≈ +10-18%) • Lipid droplets and widespread disintegrated cell systems • Swollen and vacuolated hepatocytes • Hepatic mRNA of PPARα, CYP4A1, ACOX, Cd36 • Hepatic triglycerides ↑ **Clinical chemistry:** • Serum ALP ↑ (m only, ≈ +73% at LOAEL) • Serum urea nitrogen ↑ (≈ +17% at LOAEL) • Serum glucose ↑ (≈ +9% at LOAEL) • Serum albumin ↑ (≈ +6% at LOAEL) • Creatine kinase ↑ (≈ +23% at LOAEL) • Serum lipoprotein levels ↓ (no quantification possible because data are expressed as ratio derived from NMR spectra, no statistics to determine LOAEL)	0.2 – 0.02 0.05 0.05 0.05 0.05 – – –	0.5 0.02 0.05 0.2 0.2 0.2 0.2 0.02 0.02 0.02	• kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Ding et al., 2009)
PFDoDA (95%)	110-day RDT study Rat (SD) n/sex/group = 6 (m) Exposure: oral (gavage), vehicle: 0.2% Tween-20)	**Kidney:** • Renal protein level of pyruvate carboxylase ↑ (≈ 10-18%) • Renal protein level of isovaleryl coenzyme A dehydrogenase, malate dehydrogenase 1 and dihydrolipoamide S-acetyltransferase ↑ **Remarks** Study to assess MOA of nephrotoxicity: disorders in glucose and amino acid metabolism may contribute to nephrotoxicity; α2u globulin may play a role in protecting the kidneys from PFDoDA toxicity	– 0.05	0.05 0.2	• liver, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Zhang et al., 2011)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
	Doses: 0, 0.05, 0.2, 0.5 mg/kg bw/d						
PFDoDA (purity not specified)	110-day RDT study Rat (SD) n/sex/group = 6 (m) Exposure: oral (gavage), vehicle: 0.2% Tween-20) Doses: 0, 0.2, 0.5 mg/kg bw/d	**Liver:** • Hepatic cholesterol ↑ • Hepatic triglycerides ↑ • Altered hepatic levels of signal transduction proteins (e.g. glycogen synthase kinase, insulin receptor substrate) **Remarks** Study to assess MOA of hepatotoxicity: chronic PFDoDA exposure may inhibit insulin signal pathways and inhibition of GSK3 might contribute to the observed increases of lipid levels in the liver	 – – –	 0.2 0.2 0.2	• Kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Zhang et al., 2013)
PFDoDA (97%)	42-day RDT study, OECD TG 422, 14 day recovery (0, 2.5 mg/kg bw/d) Rat (SD) n/sex/group = 7 Exposure: oral (gavage) Doses: 0, 0.1, 0.5, 2.5 mg/kg bw/d (14-day recovery group: 0, 2.5 mg/kg bw/d)	**Body weight** ↓ (≈ −33% at LOAEL) **Liver:** • Rel. liver weight ↑ (≈ +15-20% at LOAEL) • Hepatic hypertrophy ↑ • Hepatic necrosis ↑ • Inflammatory cholestasis ↑ **Kidney:** • Rel. kidney weight ↑, abs. ↓ (m only) **Immune system:** • Abs. spleen weight ↓ (≈ −14–38% at LOAEL) • White blood cells in recovery group ↓ (m only, ≈ −20%) **Thyroid:** • Abs. thyroid weight ↓ (m, ≈ −55% at LOAEL) **Haematopoietic system:** • Reticulocytes ↓ (m only, ≈ −63% at LOAEL) • Mean corpuscular haemoglobin concentration ↑ (m only, ≈ +4%) **Clinical chemistry:** • Serum ALP ↑ (m only, ≈ +54% at LOAEL) • Serum total cholesterol ↓ (m only, ≈ −33% at LOAEL) • Serum glucose ↓ (m, ≈ −27% at LOAEL) • Globulin-α2 ↓ (m, ≈ −14% at LOAEL) **Other effects:** • Pancreatic zymogen granules ↓ (m only, 5/7 animals at HD/LOAEL, 1/5 in recovery group, compared to 0/5-12 in control groups) **Reproductive Tissue (m only):** • Abs. L. Epididymis weight ↓ (m, ≈ −32% at LOAEL) • Spermatid and spermatozoa counts ↓	0.5/0.5 0.1/0.1 0.5 0.5 0.5 0.5 0.5/0.1 – 0.5 0.5 0.5 0.1 – 0.5 0.1 0.5 0.5 0.5	2.5/2.5 0.5/0.5 2.5 2.5 2.5 2.5 2.5/0.5 2.5 2.5 2.5 2.5 0.5 0.1 2.5 0.5 2.5 2.5 2.5	•		(Kato et al., 2015)
PFDoDA	110 day RDT study	**Body weight ↓ (≈ −5.5% at LOEL)**	0.2	0.5	• Kidney, nervous		(Shi et al.,

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
95%	Rat (SD) n/sex/group = 6 (m) Exposure: oral (vehicle: Tween-20) Doses: 0.02, 0.05, 0.2, 0.5 mg/kg bw/d	**Endocrine system:** • Serum testosterone ↓ (≈ –44% at LOEL) **Reproductive tissue:** • Cast-off cells in some seminiferous tubules in testes (no quant) • Testicular gene expression of StAR ↓ (≈ –35% at LOEL) • Testicular gene expression of P450scc ↓ (only at 0.05) • Testicular gene expression of IGF-I ↓ (≈ –25% at LOEL) • Testicular gene expression of IGF-IR and IL-1α ↓ (≈ –25%/-30% at LOEL) • Testicular gene expression of GnRH-R and FSH ↓ (≈ –45% at LOEL) • Testicular gene expression of LH ↓ (≈ –50%, only at 0.05mg/kg bw/d)	0.05 0.2 - 0.02 0.05 0.2	0.2 0.5 0.02 0.05 0.2 0.5	system, immune system, haematopoietic system, thyroid		2009)
PFDoDA 95%	14-days RDT Rat (SD) n/sex/group = 6 (m) Exposure: oral (gavage, vehicle: Tween-20) Doses: 1, 5, 10 mg /kg bw/d	**Body weight** ↓ (≈ –25% at LOEL) **Clinical chemistry:** • Total serum cholesterol ↑ (≈ +38.5% at LOEL) **Endocrine system:** • Serum LH ↓ (≈ –30% at LOEL)(FSH ns) • Testosterone ↓ (≈ –50% at LOEL) • Estradiol ↓ (only at 5 mg/kg bw/d, ≈ –50%) **Reproductive tissue:** • Apoptotic features present in Leydig cells, Sertoli cells and spermatogenic cells • Abs. testis weight ↓ (≈ –22% at LOEL) • Rel. testis weight ↑ (≈ +36% at LOEL) • Testicular gene expression of SR-B1 ↓ (≈ –60% at LOEL) • Testicular gene expression of StAR ↓ (≈ –50% at LOEL) • Testicular gene expression of P450scc ↓ (≈ –90% at LOEL)	1 5 5 1 5 5 1 1 1	5 10 10 5 10 10 5 5 5 5	• Kidney, nervous system, immune system, haematopoietic system, thyroid		(Shi et al., 2007)
PFDoDA	110-day RDT study Rat (SD) n/sex/group = 4-10 (m) Exposure: oral (gavage), vehicle: 0.2% Tween-20) Doses: 0, 0.05, 0.2, 0.5 mg/kg bw/d	**Liver:** • Hepatic SOD activity ↑ • TBARS in liver ↑ • Hepatic GPX activity ↑ • mRNA of PPARα/Cyp4A1↑ • mRNA of mitochondrial acyl-CoA-thioesterase 1 and hydroxyacyl-CoA-dehydrogenase ↑	0.2 0.2 0.2 0.05 –	0.5 0.5 0.5 0.2 0.05	• Kidney, nervous system, immune system, haematopoietic system, thyroid, endocrine system		(Liu et al., 2016)
PFTrDA: Perfluorotridecanoic acid **(Syn:** Pentacosafluorotridecanoic acid, **CAS no:** 72629-94-8**, EC no:** 276-745-2**, Mol. Formula:** $C_{13}HF_{25}O_2$, **MW:** 664.11; SVHC, candidate list for authorisation, no self class. available)							

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
					•		
PFTeDA: Perfluorotetradecanoic acid **(Syn:** Heptacosafluorotetradecanoic acid, **CAS no:** 376-06-7, **EC no:** 206-803-4, **Mol. formula:** $C_{14}HF_{27}O_2$, **MW:** 714.11; SVHC, candidate list for authorisation; Self class. available, but only Skin Corr. 1B)							
PFTeDA, Perfluorotetradecanoic acid	42-day repeated dose and reproductive/developmental toxicity (OECD TG 422), 14-day recovery Rat (SD) n/sex/group = 12 (m+f) Exposure: oral (vehicle control,0.5% carboxymethylcellulose sodium in water) Dose groups: 0, 1, 3, 10 mg/kg bw/day Dosing regime All animals: 14 day pre mating Males: 42 day Females: during lactation up to 5 days after parturition (app. 42 days i.e. 14 premating, 1-2 days mating 21-23 gestation, plus 5 days) Recovery Group: high dose for 42 days without mating and vehicle control, followed by a 14 day recovery period	**Body weight:** • Body weight in RDT study ↓ (m, ≈ –7% only in recovery group, f: ns) • Body weight in Repro/DevTox study ↓ (f, ≈ –6% at LOAEL on day 4 of lactation) • Body weight ↓ (f, ≈ –9% at LOAEL throughout entire lactation period) • Food consumption ↓ (f, day 5 and 10 gestation; day 4 lactation) **Liver:** • Abs. liver weight ↑ (m, ≈ +22% at LOAEL, also in recovery group) • Rel. liver weight ↑ (m/f, ≈ +19/+11% at LOAEL, also in recovery groups) • Centrilobular hepatocyte hypertrophy ↑ (m/f, also in recovery groups) • Microgranulomas ↑ (f, also in recovery group; m: microgranulomas in treatment and control group) • Focal necrosis in one female **Kidney:** • Abs. + rel. kidney weight (m/f, ns) **Immune system:** • Thymic cortex atrophy ↑ (m: NA; f: at LOAEL) • Abs.+rel. thymus weight (m/f, ns) • Abs.+rel. spleen weight (m/f, ns) • White blood cell count (m/f, ns) **Thyroid:** • Follicular cell hypertrophy ↑ (m; f: NA) **Haematopoietic system:** • Activated partial thromboplastin time ↓ (>10%) • Females: decrease in extramedullary haematopoiesis • Haemoglobin, haematocrit ↓ (f, both ≈ –8%, only in recovery group) • Shortened prothrombin time ↓ (f, both ≈ –7%, only in recovery group) **Clinical Chemistry:** • β-globulin protein fraction ↓ (m/f, ≈ –8/–13% at LOAEL) • Serum ALP ↑ (m, ≈ +43% at LOAEL, +41% in recovery group, f: ns) • BUN ↑ (m, ≈ +39% at LOAEL, not in recovery group; f: only in recovery) • Serum chloride ↑ (f, ≈ +3% at LOAEL, not in recovery group, m: ns)	1 3 3 1 1/3 1/3 – 3 1 – 1 3/3 3 3 3	3 10 10 3 3/10 3/10 10 10 3 10 3 10/10 10 10 10	• All major organs and tissues have been included, as listed in the manuscript		(Hirata-Koizumi et al., 2015)

