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A Commentary on ATSDR's Toxicological Profile for Perfluoroalkyls

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Summary

The crux of the various intermediate MRLs for ATSDR (2018), and for the PBPK modeling of EPA (2016) on which ATSDR's MRLs are based, in part, is an assumption that the proper dosimetry between mouse and human is area-under-the-curve (AUC), represented by differences in the half-lives of PFOA and related compounds. This assumption does not appear to be correct for the critical effects chosen by ATSDR (nor for those chosen by EPA). In contrast, alternative dosimetry, that is peak concentration, represented by Cmax, appears to be scientifically more defensible, since it better reflects the nature of dosing and/or the timing of the critical effects.

ATSDR needs to consider this dosimetic alternative, Cmax, in its MRL development.

Basis

ATSDR (2018) and EPA (2016) have both provided a summary of the various human and experimental animal studies on this class of chemicals and are to be commended for comprehensive and readable texts. Unfortunately, it is our opinion that both agencies may have missed the appropriate dosimetric adjustment, that of Cmax rather than AUC, for the No Observed Adverse Effect Level (NOAEL) or Lowest Observed Adverse Effect Level (LOAEL) of the critical effects.[1] This miss is surprising given the fact that Cmax is the recommended default in EPA (1991) guidelines for developmental toxicity,[2] the critical effect in EPA (2016), and is likely the better choice for ATSDR (2018) where the critical effects are also presumably from gestational exposure. EPA (2014) guidelines for data-derived extrapolation factors also confirm the use of Cmax when the critical effects are more likely evoked by short-term, peak exposures.[3]

A close reading of the Lau et al. (2006) study, which forms the basis of the EPA (2016) PFOA risk value, and similar analysis by ATSDR (2018), suggests that the effects are more likely due to Cmax and not AUC. For example,

- The dosing is by gavage; thus, a larger Cmax is expected than when PFOA administration is via drinking water or diet.
- A dose-dependent prenatal loss with neonatal survival flattening out after birth suggests that this mortality is due to *in utero* exposure, since if mortality was related to AUC, it would not flatten out after birth due to continued internal PFOA exposure.
- Other post-parturition effects are unlikely to be caused by continued PFOA exposure since milk levels are at ~10% of dam serum levels (at least in rats, see

[1] The critical effect is the first adverse effect, or its known and immediate precursor, that occurs as dose or concentration of a chemical increases.
[2] The appropriate guidelines are EPA (1991) for developmental toxicity (pages 38 and 45) where peak concentration (or Cmax—a term not used widely back then) is the default position.
[3] EPA (2014) data-derived extrapolation factor guidelines (pages 22-23) allow EPA to consider either AUC or Cmax depending on how the critical effect is likely to be evoked.

> Lau et al., 2006, page 516, column 2), and mouse dams did not receive any additional PFOA after parturition. Again, this suggests that the effects are due to in utero exposure since the continued internal PFOA exposure is much reduced.

EPA (2016) also describes six other studies found on pages 4-13 in Tables 4-8; ATSDR (2018) also describes these studies. Each of these studies also suggest Cmax findings. Four of EPA's six studies are also gavage studies during gestation – consistent with Cmax- caused effect; one study is in drinking water where the selected effect occurs after 1 day–clearly a Cmax effect–and the remaining 90-day study shows liver effects that neither EPA, nor ATSDR, thinks are adverse (see EPA, 2016, page 244 and ATSDR, page A-34).

ATSDR's critical study, Koskela et al. (2016), is also a short-term exposure (gestational exposure) with effects possibly due to Cmax rather than AUC.[4] This study is limited by small groups of animals and shows an effect in mice (females only) at 13-17 months of age, presumably attributable to gestational exposure. The effects and statistics described by ATSDR appear to be based on individual pups and not on the litter, which is not consistent with recommended analysis by EPA (1991) guidelines, nor with the conclusion drawn that the effect is due to the gestational exposure. In our opinion, whether this conclusion is correct requires further rigorous testing and analysis. ATSDR should reconsider use of this study as the basis of the MRL, including AUC and Cmax as the dosimeter for the critical effect. If this study cannot be used, then ATSDR will likely use Lau et al. (2006) as the basis of its MRLs and the issues associated with the use of this study as described above by EPA will apply to ATSDR.

Thus, depending on the ratio of Cmax between the experimental animal species and humans for the given critical effects, the ATSDR (2018) MRLs and the matching values for EPA (2016) may not be correct. ATSDR (2018) and EPA (2016) will both need to conduct a comparison of Cmax between the chosen experimental animal species and humans in developing the PBPK adjustment in lieu of a default uncertainty factor.

