Comments on ATSDR's *Toxicological Profile for Perfluoroalkyls*

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Introduction and Overview

The ATSDR *Toxicological Profile for Perfluoroalkyls* (Draft for Public Comment, June 2018) offers provisional minimal risk levels (MRLs) for four perfluoroalkyl substances (PFAS): perfluorooactanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorononanoic acid (PFNA), and perfluorohexane sulfonic acid (PFHxS).

These MRLs are, in general, more restrictive than analogous reference values set by U.S. EPA or other agencies. For example:

- U.S. EPA's proposed reference dose for PFOA is 2×10^{-5} mg/kg/day;
- Health Canada's tolerable daily intake for PFOA is similar (at 2.5×10^{-5} mg/kg/day); and
- ATSDR's provisional MRL for PFOA is an order of magnitude more restrictive, at 3×10^{-6} mg/kg/day.

Unfortunately, ATSDR's provisional MRLs are no more justifiable than previously proposed guideline-values, and cannot be said to be reliable. Among other issues, the provisional MRLs for these four PFAS:

- Are not based on evidence of adverse effects in humans;
- Are sometimes based on questionable "principal studies";
- Do not reflect known or reasonably anticipated differences in sensitivities between and among laboratory rats, laboratory mice, and humans; and
- Fail to account for many recent, relevant studies.

With regard to the first point, it remains the case that epidemiologic and/or clinical evidence has so far failed to demonstrate that any PFAS harms human health. Notably, cancer patients in a phase 1 trial have been dosed with massive amounts of PFOA (up to 1.2 grams per patient per week), as an experimental chemotherapeutic drug, with no apparent harm to their livers

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(the organ most clearly and adversely affected by PFOA in laboratory rodents) or other organs (Convertino et al., 2018).[1]

Of course, high-level exposures to various PFAS, including PFOA, clearly *do* harm the health of laboratory animals, and it is entirely appropriate to base health-protective guidelines on exposure-response data derived from laboratory animal studies.

However, doing so requires considerable toxicological judgment — both in choosing which "principal studies" and dose-response data-sets to use, and deciding how to use them. The principal studies must be well-designed and executed, and the results should have been replicated. As explained below, for some of its four provisional MRLs, ATSDR's choice of principal studies is questionable; while for others, the data-sets are reliable enough, but ATSDR's use of them appears to be unjustified.

This is especially unfortunate because the text of the *Profile* itself is often quite informative and insightful. However, none of this insight is carried over into the derivation of the MRLs. Indeed, the latter — which are derived in Appendix A — are essentially uninformed by the almost 700 pages of text that precede them. This perplexing disconnect should not carry through to the final version of the *Profile* and its MRLs.

In this draft version, Appendix A assumes, by default, and without justification, a combined "uncertainty factor" of 300 (in three cases, a factor 10 of this is termed a "modifying factor") for each of its four provisional MRLs. So doing, Appendix A fails to conform with modern, human health risk assessment practice that, among other things, encourages the application (or at least consideration) of "chemical-specific adjustment factors" to provide more biologically-based, predictive, and still protective guidance values (see, for example, Meek et al., 1999, 2002 & 2011; Edler et al., 2002; WHO/IPCS, 2005; US EPA, 2014; Bhat et al., 2017).

For example, for each of its four provisional MRLs, the Agency simply applies a default factor of 10 to account for "human variability," but fails to justify this value. Of course, humans do vary

[1] As is typical for cancer chemotherapeutic drugs, these large doses did cause fatigue, nausea, vomiting, and diarrhea, which were considered tolerable by the patients. The draft *Profile* does not cite this paper, but should. The Convertino et al. (2018) paper was available five months prior to the release of the *Profile*; and ATSDR was aware of this clinical trial of PFOA, since the *Profile* cites a 2011 poster session abstract that describes it (MacPherson et al., 2011), and the poster *per se* is included in comments to U.S. EPA (Dupont, 2014). This information is especially important for the exposure assessment sections of the *Profile*, which at present indicate that it is manufacturing workers, and perhaps people drinking highly contaminated water, who are the groups receiving the largest doses of PFAS. For PFOA, at least, that is not the case. PFOS also has anti-tumor activity (Wimsatt et al., 2016), although we know of no clinical trials using PFOS.

