

PFAS Elimination and PFAS Effects on COVID-19 Vaccine Response

**Observational and Experimental
Studies in Highly Exposed Adults.**

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UNIVERSITY OF GOTHENBURG

Gothenburg 2025

Cover illustration: Photograph of Storforsen, taken by Sandra Wallin & Ragnar Englund. To my knowledge, Storforsen is not contaminated by PFAS.

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愚公移山

The man who moves a mountain begins by carrying away small stones.

Confucius

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ABSTRACT

Background and aims: Per- and polyfluoroalkyl substances (PFAS) are persistent compounds, some of which have long serum half-lives in humans. Yet, details about how PFAS are eliminated from the human body are limited. PFAS exposure has been associated with lower vaccine responses in children, but it is unclear whether this also occurs in adults. Therefore, the first theme of this thesis was to describe PFAS elimination in humans and investigate if it can be enhanced, while the second theme was to evaluate PFAS effects on COVID-19 vaccine response in adults. The studies were conducted in Ronneby, Sweden, where drinking water had previously been highly contaminated from firefighting foams.

Methods and results: Firstly, PFAS were repeatedly measured in highly exposed individuals after the end of exposure, from which half-lives in serum were estimated. Shorter half-lives were associated with younger age, female sex during the fertile age, higher kidney function, higher gut inflammation, and lower gut permeability. Secondly, fecal and urinary elimination were estimated, both of which were found to be important, with variations between individuals and PFAS compounds. Thirdly, in an experimental, cross-over trial, bile acid sequestrants markedly increased PFAS elimination, substantially lowering serum PFAS levels.

Finally, in an mRNA COVID-19 vaccination study in adults aged 20-60 years, no associations were found between PFAS levels and serum anti-spike antibody and T cell responses.

Conclusions and implications: PFAS serum half-lives vary between individuals, and PFAS are eliminated through both urine and feces. This implies that PFAS exposure models, aiming to predict serum levels from external exposure, need to properly include population characteristics and both fecal and urinary elimination routes. PFAS elimination can be substantially enhanced; however, the net health benefits of such interventions are not yet known.

The mRNA vaccine response was not reduced in adults with high PFAS exposure. Whether this indicates a more mature immune system in adulthood, a specific effect of the mRNA vaccine, or other mechanisms needs to be further explored.

Keywords: PFAS, elimination, COVID-19 vaccine response

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SAMMANFATTNING

I december 2013 upptäcktes det att dricksvattnet från ett vattenverk i Ronneby innehöll mycket höga nivåer av miljögiftet PFAS. Föroreningarna kom från brandsläckningsskum som under lång tid hade använts vid brandövningar på flygflottiljen i Kallinge. Det förorenade vattnet ersattes genast av rent vatten från ett annat vattenverk i kommunen. En tidig kartläggning visade att många av invånarna i Ronneby hade mycket förhöjda halter av PFAS i blodet.

Avhandlingens första tre delarbeten studerade utsöndringen av PFAS ur kroppen. En del av PFAS-ämnena tar flera år att lämna kroppen, och det skiljer sig mycket mellan individer. Däremot saknas detaljer kring varför det går snabbare för vissa och hur PFAS lämnar kroppen. Det första delarbetet identifierade faktorer som kännetecknar snabb utsöndring ur kroppen – ung ålder, kvinnligt kön i fertil ålder, god njurfunktion och tarpåverkan. I det andra delarbetet mätte vi PFAS-utsöndringen ur kroppen genom urin och avföring, och fann att avföringen var minst lika viktig som urinen. I det tredje delarbetet fann vi att utsöndringen kunde påskyndas med hjälp av de två olika gallsyrebindande läkemedlen Kolestyramin och Colesevelam. Det finns dock risker och biverkningar med läkemedlen, och en risk-nyttavärdering behövs för att säkerställa om det finns hälsovinster med att sänka PFAS-halterna i kroppen.

De två sista delarbeten av avhandlingen studerade PFAS effekt på COVID-vaccinations svar hos vuxna. Tidigare studier har nämligen funnit att barn med högre PFAS-halter i blodet hade lägre antikroppshalter efter barnvaccinationer. Motsvarande studier av vuxna har inte visat lika tydliga samband. Vi genomförde därför en COVID-19 vaccinationsstudie, där 367 vuxna individer från Ronneby och grannkommunen Karlshamn vaccinerades med Modernas mRNA-vaccin. I det fjärde delarbetet utvärderades vaccinationen genom att mäta antikropps nivåer i blodet. I det femte delarbetet studerades T-cellsaktivitet. Det gjorde vi genom att plocka ut T-celler från individernas blod och utsätta dem för virusproteiner, för att sedan mäta signalsubstanserna som T-cellerna producerade. Alla som vaccinerades fick ett fullgott immunsvår, och det fanns inga tecken på att höga halter av PFAS skulle försämra vaccinationseffekten hos vuxna. Om vuxnas immunförsvar är mer moget än barns, om det är egenskaper hos mRNA-vaccin som särskiljer dem från andra vaccin, eller om det finns någon annan förklaring till varför PFAS-exponerade inte fick lägre immunsvår, är frågor som framtida forskning får svara på.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

- I. Li Y, Andersson AG, Xu Y, Pineda D, Nilsson CA, Lindh CH, Jakobsson K, Fletcher T. Determinants of Serum Half-lives for Linear and Branched Perfluoroalkyl Substances after Long-Term High Exposure – a Study in Ronneby, Sweden. *Environment International*. 2022;163:107198. doi: 10.1016/j.envint.2022.107198.
- II. Andersson AG, Fletcher T, Xu Y, Kärman A, Pineda D, Nilsson CA, Lindh CH, Jakobsson K, Li Y. The Relative Importance of Fecal and Urinary Excretion of Perfluorooctane Sulfonic Acid and Perfluorooctanoic Acid After High Exposure – an Observational Study in Ronneby, Sweden. *Environmental Research*. 2025;285:122487. doi: <https://doi.org/10.1016/j.envres.2025.122487>.
- III. Andersson AG, Xu Y, Kärman A, Cederlund J, Lindh CH, Pineda D, Fletcher T, Jakobsson K, Li Y. Serum, Urinary and Fecal Concentrations of Perfluoroalkyl Substances after Interventions with Cholestyramine/Colesevelam and Probenecid – a Cross-Over Trial in Ronneby, Sweden. *Environment International*. Accepted, in print.
- IV. Andersson AG, Lundgren A, Xu Y, Nielsen C, Lindh CH, Pineda D, Cederlund J, Pataridou E, Søgaard Tøttenborg S, Ugelvig Petersen K, Fletcher T, Lagging M, Bemark M, Jakobsson K, Li Y. High Exposure to Perfluoroalkyl Substances and Antibody Responses to SARS-CoV-2 mRNA Vaccine – an Observational Study in Adults from Ronneby, Sweden. *Environmental Health Perspectives*. 2023;131(8):87007. doi: 10.1289/ehp11847.
- V. Andersson AG, Lundgren A, Xu Y, Nielsen C, Lindh CH, Pineda D, Vallin J, Johnsson C, Fletcher T, Bemark M, Jakobsson K, Li Y. The T Cell Response to SARS-CoV-2 mRNA Vaccine in Adults with High Exposure to Perfluoroalkyl Substances from Ronneby, Sweden. *Chemosphere*. 2024;369:143770. doi: 10.1016/j.chemosphere.2024.143770.

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ABBREVIATIONS

PFAS	Per- and polyfluoroalkyl substances
PFHxA	Perfluorohexanoic acid
PFOA	Perfluorooctanoic acid
PFNA	Perfluorononanoic acid
PFBS	Perfluorobutane sulfonic acid
PFPeS	Perfluoropentane sulfonic acid
PFHxS	Perfluorohexane sulfonic acid
PFHpS	Perfluoroheptane sulfonic acid
PFOS	Perfluorooctane sulfonic acid
L-PFOS	Linear perfluorooctane sulfonic acid
1m-PFOS	Branched PFOS, perfluoro-1-methylheptane sulfonic acid
2/6m-PFOS	Branched PFOS, sum of perfluoro2/6-methylheptane sulfonic acid
3/4/5m-PFOS	Branched PFOS, sum of perfluoro3/4/5-methylheptane sulfonic acid
ADME	Absorption, distribution, metabolism, excretion
Vd	Volume of distribution
CL	Clearance
k	Serum elimination rate
PBPK	Physiologically based pharmacokinetic (modelling)

COVID-19	Coronavirus disease 2019
SARS-COV-2	Severe acute respiratory syndrome coronavirus 2
mRNA	Messenger ribonucleic acid
ATSDR	Agency for Toxic Substances and Disease Registry
EFSA	European Food Safety Agency
NHANES	National Health and Nutrition Examination Survey
US EPA	United States' Environmental Protection Agency
BAS	Bile acid sequestrants
OAT	Organic Anion Transporter

THESIS IN BRIEF

Paper	Title
I	Determinants of Serum Half-Lives for Linear and Branched Perfluoroalkyl Substances after Long-Term High Exposure – a Study in Ronneby, Sweden.
II	The Relative Importance of Fecal and Urinary Excretion of Perfluorooctane Sulfonic Acid and Perfluorooctanoic Acid after High Exposure – an Observational Study in Ronneby, Sweden.
III	Serum, Urinary and Fecal Concentrations of Perfluoroalkyl Substances after Treatment with Cholestyramine/ Colesevelam and Probenecid – a Cross-Over Trial in Ronneby, Sweden.
IV	High Exposure to Perfluoroalkyl Substances and Antibody Responses to SARS-Cov-2 mRNA Vaccine – an Observational Study in Adults from Ronneby, Sweden.
V	The T Cell Response to SARS-Cov-2 mRNA Vaccine in Adults with High Exposure to Perfluoroalkyl Substances from Ronneby, Sweden.

Aims	Methods	Main results
Measure and identify determinants to serum PFAS half-lives	Prospective cohort study (n=114) with multiple serum PFAS measurements	Half-lives associated with age, female sex in fertile age, kidney and intestinal health.
Compare fecal and urinary elimination of PFAS	Cross-sectional fecal, urine and serum samples (n=147)	Fecal elimination is just as important as urinary elimination
Measure medication effects on PFAS elimination.	Cross-over experiments (n=10): 1 week and 12 weeks treatment with medications	Cholestyramine & colesevelam lowered PFAS; probenecid did not
Evaluate mRNA COVID-19 vaccine effectiveness in PFAS exposed adults.	Prospective cohort study (n=367), vaccinated with two doses and PFAS effect evaluated with serum antibodies levels	No associations between PFAS and antibody levels
Evaluate mRNA COVID-19 vaccine effectiveness in PFAS exposed adults.	Prospective cohort study (n=116), vaccinated with two doses and PFAS effect evaluated with T cell response.	No associations between PFAS and T cell responses

1 INTRODUCTION

1.1 PFAS

1.1.1 CHEMICAL STRUCTURE

Per- and polyfluoroalkyl substances (PFAS) have been defined by the Organization for Economic Co-operation and Development (OECD) as:

“... substances that contain at least one fully fluorinated methyl or methylene carbon atom (without any H/Cl/Br/I atom attached to it)...” (1)

In other words, the general structure is a carbon backbone with attached fluoride instead of hydrogen. The carbon-fluoride bonds are synthetic, organic covalent bonds that are extraordinarily strong, rendering PFAS resistant to chemical, biological, mechanical, and thermal breakdown (2). The number of unique compounds usually ranges between 4,700 and 11,000 (e.g., in the PRIO database by the Swedish Chemicals Agency (3)), but there may be more than 1.7 million chemicals that fit the OECD definition (4).

PFAS can roughly be subdivided into two structural groups – the more studied and historically used **perfluorinated** compounds, which have all their hydrogen in their carbon chain replaced with fluoride; and the **polyfluorinated** compounds, which have some of their hydrogen atoms replaced (5). However, many polyfluorinated PFAS break down into perfluorinated compounds (5, 6) (e.g., 6:2 Fluorotelomer sulfonic acid (6:2 FTS) are converted into perfluorinated carboxylic acids in wastewater (7)), possibly even in humans (8). Although there have been *in vitro* studies showing that some bacteria and enzymes can degrade perfluorinated compounds (9), little to no degradation has been observed *in situ*, and the perfluorinated compounds are thus regarded as environmentally stable.

While polyfluorinated compounds are technically part of PFAS, this thesis refers only to perfluorinated compounds when writing PFAS. The three main PFAS in this thesis is perfluorooctanoic acid (PFOA), perfluorohexane sulfonic acid (PFHxS) and perfluorooctane sulfonic acid (PFOS) (Figure 1).

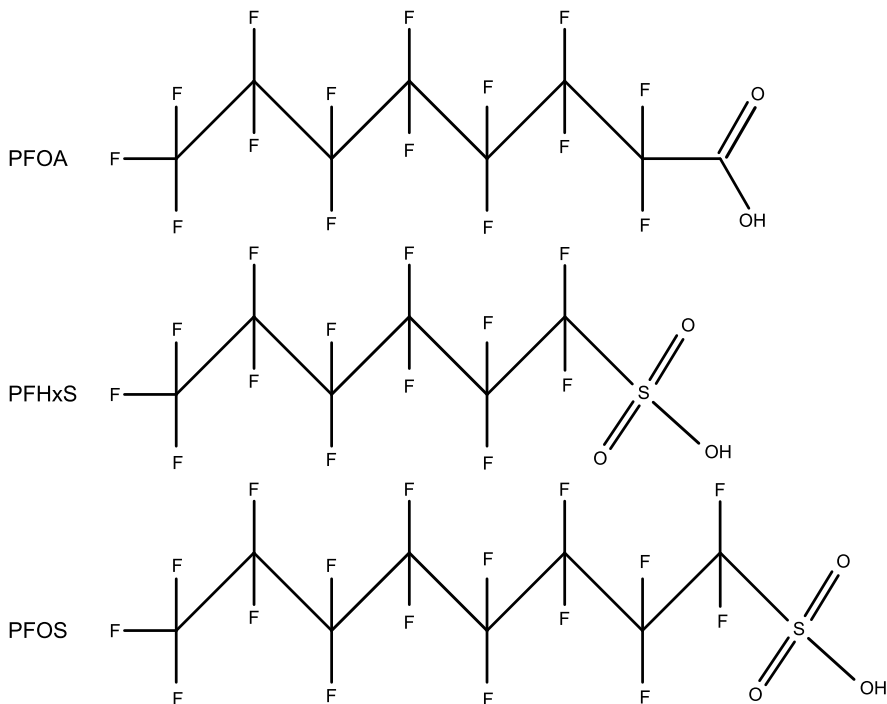


Figure 1 Chemical structure of PFOA, PFHxS, and PFOS. The sticks represent covalent bonds between atoms. Corners without letters represent carbon atoms. F = fluorine, S = sulfur, O = oxygen, H = hydrogen.

1.1.2 PFAS EXPOSURE

1.1.2.1 BACKGROUND EXPOSURE

Background exposure refers to exposure that is present for everyone, typically resulting from multiple sources. These sources involve everyday consumer goods such as food packaging, textiles, and disposable personal hygiene products (10), as well as environmental sources, such as drinking water, indoor dust, and certain types of food (11-13). Environmental contamination is widespread, with measured concentrations in surface and groundwater around the globe (14). The PFAS in the environment originate partly from industrial sites, military fire training areas, landfills, and wastewater plants (15). There also seems to be atmospheric transportation, as PFAS has been measured in rain (16), and in remote, arctic ice sheets (17, 18).

These ubiquitous exposure sources lead to elevated serum PFAS concentrations in humans, known as background exposure levels. Studies of background exposure levels in western general populations show two trends: PFOS is usually the PFAS at the highest serum concentrations, followed by PFOA, and then PFHxS or perfluorononanoic acid (PFNA) (19-22); and that the serum concentrations of PFOA, PFOS, and some other PFAS have been declining since approximately the year 2000 (20-24), reflecting that Western countries have regulated the production and use of these PFAS.

1.1.2.2 HOTSPOT EXPOSURES

PFAS hotspots are sites where PFAS exposure significantly exceeds the background exposure. One group of PFAS hotspots is occupational settings, found in workers who either work with producing PFAS (25-27), using PFAS-containing firefighting foams such as firefighters (28-30), or applying PFAS to other products such as ski wax technicians (8). Hotspot exposure can also result from heavy environmental contamination, where high PFAS levels are measured in drinking water, soil, or locally produced food.

One major environmental hotspot was the industrial PFOA contamination of drinking water in the mid-Ohio River Valley, which originated from DuPont's Washington Works PFAS manufacturing plant. As part of the C8 Health Project, 69,030 individuals were recruited for PFAS serum measurements and the collection of health-related data. In line with the drinking water contamination, the population was found to have especially elevated serum PFOA levels (31).

Another frequent source of environmental pollution is PFAS-containing aqueous film-forming foam (AFFF), which is used for extinguishing burning hydrocarbons. Two different methods have been used for production of the PFAS in AFFF – electrochemical fluorination and later (fluoro)telomerization. The electrochemical fluorination method yields mainly linear PFOS but also branched PFOS, other perfluorinated sulfonates like PFHxS, and other precursors and intermediates (5). The telomerization method, on the other hand, creates almost exclusively linear isomers (for AFFF, usually Fluorotelomer sulfonic acids are produced), which can degrade to perfluorinated compounds like PFOA and PFNA (5). Depending on the AFFF formula used, the environmental pollution profile might contain different proportions of PFAS compounds, as shown from several groundwater samples taken at five military bases in the US, where different AFFF products were used (32).

Analogously, different serum PFAS profiles have been found in populations exposed to AFFF. In Korsør, Denmark, individuals who had eaten meat from cows fed on AFFF-contaminated fields had substantially high levels of PFOS (median 181 ng/mL), while PFHxS (8.8 ng/mL) and other PFAS (e.g., PFOA, 0.92 ng/mL) were at lower concentrations (33). In contrast, firefighters from Australia (28-30) had only slightly higher median PFOS concentrations (66 ng/mL in the 2015 study and 14 ng/mL in the two 2022 studies) than PFHxS (25 and 6.5 ng/mL, respectively).

1.1.2.3 RONNEBY – LARGE PFOS AND PFHXS HOT-SPOT

In December 2013, it was discovered that one of the two waterworks in Ronneby, Sweden, was heavily contaminated with PFAS (10,380 ng/L), mainly PFOS (8,000 ng/L) and PFHxS (1,700 ng/L) (Figure 2) (34). The source of the pollution was AFFF used at the nearby military airfield from the 1980s to the 2000s. The contaminated waterworks supplied water to approximately one-third of the 28,000 inhabitants living in Ronneby, thus creating vast contrasts in exposure within the municipality. Directly after the discovery, the other waterworks in Ronneby municipality provided clean water, removing the high exposure.

A pilot study in 37 children revealed elevated serum levels in children from the area with water from the contaminated waterworks, prompting a broadening of biomonitoring to all interested inhabitants in Ronneby. A total of 3,293 members from the general population were recruited into the Ronneby Biomarker Cohort (34). In a subset of the Ronneby Biomarker Cohort, 114 individuals were followed from June 2014 to May 2018 with repeated serum PFAS measurements to estimate the serum elimination rate (k) and half-life (Paper I and Li et al. 2018 (35)).

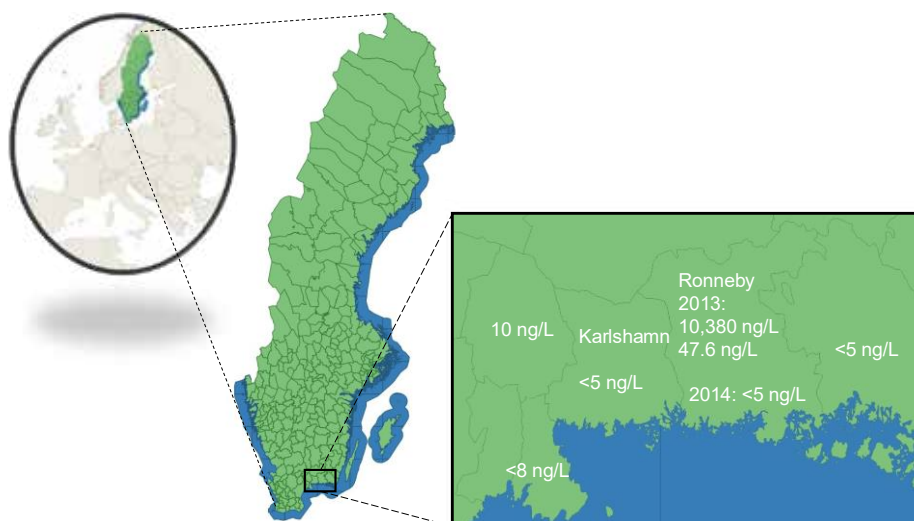


Figure 2 Localization of Ronneby and the drinking water concentrations in the two waterworks, in December 2013, when the contamination was discovered. The highly contaminated waterworks were closed, and the use of carbon filters on the remaining, slightly contaminated waterworks enabled the provision of clean water. The drinking water concentrations in neighboring municipalities, measured later, are shown for comparison.

1.2 PHARMACOKINETICS AND PFAS

Pharmacodynamics and pharmacokinetics are tools created by pharmacologists to evaluate internal outcomes and exposures, respectively, of potential drugs. Pharmacodynamics is the study of the effect of a certain amount of a chemical compound, usually a specific dose of a drug, on target cell, tissue, organ, or whole body, while pharmacokinetics is the study of the destiny of the same chemical compound within the human body (36). In other words, pharmacodynamics is the study of what a compound does to the body, while pharmacokinetics is the study of what the body does to the drug.

Many of the pharmacodynamic and pharmacokinetic tools developed for the pharmaceutical industry can also be applied to unintended exposures such as PFAS and other environmental pollutants. Typically, pharmacokinetics will refer to a “drug,” but in the following sections, “compound” will be used instead, as the concepts will be applied to PFAS (which are not drugs).

1.2.1 ADME

The different parts of pharmacokinetics can be divided into four basic, and sometimes overlapping, categories: absorption, distribution, metabolism, and excretion (ADME). These are often described as processes that occur sequentially: First, if administered orally, the compound enters the gastrointestinal system. There, a proportion of the dose passes through the intestinal mucosa, either passively through the cell membranes or actively via cellular transporters. The portal vein then transports the passed proportion to the liver. As it passes through the intestinal cells or the hepatocytes in the liver, the compound may undergo breakdown. The proportion of the administered dose that is detected in the systemic blood flow is considered **absorbed** (37).

After absorption, the compound is transported throughout the body. This enables the compound to **distribute** to peripheral tissues. The distribution depends on how well the compound can enter other tissues, either passively or actively through transporter proteins, and what the compound binds to, such as plasma proteins like albumin, phospholipids in cell membranes, or other tissue or cell structures. This is in turn determined by the chemical properties of the compound, including size, configuration, polarity, and acidity. (37)

During and after distribution, the compound can undergo chemical alteration through **metabolism**. Metabolism is primarily carried out by hepatocytes to facilitate the excretion of foreign compounds through feces or urine. This process occurs via two parallel systems: Phase 1 metabolism, which involves oxidation, reduction, and hydrolysis reactions that transform the compound into new substances called metabolites; and Phase 2 metabolism, where enzymes attach hydrophilic compounds, such as glucuronate, to the molecules. (38)

Lastly, the compound, whether metabolized or in its original form, is **excreted** in the feces (via the bile) or in the urine (37). Since metabolism and excretion, both directly and indirectly, remove the compound from the body, both processes can be summarized as **elimination** (37). Elimination determines how long a specific amount stays in the body.

It should be noted, however, that the pharmacokinetic process does not always follow this order. For example, some compounds may not be absorbed at all, such as the bile acid sequestrants cholestyramine and colesevelam (39), while others (e.g., the drug naloxone) are absorbed in the intestines but are directly metabolized in the intestinal cells or by the liver (in a process known as the

first-pass effect). In other words, for some compounds, certain aspects of ADME can be omitted or emphasized.

