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Invited Perspective: Challenges in Evaluating the Effect of Per- and Polyfluoroalkyl Substance Mixtures on Polycystic Ovarian Syndrome

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The health effects of chemical mixtures are an imperative research priority for environmental epidemiology, but there are numerous challenges in evaluating and interpreting the available evidence for these effects. New work by Zhan et al.[1] published in this issue addresses some of the key challenges related both to the specific outcome of interest and to disentangling relationships for any outcome with complex mixtures. Zhan et al. use both novel and traditional statistical models to estimate both individual and joint associations between 23 different per- and polyfluoroalkyl substances (PFAS) and risk of polycystic ovarian syndrome (PCOS)—a departure from most past human health studies, which examined coexposures to PFAS primarily as confounders or not at all.

Considerations Related to Evaluation of PCOS

Existing research on PCOS has indicated possible associations with different PFAS exposures (Table 1). Although most results are consistent with positive associations, there are multiple sources of uncertainty, including potential reverse causation, selection of participants from infertility clinic settings, and some inconsistent inverse associations for individual PFAS. Because reverse causation is likely the most difficult to address, we focus on it here.

There are multiple potential mechanisms by which reverse causation could explain the positive associations between PFAS exposure and PCOS risk reported in some studies. These include differential elimination of PFAS due to menstrual, parity, and endocrine changes associated with PCOS and conditions that co-occur with PCOS (e.g., adiposity, insulin resistance). The majority of studies measured PFAS exposure using biomarkers, which are susceptible to this potential bias because they are measured after the exposure is processed by the body. However, one study[2] used PFAS concentrations in drinking water—which cannot be affected by differential elimination—as the primary exposure metric; this study also found an association with PCOS, which reduces concern for reverse causation to some extent.

In biomarker studies, stratified analyses by these factors can provide additional insight on the likelihood of reverse causation. Looking at menstruation, women with PCOS have more irregular periods, including longer cycles and shorter bleeding times, both of which could reduce PFAS elimination.[3,4] Restricting analyses to women with normal blood volume loss did not change the overall results in studies by Wang et al.[3] or Zhan et al.[1] However, volume was based on self-report, which is anticipated to have some measurement error[5] that may differ by case status, so there is remaining potential for differential bias. In contrast, parity is straightforward to measure. Zhan et al. did not find a difference in association between the overall population and nulliparous women; conversely, Wang et al. restricted their study to nulliparous women and found inverse and null associations for some of the same PFAS associated with increased risk by Zhan et al., so uncertainty remains as to the impact of this potential bias.

Co-occurring health conditions are another possible source of reverse causation or confounding. Adiposity is a risk factor for PCOS.[6] However, adiposity may be affected by PFAS exposure—for example, if PFAS are associated with endocrine disruption leading to insulin resistance—and could affect the levels of PFAS in the body by influencing pharmacokinetics or individual behavior related to exposure. Studies of PCOS and PFAS exposure reporting body mass index (BMI) by case status found that women with PCOS tended to have higher BMI than controls,[3,7] which makes it difficult to determine whether the observed association between PCOS and PFAS exposure was actually due (at least in part) to the association between PCOS and adiposity. Looking further, Zhan et al. observed stronger associations in overweight and obese women than normal-weight women.[1] The authors suggest that adiposity may act as a moderator in the relationship, possibly via insulin resistance. However, although some studies have reported associations between PFAS exposure and both adiposity and insulin resistance, the data overall are not consistent across studies [based on draft assessments of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) by the U.S. Environmental Protection Agency[8,9] and another review[10]]. This lack of a clear relationship between PFAS exposure and adiposity and insulin resistance complicates the interpretation of the findings in Zhan et al.[1] because it decreases the likelihood of reverse causation while raising anew the question of why the association with PCOS would be stronger in overweight women.

Overall, Zhan et al.[1] have made a valuable contribution to the question of reverse causation for PCOS, but more research is needed to better understand these relationships.

Considerations Related to Evaluating Mixtures of Exposures

Existing studies of PCOS and PFAS exposure have focused mostly on legacy PFAS and have evaluated effects of single PFAS individually. Zhan et al. evaluated a larger number of PFAS than previous studies and used methods that enabled identification of nonlinear and mixture effects.[1] Their univariate analyses identified several PFAS associated with PCOS, including elevated odds for all legacy PFAS evaluated (similar to previous studies), although not all were statistically significant. When using mixture methods to account for joint effects, some associations remained significant, whereas others were attenuated and no longer significant, highlighting the importance of considering mixture effects when interpreting associations. Importantly, many relationships were nonlinear, which

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Table 1. Summary of studies examining polycystic ovarian syndrome (PCOS) and selected per- and polyfluoroalkyl substances (PFAS).

