DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

Date: September 30, 2010

From: Toxicology Group 1, Division of Food Contact Notifications (DFCN)
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Subject: Critical review of studies conducted with ≥ C8 perfluorinated compounds concerning selected endpoints. (b) (4) .

To: Regulatory Group 2, DFCN
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RELATED FILES[1]

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INTRODUCTION

This memorandum documents Toxicology's updated critical review of the available knowledge base for ≥ C8 perfluorinated compounds and our conclusions regarding the human health relevance of these findings. Toxicology's original review of the available data on the toxicity of perfluorooctanoic acid (CASRN: 335-67-1, PFOA; McDougal/Honigfort, 6/13/07, (b) (4)) noted the biopersistence of PFOA in male rats and the fact that Toxicology had previously derived a unit cancer risk value for PFOA (Twaroski/Gilliam, 10/01/2002, (b) (4)). Carcinogenicity was considered to be the most sensitive and relevant endpoint for PFOA in particular (McDougal/Honigfort, 6/13/07). In the absence of data to suggest otherwise, carcinogenicity was also considered to be the most sensitive endpoint for structurally-similar perfluorinated compounds, such as ≥ C8 perfluorinated telomer alcohols, based on the available data for PFOA, the assumption of similar modes of action and/or metabolism to PFOA, and Toxicology's conclusion that perfluorinated compounds were not potent reproductive or developmental toxicants (Gu/Rice, 11/17/07; Rice/Honigfort, 12/1/06; McDougal/Honigfort, 5/18/06, (b) (4)). Based on the data available demonstrating carcinogenic effects in rats, rats were considered to be the most sensitive and relevant species in which to assess the toxicity of this class of compounds. This rationale was applied to Toxicology's review of low molecular weight oligomeric species (LMWO) of perfluorinated food contact substances (FCSs), their precursors, and any perfluorinated degradation products in a structural activity relationship argument that considered assessment of potential carcinogenicity in male rats with representative C8 compounds to be 'worst-case' in terms of human health risk assessment.

[1] PNC: Prenotification Consultation; FCN: Food Contact Notification; FAP: Food Additive Petition; CASRN: Chemical Abstract Services Registry Number; FMF: Food Master File; NOEL: No Observed Effect Level; NOAEL: No Observed Adverse Effect Level; LOEL: Lowest Observed Effect Level; GD: Gestation Day; PND: Postnatal Day; BW: Bodyweight; PFCs: Perfluorinated Compounds; RA: Risk Assessment; HI: Hazard Identification; SAR: Structure Activity Relationship.

Since Toxicology's original review of these compounds in 2007, the U.S. EPA published a Tier 3 toxicity value for PFOA (EPA, 2009a) and has proposed initiating rulemaking for managing long-chain perfluorinated compounds under the Toxic Substances Control Act, Part 6 (EPA, 2009b). The Action Plan highlights several uncertainties regarding the pharmacokinetics and toxic modes of action for these compounds that suggest a need for updating Toxicology's original review. In addition, Toxicology's ongoing assessment of the public literature database on perfluorinated compounds has identified new information published since Toxicology's original review in 2007. Based on these data, Toxicology has identified the need to consider other possible endpoints, perfluorinated carbon chain lengths, and animal models. The purpose of this memorandum is to summarize our review of toxicity studies addressing the endpoints of endocrine-disruption, reproductive health and function, and pre- and postnatal developmental toxicity, in order to assess whether these endpoints represent sensitive targets of toxicity for perfluorinated compounds. The methods by which the review was performed, summarized results from the reviewed studies, and the conclusions from this review are stated below.

METHODS AND CRITERIA

The section describes the methods by which the review was performed. It should be emphasized that the purpose of this review was to up date the risk assessment and, in doing so, update the hazard identification endpoints of perfluorinated compounds. In addition, based on the SAR, it is assumed that conclusions derived from this review should be considered in the hazard identification for perfluorinated compounds as a class, assuming that the perfluorinated moieties common to members of this class of compounds are the primary sources of their toxicity. There is, however, a great deal of uncertainty in the above-stated assumption due to the different additional functional groups which may play a role in metabolism (i.e., alcohols, acids, etc.), the molecular weight and volume, the chain length and amount of fluorination, as well as the molecular bonds and side chains in the case of LMWO. As such, though this review attempts to provide conclusions and recommendations for these compounds as a class, these factors should be considered in any discussion of future substances with regard to linking the data discussed herein to the safety assessment of a particular perfluorinated compound..

Compounds Considered in the Review

This critical review intentionally restricted the scope of its assessment to perfluorinated impurities of ≥ C8 that are precursor compounds in the manufacture of perfluorinated FCSs, impurities in these FCSs, or possible degradation products. The compounds considered in this review included the following:

Chemical name	**CASRN**	**Acronyms**
Perfluorooctanoic acid	335-67-1	C8, PFOA
Perfluorononanoic acid	375-95-1	C9, PFNA
Perfluorodecanoic acid	335-76-2	C10, PFDA
Perfluoroundecanoic acid	2058-94-8	C11, PFUnDA
Perfluorododecanoic acid	307-55-1	C12, PFDoDA
1H, 1H, 2H, 2H-perfluorodecanol	678-39-7	8:2-telomer B alcohol; 8-2 TBA
1,1,2,2-tetrahydroperfluoro-1-dodecanol	865-86-1	10:2-telomer B alcohol: 10-2 TBA
1,1,2,2-tetrahydroperfluoro-1-tetradecanol	39239-77-5	12:2-telomer B alcohol; 12-2 TBA
Mixture of 0.34% 1,1,2,2-Tetrahydroperfluoro-1-Hexanol; 32.17% 1,1,2,2-Tetrahydroperfluoro-1-Octanol; 27.3% 1,1,2,2-Tetrahydroperfluoro-1-Decanol; 19.49% 1,1,2,2-Tetrahydroperfluoro-1-Dodecanol; 10.39% 1,1,2,2-Tetrahydroperfluoro-1-Tetradecanol; 4.33% 1,1,2,2-Tetrahydroperfluoro-1-Hexadecanol;	CASRNs: 2043-47-2; 647-42-7; 678-39-7; 865-86-1; 39239-77-5; 60699-51-6; 65104-67-8; 65104-65-6, respectively	Mixed TBA

0.59% 1,1,2,2-Tetrahydroperfluoro-1-Octadecanol; 0.06% 1,1,2,2-Tetrahydroperfluoro-1-Eicosanol		

(b) (4) [redacted]

Sources of Information Included in the Review

This review considered in-house reviews of any of the relevant compounds listed above and performed a focused search of the public databases for information on the endpoints of interest pertaining to these compounds. Public literature databases that were searched for this review included: PubMed, Google Scholar, ToxNet, ChemIDplus advanced, the U.S. EPA's website, the Agency for Toxic Substances and Disease Registry (ATSDR)'s website, Scirus, and IPCS Inchem. The dates of the literature search ranged from April 24, 2010 to July 10, 2010 (includes in-press citations). Literature databases were searched using the names of the compounds as listed above and the following additional keywords: reproductive, developmental, prenatal, postnatal, rat, mouse, estrogen, androgen, thyroid, testes, uterus, ovary, ovaries, endocrine, and mammary. Approximately 56 hits were retrieved from this search.

Endpoints Assessed

The endpoints assessed in this review were those concerned with effects of perfluorinated compounds on the endocrine system at various life stages, including reproductive health and function and pre- and postnatal development. Endpoints of interest are listed below:

- Pre- and postnatal development: Relevant parameters for this endpoint are, for the most part, the measures of developmental toxicity as specified in guideline testing (OECD, 2004; ICH, 1993; Redbook 2000) for developmental toxicity studies. There is some overlap in this area with parameters measured under reproductive function, such as birth weight, postnatal weight gain, and development; but this endpoint, as defined herein, also includes effects of the test compound on development when administered during puberty and the assessment of effects in adulthood of brief pre- or postnatal exposures;
- Reproductive health and function: Relevant parameters for this endpoint are, for the most part, the measures of reproductive function as specified in guideline testing (OECD, 2004; ICH, 1993; Redbook 2000) for reproductive toxicity studies. Additional parameters include: accessory sex organ histopathology (i.e.: mammary gland); serum sex hormones (estradiol (E), testosterone (T), progesterone (P), lutenizing hormone (LH), follicle stimulating hormone (FSH)); effects on reproductive hormone synthesis and metabolism, such as changes in enzyme activity, protein levels, or gene expression; and binding/activity of a test compound at a sex hormone receptor, such as the androgen receptor (AR) or the estrogen receptor (ER); and
- Thyroid gland: Alterations in thyroid hormone secretion and metabolism, thyroid weight parameters, and histology are considered under this endpoint. Alteration of other hormonal pathways, such as corticosteroid homeostasis or growth hormone secretion and metabolism, are not considered in this review.

Noteworthy, many of the reviewed studies focus on a subset of parameters for a given endpoint at the expense of other more general apical endpoints. These studies assess parameters that may not be assessed in more comprehensive studies and may not represent validated protocols. As such, the data presented was considered in the review as potential indicators of toxicity of the test compound at the site of interest.

Criteria for Inclusion

This review considered three tiers:

- *In vivo* studies in mammals (literature search parameter)
- Criteria for hazard identification inclusion

- Criteria for risk assessment inclusion

Of the 56 studies identified in the literature search, many were not considered suitable for use in the safety evaluation, based on application of criteria (b) (4) [redacted] These criteria were: administration; sample size (N ≥ 6/group) : method of statistical analysis (S; use of litter as statistical unit for developmental toxicity studies; use of ANOVA, with repeated measures as appropriate)[3]; end point measured (EP); plausibility; dose response (#D; use of at least three doses of the test article)[4]; consideration of environmental conditions (ENV; i.e.: housing conditions, use of proper controls for confounding environmental factors – discussed below); and repeatability. Prior to the evaluation based on these criteria, studies were triaged based on their *in vivo* nature, species evaluated, and, if available, use of the oral route of administration. Studies conducted in rodents or other mammalian species were considered to be the most relevant for this review, based on consideration of physiological similarities among mammalian species and the historical use of rodents in validated study designs used for human health risk assessment[5]. As consumer exposure to migrants from food packaging are via food and drinking water, the oral route of administration was considered to be most relevant to the assessment herein. However, as perfluorinated carboxylates are systemically-bioavailable via all routes of exposure and are not metabolized to alternate forms *in vivo*, studies conducted via alternate routes of administration with these compounds were considered relevant as hazard identification, if appropriate data from oral studies were unavailable (Kudo and Kawashima, 2003; Goecke *et al*., 1992; Ylinen and Auriola, 1990). Additional criteria that were weighted in determining the applicability of these data are discussed below:

- Choice of rodent model (M): Comparisons of the pharmacokinetics of PFOA in mice, rats, and humans suggest that mice more closely resemble humans than do rats (EPA, 2009b). As the fast kinetics of PFOA in the female rat (< 24 hours) would ensure that the conceptus is not exposed to a large, cumulative body burden of perfluorinated compound during critical windows in development, the ability of the assay to detect developmental toxicity of the perfluorinated compound would be reduced. In contrast, a human or mouse conceptus (half-lives in females of 3.8 years and 16 days, respectively) would be exposed throughout the pre- and postnatal period to the accumulated maternal body burden of the perfluorinated compound in question (Fenton *et al*., 2009; Rodriguez *et al*., 2009), resulting in a much higher total dose to the offspring. Therefore, the mouse was concluded to be the most sensitive and human-health relevant species in which to assess the developmental effects of PFOA and PFNA, where the estimated systemic half-life of 41 days in the female mouse is over 20-fold the estimated systemic half-life for PFNA in the female rat of 2 days (EPA, 2009b). Therefore, although studies in either model are acceptable for risk assessment purposes, more confidence and weight will be put in the mouse data.

- Environmental Contamination: Some studies observed detectable levels of PFOA in the serum in negative controls, indicating the presence of an environmental source for perfluorinated compound exposure (White *et al*., 2007; Wolf *et al*., 2007; Abbott *et al*., 2007). Given that perfluorinated compounds are omnipresent in food, water, and air (EPA, 2009b), it is probably not technically feasible to completely screen out all sources of environmental contamination. Therefore, while the presence of contamination in these studies is a limitation, it alone does not necessarily negate the use of the study in the assessment.

[2] Studies that had N/group ≥ 10 were considered to be RA-quality studies. Studies with 6-9 animals/group were considered HI-quality studies. Studies that had n< 6 animals/group were only considered as supplemental data.
[3] Studies that used improper statistical analytical procedures were not considered useful for either RA or HI.
[4] Studies that used less than 2 doses of the test article were considered useful for HI only.
[5] OECD Guidelines for the testing of chemicals: Summary of considerations in the report from the OECD expert groups on short-term and long-term toxicology. Accessible online at http://oberon.sourceoecd.org/vl=7935804/cl=101/nw=1/rpsv/cgi-bin/fulltextew.pl?prpsv=/ij/oecdjournals/1607310x/v1n4/s1/p1.idx.

- Cross-foster study design (CF): The cross-foster study design has been traditionally used to experimentally separate *in utero* influences from postnatal influences on development (OECD, 2004; McLaren, 1981). However, use of this study design has several disadvantages, such as difficulty in subsequently accounting for effect of litter of origin, reduction in the power of the study, the possibility of sampling errors (Chiarotti *et al*., 1987), and stress-induced alterations in maternal care (Denenberg *et al*., 1963). Cross-fostering may also induce epigenetic changes in offspring that influence adult phenotypic markers, such as bodyweight (Hager *et al*., 2009; Bartolomucci *et al*., 2004) and behavior (Lu *et al*., 2009; Bartolomucci *et al*., 2004). Study designs that include controls where the litters are raised by their own mothers may lead to a non-factorial arrangement and considerable loss of power in data analyses (Chairotti *et al*., 1987). As such, cross-foster studies will, at best, be considered suitable for hazard identification purposes only if their findings are corroborated by results from studies conducted using a conventional, non-cross-foster design. Otherwise, cross-foster studies will be considered supplemental information.

- Dosing duration during the study (DD): Validated reproductive toxicity study designs recommend that dosing of the P_0-generation commence 2-10 weeks prior to mating and continue through weaning of the offspring, indirectly via dosing of the dam or directly via gavage dosing of the pups (OECD, 2004; ICH, 1993; Redbook 2000), in order to realistically model pre- and postnatal toxicity of lifelong exposure to the substance in the human population. In addition, the compound is assumed to be at steady-state systemic concentrations during the study. However, given the long-half-lives of ≥ C8 perfluorinated compounds and the assumption of 3-4 half-lives to reach systemic steady state, the standard duration of premating and/or post-parturition exposure may be of insufficient length for the dam to reach systemic steady-state prior to mating, resulting in a lower body burden, and thus exposure, to the conceptus during gestation and postnatally and reducing the sensitivity of the study. Therefore, studies should ensure that the dam is at systemic steady-state concentration for the test substance by GD 0, and dosing of the dam should continue through gestation and lactation until weaning at PND 22. Similarly, studies conducted in adult animals should be of sufficient duration such that the test substance will reach systemic steady-state during the dosing period. Therefore, for PFOA, the dam should ideally be dosed for at least 48 days prior to mating for mice; systemic toxicity studies in male rats with PFOA should be at least 3 weeks in duration (3 x 6-7 day half-life in male rats). For systemic toxicity studies conducted with PFDA in rats, the minimum dosing duration should be 120 days in males (3 x 40-day half-life) and 180 days in females (3 x 59-day half-life). Studies with inadequate duration but meeting other criteria and having positive results can be used for RA or HI, but those with negative results may not have fully evaluated the chemical in question and are thus not considered useful for RA or HI. In addition, the quantitative estimates provided for such studies where duration was inadequate must be considered with additional uncertainty, as the points of departure may be less than determined if the study had been of adequate length to ensure steady state.

  A large number of the studies herein may be described as '*in utero* only exposure studies', where offspring are only exposed to the test compound during gestation and subsequently assessed for toxicity during the postnatal period. These studies were designed to address the hypothesis that exposures during critical windows of development may contribute to or cause adult onset of disease (Fenton, 2006; Soto *et al*., 2009; Grun and Blumberg, 2009). While this study design does not model chronic exposures to these compounds, information from these studies is considered to be relevant to the discussion herein, as these studies may highlight the action of PFCs during critical windows of development and provide mechanistic insights that other study designs may not. Therefore, a study of inadequate dosing duration may be useful for hazard identification, depending on other factors and the overall assessment of the quality of the study.

In vivo studies in mammals that were concluded to be of insufficient quality with respect to the criteria stated above for use in RA or HI are briefly cited as supplementary information for each endpoint, as deemed appropriate to enrich the dataset. Other toxicity studies conducted in non-mammalian species, epidemiological studies, and *in vitro* studies were also consulted for interpretation of the dataset. Results from these studies are also cited briefly herein, where appropriate, when common endpoints of measurement added to the understanding of animal data.

REVIEW

Findings from previously-reviewed studies and newly reviewed studies are discussed below by endpoint. In cases of studies conducted based on acceptable guidance for the endpoints examined, the limited deficiencies are noted but the strengths of these studies are not detailed in this review as they are clearly articulated in the applicable guidance documents from which they comport. As such, these studies were considered adequate for determination of NOELs or, as deemed appropriate, NOAELs and useful for the safety assessment.