1.2.2 PHARMACOKINETIC MODELING

Pharmacokinetic models are useful for summarizing data, facilitating extrapolation, and predicting pharmacokinetic parameters (37, 38).

1.2.2.1 COMPARTMENTAL MODELS

One type of model is the compartment model, where the body is simplified into one or multiple compartments with absorption, distribution, metabolism, and excretion to and from these compartments (37, 38). The simplest of these models is the one-compartment model, which represents the body as a single, central compartment (38). This model requires a mono-exponential decay, meaning a log-linear decrease in serum concentrations over time. If the decline is multi-phasic, meaning it has different log-linear slopes over time, then a multi-compartmental model is more appropriate (38).

Three key concepts in compartmental models used to measure and describe elimination are clearance, elimination rate, and half-life. **Clearance** is defined as the volume of fluid (usually plasma or serum) completely cleared of the compound per unit of time (measured in mL/min) (37). Systemic clearance, also known as total body clearance, is the combined clearance from various elimination routes, including intrinsic hepatic clearance from liver metabolism, renal clearance from urine excretion, fecal clearance from feces excretion, and clearance from other potential elimination routes that may be present (38).

Elimination rate, on the other hand, is the rate at which serum concentrations decrease (per unit of time). It can be used to estimate serum concentrations at time t , assuming a mono-exponential decay (37):

$$C_t = C_0 \cdot e^{-k \cdot t}$$

Half-life is the time it takes for C_t to become half of the original C_0 concentration, meaning how long it takes for serum concentrations to decrease by 50% (37).

In addition to these three key elimination concepts, the compartment models also estimate the **volume of distribution** (V_d), defined as “a measure of the apparent volume in the body which contains the compound” (38). It is calculated by dividing the total amount (dose) of the compound in the body by

its concentration in serum or plasma (38). The easiest way to estimate V_d is to experimentally administer a certain dose, intravenously or orally, and then measure the serum concentration after distribution. V_d s can range from the plasma volume of 3L (approximately 47.9 mL/kg in a 70 kg human) to several thousand liters if the compound primarily distributes to tissues, such as adipose tissue, instead of remaining in the plasma (37, 38). It is therefore not an actual volume, as it can extend the total body volume.

Clearance (CL), elimination rate (k), half-life ($t_{1/2}$), total amount in the body (A), serum concentrations (C) and V_d are related in a one-compartment model as follows (38):

$$A = C \cdot V_d$$

$$k = CL/V_d$$

$$t_{1/2} = \ln(2) \cdot V_d/CL$$

1.2.2.2 PBPK MODELS

Another method to model ADME is to use Physiologically based Pharmacokinetic (PBPK) models. These models are more advanced, incorporating physiological, anatomical, and biochemical factors such as organ blood flow rates, tissue and fluid volumes, blood to plasma and tissue to plasma concentration ratios, protein binding, metabolizing enzymes, and transporter activities (38). One of the most cited and used PFAS PBPK models is the one by Loccisano et al. 2011 (40) (Figure 3). In short, the model assumes eight different compartments, with blood flow from plasma to each compartment, and only the free fraction (unbound to plasma proteins) available for distribution to other compartments. In this model, the only excretion route is through urine (not through menstruation nor feces), at an elimination rate of k_{urine} (Figure 3).

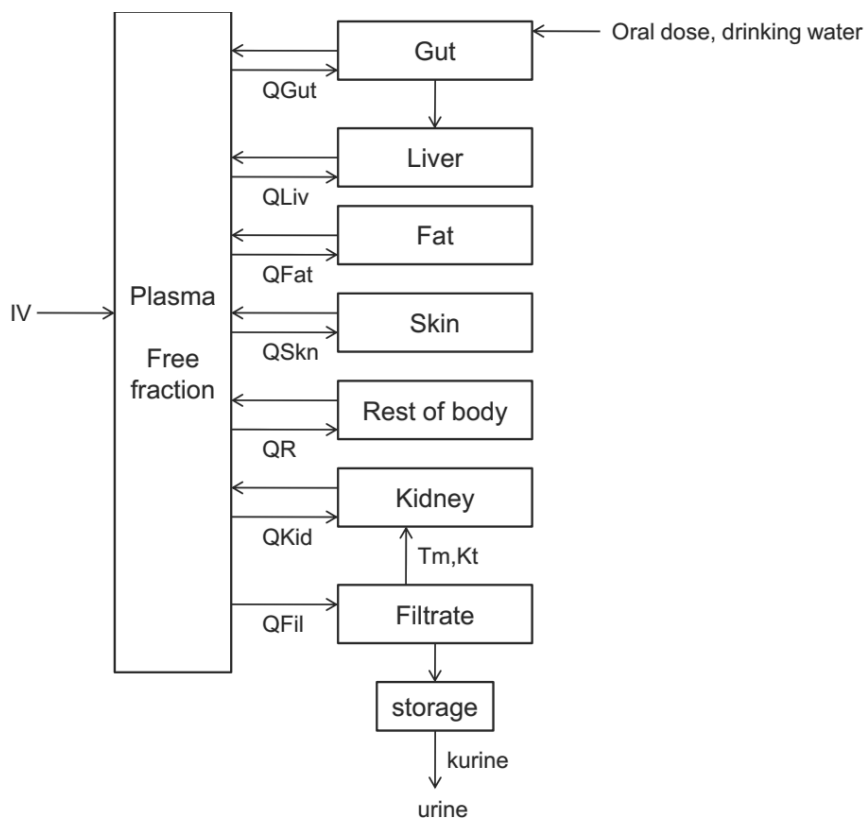


Figure 3 PBPK model for PFOA and PFOS in monkeys and humans. Q refers to blood flow to and from the different tissues, except for Q_{Fil} , which is the clearance of plasma to the Filtrate compartment. The k_{urine} refers to the elimination rate into urine. From Loccisano et al. 2011 (40). Republished with permission.

1.2.3 PFAS ADME

PFAS is completely **absorbed** in the intestines (41). After absorption, PFAS binds strongly to plasma proteins such as albumin (42, 43) and globulins (43), with less than 1% unbound in plasma (43, 44).

In addition to binding to plasma, PFAS appear to **distribute** and accumulate in mainly liver and kidney tissue, as indicated by studies of PFOA and PFOS in mice (45), rats (46-49), and humans (27, 50-52). In humans, PFAS have also been measured in bone, lung, brain (50, 53), thyroid, and pancreas tissue (52). PFAS do not accumulate in adipose tissue, unlike other Persistent Organic Pollutants (54), as PFAS have only been detected at concentrations lower than serum levels in pooled (52) and fetal adipose samples (55).

Although present in metabolizing organs such as the liver, perfluorinated PFAS, such as PFOS and PFOA, are not **metabolized** (46). Although many polyfluorinated PFAS can be metabolized, the end product of the metabolism process can be perfluorinated compounds (e.g., 8:2 Fluorotelomer alcohol (FTOH) is converted into PFOA in humans (8)).

In animals, PFAS are **excreted** through urine, as shown in rats (46, 56-58), monkeys (59), cattle (60, 61) and other animals (62). However, PFAS have also been measured in bile (46) and in feces (56, 61) of animals. The excretion of PFAS in rodents and smaller mammals is so rapid that serum half-lives typically range from a few hours (as in rats) to about a month (as in cynomolgus monkeys) (62) (Figure 4).

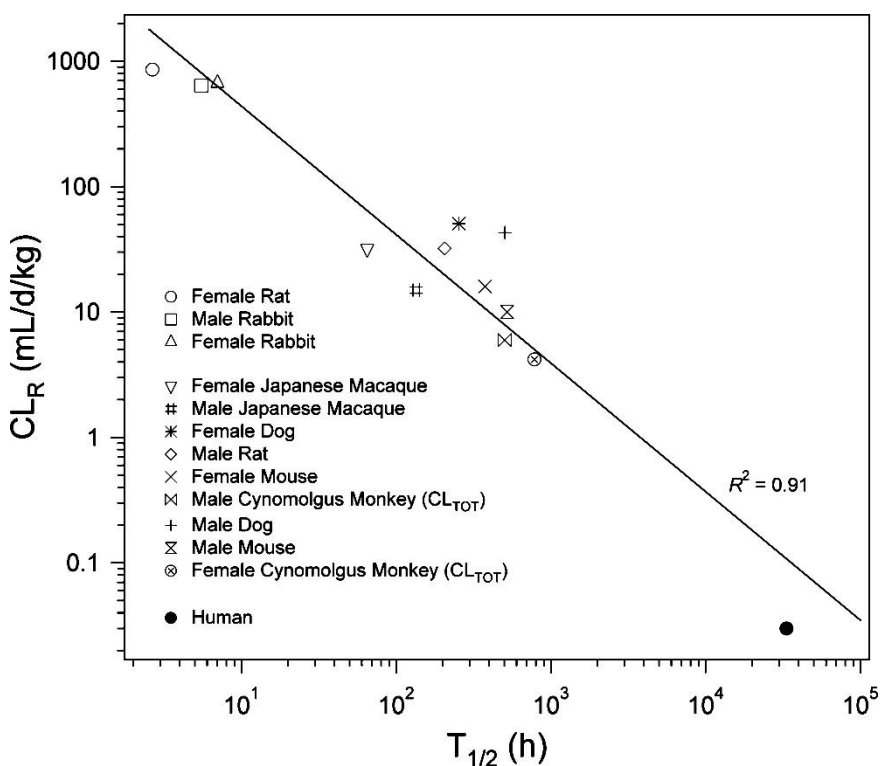


Figure 4 An almost linear correlation between renal clearance and serum half-lives of PFOA across different species. From Han et al. 2012 (62). Republished with permission.

In humans, however, the elimination of PFAS is significantly slower. For short-chained PFAS, like perfluorobutane sulfonic acid (PFBS) and

perfluorohexanoic acid (PFHxA), the serum half-lives are approximately 1-2 months (63, 64), whereas for long-chained PFAS the serum half-lives extend to several years. A recent meta-analysis estimated mean serum half-lives (with 95% confidence interval) of 2.75 (1.88, 3.63) years for PFOA, 4.77 (3.26, 6.29) years for PFOS, and 5.35 (3.16, 7.55) years for PFHxS (65).

It has been suggested that the slow elimination of PFAS in humans results from extensive tubular reabsorption of urine-excreted PFAS (62) and intestinal reabsorption of bile-excreted PFAS, in a process called the enterohepatic recirculation (66). This explanation is supported by the fact that low concentrations of PFAS, relative to serum levels, have been measured in human urine (67-71), and human fecal concentrations were non-detectable in a study of seven individuals (72) and low in a study of eight individuals (73), even though bile concentrations have been measured in relatively high concentrations (66, 68). Active transporters in the kidneys and intestines are believed to play a central role in these reabsorption processes. One key group of active transporters involved in PFAS movement is the Organic Anion Transporters (OATs), located at multiple sites in the human body and likely contributing to both the reabsorption and excretion of PFAS (74).

1.2.4 PFAS PHARMACOKINETIC MODELING

1.2.4.1 PFAS COMPARTMENT MODELS

One-compartment models have been extensively used to simulate PFAS pharmacokinetics in humans (75), as PFAS exhibit mono-exponential decay in serum (Figure 5). These models have been used for estimating serum half-life (65, 69, 76, 77), Vd (77, 78), clearance (66-68, 79), and daily intake (80).

Central to many of these models is the PFAS Vd, as it can convert serum concentrations into the total amount in the body. The PFOA and PFOS Vds estimated by Thompson et al. 2010 (78) are the most common estimates and have been widely used in other studies (76, 81-83). Thompson et al. 2010 estimated Vds for PFOA using drinking water and serum concentrations from Lubeck and Little Hocking, USA (C8 population); a PFOA half-life of 2.3 years (84); a gastrointestinal uptake of 0.91; and an assumed daily contaminated water intake of 1.4 L. Based on this, they calculated the PFOA Vd to be 170 mL/kg. They then assumed that the PFOS Vd was 35% higher, based on an animal study by Andersen et al. 2006 (85), which resulted in a Vd of 230 mL/kg (78).

However, these Vds have not been validated in human populations. This is because the total amount in the body is impossible to determine in real-world settings, where multiple exposure sources occur over extended periods, and total clearance has not been measured. The only human study that quantified both the total body amount and total clearance was an experimental case report, where a 67-year-old male ingested ^{13}C -labeled PFAS orally to measure absorption, Vds, urinary and fecal elimination, total clearance, and serum half-lives. In this study, Vds were 121 and 152 mL/kg for PFOA and PFOS, respectively (41).

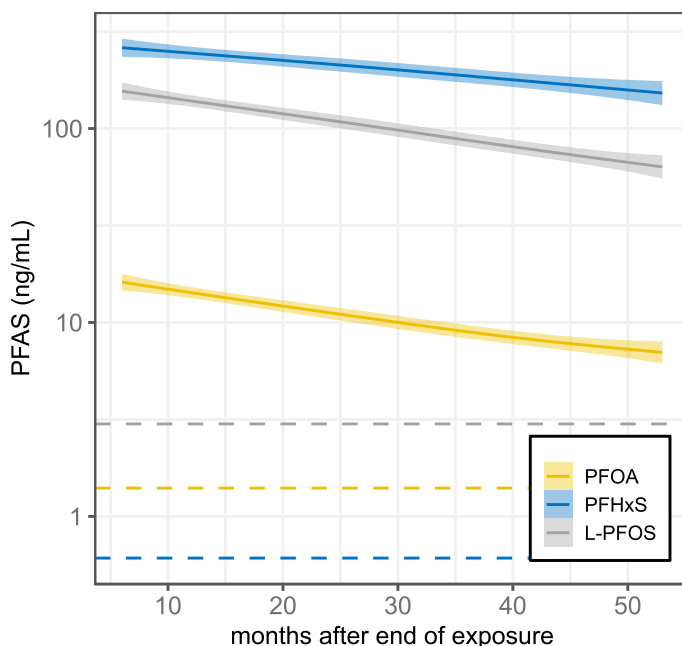


Figure 5 Mean PFAS serum levels several months after the end of drinking water exposure in Ronneby, Sweden. The solid lines and attached shaded areas represent smooth splines with 95% confidence intervals, respectively, for PFHxS, PFOA, and linear PFOS (L-PFOS). The dashed lines show the median PFAS levels in the reference group from Karlshamn, sampled in 2016. The data are from the Ronneby Panel Study ($n=114$), i.e., Paper I.

1.2.4.2 PFAS PBPK MODELS

Many of the PBPK models in humans are based on the Loccisano et al. 2011 model (Figure 3), but with the addition or optimization of specific parameters (83, 86-92). In these models, it was generally assumed that the only elimination of PFAS is through renal excretion, even though animal studies suggest that

the fecal excretion is also an important route (see section 1.2.3). In some studies, fecal elimination was included in the PBPK model but was considered negligible: Chou et al. 2019 set the fecal elimination rate to be 738 times lower than the urinary elimination rate (93), while Worley et al. 2017 set fecal elimination to be 620 times lower than urinary elimination (94).

Whether using one-compartment or PBPK models, it is crucial to have accurate estimates of the included variables (such as V_d or elimination rates) to develop models that can reliably predict the variables of interest, like serum concentrations from drinking water concentrations. Additionally, the pharmacokinetics of a compound is only relevant if there are pharmacodynamic effects, meaning if the compound has any health impacts.

1.3 PFAS HEALTH EFFECTS

Several government agencies, such as the European Food Safety Authority (EFSA), the Agency for Toxic Substances and Disease Registry (ATSDR), and the United States Environmental Protection Agency (US EPA), have compiled human epidemiological and experimental toxicological data to evaluate PFAS health effects, mainly focusing on PFOA and PFOS, the most used and studied compounds. The health effects identified by all reports include reduced childhood vaccine antibody response, lower gestational weight, increased serum cholesterol (a risk factor for cardiovascular disease), and elevated serum alanine transferase (a marker of liver damage) (95-98).

There are differences regarding the cancer assessment of PFAS. EFSA concluded that support for PFAS carcinogenicity was insufficient (95). However, since the EFSA report in 2020, several PFAS cancer studies have been published, and the US EPA concluded in 2024 that both PFOA and PFAS are “likely to be carcinogenic to humans” (97, 98). Moreover, the International Agency for Research on Cancer (IARC) categorized PFOA as “carcinogenic to humans” (Group 1) and PFOS as “possibly carcinogenic to humans” (Group 2B) in 2025 (99).

Other health outcomes, including thyroid function, liver disease, diabetes, kidney disease, as well as reproductive and developmental outcomes, have been studied and have yielded some, yet limited, evidence in humans (100).

Due to the health risks, the accumulative properties in humans, and the environmental persistence of PFAS, several PFAS have been regulated by the

Stockholm Convention on Persistent Organic Pollutants. Currently, PFOA, PFHxS, and other long-chained perfluoro carboxylic acids like PFNA have been classified as class A (meaning a recommendation to eliminate production and use), while PFOS has been classified as class B (recommending restrict production and use) (101, 102). These restrictions cover different salts and isomers of these compounds, as well as other PFAS that break down into the compounds in question (i.e., several polyfluorinated compounds). However, many PFAS compounds are still unregulated. Therefore, several national European authorities have submitted a PFAS restriction proposal to the European Chemicals Agency (ECHA), aiming to ban PFAS as a chemical group (103).

1.3.1 PFAS VACCINATION EFFECTS

The EFSA identified the effect of PFAS on childhood vaccination response as the primary adverse health concern, leading to the establishment of their total weekly intake (TWI) limit to prevent vaccine impairment (95). The evidence supporting this conclusion started with Grandjean et al. 2012, which reported significantly negative associations between PFAS exposure and antibody responses after childhood vaccinations for diphtheria and tetanus (104). Several subsequent studies have corroborated these findings across various vaccines (diphtheria, tetanus, *Haemophilus Influenzae*, Mumps, Rubella, and Measles) and vaccine types (toxoid, attenuated virus, polysaccharide-protein). Nonetheless, most research has been conducted in northern Europe (Table 1).

While there is ample evidence of PFAS effects on vaccine responses in children, the data for adults are more inconclusive, with fewer negative associations (Table 2). A challenge when studying adults is that they are rarely immunologically naïve, meaning the vaccination is usually not their first exposure. For instance, assessing the diphtheria-tetanus vaccine response from childhood vaccination programs is difficult in adults, as they may have had different numbers of previous doses depending on compliance, changes in vaccine policy over time, or additional booster doses. It is also difficult to study the influenza vaccine response, as there is significant cross-reactivity between different viral strains and vaccines, resulting in those who have had an influenza infection or vaccination previously have a more rapid and potent antibody response to new influenza strains or vaccines (105). Although a meta-analysis found a combined negative association between PFAS and vaccine response based on both childhood and adult studies (106), more research is needed to clarify vaccine responses in adults.

Table 1 Summary of PFAS childhood vaccination studies

Authors	Study design & population	Vaccination (type)	PFAS levels [ng/mL]	Main results (95% CI)
Grandjean et al. 2012 (104)	PC: 587 Faroese children followed from prenatal to year 5 and 7	DT (toxoid)	Age 5, GM: PFOS 16.7 PFOA 4.1	PFAS3: -49.4% (-66.7, -22.0) lower antibody levels per doubling of PFAS at 7 years
Granum et al. 2013 (107)	PC: 56 Norwegian children followed from birth to year 3	T (toxoid), Hib (polysaccharide-protein), Measles & Rubella (attenuated)	Maternal, median: PFOS 5.5 PFOA 1.1	Rubella: Negative associations (e.g., PFOA: -0.40 Optical Density per ng/mL), Measles: Borderline negative associations.
Mogensen et al. 2015 (108)	PC: 459 Faroese children from prenatal to year 7	DT (toxoid)	Age 5, median: PFOS 17.3 PFOA 4.1	PFAS3: ~50% decrease of joint diphtheria/tetanus per doubling of PFAS
Stein et al. 2016a (109)	CS: 1,191 aged 12-19 from NHANES 1999-2000 and 2003-2004	MMR (attenuated)	GM: PFOS 20.8 PFOA 4.13	Mumps: -7.4% (-12.8, -1.7) per doubling of PFOS Rubella: -13.3% (-6.2, -19.9) per doubling of PFOS
Grandjean et al. 2017a (110)	PC: 516 Faroese children follow to year 7 and 13	DT (toxoid)	Age 7, median: PFOS 15.3 PFOA 4.4	Diphtheria: ~25% decrease per doubling of PFAS Tetanus: non-significant, positive signals

Grandjean et al. 2017b (111)	PC: 349 Faroese children follow from prenatal to year 5	DT (toxoid)	18 months old, median: PFOS 7.1 PFOA 2.8	Tetanus: ~20% decrease per doubling of estimated PFOS, and PFOA based on breast milk intake.
Abraham et al. 2020 (112)	CS: 101 German children 1-year olds	DT (toxoid), Hib (polysaccharide-protein)	Mean breastfed (n=80); formula-fed (n=21): PFOS 15.2; 6.8 PFOA 16.8; 3.8	PFOA negative associations to anti-Hib, anti-D and anti-T, as well as IFN- γ for DT and IL-10 for PHA
Timmermann et al. 2020 (113)	PC*: 237 Guinea-Bissau children follow-up at 9 months 2 years old	Measles (attenuated)	Median: PFOS 0.77 PFOA 0.68	~10-20% decrease of antibody levels per doubling of PFAS
Timmermann et al. 2022 (114)	RC: 338 Greenlandic children follow-up at 7-12 years old	DT (toxoid)	Median: PFOS 8.68 PFOA 2.28	Diphtheria: -9% (-16, -2) per ng/mL PFOS, in subset with known vaccination doses (n = 169)
Zell-Baran et al. 2023 (115)	PC: 145 US children followed up at 4-8 years old	MMR (attenuated), varicella (attenuated)	Maternal median: PFOS: 2.6 PFOA: 1.1	Higher odds of "low" antibody titer per In- increase of PFAS, e.g., 1.77 (1.01, 3.09) for Measles and PFOA and 2.38 for Mumps and PFOA. Rubella & Varicella: no associations
Zhang et al. 2023 (116)	CS: 819 US adolescence aged 12-19 years from NHANES 2003-2004 and 2009-2010	MMR (attenuated)	GM: PFOS: 12.44 PFOA: 3.33	Rubella and Mumps: Negative associations with PFAS (~5 to 15% decrease per 2.7 fold increase), but not in the high folate tertile. Measles: no associations.

Sigvaldsen et al. 2024 (117)	PC: 880 Danish children, postnatal PFAS and follow-up at 18 months old	DT (toxoid), MMR (attenuated)	Median: PFOS 4.65 PFOA 2.44	Negative, but not statistically significant, associations.
Hong et al. 2025 (118)	348 US children follow-up after vaccination	MMR (attenuated)	Maternal median: PFOS: 4.0 PFOA: 1.6	Measles: Negative associations, yet several non-significant. Rubella: No associations. Mumps: Not studied.

PC = prospective cohort study, CS = cross-sectional study, RC = retrospective cohort study, DT = diphtheria & tetanus, MMR = measles, mumps, & rubella, HiB = *Haemophilus influenzae* type B, GM = geometric mean

*= nested cohort study within a randomized controlled trial (RCT).