Reference, study design, and population	Effect estimate	PFOA	PFOS	PFHxS	PFNA	PFDA	PFDoA
Vagi et al.[7] Case–control study in the United States with recruitment in general population $N = 52$ cases and 50 controls	OR (95% CI) vs. T1	T2: 1.65 (0.45, 6.14) T3: 6.93 (1.79, 29.92)*	T2: 3.43 (0.95, 13.31) T3: 5.79 (1.58, 24.12)*	T2: 0.85 (0.20, 3.31) T3: 1.20 (0.35, 4.07)	T2: 1.13 (0.37, 4.49) T3: 2.25 (0.67, 8.00)	NA	NA
Wang et al.[3] Case–control study in China with recruitment of nulliparous women with diagnosed infertility and PCOS $N = 180$ cases and 187 controls	OR (95% CI) vs. T1	T2: 0.56 (0.28, 1.04) T3: 0.58 (0.26, 1.26)	T2: 0.81 (0.37, 1.79) T3: 0.44 (0.14, 1.37)	T2: 1.68 (0.83, 3.37) T3: 2.08 (0.88, 4.93)	T2: 1.31 (0.55, 3.13) T3: 1.62 (0.45, 5.80)	T2: 1.15 (0.51, 2.61) T3: 2.06 (0.63, 6.78)	T2: 2.36 (1.12, 4.99)* T3: 3.04 (1.19, 7.67)*
Zhan et al.[1] Case–control study in China with recruitment of women with PCOS-related infertility $N = 366$ cases and 577 controls	OR (95% CI) for log-unit increase	1.07 (0.83, 1.36)	1.27 (1.08, 1.45)*	1.26 (0.92, 1.65)	1.13 (0.89, 1.44)	1.08 (0.85, 1.15)	1.32 (1.19, 1.52)*
Hammarstrand et al.[2] Cohort in Sweden with high PFAS contamination, cases identified through registry $N = 29{,}106$ (161 cases)	HR (95% CI) for ever high vs. never high exposure	Exposure to summed PFAS (primarily PFHxS, PFOS, PFOA) based on drinking water) 1.43 (0.99, 2.05)			NA	NA	NA
Heffernan et al.[4] Case–control study in UK with recruitment of women from IVF clinic $N = 30$ cases and 29 controls (age, BMI matched but crude analysis)	Geometric mean (95% CI) for cases; controls	2.4 (1.9, 2.9); 2.4 (2.0, 2.9)	3.9 (3.4, 4.4); 3.1 (2.6, 3.6)*	1.1 (0.9, 1.4); 0.9 (0.8, 1.2)	0.6 (0.5, 0.7); 0.5 (0.4, 0.6)	NA	NA

Note: Results for additional PFAS are reported in Wang et al.[3] and Zhan et al.,[1] including PFBS, PFHpA, PFUA (both papers), and PFHpS, PFUnDA, PFBA, PFPeS, PFPeA, isomers of PFHxS and PFOS, PFAS alternatives 6:2 Cl-PFESA, 8:2 CL-PFESA, and HFPO-DA (Zhan et al.[1]). 6:2 Cl-PFESA, 6:2 chlorinated perfluoroalkyl ether sulfonic acid; 8:2 CL-PFESA, 8:2 chlorinated perfluoroalkyl ether sulfonic acid; BMI, body mass index; CI, confidence interval; HFPO-DA, hexafluoropropylene oxide dimer acid; HR, hazard ratio; IVF, in vitro fertilization; NA, not applicable; OR, odds ratio; PFBA, perfluorobutanoic acid; PFBS, perfluorobutane sulfonate; PFDA, perfluorodecanoic acid; PFDoA, perfluorododecanoic acid; PFHpA, perfluoroheptanoic acid; PFHpS, perfluorohexane sulfonate; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonate; PFPeA, perfluoropentanoic acid; PFPeS, perfluoropentane sulfonic acid; PFUA, Perfluoroundecanoic acid; PFUnDA, perfluoroundecanoic acid; T, Tertile. $^{*}p < 0.05$.

(along with the mixtures approach) may partially account for differences between this and previous studies.

Zhan et al.[1] took an important step toward better understanding the relationships of interest, but as they noted, evaluation of chemical mixtures presents unique challenges. Traditionally, mixtures analysis has relied on whole-mixture methods or assumptions of dose additivity, which may not be justified. For example, Goodrum et al.[11] described different mixture approaches for PFAS, noting that few whole-mixture studies exist and other studies show violations of dose addition, including some cases of PFAS interacting to produce antagonistic or synergistic mixture effects. An additional complexity is the potential mismatch between the composition of the PFAS mixture as it occurs in environmental media (the sources of exposure) and as measured in biological tissues. Although no information is available to determine that this is a concern for the study population described by Zhan et al.,[1] it is possible that such a mismatch may stem in part from variation in pharmacokinetics between compounds, including different half-lives in the human body. These differences may be exacerbated if the disease process affects pharmacokinetic processes for all or a subset of the PFAS measured. Consequently, the mixture measured at the time of study may or may not adequately represent the mixture to which the individual was exposed during the time window relevant for disease development. The use of pharmacokinetic models to estimate exposure for different time windows, and the incorporation of mechanistic and toxicology data, may aid in the interpretation of findings from epidemiology studies.

The authors used a variety of analytic approaches to explore individual and joint effects of co-occurring exposures. Even when exposures are highly correlated, each may have different types and levels of measurement error and sets of potential confounders and effect modifiers. Indeed, bias amplification resulting from correlation among exposures—in combination with factors such as exposure measurement error or confounding that affects one exposure or a subset of co-occurring exposures (important when PFAS exposure arises from different sources)—can lead to greater bias in multipollutant models compared with single-pollutant models.[12] The authors' analyses partially address these concerns. Quantile g-computation may be less sensitive to bias amplification because the goal is to estimate a mixture joint effect, a particularly useful feature when PFAS are considered as a class or in groups of similar PFAS.[13] This approach may be beneficial over the long term given that it better reflects the highly correlated nature of our exposure to PFAS and avoids the delays involved when PFAS are regulated one at a time.[14,15] However, the ability to disentangle effects for individual PFAS is still useful and needed, both for biological understanding as well as for the current regulatory framework where PFAS are regulated as individual compounds. Thus, it is helpful for publications to report both types of results.

Final Thoughts

Evaluating health effects attributable to PFAS exposure is challenging, and this is particularly true for complex conditions such as PCOS. Publication of Zhan et al.[1] and future studies will increase our ability to disentangle the potential impacts of individual PFAS and mixtures of PFAS on human health.

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