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Table 2: Repeated dose toxicity (RDT) animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
		• Serum inorganic phosphorus ↓ (m, ≈ +8% only in recovery group; f: ns) • Serum triglycerides ↓ (m, ≈ –46% only in recovery group; f: ns) • Serum cholesterol ↓ (m: ns; f: ≈ –25% only in recovery group) **Other effects:** • Abs. weight pituitary gland ↓ (m) • Hindlimb grip strength ↓ (m, ≈ –20-25%) • Urinary parameters (ns) **Reproductive/developmental toxicity:** • Body weights in male and female pups ↓ • No effects on estrous cycle, copulation index, fertility index, gestation length, Corpora lutea, implantation sites, delivered pubs, sex ratio	 1 3 3 	 3 10 10 			
PFPeDA: Perfluoropentadecanoic acid (Syn: Hexacosafluoro-13-(trifluoromethyl)tetradecanoic acid**, CAS no:** 18024-09-4**, EC no:** 241-936-1 **, Mol. Formula:** $C_{15}HF_{29}O_2$ **, MW:** 764.12; no data on ECHA chem search							
					•		
PFHxDA: Perfluorohexadecanoic acid **(Syn: Perfluoropalmitic acid**, **CAS no:** 67905-19-5, **EC no:** 267-638-1, **Mol. formula:** $C_{16}HF_{31}O_2$, **MW:** 814.13; only self class. Skin Corr. 1B , no further ECHA data)							
PFHxDA, Perfluorohexadecanoic acid	42-day Repeated dose and reproductive/developmental toxicity (OECD TG 422), 14-day recovery Rat (SD) n/sex/group = 12 (m+f) Exposure: oral (vehicle control,0.5% carboxymethylcellulose sodium in water) Dose groups: 0, 4, 20, 100 mg/kg bw/day) Dosing regime: All animals: 14 day pre mating Males: 42 day Females: during lactation up to 5 days after parturition High dose recovery groups (14 days	**Body weight:** • Body weights ↓ (m, ≈ –4% at LOAEL on day 35 and 42; f: ns) • Food consumption ↓ (m in RDT experiment: at day 14 recovery period; f in DevTox experiment: day 5 - 10 gestation; day 4 lactation) **Liver:** • Abs. liver weights ↑ (m, ≈ +19% at LOAEL, also in recovery group; f: ns) • Rel. liver weights ↑ (m, ≈ +30% at LOAEL, also in recovery group; f: ns) • Centrilobular hepatocellular hypertrophy ↑ (m/f, m also in recovery group) • Centrilobular fatty changes **Kidney:** • Abs. + rel. kidney weight (m/f, ns) **Immune system:** • Abs.+rel. thymus weight (m/f, ns) • Abs.+rel. spleen weight (m/f, ns) • White blood cell count (m/f, ns) **Thyroid:** • Rel. thyroid weight ↑ (m, +26% at LOAEL, not in recovery group; f: ns) **Endocrine system:**	 20 20 20 20 20 4	 100 100 100 100 100 20	•		(Hirata-Koizumi et al., 2015)

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Table 2: Repeated dose toxicity (RDT) animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
	recovery after treatment)	• T3 ↓ (m: ns; f, ≈ –17% at LOAEL, not in recovery) • T4 ↓ (m, ≈ –24% only in recovery group; f: ns) **Haematopoietic system** (ns) **Clinical Chemistry** • Serum total bilirubin ↓ (m, ≈ –31% only in recovery group; f, ≈ –25% at LOAEL, also in recovery group) • BUN ↑ (m: ns; f, ≈ +21% at LOAEL, not in recovery group • Serum sodium ↑ (m: ns; f, ≈ +1% at LOAEL, not in recovery group) • Serum chloride ↑ (m/f, ≈ +3/+2% at LOAEL, f also in recovery group) • Serum cholesterol (m/f, ns) • Serum triglycerides (m/f, ns) **Other effects** • Hindlimb grip strength ↓ (m/f, ≈ –20/–23%, only in recovery group) • Urinary parameters (ns) • Abs. + rel. adrenal weights ↓ (m, –18-22% only in recovery group; f: ns) **Reproductive/developmental toxicity** • Slightly lower body weights in male and female pups, no other effects are reported	– 20 20 20 20/4	4 100 100 100 100/20			
PFHpDA: Perfluoroheptadecanoic acid **(Syn:**, **CAS no**: , **EC no:** , **Mol. formula:** $C_{17}HF_{33}O_2$, **MW:**)							
PFODA: Perfluorooctadecanoic acid **(Syn:** Perfluorostearic acid, **CAS no**: 16517-11-6, **EC no:** 240-582-5, **Mol. formula:** $C_{18}HF_{35}O_2$, **MW:** 914.15; only self class. Skin Corr. 1B , no further ECHA data)							
PFODA, Perfluorooctadecanoic acid	42-day Repeated dose and reproductive/developmental toxicity (OECD TG 422) Rat (SD) n/sex/group = 12 (m+f) Exposure: oral (gavage; vehicle control,0.5% carboxymethylcellulose sodium in water) Dose groups: 0, 40, 200, 1000 mg/kg bw/day) Dosing regime	**Body weight** ↓ (m/f, ≈ –20% at LOAEL at day 42/28, also in recovery groups) Food consumption ↓ at day 5 lactation Food consumption ↓ at day 35 and 42 **Liver** • Abs. liver weight ↑ (m/f, ≈ +45/+29% at LOAEL, also in recovery group) • Rel. liver weight ↑ (m/f, ≈ +42/+35% at LOAEL, also in recovery group) • Centrilobular hepatocellular hypertrophy (m/f, also in recovery group) **Immune system** • Thymic atrophy of the cortex (2 animals/24) • Abs. thymus weight ↓ (m, –40% at LOAEL, not in recovery group; f: ns) • Rel. thymus weight (m/f, ns) • Abs.+rel. spleen weight (m/f, ns) • White blood cell count ↑ (m: ns; f, ≈ +35% only in recovery group)	200 200 200 40/200 40 40/200 200	1000 1000 1000 200/1000 [redacted] [redacted] 1000	• kidney, nervous system, thyroid, endocrine system		(Hirata-Koizumi et al., 2012)

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Table 2: Repeated dose toxicity (RDT) animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	NO(A)EL (mg/kg bw/d)	LO(A)EL (mg/kg bw/d)	Key parameters / targets not addressed	Serum/tissue concentration of PFAS /metabolites (time of sampling)	Reference
	All animals: 14 day pre mating Males: 42 day Females: until 5th day of lactation (42-56 days)	**Haematopoietic system:**					
		• Erythrocytes, haemoglobin ↓ (m)	40	200			
		• Reticulocyte ratio ↓ m,↑m: recovery group	200	1000			
		• Prothrombine time ↓ (f),	40	200			
		• Basophiles ↑ (f)	200	1000			
		Clinical chemistry:					
		• Albumin protein fraction ↑ (m, ≈ +15% at LOAEL, also in recovery group; f: only in recovery group)	40	200			
		• α_1-globulin protein fraction ↓ (m/f, ≈ –41/–23% at LOAEL, remains reduced in recovery group)	200/40	1000/200			
		• α_2-globulin fraction (m+f, ns)					
		• β-globulin protein fraction ↓ (m/f, ≈ –17/–10%, only in recovery group)					
		• γ-globulin protein fraction ↑ (m/f, ≈ +68/+59% at LOAEL, not in recovery group)	200	1000			
		• AST (m+f, ns)					
		• ALT ↑ (m, ≈ +53% at LOAEL, f: ns)	200	1000			
		• ALP ↑ (m/f, ≈ +88/+72% at LOAEL, remains increased in m in recovery group)	200	1000			
		• Total bilirubin (m, ≈ +167% at LOAEL, but not in recovery group, f: ns)	200	1000			
		• BUN ↑ (m/f, ≈ +41/+41% at LOAEL, not in recovery groups)	200	1000			
		• Total cholesterol ↓ (f, ≈ –25%, not in recovery group; m: ns)	200	1000			
		• Triglycerides ↓ (m, ≈ –67% only in recovery group; f: ns)					
		Other effects					
		• pancreatic zymogen granules ↓ (m: tendency, f: significant)	200	1000			
		• Rel. brain weight ↑ (m/f, ≈ +25/+13% at LOAEL, m also in recovery group)	200	1000			
		Reproductive and developmental findings:					
		No significant differences in delivery, live birth and viability indices between control and PFODA treated groups, but a decreasing tendency in the HD-group. • body weight of male and female pups ↓	200	1000			

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Key parameters/targets

Mortality, body weight, liver, kidney, thyroid, nervous system, immune system, haematopoietic system, endocrine system, clinical chemistry, other effects

Abbreviations/Symbols

/ (forward slash): in the NOAEL and LOAEL columns indicate NOAELs and LOAELs for males/females (unless stated differently); ↑: significant increase; ↓: significant decrease; abs., rel.: absolute, relative; Acox: Acyl CoA oxidaseALP: alkaline phosphatase; ALT: Alanine aminotransferase; AST: aspartate aminotransferase; BUN: blood urea nitrogen; CYP4A1: cytochrome P4504A1 or arachidonic acid monooxygenase; C: control; LD: low dose; MD: medium dose; HD: high dose; m, f: male, female; MoA: mode of action; ns: no significant difference to control; NA: not available, i.e. not determined; T3: triiodothyronine; fT4: free thyroxine; tT4: total thyroxine; bw: body weight; WT: wildtype; KO: knock out; PPAR- peroxisome proliferator-activated receptor