Table 2 Summary of PFAS vaccination studies in adults

Authors	Study design & population	Vaccination (type)	PFAS levels [ng/mL]	Main results (95% CI)
Looker et al. 2014 (119)	PC: 411 C8 adults, serum sampled 21 days after vaccination	FLUVIRIN, trivalent influenza (attenuated)	Median: PFOS 9.1 PFOA 31.6	PFOA: Lower odds of protective ratio for A/H3N2, but not the other influenza strains PFOS: No associations
Kielsen et al. 2016 (120)	PC: 12 Danes age 23-66, multiple post-vaccination serum samples	DT (toxoid)	Median: PFOS 9.52 PFOA 1.69	Diphtheria: -11.9% (-0.33, -21.92) lower antibody levels per doubling of PFOS, similar for other PFAS.
Stein et al. 2016b (121)	PC: 78 Americans, age 21-49. Serum sampled pre-, 3 days post- and 30 days post-vaccination	FluMist, intranasal influenza (attenuated)	GM: PFOS 5.22 PFOA 2.28	Higher risk of seroconversion in higher PFAS tertiles, e.g., the highest PFOA tertile 6.8 (1.0, 48.1), although inconsistent.
Pilkerton et al. 2018 (122)	CS: 1,202 aged 19-49 and 1,012 aged 12-18 from NHANES 1999-2000 and 2003-2004.	MMR, studied only rubella (attenuated)	Mean: PFOS ~25 PFOA ~5	Rubella: Negative associations for both PFOA and PFOS in adults, but not in adolescent children.
Shih et al. 2021 (123)	PC: 281 & 399 Faroese aged 28, vaccination de novo (Twinrix) and booster doses (DT)	DT (toxoid), Hepatitis A and B (attenuated)	Median ^a : PFOS ~6-32 PFOA ~1-5	Limited evidence for associations between PFAS and antibodies, however, potentially sex-specific differences in response.

PC = prospective cohort study, CS = cross-sectional study, DT = diphtheria & tetanus, MMR = measles, mumps, & rubella, HiB = *Haemophilus influenzae* type B

^a = in Shih et al. 2021, median concentrations varied between the different samplings (age 0, 7, 14, 22 and 28)

1.4 COVID-19

In December 2019, the first cases of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) were identified in Wuhan, China, causing coronavirus disease 19 (COVID-19). Related to SARS and MERS, SARS-CoV-2 is a coronavirus thought to have originated in bats and then spread to humans (124). Within a few months, the disease spread worldwide, leading the World Health Organization (WHO) to declare a pandemic on March 11, 2020 (125).

By December 2020, less than a year into the pandemic, the first COVID-19 vaccine, Pfizer-BioNTech's Comirnaty BNT162b2, was developed. Moderna soon followed with Spikevax mRNA-1273 (126). Both vaccines are mRNA-based vaccines, containing mRNA for the SARS-CoV-2 Spike protein. The vaccine delivers the mRNA to human cells, instructing them to produce the Spike protein, which immune cells can then target to develop immunity (127). Besides the mRNA vaccines, there were other, conventional vaccines like the Johnson & Johnson vaccine. Within a few months in 2021, mass vaccination campaigns were underway worldwide.

Although vaccination efforts were swift and precise, over 778 million SARS-CoV-2 infections (128) and 7 million COVID-19 deaths (129) have been reported to the WHO at the time of writing this thesis. The death toll is likely underreported, as the global excess mortality during 2020 and 2021 has been estimated at 18.2 million people (130). The vaccines, which show great effectiveness and low risk of side effects, have been estimated to have saved over 1.6 million lives in Europe alone (131).

As societies relied on COVID-19 vaccines to return to normalcy, early concerns about impaired vaccine response due to PFAS were raised (132), based on the negative associations found in childhood vaccination studies (see section 1.3.1). These worries were strengthened by early studies linking PFAS exposure to higher rates of SARS-CoV-2 infection (133, 134) and COVID-19 severity (135, 136).

2 AIMS

This thesis consists of two separate, but related themes: **PFAS elimination** and **PFAS effects on COVID-19 vaccine response** in highly PFAS exposed adults.

The first study, Paper I, was a continuation of the previous serum half-life research in the Panel Study group in Ronneby, with a higher number of follow-ups and a broader range of PFAS compounds. The study aimed to estimate new serum half-lives and identify the determinants of these half-lives.

The second study, Paper II, examined elimination by measuring PFAS in feces and urine, with the aim of comparing the fecal and urinary elimination routes. During the comparison, it became evident that the total elimination did not align with the expected amounts based on previously reported Vds. As a result, a secondary aim for Paper II was to estimate new Vds.

With PFAS measured in serum, feces, and urine, Paper III aimed to investigate whether bile acid sequestrants (BAS) or the OAT inhibitor probenecid could enhance the slow elimination process. Paper III began with a short-term trial, comparing differences in fecal and urinary concentrations to assess intervention effects. Based on these results, a long-term trial focusing on serum concentrations was conducted.

Papers IV and V were based on the PFAS Immune Response after Vaccination against COVID-19 (PIRVACoV) cohort study, where 367 adults were vaccinated with the mRNA COVID-19 vaccine Spikevax (Moderna). The overarching aim was to assess if PFAS affected the vaccine response, measured by serum antibody levels (Paper IV), and T cell response, measured through cytokine production after stimulation (Paper V), 5 weeks and 6 months after vaccination. Initially, there was a plan to study long-term antibody and T-cell vaccine responses; however, the emergence of the Omicron SARS-CoV-2 variant, which could even infect vaccinated individuals, made it impossible to further evaluate the vaccine response.

In exploratory analyses from Paper IV, a sex-specific pattern was identified. Therefore, a secondary aim of Paper V was to determine whether similar sex-specific patterns could be observed in T cell responses.

The studies are all connected to each other through PFAS internal exposure in humans (Figure 6). However, the following chapters will be divided into two different, thematic chapters: PFAS elimination and PFAS effects on COVID-19 vaccine response.

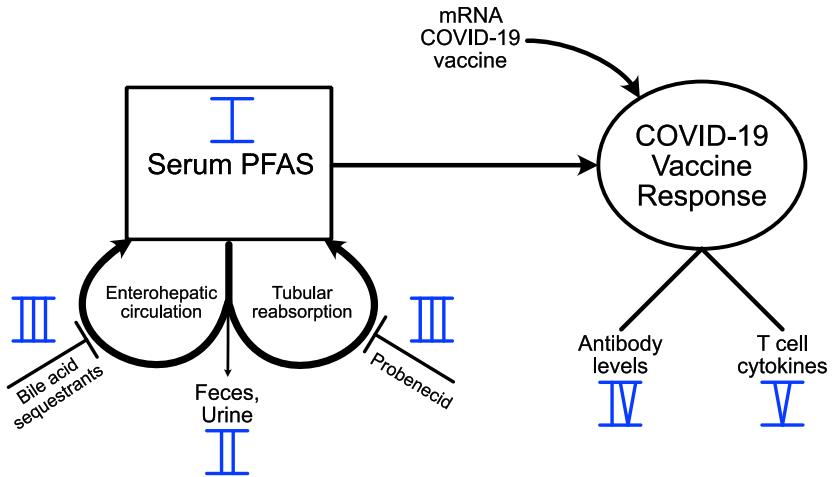


Figure 6 Flow chart, illustrating how the papers in this thesis relate to each other. The papers are referred to by Roman numerals.

3 PFAS ELIMINATION

3.1 SERUM HALF-LIFE & DETERMINANTS

Studies have identified correlations between higher serum levels and older age, greater consumption of contaminated water, male sex during fertile years, smoking, and, in some populations but not others, increased seafood and tea intake (19, 31, 35, 84, 137, 138). However, because PFAS serum levels accumulate over time and the data are cross-sectional, it is hard to determine causality – whether higher serum levels result from increased PFAS exposure, from reduced PFAS elimination, or from unadjusted confounding factors.

One approach to examine these questions is to identify determinants of serum half-life. However, studies on half-life and its determinants are less common than cross-sectional serum measurements because they require multiple serum samples from the same individuals. Existing studies show correlations between shorter serum half-life and female sex in fertile age (35), age (137, 139), lower consumption of public water and homegrown vegetables (84), and lower smoking (137). Still, other potentially important determinants have not yet been examined.

Our study in Ronneby (Paper I) estimated serum PFAS half-lives (Table 3) and identified several half-life determinants. Both younger age and female sex in fertile age were linked to shorter half-lives, supporting findings from cross-sectional and longitudinal studies. However, we also found that better renal function, indicated by higher estimated glomerular filtration rate (eGFR) and a greater proportion of PFAS in urine (compared to serum), lower gut permeability measured by fecal zonulin levels, and higher gut inflammation measured by fecal calprotectin levels, were associated with shorter serum half-lives.

Additionally, shorter serum half-lives were observed in the tertile with the lowest initial serum PFAS levels. However, since PFAS elimination occurred before the contamination was discovered, individuals with higher serum elimination rates would have started the study with lower serum concentrations. Throughout the study, we found that more than 66% of individuals who were above or below the median elimination rate (“fast” vs “slow” eliminators) remained above or below the median. In other words, differences in individual serum elimination rates tended to remain stable over

time, which could explain why lower initial elimination concentrations were linked to higher elimination rates.

A surprising finding was that serum half-lives increased over time. For example, the mean PFHxS half-life shifted from 3.85 years in the initial three samples (month 6-12 after the end of exposure) to 4.62 years in the last four samples (the final 2.5 years). The increase in serum half-lives is supported by a study of airport workers, where half-lives were significantly shorter than in other studies (e.g., PFHxS 2.8 years) shortly after the end of exposure (63). If serum half-lives actually change after exposure ends, then the assumption of mono-exponential decay used in one-compartment models would be inaccurate, implying that more complex models should be considered.

3.1.1 COMPARISONS WITH OTHER STUDIES

Many other half-life studies were conducted with limited study populations, such as only older men, making it impossible to examine determinants like sex or age. Additionally, extrapolating from these specific populations to broader groups, such as all humans, should be done with caution.

However, many of their half-lives were similar to the half-lives observed in Paper I subpopulations that resembled their study populations (Table 3). For example, Olsen et al. 2007 reported a high PFHxS half-life of 7.1 years in subjects aged 55-75 (26), which was much longer than the 4.5 years found overall in Paper I. However, it was quite similar to our data for the over-50 subpopulation, where the half-life was 6.4 years. In cases where there was not a perfect match, the remaining difference could depend on other serum determinants or ongoing PFAS exposure not being accounted for. In other words, variation between studies can partly be attributed to the specific characteristics of the study populations and settings. Nonetheless, Paper I was only able to explain 17-51% of the variance (R^2); further research on determinants is necessary for more accurate individual serum half-life predictions.

Table 3 Comparisons between apparent PFAS half-lives in other studies with subgroups within Paper I, depending on which subgroup the study population of the other studies mostly resemble.

	Study population	Mean HL (95% CI) in years	Corresponding Paper I strata	Mean HL (95% CI) in years
Paper I	114 men and women	PFOA: 2.5 (2.3, 2.7) PFPeS: 0.94 (0.86, 1.02) PFHxS: 4.5 (4.1, 5.0) PFHpS: 4.6 (4.1, 5.1) L-PFOS: 3.7 (2.6, 2.9) 1m-PFOS: 5.0 (4.6, 5.6) 2/6m-PFOS: 2.7 (2.5, 2.9) 3/4/5m-PFOS: 3.4 (3.2, 3.7)	-	-
Olsen et al. 2007 (26)	Retired workers, 24 males and 2 females, age range 55-75	GM: PFOA: 3.5 (3.0, 4.1) PFHxS: 7.1 (5.8, 9.2) PFOS: 4.8 (3.1-4.4)	>50-year-olds	PFOA: 3.4 (2.6, 4.8) PFHxS: 6.4 (5.0, 9.0) L-PFOS: 3.8 (3.1, 4.8) 1m-PFOS: 6.6 (5.0, 9.6) 2/6m-PFOS: 3.8 (3.2, 4.8) 3/4/5m-PFOS: 4.8 (3.9, 6.3)

Bartell et al. 2010 (84)	200 men and women, mean age 54.5	PFOA: 2.3 (2.1-2.4) years	15–50-year-olds; >50-year-olds	PFOA: 2.5 (2.0, 3.5); 3.4 (2.6, 4.8)
Brede et al. 2010 (139)	67 children, men, and mothers	PFOA: GM 3.26 without background adjustments	Whole study population	PFOA: 2.5 (2.3, 2.7)
Xu et al. 2020 (63)	17 airport workers, age range 24-62	PFOA 1.5 (1.2, 2.0) PFPeS 0.6 (0.5, 1.0) PFHxS 2.8 (2.1, 4.4) PFHpS: 1.35 (0.8, 5.7) L-PFOS: 1.7 (1.0, 6.0) 1m-PFOS: 0.9 (0.5, 4.8) 2/6m-PFOS: 0.7 (0.5, 1.8) 3/4/5m-PFOS: 0.8 (0.5, 2.8)	The first measurements (months 6 to 12)	PFOA 2.1 (1.8, 2.7) PFPeS 0.8 (0.7, 1.0) PFHxS 3.85 (3.15, 5.3) PFHpS: 3.65 (3.0, 4.95) L-PFOS: 2.5 (2.1, 3.0) 1m-PFOS: 4.6 (3.5, 6.3) 2/6m-PFOS: 2.6 (2.2, 3.15) 3/4/5m-PFOS: 3.3 (2.67, 4.08)
Nilsson et al. 2022a (28) Branched PFOS-isomers presented in Nilsson et al. 2022b (140)	~120 firefighters (97% males)	PFHxS: 6.0 (5.5, 6.5) PFHpS: 5.6 (5.1, 6.0) PFOS: 5.7 (5.2, 6.2) Non-adjusted: L-PFOS: 4.0 1m-PFOS: 11.5	Males 15-50; males >50	PFHxS: 5.4 (4.15, 7.7); 6.5 (5.0, 9.1) PFHpS: 5.4 (4.1, 8.0); 7.1 (5.3, 10.7) L-PFOS: 2.9 (2.4, 3.7); 3.7 (3.0, 4.6) 1m-PFOS: 6.1 (4.5, 9.5), 6.25 (4.8, 8.9) 2/6m-PFOS: 2.9 (2.4, 3.6); 3.7 (3.1, 4.6)

		6m-PFOS: 4.2 3/4/5m-PFOS: 7.5		3/4/5m-PFOS: 4.0 (3.2, 5.4); 4.7 (3.8, 6.1)
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The results from Paper I also support the hypothesis that PFAS is excreted through both the kidneys and intestines, as eGFR and urine/serum ratio are likely related to renal clearance, while gut permeability and inflammation are probably related to fecal clearance of PFAS. However, previous reviews (141) and PBPK models (see section 1.2.4) have assumed that fecal PFAS elimination in humans, even if present, is negligible. This contrasts with the fact that measured renal clearance in humans does not equal the estimated total clearance (25, 66, 67, 79). Consequently, there should be another major elimination route besides the urinary route – what are the main elimination routes in humans? Is fecal elimination one of them?

3.2 ELIMINATION ROUTES

3.2.1 RENAL ELIMINATION

Several studies in humans have measured urinary concentrations and estimated renal clearance of PFAS (Table 4). These studies have different strengths and weaknesses. Two studies measured PFAS in 24-hour urine samples, which enabled estimations of renal clearance based on actual daily urine volumes. However, these studies were small (20 and 10 individuals, respectively) (67, 68), making their results susceptible to random day-to-day or individual variation in renal clearance. The other studies collected spot urine samples from larger study populations but relied on assumed daily urine volumes of 1.4 L for men and 1.2 L for women to estimate renal clearance (25, 69, 70, 142).

In Paper II, we also collected spot urine samples but estimated renal clearance through creatinine adjustment instead of assuming daily urine volume. We achieved this by first normalizing the urine samples using urine creatinine concentrations and then estimating each individual's daily creatinine excretion based on the equation by Forni Ogna et al. 2015 (143). The results were similar to those from other studies, especially the ones using 24-hour urine (Table 4).

The mean and median renal clearance were also similar when the study population was extended from 147 to 393 urine samples (“Extended Paper II”,

Table 4). In Paper II, we limited the study population to the 147 individuals who provided both urine and fecal samples. However, 275 subjects had provided up to three urine samples each, totaling 393 samples. The consistency in results when using data from both 147 and 393 samples enhances the overall estimates, as the larger sample size increases statistical power.

Similarly, the mean and median renal clearance were comparable when estimated using the same assumption as the other spot urine studies, namely a daily urine production of 1.4 L for men and 1.2 L for women (Table 4). This “Modified” method generated variation compared to the creatinine-based estimations at the individual level, but the mean was only 11% higher. Furthermore, the variation seemed unbiased, as shown by a Bland-Altman plot (Figure 7), which showed an even distribution (i.e., a rectangular shape), with no apparent difference between the PFAS compounds. The results were similar even when urine concentrations were not density-adjusted (results not shown). In other words, while individual estimates vary between the methods (as shown by Figure 7), the mean and median values were quite similar (Table 4).

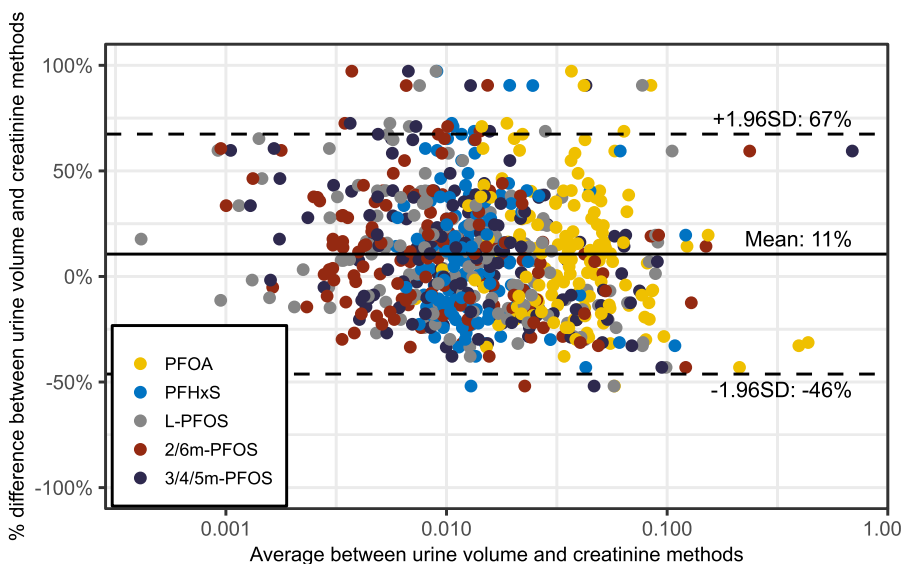


Figure 7 Bland-Altman plot comparing clearance estimated using assumed urine volume (with density adjustments) and estimated daily creatinine production. Renal clearance based on daily urine volumes was a mean 11% higher than renal clearance based on estimated daily creatinine production. Based on data from Paper II.

Table 4 Renal PFAS clearance in different studies, using different methods

Study	Study population	Urine sample	How spot urine is converted into clearance	Renal clearance (mL/day/kg)							
				PFHxS		PFOA		PFOS			
				Mean	Median	Mean	Median	Mean	Median		
Harada et al. 2005 (67)	10 females	24h	-	-	0.033	-	-	0.019	-		
	10 males		-	-	0.027	-	0.012	-			
Fuji et al. 2015 (68)	10 adults	24h	-	-	0.044	-	-	-	-		
Zhang et al. 2013 (69)	20 young females	Spot	Urine volume excreted per day males: 1.4 L/day females: 1.2 L/day	0.039	0.033	0.29 ^a	0.15 ^a	0.045 ^a	0.038 ^a		
	66 males and older females			0.027	0.015	0.79 ^a	0.18 ^a	0.031 ^a	0.019 ^a		
Zhou et al. 2014 (70)	9 controls	Spot	Urine volume: same as Zhang et al. 2013	6.47	6.47	0.146	0.121	0.026 ^a	0.016 ^a		
	39 fishery employees			0.014	0.012	0.075	0.061	0.015 ^a	0.008 ^a		
	7 fishery family members			0.009	0.006	0.065	0.079	0.016 ^a	0.015 ^a		

Gao et al. 2015 (142)	69 industry workers	Spot	Urine volume: same as Zhang et al. 2013	0.08	0.03	0.21	0.07	0.02 ^a	0.01 ^a
Fu et al. 2016 ^b (25)	46 industry workers	Spot	Urine volume: same as Zhang et al. 2013	0.042 ^a	0.031 ^a	0.11 ^a	0.08 ^a	0.025 ^a	0.012 ^a
Paper II	147	Spot	Daily creatinine	0.015	0.011	0.047	0.034	0.020 ^a	0.011 ^a
Modified			Urine volume: same as Zhang et al. 2013 ^c	0.016	0.012	0.046	0.038	0.019 ^a	0.012 ^a
Extended Paper II	275, providing 393 samples	Spot	Daily creatinine	0.016	0.012	0.046	0.037	0.022 ^a	0.013 ^a
Modified			Urine volume: same as Zhang et al. 2013 ^c	0.019	0.014	0.051	0.042	0.025 ^a	0.014 ^a

^a = linear isomer is presented; clearance of branched isomers was also estimated. ^b = median and mean values calculated from supplementary material. ^c = urine concentrations were density-adjusted prior to clearance estimations using assumed urine volumes.

Although the assumed daily urine volume method and the estimated daily creatinine method produced similar results in the Ronneby data, they may be less accurate in other spot urine studies. These other studies have used urine concentrations without density or creatinine adjustments. Such unadjusted concentrations depend on factors like fluid intake (since urine becomes more diluted with increased fluid intake) (144) and the timing of sampling (as morning urine is generally more concentrated) (145). Additionally, daily urine volume can vary between populations, possibly due to variations in overall fluid intake. Therefore, adjusting for urine density or urine creatinine could likely improve the accuracy of renal clearance estimates in the other studies.

Although variations in body mass might theoretically influence daily urine volumes, both the Zhou et al. 2014 population (70) and the Fu et al. 2016 population (25) had comparable body masses (mean weights of 68 kg and 62 kg, respectively) to the Italian population (146) from which the urine volumes were obtained (mean weight of 68 kg).

3.2.2 FECAL ELIMINATION

Although several studies have measured PFAS in urine and calculated renal clearance, fecal PFAS measurements have been conducted less frequently. Even when measured, the fecal data were either not coupled with urine measurements (72, 73) or were at too low concentrations for both renal and fecal clearance within the same compound (41). As a result, comparisons between fecal and renal elimination were not possible.

Paper II was not only the first larger study to measure fecal concentrations and estimate fecal clearance from them, but also the first to compare fecal and urinary elimination. We found that PFAS is present in feces, and that fecal elimination is the main route for linear and branched PFOS isomers (Figure 8).

Interestingly, our study is supported by studies showing higher liver-to-serum concentrations (49, 50, 52) and higher bile-to-serum concentrations (66) for PFOS compared to PFOA and PFHxS. It is therefore reasonable that we observed higher fecal concentrations of linear and branched PFOS.

The most notable difference between fecal PFOS and PFOA appears in cattle – while PFOA was rapidly excreted within 28 days through urine (60), PFOS was distributed to the liver and bile, slowly excreted via feces, and barely excreted through urine at all (61).

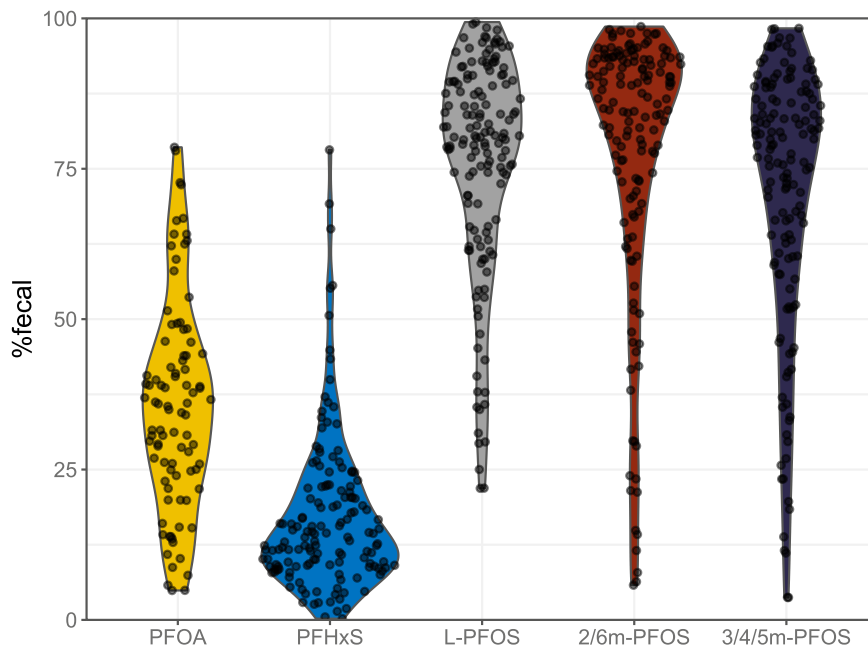


Figure 8 Percent of the total elimination that was fecal. Dots represent individual ratios between fecal and the sum of fecal and urinary elimination. Modified from Paper II, with PFHxS measurements included. Republished with permission.