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with regard to their sensitivities to the adverse effects of chemicals; but whether a factor of 10 is appropriate for accounting for populational variability *depends on the chemical and end-point at issue*.

For these four chemicals, interindividual differences in metabolic rate need not be accounted for, since these PFAS are not metabolized by either laboratory animals or humans.

Also, three of the four provisional MRLs are based on developmental effects associated with PFAS exposures of fetuses *in utero* and/or of neonates through lactational exposures. Of course these life stages are uniquely sensitive to the effects of developmental toxicants. There is no need to account for the possibility of some greater sensitivity of older children or of the elderly, for example, since for all other such subpopulations, development has already occurred.

In what senses, then, is a factor of 10 for "human variability" the "correct" value for these four PFAS MRLs? The Agency does not say, but it should. In several cases, the variability due to variation in elimination rates is known, and substantially less than a factor of 10; what would remain is only variability due to potentially differing sensitivities within the chosen, already most sensitive sub-population.

Also important, but also ignored in the derivation of the MRLs, are the *qualities* of the principal studies upon which the provisional MRLs are based.

For example, despite the availability of multiple high-quality studies on PFOA (most of which are cited in the *Profile* text), Appendix A relies for its MRL-derivation on results in rodents from a single poor-quality study[2] that fails to conform with internationally accepted study-guidelines, uses too few rodents, tests these rodents at only one dose-level, relies on unverified test-methods, has not been replicated (indeed, has been contradicted by more recent data), is strictly uncontrolled, uses the wrong basic measurement unit, and is otherwise entirely unsuitable. As detailed below, the "final" MRL for PFOA should be based on far more reliable data from guideline-based studies.

The *Profile* is based on literature searches that cover the period up until May 2016, so is now more than two years out-of-date. Had ATSDR searched for more recent literature (even for just papers that cited the principal studies that the Agency has selected), it would have found that the results of the principal study selected for the PFOA MRL, for example, *were not replicated* using standard test-methods.

[2] Two papers are cited as the principal studies (Onishchenko et al., 2001, and Koskela et al., 2016), but the laboratory mice reported on in the latter publication are simply a subset of the mice reported on in the earlier publication.

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In Appendix A, the Agency presents various approaches taken to estimate "human equivalent doses" (HEDs). Oddly, these approaches differ for the different PFAS, and they have been applied in a mutually exclusive fashion. As a result, and without justification, the Agency has ignored various high-quality studies, and relied instead on lower quality studies.

In particular, for PFOA and PFOS, the Agency relies on the use of the Wambaugh et al. (2013) modeled parameters to estimate the average serum concentration in experimental animals, thus ignoring any studies that did not use female CD-1 mice, female C57Bl6 mice, or male Sprague-Dawley rats (Table A-7). The Agency similarly ignores, for PFOS, any studies that did not use female CD-1 mice, female Sprague-Dawley rats, or Cynomolgus monkeys (Table A-15).

This unjustified approach was followed *even in cases where the serum concentrations were measured* in the cited studies, or in other studies using the same animals (but not analyzed in Wambaugh et al., 2013). However, with known dosing schedules, a good approximation to the volume of distribution, and even a single measurement at a known time point, the average serum concentration in experimental animals of PFOA and PFOS can be estimated; and in several of the studies reporting serum concentration measurements, additional information is provided that allows better estimates. The accuracy of this estimation is probably as good, for any single experiment, as the estimate obtained using the Wambaugh et al. (2013) modeled parameters. And, indeed, this approach using measured concentrations is taken for PFHxS and PFNA.

There is no reason to not use the same approach for PFOA and PFOS, relying, if necessary, on estimated parameters from other experiments on the mouse and rat strains not analyzed by Wambaugh et al. (2013). Indeed, if concentration measurements are available for the animals used in any study, then estimates using this approach should be compared with those obtained from the Wambaugh et al. (2013) modeling approach, and any discrepancies described and resolved. In particular, in Table A-7 for PFOA:

- Loveless et al., 2008 — serum PFOA concentrations were not measured in this experiment (which dosed for 29 total days), but a previous, cited experiment (Loveless et al., 2006) using the same animals at the same daily doses included measurements of serum concentration at 14 days. The previous measurements are quite sufficient to estimate average serum concentrations in Loveless et al., 2008.
- Abbot et al., 2007 — measured and reported serum PFOA concentrations.
- Cheng et al., 2013 — no suitable measurements in this report
- Albrecht et al., 2013 — measured and reported serum PFOA concentrations.