Endpoint: Pre- and Postnatal Developmental Toxicity

Conclusions from Previously Reviewed Studies

Toxicology's previous conclusions from these studies are as follows:

Study/Review Memorandum	Species/Compound Tested/Doses	Developmental NOEL/Effects at LOEL (mg/kg)	Limitations	Utility
Gortner *et al*., 1981; Gu/Rice, 11/17/07	Ammonium (APFO) perfluorooctanoate; pregnant Sprague-Dawley/CD rats (n=22/group) gavaged with 0, 0.05, 1.5, 5, 150 mg/kg bw/day on GD 6-15, sacrifice GD 20	Maternal (5/150): ↓BW (GD 9-15); ataxia and death Developmental: > 150 mg/kg bw	M	RA
Gortner *et al*., 1982; Gu/Rice, 11/17/07	APFO; pregnant New Zealand white rabbits (n=18/group) gavaged with 0, 1.5, 5, 50 mg/kg bw/day GD 6-18, sacrifice GD 29	Maternal (5/50): ↓BW gain in GD 6-9 only Developmental (50/50): Dose-related trend ↑ incidence 13th-rib (deemed not biologically significant)	M, DD(?)	RA
Staples *et al.*, 1984; Gu/Rice, 11/17/07	APFO; Sprague-Dawley/CD rats (12-25/group), 0, 0.1, 1, 10, 25 mg/m^3 (6 h/day) by inhalation. Treatment GD 6-15. Sacrifice GD 21 (Group A) or PND 23-dams & PND 35-pups (Group B)	Maternal (1/10): ↓BW gain & food consumption, clinical signs, ↑liver wt; death at 25 mg/m^3. Developmental (10/25): ↓BW.	M, route	HI
Argus Research, 2002; Gu/Rice, 11/17/07	APFO; Crl:CD (SD)IGS BR VAF/Plus rats 30/sex/dosage (F_0); 2 pups/sex/litter (60/sex/dosage; F_1), gavaged with 0, 1, 3, 10, 30 mg/kg bw/day for six weeks old to day before sacrifice (Males: DS 45 for P_0 & P_1; Females: PND 22 P_0 & P_1) or PND 22 for F_2	F_0 & F_1 repro NOEL : ≥ 30 mg/kg/d, both sexes Male F_1 Dev LOEL: 1 mg/kg: ↓BW parameters, changes in abs & BW-rel wts of liver, spleen, kidney, ↑postweaning mortality, clinical signs, histopathology in adrenal & liver. At 30 mg/kg, ↓postnatal survival & delayed attainment of puberty. F_1 Dev Females: 10/30: ↓BW & BW gain, food consumption; ↑ postnatal mortality; delayed onset of sexual maturation & ↑#s of estrous stages/21 days. F_2 NOEL: ≥ 30 mg/kg/d	M	RA

Harris and Birnbaum, 1989; Gu/Rice, 11/17/07	PFDA; pregnant C57Bl/6 mice (10-14/group), gavage, **I:** 0, 0.03, 0.1, 0.3, 1, 3, 6.4, or 12.8 mg/kg/d GD 6-15; **II:** 0, 0.25, 0.5, 1, 2, 4, 8, 16, 32 mg/kg/d GD 10-13). Sacrificed GD 18.	Maternal (0.3/1 & 0.25/0.5): ↑Abs & BW-rel liver wts Developmental (0.3/1 & 0.25/0.5): ↓Live fetal BW	DD, group-housing during pregnancy	HI
(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]

Newly-identified Studies Considered Useful for Hazard Identification

Six additional studies were identified that addressed this endpoint and met the first and second tiers of review based on the evaluation criteria herein. All of these studies, except for the Abbott *et al.* and Boberg *et al.* studies, were conducted by the same laboratory using the same basic procedures for housing, etc. Therefore, the commonalities in methodology between these 4 studies will be summarized here prior to detailed review of each study in chronological order below. The two afore-cited studies from other laboratories will be reviewed separately. All of the studies in this group were conducted in pregnant CD-1 mice and intended to elucidate various aspects of the developmental toxicity of PFOA. Timed pregnant CD-1 mice, which were obtained from Charles River Laboratories (Raleigh, NC) on GD 0, were weighed upon arrival and randomly assigned to treatment groups, the basis of randomization not stated. Dams were housed individually in polypropylene cages with Alpha-Dri nesting paper. LabDiet 5001 (PMI Nutrition International) and tap water (PFOA < unstated limit of detection) were provided *ad libitum*. Animal facilities were maintained on a 12-hour light/dark cycle and controlled for temperature (20-24 °C) and relative humidity (40-60%). PFOA (ammonium salt, > 98% purity) in deionized water was administered daily by gavage in a 10 ml/kg bw volume. There was no stated acclimatization period or clinical exam prior to study start.

1) 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. *Toxicol. Sci.*, **90**(2): 510-8.

Methods: The purpose of this study was to assess the prenatal and postnatal toxicity of PFOA in mice. Mice were gavaged with PFOA at doses of 0, 1, 3, 5, 10, 20, or 40 mg/kg bw/day on GD 1-17; the basis for choosing these doses was unstated, and the initial numbers of dams per group was unspecified. At GD18, a cohort of the dams (n=9-47/group[6]) were sacrificed and the litters subjected to a standard teratology evaluation. The remaining dams (n=7-23/group) were dosed again on GD18 and then allowed to deliver. Dams were not dosed during lactation. Numbers of live offspring per litter and pup bodyweights were assessed at birth, on PND 1-4, and at intervals of several days thereafter until PND 23.

[6] Numbers of animals per group were not explicitly stated in the 'Methods' section; numbers derived for prenatal and postnatal endpoints above were taken from Tables 2 and 3 of the text, respectively.

Surviving pups from litters of less than 3 pups were fostered with dams in the same treatment group. After weaning, pups were housed separately by gender, dams were sacrificed, and the uteri were evaluated for numbers of implantation sites. Eye opening was monitored beginning on PND 12, and puberty markers (preputial separation and vaginal opening) were monitored beginning on PND 24. Age at first estrus was monitored daily after vaginal patency was attained. Data were analyzed via ANOVA with post-hoc testing by either Duncan's multiple range test or Fisher's Exact test (full litter resorptions (FLR) only), using litter as the statistical unit. Maternal weight gain was analyzed using ANOVA with repeated measures.

Results: Dose-responsive decreases in maternal bodyweights were noted at 20 and 40 mg/kg bw/day during gestation. While there was no significant effect of PFOA on numbers of implantations, a dose dependent increased incidence of FLRs were observed at ≥ 5 mg/kg bw PFOA, with 100% FLR at 40 mg/kg. Maternal absolute liver weights at term and serum PFOA levels at term were significantly dose-responsively increased in all treated groups up to 10 mg/kg, with a dose-responsive increase in serum PFOA up to 0.25 mg/ml in the highest dose group. Significant prenatal loss and decreased numbers of live fetuses were observed only in the 20 mg/kg group. Increased incidences of enlarged fontenels and reduced ossification of several skeletal components were observed, with reduced ossification observed in all dose groups[7]. Increased incidences of minor tail and limb defects and microcardia were also observed at 10 and 20 mg/kg PFOA; decreased fetal bodyweight was noted at 20 mg/kg. Time to parturition was increased by ~ 12 hours in the high-dose group. Postnatal survival was decreased at ≥ 5 mg/kg PFOA; most pups exposed to 10 or 20 mg/kg *in utero* did not survive after PND 1. Decreased bodyweight gains (25-30%) were noted in both sexes until weaning at ≥ 3 mg/kg bw PFOA. Recovery to control values occurred by 6.5 weeks of age in females and 13 weeks of age in males. As the offspring aged, bodyweights of both genders tended to be greater than control values by ~10%. Significant delays in eye opening were observed at ≥ 5 mg/kg in both sexes, and accelerated sexual maturation in males was observed in all dose groups. A slight delay (~1.5 days) in vaginal opening was noted at 20 mg/kg in females, and age at first estrus was significantly increased at 5 and 20 mg/kg, but not at 10 mg/kg.

Strengths: The teratogenicity and postnatal phases of the study were mainly conducted according to standard guidelines for developmental toxicity studies. An adequate spread of doses and number of animals per group were used, and the route of administration and animal model used in the study were highly relevant to the assessment of chronic dietary exposure in humans. Repeated measures ANOVAs were used to assess maternal bodyweight gain.

Weaknesses: The basis for randomization of the dams was unstated, and the dams were not habituated prior to use on study. Although the dosing duration of the study is typical for teratology studies, there is a concern for adequate establishment of steady state. Furthermore, the dams were not dosed after parturition, decreasing the dose delivered postnatally to the pups and decreasing the sensitivity of the study to detect postnatal toxicity of PFOA. Since the study had positive findings, this comment is not so much a weakness, but a concern that the design may have not fully captured the potential toxicity of the compound. In addition, it is unclear from the description of the statistical methods used in the study whether repeated measures ANOVAs were used to assess postnatal bodyweight gain in the litters. The additional endpoints of histopathology and organ weight parameters were not assessed for the exposed offspring; this is not a weakness per se, but such information would have added to the overall evaluation.

Utility of study: This study is considered useful for **risk assessment** of PFOA. Toxicology concludes that there was no NOEL for prenatal toxicity to both sexes or postnatal toxicity to male offspring in this study, based on the observations of reduced ossification in fetuses of both sexes and accelerated sexual maturation in male pups, respectively. The NOEL for postnatal toxicity in female offspring is concluded

[7] Results for fetal parameters not separated by sex in Table 2 of the text.

to be 1 mg/kg, based on decreased bodyweight gains until weaning at ≥ 3 mg/kg bw PFOA.

2) Wolf, C.J., Fenton, S.E., Schmid, J.E., Calafat, A.M., Kuklenyik, Z., Bryant, X.A., Thibodeaux, J., Das, K.P., White, S.S., Lau, C.S., and Abbott, B.D. (2007) Developmental toxicity of perfluorooctanoic acid in the CD-1 mouse after cross-foster and restricted gestational exposures. *Toxicol. Sci.*, 95(2): 462-473.

Methods: This study was designed to examine the role of gestational exposure versus lactational exposure with regard to postnatal bodyweight deficits, bodyweight gain, neonatal lethality, and developmental delay. Doses were based on a previous study (Lau *et al*., 2006). PFOA was assessed in one of two study designs: a cross-foster study and a restricted-exposure study. The study was conducted in two blocks, 4 weeks apart, with 56 mice/block. In the cross-foster study, doses of PFOA used were 0 (n=48 dams), 3 mg/kg bw/day (n=28 dams), or 5 mg/kg bw/day (n=36 dams) administered on GD 1-17. Mice were monitored, but not dosed, on GD 18-19 for parturition, and the following parameters were recorded: date and time of birth, numbers of live and dead pups, sex ratio, and litter weights by sex. Litters of similar ages were then fostered to yield the following treatment groups: 1) control pups fostered to control dams (control); 2) control pups to 3 mg/kg bw dams (3L, lactational exposure only); 3) control pups to 5 mg/kg dams (5L); 4) pups dosed *in utero* with 3 mg/kg bw to control dams (3U, *in utero* exposure only); 5) pups dosed *in utero* with 5 mg/kg bw fostered to control dams (5U); 6) 3 mg/kg bw pups fostered to 3 mg/kg bw dams (3U+L); and 7) 5 mg/kg pups fostered to 5 mg/kg bw dams (5U+L) for an *n* of 14-18 litters per group. In the restricted exposure study, timed pregnant CD-1 mice were obtained and monitored as per above, but gavaged with PFOA in water (10 ml/kg) as follows: vehicle for GD 7-17 (n=12); 5 mg/kg bw/day PFOA on GD 7-17 (n=14), GD 10-17 (n=14), GD 13-17 (n=12), or GD 15-17 (n=12); or 20 mg/kg bw/day for GD 15-17 (n=6). Mice were then monitored for parturition as before and the same parameters were recorded at birth. For both study designs, litters were comprised of 10 pups of equal numbers of males and females. Data for pups were analyzed using the litter as the statistical unit via one-way ANOVA that included the effect of study block. Post-hoc analyses were conducted via Dunnett's t-test or pairwise t-test with Bonferroni's corrections for multiple comparisons. Linear regression analysis was used to test for trends in pup birth weight and PND 22 relative liver weight.

Results: Due to the complexity of the study design, results for parameters measured are presented in the following table:

Endpoint	**Cross-foster study (CF)**	**Restricted exposure study (RE)**
Maternal parameters: BWs, liver wts, & serum PFOA at PND 22. Resorptions & implantation sites for all dams via staining of uteri with 2% ammonium sulfide.	↑Incidence of whole litter loss, high-dose group. No adverse effects on maternal BW & BW gain, #s of implantations. ↑Abs & BW-rel liver wts, all dams. ↑Dose-responsive serum PFOA, all PFOA-exposed dams.	No adverse effects on maternal BW, BW gain, or #s of implantations. ↑BW-rel liver wts, all dams, except GD 15-17. ↑Dose-responsive serum PFOA, all PFOA-exposed dams; GD 15-17 had lowest serum PFOA.
Pup survival	↓5U+L (65% alive at weaning)	↓20 mg/kg; mean time to death 6.9 d
Birth weight	↓5 mg/kg, males and females	↓5 mg/kg GD 7-17, 10-17; 20 mg/kg, males only
Pup BW gain before weaning (PND 1-22): assessed PNDs 1, 2, 3, 4, 7, 10, 14, 17, and 22.	↓Males & females, 3U+L, 5U, & 5U+L; ↓BW gain, both sexes, 3U+L, 5U, & 5U+L groups.	↓All treated males and females
Pup BW gain after weaning: PND 22; BWs for one pup/sex/litter weekly after weaning PND 29-245 (CF) or PND 29-189 (RE).	↓BW; 3U+L, 5U, & 5U+L males at PND 22 & all females except 3L. Recovery of males to control within one week of weaning, except 5U+L males which recovered by PND 36. ↑BW, 3U males after PND 85 until study termination. ↓BW, 5U & 5U+L females until PND 85, after which not different from	Continued ↓BW in GD7-17 & 10-17 males after weaning until PND71 (GD10-17 group) and PND 78 (GD7-17). Females not significantly different from control, PNDs 22-161, after which GD 13-17 female ↑BW.

	controls.	
Pup liver wts: PND 22 for one pup/sex/litter.	↑BW-rel liver wts, all PFOA-exposed pups. ↑Abs liver wts, all males except 5U; only in 3U and 5U+L females.	↑BW-rel liver wts, all PFOA-exposed pups.
Serum PFOA: PND 22 for one pup/sex/litter.	Highest at 3 weeks old in 5U+L>3U+L. Similar *in utero* vs lactation. Females ↓ from weeks 3-9, but > controls at 9 weeks old.	↑Dose-responsive, all PFOA-exposed pups. Correlated with total dose; 5 mg/kg PFOA/GD 15-17 group had lowest levels.
Developmental landmarks: Eye opening monitored beginning PND 10; hair growth monitored PND 8 (RE study only), 11 (CF), and 17 (both studies).	Delayed eye opening, 3U+L, 5U, & 5U+L groups. Delayed emergence of body hair, 5U, 3U+L, & 5U+L groups.	Delayed eye opening 5 mg/kg PFOA GD7-17 & 10-17. Delayed emergence of body hair in 5 mg/kg PFOA, GD7-17 & 10-17.

Strengths: The strengths of the study include the animal model used, the route of administration, the numbers of animals used, the assessment of valid endpoints previously shown to be targets of toxicity for PFOA, internal dosimetry, the assessment of concomitant endpoints such liver weights which adds to the validity and relevance of the reported results, and the repeatability of the findings regarding PFOA's effects on neonatal survival and bodyweight gain in the Lau *et al*. and Abbott *et al*. studies.

Weaknesses: The weaknesses of the study included use of a cross-foster design, the lack of true controls for the restricted exposure phase of the study, the lack of habituation period prior to dosing for the dams, the fact that the basis of randomization to treatment groups was unreported, the inadequate dosing duration of the dams during the study, and the paucity of endpoints examined in pups at later time points. The use of only two dose levels of the test substance would be a limitation for the individual study; however, the study authors were using these doses as a follow-up to their dose response assessment in Lau *et al*., 2006. As such, though a true dose response assessment would have provided additional information, the rationale is supportable for the hypothesis being examined. In addition, it is unclear from the description of the statistical methods used in the study whether repeated measures ANOVAs were used to assess postnatal bodyweight gain in the litters.

Utility of study: Based on the weaknesses detailed above, most importantly the cross-foster design, this study is limited to use for **hazard identification** for PFOA. Based on the data presented, Toxicology concludes that there is no NOEL in the offspring for the RE study, based on the finding of increased bodyweight-relative liver weights and decreased pup bodyweight gain at the systemic LOEL of 5 mg/kg PFOA for GD 15-17. Toxicology concludes that there is no NOEL in the offspring for the CF study, based on the finding of increased bodyweight-relative liver weights at the systemic LOEL of 3 mg/kg PFOA during lactation only.

3) White, S.S., Calafat, A.M., Kuklenyik, Z., Villaneuva, L., Zehr, R.D., Helfant, L., Strynar, M.J., Lindstrom, A.B., Thibodeaux, J.R., Wood, C., and Fenton, S.E. (2007) Gestational PFOA exposure of mice is associated with altered mammary gland development in dams and female offspring. *Toxicol. Sci.*, **96**(1): 133-144.

Methods: The purpose of this study was to assess the effects of PFOA on mammary gland development and differentiation in mice. Timed pregnant CD-1 mice (n=60 dams, divided equally between two blocks) were gavaged with 0 or 5 mg/kg bw/day PFOA in water on GD 1-17 (n=14, controls and PFOA-treated), 8-17 (n=16), or 12-17 (n=16). The dose of 5 mg/kg PFOA was based on the results of Lau *et al*., 2006. Maternal bodyweight parameters were measured daily during pregnancy. At parturition, pups were individually sexed and weighed. All the litters in each treatment group were pooled, and the pups randomly fostered among all the dams in each treatment group for a total number of pups per litter of 10, with both genders equally represented. On PNDs 5, 10, and 20, litters were weighed, and average pup weights were calculated. Half of the dams with their litters were necropsied on PND 10; the others were

necropsied on PND 20. Trunk blood (dams and pups) and livers (pups only) were semi-quantitatively analyzed for PFOA. Uteri from dams were examined for implants to assess post-implantation loss. Fourth and fifth inguinal mammary glands were excised from dams and pups and prepared for microscopic examination either as whole mounts (N=3-4 dams/group; N=3-6 litters/group) or as hematoxylin and eosin (H&E)-stained 5-micron sections (N=3-6 dams/group) . Development scores were calculated for mammary gland whole mounts from offspring using a 1-4 subjective, age-adjusted developmental scale by two independent scorers, blinded to treatment. H&E-stained sections from lactating dams were similarly scored. RNA was extracted from glands from the contralateral side and assessed for changes in gene expression via real-time quantitative PCR (N=3-6 dams/group). Statistical analyses were performed by ANOVA, with bodyweight as a covariate as appropriate, with post-hoc testing using Dunnett's t-test. Experimental block was removed from the model after testing for block effects; analyses of offspring bodyweight data and mammary gland scores used the litter as the unit of measure.

Results: No significant effects of treatment on maternal bodyweight, mean numbers of implant sites, or numbers of live pups born were noted. Significant decreases in pup bodyweights were noted for all PFOA-exposed litters at all time periods measured, with reductions reaching 40% of control in the GD1-17-exposure group on PND 5. At PNDs 10 and 20, bodyweights were still significantly decreased by 25-39% (PND 10) and 26-33% (PND 20). The decreases in neonatal bodyweights were increased in severity with increasing total PFOA exposure (dose x time), such that the most severe bodyweight decrements were noted in the GD1-17-exposure group. Blood and liver PFOA concentrations in offspring were proportional to total dose at PND 20, but not at PND 10 or PND 1 (liver only), where there was no difference between blood or liver PFOA levels in offspring from the GD 1-17 and GD 8-17 groups. All exposed pups exhibited impaired mammary gland epithelial branching, and longitudinal growth was significantly stunted at PND 10 and 20. Developmental scores for exposed pups were decreased by 50% over control pups, and the decrease in score was independent of bodyweight and occurred during a period of time when serum and liver PFOA levels were decreasing in the pups.

Strengths: The strengths of this study include route of administration, use of the most sensitive animal model, assessment of internal dosimetry in dams and pups concomitantly with other endpoints, and the comprehensive nature of the evaluation of endpoints related to mammary gland development, including evaluation of the possible effect of bodyweight as a covariate on this endpoint. Another strength of the study is repeatability, as these results were confirmed in a subsequent study by this group.