8. Annex 3: Developmental and reproductive toxicity studies

Table 3: Developmental and reproductive toxicity animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	maternal NO(A)EL	maternal LO(A)EL	offspring NO(A)EL	offspring LO(A)EL	Serum/tissue concentration of PFAS /metabolites	Reference
Perfluoroalkyl carboxylic acids (PFCAs)								
PFBA: Perfluorobutanoic acid (Syn: Heptafluorobutyric acid, **CAS no:** 375-22-4, **EC no:** 206-786-3, **Mol. formula:** $C_4HF_7O_2$, **MW:** 214,xx?; pre-registration process under REACH, self classification available**)**								
PFBA, perfluorobutyrate, ammonium (98%)	17-days GD1-GD17 Mouse (CD1; timed-pregnant) Maternal n/group = 30 (3 blocks) Offspring sample size? Exposure: oral (gavage) Doses: 0, 35, 175, 350 mg/kg bw/d	**Maternal**					<u>350 mg/kg pregnant dams:</u> 2.49 µg/ml serum 0.96 µg/g liver <u>Pups PND1</u> 0.37 µg/ml serum 0.24 µg/g liver <u>Pups PND10</u> 1.12 µg/ml serum 0.04 µg/g liver	(Das et al., 2008)
		• maternal liver weight ↑ (30% est. Fig 2; reversible)	35	175				
		Fertility						
		• full litter resorptions ↑ (26.9%/350 mg/kg versus 6,8% in control)	175	350				
		• neonatal mortality (no effect)						
		• number/weight of living foetuses/number of implants (no effect) Gene expression analysis (neonatal mice): no effects on PPARa/pregnane X-receptor regulated genes						
		Developmental						
		• pups liver weight PND1 ↑ (10-20% est. Fig 5; no longer observed at PND10)			35	175		
		• slightly delayed eye opening (1-1.5 days)			0	35		
		• delayed vaginal opening (2-3 days)			35	175		
		• delayed preputial separation (350/0 mg/kg 33% vs 7%)			175	350		
PFHxA: Perfluorohexanoic acid (Syn: Undecafluorohexanoic acid, **CAS no:** 307-24-4, **EC no:** 206-196-6, **Mol. formula:** $C_6HF_{11}O_2$, **MW:** 264,xx?; under PBT assessment under REACH, self classification available)								
PFHxA ammonium 93.4% purity	12-day GD 6-18 (for both experiments?) Mouse (CD1; timed-pregnant) Maternal: n/group = 20 (for both experiments?) Exposure: oral (gavage) Two phases i.e. two experiments: Experiment (phase) 1 Doses: 0, 100, 350, 500 mg/kg bw/d	**Maternal**						(Iwai and Hoberman, 2014)
		• maternal mortality (0: 2/20; 100: 6/20; 350: 1/20; 500: 3/20)						
		Fertility						
		• Postnatal mortality (PND 0) ↑ (0:0/217; 100: 0/250; 350: 3/232; 500:21/150)			350	500		
		• Postnatal mortality ↑ (PND 1-4) (0: 2/217; 100: 2/250; 350: 25/229; 500; 20/129)			100	350		
		• decreased litter size			350	500		
		• neonatal mortality ↑			350	500		
		Developmental						
		• weight gain during lactation ↓						
		• relative liver weight in F1 ↑						
		Phase 2						
	Experiment (phase) 2	increased postnatal mortality (PND 0) (0: 0/249; 7:0/211; 35: 0/232; 175: 4/238)			35	175		

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Table 3: Developmental and reproductive toxicity animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	maternal NO(A)EL	LO(A)EL	offspring NO(A)EL	LO(A)EL	Serum/tissue concentration of PFAS /metabolites	Reference
	Doses: 0, 7, 35, 175 mg/kg bw/d							
	Statistical re-evaluation of Iwai and Hoberman 2014 confirmed 175 mg/kg as NOAEL for the reported pup mortality				175	350		(Iwai et al., 2019)
PFHxA (Na-PFHxA)	comprehensive toxicological evaluation including **A)** One generation reproduction study similar to OECD TG 415 P0 70d prior to mating until weaning 126d (f), 110d (m) Rat (SD) n/sex/group = 20 Exposure: oral (gavage) Doses: 0, 20, 100, 500 mg/kg bw/d	**Maternal** • reduced maternal body weight gain (10-20%) **Developmental** • reduced pup body weight (10-20%)	100	500	100	500		(Loveless et al., 2009)
	B) Developmental toxicity study similarto OECD TG 414 GD6-20/ analysis at GD21 Rat (SD) n/sex/group = 20 Exposure: oral (gavage)	**Maternal** • reduced maternal body weight gain (19%) **Developmental** • decreased fetal weight (10%) **no detectable effects related to reproduction**	100	500	100	500		

Table 3: Developmental and reproductive toxicity animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	maternal NO(A)EL	maternal LO(A)EL	offspring NO(A)EL	offspring LO(A)EL	Serum/tissue concentration of PFAS /metabolites	Reference
	Doses: 0, 20, 100, 500 mg/kg bw/d							
PFOA: Perfluorooctanoic acid (Syn: Pentadecafluorooctanoic acid, **CAS no:** 335-67-1, **EC no:** 206-397-9, **Mol. formula:** $C_8HF_{15}O_2$, **MW:** 414,xx?; registered under REACH, self classification available)								
PFOA ammonium 98% purity	GD1 - birth Mouse (CD1; timed-pregnant) (no information on number per dose) Exposure: oral (gavage) Doses: 0, 1, 3, 5, 10, 20,40 mg/kg	**Maternal** • liver weight ↑ (2-fold < 3 mg/kg) • decreased maternal weight gain • **Developmental** • neonatal mortality (Very high in the 20 mg/kg (60%) and 40 mg/kg (90%) dose groups) • • growth retardation and and decreased weight among surviving pups • • enlarged frontanel, reduced ossification (sternebrae, calvaria), minor • tail and limb defects	0 5	1 10	3 3 5	5 5 10	For determination of serum levels: rats 10 mg/kg 20 d mice 20 mg/kg 7 or 17d mice 20 mg/kg 17 d 199 µg/ml (m) 171 µg/ml (f) rats 10 mg/kg 20d 111 µg/ml (m) 0,69 µg/ml (f)	(Lau et al., 2006)
PFOA ammonium 98% purity	**A:** pregnant mice (CD-1) GD1-17 n/group = 10, 12, 11 Exposure: oral (gavage) Doses: 0, 1, 5 mg/kg bw/d **B:** pregnant mice (CD-1) during gestation (GD1-17/birth) 0 mg/kg (n=7) 1 mg/kg (n=10) Exposure: oral (gavage) both subgroups of **B** were also exposed to 5 ppb PFOA in drinking water (app. 0.00045 mg/kg) starting at GD7 for the duration of the study Offspring generations of **B** (F1-2) were continuously dosed with 5 ppb PFOA (drinking water) until the end of experiment	**P0: (only A)** **increased neonatal mortality** **P0: compromised weaning induced mammary involution PND22 (A)** **P0: compromised weaning induced mammary involution PND22 (B)** **F1: reduced developmental mammary scores (A)** **F1: reduced developmental mammary scores (B)** **F1 (A&B)** compromised lactational morphology at PND10 (A) (due to developmental exposure/ treatment of P0 A) (no clear evidence for diminished nutritional support) (control groups only exposed to 5ppb in drinking water were also affected) (B) **F1 (only A)** reduced uterine implants compromised lactational morphology **F2 (A)** Mammary gland scores are consistent with delayed differentiation at	0 0	1 0.00045	1 0 0 1 0 1 1	5 1 0.00045 1 0.00045 5 5	Group only exposed to 5ppb PFOA (B1) p0->F2 P0 weaning: 74,8 ng/ml F1 PND22 21.3 ng/m F1 PND63 66.33 ng/m F1 dams weaning 86.9 ng/ml F2 pups PND22 26.6 ng/ml F2 pups PND63 68,5 ng/ml Group P0 1 mg/ml/ 5ppb P0->F2 (B2) P0 weaning: 4772.0 ng/ml F1 PND22 2743.8 ng/m F1 PND63 187.0 ng/m F1 dams weaning 173.3 ng/ml F2 pups PND22 28.5 ng/ml F2 pups PND63 69.2 ng/ml	(White et al., 2011)

Table 3: Developmental and reproductive toxicity animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	maternal NO(A)EL LO(A)EL	offspring NO(A)EL LO(A)EL	Serum/tissue concentration of PFAS /metabolites	Reference
		PND 22 (ns)			group P0 only 5 mg/kg PFOA (A3) P0 weaning: 26980.0 ng/ml F1 PND22 10045 ng/m F1 PND63 760.3 ng/m F1 dams weaning 18.7 ng/ml F2 pups PND22 7.8 ng/ml F2 pups PND63 1.22 ng/ml	
PFOA 90% purity	17-days, GD1-17 Mouse (ICR; timed-pregnant) Maternal sample size: n/group = 15-19 Exposure: oral (gavage) Doses: 0, 1, 5, 10 mg/kg bw/d	**Maternal** • liver weight ↑ (relative weight 5.4-11.4%) • • Increased GGT, AST, ALP • Reduced globulins, triglycerides and cholesterol **Developmental** • reduced birth weight • neonatal mortality (100%, 10 mg/kg)	1 5 5 10 5 10 	 1 5 1 5		(Yahia et al., 2010)
PFOA	Two generation study EPA OPPTS 8703800 P0 70 day prior to mating (m) or until weaning (f) Rat (SD) n/sex/group = ? (males and females?) Parental (F0) doses: 0, 1, 3, 10, 30 mg/kg bw/d F1: dosing continued after weaning F2: was maintained through 22d lactation	**Parental** • no reproductive endpoints affected in any generation **Developmental (F1)** • birth weights ↓ in relationship with reduced viability • Reduced viability not observed in F2 • Preputial separation and vaginal opening somewhat delayed		10 30		(Butenhoff et al., 2004)
PFOA	timed pregnant mice (CD-1) Litter sample size: n/group = 11-13 Exposure: oral (gavage) 0, 1, or 5 mg/kg bw/d sacrifice timepoint GD11.5 or GD17.5	**Maternal** • abs liver weight ↑ (E11.5) (0: 2.2g; 1: 2.9g, 5: 4.5g) • rel liver weight ↑ (E11.5) (0:5.9%; 1:7.4%, 5: 11.0%) • enlarged hepatocytes (E17.5) • abnormal ultrastructure of liver cells (increased peroxisomes/mitochondria) •	0 1 0 1 0 1 0 1			(Blake et al., 2020)

Table 3: Developmental and reproductive toxicity animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	maternal NO(A)EL	maternal LO(A)EL	offspring NO(A)EL	offspring LO(A)EL	Serum/tissue concentration of PFAS /metabolites	Reference
	dosing GD1.5 until 11.5 or GD 1.5 until 17.5	• Triglycerides (E 11.5) (1 mg/kg 37%, 5 mg/kg 58%)	0	1				
		• Glu ↓ (5 mg/kg E17.5: 20% lower)	1	5				
		• AST ↑ (5 mg/kg E17.5: up 100%)	1	5				
		Fertility						
		• embryo weight↓	1	5				
		• placental lesions (nodules, labyrinth congestion or atrophy	0	1				
PFOA Ammonium salt (**APFO**) 98% purity	GD1-17 Mouse (PPARα KO -/- or 129S1/SvlmJ WT), timed-pregnant Number of litters highly variable n/group = 4-22 litters for individual doses Exposure: oral (gavage) Doses: 0.1, 1, 3, 5, 10 or 20 mg/kg bw/d	**Maternal**						(Abbott et al., 2007)
		• Maternal liver weight (WT) ↑ (more than 2-fold at 5 mg/kg)	1	5				
		• Maternal liver weight (PPARα-KO) ↑ (more than 2-fold at 5 mg/kg)	1	3				
		• Maternal liver weight not dependent on PPARα						
		Fertility						
		• litter loss wt (100%) (1 mg/kg: 70%)	1	5				
		• litter loss wt PPARα KO -/- (100%) (5 mg/kg: 86%)	10	20				
		• Comparable embryo lethality in WT and PPARα KO mice at 5 mg/kg (100% versus 86%)						
		• Embryo lethality:PPARα-independent						
		• neonatal mortality can only be addressed up to 1 mg/kg in WT mice due to total litter losses at higher exposures						
		Developmental						
		• postnatal survival ↓ (WT) (0 mg/kg: 78.9% vs. 1 mg/kg: 42.5% survival on PND 22)						
		• postnatal survival ↓ (PPARα-KO) (0 mg/kg: 92% vs. 1 mg/kg > 90%, 3 mg/kg: 87%)			0.3 3			
		•						
		• neonatal mortality ↑ (WT: Fig 2A in publication)				0.6		
		• neonatal mortality ↑ (PPARα-KO: Fig 2B in publication)				–		
		• neonatal mortality PPARα-dependent						
		•						
		• delayed eye opening (WT) (≈ 1 day)			0.3	0.6		
		• delayed eye opening (PPARα-KO)			1	3		