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And in Table A-15, for PFOS:

- Long et al, 2013 — no suitable measurements in this report
- Peden-Adams et al, 2008 — measured and reported serum PFOS concentrations.
- Guruge et al., 2009 — measured and reported plasma PFOS concentrations.
- Dong et al., 2009 — measured and reported serum PFOS concentrations.
- Dong et al., 2011 — measured and reported serum PFOS concentrations.
- Onishchenko et al, 2011 — no suitable measurements in this report.
- Wang et al., 2015c — measured and reported serum PFOS concentrations.
- Yahia et al, 2008 — no suitable measurements in this report.

Of course, the lack of measurements *within any particular report* should not end the quest for estimated serum concentrations; it is necessary to search related literature (particularly that published by, or cited by, the same authors) for experiments with serum concentration measurements in the same animals under similar experimental conditions. Why did the Agency fail to perform such a search?

In what follows, we present additional, hopefully constructive criticisms of the four provisional MRLs. We would note that assessing risks to human health for these compounds is not straightforward, and there is no one best approach. Nonetheless, we hope to explain how current evidence can be better used, and how future research may address uncertainties as to whether and how PFAS affect public health.

PFOA

The provisional, intermediate-duration MRL for PFOA is based on work in mice published by Onishchenko et al. (2011) and Koskela et al. (2016). The latter study relied on mice used in the former study, evaluated at a later age and for a different end point.

For at least the reasons detailed below, these "principal studies" fail to provide a sound basis for the derivation of an MRL. These investigations are nominally studies of developmental neurotoxicity (from prenatal exposures to PFOA or PFOS), but their methods are poor, and their results are unreliable. The Agency should choose different studies as the basis for its "final" MRL.

Groups of toxicologists, in regulatory agencies and elsewhere, have worked for decades to standardize the design of laboratory rodent studies (whether of potential drugs or other chemicals) for purposes of human health risk assessment. To investigate developmental

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neurotoxicity, the relevant guideline is *OECD Test Guideline (TG) 426* (OECD, 2007, based on U.S. EPA, 1998). As explained by Beronius et al. (2013):

> Both the US EPA and the OECD guidelines for [developmental neurotoxicity] DNT testing are structured to include investigations of developmental landmarks and behavioral ontogeny, motor activity, motor and sensory function, learning and memory, and neuropathology. For some of these categories several different validated test methods are available and the guidelines are largely flexible regarding which test method to include in the study design.

Unfortunately, the studies chosen by ATSDR for the PFOA provisional MRL fail to conform to the essential elements of the *Guideline*. For example:

- The *Guideline* calls for the use of rats as the study subjects, but Onishchenko et al. (2011) conducted their studies in mice.
- The *Guideline* calls for the use of at least three dose-groups, but Onishchenko et al. (2011) reported on only one dose-group.
- The *Guideline* calls for evaluation of 20 litters per dose-level, but Onishchenko et al. (2011) used only 6 pregnant dams in their exposed group (and 10 dams in their control group).
- The *Guideline* calls for the reporting of clinical observations of the test rodents, but Onishchenko et al. (2011) provide no such reporting.

Next, the Agency is not entirely accurate in its summarizing of the principal studies. For example, reviewing the results of Onishchenko et al. (2011), the Agency writes (page A-23), "Prenatal PFOA exposure was associated with increases in global activity and exploratory activity in adult offspring . . . ", but this is inaccurate. What was reported by the investigators was an increase in activity by male mice both during the first hour (habituation) and subsequently, but a *decrease* by female mice during the first hour of habituation, *with no change thereafter*.

