How "forever chemicals" might impair the immune system

Researchers are exploring whether these ubiquitous fluorinated molecules might worsen infections or hamper vaccine effectiveness.

Carolyn Beans, *Science Writer*

Stain-resistant carpets and nonstick pots were once the epitome of "better living through chemistry," their space-age properties conferred by molecules known as perfluoroalkyl and polyfluoroalkyl substances (PFAS). But in the early 2000s, researchers began to discover that PFAS were somehow reaching the farthest corners of the planet—from polar bears in Alaska (1) to pilot whales in the Faroe Islands of the North Atlantic (2). These molecules contain chains of carbon peppered with fluorine atoms, which together form one of the strongest known chemical bonds. That helps these chemicals excel at repelling grease and water but also makes them astonishingly resistant to degradation in the environment (3).

Amid a flurry of new studies, scientists are still figuring out what risks these ubiquitous "forever chemicals" pose to public health (see "PFAS Politics"). Epidemiologists and toxicologists point to myriad possible consequences, including thyroid disease, liver damage, and kidney and testicular cancers (4). Impacts on the immune system are a particular concern.

Animal models and human studies have provided strong evidence that PFAS alter the immune system, diminishing the ability to fight disease or respond to a vaccine. These studies have heightened urgency as nations across the globe grapple with the coronavirus disease 2019 (COVID-19) pandemic and engage in a vaccination campaign of historic proportions. Researchers are intent on better understanding how PFAS affect coronavirus and other infectious diseases—as well as the vaccinations meant to stymie them.

But many questions remain: Scientists don't know the toxicity levels of most PFAS or how mixtures of PFAS may interact to affect immune health. Even for the most commonly studied PFAS, little is known about the mechanics of how these substances interact with the immune system.

A Troubling Finding

People are exposed to forever chemicals through contaminated water, food, and air, as well as countless products including cosmetics and upholstery. In 2015, the Centers for Disease Control and Prevention (CDC) National Health and Nutrition Examination Survey reported that PFAS were found in the blood of nearly all Americans sampled (5). US companies no longer manufacture the two best-known PFAS, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). But these legacy PFAS persist in the environment, even as thousands of others remain in production.

Animal models and human studies suggest that forever chemicals, delivered through water, food, and air, alter the immune system, potentially diminishing our ability to fight disease or respond to a vaccine. Image credit: Shutterstock/Dmitry Naumov.

About a decade ago, researchers started to detect signs of immune system after-effects in humans. In 2008, environmental epidemiologist Philippe Grandjean of the University of Southern Denmark in Odense read a study on PFOS that worried him. The work, by Margie Peden-Adams, then of the Medical University of South Carolina in Charleston, and colleagues, suggested that PFOS in mice, at levels similar to those found in humans, could suppress the immune system (6).

Grandjean, who is also an adjunct professor of environmental health at the Harvard T.H. Chan School

Published under the PNAS license.
Published April 8, 2021.

Downloaded from https://www.pnas.org by 174.6.85.23 on August 15, 2022 from IP address 174.6.85.23.

Although no longer in use, an aqueous fire-fighting foam containing a mixture of PFOA, PFOS, and other PFAS ingredients remains detectable in contaminated water. Image credit: Shutterstock/Bborriss.67.

of Public Health in Boston, MA, was already studying the immune effects of other environmental toxicants on children in the Faroe Islands, who may be exposed through their traditional marine diets. He knew that this community could also be exposed to PFAS through seafood.

Using previously collected blood serum samples, Grandjean tracked PFAS levels from birth in nearly 600 children born between 1997 and 2000, as well as the children's antibodies against tetanus and diphtheria. The children had all been vaccinated against these diseases and should have had sufficient antibodies for protection. "We were completely shocked when we looked at the data," Grandjean recalls.

His team found that a doubling of PFOS exposure at birth (estimated from the mother's blood serum) was associated with a nearly 40 percent drop in diphtheria antibody concentration at age 5. A doubling of exposure to PFOS and PFOA at age 5 (estimated from the child's own blood serum) made children 2.4 to 4.2 times more likely to fall below a protective level for both tetanus and diphtheria antibodies at age 7 (7). "If they are below the protective level, it means that the vaccine had actually failed," explains Grandjean.