Table 3: Developmental and reproductive toxicity animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	maternal NO(A)EL	LO(A)EL	offspring NO(A)EL	LO(A)EL	Serum/tissue concentration of PFAS /metabolites	Reference
PFOA	GD1 - GD17 Male offspring was analyzed at PD 21 and PD70 Mouse (Kunming) n/sex/group = 10 (litters or male pups?) Exposure: oral (gavage) Doses: 0, 1, 2.5 or 5 mg/kg bw/d	**Developmental** • neonatal mortality (PD7) • decreased number of Leydig cells • altered ultrastructure, markedly increased intracellular substance • • Expression of imprinted Dlk-Dio3 gene cluster • • no difference in testicular index			2.5 1 1 1	5 2.5 2.5 2.5		(Song et al., 2018)
PFOA ammonium salt (**APFO**) >98% purity	7-17 days, GD1-GD17 (full gestation exposure) or GD10-GD17 (late gestation exposure) Mouse (CD-1), timed-pregnant Maternal n/group = 13 (both experiments) Litters n/group = 13 (equalized to 5 m and 5 f in each litter) in full gestation exposure) n/group = ca. 11 (15% of dams were not pregnant (PFOA independent); litters were mixed and each dam nursed 7-9 pups with 4-7 females) Exposure: oral (gavage) (only dams) Doses (full gestation exposure): 0, 0.3, 1.0, or 3.0 mg/kg bw/d Doses (late gestation exposure): 0, 0.01, 0.1, or 1.0 mg/kg bw/d Remark: short acclimation period (only 1 day!): stress responses might potentiate PFOA effects. On the other hand, all experiments with purchased timed-pregnant mice have this problem	**Developmental** • no effects on offspring body weight in both experiments (f+m) • abs/rel liver weight of offspring in full/late gestation exp. (f+m) ↑ • delayed epithelial growth in the mammary gland (full-gestation exp: PND 63 and 84, on PND 63 no control comparison, but comparison to historical controls…) • delayed epithelial growth in the mammary gland (late gestation exp: PND1-PND14/PND 14) • overall developmental scores ↓ in both experiments (full/late gestation exp)				0.3 0.3 0.1 0.3/ 0.01		(Macon et al., 2011)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	maternal NO(A)EL	maternal LO(A)EL	offspring NO(A)EL	offspring LO(A)EL	Serum/tissue concentration of PFAS /metabolites	Reference
PFOA ammonium salt (APFO) >98% purity	17 days, GD1-GD17 Mouse (CD-1 and C57Bl/6); timed-pregnant Maternal sample size: CD-1 mice in three blocks (block 1 n = 97, block 2 n = 40, and block 3 n = 26) C57Bl/6 mice in one block (n = 41) Litter sample size (equalized to 10 pups per dam, each 6-7 f + 3-4 m) Exposure: oral (gavage, in water) Doses: 0, 0.01, 0.1, 0.3 or 1.0 mg/kg bw/d Pregnancy rates in CD-1 females were >60% the C57Bl/6 block yielded a much lower rate of approximately 27% litter with n<5 were excluded	**Developmental** • net body weight (body weight-liver weight) (only CD-1) ↓ • no effect on abs liver weight (both strains) • rel liver weight at PND 21 ↑ (but not at PND35 and 56)(only CD-1 mice) • estradiol and progesterone (ns in both strains) • • no effects on female pubertal events (both strains) • • mammary gland developmental scores reduced on PND 35 and 56 (tbc) (CD-1) • mammary gland developmental scores reduced on PND 21 and 61 (tbc) (C57Bl/6)			1 1 0.01 0.3			(Tucker et al., 2015)
PFNA: Perfluorononanoic acid (Syn: Heptadecafluorononanoic Acid, **CAS no:** 375-95-1, **EC no:** 206-801-3, **Mol. formula:** $C_9HF_{17}O_2$, **MW:** 464,xx?; registered under REACH, self classification available)								
PFNA 97% purity	90-day study, PND25 - PND114 (prepuberty) Mouse (Parkes strain) n/sex/group = 7 (m) Exposure: oral (gavage) Doses: 0, 0.2, 0.5 mg/kg bw/day	**Males** • Body weight (ns) **Fertility** • Abs/rel testis weight (ns) • spermatozoa motility/viability/number ↓ (≈ –26%/–65%/–37% at LOEL) • serum cholesterol ↓ (≈ –33% at LOEL) • serum testosterone ↓ (≈ –31% at LOEL) • Litter size ↓ (≈ –42% at LOEL) (reproduction assay) • testicular proteins/mRNA involved in steroidogenic processes ↓ (StAR/Cyp11A1/3β-HSD/17β-HSD) (≈ –47%/–49%/–35%/–64% at LOEL) • Testes lipid peroxidation ↑ (≈ +41% at LOEL) • Antioxidative enzymes (SOD, CAT) ↓ (≈ –22%/–27% at LOEL) • number of proliferating testicular cells (PCNA) ↓ (≈ –28% at LOEL) • Testes apoptosis (Caspase-3 staining) ↑ (≈ +35% at LOEL)	0.2 0.2 0.2 0.2 0.2 0.2 – 0.2 0.2	0.5 0.5 0.5 0.5 0.5 0.5 0.2 0.5 0.5				(Singh and Singh, 2019b)