Moreover, it is not known whether these observations, such as they are, in fact represent effects caused by to PFOA. This is because:

- The PFOA-exposed male mice were not matched with their controls. The 6 PFOA-exposed males were housed 3 and 3 in two cages, but the 8 control males were housed as 4 and 4 (these distributions are not explicitly stated, but are the only possibilities given the described experimental design). Activity levels in social groups might well depend on crowding levels.

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- The authors made no correction for their multiple comparisons. A quite large number of comparisons were made (at least 34 initial comparisons[3] can be seen in the reported results) with regard to behavioral-endpoints. Given this large number, the 5 "significant results" (at "$p<0.05$") that Onishchenko et al. (2011) report as being associated with PFOA might well have arisen due to chance alone, and not to any PFOA-induced effect. It is not possible to fully evaluate this problem, since the exact set of tests performed is not described.
- Some of the analysis was clearly performed post-hoc: the authors write, "… signs of altered locomotor activity in the exposed groups prompted us to extend the analysis of behavioral data …", which further compromises any statistics-based conclusions.
- There was no accounting for litter or individual animal effects in the analysis, and, as noted above, too few litters (6 for the experimental group, 10 for the control group; apparently there was no matching on litters) were used in any case to reach valid conclusions. The 5 "significant results" among males are clearly obtained from analyses of the wrong measure. All 5 of them could be due to excess activity by one mouse, for example, which would not correspond to a statistically significant effect. The analytical units here should clearly be mouse and litter, potentially taking into account interaction effects within each cage (since an over-active mouse might induce activity in other mice).
- In a more recent paper, several of the Onishchenko et al. authors (Spulber et al., 2014) state "… we re-analyzed the data we reported earlier [19], focusing on the novelty-induced hyperactivity in mice (Fig. 2 D), and found that mice exposed to 0.3 mg/kg/day PFOS display both less locomotor activity, and faster habituation (larger negative IOC value) as compared to controls and mice exposed to 3 mg/kg/day PFOS (Fig. 2 E)." Reference 19 is to the principal study, Onishchenko et al. (2011). Since Onishchenko et al. (2011) report only on exposure at 0.3 mg/kg/day PFOS, omitting the results subsequently documented at 3 mg/kg/day, this 2014 paper raises the possibility that higher dose(s) of PFOA might have also been tested, with the results similarly omitted from the 2011 report. Importantly, the higher dose (3 mg/kg/day) of PFOS resulted *in no effect* compared with control for at least one of the results reported as positive at 0.3 mg/kg/day, suggesting that the authors of Onishchenko et al. (2011) underestimated the variability in their experiments and/or applied incorrect statistical treatments.
- The apparatus used by Onishchenko et al. (2011) — cited as Trafficage, NewBehavior, Zurich, Switzerland; http://www.newbehavior.com/products/trafficage — is unusual, indeed almost unique (used apparently by only one group), for such studies, and it has not been calibrated against standard measures. A subsequent version of the apparatus (cited as TraffiCage, TSE-Systems, Germany; https://www.tse-systems.com/product-details/trafficage) has distinct differences (6 coils in place of 5) and requires special

[3] Counting males and females together, at least the following: 2 for the locomotor tests, 6 for the novelty comparisons in Figure 2, 10 in Table 2, 12 in Table 3, 2 in Figure 4, 2 in Figure 5.

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computer code to "correct" the measurements (*e.g.* Dudek et al., 2015). No such "corrections" were applied in the cited experiment, or at least none were mentioned.

- There is no reference to a tested protocol that would eliminate potential biases (*e.g.* even if the control and test animals were housed in the same room, they might be differentially sensitive to external influences such as vibrations, even if housed on the same bench, due to resonance locations in the building or support structures) and potential corrections needed (*e.g.* the shielding effect of tissue on the transponders might affect the recorded location of the animal vary with the orientation of the animal within the cage). The need for extensive protocol testing is apparent in the results of tests carried out on similar apparatus for rats (Redfern et al., 2017).
- The manufacturers of both the originally cited and the subsequent version of the TraffiCage apparatus failed to respond to emails from us requesting technical details of their apparatuses, and no such details are provided on their web sites (the first now re-directs to TSE-Systems), so it is not possible to even theoretically evaluate the minimal experimental details provided. The "References" on the TSE-Systems site is simply a Google Scholar search. Certain technical details (the time resolution) of the apparatus used by Onishchenko et al. (2011) are given different values[4] in subsequent publications (Spulber et al. 2014, 2015).