3.2.3 DETERMINANTS FOR CLEARANCE

Similarly to Paper I, we explored several covariates as potential determinants for fecal and renal clearance in Paper II. Unlike Paper I, all estimates of these covariates in Paper II were close to zero with wide confidence intervals.

Both high fecal calprotectin and low eGFR levels were associated with shorter half-lives and higher fecal and renal clearances, respectively, indicating that the shorter half-life might result from higher elimination through feces and urine. Interestingly, the same pattern was not seen with fecal zonulin, as higher fecal zonulin was associated with longer half-lives and higher fecal clearance (Table 5). The reason behind this is unknown, whether it is spurious results or bias due to unmeasured confounding.

Table 5 Comparisons between fecal calprotectin, fecal zonulin and estimated glomerular filtration rate (eGFR) as determinants for serum half-life (Paper I) and fecal and renal clearance (Paper II)

		PFOA		L-PFOS		2/6m-PFOS		3/4/5m-PFOS	
		% diff	P	% diff	P	% diff	P	% diff	P
Fecal calprotectin	Half-life	-25	0.02	-6	0.35	-6	0.40	-7	0.35
	Fecal CL	20	0.22	9	0.29	36	0.05	33	0.07
eGFR	Half-life	-22	0.05	-12	0.13	-16	0.03	-18	0.04
	Renal CL	9	0.66	42	0.14	29	0.32	21	0.47
Fecal zonulin	Half-life	8	0.53	9	0.14	9	0.11	15	0.06
	Fecal CL	8	0.59	12	0.17	4	0.82	30	0.10

All covariates were dichotomized (above vs. below): calprotectin at 50 ng/kg, zonulin at the median, and eGFR at 90 mL/min/1.73 m² for half-life and 60 mL/min/1.73 m² for renal clearance. CL = clearance, eGFR = estimated glomerular filtration rate based on the CKD epi 2021 equation with serum creatinine and cystatin C (147).

3.2.4 TOTAL ELIMINATION

PFAS is therefore excreted through both urine and feces in humans. In Paper II, we also estimated the daily average blood loss for menstruating women (women under 51 years without hormonal contraception were assumed to excrete 30 mL of blood each month) and blood donors (assuming they donate 450 mL of blood twice a year). Assuming that the measured clearances from urine, feces, menstruation (148), and blood donation (149, 150) equal the total clearance, it can be compared to the estimated total clearance:

$$CL_{tot_{obs}}/CL_{tot_{est}} = \Sigma(CL_{renal}, CL_{fecal}, CL_{blood\ loss})/k \cdot V_d$$

Using V_ds at 170 mL/kg for PFOA (Thompson et al. 2010 (78)), 230 mL/kg for PFOS (Thompson et al. 2010 (78)), and 250 mL/kg for PFHxS (data from Sundström et al. (151), used by Chiu et al. (77)), our observed total clearance from Paper II is approximately 50% of the expected total clearance for PFOA and PFOS, and even lower for PFHxS (Figure 9).

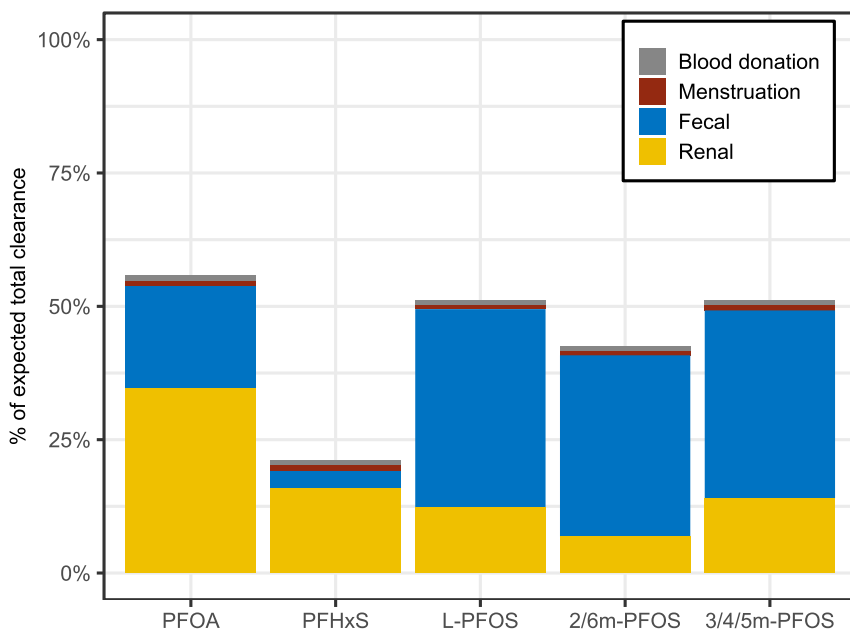


Figure 9 The mean of the ratios between expected and observed total PFAS clearance in individuals from Ronneby, Sweden, grouped by elimination route. Based on data from Paper II.

In other words, it appears that the Ronneby data either lack elimination routes or the existing ones are underestimated, assuming that the Vds and serum elimination rates (k) are accurate. If an elimination route is missing, what could it be?

PFAS have been detected in the hair and nails of populations with background exposure. The concentrations in hair ranged from a mean of 0.046 to 0.69 ng/g for PFOA and from below the limit of detection to 1.06 ng/g for PFOS. In nails, the levels ranged from a mean of 0.24 to 1.75 ng/g for PFOA and from 1.04 to 44.7 ng/g for PFOS (141). However, these concentrations should reflect small daily elimination rates, as only a minor amount of hair and nails is produced daily. Additionally, the authors note that external exposure to the nails and hair could have influenced the measured concentrations, despite the use of washing techniques (141).

PFAS are transferred over the placenta (152-154) and into breast milk (155-158), exposing the fetus/newborn while increasing elimination from the

mother. While this could influence the serum elimination rates, the Vd calculations varied little when women of fertile age were excluded (Paper II).

Finally, a study of 20 background-exposed individuals found no evidence that sweat, provoked by sauna exposure or exercise, contained any significant amount of PFAS (159).

In summary, it is unlikely that a major elimination route is missing. Instead, several factors could be biased: the clearance estimations in Paper II may be underestimated; the serum elimination rates may be overestimated; and/or the assumed Vds from previous studies may be overestimated.

3.3 NEW VOLUME OF DISTRIBUTIONS

In Paper II, we estimated Vds from observed total clearance instead of estimating total clearance from assumed Vds. This yielded substantially lower Vds compared to the Vds from Thompson et al. 2010 (Table 6). Albeit still lower, the Vds generated from Ronneby were more similar to those measured by the case report of Abraham et al. 2024 (41), where radiolabeled PFAS were ingested orally and Vds were estimated from serum concentrations after absorption and distribution . However, since the study by Abraham et al. 2024 was a case report on only one individual, it is unclear where the measured Vds fall within the population distribution of Vds.

Table 6 Volume of distribution estimated in Paper II, compared to the case-study by Abraham et al. 2024 and the commonly used Vds from Thompson et al. 2010.

PFAS	Paper II			Abraham et al. 2024 (41)	Thompson et al. 2010 (78) & Chiu et al. (77)
	N	Mean	Median (P10, P90)	Point estimate (range)	Estimated from drinking water concentrations
PFOA	36	96	74 (48, 176)	121 (118-124)	170
PFHxS	40	54	39 (23, 79)	125 (122-128)	250
L-PFOS	40	116	93 (52, 192)	152 (148-NA) ^a	230 ^a
2/6m-PFOS	40	96	85 (31, 184)		
3/4/5m-PFOS	40	119	91 (44, 230)		

^a= only Vd for total PFOS was estimated.

3.3.1 BIOLOGICALLY PLAUSIBLE VDS?

Since a compound's Vd depends on its chemical properties and its affinity to organs other than plasma (38), comparing the chemical properties and distribution of PFAS to other compounds can help evaluate whether the Vds from Paper II were plausible.

Several compounds have Vds around 100 mL/kg, similar to our estimates. A compound with a Vd of about 70 mL/kg is the anticoagulant heparin, which is mostly confined to the plasma volume (160). However, the Vds for PFAS should be larger than the plasma volume, as PFAS have been measured in other tissues such as liver and kidney tissue (50, 53). Furthermore, PFAS are highly bound to plasma proteins (42), and compounds bound to plasma proteins cannot have Vds lower than that of the plasma proteins, which are approximately 100 mL/kg (37). One example of a medication that is 99% bound to plasma proteins is warfarin, another anticoagulant. Warfarin has a Vd of 10 L (~140 mL/kg) (161). Other examples are nonsteroidal anti-inflammatory drugs, with up to 99% plasma protein binding and Vds as low as 100 mL/kg (162). In summary, there are examples of medications with similar Vds as estimated in Paper II, yet below 100 mL/kg is uncommon and should be treated with caution.

There are other aspects of the Vds estimated in Paper II that should also be treated with caution. First, the variability of our Vd estimations is wide and likely not due to interindividual variation, with 10th to 90th percentile values ranging from 48 to 176 mL/kg for PFOA and 52 to 192 mL/kg for L-PFOS. Instead, the variability was likely a product of methodological errors, such as measurement errors. Additionally, since PFAS does not accumulate in adipose tissue (52, 55), it may be better to use Vds per lean body mass instead of per total body mass (to get mL/kg body weight). This has been suggested for hydrophilic medications (163). The risk of using total body mass is that the Vds are underestimated in overweight and obese individuals with high fat percentage, shifting the Vds downwards.

3.3.2 THE PFHXS PROBLEM

In Paper II, we only presented the PFOA and PFOS results, although PFHxS was measured in all three media (serum, urine, and feces). We excluded the PFHxS data because the median Vd calculated for PFHxS was only 39 mL/kg, which is significantly below the plasma volume and therefore biologically implausible.

Given that $Vd = CL/k$, we considered whether the serum elimination rate (k) of PFHxS was too high or the total clearance (CL) of PFHxS was too low in our Vd estimation. The method for k was based on repeated serum samples, which are more robust than spot fecal concentrations. Therefore, we primarily suspected that the fecal concentrations were biased downwards.

While there is some evidence for the fecal elimination of PFHxS to be lower than PFOS and PFOA, it is unlikely to be at the magnitude shown in Paper II. The other study that measured fecal PFHxS and PFOS found similar fecal concentrations, and nearly twice as high serum levels for PFHxS compared to PFOS. This resulted in the fecal to serum ratio for PFHxS being approximately half that of PFOS. The study was small ($n=8$), and many fecal PFAS concentrations were below the detection limit (1 ng/g dry weight) (73). In contrast, the fecal to serum ratios in Paper II was more than 10 times lower for PFHxS than for PFOS and PFOA, illustrated by the PFHxS shift in Figure 10.

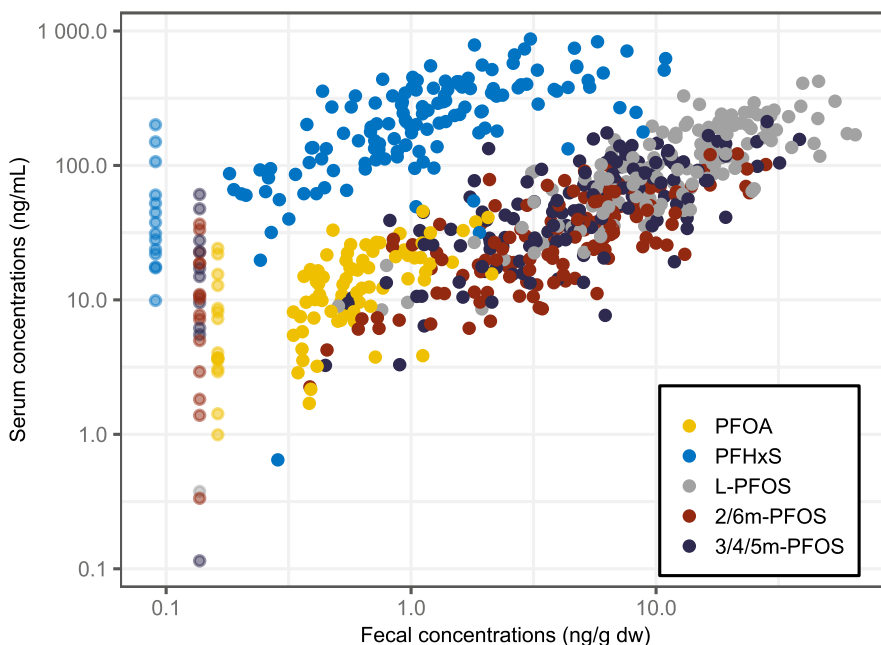


Figure 10 Fecal concentrations compared to serum concentrations of different PFAS in 147 individuals from Ronneby, Sweden. PFHxS is different from PFOA and the PFOS isomers, with a low fecal to serum ratio. Note the logarithmic scales. The slightly transparent values to the left in the figure are values below the limits of detection (LOD), presented as $LOD/2$. Based on data from Paper II.

A somewhat lower fecal excretion of PFHxS is also supported by human autopsy studies showing PFHxS at lower concentrations in the liver compared to the kidney or blood, while PFOS and PFOA had higher liver-to-kidney ratios (50, 53). Likewise, PFHxS has been measured in liver concentrations 50 times lower than PFOS, even though blood concentrations were only two times lower. However, the liver and blood samples were from different individuals, so the exact numbers should be treated with caution (51). If less PFHxS is present in hepatocytes, then less can be excreted in bile and, subsequently, in feces. However, it could also be that the lower PFHxS liver concentrations instead indicate that the PFHxS that end up in the liver gets excreted rather than accumulated.

Nevertheless, as the Vd estimations for PFHxS were not biologically plausible, we did not trust the PFHxS results and decided not to report them in Paper II.

3.4 DRUGS ENHANCING PFAS ELIMINATION

Regardless of the actual volume of distribution or total clearance, there is ample evidence that serum half-lives in humans are long and that high serum concentrations indicate long internal exposures, leading to high cumulative exposure over time. For this reason, there have been many efforts to identify interventions that could increase elimination rates, such as phlebotomy and plasma donation (149, 150), hemodialysis (164), microbiome supplementation (165, 166), and dietary fiber intake (167, 168).

A promising medical intervention for increasing PFAS elimination is the use of bile acid sequestrants (BAS), e.g., cholestyramine and colesevelam. As the name indicates, BAS bind to bile acids and sequester them, preventing their enterohepatic recirculation and resulting in fecal excretion (169). BAS have been used to treat high cholesterol levels because the liver converts cholesterol into bile acids to replace those lost through BAS.

In early rat experiments, cholestyramine caused an increase in fecal PFOA and PFOS levels, while reducing liver concentrations (170). Almost 30 years later, Genuis et al. were the first to replicate the results in humans in one (171) and eight (73) subjects. Møller et al. 2024 strengthened the evidence further by administering cholestyramine to 43 highly PFOS-exposed individuals, resulting in a 63% reduction in serum PFOS levels over 12 weeks, compared to only a 3% decrease during the 12 weeks without the intervention (33). The decreases in serum PFHxS and PFOA were also faster with cholestyramine

(19% and 22% with cholestyramine compared to 3.5% and 2.4% without), but the effect sizes were smaller than those of PFOS. Following Møller et al. 2024, a study of Australian firefighters found an annual decrease of only 36% for PFOS and 29% for PFHxS with cholestyramine (172). However, the individual daily dose ranged from 4 to 12 g, making it hard to accurately assess the intervention effect. Additionally, the mean baseline serum half-lives for the control group were lengthy, at 7.3 years for PFOS and 9.4 years for PFHxS (172). This may indicate ongoing exposure, which could lessen the apparent impact of the intervention.

Another candidate medical intervention to enhance PFAS excretion is the OAT inhibitor probenecid. Probenecid, originally used to treat gout, inhibits several cellular transporters in the kidneys (74). These transporters include those that can transport PFAS, such as Organic Anion Transporter 1 (OAT1), OAT3, OAT4, Urate transporter 1 (URAT1), and Organic Anion Transporting Polypeptides (OATPs) (173-178). Some of these, like OAT1 and OAT3, transport molecules from blood to urine and mediate the excretion of PFAS. In contrast, OAT4, URAT1, and OATPs transport molecules from the urine back to the blood and mediate the reabsorption of PFAS present in urine (74). While probenecid inhibits renal clearance of PFAS in rats (57), the effect of inhibiting both excreting and reabsorbing transporters with probenecid has not been tested in humans. However, two cross-sectional studies showed conflicting results – the first found slightly higher serum PFAS levels (179), while the second observed slightly lower serum levels (180), in probenecid users compared to non-users.

Therefore, in Paper III, we conducted a short-term, one-week cross-over experimental trial to evaluate the effects of cholestyramine and probenecid on fecal and urinary PFAS levels, respectively. In this short-term trial, we corroborated the elimination-enhancing effect of cholestyramine, with significantly higher fecal concentrations during the cholestyramine week compared to the control (left panel of Figure 11). In contrast, we found no increased urinary PFAS concentrations during the probenecid intervention (right panel of Figure 11), implying that the broad inhibition of kidney transporters did not lead to increased PFAS excretion.

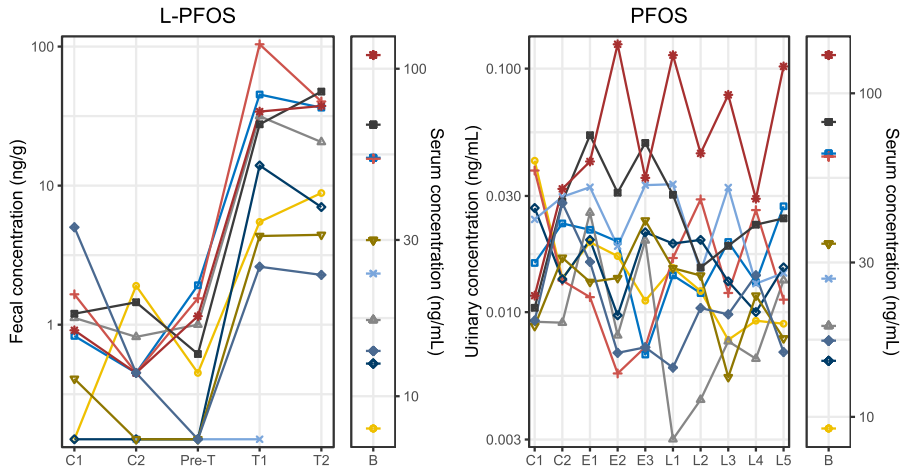


Figure 11 Before and during cholestyramine intervention (left panel) and probenecid intervention (right panel) for linear PFOS (L-PFOS) and total PFOS, respectively. Each line represents one individual's repeated measurement in feces and urine, respectively. Note the high increase in fecal concentrations with cholestyramine, compared to the similar urine concentrations with probenecid. C = control samples, T = cholestyramine treatment, E = early probenecid samples, L = late probenecid samples, B = baseline. From Paper III, cropped versions of figure 2 and 3. Republished with permission.

We also conducted a long-term, 12-week cross-over experimental trial, with the BAS colesevelam as the sole intervention. The exclusion of probenecid was based on interim analyses showing no increased elimination. The switch from cholestyramine to colesevelam was, however, due to participant experience — cholestyramine is a powder that, when mixed with water, forms a distasteful solution, which half of the study participants were not willing to take for 12 weeks. Colesevelam tablets were, therefore, considerably easier to ingest.

In the long-term trial, serum PFHxS, PFOS, and PFOA decreased by 14%, 38% and 11%, respectively, during the 12-week colesevelam intervention, significantly higher than the corresponding control periods (Figure 12).

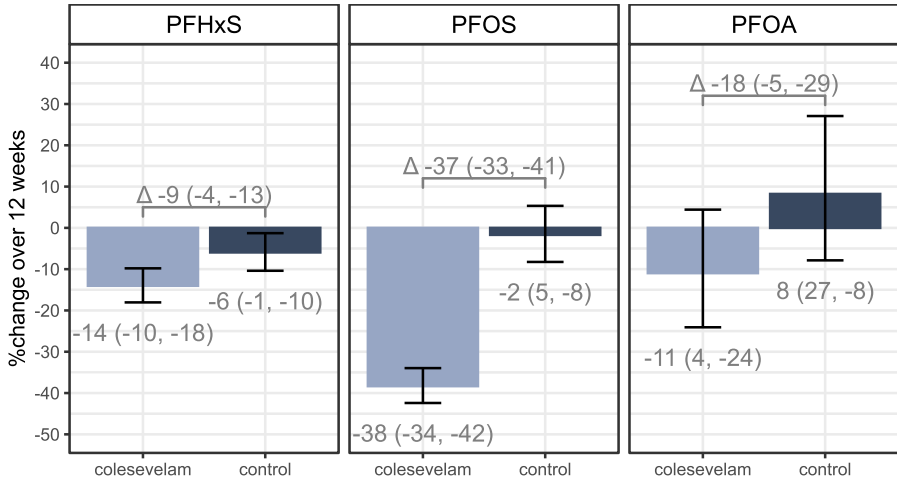


Figure 12 Percentual change in serum PFAS concentrations after 12 weeks of colesevelam versus 12 weeks of no intervention (control). The numbers below the bars correspond to the intervention effect, while the numbers above represent the modeled difference between colesevelam and control. Point estimates and 95% confidence intervals in parentheses were derived from linear mixed-effects models. From Paper III, republished with permission.

The colesevelam effect was consistently lower than the cholestyramine effect shown by Møller et al. 2024 (33), possibly due to differences in dose frequency (cholestyramine was administered three times a day, while colesevelam was only taken twice daily), differences in PFAS-binding between the compounds, or other variations between the study populations. However, the colesevelam effect was higher than the cholestyramine effect in Delaere et al. 2025 (172), possibly because of lower cholestyramine doses administered (between 4 and 12 g).

While the effect of colesevelam was lower than that of cholestyramine in Møller et al. 2024 (33) and higher than that of cholestyramine in Delaere et al. 2025 (172), the finding that PFOS was more readily excreted with BAS than PFOS and PFHxS remains consistent across the studies. This is biologically plausible, as it relates to the higher PFOS concentrations in the liver than PFOA and PFHxS (similar to the higher fecal elimination of PFOS, discussed in sections 3.2.2 & 3.3.2).

4 PFAS EFFECTS ON COVID-19 VACCINE RESPONSE

The COVID-19 pandemic, a worldwide natural experiment, created a situation where numerous immunologically naïve adults received novel vaccines within a narrow time frame. This was previously a rare setting for PFAS research. For other viruses or bacteria, most adults had either been immunologically exposed through vaccination programs or infection, such as with past influenza strains. This new scenario offered a unique chance to accurately assess the effect of PFAS on adult vaccine response.

Vaccinations aim to induce immunity, that is, immunological memory, which depends on the adaptive immune system. This system includes various cell types: B cells, which produce antibodies that neutralize pathogens or prepare them for other immune cells; CD4⁺ T cells, which secrete cytokines to support other immune cells; and CD8⁺ T cells, which directly destroy infected cells (181, 182). While it is possible to collect, count, and categorize subtypes of B cells, it is more common and easier to assess B-cell activity indirectly by measuring antibody levels in serum or plasma. The antibodies can either be specific to the antigen of interest, such as the SARS-CoV-2 spike protein, or the total amount can be measured. Similarly, cytokines secreted by T cells after stimulation with the antigen can be measured instead of counting and categorizing the T cells themselves.