Weaknesses: The weaknesses of the study were the use of only one dose of PFOA, the lack of true negative controls for the different durations of exposures to PFOA, the use of cross-fostering, the lack of acclimatization period before start of the animals on study, lack of randomization to treatment group by weight, and lack of assessment of other concomitant endpoints in pups such as attainment of developmental landmarks. Inadequate dosing duration for the dams is also a concern. In the studies evaluating mammary gland analysis in the dams using whole mount analysis, too few replicates were used to meet the hazard identification criteria for inclusion.

Utility of study: Based on the weakness of the study detailed above, most specifically statistical analysis and the use of cross-foster study design, this study is not useful for risk assessment. However, due to the repeatability of many of the findings within the database, this study is deemed useful for **hazard identification** of PFOA. Based on the data presented, Toxicology concludes that there is no NOEL in this study, based on the findings of decreased pup bodyweights at all time periods and decreased mammary development at the systemic LOEL of 5 mg/kg during GD 12-17.

4) White, S.S., Kato, K., Jia, L.T., Basden, B.J., Calafat, A.M., Hines, E.P., Stanko, J.P., Wolf, C.J., Abbott, B.D., and Fenton, S.E. (2009) Effects of perfluorooctanoic acid on mouse mammary gland development and differentiation resulting from cross-foster and restricted gestational exposures. *Reprod.*

Toxicol., 27(3-4): 289-98.

Methods: This study assessed the effects of prenatal PFOA-exposure of varying duration on mammary gland development in offspring and dams; the effects of pre- and postnatal exposure during different time periods of development are also explored using the same cross-foster or restricted exposure design as was used in the Wolf *et al.* study. The late-life cross-foster (LLCF) study used two blocks of animals, spaced 4 weeks apart, with 56 GD 0 dams/block. Mice were gavaged daily with 0 (n=48), 3 (n=28), or 5 (n=36) mg/kg bw/day PFOA on GD 1-17 in deionized water. The experimental groups were: control; 3L; 5L; 3U; 5U; and 5U+L. Litters were observed daily and weighed on PND 1, 2, 3, 4, 10, 14, 17, and 22. Eye opening was monitored beginning on PND 10. Hair growth was recorded on PND 8, 11, and 17. Pups were weaned and weighed on PND 22, and males and females were housed separately (housing conditions unstated). Female offspring were then necropsied on PND 22, 29, 32, 42, and 62, as well as at 18 months of age (n=9-18 adult females/group/time point). The restricted exposure (RE) phase used a single block of 64 pregnant mice. Mice were gavaged with PFOA as follows: 0 on GD 7-17 (control; n=12); 5 mg/kg PFOA in deionized water on GD 7-17 (n=14); 5 mg/kg PFOA on GD 10-17 (n=14); 5 mg/kg PFOA on GD 13-17 (n=12); or 5 mg/kg PFOA on GD 15-17 (n=12). The date and time of birth, numbers of live and dead pups, and numbers of pups of each sex were recorded. Each litter was weighed by sex and then culled to 10 pups per litter, with equal representation of male and female. Litters and weaned females were then treated the same as for the LLCF study, with n=10-21 adult female offspring/group/time point. Data were analyzed via ANOVA, with study block were removed from the model after no significant effect of block was detected. Bodyweight and maternal effects were analyzed on a litter basis via ANOVA, which included effects of sex, litter, pup age, and treatment, with Dunnett's T-test or pairwise t-test post-hoc testing and Bonferroni's corrections for multiple comparisons. Trends in pup birth weight and PND 22 relative liver weights were assessed via linear regression analysis. Mean mammary gland scores were analyzed via ANOVA using bodyweight at time of collection as a random effect, with litter as the unit of measure for neonatal scores and Dunnett's or Tukey's t-tests for post-hoc analyses.

Results:

Endpoint Assessed	**Pups**
Bodyweight	LLCF: ↓All on PND 22, except 3L. Recovery by PND 29 (5U, 3U, 3U+L) or 85 (5U & 5U+L). RE: Recovery from pre-weaning BW deficits within one week of weaning except for GD 10-17 group[8]. BW data not presented.
Serum PFOA	LLCF: Highest levels on PND 22 in 5U+L (~20,000 ng/ml), with 12-15,000 ng/ml in 5U and 5L. PFOA still ~4,000 ng/ml in 5U+L on PND 42. PFOA < LOD all groups by PND 63. RE: Not measured
Bodyweight-relative liver weight	LLCF: ↑All exposed groups at weaning RE: Not measured
Mammary gland development: 4th & 5th inguinal mammary glands from pup or dams at necropsy. Assessed one of 3 ways: 1) 2 blinded, independent scorers using 1-4 subjective, age-adjusted, developmental scale: whole mount preparations from pups between PND 1 and 63; 2) pathologist with respect to concurrent controls: H&E sections from lactating dams & 18-month-old offspring; or 3) pathologist: whole mounts from 18-month-old offspring or dams.	LLCF: ↓ PNDs 22, 42, & 63, all PFOA-exposed pups, except 3L at PND 22 & 3U+L at PND 42: Delayed ductal elongation, TEB appearance, & ↓ secondary & tertiary branching. ↓Epithelial densities with peripheral, localized ↑epithelial density, all 18-month-old PFOA-exposed, especially 3U+L & 5U+L. ↑Numbers of darkly-staining foci due to inflammatory infiltration into ductal tissue. Hyperplasia of the ductal epithelium, ↑ stromal epithelial densities, or inappropriate differentiation of ductal tissue. RE: Same findings as LLCF

[8]Based on a reference to this study that states that all prenatally-exposed offspring in the RE section of the study, except for the GD 10-17 group, had recovered from bodyweight deficits within one week of weaning as previously reported (citing Wolf *et al*. at the end of the sentence). It is unclear from this statement if the author meant that they used the same animals as in the Wolf *et al*. study or simply observed the same phenomena RE pup BW after weaning as Wolf did.

Strengths: The strengths of this study include the animal model used, the route of administration, the assessment of concomitant endpoints such as bodyweight and liver weight parameters, internal dosimetry, the study duration and assessment of later-life effects of pre- and early postnatal exposure, and the comprehensive nature of the assessment of mammary gland effects. An additional strength was the repeatability of the reported findings herein in a previous study by this group.

Weaknesses: The weaknesses of the study included: lack of habituation period for the dams prior to start of the study, the fact that the basis of randomization to treatment prior to study start was not reported, lack of exposure after weaning, low numbers of replicates used in the RE and LLCR studies, use of the cross-foster study design, and lack of true negative controls for the RE phase of the study. In addition, it is unclear from the description of the statistical methods used in the study whether repeated measures ANOVAs were used to assess postnatal bodyweight gain in the litters. Inadequate dosing duration for the dams is also a concern. The use of only two dose levels of the test substance would be a limitation for the individual study; however, the study authors were using these doses as a follow-up to their dose response assessment in Lau *et al.,* 2006. As such, though a true dose response assessment would have provided additional information, the rationale is supportable for the hypothesis being examined.

Utility of study: Based on the weakness of the study detailed above, most specifically statistical analysis and the use of cross-foster study design, this study is not useful for risk assessment. However, due to the repeatability of many of the findings within the database, this study is deemed useful for **hazard identification** of PFOA. From the data presented, Toxicology concludes that there is no NOEL for this study, based on the finding of increased bodyweight-relative liver weights at weaning in the 3L group, the group with the least systemic exposure to PFOA.

5) 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. *Toxicol. Sci.*, **98**(2): 571-581.

Methods: The purpose of this study was to determine the role of PPAR-α in the developmental toxicity of PFOA in mice. Pregnant wild-type (WT) or PPAR-α knock-out (KO) 129S1/SvlmJ mice were obtained from in-house breeding colonies on GD 0 (sperm-plug confirmed). All mice, including the mice used in the study, were maintained under a 12-hour light-dark cycle, controlled for temperature (20-24°C) and relative humidity (40-60%), with LabDiet 5001 chow and tap water *ad libitum*. Mice were housed in ventilated Techniplast cages until breeding overnight to produce WT, KO, or heterozygous (HET) litters. Plug-positive females were then weighed, randomly assigned to treatment groups, and housed individually in polypropylene cages. Pregnant mice were weighed each morning and gavaged with 0, 0.1, 0.3, 0.6, 1, 3, 5, 10, or 20 mg/kg bw/day PFOA (ammonium salt, > 98% purity) in deionized water in a volume of 10 ml/kg (n=7-22 WT/group; 4-23 KO/group)[9] on GD 1-17. At parturition, numbers of live and dead pups were recorded, and male and female pups were weighed for each litter, grouped by sex. On PNDs 1-10, 14, 17, and 22, numbers of live pups per litter were enumerated, and pups were weighed by sex. Eye opening was monitoring beginning on PND 12. At weaning on PND 22, pups were weighed. One pup from each litter and all adult females were weighed and killed for analysis of serum PFOA and enumeration of implantation sites in uteri. The remainder of the weaned pups were housed by sex and weighed monthly. Data for each strain were analyzed separately via one-way ANOVA, with post-hoc analyses correcting for multiple comparisons. Linear regression analysis was used to test for trends in pup birth weight and PND 22 relative liver weight. Analyses of data for offspring used the litter as the

[9] Numbers taken from Table 1 of the text. Numbers of dams initially assigned to each group were unstated.

statistical unit.

Results: Maternal weight, weight gain, total numbers of pups at birth, numbers of implantation sites, and pup birth weights were unaffected by PFOA administration in either WT or KO dams. Incidences of FLR in early gestation were significantly increased at ≥ 5 mg/kg bw/day PFOA in both WT and KO dams. The percent litter loss, excluding FLR, increased with dose in WT females and was statistically-significant at ≥ 0.6 mg/kg bw/day PFOA; no effect of PFOA treatment was noted in KO females. Survival in WT litters was significantly decreased at 0.6-1 mg/kg PFOA (~42-43% of pups alive on PND 22 compared to control) and at ≥1 mg/kg PFOA of WT and HET pups in mixed litters; postnatal survival of KO pups was unaffected by PFOA, even at doses as high as 3 mg/kg, indicating that a functioning PPAR-α allele was essential for this effect. WT, but not KO, litters had a dose-related trend for delayed eye opening, which was statistically significant at 1 mg/kg PFOA. WT pups had significantly decreased bodyweights on PNDs 9, 10, and 12 (males) and PND 7-10 and 22 (females) at 1 mg/kg PFOA. Dose-related trends for reduced bodyweights on some days and bodyweight gain were detected in both WT and KO pups. Bodyweight differences from control were insignificant after weaning for both WT and KO mice out to 28 weeks (male) or 52 weeks (female). Serum PFOA in dams and offspring at weaning increased with dose in both KO and WT mice. Bodyweight-relative liver weights also dose-responsively increased in WT and KO dams starting at 1 mg/kg and 3 mg/kg PFOA, respectively. Bodyweight-relative liver weights were significantly and dose-responsively increased in all PFOA-treated WT pups but were only significantly increased at the highest dose evaluated in the KO pups of 3 mg/kg PFOA.

Strengths: The strengths of this study were the route of administration used, the animal model used, the exploration of the role of PPARα in the observed toxicity, the statistical design and conduct of the study, the large number and range of doses of PFOA used, and the repeatability of the results in the context of the results of Lau *et al*. The numbers of dams assigned per treatment group were unstated in the article, but the higher dose groups appear to be underpowered for some endpoints. However, this may be due to toxicity, i.e.: early implantation loss, noted during the study, instead of inadequate study design.

Weaknesses: The weaknesses of the study were the lack of assessment of other developmental endpoints, such as attainment of puberty, and the inadequate dosing duration of the study. It is unclear from the description of the statistical methods used in the study whether repeated measures ANOVAs were used to assess postnatal bodyweight gain in the litters. In addition, the study did not use the common strains of mouse normally used in toxicity testing (CD-1 mice or C57Bl/6); while this is not necessarily a weakness, this may have influenced the sensitivity of the study.

Utility: This study is useful for **risk assessment** of PFOA, especially as it relates to mode of action through PPARα. Based on the reported results, Toxicology concludes that the NOEL for reproductive toxicity and postnatal developmental in WT mice in this study is 0.3 mg/kg PFOA, based on increased litter loss for all litters and decreased numbers of pups alive on PND 22 at 0.6 mg/kg PFOA.

6) Boberg, J., Metzdorff, S., Wortziger, R., Axelstad, M., Brokken, L., Vinggaard, A.M., Dalgaard, M., and Nellemann, C. (2008) Impact of diisobutyl phthalate and other PPAR agonists on steroidogenesis and plasma insulin and leptin levels in fetal rats. *Toxicology*, **250** (2-3): 75-81.

Methods: The purpose of this study was to assess the effects of PFOA, diisobutyl phthalate, and butylparaben on reproductive organ and hormonal parameters in male and female rat fetuses. Time-mated Wistar female rats (Han-Tac: WH, Taconic M&B, Denmark; BW ~ 220 g) were supplied on GD 3, randomized by bodyweight into treatment groups (n=8/group; 4 blocks mated on consecutive days), and pair-housed in semi-transparent plastic cages with Tapvei aspen bedding in a temperature (21 ± 2°C)- and humidity (45-55%)-controlled room on a 12-hour light/dark cycle, with Altromin Standard Diet 1324 and tap water available *ad libitum*. Dams were gavaged daily with PFOA (purity 96%) at 0 or 20 mg/kg bw/d

in unstated vehicle from GD 7 until necropsy on either GD 19 or GD 21. Dams were inspected for general signs of toxicity twice daily until necropsy, when dams were sacrificed via decapitation and fetuses were removed. Fetal bodyweights and anogenital distances were recorded; pooled fetal trunk blood from each litter by sex was analyzed for serum insulin, leptin, MCP-I, IL-1B, PAI-1 active, IL-6, TNFα, E, T, and adipokines. Livers, adrenals, and testes from GD 19 and 21 males were excised and assessed for histopathology; immunohistochemistry, and gene expression (n=10/group) via quantitative RT-PCR; ovaries from GD 21 females were assessed for histopathology, immunohistochemistry, gene expression, or E content. Logarithmically-transformed data for hormone and gene expression were tested for normality and homogeneity of variance prior to analysis by mixed-model ANOVA with litter as the statistical unit, with post-hoc testing via Dunnett's test.

Results: There were no effects of PFOA on any of the parameters measured or on bodyweight gain in the dams during gestation.

Strengths: The strengths of the study were the route of administration, the numbers of animals used, the environmental conditions used during the study (except for pair-housing the dams), the statistical methods used for the study, and the variety and depth of the analyses of the effects of prenatal exposure to PFOA on gonadal development and fetal reproductive hormone synthesis and excretion. Given the pharmacokinetics of PFOA in the female rat, the dosing duration was also sufficient to ensure measurement of the stated endpoints under relative steady-state conditions.

Weaknesses: The main weaknesses included use of the rat as the animal model, the use of only one dose of the test article, and the stated error in necropsy day assignment wherein 1/4th of the dams sacrificed at the GD 21 time point were actually only at GD 20. While the study authors stated that the effects of this error were taken into account in their statistical analyses, the fact that a fourth of the fetuses at GD 21 were actually a day younger than the intended age may have introduced significant error into measurements of fetal parameters at that time point and affected the sensitivity of the study. In addition, this study was conducted in the same range of reported results as studies in mice and rats (see above), as such and as detailed in the criteria; these findings are in conflict with the dataset as a whole (goes toward repeatability), further suggesting a concern for insensitivity of the rat.

Utility: As noted above, there were serious errors in the study which, along with the fact that only 8 dams/dose were used and only one dose of PFOA, discount its utility for risk assessment. However, the study is useful for **hazard identification**. Based on the data presented, the NOEL is 20 mg/kg PFOA.

Supplementary Information

Several epidemiological studies have examined the association between PFOA exposure and effects on neonatal outcome variables, including birth weight and length, ponderal index, head circumference, gestational age, Apgar scores, and developmental milestones at 6 and 18 months of age. One study reported negative associations between umbilical cord serum PFOA levels and head circumference for vaginal births and between serum PFOA and ponderal index, regardless of method of delivery, in babies born to non-occupationally-exposed women (Apelberg *et al*., 2007); non-significant associations of serum PFOA with decreased birth weight were also noted. Another study conducted in non-occupationally exposed women noted an association of maternal first trimester serum PFOA levels and birth weight, abdominal circumference, and birth length (Fei *et al*., 2007, 2008a); non-significant negative associations between maternal PFOA levels and head circumference were also noted. In contrast, other studies conducted in occupationally-exposed and non-occupationally exposed populations have reported no association between maternal or umbilical cord serum PFOA levels and gestational age at birth, birth weight, birth length, head or chest circumference, risk for preterm birth, or risk for low birth weight (as summarized in Olsen *et al*., 2009; Fei *et al*., 2007; Nolan *et al.*, 2009; Washino *et al*., 2009). An

additional study examining attainment of developmental landmarks found no association between maternal or umbilical cord serum PFOA levels and Apgar scores at birth or the timing of attainment of developmental milestones at 18-months-old (Fei *et al*., 2008b). In contrast, studies conducted in chickens report that prenatal exposure to PFOA decreases hatchability, interferes with pigmentation, decreases imprinting, and causes pathological changes in the liver in chicks exposed *in ovo* (Yanai *et al*., 2008; Pinkas *et al*., 2010). Several additional *in vivo* studies were identified in the literature which did not meet the criteria for hazard identification. For completeness, these studies are tabulated in Attachment 2.

Section Summary

In this section, Toxicology has evaluated the available evidence concerning the effects of perfluorinated compounds on prenatal and postnatal endpoints. For the endpoint of prenatal toxicity, Toxicology has concluded that the stated perfluorinated compounds have adverse effects on the following parameters:

- Pregnancy maintenance/fetal loss: PFOA treatment increased incidences of percent litter loss and/or FLR in mice with NOELs in the range of 0.3-3 mg/kg bw during gestation; the effects of PFOA-treatment of early FLR appeared to be PPARα-independent, as PFOA also increased FLR in KO mice, although at a higher dose than was noted in WT mice. Dose-related decreased fetal survival was also noted in mice treated with PFOA ≥ 20 mg/kg. In contrast, no effects on pregnancy maintenance or fetal survival were observed in rats or rabbits treated with PFOA or in rats treated with the telomer alcohols. It is also noteworthy that the dose ranges inducing FLR in mice are lower than those inducing overt fetal death, indicating that PFOA may be inducing FLR through indirect effects instead of via direct toxicity to the conceptus. Additional data from the Harris and Birnbaum PFDA study conducted in mice suggests that PFDA may also have adverse effects on fetal bodyweight as well, possibly at doses lower than PFOA.