Further doubt on the validity of any causal connection between exposure to PFOA and the effects claimed in Onishchenko et al. (2011) is raised by (i) the complete lack of agreement between effects claimed in male and female mice in the results obtained, and (ii) the subsequent failure to replicate the results (Goulding et al., 2017).

Moreover, the experiments of Abbott et al. (2007) on PPARα-null mice, and of Albrecht et al. (2013) on PPARα-null and PPARα-humanized mice, showed that developmental toxicity in the mouse is dependent on the expression of mouse PPARα and not human PPARα. Thus, even were the effects reported by Onishchenko et al. (2011) in the mouse actually caused by PFOA, *human fetuses and neonates would be expected to be relatively resistant* to such effects.

The second paper (Koskela et al., 2016) selected as the basis for the provisional MRL examined an outcome in the female mice used in Onishchenko et al. (2011) when they had grown to 13 or 17 months old. This experiment was again uncontrolled, in that the dosed and control groups were of different weights, and the outcome measures were such that, as stated by the authors, "[t]he mild effects seen here are probably explained to some degree by increased body weight and thus increased load on the long bones …" although of course without the necessary

[4] 50 ms and 20 ms respectively. However, while the time-stamp provided by the apparatus might provide such resolution, the actual measurements using the RFID transponder types described take somewhat longer, and a full measurement cycle over the 5 coils would take longer still (typically about 60 ms and 500 ms respectively, based on Redfern et al., 2017).

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controls it is impossible to rule out (or rule in) some effect of PFOA exposure. Once again, the observations of Abbott et al. (2007) and Albrecht et al. (2013) on the PPARα-dependence of developmental effects in mice is relevant (Table 2-3 classifies the outcome claimed by Koskela et al., 2016, as developmental).

In neither experiment were the reported outcomes determined, *by ATSDR itself*, to be serious effects. Table 2-3 of the *Profile* classifies the claimed "Increased locomotor activity in adult offspring" listed for Onishchenko et al. (2011) as a "Less serious" effect. Table 2-3 fails to note that this effect was only seen in male offspring, and the opposite effect was transiently seen in female offspring, and only during novelty induced activity. The bone differences seen by Koskela et al. (2016) were also classified in Table 2-3 as "Less serious" effects. Why does Appendix A fail to mention these caveats? Surely MRLs should be based on effects deemed to be seriously adverse: if not, why bother making this distinction throughout the text?

PFOS

The Agency chooses to base its provisional MRL for PFOS on "[D]elayed eye opening and decreased pup body weight," as reported in a two-generation rat study by Luebker and colleagues, 2005 (page A-36). This is a questionable choice, given what the study authors themselves write about these two "effects". In particular, Luebker et al. (2005) note (*emphases added*):

> The *slight* delay in eye opening (0.6 days compared to control) in the 0.4 mg/(kg day) dose group *was not considered an adverse outcome*. . . .
>
> *Only transient* reductions in body weights occurred during mid-lactation in the F_2 generation pups at the 0.4 mg/(kg day) dose level. *This observation was not considered toxicologically significant* because the small reductions in pup body weights were associated with minimally larger live litter sizes at birth and on LD 4 pre-culling, as compared with the control group, and body weights in this dose group were comparable to controls at the end of lactation.

Nowhere in its discussion of this "principal study" (pages A-41-A-42) does ATSDR mention these important caveats. Why? If ATSDR maintains this study as the basis for the PFOS MRL, then it should provide experimental evidence (a) that a 0.6 day delay in eye opening is not within normal variability for this strain of rats, and (b) that the larger litter size cannot explain the reductions in body weight.