4.1 SERUM ANTIBODIES

Five studies have evaluated PFAS on COVID-19 vaccination anti-spike antibody response (Table 7). Two studies were conducted in the general population, as shown by the low background PFAS levels (183, 184). Even though Porter et al. 2022 (185) and Bailey et al. 2023 (186) included individuals with elevated PFAS exposure through occupational and drinking water exposures, respectively, their median and geometric mean PFAS serum levels were only slightly elevated and would be regarded as background exposure populations 20-30 years ago (20, 22-24). While Porter et al. 2022 (185) included some individuals with very high PFOS, PFOA, and PFHxS levels (>100 ng/mL), our Paper IV had both higher median levels and higher 95th percentiles. In other words, the studies covered a wide range of PFAS exposure levels.

The main results of these five studies, regardless of the PFAS spectrum, reported no associations between PFAS exposure and anti-spike antibody response (illustrated by Figure 13, where different exposure groups in Paper IV had similar antibody levels 5 weeks and 6 months after vaccination). Although Porter et al. 2022 (185) noted a borderline significant -3.45% (95%CI: -7.03, 0.26) change in IgG per IQR PFOS, this was not replicated in the other studies. Porter et al. (185) had a completely different vaccination design, with nine different “antigenic stimulus groups,” defined as various combinations of 1-2 doses of Moderna, Pfizer, or J&J vaccination, or SARS-CoV-2 infection. Even though these groups were adjusted for, the complexity of the study design could introduce spurious associations.

Timmermann et al. 2024 (184) also reported a borderline significant association, with an -802 BAU/mL (95% CI: -1812; 208) difference between the second and third vaccination per doubling of PFAS. However, these results could be due to regression to the mean, as the median antibody levels after the second vaccination were 436 BAU/mL (95% CI: -669; 1541) higher per doubling of PFAS, which then reverted to null after the third vaccination. Additionally, neither Bailey et al. 2023 nor Paper IV, which both had several follow-ups, could replicate these results.

One key difference between studies is how information on SARS-CoV-2 infections was collected. While all studies collected self-reported data on previous infection, some (Bailey et al. 2023, Timmermann et al. 2024, and Paper IV) also measured pre-vaccination anti-spike antibody levels, and a few (Bailey et al. 2023 and Paper IV) tracked anti-nucleoid antibodies continuously throughout the trial. Anti-nucleoid antibodies are specific biomarkers for SARS-CoV-2 infection, as the mRNA vaccines only contain mRNA for the spike protein. Using anti-nucleoid antibodies, we identified and excluded infected individuals who had not reported SARS-CoV-2 infection, likely because they were asymptomatic.

There were also differences in how infections were handled in the statistical analysis. While SARS-CoV-2 infection cannot be considered a confounder, as it is not a source of PFAS exposure, it should be considered as an important source of anti-spike antibodies and thus of outcome variation (see section 5). Therefore, some statistical considerations for SARS-CoV-2 infection are reasonable to reduce variation and to increase statistical power. Two of the studies (Porter et al. 2022 and Bailey et al. 2023) adjusted for SARS-CoV-2 infection, while the other two excluded infected individuals (Hollister et al.

2023 and Timmermann et al. 2024). In Paper IV, we used both approaches, with no associations regardless of method.

It should be noted that a pre-vaccination immune response does not necessarily mean a prior SARS-CoV-2 infection, as there have been reports of non-specific antibody and T cell responses against the SARS-CoV-2 virus, probably produced from a previous encounter with a common coronavirus (182, 187, 188). However, a previous immune response would still introduce variation, and arguments can thus be made to exclude individuals from the main analyses.

In summary, despite differences in study design, study populations, and statistical methods, such as how SARS-CoV-2 infection was handled, no negative associations were observed. This contrasts with the childhood vaccination studies (section 1.3.1).

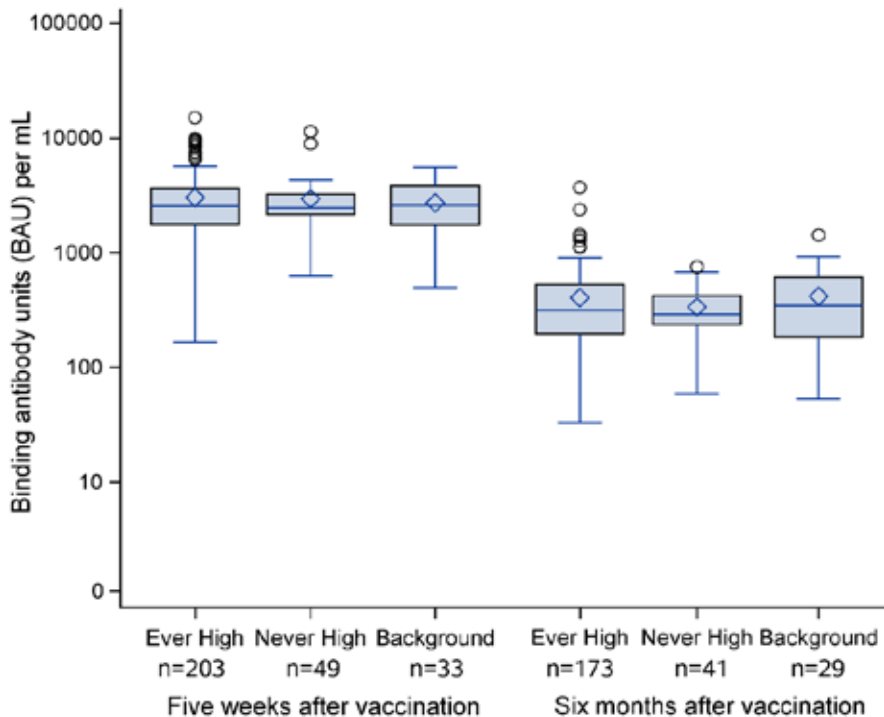


Figure 13 Box plot showing similar serum antibody levels five weeks and six months post COVID-19 vaccination in non-infected individuals from three exposure groups: those who lived with contaminated drinking water at home (Ever high), those who lived or worked in Ronneby, but did not have contaminated drinking water at home (Never high); and background exposed individuals from the neighboring municipality of Karlshamn (Background). Republished with permission from Paper IV.

Table 7 PFAS COVID-19 vaccination studies that measure serum antibody levels

Authors	Study design & population	Vaccination	PFAS levels [ng/mL]	Main results (95% CI)
Porter et al. 2022 (185)	PC: 415 current and retired workers at 3M, USA	1-2 doses of Moderna, Pfizer BioNTech or J&J	Median PFOS: 7.46, PFOA: 1.63 PFHxS: 2.20	Borderline significant, negative associations, e.g., -3.45% (-7.03, 0.26) IgG per IQR PFOS.
Bailey et al. 2023 (186)	PC: 226 aged 12-90 from Michigan, USA	Pfizer BioNTech/Moderna, 2 doses	GM PFOS: 10.49 PFOA: 3.90	No associations between PFAS and vaccination effect
Hollister et al. 2023 (183)	PC: 860 health-care workers aged 21-79 from several US states	564 Pfizer BioNTech, 296 Moderna, 2 doses	Median PFOS: 3.3 PFOA: 1.1	No associations between PFAS and vaccination effect
Timmermann et al. 2024 (184)	PC: 371 Danish adults age 50-69	Pfizer BioNTech, 3 doses	Median PFOS: 5.32 PFOA: 1.08	No associations, possibly steeper decrease between second and third vaccination: -802 BAU/mL (-1812, 208) per doubling of Σ PFAS.
Paper IV	PC: 367 adults age 20-60 from Ronneby, Sweden	Moderna, 2 doses	Median PFOS: 36 PFOA: 2 PFHxS: 34	No associations between current, historic or prenatal PFAS exposure and antibody levels.

PC = prospective cohort study, GM = geometric mean, IQR = interquartile range, BAU = binding antibody unit

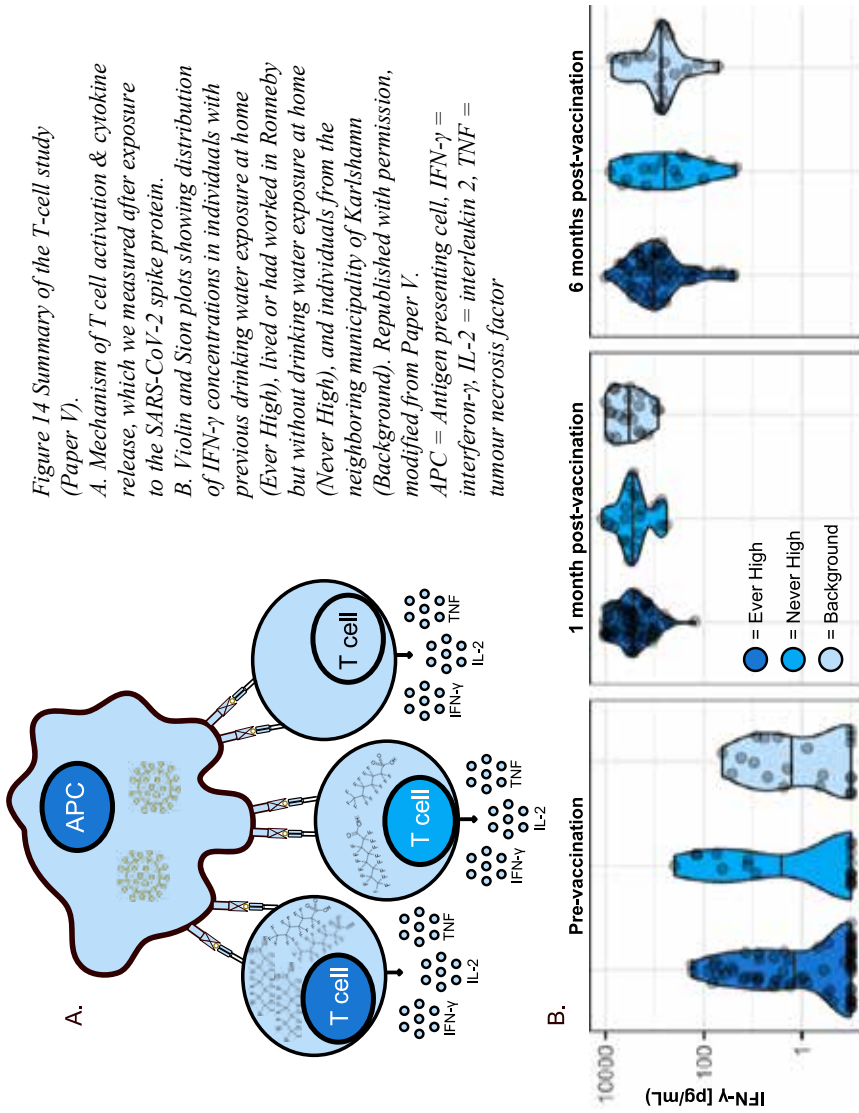
4.2 T CELL RESPONSE

An adequate T cell response might be even more important than an antibody response for protection against SARS-CoV-2 infection and COVID-19 severity, as humans and animals with impaired antibody production can still develop some immunity after SARS-CoV-2 infection or vaccination (182, 189, 190). Vaccinated patients taking the B-cell-inhibiting medication Rituximab had low antibody responses, yet adequate T cell responses (191). Furthermore, Rituximab users had a lower post-vaccination incidence of SARS-CoV-2 infection compared to non-vaccinated users, suggesting that T cell response may be sufficient for protection against COVID-19 (192).

Although several studies have evaluated the association between PFAS and COVID-19 serum antibody levels (Table 7), the effect of PFAS on COVID-19-specific T cell activation has only been examined in Ronneby (Paper V, summarized in Figure 14). There are several different techniques to assess T cell response (189), and we applied a cytokine release assay to evaluate T cell response before and after vaccination. In brief, we isolated T cells from whole blood, stimulated them with SARS-CoV-2 spike protein, and then measured the cytokines Interferon γ (IFN- γ), Tumor Necrosis Factor (TNF), and Interleukin 2 (IL-2) (Paper V). These cytokines were selected because they have been correlated with the response to SARS-CoV-2 infection and COVID-19 vaccination (181, 189, 190, 193). In summary, we found no associations between PFAS and the T cell cytokines.

In addition to the SARS-CoV-2-specific T cell response, we also conducted a general immune evaluation using stimulated phytohemagglutinin (PHA) T cell response and total serum immunoglobulin levels. These analyses aimed to determine if the maximum, non-specific immune response was impacted by PFAS exposure. However, no associations with PFAS were observed.

While no other study has examined the effect of PFAS on T cell response in adults, the childhood vaccination study by Abraham et al. 2020 used a similar cytokine release assay after diphtheria/tetanus vaccination (112). They found negative correlations between PFOA/PFOS and tetanus/diphtheria toxoid-stimulated IFN- γ production, as well as between PFOA levels and PHA-induced IL-10 levels (112). However, the authors noted that IL-10 had low biological reproducibility (112).



4.3 SEX-SPECIFIC VACCINE RESPONSE

It is well known that the immune system differs between males and females. Females generally have a more active immune system, which has been hypothesized to explain the higher incidence of autoimmune diseases and lower incidence of malignant cancers in females compared to males (194). Furthermore, females generate higher vaccine responses than males (194).

Sex differences in the effect of PFAS on vaccine response have also been investigated in individuals with PFAS background exposure. One study found a negative association between serum PFAS levels and anti-rubella antibodies in men but not in women (122), and another study showed a different (yet mostly non-significant and non-consistent) antibody response between males and females after vaccination against hepatitis A and B (123).

In Paper IV, we found higher anti-spike antibody levels per interquartile range (IQR) of PFAS (e.g., 16% higher per IQR PFOA) for females, compared to the negative, non-significant point estimate for males (Figure 15). A similar, yet weaker, pattern was observed in Paper V, with higher TNF levels per IQR PFAS for women and lower for men (Figure 16). The associations were statistically significant at 6 months for women and at 1 month for men, but not vice versa. In contrast, no sex difference in the effect of PFAS was observed for IFN- γ or IL-2 (Figure 16) or measurements of the general immune response (i.e., phytohemagglutinin-induced serum cytokine levels and total immunoglobulin levels).

In summary, we observed positive associations between PFAS and serum anti-spike antibody and TNF levels in women, and possibly the opposite in men. Future research should confirm the sex-specific effects of PFAS, explore the underlying biological mechanisms, and assess the implications of such effect modifications.

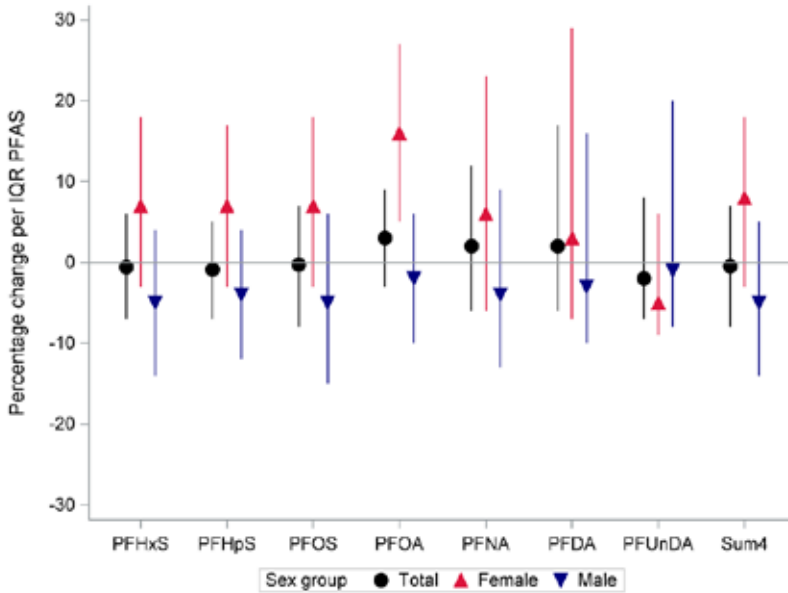


Figure 15 Percentual change in serum anti-spike antibodies per interquartile range (IQR) of PFAS in the entire study population and stratified by sex. Point estimates (symbols) and 95% confidence intervals (lines) were derived from adjusted mixed-effects regression models. From Paper IV. Republished with permission.

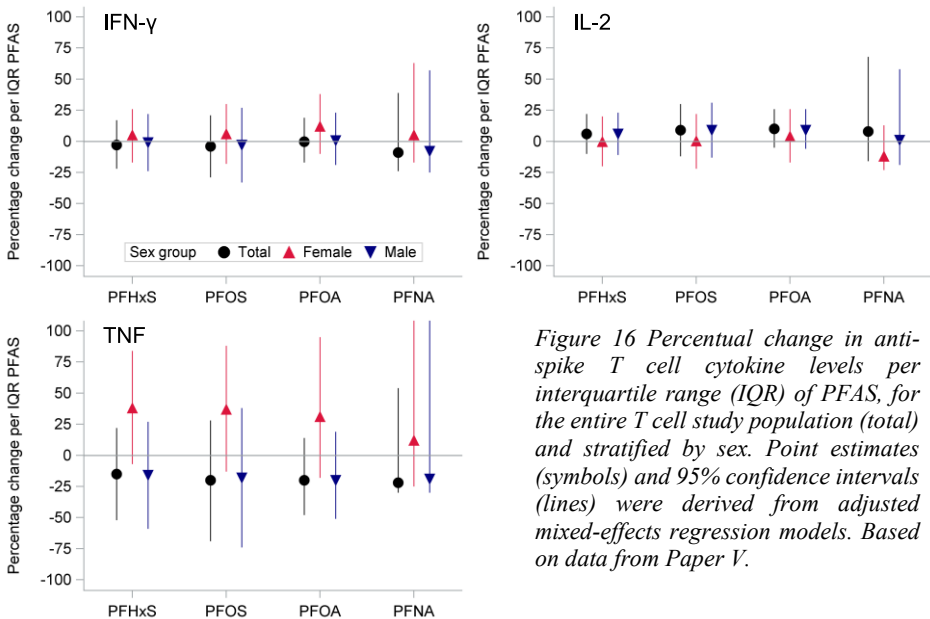


Figure 16 Percentual change in anti-spike T cell cytokine levels per interquartile range (IQR) of PFAS, for the entire T cell study population (total) and stratified by sex. Point estimates (symbols) and 95% confidence intervals (lines) were derived from adjusted mixed-effects regression models. Based on data from Paper V.

5 METHODS, STRENGTHS, AND WEAKNESSES

The methods of the five studies in this thesis are summarized in Table 8.

Table 8 Summary of methods Paper I-V

	Study design	Study population	Exposure	Outcome
I	Prospective cohort study	n = 114, aged 4-84	Potential serum determinants	PFAS serum levels
II	Cross-sectional, subset longitudinal	n = 147, aged 16-85	-	PFAS fecal/urine levels relative to serum
III	Cross-over, short-term trial	n = 10, aged 25-46	Cholestyramine or probenecid, 1 week	
	Cross-over, long-term trial	n = 10, aged 26-47	Colesevelam, 12 weeks	PFAS serum levels
IV	Prospective cohort study ^a	n = 367, age 20-59	Current & historic PFAS exposure	Antibody levels
V		n = 116, age 20-59		T cell cytokine levels

^a = Even though a medical intervention was involved with the COVID-19 vaccination, the studies were considered prospective cohort studies, as all participants were vaccinated, and it was rather the PFAS exposure that was evaluated.

5.1 STUDY DESIGNS AND POPULATIONS

5.1.1 STUDY DESIGNS

This thesis comprises three prospective cohort studies, one cross-sectional study, and two clinical cross-over trials. The research question guided the choice of study design – when the exposure of interest was non-malleable (i.e., not ethically or practically feasible to administer), such as potential serum determinants (sex, age, kidney function, etc., in Paper I) or PFAS levels (Papers IV & V), prospective cohort studies were employed. In contrast, when exposure could be controlled – for instance, through medication intervention in Paper III – an experimental, clinical trial was deemed more suitable. The

cross-sectional design for Paper II was selected because the goal was to explore the descriptive relationship between urinary and fecal elimination, with only one fecal sample collected per individual.

In Paper III, we specifically chose randomized cross-over trials over parallel-group randomized clinical trials (meaning separate control and intervention groups). The main advantage of the cross-over design is that each participant is their own control, decreasing individual variability and thus increasing statistical power (195). As we had difficulties recruiting participants (we aimed at 20 individuals but were only able to recruit 10 per trial), the cross-over design allowed us to gather more data from each participant.

A weakness of cross-over designs is the carryover effect (195). However, the risk of carryover effects in Paper III was low – both cholestyramine and colesevelam are not absorbed into the systemic circulation, and probenecid has a short serum half-life of 4-12 hours (196). To be cautious, we included a wash-out week in the short-term trial; in the long-term trial, we deemed the carryover effect to be minor compared to the 12-week intervention periods.

We also chose not to include blinding of the interventions because the outcomes of PFAS in urine, feces, and serum are unlikely to be influenced by placebo or nocebo effects. Additionally, it would be difficult to blind participants from cholestyramine treatment in the short-term trial, as the cholestyramine powder needs to be dissolved in water and then ingested.

5.1.2 STUDY POPULATIONS

The study populations ranged in size from 10 to 367 individuals and varied in composition depending on the aims of each study. The goal of Paper I was to identify characteristics that could predict individual serum PFAS half-lives in PFAS-exposed individuals. Similarly, Paper II aimed to explore the relationship between fecal and urinary elimination in PFAS-exposed individuals. Since the target population in both studies was PFAS-exposed, it was important to include a wide age range and both sexes to ensure that the findings could be generalized to the larger population. Although we initially intended to include children in Paper II, we ultimately chose not to. While we had a biologically plausible method to estimate creatinine production based on age-specific data from Remer et al. 2002 (196), we believed that the body weight-adjusted fecal estimates were not accurate in children. For example, the method would estimate that a 20 kg child would produce approximately $29 \times 20 \div 76 \approx 7.6$ grams of dry feces daily, which we believed would be too low.

For Paper III, the aim was to evaluate cholestyramine and probenecid intervention on PFAS elimination. We restricted the source population to young adults aged 20-45 for several reasons. Firstly, experimental and medicinal studies involving children, pregnant women, and other vulnerable groups require special ethical considerations, so we chose to exclude them. Secondly, by focusing on young adults, we avoided many age-related diseases and general frailty. To ensure healthy participants, we also excluded comorbidities that we hypothesized could impact drug tolerability or intervention effectiveness, such as kidney disease, liver disease, and inflammatory bowel diseases. Thirdly, we believed that the group most likely to benefit from a medical intervention aimed at reducing PFAS levels would be future mothers, since a treatment before pregnancy could interrupt the transgenerational transfer of PFAS from mother to fetus and child. Finally, previous study participation was also an inclusion criterion, allowing us to include participants with still elevated PFAS serum levels. We aimed to recruit 20 participants but managed only to recruit 10 for each trial, even though more than 500 letters were sent.

For Paper IV, the aim was to assess COVID-19 vaccine effectiveness in adults exposed to PFAS. Therefore, the target population was adults. However, part of this group could not be recruited because the Public Health Agency of Sweden (Folkhälsomyndigheten) prioritized vaccinating individuals over 60, adults with chronic systemic diseases (such as cardiovascular disease, COPD/asthma, impaired lung function, chronic liver or kidney failure, diabetes mellitus, severely impaired immune systems, and Down's syndrome), and healthcare personnel (197); these groups had already been vaccinated when the study began. Notably, children were not vaccinated against COVID-19, which limited their inclusion. In summary, the source population was limited to adults aged 20-60, without immuno-compromised patients and healthcare workers. Excluding vulnerable groups may have decreased variability in the outcome but could also impact how well the results apply to the broader target population.