- Reduced skeletal ossification and/or skeletal variations: Except for the developmental toxicity study conducted in rats with PFOA by Gortner *et al*., the other studies have demonstrated increased incidences of skeletal variations or ossification delays. Increased incidences of 13th rib were noted at a dose of PFOA associated with maternal toxicity in rabbits but were not considered adverse. The other studies noted reduced ossification at doses as low as 1 mg/kg PFOA in mice and at doses of 200 mg/kg in rats with the telomer alcohols; these doses were associated with maternal toxicity in the telomer alcohol studies but occurred at doses lower than the maternal systemic LOEL in the Lau *et al*. study, indicating that the mouse fetus is highly and specifically sensitive to the effects of PFOA. These effects did not follow an overall dose-response pattern but were present at all doses ≥ 1 mg/kg, possibly indicating that 1 mg/kg was above the linear part of the dose response-curve. Noteworthy, the 1 mg/kg dose is a LOEL; given the low sensitivity of the study, it is entirely possible that the actual NOEL for this effect of PFOA-treatment in mice is substantially lower than 1 mg/kg. In addition, it is also possible that testing of telomer alcohols in mice may produce lower NOELs than those established in the rat studies.

- Decreased fetal bodyweight: This effect was noted with the mixed TBA in rats, and PFOA in mice. The LOELs for this endpoint were 200 mg/kg for TBA and 20 mg/kg for PFOA. While this was the most sensitive adverse effect on fetal parameters with the mixed telomers, this effect occurred only at doses associated with clear maternal and fetal toxicity (i.e.: fetal death) in mice with PFOA. Additional data from the Harris and Birnbaum study conducted in mice with PFDA suggests that PFDA may also have adverse effects on fetal bodyweight as well, possibly at doses lower than PFOA. In addition, it is also possible that testing of telomer alcohols in mice may produce lower NOELs than those established in the rat studies.

For the endpoint of postnatal toxicity, Toxicology has concluded that the stated compounds have adverse effects on the following parameters:

- Neonatal survival: Decreased neonatal survival was noted in studies conducted in rats with the mixed TBA and PFOA and in mice with PFOA. LOELs ranged from 1-5 mg/kg PFOA in mice to 30 mg/kg PFOA in rats, indicating a 6-30-fold increase in sensitivity of mice to PFOA for this endpoint. This effect appears to be PPARα-dependent, as PFOA had no adverse effect on neonatal survival in KO mice. As noted in various reviews, PPARα-dependent findings add additional uncertainty with regard to human relevance (EPA, 2005). Noteworthy, the LOELs for decreased neonatal survival in rats for the mixed TBA are > 3 times the LOEL in rats for this endpoint with PFOA (100 mg/kg versus 30 mg/kg), which suggests that these compounds are less potent, at least for this measurement. However, the effects at this dose of the mixed telomers cannot be explained by assumed metabolism to PFOA. The LOEL for the mixed TBA is equivalent to ~27.3 mg/kg of the 8-2 TBA, which, via Toxicology's previously estimated 6% conversion rate for 8-2 TBA to PFOA *in vivo* (Roth/File, RE: (b) (4) 2/28/06), is equivalent to ~1.64 mg/kg PFOA. This dose of PFOA is clearly below the level in rats at which decreased neonatal survival would be expected.

- Decreased postnatal bodyweight gain prior to weaning: This effect was noted in studies conducted with PFOA in mice and in rats and in the study conducted with the mixed TBA in rats. While this effect was only noted at the highest test article dose in female rats exposed to PFOA (30 mg/kg) and rats of both sexes exposed to the mixed TBA (250 mg/kg), this effect was noted in mice of both sexes and male rats exposed to PFOA at doses as low as 1 mg/kg. Although a significant trend for reduced weight gain during weaning was noted in both PPAR-α WT and KO pups in the Abbott *et al*. study, statistically-significant decreases in postnatal weight gain were only observed in WT pups, indicating that this effect is at least partially PPAR-α-dependent. Unlike rats, adverse effects on postnatal weight gain were noted in mice of both sexes, although the results of the Wolf *et al*. study suggested that males may be more sensitive than females. Moreover, the Wolf *et al*. study suggests that the prenatal period is a window of susceptibility to the toxic effects of perfluorinated compounds and that pup bodyweight is a highly sensitive endpoint for toxicity, as even brief prenatal exposures to extremely low total doses of PFOA (5 mg/kg on GD 10-17) are sufficient to produce adverse effects on growth in male mice. The overall effect of PFOA on postnatal bodyweight gain appeared to be dependent upon the total dose received by the offspring, with pups exposed to PFOA both *in utero* and during lactation having more severe bodyweight gain deficits than pups exposed either *in utero* alone or during lactation alone. These two results together indicate that the prenatal period constitutes a sensitive time period in which even brief, low-dose exposures to perfluorinated compounds may have marked effects on this postnatal growth, even after cessation of dietary exposure to PFOA.

- Delayed attainment of eye-opening and hair growth: This effect was only noted after PFOA-administration in mice, where LOELs for these effects ranged from 1-5 mg/kg PFOA administered to mice on GD 1-17. As was the case for postnatal bodyweight gain, PFOA produced adverse effects even after brief, relatively low-dose exposures during the last week of gestation, and *in utero* exposure was necessary and sufficient to induce significant delays in development. Effects on this parameter were also related to total dose administered to the pups, where pups with higher total exposures (dose x time) experienced more severe delays than pups with lower total exposures. This effect appears to be PPAR-α-dependent, developmental delays were not noted in PPAR-α KO mice; as such, given the uncertain relevance of PPAR-α-dependent toxicity to humans, the relevance of this finding to human health risk assessment is also uncertain.

- Stunted mammary gland development in females: Mammary gland parameters were only assessed in studies conducted in mice with PFOA, both of which administered PFOA during gestation. Both studies observed treatment-related decreases in mammary gland development. As

with the effects on bodyweight and attainment of developmental landmarks, this effect occurs in mice after even brief, low-dose prenatal exposures to PFOA. Noteworthy, lactational exposure alone was also sufficient to induce decreased mammary development; the severity of the decrease was dependent upon total exposure to PFOA, as with the effects on attainment of developmental landmarks and growth. The adverse effects of PFOA on mammary gland development persisted out to 18-months, long after cessation of systemic PFOA exposure, in the White *et al*., 2009 study, indicating that this adverse effect of PFOA may be non-reversible. Although the available bioassay data in rats do not indicate treatment-related effects on the mammary gland, the pharmacokinetics of PFOA in the female rat may make it less sensitive than the female mouse to the long-term toxicity of PFOA at this endpoint. Moreover, the rats in the bioassay were not dosed beginning *in utero*, possibly further diminishing the sensitivity of that study to detect toxicity at that endpoint.

Changes in the timing of attainment of puberty have also been noted in the above-cited studies; however, the direction of the change (acceleration in males in the Lau *et al*., study; delay in males and females in the Argus, 2002 study and a slight delay in females in the Lau *et al.* study) indicates that the effects of PFOA on this parameter have not been adequately characterized by the aggregate of the data. Further, more robustly-designed studies will be needed in order to provide conclusive data on this parameter. Other adverse effects on pre- and postnatal development were reported in studies considered as supplementary information, including: induction of malformations in birds, neurotoxicity in mice and birds, and increased bodyweight and altered body composition parameters during later-life in mice. While this reviewer's confidence in the validity and applicability of these findings to human health risk assessment is relatively low, the data suggest that developmental exposure to perfluorinated compounds may have effects on neurodevelopment and bodyweight homeostasis in adulthood which more robustly-designed studies could adequately characterize. Based on a comparison of the severity of the effects in male rat (2-generation study) versus mouse offspring (Lau *et al*. study) at the LOEL of 1 mg/kg bw PFOA, the mouse appears to be more sensitive to the developmental toxicity of PFOA. Moreover, the White *et al*., 2009 study suggests that toxicity resulting from brief prenatal exposure persists even into late adulthood in the absence of continued systemic exposure to PFOA.

Although these studies were conducted on the stated compounds, Toxicology concludes that the endpoints cited above are of concern for hazard identification for all perflurorochemicals. The available data in aggregate suggest that mice may be more sensitive to the adverse pre- and postnatal effects of these compounds than rats, due to pharmacokinetic considerations, and that brief *in utero* exposure alone is sufficient to induce postnatal toxicity into adulthood, even in the absence of continued exposure to the compound. Although additional data on the effects of perfluorinated telomer alcohols in mice are needed, comparison of the postnatal effects of perfluorocarboxylates versus telomer alcohols in rats suggest that the telomer alcohols may be slightly less toxic during the postnatal period than the PFOA and PFDA. These studies in aggregate emphasize the need to assess the possibility of delayed toxicity in adult life arising from brief prenatal exposure in appropriately-designed risk assessment-quality studies.

Endpoint: Reproductive Health and Function in Males

Conclusions from Previously Reviewed Studies

Toxicology's conclusions from the studies that assessed male reproductive health parameters are as follows:

Study/Review memorandum	Study design	Effects on male reproductive endpoints	Limitations	Utility
Argus Research, 2002; Gu/Rice, 11/17/07;	APFO; See description in previous section	No effects fertility, sperm parameters, histology in adult F_0/F_1 rats. ↑BW-rel only	None for adults;	RA

York *et al*., 2010.		reproductive organ wts, F1, all doses. ↓Abs epididymes, seminal vesicles (+/- fluid), & prostate wts, 30 mg/kg P_0; ↑abs seminal vesicle wts-fluid, 10 mg/kg. ↑BW-rel reproductive organ wts, all organs, 30 mg/kg P_0 & all organs but prostate & cauda epididymes at 10 mg/kg. ↑BW-rel seminal vesicle + fluid wts, 3 mg/kg. ↓ & ↑Brainwt-rel wts seminal vesicles + fluid & testes, respectively, at ≥ 1 mg/kg & ≥ 3 mg/kg, respectively.	animal model for offspring	
(b) (4)				
Biegel *et al*., 2001; Twaroski/Gilliam, 10/1/02, RE: FCN 260.	Sprague-Dawley male rats; 156 rats/dose; APFO in diet 0 or 300 ppm (13.6 mg/kg bw/day) for 2 years	↑Incidence Leydig cell adenomas & hyperplasia & abs. testes wt at 2 years. ↑Serum E at 1, 2, 6, 9, 12, 15, 18, & 21 months. No changes: accessory sex organ wts; serum T, FSH, prolactin, LH, rates of beta-oxidation or cell proliferation in testes.	Only 1 dose examined	HI (RA for neoplastic findings)

Newly-identified Studies Considered Useful for Hazard Identification

1) Bookstaff, R.C., Moore, R.W., Ingall, G.B., and Peterson, R.E. (1990) Androgenic deficiency in male rats treated with perfluorodecanoic acid. *Toxicology and Applied Pharmacology*, **104**: 322-333.

Methods: The purpose of the study was to assess the effect of PFDA on reproductive hormones and organ histology in male rats. Male adult Sprague-Dawley rats (weight ~295 g) were supplied from Harlan Sprague-Dawley and housed individually in stainless-steel cages in a temperature-controlled room (19-23°C) under a 12-hour light/dark cycle and provided with ground feed (Rat Chow, Purina Mills) on either an *ad libitum* basis or as pair-fed controls. Tap water was available to all rats *ad libitum*. The study did not specify a habituation period. Rats (n=10/group) were dosed once with PFDA (96% purity) at 0, 20, 40, or 80 mg/kg in propylene glycol vehicle or an equal volume of vehicle via intraperitoneal (i.p.) injection and sacrificed 7 days after dosing. *Ad libitum* and weight-matched pair-fed controls were included. Serum T, LH, and 5-dihydrotestosterone (DHT) were measured from trunk blood collected at necropsy. Testis, seminal vesicle, and ventral prostate weights were recorded; and reproductive organs were fixed (testis in Bouin's solution) and sectioned for microscopic examination, where epithelial height in seminal vesicles was quantified. Human chorionic gonadotropin (hCG)-stimulated T production was assessed from decapsulated testis from treated rats *ex vivo*. In a separate section of the study, two hours after treatment with PFDA or vehicle, rats were anesthetized, castrated, and T capsules were implanted subcutaneously (s.c.) to assess the effects of PFDA on reproductive organ parameters in the absence of effects on serum hormones. Parametric data were analyzed via one-way ANOVA with post-hoc significance testing. Non-parametric data were analyzed via the Kruskal-Wallis test.

Results: PFDA caused a dose-related decrease in feed consumption and body weight, with effects being particularly severe at 80 mg/kg (decreased 44% and 72% relative to control, respectively). Significant dose-related decreases in T and DHT were noted over both control groups at ≥ 40 mg/kg bw, with T and DHT being decreased to 12 and 18%, respectively of *ad libitum* control values, compared to 58-59% of control in the same parameters for the pair-fed control group. No effect of treatment was noted in plasma LH, nor were effects on spermatogenesis noted. Dose-related decreases in seminal vesicle and ventral prostate weight parameters were noted versus pair-fed and *ad libitum* controls for all doses, with decreases of 42% and 49%, respectively, noted at 80 mg/kg. Slight decreases in testicular weights were also noted at 80 mg/kg bw. Marked atrophy of the seminal vesicle epithelium was noted at ≥ 40 mg/kg bw, with epithelial height decreased to half of *ad libitum* and pair-fed control values at 80 mg/kg. Castration and supplementation with T abolished PFDA's effects on reproductive organ weights and seminal vesicle epithelial height. Decreased hCG-mediated *ex vivo* stimulation of T output from decapsulated testis was noted from testis from PFDA-treated males at ≥ 40 mg/kg bw PFDA.

Strengths: The strengths of this study include adequate numbers of animals used for the short-term nature of the study, the in-depth nature and sensitivity of the endpoints measured, the inclusion of a pair-fed control to assess the possibility of secondary effects of bodyweight decreases on hormonal parameters, and the animal model used. Also, as similar effects of PFDoDA were noted in the testes of rats (Shi *et al*., 2007), one of the strengths of this study is repeatability.

Weaknesses: The weaknesses of the study include the apparent lack of a habituation period, the route of administration used, the fact that the test substance was not repeatedly administered, and the lack of a positive control for the *ex vivo* T production study. Although the study had an adequate number of doses, it would have been better designed if it also included a lower range of doses.

Utility of Study: This study is useful for **hazard identification**, but is limited to range finding for any type of repeated exposure measures. From the data presented, Toxicology concludes that there is no NOEL for this study, based on decreased seminal vesicle and prostate weights at the LOEL of 20 mg/kg

bw/day.

2) 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. *Toxicology and Pharmacology*, **113**: 209-217.

Methods: The purpose of the study was to determine the mechanism responsible for the increased incidences of Leydig cell adenomas seen in the rat bioassay performed with PFOA (Biegel *et al*., 2001). Twelve-week-old male CD rats were obtained from Charles River Laboratories and individually-housed in stainless-steel wire-mesh cages in a temperature- (23±2°C) and humidity- (40-60%) controlled room maintained on a 12-hour light/dark cycle, with Purina Rodent Chow 5002 meal and tap water *ad libitum*. Rats were habituated for one week, during which rats were observed with respect to weight gain and gross signs of disease or injury and certified to be clinically normal and free of disease prior to study. Rats (n=15/group) were gavaged with 0, 1, 10, 25, or 50 mg/kg bw/day of PFOA (> 99.9% purity) in water in a volume of 2 ml/kg for 14 days. The highest dose was based on the results of a 14-day pilot study, in which no mortality was observed when rats were dosed with 50 mg/kg PFOA (data not shown). A pair-fed control group was included for the 50 mg/kg PFOA group. Rats were necropsied on Day 15, and the following endpoints were assessed: serum T, E, and LH; absolute and bodyweight-relative weights for liver, testes, epididymes, accessory sex organ units including fluids but with the bladder removed, prostate, coagulating glands, seminal vesicles, and levator ani muscle; histologic evaluation of one testis (Bouin's fluid); T content in testicular insterstitial fluid; and peroxisomal beta-oxidation in liver homogenates (5 rats/group). In a second experiment, 30 rats/PFOA dose were dosed with 0 or 50 mg/kg PFOA for 14 days, followed by injection with either human chorionic gonadotropin hormone (100 IU s.c., n=10 rats/group), naloxone (2 mg/kg, s.c., n=10 rats/group), or GnRH (3 µg/kg, i.p.) one hour prior to sacrifice. Serum T and LH was assessed for all animals; for hCG-injected animals, serum progesterone, 17-alpha-hydroxyprogesterone, and androstenedione were also assessed. Parametric data were analyzed via one-way ANOVA, with post-hoc testing via Dunnett's test. Non-parametric data were analyzed via Kruskal-Wallis test and Mann-Whitney U-test. Trend testing via Jonckheere and Cochran-Armitage tests was also performed.

Results: Significant, dose-dependent decreases in final bodyweights and bodyweight gain were noted at ≥ 10 mg/kg PFOA compared to *ad libitum* control. The following statistically-significant effects on organ weight parameters were observed: increased relative liver weight at ≥ 10 mg/kg PFOA versus *ad libitum* control; increased relative liver weight in 50 mg/kg PFOA group over pair-fed control; increased relative testes weight at ≥ 25 mg/kg PFOA versus *ad libitum* but not pair-fed control; decreased relative accessory organ unit weight at ≥ 25 mg/kg PFOA versus *ad libitum* control; and decreased relative accessory organ unit and ventral prostate weights at 50 mg/kg PFOA versus pair-fed control. No individual differences in absolute testes, prostate, seminal vesicles, or coagulating glands were noted. Hepatic beta-oxidation rates were increased in the livers of rats at ≥ 10 mg/kg PFOA versus *ad libitum* control, with no differences between the two control groups in beta-oxidation rate. Serum E levels were significantly increased at ≥ 10 mg/kg PFOA versus *ad libitum* control, and serum E levels were significantly increased in the 50 mg/kg PFOA group versus the pair-fed control. There were no significant differences in serum or interstitial fluid T or serum LH, although a significant downward trend with dose was noted for serum T. No effects on testicular histopathology were mentioned. Non-significant decreases in serum T and significantly decreased serum androstenedione levels were noted after hCG-challenge.

Strengths: The strengths of the study include the route of administration, the numbers of animals used, the robust design and conduct of the study, the use of a range of doses of PFOA, the repeated-dose design of the study, and the assessment of multiple measurements relevant to male reproductive health.

Weaknesses: The length of the study, the inadequate dosing duration, the lack of reporting of effects of

the test article on histopathology, and the lack of positive controls for the challenge studies were the weaknesses of this study.

Utility of Study: This study is useful for **hazard identification**, but is limited to range finding for any type of repeated exposure measures. Based on the data reported for effects on liver weight parameters, beta-oxidation rates, bodyweights and bodyweight gain, and serum E, Toxicology concludes that the NOEL for this study is 1 mg/kg PFOA with a LOEL of 10 mg/kg bw/day.

3) Liu, R.C.M., Hurtt, M.E., Cook, J.C., and Biegel, L.B. (1996) Effect of peroxisome proliferator, ammonium perfluorooctanoate (C8), on hepatic aromatase activity in adult male Crl:CD BR (CD) rats. *Fund. Appl. Toxicol.*, **30**: 220-8.