Not only does ATSDR fail to explain why it disagrees with the study authors concerning the lack of toxicological significance of the relied-upon PFOS-associated effects: the Agency also fails to explain what it believes these effects mean for the development of human infants. Indeed, while eye opening is one developmental milestone in rodents, there is of course no direct

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analogue for humans. Further, there are many other developmental milestones, and/or indications of developmental toxicity, typically measured in two-generation rodent studies; and these are not, apparently, affected by PFOS at the effect-level chosen by ATSDR from Luebker et al (2005). Such typical rodent developmental effects include: olfactory discrimination, swimming performance, nocioception (measured by the tail flick test), sensorimotor gating-prepulse inhibition, exploratory behavior, and social (play) behavior (see, for example, Schneider & Przewlocki, 2005). Is it significant that PFOS, at the point of departure, is not known to affect any of these developmental and/or neurobehavioral endpoints? ATSDR does not say.

Separately, and also perplexingly, ATSDR derives its MRL (based, nominally, on Luebker et al., 2005) by applying a "modifying factor" of 10 to account (page A-42) "for concern that immunotoxicity may be a more sensitive endpoint of PFOS toxicity than developmental toxicity. This seems poorly justified, at best. The Luebker et al. (2005) is not a study of immunotoxicity, and no "modification" of dose-response data from it can be used to predict immunotoxicity even in rats, let alone in humans.

If the Agency believes that PFOS is immunotoxic at or near environmentally-relevant exposures, then it should rely directly on other studies that actually address immunotoxicity. But if the Agency believes instead that such studies are no more than suggestive, then it should discount them.

At the least, the effect-levels in such immunotoxicity studies should be compared with those in the principal, currently selected study: if the effect-levels in the immunotoxicity studies are comparable or larger than those in the principal study, then clearly no further "modifying factor" is necessary; while if the effect-levels are smaller, then any "modifying factor" need not exceed the ratio of the effect-levels (and in this case the immunotoxicity study would effectively become the principal study).

If ATSDR is concerned that PFOS might be immunotoxic at environmentally-relevant exposures, then it should propose specific additional research aimed to uncover such an adverse effect, which, if found, could provide a reliable, relevant data-set for purposes of human health risk assessment.

As it stands, though, the Agency bases its provisional MRL for PFOS on "critical effects" (page A-36) in neonatal rats that the study-investigators themselves deem to be "slight", "transient", and not "toxicologically significant;" and then compounds its questionable choice by dividing by an arbitrary factor of 10 that appears to be more "precautionary" than it is scientific.

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PFNA

The Agency bases its provisional MRL for PFNA on "[D]ecreased body weight and developmental delays" as reported in a two-generation mouse study by Das et al., (2015; page A-57). This study utilized four dose-rates (1, 3, 5, and 10 mg PFNA/kg-day), and 8 to 10 dams per dose-group. The authors report, "Mouse pups were born alive and postnatal survival in the 1 and 3 mg/kg PFNA groups was not different from that in controls." Offspring that had been exposed at 3 mg/kg (but not at 1 mg/kg) gained weight at reduced rates (starting at postnatal day 7); and both eye-opening and vaginal opening separation were delayed in offspring at 3 mg/kg (but not at 1 mg/kg).

With regard to mechanism of action, Das et al. (2015) note the "robust activation of peroxisome proliferator-activated receptor-alpha (PPARα) target genes by PFNA that resembled the responses of PFOA." And as expected and reported by Wolf et al. (2010), PFNA failed to induce these adverse effects in mice that had been genetically engineered to lack this important receptor.

Accordingly, as noted above, human fetuses and neonates would be expected to be *considerably less*, not more, sensitive to these PFNA-induced, PPARα-mediated effects.[5] But again, the Agency derives its MRL by assuming that human offspring could be up to 30 times more sensitive than the "average" mouse. The Agency again fails to even discuss the genuine uncertainties in its 30-fold "uncertainty factor," let alone to justify its choice of this precise and,

[5] PPARs are present throughout the plant and animal kingdoms: many forms of these receptors have so far been identified (see, for example, Tyagi, 2011, for an authoritative review). The specific molecular forms and structures of these receptors differ among rats, mice, monkeys, and humans; and some of these differences profoundly affect how PFAS and other PPAR-agonists affect rodents, for example, as opposed to humans. Tyagi, 2011 note (emphases added):