Table 3: Developmental and reproductive toxicity animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	maternal NO(A)EL	maternal LO(A)EL	offspring NO(A)EL	offspring LO(A)EL	Serum/tissue concentration of PFAS /metabolites	Reference
PFNA 97% purity	14-day study, PND25 -PND38 (prepuberty) Mouse (Parkes strain) n/sex/group = 10 (m) Exposure: oral (gavage) Doses: 0, 2, 5 mg/kg bw/day	**Males** • Body weight gain ↓ **Fertility** • intratesticular/serum testosterone ↓ (≈ –20%/–72% at LOEL) • Rel no. affected seminiferous tubules ↑ (≈ +424% at LOEL) testicular proteins/mRNA involved in steroidogenic processes (SF-1/StAR/Cyp11A1/3β-HSD/17β-HSD) ↓ (≈ –23%/–57%/–50%/–45% /–46% at LOEL) • Testes lipid peroxidation ↑ (≈ +74% at LOEL) • Antioxidative enzymes ↓ (SOD/CAT/GST) (≈ –31%/–35%/–34% at LOEL) • number of proliferating testicular cells (PCNA) ↓ (≈ –13% at LOEL) • Testes apoptosis (Caspase-3 staining) ↑ (≈ +100% at LOEL)	2 – – – – – – –	5 2 2 2 2 2 2 2				(Singh and Singh, 2019d)
PFNA 97% purity	14-day study GD12 – parturition Mouse (Parkes strain) **Maternal (pregnant females):** n/sex/group = 10 **Offspring** (male neonates only, killed on PND3): n/group = 20 (2 per litter) n/group = 5 (1 per litter) for testes sampling Exposure: oral (gavage) from GD 12 Doses: 0, 2, 5 mg/kg bw/day	**Maternal** • Body weight, birth rate, number of pups per dam, weight of male pups) (ns) **Developmental** • Abs testis weight of neonatal offspring (ns) Testis histology (ns) • Intratesticular level of testosterone in neonatal offspring ↓ (≈ –42 % at LOEL) • testicular proteins/mRNA involved in steroidogenic processes (SF-1/StAR/Cyp11A1/3β-HSD/17β-HSD) ↓ (≈ –23%/–33%/–37%/–31%/–25% at LOEL) • testicular proteins/mRNA involved in gonad development (WT1/SF1) ↓ (≈ –67%/–23% at LOEL) • Testes PCNA expression ↓ (≈ –32% at LOEL)			2 2/–/ 2/ 2/ 2 –/ 2 2	5 5/2/5/ 5/ 5 2/ 5 5		(Singh and Singh, 2019c)
PFNA 97% purity	14-day study, PND25 -PND38 (prepuberty) Mouse (Parkes strain) n/sex/group = 10 (m) Exposure: oral (gavage)	**Males** • Abs/rel liver weight (≈ +100/42% at LOEL) • Hepatocellular hypertrophy • PPARα expression ↑ (80-fold change at LD, only 5-fold change at HD) • Hepatic liver peroxidation ↑ (≈ +26% at LOEL) **Fertility** • Testicular glucose ↓ (≈ –22% at LOEL) • Testicular lactate ↓ (≈ –1% at LOEL) • Serum cholesterol ↓ (≈ –19% at LOEL) • Fasting blood glucose ↑ (≈ +16% at LOEL)	– – – – – – 2 –	2 2 2 2 2 2 5 2				(Singh and Singh, 2019a)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	maternal NO(A)EL	maternal LO(A)EL	offspring NO(A)EL	offspring LO(A)EL	Serum/tissue concentration of PFAS /metabolites	Reference
	Doses: 0, 2, 5 mg/kg bw/day							
PFNA (97%)	14-day-study Rat (SD) n/sex/group = 6 (m) Exposure: oral (gavage) Doses: 0, 1, 3, 5 mg/kg bw/d	**Fertility** • Serum testosterone ↓ (≈ –85% at LOEL) • Serum estradiol ↑ (≈ +104% at LOEL) • Apoptotic cells in testes ↑ (≈ +367% at LOEL) • Fas gene ex ↑ (≈ +90% at LOEL) • Bax gene ex ↑ (≈ +36% at LOEL) • Bcl-2 gene ex ↓ (≈ –27% at LOEL) • Vacuolization between Sertoli cells and spermatogonia ↑ • Testicular WT1 protein levels ↑ (≈ +59% at LOEL) • Testicular Transferrin protein levels ↓ (≈ –33% at LOEL) • Serum MIS ↑ (≈ +22% at LOEL) Serum inhibin B ↓ (≈ –10% at LOEL)	3 3 1 3 3 1 1 – – 3 –	5 5 3 5 5 3 3 1 1 5 1				(Feng et al., 2010; Feng et al., 2009)
PFNA **Purity and salt not specified**	GD 1-20 Rat (SD) **Maternal (pregnant females):** n/sex/group = not specified, but since there were 18-19 litters, there were probably 18-19 dams… (?) **Offspring:** n/group = 18-19 litters for birth weight n/group = 10-12 pups/litter (litter size was standardized) n/group/sex = 1-2 per litter (not specified how many litters) for systolic blood pressure n/group = 5 (1 per litter from 5 litters) for nephrons (males only) Exposure: oral (gavage) (only dams)	**Maternal** • Body weight ↓ b(≈ –25%/–11% at GD10/GD21) **Fertility** • Birth weight ↓ (≈ –10% for females) **Developmental** • Systolic blood pressure at 10 weeks of age ↑ (≈ +7/10% for m/f) • Systolic blood pressure at 26/56 weeks of age (ns) • Nephrons per kidney (m) ↓ (≈ –19%) **Remark:** Offsping was weaned by non-treated foster-dams! No maternal toxicity effects and no lactational effects Also arsenic trioxide and nicotine bitartrate were also tested as comparative substances (to PFNA. A second experiment with separate a control comparing Dex (as positive control; known to increase blood pressure), Atrazine, and PFOS, is also published in this paper. Body weight gain in PFNA treated animals below those treated with arsenic. Lowest bw gains were observed with nicotine.						(Rogers et al., 2014)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	maternal NO(A)EL	maternal LO(A)EL	offspring NO(A)EL	offspring LO(A)EL	Serum/tissue concentration of PFAS /metabolites	Reference
	Doses: 0, 5 mg/ kg bw/d							
PFNA >98% purity	28-day RDT study, NTP study, similar to OECD TG 407 Rat (SD) n/sex/group: 10 Exposure: oral (gavage, in distilled water) Doses: males: 0, 0.625, 1.25, 2.5, 5, 10 mg/kg bw/d; females: 0, 1.56, 3.12, 6.25, 12.5, 25 mg/kg bw/d	**Fertility** • Plasma testosterone in m ↓ (≈ –81% at LOEL) • Plasma testosterone in f ↑ (≈ +30% at LOEL) • L. Cauda Epididymis weight ↓ (m, ≈ –11% at LOAEL) • L. Epididymis weight ↓ (m, ≈ –7% at LOAEL) • Testis weight ↓ (m, ≈ –7% at LOAEL) • Sperm count (10^6/g cauda epididymis) ↓ (m, ≈ –18% at LOAEL) • Germinal epithelium degeneration ↑ • Interstitial cell atrophy in testes ↑ • Seminiforous tubule spermatid retention ↑	1.25 – 0.625 – 0.625 0.625 1.25 1.25 1.25	2.5 1.56 1.25 0.625 1.25 1.25 2.5 2.5 2.5				(NTP, 2019b)
PFNA 97% purity	17 days ReproDevTox study (GD1-17), non-guideline study Mouse (CD-1) **Maternal sample size:** n/group = 1-10 (for pregnancy outcome) n/group = 19-27 (maternal body weight gain) n/group = 5-15 (liver weights) **Offspring sample size:** n/group = 11-17 litters (postnatal body weight and neonatal survival) n/group=6-13 litter (offsping body and liver weight & developmental delay)	**Maternal** • Maternal body weight at GD13 ↓ (≈ –30% at 10mg/kg bw/d) • Maternal body weight between GD11-GD17 ↑ (≈ +10% at LOEL) • Abs/rel liver weight (non-pregnant) ↑ (≈ +83%/88% at LOEL) • Abs/rel liver weight (pregnant) ↑ (≈ +45%/41% at LOEL) • Abs/rel liver weight (post-weaning) ↑ (≈ +33%/19% at LOEL) **Fertility** • Pregnancy not carried to term (full litter resorption) at LOEL • Pregnancy outcome (Implants per litter, abs/rel live foetuses per litter, fetal weight, skeletal or visceral examinations) (ns) **Developmental** • Abs/rel fetal liver weight ↑ (≈ +29/22% at LOEL) • Postnatal survival ↓ (≈ 20% survival at LOEL from PND10 onwards) • Postnatal body weight at PND 7/14/21↓ (≈ –17/24/23% at LOEL; effects persist in male pups until PND 287, but recovers in female pups after PND 49) • Rel postnatal liver weight at PND10/24↑ (≈ +37/27% at LOEL)	5 1 – – – 5 3 1 –	10 3 1 1 1 10 1 5 3 1			Serum concentrations (µg/ml): Pregnant at term: Control: 0.015 ± 0.003 1 mg/kg bw/d: 15 3 mg/kg bw/d: 25 5 mg/kg bw/d: 80 Not pregnant: Control: 0.19 ± 0.06 1 mg/kg bw/d: 30 3 mg/kg bw/d: 40 5 mg/kg bw/d: 200 Post-weaning: Control: 0.020 ± 0.001 1 mg/kg bw/d: 10 3 mg/kg bw/d: 15 5 mg/kg bw/d: 90	(Das et al., 2015)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	maternal NO(A)ELLO(A)EL	offspring NO(A)EL LO(A)EL	Serum/tissue concentration of PFAS /metabolites	Reference
	Exposure: oral (gavage, vehicle: deioinized water) Doses: 0, 1, 3, 5, (10, only till GD13) mg/kg bw/d Remarks: Since overt toxicity was indicated at 10 mg/kg bw/d, this group was sacrificed at GD13 and not included for further analysis in this study.	• Rel postnatal liver weight at PND 42 ↑ (≈ +18% at LOEL) • Delayed development (Eye opening ≈ +13%, preputial separation ≈ +11%, vaginal opening ≈ +10% at LOEL) • Gene expression heatmapà authors: "PFNA induced a clear PPARα-dependent gene expression signature in both fetal and neonatal mouse liver." (Rather complex heatmap. PPARα not measured directly, but only PPARα dependent genes. **Remarks:** Increased liver weight of offspring persistent into adulthood. Absolute liver weights were significantly increased in the 1, 3 and 5 mg/kg dose groups. However, this effect was not dose dependent, as the 5 mg/kg group was less affected "(…) at 43 weeks of age PFNA was still detectable in the liver and serum (data not shown), indicating the exceptionally slow elimination of this fluorochemical." EFSA Opinion: "The authors also provided benchmark dose estimates. The most sensitive ones were BMD_5/$BMDL_5$ values of 0.43/0.27 mg/kg bw/day for increased relative liver weights in mothers and BMD_5/$BMDL_5$ of 0.24/0.19 mg/kg bw per day for increased relative liver weight of pups at PND1."		1 3 1 3	Liver concentrations (µg/g) Pregnant at term: Control: 0.1 ± 0.01 1 mg/kg bw/d: 100 3 mg/kg bw/d: 260 5 mg/kg bw/d: 330 Not pregnant: Control: 0.67 ± 0.18 1 mg/kg bw/d: 170 3 mg/kg bw/d: 330 5 mg/kg bw/d: 450 After weaning: Control: 0.07 ± 0.01 1 mg/kg bw/d: 20 3 mg/kg bw/d: 120 5 mg/kg bw/d: 200 Fetal liver at term (µg/g): Control: 0.011 ± 0.007 1 mg/kg bw/d: 10 3 mg/kg bw/d: 35 5 mg/kg bw/d: 70 Serum offspring (µg/ml) (at PND1àPND10àPND24): 1 mg/kg bw/d: ≈20à10à1 3 mg/kg bw/d: ≈50à20à5 5 mg/kg bw/d: ≈75à80à25 Liver offspring (µg/g) (at PND1àPND10àPND24àPND42àPND70): 1 mg/kg bw/d: ≈55à60à20à10à5 3 mg/kg bw/d: ≈150à150à55à30à20	

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							5 mg/kg bw/d: ≈205à210à175à35à40	
PFNA (97%)	GD 1-PND21 (42-days for nonpregnant females) Mouse (129S1/SvImJ WT and PPARα KO on 129S1/SvImJ background (Ppara-tm1Gonz/J)) n/sex/group = 9-18 pregnant females, 8-16 litters Exposure: oral (gavage) Doses: 0.83, 1.1, 1.5, 2 mg/kg bw/d	**Maternal** • Body weight (ns) • Abs/rel liver weight (non-pregnant WT) ↑ (≈ +73/72% at LOEL) • Abs/rel liver weight (pregnant WT) ↑ (≈ +26/12% at LOEL) • Abs/rel liver weight (non-pregnant KO) ↑ (≈ +46/26% at LOEL) **Fertility** • Implantation and total litter size (ns) • Number of live pups at birth (WT) ↓ (at LOEL) • Full litter resorption or whole litter loss (WT) ↑ (35% litter loss at LOEL) • Pregnancy rate (KO) ↓ **Developmental** • Survival of pups (PND0-PND21) (WT) ↓ (≈ –64% at LOEL) • Eye opening ↑ (i.e. delay) (WT) (+2 days at LOEL) • Pup birth weight (ns) • Pup weight gain (PND7-post-weaning) (WT f) ↓ (≈ –25% at LOEL) • Pup body weight (PND21, WT, sexes combined) ↓ (≈ –21% at LOEL) • Pup abs/rel liver weight (PND 21, WT, sexes combined) ↑ (≈ +45/42% at LOEL) • Pup rel liver weight (PND 21, KO, sexes combined) ↑ (≈ +22% at LOEL) **Remarks:** non pregnant = mice that had a vaginal plug, but had full litter resorption KO mice with no live pups take up more PFNA than WT but show less/weaker effects KO mice with live pups have less PFNA in serum than WT KO pups show higher PFNA serum levels compared to WT (Pregnant KO mice transfer more PFNA to offspring?) Mice with live pups showhigher variance in serum PFAN than mice with no live pups (WT and KO)	– 0.83/– 1.1/0.83 0.83 1.5	0.83 1.1/0.83 1.5/1.1 1.1 2	 0.83 1.5 1.5 1.5 – 1.5	 1.1 2 2 2 0.83 2	Serum PFNA (µg/ml) in females with no live pups (WT/KO): Control: 0.067 ± 0.005/ 0.048 ± 0.008 0.83 mg/kg bw/d: 28.5 ± 1.22/ 38.4 ± 2.34 1.1 mg/kg bw/d: 39.7 ± 1.26/ 53.9 ± 2.51 1.5 mg/kg bw/d: 48.4 ± 1.54/ 72.1 ± 2.91 2 mg/kg bw/d: 64.0 ± 2.46/ 83.4 ± 2.93 Serum PFNA (µg/ml) in females with live pups (WT/KO): Control: 0.022 ± 0.004/ 0.016 ± 0.001 0.83 mg/kg bw/d: 8.91 ± 1.51/ 2.76 ± 0.172 1.1 mg/kg bw/d: 23.2 ± 2.57/ 4.17 ± 0.310 1.5 mg/kg bw/d: 21.0 ± 3.01/ 11.8 ± 5.71 2 mg/kg bw/d: 35.3 ± 3.90/ 22.6 ± 5.69 Serum PFNA (µg/ml) in pups (WT/KO): Control: 0.033 ± 0.008/ 0.068 ± 0.027 0.83 mg/kg bw/d: 9.60 ± **9.37**/ 15.2 ± 1.01	(Wolf et al., 2010)