At age 5, more than one-quarter of the children were indeed below this protective level for tetanus, and more than one-third were below the protective level for diphtheria (7). Grandjean notes that although children normally experience some reduction in antibody levels before getting their recommended booster shots, this number of children below the protective level was more than expected.

Grandjean's team is not monitoring the children for upticks in diphtheria and tetanus, given the rarity of these diseases. But they do view the antibody levels produced in response to these vaccines as a proxy for the immune system's ability to respond to disease in general. And the researchers have now gone on to investigate the link between PFAS exposure in the Faroe Islands and common childhood infectious diseases, tracking a new group of newborns by asking mothers about fever and symptoms every two weeks. In a similar study in Denmark, Grandjean and colleagues recently reported that prenatal exposure to PFAS was associated with an increased risk of children later being hospitalized for infectious diseases (8).

Other recent work suggests that the links between prenatal PFAS exposure and immune suppression extend to other ailments as well. Immunotoxicologist Berit Granum of the Norwegian Institute of Public Health in Oslo, Norway, and colleagues looked at relationships between PFAS in pregnant Norwegian mothers and their children's likelihood of contracting common infectious diseases. After monitoring almost 1,000 children up to age 7, the team reported that exposure to PFOA and another PFAS called perfluorohexane sulfonic acid (PFHxS) was associated with higher rates of diarrhea or gastric flu. By age 3, children with higher exposure to PFOS, PFOA, PFHxS, or perfluoroheptane sulfonic acid (PFHpS) had higher rates of bronchitis or pneumonia (9). In contrast, other studies have hinted that PFAS may at times overactivate the immune system (10).

"What is really important is: what does the overall body of evidence tell us?" says environmental toxicologist Jamie DeWitt of East Carolina University in Greenville, NC. In a 2020 review, she and coauthors concluded that PFAS can suppress the human immune response (4). Similarly, in 2016, the National Toxicology Program concluded that PFOS and PFOA are "presumed to be an immune hazard to humans" (11).

For a given individual, the consequences of altered immune function may be subtle, notes immunotoxicologist Dori Germolec of the Division of the National Toxicology Program at the National Institute of Environmental Health Sciences, Durham, NC. It could mean that someone catches an extra cold in a given year or takes slightly longer to clear an infection. But at a population level, she says, there could be "an economic impact in terms of more doctor visits, more parent days off of work because the kids are sick."

Animal Model Inroads

Cell and animal research into the immune system effects of forever chemicals offers further hints at the chemicals' impact and reach, but that work is also beset with a host of challenges.

Germolec began studying a PFAS known as perfluoro-*n*-decanoic acid (PFDA) in mice after CDC data showed rising levels of several PFAS in humans (12). Her team reported in 2018 that mice exposed to PFDA had fewer key immune cells in their spleens, although their exposure levels were higher than those typical for humans (13). Through in vitro analyses, Germolec is now exploring the effects of PFDA and other PFAS on human immune cells.

Meanwhile, chemical and biological engineer Carla Ng of the University of Pittsburgh, PA, is collaborating

Downloaded from https://www.pnas.org by 174.6.85.23 on August 15, 2022 from IP address 174.6.85.23.

Researchers found that children in the Faroe Islands exposed via diet to PFAS chemicals experienced drops in antibody concentrations against tetanus and diphtheria, diseases that the children had been vaccinated against. Image credit: Shutterstock/Fexel.

with toxicologists to screen hundreds of PFAS by using zebrafish and mice. First, Robyn Tanguay of Oregon State University in Corvallis exposes zebrafish embryos to each chemical, to look for toxic effects. Then DeWitt will test any toxic PFAS in mice, to assess their impact on the immune system. Drawing on these data, Ng will create computer models that predict concentrations of individual PFAS in the organs of mice and zebrafish based on chemical structure and exposure dose. Ultimately, these models could more rapidly predict the toxicity of other PFAS without the need for animal studies.

But translating animal-based findings to humans, a persistent challenge, can be especially problematic in PFAS studies, explains Mark Johnson, director of toxicology at the Army Public Health Center at Aberdeen Proving Ground, Maryland. There can be huge differences in the time it takes different species to clear PFAS from their systems. The half-life for PFOA in mice, for example, is on the order of days. For humans, it's years (4).

Ng is developing models to explore how PFAS bioaccumulation may differ across animal species—both to identify good model species for human toxicity studies and to better understand which species may be especially vulnerable to PFAS in the environment. The models aim to predict how well a PFAS will bind to particular proteins, whose structures could vary in different animals.