Methods: The purpose of this study was to assess the effect of PFOA on hepatic and testicular aromatase activity in male rats. 11-Week-old male Crl:CD BR (CD) rats were obtained from Charles River Laboratories (Raleigh, NC) and singly-housed in a temperature- (23 ± 2°C) and humidity- (40-60%) controlled room maintained on a 12-hour light/dark cycle, with Purina Certified Rodent Chow #5002 meal and tap water provided *ad libitum*. Rats were quarantined for one week prior to random assignment to treatment groups, stratified by bodyweight. Rats (n=15/group) were gavaged with 0, 0.2, 2, 20, or 40 mg/kg PFOA (> 99.9% purity) in deionized water in a volume of 2 ml/kg for 14 days; *ad libitum* and pair-fed control groups were included. Doses in the study were chosen based on the results of a 14-day pilot study (data not shown). Serum E; testicular weights; hepatic microsome beta-oxidation activity (n=6/group); hepatic and testicular microsomal aromatase activity; and total hepatic microsomal P450 content (n=6/group) were determined. The effects of PFOA on aromatase enzyme kinetics in isolated liver microsomes from treated rats (n=2/group) and in isolated microsomes from untreated rats incubated with PFOA *ex vivo* were determined. Direct effects of PFOA on hepatocyte aromatase were also determined via incubation of isolated hepatocytes from untreated rats with PFOA *ex vivo*. Data were analyzed via one-way ANOVA, with post-hoc testing via Dunnett's test.

Results: Final bodyweights were decreased at ≥ 20 mg/kg PFOA compared to *ad libitum* controls; bodyweights at 40 mg/kg PFOA were decreased compared to both controls. Absolute and bodyweight-relative liver weights were dose-responsively increased over both controls at ≥ 2 mg/kg PFOA, and the protein yields of the hepatic microsomal fraction were significantly and dose-responsively increased at all doses of PFOA over both controls. Bodyweight-relative testes weights were decreased compared to *ad libitum* controls for both the pair-fed group and the 20 mg/kg group; relative testes weights were decreased in the 40 mg/kg group compared to both controls. There were no significant differences in absolute testes weights or in testicular microsomal aromatase activity. In contrast, hepatic aromatase activity was dose-responsively and significantly increased compared to both controls at ≥ 2 mg/kg PFOA. Serum E was also significantly and dose-responsively increased at ≥ 2 mg/kg PFOA compared to both controls, with a significant linear correlation between hepatic aromatase activity and serum E. Hepatic beta-oxidation and total P450 were also dose-responsively and significantly increased compared to both controls at ≥ 2 mg/kg PFOA and at ≥ 20 mg/kg PFOA, respectively. In contrast, direct treatment of hepatocyte cultures with PFOA decreased aromatase activity in a dose-responsive fashion at concentrations ≥ 100 μM.

Strengths: The strengths of the study are the animal model, the route of administration, the numbers of animals and doses of PFOA used in the study, the environmental conditions and method of conduct of the study, and the statistical methods. The use of a pair-fed control to examine the role of effects of bodyweight on the measured endpoints also added confidence to the study. Additionally, the assessment of the specificity of the microsomal aromatase assays using the inhibitors DHT and aminoglutethimide increased confidence in the validity of those results.

Weaknesses: The main weaknesses of the study were the dosing duration and the paucity of testicular and hormonal endpoints examined, although it should be pointed out that the main focus of the study was specifically the effects of PFOA on hepatic aromatase and serum E. In addition, some of the parameters measured either used very low n or were not generally validated.

Utility: Based on the short dosing duration and restricted numbers of endpoints examined, the study is unsuitable for risk assessment. Based on the numerous strengths in study design as enumerated above, the study is useful for **hazard identification** for PFOA and for perfluorinated compounds in general, specifically with regard to indirect endocrine disruptive effects of perfluorinated compounds in the liver. From the reported study results, this reviewer concludes that the NOAEL for systemic toxicity is 0.2 mg/kg/day, based on increased serum E and liver weight parameters at 2 mg/kg.

4) Shi, Z., Zhang, H., Liu, Y., Xu, M., and Dai, J. (2007) Alterations in gene expression and testosterone synthesis in the testes of male rats exposed to perfluorododecanoic acid. *Toxicol. Sci*., **98**(1): 206-215.

Methods: Male Sprague-Dawley rats (age unknown; bodyweight range: 230-240 g) were obtained from Weitong Lihua Experimentary Animal Central in Beijing, China, ranked by bodyweight, and assigned to treatment groups (n=10/group). Rats were housed 2/cage in a temperature- (20-26°C) and humidity- (30-70%) controlled room on a 12-hour light/dark cycle, provided feed and water *ad libitum*, and acclimatized for 1 week prior to study start. Rats were gavaged with 0, 1, 5, or 10 mg/kg bw/d of PFDoDA (CASRN: 307-55-1, 95% purity) in 0.5% Tween-20 vehicle for 14 days in a volume of 6 ml/kg bw. The doses for the study were chosen based on results of a preliminary 14-day trial (data not shown). All rats were weighed at the end of treatment. Six rats/group were decapitated, and trunk blood was collected. Testes and epididymes were weighed, and the epididymes and one part of the testes were fixed in 2.5% glutaraldehyde and processed for analysis via electron microscopy. The other part of the testis was frozen in liquid N_2 for total RNA extraction. Serum T, E, total cholesterol, FSH, and LH were assessed. Gene expression levels in testis were quantified via real-time quantitative PCR. Bodyweight-relative testes weight was calculated. Data were analyzed by a one-way ANOVA or general linear model and post-hoc testing via Dunnett's two-sided t-test. Histopathological data were not analyzed for statistical significance.

Results: Bodyweights were significantly reduced in a dose related manner by ≥ 25% at the two highest dose levels, with no effect on bodyweight noted at 1 mg/kg. Absolute testes weight was significantly decreased at the highest dose (-22%), and a degressive trend was noted in the two lower dose groups. Relative testes weights were increased at the two highest doses by 36% for both. The following differences in serum parameters were noted: increased total cholesterol at the highest dose (+35%); decreased T at the two highest doses (-62% and -84%, dose-responsive); and decreased LH levels at the highest dose. No treatment-related effects on FSH or E were noted. Several ultrastructural changes to the testes were noted, with dose-responsive increases in severity starting at 5 mg/kg bw/day, including apoptotic changes in Leydig and Sertoli cells and in spermatogonia. Severe decreases in gene expression in testes for hormones involved in steroid synthesis and metabolism were also noted at doses greater than 5 mg/kg. No changes in histopathology or gene expression were noted at 1 mg/kg.

Strengths: The strengths of this study included the animal model used, the route of administration, the use of multiple doses of the test substance, the control of environmental conditions in the study, and the extensive array of endpoints correlated to male reproductive health measured in the study. While the numbers of animals used in the study were quite low and may have affected the sensitivity of the study, the study was still sufficiently powered to have confidence in the validity of the reported positive results.

Weaknesses: The weaknesses of the study include the uncertain validity and relevance of the assay for gene expression changes in the testes, and the probability that the dosing duration of the study was

inadequate.

Utility of study: This study is useful for **hazard identification**, but is limited to range finding for any type of repeated exposure measures. Based on the data reported, Toxicology concludes that there is no NOAEL for this study, based on a trend of decreased testis weights at the LOEL of 1 mg/kg bw/day.

5) 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**: 192-200.

Methods: The purpose of this study was to assess the effects of chronic exposure to PFDoDA on testicular parameters in rats. 4-Week old male Sprague-Dawley rats (Weitong Lihua Experimental Animal Center, Beijing, China; n=6/group) were housed as described in Shi *et al.*, 2007 and gavaged with 0, 0.02, 0.05, 0.2, or 0.5 mg/kg bw/day of PFDoDA (95% purity) in 0.2% Tween-20 for 110 days at 6 ml/kg bw. At the end of treatment, all rats were decapitated, and trunk blood was collected and processed for serum. The testes, prostate, seminal vesicles, and vas deferens of each animal were weighed. Testes were examined microscopically. The following serum hormones were measured: T, E, FSH, LH, GH, insulin, and total cholesterol. Total RNA from testes, hypothalamus, pituitary, and liver were analyzed for changes in gene expression via real-time quantitative PCR. Protein levels of StAR and P450SCC were analyzed in testes samples via Western blot. Data were analyzed by a one-way ANOVA and post-hoc testing via Dunnett's two-sided t-test.

Results: PFDoDA decreased bodyweight by 7.2% at 0.5 mg/kg and caused histopathological changes in the seminiferous tubules of the testes (appearance of cast-off cells), but no changes in reproductive organ weight parameters or total cholesterol were noted. Serum T was dose-related decreased by 44% and 60% at ≥ 0.2 mg/kg, but no other changes in serum hormones were noted. StAR mRNA in the testes was decreased at all doses, and StAR protein levels were also decreased at ≥ 0.05 mg/kg in a roughly dose-responsive manner (levels were 62.6%, 50.6%, and 53% of controls). Decreased testicular IGF-1 mRNA was also noted at ≥ 0.05 mg/kg, and decreased testicular IGF-IR and IL-1alpha mRNA was noted at ≥ 0.2 mg/kg. The highest dose of PFDoDA significantly decreased P450SCC protein levels but had no effect on mRNA levels. Decreased FSH and GNRH-R mRNA levels were noted in the pituitary at 0.5 mg/kg. Additional, non-dose-responsive changes in mRNA were noted in the testes, hypothalamus, and pituitary.

Strengths: The strengths of this study were the animal model and route of administration used, the housing conditions, the length of the dosing period, the statistical analyses used, and the use of a wide range of doses for the test article. Although not validated, the authors attempted to measure gene expression and protein changes as an upstream event leading to some of the apical endpoints identified, which adds to the knowledge of mechanisms of action. Another strength is the repeatability of the results with regards to the results of Shi *et al.*, 2007 and Bookstaff *et al.*, 1990.

Weaknesses: The dosing duration being insufficient to ensure that the compound was at steady-state, and the number of replicates in this study is considerably lower than recommended.

Utility of study: This study is useful for **hazard identification**, but is limited to range finding for any type of repeated exposure measures. Based on the data reported, Toxicology concludes that the NOAEL for traditional endpoints is 0.05 mg/kg, based on decreased serum T at ≥ 0.2 mg/kg. More information would be needed to consider the molecular changes observed as adverse.

6) Feng, Y., Shi, Z., Fang, X., Xu, M., Dai, J. (2009) Perfluorononanoic acid induces apoptosis involving the Fas death receptor signaling pathway in rat testis. *Toxicol. Lett.*, **190**: 224-230.

Methods: The purpose of this study was to examine the effects of PFNA on male reproductive parameters in rats. Seven-week-old Sprague-Dawley rats (bodyweights of 230-240 g) were purchased from Weitong Lihua Experimentary Animal Central in Beijing, China and assigned to treatment and control groups (n=6/group, basis of assignment unstated). Animals were singly-housed in polycarbonate cages with wire lids and solid bottoms in a temperature- (20-26°C) and relative humidity- (30-70%) controlled room on a 12-hour light/dark cycle, with access to feed and water *ad libitum*. Rats were habituated for one week prior to study. Rats were gavaged with PFNA (97% pure) in 0.2% Tween-20 at doses of 0, 1, 3, or 5 mg/kg bw/day for 2 weeks in a volume of 6 ml/kg bw; doses were based on the results of a preliminary experiment (data not shown), where mortality was noted at 20 mg/kg bw/day. At necropsy, trunk blood was collected for analysis of serum T, E, FSH, and LH. Testes were excised, and one part of the right testis was fixed in Bouin's, sectioned and stained with H&E or re-processed and stained for TUNEL assay, and analyzed microscopically. The other part of the right testis was fixed in 70% ethanol and minced; cells isolated from testis were then stained with propidium iodide and analyzed via flow cytometry. Gene expression in testis was analyzed via real-time RT-PCR; protein expression was analyzed by Western blot. Data were statistically analyzed via one-way ANOVA, followed by post-hoc testing via the LSD multiple range test.

Results: PFNA significantly increased serum E by 2-fold and significantly decreased serum T by 85.4% at the highest dose. Serum T was significantly increased at 1 mg/kg by 87.5% of control, with no significant differences noted in the mid-dose group. No significant effects of PFNA were noted in serum FSH or LH. In the high dose group, crescent chromatin condensation and margination and sloughing of germ cells into the lumen of seminiferous tubules was noted; no histopathological changes were noted at lower doses. Significant and dose-related increases in numbers of TUNEL-positive cells and caspase-8 protein were noted in testes at ≥ 3 mg/kg, with non-statistically-significant increases also noted at 1 mg/kg. Significantly decreased expression of Bcl-2 in the testes was also noted at ≥ 3 mg/kg, and significantly increased expression of Bax and Fas in testes were noted at the high dose. Although significantly decreased Fas-L expression was noted at 3 mg/kg only, this effect did not appear to be treatment-related due to lack of dose-response. No effect of PFNA on caspase-9 protein content in testes was noted.

Strengths: The strengths of this study include the animal model used, the route of administration, the spread of doses used in the study, the measurement of concomitant endpoints related to reproductive hormone homeostasis and testicular pathology, and repeatability with regard to confirmation of testicular effects seen with structurally-related compounds (Shi *et al*., 2007; Bookstaff *et al*., 1990). In addition, the use of gene and protein expression to look for early makers of apopotosis is also interesting, though not validated.

Weaknesses: The weaknesses of the study include the inadequate nature of the dosing period, and the lack of measurement of concomitant endpoints, such as bodyweight or organ weight. The numbers of animals used in the study are too low and may have affected the sensitivity of the study.

Utility of study: This study is useful for **hazard identification**, but is limited to range finding for any type of repeated exposure measures. A tentative NOAEL of 1 mg/kg PFNA may be derived from this study, based on pathological changes in the testes at 5 mg/kg. The increased serum T noted at 1 mg/kg is of unknown biological significance, based on the lack of relationship of the response to dose.

Supplementary Information

A number of studies were identified that examined the effects of perfluorinated compound administration on Leydig cell function *in vitro* and *ex vivo*. PFOA, PFDA, and PFDoDA reportedly inhibited steroidogenesis in primary Leydig cells derived from adult rats and Leydig cell tumor lines (Shi *et al*.,

2010; Biegel *et al*., 1995; Boujrad *et al*., 2000; Zhao *et al*., 2009) via competitive inhibition of steroidogenic enzymes and also possibly downregulation of steroidogenic gene expression. Indicators of cell damage, such as lipid accumulation and reactive oxygen species (ROS) accumulation in mitochondria, were also noted in Leydig cells treated with perfluorinated compounds (Boujrad *et al*., 2000; Shi *et al*., 2010). Impaired reproductive hormone synthesis, induction of the female-specific sex marker vitellogenin in the liver, and impaired fecundity have been reported in adult male zebrafish exposed to the 8-2 telomer B alcohol for 4 weeks (Liu *et al*., 2010).

In contrast to some of the laboratory data, impaired semen quality was associated with high levels of multiple perfluoroalkyl compounds in non-occupationally exposed males in a Danish study (Joensen *et al*., 2009). No significant associations between blood PFOA levels and reproductive hormones were noted in an occupationally-exposed male population (Olsen *et al*., 1998). Retrospective cohort mortality analyses of occupationally exposed males have reported conflicting findings regarding the association of PFOA exposure and incidences of reproductive organ cancers, with one study reporting a statistically-significant 3.3-fold increase in prostate cancer mortality in highly-exposed workers as opposed to workers with low or no exposure to PFOA (Gilliland and Mandel, 1993). Another study found no association between levels of PFOA exposure in worker populations and reproductive cancer mortality rates (Leonard *et al*., 2008). Results from studies conducted in mammals with perfluorinated compounds that met the first tier of review but did not meet the criteria for hazard identification are summarized in Attachment 1.

Section Summary

In this section, Toxicology has evaluated the available evidence concerning the effects of perfluorinated compounds on male reproductive health endpoints. Toxicology has concluded that the stated perfluorinated compounds have adverse effects on the following validated parameters:

- Fertility and spermatogenesis: Spermatogenesis in rats was unaffected by PFOA, PFDA, or mixed TBA administration at the highest dose levels used in these studies of 30 mg/kg by gavage, 80 mg/kg i.p., and 250 mg/kg by gavage, respectively. PFOA and mixed TBA also had no effect on male fertility parameters at the stated doses above;
- Testicular and accessory sex organ weight parameters: PFOA had inconsistent effects on testes and accessory sex organ weight parameters in the available studies conducted in rats (increased, decreased, no change), and effects were unrelated to dose in the cynamolgous monkey study. In contrast, decreased accessory sex organ weight parameters were noted in rats after a single i.p. dose of PFDA at ≥ 20 mg/kg. Decreased testes weight parameters were noted in rats after a single i.p. dose of 80 mg/kg PFDA and after gavage administration of 10 mg/kg PFDoDA for 14 days. Increased bodyweight-relative testes weights were noted at ≥ 5 mg/kg PFDoDA, which probably reflects the large decrement in bodyweight noted at these doses (26-38% decrease compared to controls). Similarly, the decreased testes and epididymal parameters noted in F_1 rats with 250 mg/kg mixed TBA was concluded to be secondary to effects on bodyweight at that dose. No effects on testes or accessory sex organ weight parameters were noted in rats with the 8-2 TBA;
- Testicular and/or accessory sex organ histopathology: PFOA increased incidences of Leydig cell hyperplasia and adenomas in rats when administered in the diet at ≥ 13.6 mg/kg for 2 years. In contrast, oral administration of 20-30 mg/kg PFOA for 6 months produced testicular atrophy and degeneration in one cynamolgous monkey in this dose group. In rats, PFNA and PFDoDA also produced degenerative changes in the testes at doses of 5 mg/kg for two weeks and 0.5 mg/kg for 110 days, respectively. These degenerative changes appeared to be apoptotic in nature, based on histopathological characteristics of the changes. A single i.p. dose of ≥ 40 mg/kg was sufficient to produce atrophy of the seminal vesicle epithelium in rats. In contrast, no histopathological changes were noted in the testes or accessory sex organs in rats orally-administered the 8-2 TBA or the mixed TBA for 90 days; and
- Serum hormones:

- Serum E: Contrasting effects of oral PFOA administration on serum E have been noted in rats versus monkeys, with increased serum E in rats noted at ≥ 0.63 mg/kg after dosing for 90 days and decreased serum E in monkeys noted at ≥ 10 mg/kg after dosing for 6 months. This difference in the direction of the effect could be due to either dosing duration or the animal model used, although the available evidence from the Liu *et al*., 1996 study and the two carcinogenicity studies indicates that the increased serum E noted in the rats at 90 days is a persistent effect and most likely due to induction of increased aromatase expression in the liver, but not the testes. Similarly, PFNA administration for 2 weeks also increases serum E at 5 mg/kg. In contrast, there was no effect in rats on serum E after gavage with PFDoDA for 14 days at doses of up to 10 mg/kg;
- Serum T: No effect on serum T was noted in rats after being administered PFOA in the diet for 90 days at doses up to 6.15 mg/kg. Decreased serum T was noted in monkeys after oral administration of 20-30 mg/kg PFOA for 6 months; in rats after gavage with 5 mg/kg PFNA for 14 days; in rats after a single i.p. dose of ≥ 40 mg/kg PFDA; and in rats after gavage with ≥ 0.2 mg/kg PFDoDA for 110 days. Noteworthy, the LOELs for this effect in the 14-day PFDoDA and PFNA studies are the same: 5 mg/kg, indicating that the decreased LOEL for this effect noted in the 110-day PFDoDA study was due to the longer exposure period, not an increase in the potency of PFDoDA compared to PFNA. The supplemental gene expression data in the testes of rats administered PFDA and PFDoDa suggest the possibility of interference with T biosynthesis at the level of the testes, possibly via downregulation of expression of genes for steroid biosynthetic enzymes. Support for the hypothesis comes from the cited studies in primary cells and cell lines demonstrating decreased steroidigenesis after exposure to PFOA, PFDA, or PFDoDA; and
- Other hormones: The only effects on other reproductive hormones noted were decreased DHT in rats after a single i.p. dose of ≥ 40 mg/kg PFDA and decreased LH after gavage with 10 mg/kg PFDoDA for 14 days. No effects of PFOA administration on FSH, LH, or prolactin were noted in rats. No effects on FSH or LH were noted after PFNA administration at doses up to 5 mg/kg for 14 days. No effects of PFDA on LH were noted in rats after a single i.p. dose of PFDA at levels up to 80 mg/kg or on FSH after gavage with PFDoDA for 110 days at doses of up to 0.5 mg/kg/day.