Inconsistency with Other Authorities

Conducting such an analysis is especially important in light of the large disparity between the EPA and ATSDR positions and that of the Committee on Toxicology (2009), the United Kingdom's top advisory body. A comparable PFOA drinking water level by the COT (2009), for example, would be about 10,000 ppt using the same assumptions as EPA (2016) [UK PFOA TDI of 1.5 µg/kg bw-day x 70 kg bw x 0.2 RSC ÷ 2 L/day ~ 10 µg/L or 10,000 ppt]. The principle reason for this disparity appears to be in the assumption that AUC can be worked into the assessment by ATSDR (2018) and EPA (2016), where the COT (2009) does not consider this assumption to be scientifically justified, based in part, on the determination of half-lives in the US on the basis of water-only consumption.

[4] The use of the word "possibly" here reflects that dietary nature of the exposure in Koskela et al. (2016) rather than gavage exposure found in Lau et al. (2006).

This COT (2009) rejection of the AUC/half-life approach seems reasonable based on Emmett et al. (2006) who state, "Our results thus lead us to question whether the serum PFOA half-life in the general community is as long as that published for the small retired worker group." Emmett et al. (2006) further suggest that other sources of PFOA are possible. For example, on page 12 Emmett et al. (2006) state "The reason for the higher serum PFOA levels in those aged 60 and above is not entirely clear, multivariate analysis shows the increased consumption of drinking water in this group does not fully explain the observed increase." Finally, Emmett et al. (2006) show on page 23 a blood serum level of 374 ng/mLof PFOA in 20 humans without any tap water consumption (Table 5, first row). This no exposure group had more serum PFOA than other groups who stated consumption of 1 to 2 tap water drinks per day. This suggests that a significant level of PFOA may be coming from sources other than water as **Figure 1 of these comments**, adapted from the data of Emmett et al (2006) (Table 5), shows. **Figures 2 and 3 of these comments**, also derived from the data of Emmett et al. (2006) Table 5, directly demonstrate that sources other than water are contributing to the serum PFOA concentrations.

These statements by Emmett et al. (2006) and Figures 1, 2, and 3 weaken ATSDR's use of AUC for its PFOA MRLs, since drinking water is not the sole source, and may not be even the principal source of PFOA in human serum. Therefore, ATSDR's use of half-lives, exemplified by reliance on AUC, may not be appropriate or supportive for the MRLs.

A scientific resolution of this dilemma in both the ATSDR and EPA PFOA assessments is obvious. Kinetic information for this class of chemicals is needed. For ATSDR's MRLs, kinetics in pregnant mice to discern whether Cmax or AUC is evoking the critical effects (which are primarily immunological and/or developmental) are needed. Unfortunately, there is not a kinetic study in pregnant mice. However, there are three studies from which one can construe a variety of half-lives. These half-lives are not consistent as the following text, adapted from Lau et al. (2006) or EPA (2016) will demonstrate.

- [Lau et al., page 512] For example, Lau et al. (2006) conducted an experiment to compare the body burdens of PFOA between adult rats and mice after subchronic exposure. After 20 daily treatments, a serum level of 111 µg PFOA/mL was reached in male rats while only traces of PFOA were detectable in female rats, indicating a clear sex difference in PFOA accumulation was observed in the rats (Lau et al., Table 1). In contrast, no significant differences were seen between serum PFOA concentrations of male and female mice. Additionally, a steady-state level of serum PFOA was apparently reached in mice within 1 week of chemical exposure, as serum levels in both male and female mice did not change appreciably between 7 and 17 days of treatment. [**Note: Since reaching steady state takes 4 to 5 half-lives, this implies a half-life of about 2 days**]

- [EPA, page 2-27] Hundley et al. (2006) examined excretion of PFOA in CD-1 mice, BIO-15.16 hamsters, and New Zealand White rabbits. This study is limited as only one male and one female of each species was given a single dose of 10-mg/kg ^{14}C-PFOA and housed in metabolism cages. Urine and feces were collected at 12, 24, 48, 72, 96, and 120 hours post-dose. Additional samples were collected from rabbits at 144 and 168 hours post-dose. Over 120 hours, the male mouse excreted 3.4% ^{14}C-PFOA in urine and 8.3% ^{14}C-PFOA in feces, and the female mouse excreted 6.7% ^{14}C-PFOA in urine and 5.7% ^{14}C-PFOA in feces. The male and female mice were similar in the amounts excreted. [**Note: The half-life implied here is around 20 days, but it reflects only one mouse of each sex**.]