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	maternal NO(A)ELLO(A)EL	offspring NO(A)EL LO(A)EL	Serum/tissue concentration of PFAS /metabolites	Reference
					1.1 mg/kg bw/d: 15.7 ± 1.42/ 19.4 ± 0.69 1.5 mg/kg bw/d: 17.5 ± 1.15/ 26.4 ± 1.39 2 mg/kg bw/d: 25.3 ± 2.70/ 38.4 ± 1.80	
S-111-S-WB (mixture, main component PFNA) (15.2% in water, doses already corrected) **CAS No. of mixture: 72968-38-8**	2-generation (>70 days before mating until weaning), OECD TG 416 (for repro tox) Rat (SD) n/sex/group = 30 (F0 and F1 generation) Exposure: Oral (gavage) Doses: 0, 0.025, 0.125 and 0.6 mg/kg bw/day	**Mortality** (1 F0 male died in study week 14, "moribund condition of this animal was attributed to test article administration) **Parental (F0+F1)** • Body weight (F0, m) (study weeks 0-7) (ns) • Body weight (F0, m) (study week 7-18) ↓ (≈ –25% after study week 18 at LOEL) • Food consumption (m, pre-mating weeks 0-10) ↓ (data not shown) • Abs/rel liver weight (F0, m) ↑ (≈ +22/22% at LOEL) • Abs/rel liver weight (F0, f) ↑ (≈ +16/14% at LOEL) • Abs/rel liver weight (F1, m) ↑ (≈ +16/17% at LOEL) • Abs/rel liver weight (**F1**, f) ↑ (≈ +16/14% at LOEL) • Abs/rel kidney weight (F0, m) ↑ (≈ +9/9% at LOEL) • Abs/rel kidney weight (F0, f) ↑ (≈ +7/7% at LOEL) • Abs/rel kidney weight (**F1**, m) ↑ (≈ +10/11% at LOEL) • • Hepatocellular hypertrophy (F0, m) ↑ (mild/moderate) • • Hepatocellular hypertrophy (F0, m) ↑ (mild/moderate) • Hepatocellular necrosis (F0, m) ↑ (minimal/mild) • • Hepatocellular centrilobular vacuolation (minimal and mild) • Renal tubule cell hypertrophy (F0, m) ↑ (minimal/mild) • Renal tubule cell hypertrophy (F0, f) ↑ (minimal and mild) **Fertility (F0+F1)** • No effects on fertility indices observed (F0+F1, m+f) • Sperm motility/progressive motility (F0, m) ↓ (≈ –5/6% at LOEL) • Epididymis weight (F0/F1, m) ↓ (≈ –12/10% at LOEL) • Epididymal sperm concentration (F0, m)↓ (≈ –13% at LOEL) • No effects on spermatogenic endpoints (testicular sperm numbers and sperm production rate, and morphology) **Developmental (F1+F2)** • No effects on pups (number of litters,, litter size, pup weight, vaginal patency/balanopreputial separation)	0.6 0.6 0.125 0.6 0.125 0.6 0.125 0.125 0.125 0.025/0.125 0.6 0.025/0.125 0.025 0.125/0.6 0.6 0.6 0.6 0.6		Serum concentrations analysed for C8 (PFOA9, C9 (PFNA), C11 (PFUnDA) and C13 (PFTrDA) (ng/ml): **Cmax (peak 0-16 h)** F0 females (Day 64): Control: ≈ 100 (?) 0.125 mg/kg bw/d: ≈ 3000 0.6 mg/kg bw/d: ≈ 16000 F0 females (GD19): Control: ≈ 100 (?) 0.125 mg/kg bw/d: ≈ 2500 0.6 mg/kg bw/d: ≈ 1400 Mean concentration on LD13 at 2 h post-dosing: Dams: Control: ≈ 500 (?) 0.125 mg/kg bw/d: ≈ 1500 0.6 mg/kg bw/d: ≈ 7500 Pups (m+f similar) Control: ≈ 500 (?) 0.125 mg/kg bw/d: ≈ 2000 0.6 mg/kg bw/d: ≈ 10000	Stump et al. 2008

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Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	maternal NO(A)ELLO(A)EL	offspring NO(A)EL LO(A)EL	Serum/tissue concentration of PFAS /metabolites	Reference
		• Body weight (F1, m) (post-weaning week 1-10) (ns) • Body weight (F1, m) (post-weaning week 11-22) ↓ (≈–32% in post-weaning week 22) • Body weight (F1+F2, m+f) (final (ns) • Rel liver weight of pups (F1, m/f) ↑ (≈ +7/8% at LOEL) • Rel liver weight of pups (F2, m/f) ↑ (≈ +13/11% at LOEL) • • Subacute liver inflammation (F1, m) ↑ (minimal) • Hepatocellular hypertrophy (F1, m) ↑ (minimal and mild/moderate) • • Minimal lymphocyte infiltrate (F1, m) • Hepatocellular necrosis (F1, m) ↑ (minimal) • Renal tubule cell hypertrophy (F1, m) ↑ (mild) **Remark: additional 8 females of F0 for toxicokinetics from > 70 days prior to mating till GD19** **Weaning on lactation day (LD) 21, afterwards selected F1 animals were continued on oral gavage (same doses as parents)** **Litters were reduced to 10 pups per litter (5 f, 5 m, if possible) on PND 4, on PND 13 8 per litter (4 f, 4 m if possible)** **Food efficiency was statistically significantly lower. Reduced body weight gain is not (only) a result of reduced food uptake.**		0.6 0.125 0.6 0.025 0.025/0.125 0.6 0.025 0.6		
In summary, the most sensitive endpoint after gestational exposure to PFNA was **increased liver weight** in both maternal and offspring CD-1 mice, and **a reduction in postnatal weight gain** in F1, with an **LOAEL of 1 mg/kg bw per day**, with corresponding concentration in serum from the dams at term of 20 lg/mL. **Delay in development was seen at 3 mg/kg bw per da**y, and **at 5 mg/kg bw per day, there was an increase in neonatal mortality**. A 90-day male reproductive study reported decreased sperm production, decrease in cholesterol, steroidogenic enzymes and testosterone, as well as decreased number of pups in the next generation, with an NOAEL and LOAEL of 0.2 and 0.5 mg/kg bw per day, respectively. Effects on male reproduction parameters were also reported by NTP for rats at somewhat higher exposure levels, and it was noted that **28 days** is shorter than one spermatogenic cycle and **too short to fully assess male reproductive parameters**.						EFSA 2020 (summary at the end of Repro effects of PFNA)
PFDA: Perfluorodecanoic acid (Syn: Nonadecafluorodecanoic acid, **CAS no:** 335-76-2, **EC no:** 206-400-3, **Mol. formula:** $C_{10}HF_{19}O_2$, **MW:** 514,xx?; registered under REACH, self classification available)						

Table 3: Developmental and reproductive toxicity animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	maternal NO(A)EL	maternal LO(A)EL	offspring NO(A)EL	offspring LO(A)EL	Serum/tissue concentration of PFAS /metabolites	Reference
PFDA 96%	4/10-day DevTox Study, non-guideline Mouse (C57BL/6N) n/group (maternal) = 10-13/10-14 n/group (litters) = 10-13/7-14 n/group (foetuses) = 83-106/23-53 Exposure: oral (gavage, in corn oil) Doses: 0, 0.25, 0.5, 1, 2, 4, 8, 16, 32 mg/kg bw/day on gestation days (GD) 10-13 (4 consecutive days) or 0, 0.03, 0.1, 0.3, 1, 3, 6.4, or 12.8 mg/kg bw/day, on GD 6-15 (10 consecutive days)	**Maternal (GD 10-13):** • maternal body weight gain ↓ (≈ –61% at LOEL) • Abs/rel liver weight ↑ (≈ +10/3% at LOEL) **Maternal (GD 6-15):** • Maternal body weight gain ↓ (≈ –92% at LOEL) • Abs/rel liver weight ↑ (≈ +61%/+127% at LOEL) **Fertility (GD 10-13):** • Live foetuses per litter ↓ (≈ –32% at LOEL, but ns) • Resorptions per litter ↑ (ns) (≈ +170% at LOEL) **Fertility (GD 6-15):** • Live foetuses per litter ↓ (≈ –46% at LOEL) • Resorptions per litter ↑ (≈ +344% at LOEL, but p=0.06) **Developmental (GD 10-13):** • Fetal body weight ↓ (≈ –10% at LOEL) **Developmental (GD 6-15):** • Fetal body weight ↓ (≈ –3% at LOEL) • Absence of fifth sternabrae (15% of examined foetuses at LOEL) • Delay in braincase ossification (overall) (26% of examined foetuses at LOEL) Delay in phalanges ossification (18% of examined foetuses at LOEL)	8 0.5/0.25 3 0.3	16 1/0.5 6.4 1	 16 16 6.4 6.4 0.25 0.03 3.0 – 3.0	 32 32 12.8 12.8 0.5 0.1 6.4 0.03 6.4		(Harris and Birnbaum, 1989)
PFDA (>98%)	28-day RDT study, NTP study, similar to OECD TG 407 Rat (SD) n/sex/group = 10 Exposure: oral (gavage, in distilled water) Doses: males: 0, 0.156, 0.312, 0.625, 1.25, or 2.5 mg/kg g bw/d	**Fertility** • Plasma testosterone in m ↓ (≈ –64% at LOEL) • Plasma testosterone in f ↑ (≈ +32-355% at LOEL) • L. Cauda Epididymis weight ↓ (m, ≈ –11% at LOAEL) • L. Epididymis weight ↓ (m, ≈ –10% at LOAEL) • Testis weight ↓ (m, ≈ –11% at LOAEL) • Sperm number?(10^6/g cauda epididymis) ↓ (m, ≈ –30% at LOAEL) Interstitial cell atrophy in testes ↑ (m, 80% incidence at LOAEL)	0.625 0.156 0.625 0.625 1.25 1.25 0.625	1.25 0.312 1.25 1.25 2.5 2.5 1.25				(NTP, 2019b)
PFUnDA: Perfluoroundecanoic acid (Syn: Henicosafluoroundecanoic acid, **CAS no:** 2058-94-8, **EC no:** 218-165-4, **Mol. formula:** $C_{11}HF_{21}O_2$, **MW:** 564,xx?; SVHC, candidate for authorisation under REACH, self classification available)								