There is no information available regarding perfluorinated compound activity at the androgen receptor, and the available information regarding direct activity of perfluorinated compounds at the estrogen receptor is not conclusive (see discussion in the next section). However, from the results of studies conducted on the stated compounds above, ≥ C8 perfluorinated carboxylic acids appear to have direct adverse effects on reproductive hormone homeostasis at the level of the liver and the testes and may therefore be considered as endocrine disruptors in male rats. Regarding application of these conclusions to perfluorinated compounds as a class, the available evidence regarding effects on the male reproductive system of telomer alcohols is sparse, but the data indicate that either the telomer alcohols may not be toxic to the male reproductive tract or they are significantly less potent at these endpoints than perfluorocarboxylates. This conclusion, however, should be regarded as highly tentative, given the limited number of endpoints of male reproductive toxicity measured in the TBA studies and the equally limited duration of these studies. It is possible that studies conducted for a longer duration with a more comprehensive set of endpoints measured may demonstrate that telomer alcohols have adverse effects on the male reproductive tract in rodents.

It should also be noted that the observed effects on the male reproductive tract with perfluorocarboxylates in rats do not appear to translate into direct effects on fecundity under the test conditions of standard reproductive toxicity studies. These studies in aggregate indicate that toxicity to the male reproductive tract is a hazard identification endpoint that should be included in appropriately-designed risk assessment-

quality studies for this class of compounds.

Endpoint: Reproductive Health in Females

Conclusions from Previously Reviewed Studies

Study/Review memorandum	Study design	Effects on female reproductive endpoints	Limitations	Utility
Argus Research, 2002; Gu/Rice, 11/17/07; York et al., 2010	APFO; See description in previous section	No effects on fertility, F0 or F1 adults. No effect on reproductive organ wts in F1 rats.	As previous +, no assessment of serum hormones	RA
(b) (4)				

Newly-identified Studies Considered Useful for Hazard Identification

1) Shi, Z., Zhang, H., Ding, L., Feng, Y., Xu, M., and Dai, J. (2009) The effect of perfluorododecanoic acid on endocrine status, sex hormones, and expression of steroidogenic genes in pubertal female rats. *Reprod. Toxicol.*, **27**: 352-359.

Method: The purpose of the study was to assess the effects of PFDoDA on reproductive parameters in female rats. 21-Day old Sprague-Dawley female rats were obtained from Weitong Lihua Experimental Animal Center in Beijing, China and singly-housed stainless steel cages in a temperature- (20-26°C) and humidity- (40-60%) controlled room maintained on a 12-hour light-dark cycle, with feed and water provided *ad libitum*, for a 3-day acclimatization period prior to use in the study. 24-Day-old female Sprague-Dawley rats (35-40 g, n=8/group) were gavaged with 0, 0.5, 1.5, or 3 mg/kg bw/day of PFDoDA (95% purity) in 0.5% Tween-20 vehicle for 28 days. Rats were monitored for the day of vaginal opening (VO) on a daily basis, and daily vaginal smears were collected for each female after VO until sacrifice. The day of VO and bodyweight on the day of VO were recorded. Smears were analyzed under a low-power microscope to determine cyclicity, and irregularly cycling females were identified. At the end of 28 days of treatment, diestrus females (PND 52-55) were weighed and decapitated, and trunk blood for analysis of serum hormone concentrations was collected and stored until analysis. Concentrations of the following hormones were determined: E, FSH, LH, and total cholesterol for all rats. Both ovaries and the uteri from these animals were weighed, and the left ovary and part of the uterus were fixed in 10% buffered formalin for histopathology. The right ovary and remainder of the uterus were assessed for gene expression of steroid metabolic enzymes and other proteins via real-time quantitative PCR for all rats. For

3 rats per group, numbers of primary, primordial, preantral, and antral follicles in each ovary were enumerated and uterine histopathology assessed. Data were analyzed via the Shapiro-Wilk test for normality, followed by a one-way ANOVA and post-hoc testing via Dunnett's two-sided t-test. Differences in bodyweight at VO and first estrus were analyzed via a general linear model.

Results: The only significant, treatment-related differences noted in the study were in the highest dose group as follows: decreased bodyweight at the end of treatment (-6.4%), increased cholesterol; decreased serum E (-40%); downregulated gene expression of steroid biosynthetic hormones; and decreased expression of ER-β. Decreased ERα was noted in the two highest dose groups.

Strengths: The strengths of the study were the route of administration, the repeated-dose nature of the study, the conduct of the study, the number and variety of endpoints assessed, and the method of conduct of the statistical analyses.

Weaknesses: The duration of the study and the dosing duration may have been inadequate. The use of a low number of replicates for some of the endpoints was also a weakness of the study, and the increased cholesterol contradicts some of the previous reported findings.

Utility of study: This study is useful for **hazard identification** for serum hormone endpoints, but is limited to range finding for any type of repeated exposure measures. The NOAEL for this study is 1.5 mg/kg for decreased bodyweight, increased cholesterol, and decreased serum E at 3 mg/kg.

3) White *et al*., 2007

Methods: The methods of this study are discussed in the 'Developmental Toxicity' section.

Results: Results for the pups have been previously discussed. Maternal mammary gland morphological differentiation was also significantly decreased in the GD 1-17 and GD 8-17 groups at PND 10, the peak of lactation, in a dose-responsive manner at the same time as serum PFOA-levels were dose-responsively increased in all three treatment groups. At PND 20, when control gland showed signs of involution, glands from all PFOA-exposed dams appeared similar to control glands at peak lactation. Serum PFOA levels in dams at PND 20 were unchanged from serum levels at PND 10. Non-dose-responsive changes in milk protein gene expression were noted on PND 10 and 20, with significant decreases in lactoferrin expression on PND 20 in all PFOA-exposed dams. Significantly-increased EGF expression was noted in GD 1-17 dams on PNDs 10 and 20, and in GD 8-17 dams on PND 10.

Strengths and Weaknesses: The same as discussed in the 'Developmental Toxicity' section.

Utility of study: This study is useful for **hazard identification** for mammary gland morphology endpoints, but is limited to range finding for any type of repeated exposure measures. There is no NOAEL for this study, as adverse effects on differentiation were noted after all exposure periods.

Supplementary Information

A number of studies were identified that examined the effects of perfluorinated compound administration on E-sensitive primary cells or cell lines. The 8-2 TBA reportedly demonstrated dose-dependent activation of hERα and β isoforms in a yeast two-hybrid assay (Ishibashi *et al*., 2007, 2008), while PFOA was negative for estrogenic activity in this assay. The 8-2 TBA at high doses also induced proliferation of E-sensitive MCF-7 cells, upregulation of E-responsive genes, and upregulation of expression of ER; this activity was blocked by addition of an ER antagonist (Maras *et al*., 2006; Vanparys *et al*., 2006). Another

study also reported upregulated vitellogenin and ERα in male medaka livers after exposure to 8-2 TBA (Ishibashi *et al*., 2008). Similarly, PFOA, but not the 8-2 TBA, upregulated vitellogenin in primary hepatocytes from male tilapia in a dose-responsive manner that was inhibited by tamoxifen (Liu *et al*., 2007). PFOA increased expression of E-responsive genes in the livers of male and female freshwater rare minnows and induced ovarian degeneration in minnow females (Wei *et al*., 2007). Impaired fecundity, measured as increased time-to-pregnancy, was associated with high levels of serum PFOA in non-occupationally exposed pregnant females in a Danish study (Fei *et al*., 2009). In contrast, studies of occupationally-exposed women have found no association between PFOA-exposure and mortality rates from breast cancer (Leonard *et al*., 2008) or other causes (Gilliland and Mandel, 1993). Results from studies conducted in mammals with perfluorinated compounds that met the first tier of review but did not meet the criteria for hazard identification are summarized in Attachment 1.

Section Summary

In this section, Toxicology has evaluated the available evidence concerning the effects of perfluorinated compounds on female reproductive health endpoints. Toxicology has concluded that the stated perfluorinated compounds have adverse effects on the following validated parameters:

- Fertility and estrous cycle parameters: PFOA had no adverse effects on fertility parameters in female rats or mice at doses of up to 30 mg/kg and 40 mg/kg, respectively, although increased numbers of estrous cycles/21 days were noted in rats prenatally-exposed to 30 mg/kg PFOA. Although the mixed TBA also had no effect on fertility indices, gestation length, implantation site numbers, and estrous cycles in rats, decreased implantation efficiency was noted at doses of ≥ 100 mg/kg bw/day. However, it is unclear whether this effect represents an adverse effect of the test compound on female reproductive function or a direct toxic effect on the conceptus. In contrast, PFDoDA had no effect on estrous cyclicity in female rats at doses of up to 3 mg/kg bw/day for 28 days;
- Ovarian and accessory sex organ weight parameters: No effects on reproductive organ weight parameters were noted in rats administered PFOA at doses of up to 16.1 mg/kg bw/day in the diet for 2 years, 8-2 TBA by gavage at doses of up to 125 mg/kg bw/day for 90 days, or the mixed TBA at doses of up to 250 mg/kg bw/day for 90 days;
- Ovarian and/or accessory sex organ histopathology: PFOA administration in mice decreased mammary gland development in prenatally-exposed offspring and delayed differentiation into a lactating phenotype when administered during pregnancy at doses as low as 3 mg/kg bw/day from GD 1-17 in offspring or when administered during pregnancy to adults at 5 mg/kg bw/day on GD 1-17 or GD 8-17. At 18 months of age, abnormal histopathological findings were noted in the mammary glands of prenatally-exposed mice, including increased numbers of darkly-staining foci, hyperplasia of the ductal epithelium, increased stromal epithelial densities, and/or inappropriate differentiation of ductal tissue. Interestingly, non-dose-responsive increases in the incidences of mammary gland fibroadenomas were also noted in rats in the dietary carcinogenicity study with PFOA; however, this finding was concluded to be not biologically-significant and/or not treatment-related. In addition, increased incidences of ovarian tubule hyperplasia with decreased incidences of ovarian adenomas were noted in rats at doses as low at 1.6 mg/kg bw/day PFOA in the diet for two years. No histopathological findings were noted in female rats after PFDoDA, 8-2 telomer B alcohol, or mixed TBA administration; and
- Serum hormones: The only study to assess effects on hormone production in females noted decreased serum E at the highest dose of PFDoDA of 3 mg/kg bw/day after 28 days of treatment. No effect of PFDoDA on FSH or LH was noted.

From these data, Toxicology concludes that PFOA has effects on mammary gland development and differentiation in mice. The mechanism of action of the observed effects is also unclear. Supplementary

data reporting estrogenic activity of the 8-2 TBA or PFOA in fish, or, in the case of the 8-2 TBA, E-sensitive cell lines, suggests a possible mode of action for the observed effects of interaction of perfluorinated compounds with E-mediated pathways. Moreover, given the paucity of data for this endpoint, particularly the lack of toxicity data in the most sensitive species, the mouse, with the telomer alcohols, it is not possible at this time to determine whether effects on the mammary gland are characteristic of perfluorinated compounds as a class. Similarly, only two studies noted effects in the ovary in rats after either life-long exposure to PFOA or 28-days exposure to PFDoDA administration; no effects of treatment in the ovary were noted in rats in the telomer alcohol studies. This may be due to insufficient duration of the studies, lack of assessment of concomitant endpoints such as serum E, or compound-specific effects of PFOA and PFDoDA. The only test compound in which possible effects on fertility were noted were the mixed TBA; it is unclear from the data whether the site of action of the test compound is indeed the female reproductive tract, and this effect was not noted in either rats or mice with PFOA, indicating a possible compound-specific effect. In summary, there is insufficient data covering concomitant endpoints in rats and mice for sufficient numbers of perfluorinated compounds to come to a definitive conclusion as to whether the observed effects cited above are characteristic of perfluorinated compounds as a class. However, the above data are sufficient to raise concerns that PFOA, and possibly PFDoDA, are endocrine disruptive and suggest that effects of perfluorinated compounds on the female reproductive tract should be investigated in risk assessment quality studies.

Endpoint: Effects on the Thyroid

Conclusions from Previously Reviewed Studies

Study/Review memorandum	Study design	Effects on thyroid endpoints	Limitations	Utility
Argus Research, 2002; Gu/Rice, 11/17/07; York *et al*., 2010.	APFO; See description in previous section	No effects on thyroid weight parameters or histology.	None	RA
(b) (4)	[redacted]	[redacted]	[redacted]	[redacted]

Newly-identified Studies Considered Useful for Hazard Identification

1) Van Rafelghem, M.J., Inhorn, S.L., and Peterson, R.E. (1987) Effects of perfluorodecanoic acid on thyroid status in rats. *Toxicol. Appl. Pharmacol*., **87**: 430-439.

Methods: The purpose of the study was to assess the effects of PFDA on the rat thyroid. Male Sprague-Dawley rats (300-350 g) were purchased from Harlan Sprague-Dawley and housed individually in suspended stainless-steel cages in a temperature-controlled (21°C) room on a 12-hour light/dark cycle. Rats were quarantined for 7-10 days prior to use, with ground Purina Rat Chow available *ad libitum*. Rats were i.p. injected with 20, 40, or 80 mg/kg PFDA (87.4% purity by gas chromatography) or propylene glycol:water (1:1 v/v) vehicle in a volume of 1 ml/kg bw (n=8-16 rats/group). Each PFDA-treated rat had a pair-fed control partner, and *ad libitum* controls were also included. Bodyweights and feed intakes were

measured daily for 7 days after treatment. On Day 7, 8 rats/group were necropsied. Serum T4 and T3 and unsaturated binding capacities of thyroid-binding proteins in plasma, via assessment of T3 uptake, were determined from trunk blood collected at necropsy (n=8/group). Thyroids were excised, weighed, and fixed in 10% formalin; thyroids from 4-8 rats/group were subjected to microscopic examination. Basal metabolic rates (3-8 rats/group) were measured, and body temperature data were collected on Days 1, 3, 5, and 7 for 4-8 rats/group. Data were analyzed via ANOVA with post-hoc testing; the presence of a dose-response was determined using trend testing.

Results: PFDA caused a dose-dependent decrease in bodyweight and feed intake at ≥ 40 mg/kg compared to *ad libitum* controls, with a small, non-significant decrease in bodyweight compared to pair-fed controls. Significantly reduced thyroid weight was noted at 80 mg/kg PFDA compared to both controls, although the pair-fed control thyroid weight was also decreased compared to the *ad libitum* control. No differences in thyroid weight were noted in the two lower PFDA dose groups. Marked decreases in plasma T4 were noted at all doses of PFDA compared to both control groups; total T3 was unaffected by PFDA treatment. Plasma unsaturated binding capacity was significantly decreased only at 80 mg/kg PFDA with respect to both control groups. Total oxygen consumption was dose-dependently decreased by PFDA treatment compared to *ad libitum*, but not pair-fed, controls, reaching statistical significance at 80 mg/kg. However, when total O_2-consumption was adjusted to body size, no significant differences from control were noted. Resting absolute and relative O_2-consumption were also dose-dependently decreased with PFDA-treatment compared to *ad libitum*, but not pair-fed, controls, reaching statistical significance at 80 mg/kg. For relative resting O_2, a slightly greater decrease was noted in pair-fed controls versus 80 mg/kg PFDA. Core body temperatures were decreased relative to *ad libitum*, but not pair-fed, controls over the 7-day period, with a significant linear dose trend for decreased core temperature noted for PFDA but not pair-fed controls.

Strengths: The strengths of the study included the study design, the large numbers of related and concomitant endpoints, the use of multiple doses of the test article, the use of pair-fed controls, and, in the context of the other studies on this endpoint (as listed in Attachment 1), repeatability.

Weaknesses: The weaknesses of the study included the route of administration, the low n for some measured endpoints, the fact that the study was not a repeated-dose study, the dosing duration, the use of non-traditional endpoints which are difficult to interpret in the evaluation.

Utility of study: This study is useful for **hazard identification** for thyroid weight and serum hormone endpoints, but is limited to range finding for any type of repeated exposure measures. Based on the decreases in plasma T4 noted at all doses, there is no NOAEL for this study; the LOEL is 20 mg/kg.

Supplementary Information

Epidemiological studies of occupationally-exposed populations have reported no association of high serum PFOA levels with serum thyroid hormone levels in healthy subjects (Olsen and Zobel, 2007; Olsen *et al*., 2003); however, a recent study has suggested a link between an increased prevalence of thyroid disease in the general population to increased serum PFOA levels (Metzer *et al*., 2010). Additional *in vitro* studies of the binding of perfluorinated compounds to the human thyroid hormone transport protein transthyretin (TTR), the main transport protein for thyroid hormone in humans, report that PFOA is able to displace T4 from thyroid-binding globulin at high concentrations. This *in vitro* finding is suggestive that perfluorochemcials could potentially alter thyroid hormone half-life in blood and, concomitantly, thyroid hormone homeostasis in humans (Weiss *et al*., 2009). Results from studies conducted in mammals with perfluorinated compounds that met the first tier of review but did not meet the criteria for hazard identification are summarized in Attachment 1.