In a recent collaboration with the EPA, Ng's models suggested that, for a specific type of liver protein, nine PFAS had a similar bioaccumulation potential in humans, rats, chicken, and rainbow trout. Some PFAS, though, had a higher bioaccumulation potential in humans than in Japanese medaka and fathead minnow, both commonly used in laboratory toxicology studies (14). Ng says that a similar technique could also be used to predict bioaccumulation potential or toxicity using other PFAS target receptors, including those critical to the immune system.

A Black Box

Although researchers are learning more about the toxicity of individual PFAS, they still don't know how PFAS chemicals interact. "Mixtures are going to be more environmentally relevant," says DeWitt. "People get exposed to PFAS in drinking water and food. Very rarely are they exposed to a single compound."

DeWitt recently studied the effects of a mixture of PFOA, PFOS, and other PFAS ingredients in an aqueous film-forming foam that was developed to put out fires. This foam is no longer produced, but it remains in the environment in contaminated water. The mixture contains fairly low levels of PFOA, yet DeWitt found that mice exposed to the foam experienced a drop in antibody production that was similar to mice exposed to a much higher concentration of PFOA alone (15). The implication is that PFAS mixtures could pose a greater risk than a single PFAS, although DeWitt acknowledges that PFOS in the foam may also have played a major role in antibody suppression.

Downloaded from https://www.pnas.org by 174.6.85.23 on August 15, 2022 from IP address 174.6.85.23.

Perhaps the biggest unknown is the actual mechanics of how PFAS chemicals alter the immune system. Revealing a molecular mechanism might help determine the relevance of animal studies to human health, says Johnson. "If you have that mechanism and know that the same pathway is conserved across species," he says, "then you're on firm ground."

But that mechanism is still largely a "black box," says Germolec, adding that different PFAS may act in very different ways. Still, DeWitt is beginning to lay the groundwork. Her work in mice suggests that PFOA targets crucial immune cells called B cells, which produce antibodies (16). "We've identified the cell that we think is targeted, but we haven't identified the molecular changes in that cell that lead to the deficiency in antibody production. We're hinting at mechanism," DeWitt says, "but we're not there yet."

PFAS in a Pandemic

As the body of PFAS research continues to grow, we may be living through a de facto experiment testing the effects of forever chemicals on the immune system. Researchers are now questioning whether the cumulative global impact of PFAS-impaired immune function could make the dire pandemic situation even worse.

"A lot of PFAS researchers have had this question on their mind, especially in communities that have had high exposures from contaminated drinking water or other sources," says environmental chemist Laurel Schaider of Silent Spring Institute in Newton, MA, a science research nonprofit. She and other PFAS researchers, including DeWitt, expressed their concerns in a July 2020 opinion piece (17).

Drawing on Danish biobanks, Grandjean recently analyzed PFAS levels in blood plasma samples from adults infected with COVID-19 (18). His team found a strong association between disease outcome and perfluorobutanoic acid (PFBA), a small PFAS molecule that accumulates in the lungs. In a group of more than 300 samples, the presence of PFBA in blood serum was associated with a near doubling of the likelihood of hospitalization. In a subset of patients whose PFBA levels were measured around the time of their COVID-19 diagnosis, hospitalized individuals with PFBA exposure were more than five times more likely to progress to intensive care or death (18).

PFAS Politics

The challenge of sorting out how best to regulate forever chemicals is almost as complex as the PFAS research itself.

Perhaps the biggest hurdle is the paucity of data. Although PFAS studies in humans point to a range of worrying associations, the research is largely designed to demonstrate correlations rather than direct causation, and the risks of specific exposure levels are unknown for most PFAS. "There are pushing 10,000 substances that we call PFAS," says environmental toxicologist Jamie DeWitt of East Carolina University. "Only a handful have been studied for their health effects."

Researchers often don't even know which PFAS chemicals to look for in the environment and our bodies. DeWitt's recent review notes that companies often treat the identity and quantity of PFAS chemicals used in products and processes as confidential business information (4).

And yet, new PFAS chemicals are regularly approved, often with little government review, says Laurel Schaider of Silent Spring Institute. "The challenge with PFAS has really highlighted the shortcomings in our approach to regulating chemicals."