Table 3: Developmental and reproductive toxicity animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	maternal NO(A)EL	maternal LO(A)EL	offspring NO(A)EL	offspring LO(A)EL	Serum/tissue concentration of PFAS /metabolites	Reference
PFUnDA (98.5%)	42-day combined RDT with the Repro/DevTox Screening Test (OECD TG 422) 14 days before mating until day 4 of lactation Rat Crl:CD (SD) n/sex/group = 12 (+5 of highest dose and control for 14-day recovery) Exposure: oral (gavage, vehicle: corn oil) Doses: 0, 0.1, 0.3, 1.0 mg/kg bw/d	**Parental (m/f)** • Grip strength of forefoot ↓ (no data shown) • Body weight (female satellite group) ↓ (day 38-41+ 14-day recovery; similar in males, but ns) • abs. liver weight ↑ • rel. liver weight ↑ • Hepatocyte hypertrophy ↑ (≈ 58-52% incidence at LOAEL) • rel./abs. spleen weight ↓ (m only, ≈ −19-23% at LOAEL) • serum ALP, ALT ↑ (m only, ≈ +26% for ALT and +139% for ALP) • serum urea nitrogen ↑ (≈ +46-61% at LOAEL) • serum albumin ↓ (m only, ≈ −7% at LOAEL) • total protein ↓ (≈ −10-11% at LOAEL) **Fertility (m/f)** • No effects on epididymis, testis/uterus, estrous cycle or delivery/nursing (ns) **Developmental** • No abnormal findings in general appearance of pups • Body weights (PND0) (m/f) ↓ (≈ −13/12% at LOEL) • Body weights (PND4) (m/f) ↓ (≈ −19/16% at LOEL)	0.3 – 0.3/0.3 0.1/0.3 0.3/0.3 0.3/0.3 0.3 0.3/0.3 0.3 0.3/0.3 0.3 0.3	1 1 1/1 0.3/1 1/1 1/1 1 1/1 1 1/1 1 1				(Takahashi et al., 2014)
PFDoDA: Perfluorododecanoic acid **(Syn:** Tricosafluorododecanoic acid, **CAS no:** 307-55-1, **EC no:** 206-203-2, **Mol. formula:** $C_{12}HF_{23}O_2$, **MW:** 614.10; SVHC substance, candidate list for authorisation, self class.available)								
PFDoDA (97/%)	42-day RDT study, OECD TG 422, 14 day recovery (0, 2.5 mg/kg bw/d) Rat (SD) n/sex/group = 12 Exposure: oral (gavage) Doses: 0, 0.1, 0.5, 2.5 mg/kg bw/d (14-day recovery group: 0, 2.5 mg/kg bw/d)	**Maternal** • mortality of pregnant females (7/12 dead) **Fertility** • abs. L. Epididymis weight ↓ (m, ≈ −32% at LOAEL) • spermatid and spermatozoa counts (m) ↓ • discontinuous dioestrus (f)	0.5 0.5 0.5 0.5	2.5 2.5 2.5 2.5				(Kato et al., 2015)
PFDoDA	110 day RDT study	**Paternal** • Body weight ↓ (≈ −5.5% at LOEL)	0.2	0.5				(Shi et al.,

Table 3: Developmental and reproductive toxicity animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	maternal NO(A)EL	maternal LO(A)EL	offspring NO(A)EL	offspring LO(A)EL	Serum/tissue concentration of PFAS /metabolites	Reference
95%	Rat (SD) n/sex/group = 6 (m) Exposure: oral (vehicle: Tween-20) Doses: 0.02, 0.05, 0.2, 0.5 mg/kg bw/d	**Fertility** • Abs/rel weight of testis, prostate, seminal vesicle and vas deferens (ns) • Serum testosterone ↓ (≈ −44% at LOEL) • Serum LH, FSH, cholesterol (ns) • Cast-off cells in some seminiferous tubules in testes (no quant) • Testicular gene expression of StAR ↓ (≈ −35% at LOEL) • Testicular gene expression of P450scc ↓ (only at 0.05) • Testicular gene expression of IGF-I ↓ (≈ −25% at LOEL) • Testicular gene expression of IGF-IR and IL-1α ↓ (≈ −25%/–30% at LOEL) • Testicular gene expression of GnRH-R and FSH ↓ (≈ −45% at LOEL) Testicular gene expression of LH ↓ (≈ – 50%, only at 0.05mg/kg bw/d)	0.05 0.2 - 0.02 0.05 0.2 0.2	0.2 0.5 0.02 0.05 0.2 0.5				2009)
PFDoDA 95%	28 day RDT study PND24 till PND52-55 (i.e 28-31-days, sample collection in dietrous stage, pubertal females) Rat (SD) n/sex/group = 8 (f) Exposure: oral (vehicle: Tween-20) Doses: 0, 0.5, 1.5, 3 mg/kg bw/d	**Maternal** • Body weight ↓ (-6% at LOEL) **Fertility** • Serum total cholesterol ↑ (≈+32% at LOEL) • Serum estradiol ↓ (≈–37% at LOEL) • Serum FSH+LH (ns) • Ovarian gene expression ↓ (≈–35% for LHR, ≈–40% for StAR, ≈–36% for P450SCC, ≈–36% for ER-β at LOEL) • Ovarian gene expression ER-α ↓ (≈–36% at LOEL) • Ovarian gene expression 17β-HSD ↑ (≈+110% at LOEL) **Remark: "**P450SCC is a rate limiting enzyme responsible for the conversion of cholesterol to pregnenolone in estrogen biosynthesis."	1.5 1.5 1.5 1.5 0.5 –	3 3 3 1.5 0.5				(Shi et al., 2009)
PFDoDA 95%	14-days RDT study Rat (SD) n/sex/group = 6 (m) Exposure: oral (gavage, vehicle: Tween-20) Doses: 1, 5, 10 mg /kg bw/d	**Males** • Body weight ↓ (≈ −25.2% at LOEL) **Fertility** • Total serum cholesterol ↑ (≈ +38.5% at LOEL) • Serum LH ↓ (≈ −30% at LOEL)(FSH no effect) • Testosterone ↓ (≈ −50% at LOEL) • (Estradiol ↓ (only at MD, ≈ –50%)) • Apoptotic features present in Leydig cells, Sertoli cells and spermatogenic cells • Abs testis weight ↓ (≈ −22.4% at LOEL) • Rel testis weight ↑ (≈ +36% at LOEL) • Testicular gene expression of SR-B1 ↓ (≈ −60% at LOEL) • Testicular gene expression of StAR ↓ (≈ −50% at LOEL) Testicular gene expression of P450scc ↓ (≈ −90% at LOEL)	1 5 5 1 5 5 1 1 1 1	5 10 10 5 10 10 5 5 5 5				(Shi et al., 2007)

Table 3: Developmental and reproductive toxicity animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	maternal NO(A)EL	maternal LO(A)EL	offspring NO(A)EL	offspring LO(A)EL	Serum/tissue concentration of PFAS /metabolites	Reference
PFDoDA	14-day RDT study Rat (SD) n/sex/group = 8 (m) Exposure: oral (gavage) Doses: 0, 5, 10mg/kg bw/d	**Males** • Body weight ↓ (≈ −21% at LOEL) **Fertility** • Abs. testis weight ↓ (≈ –8% at LOEL) • Serum testosterone ↓ (≈ –82% at LOEL) • Serum LH ↓ (≈ –36% at LOEL) • Serum FSH ↓ (≈ –9% at LOEL) • Gene expression of Leydig cell genes from testis ↓ (≈ –29% for Lhcgr, ≈–47% for Cyp11a1 at LOEL) • Gene expression of Leydig cell genes from testis ↓ (≈ –57% for Scarb1, ≈–59% for Star, ≈ –61% for Cyp17a1, ≈ –53% for Hsd11b1 at LOEL) • Gene expression in sertoli cells (ns) • Protein levels of Leydig cells (similar to gene expression) • Sirt1 signalling ↓ (≈ –33% at LOEL) PGC-1α signalling ↓ (≈ –35% at LOEL)	5 5 – – – 5 – 5	10 10 5 5 5 10 5 10				(Chen et al., 2019)
In **summary**, exposure of rats to PFDoDA prior to and during gestation induced **maternal and reproductive effects (continuous dioestrus and fetal loss)** with an **NOAEL of 0.5 mg/kg bw per day**. **Male reproductive effects (decreased spermatid and spermatozoa counts**) were seen at a similar **NOAEL of 0.5 mg/kg bw per day**, which is higher than the NOAEL of 0.1 mg/kg per day observed for repeated dose toxicity in the same experiment.								EFSA (summary at the end of Repro effects of PFDoDA)
PFTeDA: Perfluorotetradecanoic acid **(Syn:** Heptacosafluorotetradecanoic acid, **CAS no:** 376-06-7, **EC no:** 206-803-4, **Mol. formula:** $C_{14}HF_{27}O_2$, **MW:** 714.11; SVHC, candidate list for authorisation; Self class. available, but only Skin Corr. 1B)								
PFTeDA, Perfluorotetradecanoic acid	Repeated dose and reproductive/developmental toxicity (OECD TG 422) Rat (SD) n/sex/group = 12 Exposure: oral (gavage, vehicle 0.5% carboxymethylcellulose sodium in water) Doses: 0, 1, 3, 10 mg/kg bw/day) Dosing regime	**Parental** • Males: ↓ body weights on day 7 and day 14 (treated animals versus recovery group) • Females: ↓ body weight on day 4 of lactation • Females: ↓ body weight during lactation period • Males: ↑ absolute and relative liver weight • Females: ↑ relative liver weight **Fertility** • No effects on estrous cycle, copulation index, fertility index, gestation length, Corpora lutea, implantation sites, delivered pubs, sex ratio **Developmental effects** • Body weight of pups (m/f) ↓ (≈–14/13% on PND 1, ≈–18/17% on PND4 at LOEL)	3 1 3 1 3	10 3 10 3 10	3	10		(Hirata-Koizumi et al., 2015)

Table 3: Developmental and reproductive toxicity animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	maternal NO(A)EL	LO(A)EL	offspring NO(A)EL	LO(A)EL	Serum/tissue concentration of PFAS /metabolites	Reference
	All animals: 14 day pre-mating Males: 42 day Females: during lactation up to 5 days after parturation (app. 42 days i.e. 14 premating, 1-2 days mating 21-23 gestation, plus 5 days) High dose recovery groups (14 days recovery after treatment)							
PFHxDA: Perfluorohexadecanoic acid **(Syn: Perfluoropalmitic acid**, **CAS no:** 67905-19-5, **EC no:** 267-638-1, **Mol. formula:** C16HF31O2, **MW:** 814.13; only self class. Skin Corr. 1B , no further ECHA data)								
PFHxDA, Perfluorohexadecanoic acid	Repeated dose and reproductive/developmental toxicity (OECD TG 422) Rat (SD) n/sex/group = 12 Exposure: oral (gavage, vehicle 0.5% carboxymethylcellulose sodium in water) Doses: 0, 4, 20, 100 mg/kg bw/day) Dosing regime: All animals: 14 day pre mating Males: 42 day Females: during lactation up to 5 days after parturition High dose recovery groups (14 days recovery after treatment)	**Parental** • Males: ↓ body weights on day 35 and day 42 of administration period • Males: ↓ food consumption decreased at day 14 of recovery period • Females: ↓ food consumption GD 5 - 10 gestation; LD 4 **Developmental** • Body weight of offsping (m/f) ↓ (≈–4/5% on PND1, ≈–6/6% on PND4 at 100 mg/kg bw/d, but not significant) • no other effects are reported	20 20 20	100 100 100				(Hirata-Koizumi et al., 2015)
PFODA: Perfluorooctadecanoic acid **(Syn:** Perfluorostearic acid, **CAS no**: 16517-11-6, **EC no:** 240-582-5, **Mol. formula:** C18HF35O2, **MW:** 914.15;)								

Table 3: Developmental and reproductive toxicity animal studies (abbreviations, symbols and key parameters/targets explained underneath the table)