Paper V was a nested cohort semi-randomly selected from the entire antibody cohort. The reason for limiting the number of participants was purely practical: harvesting and stimulating T cells was labor-intensive and costly, and it had to be completed within 24 hours after sampling. To ensure variation in PFAS serum levels, we aimed to include as many background individuals as possible for the necessary whole blood sample, as we only had 40 background individuals in the antibody cohort. After that, we included individuals who

visited the health care center on either of the two Sundays when sampling occurred, as samples needed to be transported from Ronneby to the immunology lab in Gothenburg, and lab technicians preferred to work on Mondays. Additionally, we restricted our analysis to individuals without a previous COVID-19 infection, focusing on vaccine-specific T cell results. Consequently, we ended up with 116 participants.

5.2 PFAS AS EXPOSURES & OUTCOMES

In Papers IV and V, PFAS serum levels were the main exposure. Three different temporal forms of PFAS were evaluated – current exposure, historical exposure, and prenatal exposure. The current exposure was the simplest form, namely PFAS serum levels before vaccination. Historical exposure was more complex. In Paper IV, part of the analysis involved PFAS serum levels measured between 2014 and 2016. However, some individuals had not provided serum samples previously and were therefore not included in the analysis. Historical exposure was also estimated in Papers IV and V through yearly address data from Statistics Sweden and self-reported occupational history. Based on this information, participants were categorized into those who had ever lived with polluted drinking water at home (Ever High), those who had lived in Ronneby without polluted drinking water at home (Never High), and controls from the neighboring municipality of Karlshamn (Background). Seven individuals from Karlshamn who had worked in Ronneby were excluded to avoid misclassification. Lastly, prenatal exposure was categorized based on prenatal address information, by asking participants born in 1985 and later if their mother lived in the water-polluted area during the pregnancy.

The address-based modelling of historical PFAS exposure was advantageous for two reasons. First, since participants' serum PFAS levels were decreasing after the end of drinking water exposure in 2014, and some had returned to background levels by the time of the vaccination study, the historical PFAS exposure helped determine whether individuals with previously high PFAS levels were still affected. Second, because historical PFAS exposure was causally correlated with current PFAS levels, it served as an instrumental variable (more thoroughly discussed in the section 5.4.1.1).

While PFAS exposure was the primary independent variable in Papers IV and V, the exposures in the other studies served as predictors of PFAS elimination. In Paper I, the exposure was the potential determinants of serum half-life, such

as age, sex, kidney function, and gut biomarkers. In Paper II, we conducted exploratory models to investigate potential determinants of fecal and renal clearance; however, this was not the main aim of the study. For Paper III, the exposure was the interventions (probenecid, cholestyramine, and colesevelam). In other words, PFAS concentrations were instead the outcome rather than the exposure in Papers I, II, and III.

5.2.1.1 ESTIMATING HALF-LIVES FROM LONGITUDINAL SERUM CONCENTRATIONS, OR FROM TOTAL CLEARANCE?

There have been at least two different methods used to estimate serum PFAS half-lives: one involves repeatedly measuring serum levels to calculate apparent half-lives, and the other uses cross-sectional serum levels, total clearance, and assumed Vds to estimate half-lives. In Paper I, we used the former method.

Apparent half-life estimations are prone to measurement error caused by ongoing exposure – if the population is exposed during the study, an upward bias will occur, resulting in higher apparent half-lives (198). This bias exists in situations where individuals with high serum concentrations are still highly exposed, like in the PFAS manufacturing population described in Fu et al. 2016 (25). Additionally, it could also affect half-life estimations in populations where serum PFAS levels are close to background exposure levels.

The strength of the apparent half-life estimates from the Ronneby Panel Study (Paper I and longitudinal data, Paper II) lies in the fact that serum concentrations of PFHxS and PFOS were high, while external exposure was reduced to background levels with the introduction of clean water in late 2013. This is an ideal situation when ongoing (background) exposure is low relative to the serum concentrations, thereby limiting the impact on apparent half-lives.

The effects of the relatively low ongoing exposure can be demonstrated by background exposure adjustments (using the method presented in Russel et al. 2015 (198)), where half-lives were calculated before and after subtracting background serum levels. The non-adjusted versus background-adjusted half-lives calculated this way were very similar for PFHxS and PFOS, while the PFOA half-life, based on serum concentrations closer to background levels, decreased by 17% after adjustment (Table 9).

Table 9 Non-adjusted versus background-adjusted apparent half-life estimates in 114 individuals from Ronneby. Data from Paper I.

	Geometric mean serum levels at the start of the study		Mean half-life (95% CI)		% change
	Panel Study	Reference	Non-adjusted	Background-adjusted	
PFOA	16	1.4	2.99 (2.79, 3.21)	2.47 (2.27, 2.7)	-17%
PFPeS	6.2	<0.02	0.96 (0.89, 1.03)	0.94 (0.86, 1.02)	-2.1%
PFHxS	260	0.54	4.55 (4.17, 5.01)	4.52 (4.14, 4.99)	-0.7%
PFHpS	13	0.059	4.61 (4.2, 5.12)	4.55 (4.14, 5.06)	-1.3%
L-PFOS	150	3.0	2.87 (2.7, 3.06)	2.73 (2.55, 2.92)	-4.9%
1m-PFOS	23	0.17	5.01 (4.56, 5.55)	5.01 (4.56, 5.55)	0%
3/4/5m-PFOS	73	0.76	3.43 (3.19, 3.71)	3.43 (3.19, 3.71)	0%
2/6m-PFOS	49	0.47	2.67 (2.51, 2.85)	2.67 (2.51, 2.85)	0%

The goal of adjusting for background exposure was to simulate a situation where external exposures were completely removed, enabling the calculation of true apparent half-lives. We assumed that the serum concentrations of 68 individuals aged 20-50 in Karlshamn, the neighboring municipality, represented the counterfactual scenario we aimed to simulate – specifically, what the serum concentrations of our study population would be if they had not been exposed to contaminated drinking water. This assumption was quite reasonable given the geographic proximity of the two municipalities. The background exposure assessment could potentially have been improved by matching individuals on determinants identified in Paper I, such as sex, age, and eGFR. However, the bias from under- or overestimating background exposure is likely minimal for PFHxS and PFOS, since the serum concentrations were high in the Panel Study group.

The other method for estimating serum half-lives is to use cross-sectional serum concentrations, total clearance, and V_d s. The main benefit of this

approach is that ongoing external PFAS exposure is not biasing the half-life estimates (25, 69). However, it should be noted that these half-lives heavily depend on the accuracy of estimated total clearance and Vd assumptions, and studies applying this method have reported much longer half-lives compared to those derived from longitudinal studies (25, 69). As a result, we calculated apparent half-lives for Paper I and decided not to estimate half-lives from total clearance in Paper II.

5.3 STATISTICAL METHODS

A recurring theme in this thesis was the collection of repeated measurements over time from study participants. This included up to ten serum samples from the half-life panel cohort (Paper I); up to ten urine samples, five fecal samples, and seven serum samples from the experimental crossover trials (Paper III); and up to three serum samples to evaluate pre- and post-vaccine immune responses (Papers IV and V). The multiple samples collected prompted us to employ mixed-effects linear regression models in these studies, as they could incorporate all data by adding random effects for each subject. In contrast, multiple linear regression models were utilized in Paper II, since each subject contributed only one fecal and one urine sample, and in Paper IV, where we initially used simple models and gradually increased complexity.

In all analyses, the dependent variables (PFAS in serum, feces, and urine, as well as serum antibody and T cell cytokine levels) were positively skewed. Therefore, the data were log-transformed to avoid violating the assumption of normally distributed random errors and to improve the model fit. In many PFAS vaccine studies, it is common to also log₂-transform the independent variable (PFAS) to estimate the percent change in antibody levels per doubling of PFAS (Tables 1 & 2). However, we chose not to log-transform PFAS in Papers IV and V, since we did not hypothesize a curvilinear relationship between PFAS and antibody levels. A curvilinear model would attenuate the slope at higher PFAS levels, while we hypothesized that increasing PFAS levels would further impair the antibody response.

In the peer review for Paper IV, it was suggested to use a Bayesian Kernel Regression Model (BKMR) or Weighted Quantile Sum (WQS) regression model to estimate the combined effects of PFAS and potentially identify individual PFAS contributions in a multiple-exposure setting. However, since we did not find any associations between PFAS and serum antibody levels – neither in multiple nor mixed-effects linear regression models, nor with the

independent variable as continuous PFAS, PFAS in quartiles, or as address-based categories – we concluded that employing a BKMR or WQS would provide little additional value. Moreover, the correlations between PFOS, PFOA, PFHxS, and perfluoroheptane sulfonic acid (PFHpS) were very high (R between 0.8 and 0.99), so it was unlikely that these advanced models would be able to estimate the combined effect of PFAS reliably.

5.4 VALIDITY

In epidemiology, validity can be divided into two categories: internal validity, which refers to the strength of the study method, and external validity, which pertains to how well the results can be generalized to the target population (199). Internal validity is vulnerable to bias. Although there are many types of bias, they can generally be categorized into confounding bias, selection bias, and information bias (200).

5.4.1 INTERNAL VALIDITY

5.4.1.1 CONFOUNDING BIAS

In Paper I and II, determinants for serum and elimination routes were examined. In Paper I, multiple regression models were used to control potential confounders. Ultimately, sex, age, BMI, and the interaction between elapsed time and age groups were included in all models to improve model fit and prevent confounding. In contrast, no multivariate regression model was used in Paper II, as we did not find significant associations in the univariate models.

In Paper III, the cross-over design reduced the risk of confounding bias because each participant served as their own control. This is reflected in the statistical methods of Paper III, where no covariate adjustment was necessary, as we did not anticipate time-varying confounding such as seasonal effects. Additionally, we randomly assigned the order in which participants received the interventions, which should limit time-varying confounding.

The highest risk of confounding in this thesis was for Paper IV and V due to the complexity of the immunological outcome. We identified potential confounders beforehand by determining factors that could generally influence antibody and T cell responses – age, sex, smoking, SARS-CoV-2 infection, pregnancy, systemic diseases, and immunomodulating treatments like cortisone or methotrexate. Some of these factors were adjusted for in the regression models (age, sex, smoking, and, in some analyses, SARS-CoV-2

infection), while certain participants with other conditions were excluded from the study (e.g., pregnant women, those receiving immunomodulating treatments, and, in some analyses, SARS-CoV-2 infected participants). Additionally, patients with chronic systemic diseases had already been vaccinated and were thus ineligible for the trial.

Age, sex, smoking, and pregnancy have been shown to be associated with serum PFAS levels (section 3.1), and they were thus considered potential confounders. However, the relationships between serum PFAS levels and both SARS-CoV-2 infection and immunomodulating treatment were less clear. Since these covariates were considered ancestors of the outcome but not of the exposure, we did not treat them as confounding factors. Instead, SARS-CoV-2 infection and immunomodulating treatment were regarded as competing exposures (or possibly mediators) and were statistically managed to focus on the direct PFAS vaccine response.

Unmeasured confounding is a bane of epidemiological studies. Therefore, as mentioned above in section 5.2, we used address-based, historical PFAS exposure as an instrument variable to bypass some of the unmeasured confounding. This was possible because historical residence history met the three criteria for an instrument variable (201): 1) a causal effect on the exposure, 2) the instrument only affected the outcome through the exposure, and 3) no confounding between the instrument and the outcome. The first criterion was satisfied since living with polluted drinking water resulted in higher PFAS levels, but the second and third criteria could theoretically have been influenced by socioeconomic factors. However, the Ronneby (Ever High & Never High) and Karlshamn (Background) categories should be socioeconomically quite similar, and the municipalities are geographically close. Additionally, other potential confounders such as smoking, age, and sex were also adjusted for.

The relationship between historical address categorization, PFAS, and vaccine response is illustrated in Figure 17.

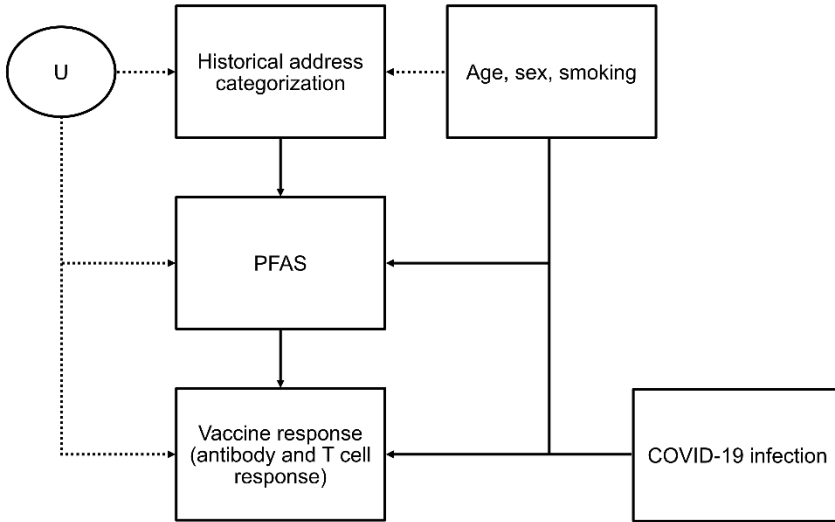


Figure 17 Directed acyclic graph illustrating the instrument variable relationship between PFAS exposure and historical address categorization. The dashed lines indicate unknown associations. U = unmeasured confounding

5.4.1.2 SELECTION BIAS

Selection bias may occur when the study population selected does not match the target population (200). The selection process for all five studies involved inviting individuals to participate, with some agreeing to take part. Additionally, some individuals were recruited through open convenience sampling, where volunteers (often family members of already included participants) who met the inclusion criteria could join without prior invitation, generating concern for self-selection bias. However, selection bias requires that participation is influenced by both the exposure and the outcome (199).

The study participants knew their exposure level beforehand, based on the water they had consumed and their results from the initial biomonitoring study in 2014. It is likely that individuals with high PFAS exposure were more interested in participating, and that the study population in Paper III may have had higher compliance with the intervention than the target population

However, no participant knew in advance what their outcome would be—whether it was serum half-lives (Papers I and III), fecal and urinary elimination rates (Papers II and III), serum antibody levels (Paper IV), or T cell responses (Paper V). Therefore, it is unlikely that any significant selection bias occurred.

5.4.1.3 INFORMATION BIAS

There is possibly misclassification in Papers IV and V regarding the historical residential exposure assessment. While data from Statistics Sweden on historical residential addresses probably have minimal misclassification, participants who did not live with polluted drinking water at home could have been exposed at work or at school. Similarly, even if a person's home address is within the contaminated area, they may have spent most of their time elsewhere or may not have consumed tap water in large quantities. However, this misclassification should be non-differential.

5.4.2 EXTERNAL VALIDITY

External validity provides the ability to extrapolate (generalize) the results from the study population to the target population, enabling the results to be applied to other populations (202).

One key aspect for external validity in Papers I, II, and III, beyond the study demographics, is whether the pharmacokinetics of PFAS are dose- and time-dependent. If they are, then extrapolation to other populations could be affected by when exposure ended and the internal level of exposure in the other population. More high-quality studies are needed to confirm or refute these findings.

For Paper IV, the exposure ranges of PFOS and PFHxS were broad. We had a relatively small study group at background exposure levels ($n = 40$), which could limit the generalizability to typical background exposure settings. However, by combining the null results from Paper IV with those from the Danish COVID-19 vaccine trial in PFAS background-exposed individuals by Timmermann et al. 2024 (184), a wide spectrum of PFAS exposure is covered within similar geographical and socioeconomic contexts. The combined findings, along with other PFAS COVID-19 vaccine response studies (Table 7), should therefore be applicable to both low- and high-PFAS-exposed adults.

5.5 ETHICAL CONSIDERATIONS

All studies included in this thesis involve human participants, with the collection of biological samples and sensitive personal data such as occupation, health history, and medication use. This requires ethical considerations. Therefore, standard ethical procedures in accordance with the Declaration of Helsinki have been followed: permissions from the ethical review authorities

have been obtained before recruiting participants; individuals were informed about the study and had the opportunity to ask questions prior to giving their informed consent; participants have been informed of their own results and the overall study findings through personalized letters; and financial compensation has been offered after the trials' completion. Participants have also had the opportunity to contact both the healthcare units where the sampling was conducted and the researchers regarding the planning and academic aspects of the studies.

The two trials involving medical products (the elimination trial, Paper III; and the vaccination trial, Papers IV & V) were classified as clinical trials and therefore adhered to stricter ethical standards according to ICH's Good Clinical Practice (GCP) (203). These international guidelines incorporate additional safety measures and responsibilities into the planning and execution of the trial. Central to GCP is the Study Protocol, which outlines a carefully detailed design of the trial. The Study Protocol must be approved by the national medicine agency (in our case, the Swedish Medical Products Agency) before the trial starts. To ensure compliance with the Study Protocol and participant safety, one medical doctor at each site (Ronneby and, for controls during the vaccine trial, Karlshamn) has served as investigators.

Research is pointless if its results are not shared with the scientific community, the affected populations, and society at large. Therefore, all studies have been published in peer-reviewed journals and presented at scientific conferences. This approach aims to guide future research, health policies, and societal interventions. The results of the studies have also been sent to all participants via letters after each study, with the possibility for them to contact us for more information. Finally, our PFAS research is continuously communicated through the Ronneby PFAS Research Project website¹, in the form of brief summaries and reports.

¹ <https://pfas.blogg.lu.se/>

6 IMPLICATIONS AND FUTURE PERSPECTIVES

6.1 UPDATING PHARMACOKINETIC MODELS

The pharmacokinetic models need to be accurate to yield accurate results. This is illustrated by Vaccari et al. 2024, where all studied pharmacokinetic models (both one-compartment and PBPK models) underestimated PFOA and overestimated PFOS serum levels in highly PFOA-exposed individuals in Veneto, Italy (83). It is possible that the pharmacokinetic parameters generated in Papers I and II, namely the apparent serum half-lives and their determinants, the fecal and renal clearance, and the new Vds, could be used to improve the models.

Government agencies such as EFSA, US EPA, California EPA, and ATSDR use pharmacokinetic models to determine safe exposure limits. They apply either PBPK models or one-compartment models to convert serum concentrations where no or low adverse effects of PFAS are expected (i.e., No-observed-adverse-effect levels, NOAEL, or Benchmark Dose, BMD) into safe exposure limits (measured in ng PFAS/kg body weight per day). Updating these pharmacokinetic models with the findings from this thesis will also change the safe exposure limits.

The safe exposure limit set by EFSA (Total Weekly Intake, TWI) (95) would probably be higher if it was updated. The TWI was estimated using a modified version of the Loccisano et al. 2011 PBPK model (40) (Figure 3). In both the original and the EFSA models, the fecal elimination route was not included. However, our results in Paper II indicate that the fecal route is on par with the renal route and should, therefore, be included. An updated model – differing only by the inclusion of fecal elimination – would thus estimate higher PFAS elimination. The updated model would also estimate a higher safe exposure limit, as a greater intake would be necessary to balance the increased elimination.

On the other hand, the safe exposure limits set by the US EPA (“Reference Doses”, RfD), California EPA (“Public Health Goals”, PHG), and ATSDR (“Minimal Risk Levels”, MLR) would instead be lowered if updated. These models were calculated using one-compartment models. In the models, serum elimination rates (ks) and Vds from the literature were used to estimate total

clearance (mL/kg body weight/day), which then served to convert the BMD/NOAEL serum concentrations (ng/mL) into safe exposure limits (ng/kg body weight/day). Different agencies employed different k_s and V_d s, ending up with different estimates of total clearance: for PFOA, 0.12, 0.28, and 0.099 mL/kg/day, respectively; for PFOS, 0.128, 0.39, and 0.0694 mL/kg/day, respectively. All these values were higher than the sum of renal and fecal clearance estimates from Paper II (PFOA: mean 0.069, median 0.051; L-PFOS: mean 0.073, median 0.059). Updating the safe exposure limits with the total clearance values from Paper II would significantly lower the limits for the US EPA and California EPA, as these agencies have likely overestimated the total elimination.

6.2 IDEAL STUDY DESIGNS FOR CLEARANCE AND V_D ESTIMATIONS

The pharmacokinetic parameters estimated in Paper II could be improved further by designing more controlled studies. However, conducting an experimental trial like Abraham et al. 2024 (41) in a larger study group would not be feasible, as it is unethical to administer PFAS to participants. Instead, calculating half-life, clearance, and V_d s from natural experiments (i.e., hot-spot populations), where populations have unintentionally been exposed to PFAS through environmental or occupational sources, is more ethical.

Therefore, the study setting in Papers I and II is appropriate for estimating these variables, but improvements could be made to the study design. Firstly, the need for estimated total daily creatinine production based on age, weight, and sex to convert spot urine to daily elimination could be eliminated by using 24-hour urine sampling. However, logistical challenges accompany 24-hour urine collection, particularly the large volume of urine that must be properly collected on site and transported to the laboratory.

Secondly, the fecal clearance estimates would be significantly improved by, similarly, sampling total feces over longer periods. Since the frequency of defecation varies greatly between individuals (204), the sampling period would likely need to be longer than 24 hours to capture each person's daily fecal elimination. However, collecting all feces would also be logistically challenging, which could limit the number of individuals participating.

Similarly, menstrual blood volume varies among individuals, and individual total menstrual PFAS elimination could be assessed by collecting tampons or pads during a complete menstrual cycle (205). However, as discussed in section 3.2.4, menstrual PFAS elimination is likely not a significant elimination route compared to fecal and urinary routes. Additionally, information on blood donation frequency, pregnancy, and breastfeeding would also be valuable.

Another requirement would be to recruit individuals with high exposure, as background-exposed individuals might have urine and fecal concentrations below the limit of detection. This was observed in Abraham et al. 2024, where several PFAS compounds were below the LOD in urine and feces (41).

If a perfect study was conducted, our methodological assumptions in Paper II (such as a specific weight-adjusted dry fecal weight and the estimated total daily creatinine production) would be avoided, and potentially more accurate parameters could be estimated. However, logistical issues with collecting more feces and urine from each individual would limit the number of participants, risking reduced external validity.

6.3 UPDATED ELIMINATION PROGNoses

In addition to improved modelling, the results from Paper I could be used to inform new hot-spot populations about their expected serum half-lives after the end of external exposure. This information could be tailored from the stratified serum half-lives from different subpopulations in Paper I. For example, if a new hot-spot population consists of older men, then the longer serum half-lives of older men (e.g., 3.67 years for L-PFOS) can be used instead of serum half-lives from the broader population (e.g., 2.73 years for L-PFOS). Conversely, if the new hot-spot population is diverse, then either the whole population estimates can be used, or different serum half-lives for different subpopulations can inform expectations for various groups within the population.

It should be noted, however, that the serum determinants in Paper I only explained 17-51% of the variance, so individual half-life predictions would still be uncertain.

6.4 BAS AS ELIMINATION INTERVENTION?

Both Paper III and the larger study by Møller et al. 2024 (33) showed large intervention effects with colestevlam and cholestyramine, respectively. While other interventions have been studied pre-clinically (see section 3.4), only BAS and plasma/blood donation have been studied in controlled trials. BAS appears to have a large effect on PFOS, but a smaller one for PFHxS, while the differences for plasma and blood donation are less pronounced, with the latter two being less effective (Figure 18). Notably, it is unclear whether the BAS intervention effect on serum elimination rates remains constant over time, as BAS has only been studied for 3 months and plasma/blood donation for 12 months (Figure 18).