Section Summary

In this section, Toxicology has evaluated the available evidence concerning the effects of perfluorinated compounds on thyroid endpoints. Findings for specific compounds by endpoint may be summarized as follows:

- Thyroid weights: No effects were noted with PFOA in rats or monkeys or the 8-2 TBA in rats. PFDA decreased thyroid weights after one injection of 80 mg/kg;
- Thyroid hormones: No effects were noted with PFOA in rats. PFOA decreased free T4 in cynamolgous monkeys at all doses and serum T3 at the highest dose. PFDA decreased serum T4 at doses as low as a single injection of 20 mg/kg. Serum hormones weren't assessed for the 8-2 TBA;
- Thyroid histopathology: No treatment-related histopathology was noted in rats with PFOA. With the 8-2 TBA, altered collagen was noted, with a dose-related increase in severity of this finding at the highest dose. Histopathology was not assessed for PFDA;

From these data, Toxicology concludes that the evidence for effects of perfluorinated compounds as a class on thyroid parameters is mixed. PFDA appears to have clear adverse effects on thyroid parameters in rodents. However, the available data concerning the effects of PFOA and other perfluorinated compounds on thyroid function is unclear at this point and requires further study before firm conclusions can be reached. It should also be noted that, with the exception of the above-cited monkey study, all of the available data for this endpoint are from studies conducted in rats, which are known to be peculiarly sensitive to thyroid toxicants and possibly not a relevant model for this endpoint if the mechanism of action of the toxicant is related to increased metabolism and clearance of thyroid hormone in rats (Wu and Farrelly, 2006). Given the fact that the available data on perfluorinated compounds indicate that Phase I enzyme induction in the liver is one of the most sensitive and robust effects of these compounds, it is possible that the observed effects of PFDA and the 8-2 TBA in the rat are related to increased clearance of thyroid hormone via hepatic enzyme induction and thus are rat-specific phenomena.. In contrast, the finding of decreased serum thyroid hormones in monkeys with PFOA may indicate a human-relevant effect of PFOA on the thyroid in humans. However, interpretation of the cynamolgous study is complicated by the issue of excess mortality, indicating that the decreased thyroid hormones may be indicative of overall moribundity instead of an organ-specific toxic effect. Further studies will be needed to fully-assess the toxic effects of perfluorinated compounds on the thyroid.

CONCLUSIONS

Toxicology has evaluated the available data from toxicity studies conducted with ≥ C8 perfluorinated carboxylic acids and telomer alcohols to assess whether pre- and postnatal toxicity are sensitive endpoints of perfluorinated compound toxicity and also to assess whether the male and female reproductive systems and the thyroid are target organs for perfluorinated compounds. Criteria stated in the review established appropriately-designed oral toxicity studies performed in rodents as the most relevant for the assessment of the risk to human health to these compounds. Information derived from studies considered useful for hazard identification provided additional information concerning mode-of-action or contributed to a weight-of-the-evidence conclusion regarding the effect of a compound on that endpoint and future study design. The NOELs and findings at the LOELs for each endpoint and compound tested from the most sensitive studies for each species (as applicable) that were considered to be risk assessment quality are tabulated below:

Compound	**Prenatal toxicity (NOEL/LOEL) mg/kg bw/d**	**Postnatal toxicity (NOEL/LOEL) mg/kg bw/d**	**Male reproductive toxicity (NOEL/LOEL) mg/kg bw/d**	**Female reproductive toxicity (NOEL/LOEL) mg/kg bw/d**	**Thyroid (NOEL/LOEL) mg/kg bw/d**

PFOA	Rat: > 150 mg/kg, no findings	Rat: < 1 mg/kg, F_1 males only, changes in organ wts	Rat: 0.06/0.63: ↑Serum E, Weeks 8 & 14	Rat: < 1.6 mg/kg, Non-dose-responsive ↑incidence & severity ovarian tubular hyperplasia, all doses	Rat: > 14.2 mg/kg, no effects noted
	Mouse: 0.3/0.6: increased %litter loss	Mouse: < 1 mg/kg, males only, accelerated puberty	Monkey: 3/10: ↓Serum E1 & E2, D 94 &183		Monkey: < 3 mg/kg, ↓Serum free T4 all doses
	Rabbit: 50/50: ↑Incidence 13th-rib, not biologically significant)				
PFNA	No data	No data	No RA-quality data	No data	No data
PFDA	No RA-quality data	No data	No RA-quality data	No data	No data
PFDoDA	No data	No data	No RA-quality data	No RA-quality data	No data
8-2 TBA	Rat: 50/200: ↑Incidence delayed skull bone ossification	No data	Rat: > 125 mg/kg bw, no findings	Rat: > 125 mg/kg bw, no findings	Rat: < 5 mg/kg, Non-dose-response ↑incidence altered colloid, all treated males
Mixed TBA	Rat: 50/200: ↓Fetal growth & ↑ incidences of reduced skull ossification	Rat: 25/100: ↓ Numbers of pups born, born alive, & alive on Day 4 of lactation	Rat: > 250 mg/kg bw/d, no findings	Rat: 25/100: ↓Implantation efficiency	No RA-quality data

Additional findings from HI-quality studies that identified target organs and/or effects of interest for future studies are tabulated below for specific compounds and endpoints:

Compound	**Prenatal toxicity: target organ/effect**	**Postnatal toxicity: target organ/effect**	**Male reproductive toxicity: target organ/effect**	**Female reproductive toxicity: target organ/effect**	**Thyroid toxicity: effect**
PFOA	Not applicable	Mice: ↓Mammary gland development	Rats: ↓BW-rel accessory sex unit wts; hepatic aromatase induction; Leydig cell adenomas	Mice: Suppression of lactating phenotype in mammary gland	No data
PFNA	No data	No data	Rats: ↑Serum E, ↓serum T, apoptotic protein induction and pathology in testes	No data	No data
PFDA	Mice: ↓Live fetal BW	No data	Rats: ↓Serum T & DHT, accessory sex organ wts, seminal vesicle epithelial height	No data	Rat: ↓Serum T4, thyroid wts, plasma binding capacity, resting metabolic rate, body temperature
PFDoDA	No data	No data	Rats: ↓Serum T & LH & testes wts; apoptotic changes in testes	Rats: ↓Serum E, steroid biosynthesis hormones in ovary	No data

Based upon consideration of the available data for specific compounds and the totality of the evidence, Toxicology concludes that pre- and postnatal development are endpoints of concern for both the tested compounds above and for ≥ C8 perfluorinated compounds as a class. While the available data on the developmental effects of > C8 perfluorinated compounds is scarce, the known increase in biopersistence,

and hence potency, with perfluorinated chain length supports the generalization of the results from the C8-homologues to the entire class. Moreover, for PFOA specifically, the data suggest that effects on pre- and postnatal toxicity may be in the same range as cancer potency. Moreover, using the Abbott *et al*. study NOEL of 0.3 mg/kg for the severe finding of increased percent litter loss, and applying a safety factor of 1000[10], an estimated daily intake of > 0.3 µg/kg bw/day or a dietary concentration of > 6 ppb would indicate a concern[11]. Noteworthy, the previously estimated unit risk value of 0.02 (mg/kg bw/day)$^{-1}$ results in a virtually safe DC of 1 ppb, suggesting that the effects observed are within the same range of exposures given the data currently available.

Based on the available data as reviewed above, Toxicology also concludes that ≥ C8 perfluorocarboxylates as a class have effects on the male, and possibly the female, reproductive system. As the majority of the studies examining these endpoints in detail were only HI-quality studies, an overall comparison of the sensitivity of the observed effects to other endpoints is not possible at this time. However, it should be noted that adverse effects on reproductive parameters in males were observed after fairly short dosing periods (90-110 days) at doses of less than 1 mg/kg for PFOA and PFDoDA, with the severity of the observed effects increasing with chain length. This indicates that, particularly for >C8 perfluorocarboxylates, the testes and accessory sex organs are fairly sensitive target organs for these compounds. In contrast, the available data for the telomer alcohols does not indicate that these substances have any adverse effects on male reproductive health even at high doses that are associated with significant bodyweight loss and other toxicity, indicating that the male reproductive system toxicity may be specific to perfluorinated carboxylates. In females, both the mixed TBA and PFDoDA had adverse effects on the reproductive system. Accordingly, Toxicology considers the male and female reproductive tract potential target organs for perfluorinated carboxylate and possibly for perfluorinated compounds in general.Although studies indicate that specific perfluorinated compounds may have toxic effects on the thyroid, available data are insufficient to generalize this tentative conclusion to perfluorinated compounds as a whole. In fact, there were very few RA- or HI-quality studies that examined thyroid endpoints in sufficient detail such that confidence in the presence or absence of an effect could be established.

It should also be emphasized that a considerable amount of uncertainty remains with regard to the effects of perfluorinated compounds as a class on the endpoints discussed above. This uncertainty is due to numerous factors, such as: the fact that almost all of the available data are from studies conducted with PFOA; lack of information on the pharmacokinetics of > C8 perfluorocarboxylic acids and the 8-2 TBA in species other than rats; lack of information on the pharmacokinetics of the > C8 telomer alcohols; and the paucity of toxicity data appropriate for use in human health risk assessment for > C8 acids and telomer alcohols, particularly telomer alcohols > C8. Therefore, Toxicology's confidence in the applicability of the conclusions herein to the assessment of perfluorinated compounds as a whole is lower than for the assessment of PFOA alone. Further data from appropriate studies conducted with > C8 acids and telomer alcohols are necessary in order to assess the effects of these compounds on these endpoints with a high degree of reliability.

To that end, Toxicology is changing its testing recommendations for this class of compounds from conduct of a focused, targeted one-year study with appropriate test substances to conduct of a full, Redbook-compliant, one-year study with an *in utero* phase, as this study design will provide the most comprehensive assessment of the endpoints of concern. This study design will assess chronic toxicity and the possibility of delayed toxicity in adulthood derived from developmental exposure, as well as assessing effects on the developing and mature endocrine system. Moreover, as per the discussion above regarding

[10] Total safety factor = 10 for intra-species variability x 10 for inter-species variability x 10 for severity of effect

[11] As detailed in the Table above, there is a lower NOEL of 0.06 mg/kg in the rat for increased E. However, decreased E was observed in the monkey study with a NOEL of 3 mg/kg. As such, the 0.06 mg/kg from the rat was not cited as the most appropriate point of departure for use in the safety assessment.

the appropriate model for use in risk assessment of these compounds, Toxicology recommends that the one-year study with *in utero* phase be conducted in mice, due to pharmacokinetic considerations.

Lastly, based on this assessment of the totality of information and the risk assessment and hazard identification studies discussed above, these compounds appear to be efficacious with regard to the endpoints identified herein at the exposures previously limited to carcinogenicity. However, there are a number of uncertainties that must be considered in this analysis such as the structural differences in various PFCs, the concern that some modes of action may not be relevant to humans, and the limited information in the dataset. Due to the considerable uncertainties remaining regarding the toxic effects of perfluorinated compounds as a class in humans, significant questions remain regarding the safe levels of dietary exposure to ≥ C8 perfluorinated compound such that additional testing is recommended to ensure safety.

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INIT: MLTwaroski: 9/30/10

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Attachment 1: Summarized *in vivo* studies which do not meet HI inclusion criteria

A. Developmental Toxicity

Study	Species/Compound Tested/Doses	Reported Results	Reasons for Exclusion
White *et al*., 2009 ELCF, pups	Mouse/ CD-1/Charles River; PFOA in water, 0 or 5 mg/kg bw/day on GD 8-17, gavage to dams; n=4 litters/dose/time point	↓BW in 5U+L PND 3-10; ↓BW all groups, PNDs 5 & 10; ↑abs. liver wts 5U+L & 5U pups on PNDs 1-10; ↑5L, PNDs 5 &10; ↑serum PFOA; ↓ mammary development, all PFOA-exposed pups, PNDs 1-10.	N; ENV; basis for randomization unstated; #D; CF; DD
Johansson *et al.*, 2008	Ten-day-old male NMRI mice (B&K, Sweden; N=3-4 litters), gavaged once with 0, 0.58, or 8.7 mg/kg bw PFOA (96% purity) or 0.72 mg/kg bw or 10.8 mg/kg bw PFDA (98% purity) in a 20% aqueous emulsion of 10:1 peanut oil:egg lecithin vehicle	↓Spontaneous habituation ratios, 2- and 4-months of age & hypoactive response to nicotine at 8.7 mg/kg PFOA. No effect of PFDA on habituation.	Small N; DD; S; lack of positive controls for the neurobehavioral studies; EP
Johanssen *et al*., 2009	Ten-day-old male NMRI mice (B&K, Sweden; n=7-8 mice/group from N=3-4 litters), gavaged once with 0 or 8.7 mg/kg bw PFOA (96% purity) in 20% aqueous emulsion of 10:1 peanut oil:egg lecithin vehicle	In 11-day old mice, ↑CaMKII, GAP-43, synaptophysin, & tau in hippocampus (58%, 17%, 92%, and 92%), respectively. ↑Synaptophysin & tau in cortex (82% and 142%). No change in cortical CaMKII & GAP-43.	#D; DD; S; N; EP
Henderson *et al*., 2007	GD 5 pregnant CD-1 mice (Charles River), n=4-5 dams/group/time point in gestational portion of study; n=5 dams/cross-foster group in cross-foster postnatal toxicity portion of study, gavaged once with 0 or 30 mg/kg 8-2 TBA (>98% purity) on GD 8	No effects mortality, dam BW, gestation BW gain, clinical signs, litter size, implantations, resorptions, fetal deaths. ↑Percentages of dams with ≥ 1 nonviable neonate & ↑percentages of anencephaly or exencephaly in non-viable neonates. No effects pup BW gain through PND 15.	N; #D; DD; CF; EP
Hinderliter *et al*., 2005	GD 0 pregnant rats (Charles River, n=5 rats/dose/time point) gavaged with 0, 3, 10, or 30 mg/kg PFOA in water from GD 0-LD 21.	No mortality or clinical toxicity. ↓BW gain GD 4-15, overall gestation wt gain, & BW during lactation at 30 mg/kg. No effects on pup survival, BW gain. 2 small litters at 30 mg/kg.	M; N; EP
Hines *et al*., 2009	Study conducted in 2 blocks. Dams gavaged with PFOA at 0 (n=5), 1 (n=8), 3 (n=7), or 5 mg/kg bw/day (n=5) (Block 1) and 0 (n=14), 0.01 (n=14), 0.1 (n=14), 0.3 (n=14), 1 (n=14), & 5 mg/kg bw/day (n=10) (Block 2) for GD 1-17. Pups fostered within each treatment group at	↑ Mortality rates, intact mice after Week 36. No significant differences: survival at specific times in late life, survival across time. ↓BWs at 5 mg/kg, PND 1-18 months of age. ↓BWs at weaning, at 1 mg/kg. ↑BW in 10-19 week-old intact 0.1 & 0.3 mg/kg. DR ↑BW at 20-29 week old intact 0.01-0.3 mg/kg (+11-15% of control), persistent to 40 weeks old at 0.01 & 0.1 mg/kg. ↑BW gain rates, intact 0.01-0.3 mg/kg, Weeks 6-37; ↑Rates BW loss, intact 0.1 & 0.3 mg/kg after Week 37. No effect 18-month BWs.	CF; DD; low N after 36 weeks; relative timing of the measurements of concomitant endpoints; excess mortality after 36 weeks in treated animals; lack of habituation period for dams; unstated basis

	birth, weaned at 3 weeks old, & group-housed (3-5 female mice/cage). Subset ovx at 21-22 days old (control (n=8); 0.01 mg/kg (n=15); 0.1 mg/kg (n=11); 0.3 mg/kg (n=14); 1 mg/kg (n=6); & 5 mg/kg (n=7)). Separate cohort of 8-week-old female mice not exposed *in utero* gavaged with PFOA at 0 (n=8), 1 (n=14), or 5 mg/kg bw/day (n=14) for 17 days.	↑Serum insulin & leptin (intact 0.01& 0.1 mg/kg) at 21-33 weeks; non-significantly ↑, intact 0.3 & 1 mg/kg. Non-significant ↑fat:bodyweight ratio (+12%) & fat:lean ratio (+14%), intact 0.1 mg/kg at 24 weeks. At 18-months, BW-rel abdominal white fat pad wts & interscapular brown fat pad wts ↓ in intact 1 & 5 mg/kg & ↑ in intact 1 & 3 mg/kg. ↓BW-rel spleen wt, 18-month old intact 3 mg/kg. No effect BW-rel liver wts at 18 months. No effects: feed consumption at 17 months, glucose tolerance at 15-16 weeks or 17 months. Serum PFOA < LOD at 18 months. No effects in OVX animals, except ↑BW-rel brown fat pad wts. No effects in adult mice.	of randomization of dams at start of study; lack of sham-operated controls for OVX mice; lack of corroboration of study results from non-cross-foster study; use of two separate blocks of animals for same experiment; unusual dose-response pattern
Staples *et al.*, 1984; Gu/Rice, 11/17/07,	APFO; Sprague-Dawley/CD rats (12-25/group), 0 or 100 mg/kg by gavage.. Treatment GD 6-15. Sacrifice GD 21 (Group A) or PND 23-dams & PND 35-pups (Group B)	No findings	M; #D; N

B. Male Reproductive Toxicity

Study	Species/Compound Tested/Doses	Reported Results	Reasons for Exclusion
Vanden-Heuvel *et al.*, 1992	Harlan Sprague-Dawley rats once i.p. with 9.4 µmol/kg PFOA or PFDA	PFOA & PFDA bound to proteins in the testes	Route; #D; DD; EP
Mehrota *et al.*, 1997	Sprague-Dawley rats, PFOA in the diet (0 or 0.05%; normal or induced hypothyroid, n=5/group) for 10 days.	↓BW (-40%), normal & hypothyroid rats; no effect testes wts. ↓Some enzymes in testes, normal & hypothyroid rats, but ↑levels of others.	N; #D; EP; DD
Mehrota *et al.*,1999	Sprague-Dawley rats, PFOA in the diet (0 or 0.05%; normal or hypophysectomized (HPX), n=5/group) for 10 days.	↓ BW, normal & HPX rats; no effect testes wts, normal rats; ↑BW-rel testes wts, HPX rats. ↓Some enzymes in testes, normal rats. No effect on enzymes in HPX rats.	N; #D; EP; DD
Kennedy, 1985	Crl:CD rats (n=5/group/time point) APFO at 0, 20, 200, or 2000 mg/kg, 10 applications total over 14 days	No alteration in testicular morphology	N; route; EP; availability of information from studies conducted via oral route; DD
Kennedy *et al.*, 1986	Crl: CD rats (n=5/group/time point) APFO dust by inhalation at 0, 1, 8, or 84 mg/m^3, 6 h/day, 5 d/wk for two weeks	No alteration in testicular or epididymal morphology	N; route; EP; availability of information from studies conducted via oral route; DD
(b) (4) [redacted]	[redacted]	[redacted]	[redacted]