With thousands of PFAS out in the environment, and limited information on health effects, it's extremely difficult to determine acceptable levels of exposure. The US Environmental Protection Agency (EPA) has set a lifetime health advisory for PFOA and PFOS, suggesting that combined levels of these chemicals in drinking water should not exceed 70 parts per trillion (19). But the health advisory isn't a regulation and isn't enforceable. "The current status is that, unless there are state requirements, public water suppliers don't currently have to test for PFAS," says Schaider. Complicating matters are the unusual characteristics of PFAS chemicals—some argue that they deserve a special regulatory category because they are so mobile and persistent. "It makes sense to be cautious about using new chemicals that are highly persistent and mobile in the environment, even in the absence of toxicity data, since if a health concern is raised later, the chemicals will already disperse readily into the environment where they will last for a long time," Schaider notes.

Under the Trump administration, the EPA recently took steps to set regulatory limits on PFOS and PFOA as part of the Safe Drinking Water Act, but the process of finalizing a legally enforceable limit will likely take years. Schaider says that the action is a "positive step," although she notes that the regulation would only apply to these two PFAS and it isn't yet clear what exposure limit would be set.

During his campaign, President Biden pledged to "tackle PFAS pollution by designating PFAS as a hazardous substance, setting enforceable limits for PFAS in the Safe Drinking Water Act, prioritizing substitutes through procurement, and accelerating toxicity studies and research on PFAS (20)." Designating PFAS as a hazardous substance would be an important step toward remediating contaminated sites, because it would "help accelerate the process of getting funding for cleanup," Schaider says, "and making the responsible parties pay."

Downloaded from https://www.pnas.org by 174.6.85.23 on August 15, 2022 from IP address 174.6.85.23.

Other PFAS studied, including PFOS and PFOA, did not show an association with disease outcome. But PFAS levels in most individuals studied were quite low, says Grandjean, so results may differ in regions like the United States where PFAS exposure is greater.

Additional studies are likely to offer some important clues. About two years ago, Schaider launched a study to explore the relationship between PFAS exposure and health outcomes in children and adults in two Massachusetts communities exposed to high levels of PFAS through drinking water—it's part of a CDC and Agency for Toxic Substances and Disease Registry (ATSDR) study of about a dozen exposed communities across the country. Schaider is also investigating how PFAS exposure may affect the immune systems of preschool-age children in two New England communities. In both projects, she now plans to look for associations between elevated PFAS levels and COVID-19 infections.

Seeking to gauge effects in a particularly hard hit population, a CDC-supported study called AZ HEROES is tracking COVID-19 infections and antibody levels in Arizona healthcare workers, emergency responders, and other essential workers. Volunteers provide weekly nasal swabs for COVID-19 testing, as well as blood samples for antibody analysis at multiple time points throughout the study. For some analyses, project lead Jeff Burgess, an associate dean for research and professor of environmental health sciences at the University of Arizona Mel and Enid Zuckerman College of Public Health in Tucson, will combine these data with another CDC-funded effort called RECOVER, which tracks COVID-19 infections in essential workers in different parts of the country. Burgess, an occupational medicine physician, is also teaming up with Alberto Caban-Martinez of the University of Miami Miller School of Medicine, FL, to recruit RECOVER participants directly in both Arizona and Florida.

The research team will then measure PFAS concentrations in blood samples to look for an association between PFAS and COVID-19. Within the AZ HEROES group, researchers will also look for associations between PFAS exposure and levels of antibodies produced in response to COVID-19 vaccines. Even with the vaccines now available, COVID-19 will continue to have health impacts, notes Burgess. "PFAS and COVID-19 is an important issue to understand better."