> PPARs were identified in rodents in 1990 and these belong to a nuclear hormone receptor superfamily containing 48 members. *But, these agents are associated with no proliferation in the human beings*. Structurally, PPARs are similar to steroid or thyroid hormone receptor and are *stimulated in response to small lipophilic ligands*. *In rodents*, a large class of structurally related chemicals including herbicides, industrial solvents, and hypolipidemic drugs lead to significant increase in the number and size of peroxisomes in the liver and may cause liver hypertrophy, liver hyperplasia, hepatocarcinogenesis, and transcription of genes encoding proximal enzymes. PPARs mainly exist in three subtypes; α, β/δ, and γ, each of which mediates the physiological actions of a large variety of FAs and FA-derived molecules. Activated PPARs are also capable of transcriptional repression through DNA-independent protein-protein interactions with other transcription factors such as NFκB signal activators and transducers of transcription STAT-1 and AP-1 signaling.

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based on the evidence, overly large value. This failure should not carry through to the final version of the *Profile*.

Some 10 epidemiological studies have addressed the hypothesis that PFNA affects birth outcomes: results from these studies have generally failed to support this hypothesis.

In particular, PFNA exposure-levels have been found to not correlate with birth weights (Monroy et al., 2008; Chen et al., 2012; Arbuckle et al., 2013; Robledo et al., 2015; Bach et al., 2016; Lee et al., 2016; Lenters et al., 2016; Shi et al. 2017) or with other developmental indices, such as birth length or ponderal index (Bach et al., 2016; Shi et al., 2017). Wang et al. (2016) reported that PFNA and four other PFAS all correlated inversely with the birth weight of girls, but not of boys.

The text of the *Profile* (page 377) does note:

> No consistent associations for alterations in birth weight were found for . . . PFNA . . . Overall, no associations were found between serum PFOA, PFOS, PFHxS, PFNA, or PFUA and increases in the risk of low birth weight or small for gestational age infants. No consistent results for risks of birth defects have been found . . .The available epidemiology data do not suggest associations between perfluoroalkyls and IQ or scholastic achievement for PFOA, PFOS, PFHxS, PFNA, PFDeA, PFUA, or PFDoA. Similarly, no associations were found between PFOA, PFOS, PFHxS, PFNA, or PFDeA and increased risk of ADHD; several studies have found decreased risk of ADHD.

Yet Appendix A, in deriving an MRL for PFNA based on the developmental endpoints in mice noted above, fails to note a lack of support from the rather abundant epidemiologic database. This seems an important omission, and should be rectified in the final version of the *Profile*.

PFHxS

The Agency bases its provisional MRL for PFHxS on "[T]hyroid follicular cell damage" supposedly reported in rats, citing Butenhoff et al. (2009) and Hoberman and York (2003). The 2003 report is unpublished, and although cited several times in the text of the *Profile*, not discussed. It apparently forms the basis of the published, 2009 paper. The agency should provide a reference to this unpublished paper that allows an interested person to locate it: a suitable form would be something such as, "Available in EPA Administrative Record AR-226, copies of which may be requested on CD-ROM from the EPA Docket Office by calling 202-566-0280 or sending an email request to: oppt.ncic@epa.gov."

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For at least two reasons, ATSDR's choice is questionable.

First, there is no evidence that the rats' thyroid follicular cells were "damaged" by PFHxS. Instead, as Butenhoff et al. (2009) note, high doses of PFHxS did, as expected, affect exposed rats' livers – effects that the Agency itself clearly rejects as irrelevant for purposes of human health risk assessment (see page A-49). The effects seen in the thyroid glands of the male (and not female) rats were (i) only indirect, being secondary to induction of the rats' livers' microsomal enzymes and, in any event, (ii) not "damage". The Butenhoff et al. (2009) study makes this point clear: but Appendix A obscures it.

Second, follow up studies in mice (Chang et al., 2018, not cited by ATSDR) found no such effects in the thyroid of either male or female rodents, neither in adults nor in the offspring. The Chang et al. (2018) study examined reproductive and developmental toxicity in CD-1 mice, with additional mice added for toxicokinetic studies. The authors report (emphasis added):

> In the current study of PFHxS, there was no effect on TSH in the adult F_0 mice or in the F_1 pups when serum TSH was measured at multiple times during their development; and, most importantly, there were no effect on thyroid histopathology. Therefore, there is *no evidence to suggest that perfluoroalkyl sulfonates such as PFHxS and PFOS impact thyroid homeostasis*.