C. Female Reproductive Toxicity

Study	Species/Compound Tested/Doses	Reported Results	Reasons for Exclusion
Yang *et al*., 2009	3-week-old C57Bl/6 or Balb/c mice (Charles River Laboratories) gavaged with 0, 1, 5, or 10 mg/kg (n=5/strain/group) once daily, 5 days per week for 4 weeks at 21-50 days of age	↓BW, 10 mg/kg after 19 doses. DR ↑abs & BW-rel liver wts, all doses both strains & hepatocyte hypertrophy. Delayed VO, 1 mg/kg in BALB/c mice & 5 mg/kg in C57Bl/6 mice; VO absent at higher doses, both strains. DR ↓ mammary gland growth, Balb/c, significant at ≥ 5 mg/kg. ↓BRDU-labeling of TEBs & stimulated TDs in BALB/c, ≥ 1 mg/kg. DR ↓uterine wts, all doses & ↓endometrial development, ≥ 5 mg/kg, Balb/c only. ↑Mammary gland & uterus growth, 5 mg/kg in C57Bl/6; ↓development, both organs, 10 mg/kg. No changes, cell proliferative indices.	N; lack of habituation period; DD; unusual DR in effects in C57 Bl/6 mice
Zhao *et al*., 2010	C57Bl/6 WT or PPARα KO mice (from different vendors) 3-week-old intact or 4-week-old ovx mice gavaged, 0 or 5 mg/kg bw (n=5-10/dose) PFOA in water 5 days/week for 4 weeks. 2nd group of intact 3-week-old mice gavaged with PFOA, 4 weeks prior to ovx. One week after ovx, injected with s.c. with vehicle, 1 µg/mouse E, 1 mg/mouse P, or E+P for 5 days	No effects mammary gland parameters, ovx mice. ↑Serum P, estrus or proestrus. ↑Mammary gland development with E, P, or E+P after ovx. ↑Mammary gland stimulation, KO &WT. ↑Steroid hormone synthesis proteins, WT & KO ovary & HSD17beta4 mRNA in WT liver only. ↑Mammary growth factor protein, ER-α, cyclin-D1, & proliferating cell nuclear antigen, WT&KO.	Use of WT and KO mice from different suppliers; S; lack of habituation period; effects on bodyweight not assessed; #D; N; DD; OVX and intact mice were different ages
White *et al*., 2009; ELCF dams	Pregnant mice (CD-1/Charles River), PFOA in water at 0 or 5 mg/kg bw/day on GD 8-17, gavage to dams, sacrifice on PNDs 1, 3, 5, 10 (n=4/group/time point)	↑BW gain gestation 5 mg/kg. Serum PFOA highest at PND 1, ↓ by LD 10. ↑BW-rel liver, 5L & 5U+L, no significant time trend. Diminishment of lactational morphology; all, 5U+L most severe, through LD 10.	N; ENV; basis for randomization unstated; #D; CF; DD
White *et al*., 2007, GD18 pregnant dams	Pregnant mice (CD-1/Charles River), PFOA in water at 0 or 5 mg/kg bw/day (n=5/group) for GD 1-17, sacrifice on GD 18	Delayed differentiation at GD18.	N; ENV; basis of randomization unstated; #D; S; DD
(b) (4) [redacted]	[redacted]	[redacted]	[redacted]

D. Thyroid Toxicity

Study	Species/Compound Tested/Doses	Reported Results	Reasons for Exclusion
Langley and Pilcher, 1985	Wistar male rats i.p. once with PFDA, 0 or 75 mg/kg; assessed for 8 days, (n=5/group/time point)	↓Feed consumption & BW beginning 24 hours after treatment; feed consumption close to nil by Day 8. ↓Body temps by Days 3-8. ↓Resting heart rate (Days 6-8). ↓Serum T4 Days 1-8. ↓Serum T3 Days 0.5-2.	Route; #D; DD; use of dose near the reported LD_{50} for PFDA; EP; S

Gutshall *et al.*, 1988	Wistar male rats pretreated with doses of T4 (Experiment I) or 0.2 mg/kg T4 (Experiment II) 7 days prior to single i.p. injection PFDA at 0 or 75 mg/kg, assessed over 14-day (Exp I) or 30-day (Exp II) period (n=8-10/group/time point, Exp I, 4-8 rats/group, Exp II)	Exp I: ↓BW, T4-treated & PFDA-alone. No effect of T4 on hypophagia. Excess mortality, all PFDA-treated groups. Exp II: ↓PFDA-related hypophagia by 0.2 mg/kg T4; no effect of T4 on PFDA-related BW loss. ↓ Serum T4 by Day 14. ↓Core temperature Days 2-14, both PFDA-treated groups, PFDA alone < PFDA + T4, Days 2 and 6.	Route; #D; DD; dose near the reported LD_{50}; excess mortality; EP; S
Gutshall *et al.*, 1989	Male Sprague-Dawley rats ± 0.2 mg/kg T4 7 days prior to study & for duration of study i.p. injected once with PFDA at 0 or 75 mg/kg (n=6/group/time point)	No effect on radiolabeled I uptake. ↓Serum T4 & T3 response to TRH challenge 24-h after PFDA. ↓Serum T4, T3, & rT3, 12 & 14-h after PFDA. PFDA + T4 had similar effects, except for no effect on rT3 at 12-h. Similar PFDA and T4 binding to serum albumin. ↑Liver enzyme activities 7 &14 days after PFDA & additional ↑ with T4.	Route; #D; DD; dose near the reported LD_{50}; EP; S; no positive control for TRH challenge study.
Kennedy, 1985	As previously described	No alteration in thyroid morphology	As previously described
Kennedy *et al.*, 1986	As previously described	No alteration in thyroid morphology	As previously described
(b) (4) [redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]

Attachment 2: Studies Reviewed in Depth that Meet HI Criteria

Study	Species/ Strain	Compound/Dose/ Route	N	Observations	Limitations	Utility
Lau *et al*., 2006	Mouse/ CD-1/Charles River	PFOA in water, 0, 1, 3, 5, 10, 20, or 40 mg/kg bw/day in water by gavage to dams, GD 1-18	N=9-47 dams, teratology N=7-23 dams, postnatal	↓Dam BWs, 20 & 40 mg/kg bw/day during gestation, ↑FLRs ≥ 5 mg/kg, 100% resorption at 40 mg/kg. ↑Dam abs liver wts at term & serum PFOA, all groups. At 20 mg/kg, ↑prenatal loss & ↓ #s of live fetuses. ↑Incidences enlarged fontenels & reduced ossification, several skeletal components, all dose groups. ↑Incidences minor tail & limb defects & microcardia, ≥10 mg/kg. ↑Time to parturition, 20 mg/kg. ↓Postnatal survival at ≥ 5 mg/kg PFOA. ↓BW gains (25-30%), both sexes until weaning at ≥ 3 mg/kg bw PFOA. Delays in eye opening at ≥ 5 mg/kg, both sexes. Accelerated sexual maturation in males, all dose groups. Slight delay in VO, 20 mg/kg females.	EP; Basis for randomization unstated; ENV; DD	RA
Abbott *et al*., 2007	Pregnant WT or PPARα KO 129S1/SvlmJ mice	0, 0.1, 0.3, 0.6, 1, 3, 5, 10, or 20 mg/kg bw/day PFOA in water by gavage for GD1-17	n=7-22 WT/dose; 4-23 KO/dose	No effect WT or KO: maternal BW, BW gain, #s of pups at birth, #s of implantation sites, pup birth wts. ↑FLR incidences early gestation at ≥ 5 mg/kg, WT & KO. DR ↑ percent litter loss, excluding FLR, in WT, but not KO; statistically-significant at 0.6 mg/kg bw/day PFOA. ↓Survival WT litters at 0.6-1 mg/kg PFOA & at ≥1 mg/kg PFOA of WT & HET pups, mixed litters; postnatal survival of KO pups unaffected. DR trend delayed eye opening, WT, not KO, litters, significant at 1 mg/kg PFOA. ↓BWs, WT pups, 1 mg/kg, PNDs 9, 10, & 12 (males) & PND 7-10 & 22 (females). No effect BW WT & KO, weaning-28 weeks (male) or 52 weeks (female). ↑Serum PFOA, dams & offspring at weaning with dose, KO & WT mice. DR ↑BW-rel liver wts, WT & KO dams, 1 mg/kg & 3 mg/kg. DR ↑BW-rel. liver wts, all PFOA-treated WT pups; only significantly ↑ in KO pups at 3 mg/kg.	EP, DD	RA
Wolf *et al*. 2007 Part 1 (CF)	Mouse/CD-1/Charles River	PFOA in water; 0, 3, 5 mg/kg, GD 1-17, gavage to dams	14-18	Dam: ↑FLR, high-dose group. No effects BW & BW gain, #s of implantations. ↑Abs & BW-rel liver wts, all. ↑DR serum PFOA, all. Pups: ↑Rel liver wt, all; ↓BW ♂3U+L, 5U, & 5U+L, all ♀;↓birth wt, both sexes; ↓survival, 5U+L; delayed eye opening 3U+L, 5U, 5U+L; delayed body hair 5U, 3U+L, 5U+L; ↓BW (PND 1-22) gain 5U, 5U, 5U+L; ↓BW gain 5U, 5U+L up to PND 85 (♀);↑BW >PND 85 3U ♂	ENV; basis randomization unstated; #D; CF;DD; EP	HI
Wolf *et al*. 2007 Part 2 (RE)	Mouse/CD-1/Charles River	PFOA in water; 0, 5 or 20 mg/kg, gavage to dams, GD7-17 (0, 5); GD 10-17, 13-17, (5); GD 15-17 (5, 20)	12-14	Dam: ≈ weight & weight gain, uterine implantation site, % litter loss, ↑Rel liver wt, all except GD17-15 (5); #pups/L/B; Pups: ↓BW at birth GD 7/10-17 (5) & 10-17 (20) ♂; ↓ survival GD15-17 (20), ↑Rel liver wt, eye & hair markers delayed in 7/10-17 (5), ↓ bw (PND 1-22), ↓ bw ♂ post weaning, ↑ bw > PND 161 ♀ GD13-17	ENV; basis randomization unstated; #D; S; DD; EP	HI
White *et al*., 2007 (pups)	Mouse/ CD-1/Charles River	0 or 5 mg/kg bw/day PFOA in water on GD 1-17, 8-17, or 12-17, gavage to dams	N=7-8/time point/dose on PNDs 10 & 20; N=14-18	↓Pup BW, all. Blood & liver PFOA proportional to dose at PND 20, not at PND 10 or PND 1 (liver only). Impaired mammary gland epithelial branching, & longitudinal growth at PND 10 & 20. ↓Developmental scores, independent of BW.	ENV; basis of randomization unstated; #D; S; DD; EP	HI
Boberg *et al*., 2008	Wistar HanTac: WH rats	0 or 20 mg/kg bw/day of PFOA in vehicle by	n=8 dams/group	No significant findings	#D; M; serious error in sacrifice	HI

		gavage from GD 7-19 or 21			day assignment	
White *et al*., 2009, LLCF	Mouse/CD-1/Charles River	0 (n=48), 3 (n=28), or 5 (n=36) mg/kg bw/day PFOA in water on GD 1-17, gavage to dams,	N=9-18/ group/time point	↓BW, all on PND 22, except 3L. Recovery by PND 29 (5U, 3U, 3U+L) or 85 (5U & 5U+L); ↑abs liver wts & serum PFOA, all at weaning; ↓mammary gland development, PNDs 22, 42, & 63, all except 3L at PND 22 & 3U+L at PND 42. ↓ Epithelial densities with peripheral, localized ↑ epithelial density at 18 months, all. ↑ #s darkly-staining foci in ductal tissue. Hyperplasia of the ductal epithelium, ↑stromal epithelial densities, or inappropriate differentiation of ductal tissue.	ENV; basis randomization unstated; #D; CF; DD	HI
White *et al*., 2009, RE, pups	Mouse/CD-1/Charles River	0 or 5 mg/kg PFOA on GD 7-17; GD 10-17; GD 13-17; or GD 15-17, gavage to dams,	n=10-21/ dose/time point	Mammary gland findings same as late-life cross-foster study	ENV; basis of randomization unstated; #D; S; DD; EP	HI
Booksta ff *et al*., 1990	Male adult Sprague-Dawley rats	0, 20, 40, or 80 mg/kg PFDA in propylene glycol vehicle via single i.p. injection. Sacrificed 7 days after dosing	10/group	DR ↓feed consumption & BW at ≥ 40 mg/kg. DR ↓T & DHT at ≥ 40 mg/kg bw. No effect LH or spermatogenesis. DR ↓seminal vesicle & ventral prostate wts at ≥ 40 mg/kg bw. ↓Testes wts at 80 mg/kg bw. Atrophy of seminal vesicle epithelium at ≥ 40 mg/kg bw; 50%↓epithelial height at 80 mg/kg. Castration & T-supplementation abolished effects. ↓hCG-mediated *ex vivo* stimulation of T output from decapsulated testis at ≥ 40 mg/kg bw PFDA.	EP; R; DD;	HI
Cook *et al*., 1992	Twelve-week-old male CD rats	Exp I: 0, 1, 10, 25, or 50 mg/kg bw/day of PFOA in water by gavage for 14 days. Exp II: 0 or 50 mg/kg PFOA for 14 days, followed by hCG s.c., naloxone s.c., or GnRH i.p. one hour prior to sacrifice	Exp I: 15/gp Exp II: 10/gp	DR ↓final BW & BW gain at ≥ 10 mg/kg; ↑rel liver wt at ≥ 10 mg/kg PFOA; ↑rel testes wt at ≥ 25 mg/kg PFOA versus *ad libitum* but not pair-fed control; ↓rel accessory organ unit wt at ≥ 25 mg/kg PFOA. No differences abs testes, prostate, seminal vesicles, or coagulating glands. ↑Hepatic beta-oxidation rates in livers at ≥ 10 mg/kg PFOA. ↑Serum E at ≥ 10 mg/kg. No effects on serum or interstitial fluid T or serum LH; significant downward trend with dose, serum T. No effects on histopathology mentioned. Non-significant ↓serum T & significantly ↓serum androstenedione after hCG-challenge.	DD, EP	HI
Liu *et al*., 1996	12-week old male Crl: CD BR rats	PFOA in water at 0, 0.2, 2, 20, or 40 mg/kg for 14 days. *Ad libitum* and pair-fed controls included.	15/group	↓BW & BW-rel testes wts at ≥ 2 mg/kg. No change abs testes wts or testes aromatase. 2-fold ↑serum E & ↑ hepatic aromatase activity at ≥ 2 mg/kg; linear correlation, serum E & liver aromatase. Aromatase inhibited *in vitro* by APFO.	DD; EP	HI
Shi *et al*.,	Male Sprague-	0, 1, 5, or 10 mg/kg bw/d of	6/group	↓BWs at ≥ 5 mg/kg. ↓Abs testes wt at 10 mg/kg, degressive trend at 1 & 5 mg/kg. ↑Rel testes wts at ≥ 5 mg/kg. ↑Total cholesterol at 10 mg/kg. DR ↓T at ≥ 5 mg/kg.	Lack of pair-fed control;	HI

2007	Dawley rats (BW range: 230-240 g)	PFDoDA in 0.5% Tween-20 by gavage for 14 days		↓LH levels at 10 mg/kg. No effects FSH or E. DR ↑ severity at ≥ 5 mg/kg in apoptotic changes in Leydig & Sertoli cells & in spermatogonia. Severe ↓gene expression in testes for steroid synthesis & metabolism proteins at ≥ 5 mg/kg.	EP, DD	
Shi *et al*., 2009	4-week old Sprague-Dawley rats	0, 0.02, 0.05, 0.2, or 0.5 mg/kg bw/day PFDoDA by gavage for 110 days	6/group	↓BW & histopathological changes in the seminiferous tubules of the testes (appearance of cast-off cells) at 0.5 mg/kg. No changes in reproductive organ wts or total cholesterol. DR ↓serum T at ≥ 0.2 mg/kg; no other changes in serum hormones. DR ↓testes StAR mRNA, all doses & StAR protein levels at ≥0.05 mg/kg. ↓Testicular IGF-1 mRNA ≥0.05 mg/kg; ↓testicular IGF-IR & IL-1α mRNA ≥0.2 mg/kg. ↓P450SCC protein at 0.5 mg/kg, no effect on mRNA. ↓Pituitary FSH & GNRH-R mRNA at 0.5 mg/kg.	Possibly DD, lack of pair-fed control	HI
Feng *et al*., 2009	Seven-week-old Sprague-Dawley rats	PFNA in 0.2% Tween-20 at 0, 1, 3, or 5 mg/kg bw/day by gavage for 2 weeks	6/group	↑Serum E & ↓serum T by 85.4% at 5 mg/kg. No effects FSH or LH. Crescent chromatin condensation & margination & sloughing of germ cells into the lumen of seminiferous tubules, 5 mg/kg only. DR ↑#s of TUNEL-+ cells & caspase-8 protein in testes at ≥ 3 mg/kg, with non-statistically-significant ↑ at 1 mg/kg. ↓Bcl-2 expression in testes at ≥ 3 mg/kg. ↑Testes Bax & Fas at 5 mg/kg. No effect caspase-9 protein in testes.	DD, EP	HI
White *et al*., 2007 (dams)	Mouse/ CD-1/Charles River	0 or 5 mg/kg bw/day PFOA in water gavage on GD 1-17, 8-17, or 12-17	N=7-8/dose/time point, PND 10 & 20	DR ↓Mammary gland differentiation, GD 1-17 & GD 8-17 groups, at peak of lactation, concurrent with ↑serum PFOA, all. Delayed involution at PND 20. ↓Lactoferrin expression, PND 20, all. ↑EGF expression in GD 1-17 dams on PNDs 10 & 20, & in GD 8-17 dams on PND 10.	ENV; basis of randomization unstated; #D; S; DD	HI
Shi *et al*., 2009	24-Day-old female Sprague-Dawley rats (35-40 g)	0, 0.5, 1.5, or 3 mg/kg bw/day PFDoDA in 0.5% Tween-20 gavage for 28 days	8/group	At 3 mg/kg: ↓ BW, ↑cholesterol; ↓serum E; ↓gene expression of steroid biosynthetic hormones; & ↓expression ER-β. ↓ER-α at ≥ 1.5 mg/kg. No changes day of vaginal opening, cyclicity, uterine or ovarian histopathology, or serum LH or FSH.	DD	HI
Van Rafelghem *et al*., 1987	Male Sprague-Dawley rats (300-350 g), Harlan Sprague-Dawley	0, 20, 40, or 80 mg/kg PFDA in propylene glycol:water (1:1 v/v) i.p.	8-16 rats/group	DR ↓BW & feed intake at ≥ 40 mg/kg vs. *ad libitum* controls, non-significant ↓BW vs. pair-fed controls. ↓Thyroid wt at 80 mg/kg PFDA vs. both controls. No change thyroid wt at lower doses. Marked ↓plasma T4, all vs. both controls; total T3 unaffected. ↓Plasma unsaturated binding capacity at 80 mg/kg vs. both controls. DR ↓total O_2 consumption vs. *ad libitum*, but not pair-fed controls, significant at 80 mg/kg. No effect BW-adjusted total O_2-consumption. DR ↓resting abs and BW-rel O_2 consumption versus *ad libitum*, not pair-fed controls, significant at 80 mg/kg. For BW-rel resting O_2, a slightly greater ↓ vs. *ad libitum* controls in pair-fed controls vs. 80 mg/kg PFDA. ↓Core temperatures vs. *ad libitum*, but not pair-fed, controls, significant trend.	R; DD	HI