1 J. P. Giesy, K. Kannan, Global distribution of perfluorooctane sulfonate in wildlife. *Environ. Sci. Technol.* **35**, 1339–1342 (2001).
2 U. Berger, U. Järnberg, R. Kallenborn, Perfluorinated alkylated substances (PFAS) in the European Nordic environment. *Organohalogen Compd.* **66**, 3996–4002 (2004).
3 National Institute of Environmental Health Sciences, Perfluoroalkyl and polyfluoroalkyl substances (PFAS) (2019). https://www.niehs.nih.gov/health/materials/perfluoroalkyl_and_polyfluoroalkyl_substances_508.pdf. Accessed 15 January 2021.
4 S. E. Fenton *et al.*, Per- and polyfluoroalkyl substance toxicity and human health review: Current state of knowledge and strategies for informing future research. *Environ. Toxicol. Chem.* **40**, 606–630 (2021).
5 R. C. Lewis, L. E. Johns, J. D. Meeker, Serum biomarkers of exposure to perfluoroalkyl substances in relation to serum testosterone and measures of thyroid function among adults and adolescents from NHANES 2011–2012. *Int. J. Environ. Res. Public Health* **12**, 6098–6114 (2015).
6 M. M. Peden-Adams *et al.*, Suppression of humoral immunity in mice following exposure to perfluorooctane sulfonate. *Toxicol. Sci.* **104**, 144–154 (2008).
7 P. Grandjean *et al.*, Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. *JAMA* **307**, 391–397 (2012).
8 L. Dalsager *et al.*, Exposure to perfluoroalkyl substances during fetal life and hospitalization for infectious disease in childhood: A study among 1,503 children from the Odense Child Cohort. *Environ. Int.* **149**, 106395 (2021).
9 A. Impinen *et al.*, Maternal levels of perfluoroalkyl substances (PFASs) during pregnancy and childhood allergy and asthma related outcomes and infections in the Norwegian Mother and Child (MoBa) cohort. *Environ. Int.* **124**, 462–472 (2019).
10 M. S. Jackson-Browne, M. Eliot, M. Patti, A. J. Spanier, J. M. Braun, PFAS (per- and polyfluoroalkyl substances) and asthma in young children: NHANES 2013-2014. *Int. J. Hyg. Environ. Health* **229**, 113565 (2020).
11 National Toxicology Program, NTP monograph: Immunotoxicity associated with exposure to perfluorooctanoic acid or perfluorooctane sulfonate (2016). https://ntp.niehs.nih.gov/ntp/ohat/pfoa_pfos/pfoa_pfosmonograph_508.pdf. Accessed January 15, 2021.
12 K. Kato, L. Y. Wong, L. T. Jia, Z. Kuklenyik, A. M. Calafat, Trends in exposure to polyfluoroalkyl chemicals in the U.S. Population: 1999-2008. *Environ. Sci. Technol.* **45**, 8037–8045 (2011).
13 R. P. Frawley *et al.*, Immunotoxic and hepatotoxic effects of perfluoro-n-decanoic acid (PFDA) on female Harlan Sprague-Dawley rats and $B_6C_3F_1$/N mice when administered by oral gavage for 28 days. *J. Immunotoxicol.* **15**, 41–52 (2018).
14 W. Cheng, J. A. Doering, C. LaLone, C. Ng, Integrative computational approaches to inform relative bioaccumulation potential of per- and polyfluoroalkyl substances (PFAS) across species. *Toxicol. Sci.* kfab004 10.1093/toxsci/kfab004 (2021).
15 C. A. McDonough *et al.*, Immunotoxicity of an electrochemically fluorinated aqueous film-forming foam. *Toxicol. Sci.* **178**, 104–114 (2020).
16 J. C. DeWitt, W. C. Williams, N. J. Creech, R. W. Luebke, Suppression of antigen-specific antibody responses in mice exposed to perfluorooctanoic acid: Role of PPARα and T- and B-cell targeting. *J. Immunotoxicol.* **13**, 38–45 (2016).
17 J. DeWitt *et al.*, Op-ed: PFAS chemicals—the other immune system threat. *Environmental Health News* (6 June 2020). https://www.ehn.org/pfas-and-immune-system-2646344962.html. Accessed 4 February 2021.
18 P. Grandjean *et al.*, Severity of COVID-19 at elevated exposure to perfluorinated alkylates. *PLoS One* **15**, e0244815 (2020).
19 United States Environmental Protection Agency, Drinking water health advisories for PFOA and PFOS. https://www.epa.gov/ground-water-and-drinking-water/drinking-water-health-advisories-pfoa-and-pfos. Accessed 4 February 2021.
20 The Biden plan to secure environmental justice and equitable economic opportunity. https://joebiden.com/environmental-justice-plan/. Accessed 4 February 2021.

Downloaded from https://www.pnas.org by 174.6.85.23 on August 15, 2022 from IP address 174.6.85.23.

https://doi.org/10.1073/pnas.2105018118