Substance (%), EC/CAS, formula	study design, species, route of exposure, doses (guideline/similar to guideline/non-guideline)	Observed effects Remarks	maternal NO(A)EL	maternal LO(A)EL	offspring NO(A)EL	offspring LO(A)EL	Serum/tissue concentration of PFAS /metabolites	Reference
PFODA, Perfluorooctadecanoic acid	Repeated dose and reproductive/developmental toxicity (OECD TG 422) Rat (SD) n/sex/group = 12 Exposure: oral (gavage, vehicle 0.5% carboxymethylcellulose sodium in water) Doses: 0, 40, 200, 1000 mg/kg bw/day Dosing regime: All animals: 14 day pre mating Males: 42 day Females: until 5th day of lactation (42-56 days)	**Parental** • Females: ↓ body weight after 14 days (~20% at day 28) ↓ lower food consumption at day 5 lactation • Males: ↓body weight after 28 days (~20% at day 42) • abs. and rel.liver weight (f+m) ↑ (≈ +66% abs. in HD) **Fertility** • number of corpora lutea ↓ (≈–16% at LOEL) • number of implantations ↓ (≈–15% at LOEL) • Total number of pups born ↓ (≈–28% at LOEL) • No of live pups on PND0/PND4↓ (≈–35/5% at LOEL) • No significant differences in delivery index and viability indices between control and PFODA treated groups, but a decreasing tendency in the HD-group. **Developmental** • Body weight pups (m/f) ↓ (=–23/23% on PND0, ≈–27/26% on PND1, ≈–28/26% on PND 4 at LOEL) Body weight gain (m/f) ↓ (≈–37/32% at LOEL)	200 200 200 40 200 200 200 200 200 200 200	1000 1000 1000 200 1000 1000 1000 1000 1000 1000 1000				(Hirata-Koizumi et al., 2012)

9. Annex 4: Carcinogenicity studies

Table 4: Animal studies in relation to carcinogenic effects (abbreviations, symbols and key parameters/targets explained underneath the table)

PFCA (%)	study design	Observed effects	NO(A)EL	LO(A)EL	Reference
PFHxA	104-week RDT carcinogenicity study Rat (SD) n/sex/group = 60 (except HD with 70) Exposure: oral (gavage) Doses: 0, 2.5, 15, 100 (m); 0, 5, 30, 200 (f) mg/kg bw/d	**no indication for neoplasia/hyperplasia**			(Klaunig et al., 2015)
PFOA	24-months RDT study Rat (SD), n/sex/group = 50-65 Exposure: oral (dietary, ad libitum) Doses: 0, 30, 300 ppm; corresponding to approximately 0, 1.5, 15 mg/kg bw/d (0, 1.3 or 14.2 mg/kg bw/d for males and 0, 1.6 or 16.1 mg/kg bw/d for females)	**Neoplasia/hyperplasia** **Liver** • Liver adenoma: 6%, 2%, and 10% in males and 0%, 0%, and 2% in females in C, LD, HD **Reproductive tissue** • Leydig cell adenoma ↑ (0, 4, 14% in C, LD, HD) • mammary gland adenoma in females: 15%, 31%, and 11% in C, LD, HD • fibroadenomas in mammary glands of females: 22%, 42%, and 48% in C, LD, HD **Pancreas** • pancreatic acinar cell hyperplasia: 0%, 4%, 4% in C, LD, HD from original study; and after re-evaluation of male tissue samples by Caverly-Rae et al. (2014): 6.5%, 2%, and 21% in C, LD, HD with significant increase in HD Caverly-Rae et al. (2014): "A pathology peer review of male exocrine pancreatic tissues from the earlier study, conducted in 1981–1983 by Butenhoff et al., was undertaken. This review identified an increase in acinar cell hyperplasia but not adenoma or carcinoma in the earlier study." The only statistically significant neoplastic lesions were an increase in testicular Leydig cell adenomas and fibroadenomas in mammary glands (Butenhoff et al., 2012b); a re-evaluation of the original slides by a Pathology Working Group resulted in no significant increase of fibroadenomas with following incidences: 36, 44, 46% in C, LD, HD (Hardisty et al., 2010)	1.5/– – 1.5 1.5/–	15/– – 15 15/–	(3M, 1983) (Sibinski, 1987) (Butenhoff et al., 2012b) (Hardisty et al., 2010) (Caverly Rae et al., 2014)
PFOA (98-100%)	24-months RDT study Rat (Crl:CD BR (CD)) n/sex/group = 156 (males only) n/group (neoplasia/hyperplasia) = 76-80 Exposure: oral (dietary) Doses: 0, 300 ppm (equivalent to 13.6 mg/kg bw/d)	**Neoplasia/hyperplasia** **Liver** • Liver adenoma ↑ (+1200% (13% vs. 1% in C)) **Pancreas** • Acinar cell hyperplasia ↑ (+290% (39% vs. 10% in C)) • Acinar cell adenoma ↑ (+800% (9% vs. 1% in C)) **Reproductive tissue** • Leydig cell hyperplasia ↑ (+39% (46% vs. 33% in C)) • Leydig cell adenoma ↑ (+267% (11% vs. 3% in C))	– – – – –	13.6 13.6 13.6 13.6 13.6	(Biegel et al., 2001)

Table 4: Animal studies in relation to carcinogenic effects (abbreviations, symbols and key parameters/targets explained underneath the table)

PFCA (%)	study design	Observed effects	NO(A)EL	LO(A)EL	Reference
PFOA (>98%)	107-weeks RDT study with perinatal and post-weaning exposure Rat (SD) n/sex/group: study 1: F0 females: 103 (C) or 36 (150 and 300 ppm groups); F1 rats: 60 m and 60 f; study 2: F0 females: 147, F1 males: 60 (no females) Exposure: oral (diet) Doses: study1: perinatal: 0, 150, 300 ppm; post-weaning: m: 0, 150, 300 ppm (≈ 0, 16, 32 mg/kg bw/d), f: 0, 300, 1000 ppm (≈ 0, 30, 100 mg/kg bw/d) study 2: perinatal: 0, 300 ppm; post weaning: 0, 20, 40, 80 ppm (≈ 0, 1.1, 2.2, 4.6 mg/kg bw/d)	**Neoplasia/hyperplasia** Non-neoplastic lesions were only observed in the liver and pancreas of male rats **Liver** • Hepatocellular adenoma and carcinoma in males of 0/40, 300/40, 0/80, and 300/80 ppm groups ↑ (0/0, 0/20, 0/40, 0/80: 0%, 0%, 14%, 22%; 300/0, 300/20, 300/40, 300/80: 0%, 2%, 10%, 24%) • higher incidences of hepatocellular carcinomas in the 300/80 ppm group compared to the 0/80 group **Pancreas** • acinar cell adenomas and adenocarcinomas ↑ in males of all groups exposed post-weaning (0/0, 0/20, 0/40, 0/80: 6%, 58%, 52%, 64%; 300/0, 300/20, 300/40, 300/80: 14%, 40%, 60%, 60%) • occurrence of acinar cell adenomas and adenocarcinomas in the 0/1,000 and 300/1,000 ppm female groups (not statistically significant) clear evidence of carcinogenic activity of PFOA in male SD rats based on the increased incidence of hepatocellular neoplasms (predominately hepatocellular adenomas) and increased incidence of acinar cell neoplasms (predominately acinar cell adenomas) of the pancreas; there was no increase in the incidence of Leydig cell neoplasms observed in this study, possibly due to lower doses compared to two previous studies (3M, 1983; Biegel et al., 2001)	1.1 -	2.2 1.1	(NTP, 2019a)
PFOA (purest available analytical grade)	52-week RDT study to assess tumor-promoting activity of PFOA Rat (Wistar, 180 g) n/sex/group = 15 (m) Exposure: oral (diet, ad libitum) Doses: 0, 0.005, 0.02% PFOA (Group A, B) or 0.015% (triphasic protocol) in diet initiation: Group A – no initiation; Group B - 200 mg/kg diethylnitrosamine; triphasic protocol - 200 mg/kg diethylnitrosamine + 0.03% 2-AAF in diet for 2 weeks + single oral dose of CCl4 (2 mL/kg) positive control: phenobarbital (0.05%)	**Neoplasia/hyperplasia** **Liver** • Hepatocellular carcinoma of Group B (C: 0/7, 0.005%: 1/7, 0.02% PFOA: 5/9) and in triphasic protocol ↑ (C: 0/7; 0.015%: 4/12) • No liver tumours occurred in the non-initiated rats receiving PFOA • PFOA also selectively induced the peroxisomal acyl-CoA oxidase activity and, to a lesser extent, catalase activity PFOA increased the incidence of malignant hepatocellular carcinoma and also selectively induced the peroxisomal acyl-CoA oxidase activity and catalase activity			(Abdellatif et al., 1991)
PFNA, PFOS, PFOA,	6 months RDT study trout A two-stage chemical carcinogenesis model in trout to evaluate PFOA, PFNA,	**Neoplasia/hyperplasia**			(Benninghoff et al., 2012)

Table 4: Animal studies in relation to carcinogenic effects (abbreviations, symbols and key parameters/targets explained underneath the table)

PFCA (%)	study design	Observed effects NO(A)EL LO(A)EL	Reference
PFDA or 8:2FTOH	PFDA, PFOS, and 8:2 fluorotelomer alcohol (8:2FtOH) as complete carcinogens or promoters of aflatoxin B1 (AFB1)- and/or N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced liver cancer. Dose groups for initiation: 10 ppb AFB1 or 35 ppm MNNG, for both initiation groups: Cohort 1: diets containing 5 ppm E2, 2000 ppm PFOA(approximately 50 mg/kg body weight/day), 2000 ppm FtOH, 1000 ppm PFNA, 200 ppm PFDA or 2000 ppm CLOF ad libitum Cohort 2: (AFB1 at 15 weeks), trout were fed 100 ppm PFOS. Finally, MNNG-initiated trout were fed 5 ppm E2 or 2000 ppm PFOA.	Incidence, multiplicity, and size of liver tumors in trout fed diets containing E2, PFOA, PFNA, and PFDA were significantly higher compared to AFB1-initiated animals fed control diet, whereas PFOS caused a minor increase in liver tumor incidence. E2 and PFOA also enhanced MNNG-initiated hepatocarcinogenesis. PFNA and PFDA enhanced incidence and size of liver tumors when compared with AFB1-initiated animals fed a control diet. In the groups receiving PFNA or PFDA only, there were no tumors or a few liver adenomas, respectively. This indicates a tumor promoting capacity of both compounds in this animal species.	
PFOA, PFOS	EFSA Opinion 2018: Risk to human health related to the presence of PFOS and PFOA in food	**PFOS** • PFOS was found to cause tumours in the liver of rats. Mechanistic studies suggested that the compound may act as a tumour promoter. **PFOA** • In Sprague–Dawley rats, PFOA induced Leydig cell tumours. Hormonal dysbalance appears to be the underlying mechanism. Inconsistent effects were noted for pancreatic hyperplasia or tumours in mammary gland and liver.	(EFSA, 2020b)