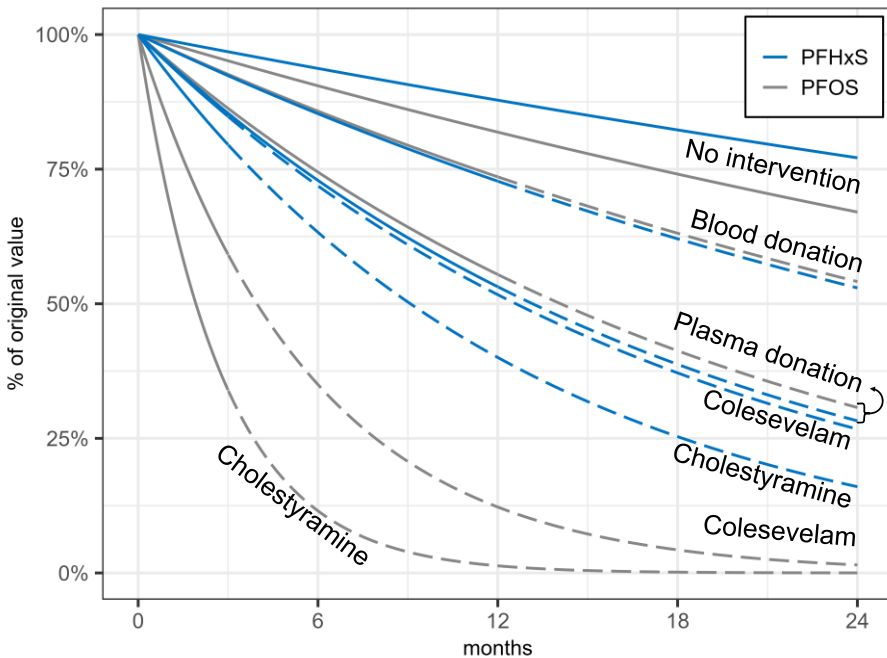


Figure 18 Mean effects of PFAS interventions compared to no intervention for serum PFOS (gray lines) and PFHxS (blue lines). The interventions were: cholestyramine, 4g three times daily for 12 weeks; colesevelam, 1.25g twice daily for 12 weeks; blood donation, every 12 weeks for 52 weeks; and plasma donation, every 6 weeks for 52 weeks. The data are extrapolated from Møller et al. 2024 for cholestyramine (33), Paper III for colesevelam, Gasiorowski et al. 2022 (with adjustments for ongoing exposure) for plasma and blood donation (150), and Li et al. 2018 for no intervention (35). The solid lines indicate the observed intervention effect, and dashed lines show an extrapolation forward in time, assuming a constant log-linear intervention effect.

While it is now known that it is possible to enhance PFAS elimination, it remains whether a PFAS-lowering intervention will affect health – will lowering of serum PFAS concentrations provide health benefits, or are the disease risks caused by PFAS exposure constant over time?

Central to this question is how PFAS exposure affects health (Figure 19). For some outcomes, exposure during critical time windows, such as prenatal exposure, is essential. This is likely relevant for early life outcomes like lower gestational weight and reduced childhood vaccination response. It could also be the mechanism for other outcomes later in life (in accordance to the developmental origin of health and disease (DOHaD) theory (206)).

For other outcomes, it could be serum PFAS levels exceeding a certain threshold that pose health risks. An analogy is paracetamol toxicity – it is only at high concentrations of paracetamol that life-threatening liver damage occurs. Threshold levels could explain the non-linear associations observed with PFAS and elevated serum lipids, where the largest increase in lipids occurs at lower serum PFAS levels (207).

Current or cumulative serum PFAS levels could also be the primary driver of outcomes. An analogous comparison is smoking, where both current and past smoking habits increase the risk of lung diseases like lung cancer and chronic obstructive pulmonary disease. Quitting smoking can improve some lung health results, but some lifelong risks remain higher compared to never-smokers. For PFAS and related health outcomes, either or both of these mechanisms may be involved (Figure 19).

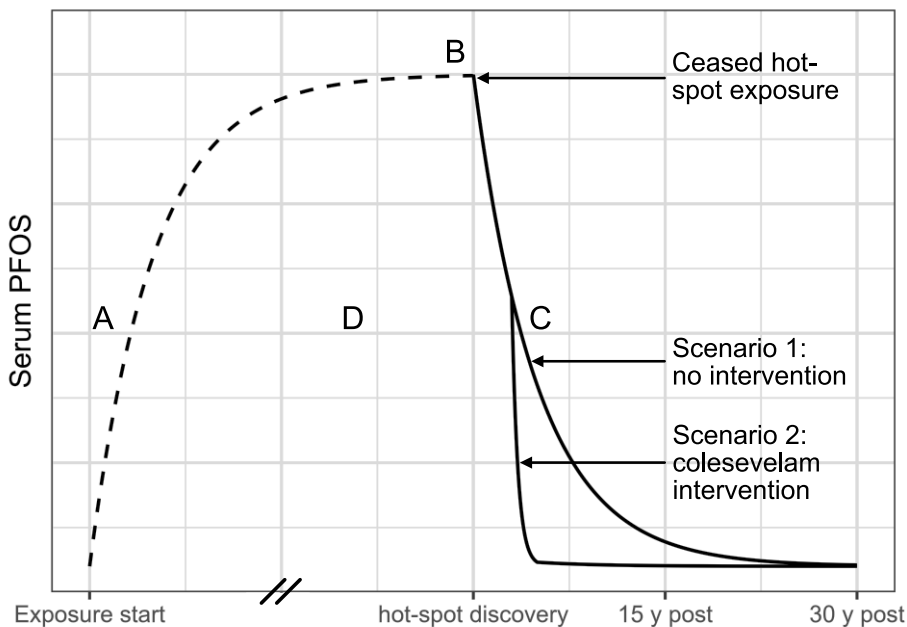


Figure 19 Schematic depiction of how different PFAS exposures could impact health. Health may be compromised by exposure during critical time windows (A), by reaching a specific threshold (B), through current exposure (C), or by cumulative exposure over time (D). The dashed line indicates the uncertainty in historic serum PFAS levels. Two scenarios demonstrate that only C and D are affected by a two-year colesevelam intervention.

Depending on the mechanisms, different intervention strategies vary in effectiveness. If damage occurs during critical time windows (e.g., prenatal exposure), then PFAS-lowering interventions should target future mothers. For the remaining three mechanisms, the key question is whether the effects are reversible or not. If they are, then different prioritization strategies could be used. Older individuals might be prioritized if current levels are critical, because they tend to have higher serum PFAS levels, are more likely to develop diseases like cardiovascular disease, and are frailer than younger people. If cumulative exposure is the main factor for disease risk, then interventions in young individuals can significantly reduce their lifetime cumulative exposure, while interventions in older individuals would only have a small impact on their total lifelong exposure. Conversely, if the health effects are permanent, then the interventions would not reduce PFAS-related health risks.

Regardless of whether PFAS-lowering interventions improve health, it is likely that lowering serum PFAS in future mothers with high levels will benefit their future children. This is because early life PFAS exposure to children, through transfer from the placenta and breast milk, would be significantly reduced. This could lead to a life free from high PFAS exposure, provided that external exposure is eliminated.

6.5 EFFECTIVE MRNA VACCINES IN ADULTS

There is now evidence indicating that PFAS negatively affect vaccine response in children, limited evidence for PFAS affecting adults being vaccinated with traditional vaccines (section 1.3.1), and no evidence that PFAS negatively affect adults being vaccinated with mRNA-based COVID-19 vaccines.

There are at least two important hypotheses behind these differences in associations, assuming that the associations are causal. Firstly, the mRNA vaccines may elicit a more robust immune response than other vaccine types, possibly using different biological pathways. This hypothesis is supported by the fact that mRNA COVID-19 vaccines stimulate a higher immune response than the non-mRNA COVID-19 counterparts (193). Secondly, it could be that the adult immune system is more mature and apt at developing a vaccine response regardless of PFAS exposure. This hypothesis is supported by the study by Shih et al. 2021, where no overall PFAS effect on diphtheria, tetanus, and hepatitis vaccine response was observed in 28-year-olds from the same Faroese cohort in which negative associations were found in childhood (123). It is also possible that both hypotheses could be present simultaneously.

One way to test these hypotheses is to administer mRNA vaccines to children and assess their vaccine response. If no associations are found, this would support the first hypothesis; however, if PFAS are linked to a lower mRNA vaccine response, this would support the second hypothesis. Unfortunately, since the COVID-19 mRNA vaccines are currently the only mRNA vaccines used in clinical practice, and SARS-CoV-2 has become part of the common flora of respiratory viruses, it is likely impossible to evaluate the naïve vaccine response. Additionally, because the COVID-19 vaccines were not widely given to children during the pandemic, it is also difficult to investigate retrospectively.

7 CONCLUSIONS

This thesis explored and provided valuable information about PFAS elimination in humans. Sex, age, kidney function, gut inflammation, and gut permeability were identified as determinants of individual half-lives. Furthermore, this thesis was the first large study to show that PFAS are eliminated not only through urine but also through feces. The slow elimination observed did not align with the traditionally used volume of distribution for PFAS, so new volumes were estimated. These findings, measurements, and estimations should be incorporated into PFAS pharmacokinetic models to improve the accuracy of risk assessments and related exposure guidelines.

This thesis also confirmed that the slow elimination can be significantly accelerated with bile acid sequestrants colesevelam and cholestyramine, but not with the gout medication probenecid. However, the overall health impact of bile acid sequestrant intervention remains unclear. Future studies are needed to determine the health effects of implementing PFAS-lowering interventions in PFAS hot-spot populations.

Finally, this thesis found an ample production of SARS-CoV-2 spike protein-specific serum antibodies and T cell cytokine responses in a strictly controlled, prospective cohort study in adults with high PFAS exposure, with no negative correlations observed with different PFAS exposure assessments. While a female-specific, positive association between serum PFOA and serum antibodies was suggested, it was not replicated in T cell cytokine responses. In other words, there was no evidence of a negative effect of PFAS on vaccine response after mRNA COVID-19 vaccination.

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Ej för de starka i världen, men för de svaga.

Ej för krigare, men för bönder som plöjt

sin jordlott utan att klaga

spelar en gud på flöjt.

Det är en grekisk saga...

Förklädd Gud,

text Hjalmar Gullberg, setting Lars-Erik Larsson

REFERENCES

1. Wang Z, Buser AM, Cousins IT, Demattio S, Drost W, Johansson O, et al. A New OECD Definition for Per- and Polyfluoroalkyl Substances. *Environ Sci Technol.* 2021;55(23):15575-8. doi: 10.1021/acs.est.1c06896.
2. Parsons JR, Sáez M, Dolfing J, de Voogt P. Biodegradation of Perfluorinated Compounds. In: Whitacre DM, editor. *Reviews of Environmental Contamination and Toxicology Vol 196.* New York, NY: Springer US; 2008. p. 53-71.
3. Swedish Chemicals Agency (KEMI). What substances are in PRIO? : Swedish Chemicals Agency; 2025 [updated 25 February 2025; cited 2025 May 20th]. Available from: <https://www.kemi.se/prioguiden/english/start/what-substances-are-in-prio>.
4. Barnabas SJ, Böhme T, Boyer SK, Irmer M, Ruttkies C, Wetherbee I, et al. Extraction of chemical structures from literature and patent documents using open access chemistry toolkits: a case study with PFAS. *Digital Discovery.* 2022;1(4):490-501. doi: 10.1039/D2DD00019A.
5. Buck RC, Franklin J, Berger U, Conder JM, Cousins IT, de Voogt P, et al. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. *Integr Environ Assess Manag.* 2011;7(4):513-41. Epub 2011/07/28. doi: 10.1002/ieam.258. PubMed PMID: 21793199; PubMed Central PMCID: PMCPMC3214619.
6. Young CJ, Mabury SA. Atmospheric Perfluorinated Acid Precursors: Chemistry, Occurrence, and Impacts. In: De Voogt P, editor. *Reviews of Environmental Contamination and Toxicology Volume 208: Perfluorinated alkylated substances.* New York, NY: Springer New York; 2010. p. 1-109.
7. Wang N, Liu J, Buck RC, Korzeniowski SH, Wolstenholme BW, Folsom PW, et al. 6:2 Fluorotelomer sulfonate aerobic biotransformation in activated sludge of waste water treatment plants. *Chemosphere.* 2011;82(6):853-8. doi: <https://doi.org/10.1016/j.chemosphere.2010.11.003>.
8. Nilsson H, Kärman A, Rotander A, van Bavel B, Lindström G, Westberg H. Biotransformation of fluorotelomer compound to perfluorocarboxylates in humans. *Environ Int.* 2013;51:8-12. doi: <https://doi.org/10.1016/j.envint.2012.09.001>.
9. Smorada CM, Sima MW, Jaffé PR. Bacterial degradation of perfluoroalkyl acids. *Curr Opin Biotechnol.* 2024;88:103170. Epub 2024/07/17. doi: 10.1016/j.copbio.2024.103170. PubMed PMID: 39013276.
10. Yang Y, Wang J, Tang S, Qiu J, Luo Y, Yang C, et al. Per- and Polyfluoroalkyl Substances (PFAS) in Consumer Products: An Overview of the Occurrence, Migration, and Exposure Assessment. *Molecules.* 2025;30(5).

Epub 2025/03/13. doi: 10.3390/molecules30050994. PubMed PMID: 40076219; PubMed Central PMCID: PMCPMC11901761.

11. Domingo JL, Nadal M. Per- and Polyfluoroalkyl Substances (PFASs) in Food and Human Dietary Intake: A Review of the Recent Scientific Literature. *J Agric Food Chem.* 2017;65(3):533-43. Epub 2017/01/05. doi: 10.1021/acs.jafc.6b04683. PubMed PMID: 28052194.

12. Vestergren R, Cousins IT. Tracking the pathways of human exposure to perfluorocarboxylates. *Environ Sci Technol.* 2009;43(15):5565-75. Epub 2009/09/08. doi: 10.1021/es900228k. PubMed PMID: 19731646.

13. De Silva AO, Armitage JM, Bruton TA, Dassuncao C, Heiger-Bernays W, Hu XC, et al. PFAS Exposure Pathways for Humans and Wildlife: A Synthesis of Current Knowledge and Key Gaps in Understanding. *Environ Toxicol Chem.* 2021;40(3):631-57. doi: 10.1002/etc.4935.

14. Ackerman Grunfeld D, Gilbert D, Hou J, Jones AM, Lee MJ, Kibbey TCG, et al. Underestimated burden of per- and polyfluoroalkyl substances in global surface waters and groundwaters. *Nature Geoscience.* 2024;17(4):340-6. doi: 10.1038/s41561-024-01402-8.

15. Hu XC, Andrews DQ, Lindstrom AB, Bruton TA, Schaidler LA, Grandjean P, et al. Detection of Poly- and Perfluoroalkyl Substances (PFASs) in U.S. Drinking Water Linked to Industrial Sites, Military Fire Training Areas, and Wastewater Treatment Plants. *Environmental Science & Technology Letters.* 2016;3(10):344-50. doi: 10.1021/acs.estlett.6b00260.

16. Berthou M, Gérard V, Pélingre M, Bagard A, Batteux TL, Losfeld G. Is it raining PFAS in France? An analysis of 52 PFAS at nanogram per liter levels in French rainwaters during autumn season. *J Environ Qual.* 2024;53(1):123-32. Epub 2023/10/27. doi: 10.1002/jeq2.20525. PubMed PMID: 37888768.

17. Hartz WF, Björnsdotter MK, Yeung LWY, Hodson A, Thomas ER, Humby JD, et al. Levels and distribution profiles of Per- and Polyfluoroalkyl Substances (PFAS) in a high Arctic Svalbard ice core. *Sci Total Environ.* 2023;871:161830. Epub 2023/01/31. doi: 10.1016/j.scitotenv.2023.161830. PubMed PMID: 36716880.

18. Hartz WF, Björnsdotter MK, Yeung LWY, Humby JD, Eckhardt S, Evangeliou N, et al. Sources and Seasonal Variations of Per- and Polyfluoroalkyl Substances (PFAS) in Surface Snow in the Arctic. *Environ Sci Technol.* 2024;58(49):21817-28. Epub 2024/11/26. doi: 10.1021/acs.est.4c08854. PubMed PMID: 39588978; PubMed Central PMCID: PMCPMC11636200.

19. Richterová D, Govarts E, Fábelová L, Rausová K, Rodriguez Martin L, Gilles L, et al. PFAS levels and determinants of variability in exposure in European teenagers - Results from the HBM4EU aligned studies (2014-2021). *Int J Hyg Environ Health.* 2023;247:114057. Epub 2022/11/04.

doi: 10.1016/j.ijheh.2022.114057. PubMed PMID: 36327670; PubMed Central PMCID: PMC9758614.

20. Olsen GW, Lange CC, Ellefson ME, Mair DC, Church TR, Goldberg CL, et al. Temporal trends of perfluoroalkyl concentrations in American Red Cross adult blood donors, 2000-2010. *Environ Sci Technol.* 2012;46(11):6330-8. Epub 2012/05/05. doi: 10.1021/es300604p. PubMed PMID: 22554481.

21. Toms LML, Bräunig J, Vijayasarathy S, Phillips S, Hobson P, Aylward LL, et al. Per- and polyfluoroalkyl substances (PFAS) in Australia: Current levels and estimated population reference values for selected compounds. *Int J Hyg Environ Health.* 2019;222(3):387-94. doi: <https://doi.org/10.1016/j.ijheh.2019.03.004>.

22. Haug LS, Thomsen C, Becher G. Time Trends and the Influence of Age and Gender on Serum Concentrations of Perfluorinated Compounds in Archived Human Samples. *Environ Sci Technol.* 2009;43(6):2131-6. doi: 10.1021/es802827u.

23. Yeung LW, Robinson SJ, Koschorreck J, Mabury SA. Part I. A temporal study of PFCAs and their precursors in human plasma from two German cities 1982-2009. *Environ Sci Technol.* 2013;47(8):3865-74. Epub 2013/03/15. doi: 10.1021/es303716k. PubMed PMID: 23484973.

24. Yeung LW, Robinson SJ, Koschorreck J, Mabury SA. Part II. A temporal study of PFOS and its precursors in human plasma from two German cities in 1982-2009. *Environ Sci Technol.* 2013;47(8):3875-82. Epub 2013/03/15. doi: 10.1021/es4004153. PubMed PMID: 23484930.

25. Fu J, Gao Y, Cui L, Wang T, Liang Y, Qu G, et al. Occurrence, temporal trends, and half-lives of perfluoroalkyl acids (PFAAs) in occupational workers in China. *Sci Rep.* 2016;6:38039. Epub 2016/12/03. doi: 10.1038/srep38039. PubMed PMID: 27905562; PubMed Central PMCID: PMC5131319.

26. Olsen GW, Burris JM, Ehresman DJ, Froehlich JW, Seacat AM, Butenhoff JL, et al. Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. *Environ Health Perspect.* 2007;115(9):1298-305. Epub 2007/09/07. doi: 10.1289/ehp.10009. PubMed PMID: 17805419; PubMed Central PMCID: PMC964923.

27. Olsen GW, Hansen KJ, Stevenson LA, Burris JM, Mandel JH. Human donor liver and serum concentrations of perfluorooctanesulfonate and other perfluorochemicals. *Environ Sci Technol.* 2003;37(5):888-91. Epub 2003/04/02. doi: 10.1021/es020955c. PubMed PMID: 12666917.

28. Nilsson S, Smurthwaite K, Aylward LL, Kay M, Toms LM, King L, et al. Serum concentration trends and apparent half-lives of per- and polyfluoroalkyl substances (PFAS) in Australian firefighters. *Int J Hyg*

- Environ Health. 2022;246:114040. Epub 2022/09/27. doi: 10.1016/j.ijheh.2022.114040. PubMed PMID: 36162311.
29. Nilsson S, Smurthwaite K, Aylward LL, Kay M, Toms LM, King L, et al. Associations between serum perfluoroalkyl acid (PFAA) concentrations and health related biomarkers in firefighters. *Environ Res.* 2022;215(Pt 3):114370. Epub 2022/09/30. doi: 10.1016/j.envres.2022.114370. PubMed PMID: 36174755.
30. Rotander A, Toms LM, Aylward L, Kay M, Mueller JF. Elevated levels of PFOS and PFHxS in firefighters exposed to aqueous film forming foam (AFFF). *Environ Int.* 2015;82:28-34. Epub 2015/05/24. doi: 10.1016/j.envint.2015.05.005. PubMed PMID: 26001497.
31. Frisbee SJ, Brooks AP, Jr., Maher A, Flensburg P, Arnold S, Fletcher T, et al. The C8 health project: design, methods, and participants. *Environ Health Perspect.* 2009;117(12):1873-82. Epub 2010/01/06. doi: 10.1289/ehp.0800379. PubMed PMID: 20049206; PubMed Central PMCID: PMCPMC2799461.
32. Backe WJ, Day TC, Field JA. Zwitterionic, Cationic, and Anionic Fluorinated Chemicals in Aqueous Film Forming Foam Formulations and Groundwater from U.S. Military Bases by Nonaqueous Large-Volume Injection HPLC-MS/MS. *Environ Sci Technol.* 2013;47(10):5226-34. doi: 10.1021/es3034999.
33. Møller JJ, Lyngberg AC, Hammer PEC, Flachs EM, Mortensen OS, Jensen TK, et al. Substantial decrease of PFAS with anion exchange resin treatment - A clinical cross-over trial. *Environ Int.* 2024;185:108497. Epub 2024/02/18. doi: 10.1016/j.envint.2024.108497. PubMed PMID: 38367552.
34. Xu Y, Nielsen C, Li Y, Hammarstrand S, Andersson EM, Li H, et al. Serum perfluoroalkyl substances in residents following long-term drinking water contamination from firefighting foam in Ronneby, Sweden. *Environ Int.* 2021;147:106333. Epub 2020/12/29. doi: 10.1016/j.envint.2020.106333. PubMed PMID: 33360412.
35. Li Y, Fletcher T, Mucs D, Scott K, Lindh CH, Tallving P, et al. Half-lives of PFOS, PFHxS and PFOA after end of exposure to contaminated drinking water. *Occup Environ Med.* 2018;75(1):46-51. Epub 2017/11/15. doi: 10.1136/oemed-2017-104651. PubMed PMID: 29133598; PubMed Central PMCID: PMCPMC5749314.
36. Abdel-Rahman SM, Kauffman RE. The integration of pharmacokinetics and pharmacodynamics: understanding dose-response. *Annu Rev Pharmacol Toxicol.* 2004;44:111-36. Epub 2004/01/28. doi: 10.1146/annurev.pharmtox.44.101802.121347. PubMed PMID: 14744241.
37. Derendorf H. Rowland and Tozer's clinical pharmacokinetics and pharmacodynamics : concepts and applications. 5th edition ed. Schmidt S, Rowland M, Tozer TN, editors. Philadelphia: Wolters Kluwer Health/Lippincott William & Wilkins; 2020.