- [EPA, page 2-21] White et al. (2011) measured serum PFOA concentrations in three generations of CD-1 mice (EPA Table 2-18). Pregnant mice (F0, n = 10–12 dams/group) were gavage-dosed with 0, 1, or 5 mg PFOA/kg from GD 1–17. A separate group of pregnant mice (n = 7–10 dams/group) were gavage-dosed with either 0 (controls) or 1 mg PFOA/kg from GD 1–17 and received drinking water containing 5 parts per billion (ppb) PFOA beginning on GD 7 and continuing until the end of the study for their offspring, except during breeding and early gestation, to simulate a chronic low dose exposure. An increase in serum PFOA concentration was observed in the control + 5 ppb PFOA groups in the F1 and F2 generations and in the 1-mg/kg + 5-ppb PFOA group of the F2 generation. A decrease was observed for the remaining groups. [**Note: The half-life implied here based on gavage dosing only and in PND22 pups of F1 and F2 generations is 10 days, as per attached spreadsheet "…Serum PFOA Levels in mice" columns I through L**.]

All of these mice were of the CD-1 strain, so most likely strain differences do not account for the disparity. Lau et al. is the critical study from which the developmental effects are used for the EPA (2016) health advisory. Using Lau et al. favors the Cmax dosimetric adjustment, rather than the use of AUC, particularly as the study was gavage.

Additional studies are needed to draw definitive conclusions about PFOA kinetics in animals and humans, as well as pregnant and non-pregnant animals. A kinetic study of PFOA in pregnant mice would discern the scientific basis for the appropriate dosimetric. In addition, a clearance study in humans with PFOA and related compounds would shed light on relevant dosimetry, i.e., is it AUC or Cmax? If AUC is the appropriate dosimeter, then a determination of human clearance is needed. In light of the fact that several groups are now using a 50% RSC in their determinations of advisories in the US, previous estimates of human half-lives that do not consider multiple sources of exposure, the very ones being used to support the choice of AUC as a dosimeter, are suspect.

Note on the recent PFOA assessment by EFSA (2018)

EFSA (2018) also appears to also be depending on half-life estimates, exemplified by use of AUC, for the lowering of their safe dose. This would not be necessary, however, if the dosimeter of the critical effect is Cmax. For example, EFSA states:

> "For PFOS, the increase of serum total cholesterol in adults, and the decrease in antibody response at vaccination in children were identified as the critical effects. For PFOA, the increase in serum total cholesterol was the critical effect. Also reduced birth weight (for both compounds) and increased prevalence of high serum levels of the liver enzyme ALT (for PFOA) were considered."

However, the decrease in antibody response after a one-shot vaccination is clearly not an AUC event, but rather one related to Cmax. Reduced birth weight may or may not be an AUC event, but a recent presentation (Dhingra et al., 2017) raises the hypothesis of reverse causation for the observed lower birth body weight found in the epidemiology studies that EFSA is using as a basis, in part, of its lowered safe dose. If this hypothesis is correct, that is, if lower birth weight babies will cause more PFOA to accumulate due to lower kidney filtration when compared with expectant mothers with normal birth weight babies, then the basis of EFSA's recent lowering of the tolerable weekly intake of PFOA/PFOS based on lower body weights is further eroded. Finally, it is suggested that ATSDR also look at Tox Science 163 (1) May, 2018, page 293 where a DECREASE in cholesterol is found in humans with high levels of PFOA, calling into question the use of an increase in cholesterol as a critical effect by EFSA.

We encourage ATSDR to carefully consider the above discussion and, specifically, show how the use of Cmax rather than AUC affects its determination of MRLs, which are otherwise extraordinarily low. Specific comments follow.

Specific Comments

Page 13. Text: PFOS significantly decreased birth weight and survival in neonatal rats exposed *in utero* (Chen et al. 2012b; Lau et al. 2003; Xia et al. 2011), and cross-fostering exposed pups with unexposed dams failed to improve survival rates (Lau et al. 2003).

> This text support the use of Cmax rather than AUC as a dosimeter because it clearly shows that continued exposure in dam milk does not further decrease survival rates.

Page 13. Text: Dosing rats during late gestation (GDs 17–20) caused significantly more pup lethality than dosing early in gestation (GDs 2–5) (Grasty et al., 2003). Early gestational exposure (GD 2-5) had 60% mortality by PND 10. Late gestational exposure (GD 17-20) approached 100% mortality by PND 10.

It is not clear if this difference is attributable to differences in Cmax, or AUC, or period of susceptibility. It clearly shows that the timing of exposure is an important factor in outcome, however. This study needs more analysis.

Page 19. Tables 1-3 and 1-4.

These results appear to be based on pup-based statistics and not litter- based statistics. (See also page A-23). If pup-based statistics have been used to determine the critical effects, these studies will need to be reconsidered as the basis of the MRLs.

Page A-24. Text: several places on the page referring to time-weighted averages (TWA).

Using a TWA assumes that the appropriate dosimetric adjustment between mice and humans is AUC. In contrast, EPA's DDEF guidelines state that the dosimetric adjustment should be based on the critical effect. Cmax should also be considered as a possible dosimeter. It would be helpful if ATSDR shows a calculation based on Cmax and acknowledges that the appropriate dosimeter needs to be further explored.