This paper is not cited in the *Profile*, but should be.

In estimating an HED from the study of Butenhoff et al. (2009), the Agency used the half-life derived by Olsen et al. (2007) in 26 retired occupationally exposed workers, only two of whom were women (both likely past menopause). The Profile should note that Li et al. (2017a,b) have derived half-lives for PFOS, PFHxS, and PFOA in 106 members of the general population, with separate estimates for men and women ages 15–50. For PFHxS (and for PFOS), the half-life for younger women was significantly less than for men, with menstrual blood loss potentially accounting for some of that difference. If the final MRL were to be based on reproductive effects, then the extrapolation to humans should be based on this smaller half-life, since women of child-bearing age would be the susceptible population. Of course, HEDs for other end-points in the rodents should be compared, and use of a longer half-life might be appropriate for estimating HEDs for such other end-points.

We note that Ramhøj et al. (2018) examined the effect of PFHxS and a mixture of PFHxS and twelve endocrine disrupting chemicals on reproductive toxicity in Wistar rats, but the F_0 generation was limited to the dams. Evaluation of these studies should be added to the *Profile*.

Overall, though, there are so far rather few published toxicologic studies on PFHxS. Perhaps deriving *any* MRL for this specific PFAS is premature? Alternatively, perhaps additional,

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unpublished studies could be located (and made publicly available): if relevant and reliable, could they be used to derive a more reliable MRL?

Additional observations

This set of compounds is typically referred to as "perfluorinated alkyl substances," and so abbreviated as PFAS. The Agency chooses instead to refer to them as "perfluorinated alkyls," which is both nonstandard and ungrammatical, the correct term in chemistry being, for example, "alkyl group". Why did ATSDR make this odd choice? We recommend against it.

We noted two typos in Appendix A in connection with PFHxS. At page A-9, the paragraph beginning "PFHxS," at line 3, the estimated half-life given by Olsen et al. (2007) was 3,109 days, not 3,102; and at line 5, the highest final concentration was 791 ng/mL, not 1,740 ng/mL (which was the highest final concentration of PFOS, not PFHxS).

Concluding remarks

Through no fault of the Agency's, ATSDR's *Toxicological Profiles*, and especially its MRLs, often stir controversy. The current *Profile* and set of MRLs are no exception. Even mainstream science news reports contained headlines and stories such as (*E&E News*, Jun. 20, 2018):

> ***After controversy, U.S. releases report showing elevated health risks from nonstick chemicals***
>
> President Donald Trump's administration has released a politically charged toxicology report about nonstick chemicals showing they can endanger human health at significantly lower levels than the Environmental Protection Agency (EPA) has previously called safe. . . .

Of course, the statements are inaccurate, in several respects, but perhaps that is to be expected. Press releases from various activists' groups were more alarmist still.

Because of their importance, the Agency's MRLs, even just the "provisional" MRLs, must be strongly evidence-based. Moreover, the ATSDR must take special care to succinctly and transparently convey the many uncertainties that surround its MRLs: U.S. EPA does this with regard to its reference dose-values; but ATSDR's standard explanations of its MRLs fall short.

For example, ATSDR must make plain, to the public, which of its MRLs are based directly on evidence from human studies, and which (all four in this case) are instead extrapolated solely from evidence in laboratory rats and/or mice. Perhaps a simple designation could be devised to mark the MRLs: such as, "Acute MRL; based on human studies"; or "Chronic MRL; based on studies in rats."

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As detailed herein, the Agency's provisional MRLs for all four PFAS should be revised. In some cases, as noted above, the Agency chose unreliable studies as their sole basis for an MRL. The Agency should choose differently going forward. In other cases, the chosen studies are reliable, but the Agency's uses of them are questionable.

We expect that ATSDR has many constraints, resource-wise and otherwise, and recognize that objective analysts may differ among themselves as to the "correct" way to assess risks to human health from given chemical contaminants. Nonetheless, there is now considerable scientific knowledge regarding at least PFOA and PFOS, if not the other two PFAS. ATSDR can and should do better as it works to finalize its MRLs.

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