38. Fan J, de Lannoy IA. Pharmacokinetics. *Biochem Pharmacol.* 2014;87(1):93-120. Epub 2013/09/24. doi: 10.1016/j.bcp.2013.09.007. PubMed PMID: 24055064.
39. Steinmetz KL. Colesevelam hydrochloride. *Am J Health Syst Pharm.* 2002;59(10):932-9. Epub 2002/06/04. doi: 10.1093/ajhp/59.10.932. PubMed PMID: 12040732.
40. Loccisano AE, Campbell JL, Jr., Andersen ME, Clewell HJ, 3rd. Evaluation and prediction of pharmacokinetics of PFOA and PFOS in the monkey and human using a PBPK model. *Regul Toxicol Pharmacol.* 2011;59(1):157-75. Epub 2010/12/21. doi: 10.1016/j.yrtph.2010.12.004. PubMed PMID: 21168463.
41. Abraham K, Mertens H, Richter L, Mielke H, Schwerdtle T, Monien BH. Kinetics of 15 per- and polyfluoroalkyl substances (PFAS) after single oral application as a mixture - A pilot investigation in a male volunteer. *Environ Int.* 2024;193:109047. Epub 2024/10/31. doi: 10.1016/j.envint.2024.109047. PubMed PMID: 39476597.
42. Bischel HN, MacManus-Spencer LA, Zhang C, Luthy RG. Strong associations of short-chain perfluoroalkyl acids with serum albumin and investigation of binding mechanisms. *Environ Toxicol Chem.* 2011;30(11):2423-30. doi: 10.1002/etc.647 %J Environmental Toxicology and Chemistry.
43. Fischer FC, Ludtke S, Thackray C, Pickard HM, Haque F, Dassuncao C, et al. Binding of Per- and Polyfluoroalkyl Substances (PFAS) to Serum Proteins: Implications for Toxicokinetics in Humans. *Environ Sci Technol.* 2024;58(2):1055-63. Epub 2024/01/03. doi: 10.1021/acs.est.3c07415. PubMed PMID: 38166384; PubMed Central PMCID: PMCPMC11149785.
44. Smeltz M, Wambaugh JF, Wetmore BA. Plasma Protein Binding Evaluations of Per- and Polyfluoroalkyl Substances for Category-Based Toxicokinetic Assessment. *Chem Res Toxicol.* 2023;36(6):870-81. Epub 2023/05/15. doi: 10.1021/acs.chemrestox.3c00003. PubMed PMID: 37184865; PubMed Central PMCID: PMCPMC10506455.
45. Bogdanska J, Borg D, Sundström M, Bergström U, Halldin K, Abedi-Valugerdi M, et al. Tissue distribution of ³⁵S-labelled perfluorooctane sulfonate in adult mice after oral exposure to a low environmentally relevant dose or a high experimental dose. *Toxicology.* 2011;284(1-3):54-62. Epub 2011/04/05. doi: 10.1016/j.tox.2011.03.014. PubMed PMID: 21459123.
46. Vanden Heuvel JP, Kuslikis BI, Van Rafelghem MJ, Peterson RE. Tissue distribution, metabolism, and elimination of perfluorooctanoic acid in male and female rats. *J Biochem Toxicol.* 1991;6(2):83-92. Epub 1991/01/01. doi: 10.1002/jbt.2570060202. PubMed PMID: 1941903.
47. Cui L, Zhou QF, Liao CY, Fu JJ, Jiang GB. Studies on the toxicological effects of PFOA and PFOS on rats using histological observation

and chemical analysis. *Arch Environ Contam Toxicol.* 2009;56(2):338-49. Epub 2008/07/29. doi: 10.1007/s00244-008-9194-6. PubMed PMID: 18661093.

48. Kudo N, Sakai A, Mitsumoto A, Hibino Y, Tsuda T, Kawashima Y. Tissue distribution and hepatic subcellular distribution of perfluorooctanoic acid at low dose are different from those at high dose in rats. *Biol Pharm Bull.* 2007;30(8):1535-40. Epub 2007/08/02. doi: 10.1248/bpb.30.1535. PubMed PMID: 17666816.

49. Kim SJ, Heo SH, Lee DS, Hwang IG, Lee YB, Cho HY. Gender differences in pharmacokinetics and tissue distribution of 3 perfluoroalkyl and polyfluoroalkyl substances in rats. *Food Chem Toxicol.* 2016;97:243-55. Epub 2016/10/30. doi: 10.1016/j.fct.2016.09.017. PubMed PMID: 27637925.

50. Nielsen F, Fischer FC, Leth PM, Grandjean P. Occurrence of Major Perfluorinated Alkylate Substances in Human Blood and Target Organs. *Environ Sci Technol.* 2024;58(1):143-9. doi: 10.1021/acs.est.3c06499.

51. Kärrman A, Domingo JL, Llebaria X, Nadal M, Bigas E, van Bavel B, et al. Biomonitoring perfluorinated compounds in Catalonia, Spain: concentrations and trends in human liver and milk samples. *Environ Sci Pollut Res Int.* 2010;17(3):750-8. Epub 2009/05/22. doi: 10.1007/s11356-009-0178-5. PubMed PMID: 19458971.

52. Maestri L, Negri S, Ferrari M, Ghittori S, Fabris F, Danesino P, et al. Determination of perfluorooctanoic acid and perfluorooctanesulfonate in human tissues by liquid chromatography/single quadrupole mass spectrometry. *Rapid Commun Mass Spectrom.* 2006;20(18):2728-34. Epub 2006/08/18. doi: 10.1002/rcm.2661. PubMed PMID: 16915561.

53. Pérez F, Nadal M, Navarro-Ortega A, Fàbrega F, Domingo JL, Barceló D, et al. Accumulation of perfluoroalkyl substances in human tissues. *Environ Int.* 2013;59:354-62. Epub 2013/07/31. doi: 10.1016/j.envint.2013.06.004. PubMed PMID: 23892228.

54. Moriceau MA, Cano-Sancho G, Kim M, Coumoul X, Emond C, Arrebola JP, et al. Partitioning of Persistent Organic Pollutants between Adipose Tissue and Serum in Human Studies. *Toxics.* 2022;11(1). Epub 2023/01/21. doi: 10.3390/toxics11010041. PubMed PMID: 36668767; PubMed Central PMCID: PMC9866963.

55. Mamsen LS, Björvang RD, Mucs D, Vinnars M-T, Papadogiannakis N, Lindh CH, et al. Concentrations of perfluoroalkyl substances (PFASs) in human embryonic and fetal organs from first, second, and third trimester pregnancies. *Environ Int.* 2019;124:482-92. doi: <https://doi.org/10.1016/j.envint.2019.01.010>.

56. Cui L, Liao CY, Zhou QF, Xia TM, Yun ZJ, Jiang GB. Excretion of PFOA and PFOS in male rats during a subchronic exposure. *Arch Environ Contam Toxicol.* 2010;58(1):205-13. Epub 2009/05/27. doi: 10.1007/s00244-009-9336-5. PubMed PMID: 19468665.

57. Kudo N, Katakura M, Sato Y, Kawashima Y. Sex hormone-regulated renal transport of perfluorooctanoic acid. *Chem Biol Interact.* 2002;139(3):301-16. Epub 2002/03/07. doi: 10.1016/s0009-2797(02)00006-6. PubMed PMID: 11879818.
58. Ohmori K, Kudo N, Katayama K, Kawashima Y. Comparison of the toxicokinetics between perfluorocarboxylic acids with different carbon chain length. *Toxicology.* 2003;184(2-3):135-40. Epub 2002/12/25. doi: 10.1016/s0300-483x(02)00573-5. PubMed PMID: 12499116.
59. Butenhoff JL, Kennedy GL, Jr., Hinderliter PM, Lieder PH, Jung R, Hansen KJ, et al. Pharmacokinetics of perfluorooctanoate in cynomolgus monkeys. *Toxicol Sci.* 2004;82(2):394-406. Epub 2004/10/08. doi: 10.1093/toxsci/kfh302. PubMed PMID: 15470233.
60. Lupton SJ, Huwe JK, Smith DJ, Dearfield KL, Johnston JJ. Absorption and excretion of ¹⁴C-perfluorooctanoic acid (PFOA) in Angus cattle (*Bos taurus*). *J Agric Food Chem.* 2012;60(4):1128-34. Epub 2012/01/10. doi: 10.1021/jf2042505. PubMed PMID: 22224442.
61. Lupton SJ, Huwe JK, Smith DJ, Dearfield KL, Johnston JJ. Distribution and excretion of perfluorooctane sulfonate (PFOS) in beef cattle (*Bos taurus*). *J Agric Food Chem.* 2014;62(5):1167-73. Epub 2014/01/22. doi: 10.1021/jf404355b. PubMed PMID: 24443932.
62. Han X, Nabb DL, Russell MH, Kennedy GL, Rickard RW. Renal elimination of perfluorocarboxylates (PFCAs). *Chem Res Toxicol.* 2012;25(1):35-46. Epub 2011/10/12. doi: 10.1021/tx200363w. PubMed PMID: 21985250.
63. Xu Y, Fletcher T, Pineda D, Lindh CH, Nilsson C, Glynn A, et al. Serum Half-Lives for Short- and Long-Chain Perfluoroalkyl Acids after Ceasing Exposure from Drinking Water Contaminated by Firefighting Foam. *Environ Health Perspect.* 2020;128(7):77004. Epub 2020/07/11. doi: 10.1289/ehp6785. PubMed PMID: 32648786; PubMed Central PMCID: PMC7351026.
64. Russell MH, Nilsson H, Buck RC. Elimination kinetics of perfluorohexanoic acid in humans and comparison with mouse, rat and monkey. *Chemosphere.* 2013;93(10):2419-25. Epub 2013/09/21. doi: 10.1016/j.chemosphere.2013.08.060. PubMed PMID: 24050716.
65. Rosato I, Bonato T, Fletcher T, Batzella E, Canova C. Estimation of per- and polyfluoroalkyl substances (PFAS) half-lives in human studies: a systematic review and meta-analysis. *Environ Res.* 2024;242:117743. Epub 2023/11/27. doi: 10.1016/j.envres.2023.117743. PubMed PMID: 38008199.
66. Harada KH, Hashida S, Kaneko T, Takenaka K, Minata M, Inoue K, et al. Biliary excretion and cerebrospinal fluid partition of perfluorooctanoate and perfluorooctane sulfonate in humans. *Environ Toxicol*

- Pharmacol. 2007;24(2):134-9. Epub 2007/09/01. doi: 10.1016/j.etap.2007.04.003. PubMed PMID: 21783801.
67. Harada K, Inoue K, Morikawa A, Yoshinaga T, Saito N, Koizumi A. Renal clearance of perfluorooctane sulfonate and perfluorooctanoate in humans and their species-specific excretion. *Environ Res.* 2005;99(2):253-61. Epub 2005/10/01. doi: 10.1016/j.envres.2004.12.003. PubMed PMID: 16194675.
68. Fujii Y, Niisoe T, Harada KH, Uemoto S, Ogura Y, Takenaka K, et al. Toxicokinetics of perfluoroalkyl carboxylic acids with different carbon chain lengths in mice and humans. *Journal of Occupational Health.* 2015;57(1):1-12. doi: 10.1539/joh.14-0136-OA %J Journal of Occupational Health.
69. Zhang Y, Beeson S, Zhu L, Martin JW. Biomonitoring of perfluoroalkyl acids in human urine and estimates of biological half-life. *Environ Sci Technol.* 2013;47(18):10619-27. Epub 2013/08/29. doi: 10.1021/es401905e. PubMed PMID: 23980546.
70. Zhou Z, Shi Y, Vestergren R, Wang T, Liang Y, Cai Y. Highly elevated serum concentrations of perfluoroalkyl substances in fishery employees from Tangxun lake, china. *Environ Sci Technol.* 2014;48(7):3864-74. Epub 2014/03/05. doi: 10.1021/es4057467. PubMed PMID: 24588690.
71. Zhang T, Sun H, Qin X, Gan Z, Kannan K. PFOS and PFOA in paired urine and blood from general adults and pregnant women: assessment of urinary elimination. *Environ Sci Pollut Res Int.* 2015;22(7):5572-9. Epub 2014/11/05. doi: 10.1007/s11356-014-3725-7. PubMed PMID: 25367642.
72. Beeson S, Genuis SJ, Benskin JP, Martin JW. Exceptionally High Serum Concentrations of Perfluorohexanesulfonate in a Canadian Family are Linked to Home Carpet Treatment Applications. *Environ Sci Technol.* 2012;46(23):12960-7. doi: 10.1021/es3034654.
73. Genuis SJ, Curtis L, Birkholz D. Gastrointestinal Elimination of Perfluorinated Compounds Using Cholestyramine and *Chlorella pyrenoidosa*. *ISRN Toxicol.* 2013;2013:657849. Epub 2013/10/10. doi: 10.1155/2013/657849. PubMed PMID: 24106616; PubMed Central PMCID: PMC3782832.
74. Niu S, Cao Y, Chen R, Bedi M, Sanders AP, Ducatman A, et al. A State-of-the-Science Review of Interactions of Per- and Polyfluoroalkyl Substances (PFAS) with Renal Transporters in Health and Disease: Implications for Population Variability in PFAS Toxicokinetics. *Environ Health Perspect.* 2023;131(7):76002. Epub 2023/07/07. doi: 10.1289/ehp11885. PubMed PMID: 37418334; PubMed Central PMCID: PMC3782832.
75. East A, Dawson DE, Brady S, Vallero DA, Tornero-Velez R. A Scoping Assessment of Implemented Toxicokinetic Models of Per- and Polyfluoro-Alkyl Substances, with a Focus on One-Compartment Models.

Toxics. 2023;11(2). Epub 2023/03/01. doi: 10.3390/toxics11020163. PubMed PMID: 36851038; PubMed Central PMCID: PMCPCMC9964825.

76. Worley RR, Moore SM, Tierney BC, Ye X, Calafat AM, Campbell S, et al. Per- and polyfluoroalkyl substances in human serum and urine samples from a residentially exposed community. *Environ Int.* 2017;106:135-43. Epub 2017/06/24. doi: 10.1016/j.envint.2017.06.007. PubMed PMID: 28645013; PubMed Central PMCID: PMCPCMC5673082.

77. Chiu WA, Lynch MT, Lay CR, Antezana A, Malek P, Sokolinski S, et al. Bayesian Estimation of Human Population Toxicokinetics of PFOA, PFOS, PFHxS, and PFNA from Studies of Contaminated Drinking Water. *Environ Health Perspect.* 2022;130(12):127001. Epub 2022/12/02. doi: 10.1289/ehp10103. PubMed PMID: 36454223; PubMed Central PMCID: PMCPCMC9714558.

78. Thompson J, Lorber M, Toms LL, Kato K, Calafat AM, Mueller JF. Use of simple pharmacokinetic modeling to characterize exposure of Australians to perfluorooctanoic acid and perfluorooctane sulfonic acid. *Environ Int.* 2010;36(4):390-7. Epub 2010/03/20. doi: 10.1016/j.envint.2010.02.008. PubMed PMID: 20236705.

79. Fujii Y, Harada KH. Per- and polyfluoroalkyl substances: toxicokinetics, exposure and health risks. *J Toxicol Sci.* 2025;50(3):97-104. Epub 2025/03/03. doi: 10.2131/jts.50.97. PubMed PMID: 40024759.

80. Li X, Zhang J, Liu W, Li X, Zhang X, Jiang Y, et al. Serum levels of perfluorinated compounds in the general population in Shenzhen, China. *Chinese Science Bulletin.* 2011;56(28):3092. doi: 10.1007/s11434-011-4616-7.

81. Verner M-A, Ngueta G, Jensen ET, Fromme H, Völkel W, Nygaard UC, et al. A Simple Pharmacokinetic Model of Prenatal and Postnatal Exposure to Perfluoroalkyl Substances (PFASs). *Environ Sci Technol.* 2016;50(2):978-86. doi: 10.1021/acs.est.5b04399.

82. East A, Egeghy PP, Hubal EAC, Slover R, Vallero DA. Computational estimates of daily aggregate exposure to PFOA/PFOS from 2011 to 2017 using a basic intake model. *J Expo Sci Environ Epidemiol.* 2023;33(1):56-68. doi: 10.1038/s41370-021-00374-w.

83. Vaccari L, Ranzi A, Canova C, Ghermandi G, Giannini S, Pitter G, et al. Reliability of toxicokinetic modelling for PFAS exposure assessment in contaminated water in northern Italy. *Heliyon.* 2024;10(15):e35288. doi: <https://doi.org/10.1016/j.heliyon.2024.e35288>.

84. Bartell SM, Calafat AM, Lyu C, Kato K, Ryan PB, Steenland K. Rate of decline in serum PFOA concentrations after granular activated carbon filtration at two public water systems in Ohio and West Virginia. *Environ Health Perspect.* 2010;118(2):222-8. Epub 2010/02/04. doi: 10.1289/ehp.0901252. PubMed PMID: 20123620; PubMed Central PMCID: PMCPCMC2831921.

85. Andersen ME, Clewell HJ, 3rd, Tan YM, Butenhoff JL, Olsen GW. Pharmacokinetic modeling of saturable, renal resorption of perfluoroalkylacids in monkeys--probing the determinants of long plasma half-lives. *Toxicology*. 2006;227(1-2):156-64. Epub 2006/09/19. doi: 10.1016/j.tox.2006.08.004. PubMed PMID: 16978759.
86. Brochot C, Quindroit P. Modelling the Fate of Chemicals in Humans Using a Lifetime Physiologically Based Pharmacokinetic (PBPK) Model in MERLIN-Expo. 2018. p. 215-57.
87. Fàbrega F, Kumar V, Schuhmacher M, Domingo JL, Nadal M. PBPK modeling for PFOS and PFOA: validation with human experimental data. *Toxicol Lett*. 2014;230(2):244-51. Epub 2014/01/21. doi: 10.1016/j.toxlet.2014.01.007. PubMed PMID: 24440341.
88. Fàbrega F, Nadal M, Schuhmacher M, Domingo JL, Kumar V. Influence of the uncertainty in the validation of PBPK models: A case-study for PFOS and PFOA. *Regul Toxicol Pharmacol*. 2016;77:230-9. Epub 2016/03/20. doi: 10.1016/j.yrtph.2016.03.009. PubMed PMID: 26993749.
89. Fàbrega F, Kumar V, Benfenati E, Schuhmacher M, Domingo JL, Nadal M. Physiologically based pharmacokinetic modeling of perfluoroalkyl substances in the human body. *Toxicol Environ Chem*. 2015;97(6):814-27. doi: 10.1080/02772248.2015.1060976.
90. Loccisano AE, Longnecker MP, Campbell JL, Jr., Andersen ME, Clewell HJ, 3rd. Development of PBPK models for PFOA and PFOS for human pregnancy and lactation life stages. *J Toxicol Environ Health A*. 2013;76(1):25-57. Epub 2012/11/16. doi: 10.1080/15287394.2012.722523. PubMed PMID: 23151209; PubMed Central PMCID: PMC3502013.
91. Verner MA, Loccisano AE, Morken NH, Yoon M, Wu H, McDougall R, et al. Associations of Perfluoroalkyl Substances (PFAS) with Lower Birth Weight: An Evaluation of Potential Confounding by Glomerular Filtration Rate Using a Physiologically Based Pharmacokinetic Model (PBPK). *Environ Health Perspect*. 2015;123(12):1317-24. Epub 2015/05/27. doi: 10.1289/ehp.1408837. PubMed PMID: 26008903; PubMed Central PMCID: PMC3502013.
92. Sweeney LM. Physiologically based pharmacokinetic (PBPK) modeling of perfluorohexane sulfonate (PFHxS) in humans. *Regul Toxicol Pharmacol*. 2022;129:105099. Epub 2021/12/22. doi: 10.1016/j.yrtph.2021.105099. PubMed PMID: 34933042.
93. Chou WC, Lin Z. Bayesian evaluation of a physiologically based pharmacokinetic (PBPK) model for perfluorooctane sulfonate (PFOS) to characterize the interspecies uncertainty between mice, rats, monkeys, and humans: Development and performance verification. *Environ Int*. 2019;129:408-22. Epub 2019/06/04. doi: 10.1016/j.envint.2019.03.058. PubMed PMID: 31152982.

94. Worley RR, Yang X, Fisher J. Physiologically based pharmacokinetic modeling of human exposure to perfluorooctanoic acid suggests historical non drinking-water exposures are important for predicting current serum concentrations. *Toxicol Appl Pharmacol.* 2017;330:9-21. Epub 2017/07/08. doi: 10.1016/j.taap.2017.07.001. PubMed PMID: 28684146; PubMed Central PMCID: PMC5664934.
95. EFSA, Schrenk D, Bignami M, Bodin L, Chipman JK, Del Mazo J, et al. Scientific Opinion on the risk to human health related to the presence of perfluoroalkyl substances in food. *Efsa j.* 2020;18(9):e06223. Epub 2020/10/01. doi: 10.2903/j.efsa.2020.6223. PubMed PMID: 32994824; PubMed Central PMCID: PMC5664934.
96. Buser MC, Jones D, Pohl RH, Ruiz P, Scinicariello F, Chou S, et al. Toxicological profile for Perfluoroalkyls. Agency for Toxic Substances and Disease Registry (ATSDR). 2021. doi: 10.15620/cdc:59198.
97. US Environmental Protection Agency (EPA). FINAL Human Health Toxicity Assessment for Perfluorooctane Sulfonic Acid (PFOS) and Related Salts. Office of Water (4304T), Health and Ecological Criteria Division, Washington, DC 20460, 2024.
98. US Environment Protection Agency (EPA). FINAL Human Health Toxicity Assessment for Perfluorooctanoic Acid (PFOA) and Related Salts. Office of Water (4304T), Health and Ecological Criteria Division, Washington, DC 20460; 2024.
99. International Agency for Research on Cancer (IARC). Perfluorooctanoic Acid (PFOA) and Perfluorooctanesulfonic Acid (PFOS): IARC Monographs on the Identification of Carcinogenic Hazards to Humans. Lyon (FR): International Agency for Research on Cancer; 2025.
100. Fenton SE, Ducatman A, Boobis A, DeWitt JC, Lau C, Ng C, et al. Per- and Polyfluoroalkyl Substance Toxicity and Human Health Review: Current State of Knowledge and Strategies for Informing Future Research. *Environ Toxicol Chem.* 2021;40(3):606-30. Epub 2020/10/06. doi: 10.1002/etc.4890. PubMed PMID: 33017053; PubMed Central PMCID: PMC5664934.
101. United Nations Environment Programme (UNEP). Stockholm Convention on Persistent Organic Pollutants (POPs) 2023 [cited 2025 August 15th]. Available from: <https://www.pops.int/Portals/0/download.aspx?e=UNEP-POPS-COP-CONVTEXT-2023.English.pdf>.
102. United Nations Environment Programme (UNEP). Twelfth meeting of the Conference of the Parties to the Stockholm Convention 2025 [cited 2025 May 20th]. Available from: <https://chm.pops.int/TheConvention/ConferenceoftheParties/Meetings/COP12/tabid/9744/Default.aspx>.

103. European Chemicals Agency (ECHA). ECHA publishes PFAS restriction proposal 2023 [cited 2025 August 7th]. Available from: <https://echa.europa.eu/en/-/echa-publishes-pfas-restriction-proposal>.
104. Grandjean P, Andersen EW, Budtz-Jørgensen E, Nielsen F, Mølbak K, Weihe P, et al. Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. *JAMA*. 2012;307(4):391-7. Epub 2012/01/26. doi: 10.1001/jama.2011.2034. PubMed PMID: 22274686; PubMed Central PMCID: PMC4402650.
105. Dormitzer PR, Galli G, Castellino F, Golding H, Khurana S, Del Giudice G, et al. Influenza vaccine immunology. *Immunol Rev*. 2011;239(1):167-77. Epub 2011/01/05. doi: 10.1111/j.1600-065X.2010.00974.x. PubMed PMID: 21198671.
106. Crawford L, Halperin SA, Dzierlenga MW, Skidmore B, Linakis MW, Nakagawa S, et al. Systematic review and meta-analysis of epidemiologic data on vaccine response in relation to exposure to five principal perfluoroalkyl substances. *Environ Int*. 2023;172:107734. Epub 2023/02/11. doi: 10.1016/j.envint.2023.107734. PubMed PMID: 36764183.
107. Granum B, Haug LS, Namork E, Stølevik SB, Thomsen C, Aaberge IS, et al. Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood. *J Immunotoxicol*. 2013;10(4):373-9. Epub 2013/01/29. doi: 10.3109/1547691x.2012.755580. PubMed PMID: 23350954.
108. Mogensen UB, Grandjean P, Heilmann C, Nielsen F, Weihe P, Budtz-Jørgensen E. Structural equation modeling of immunotoxicity associated with exposure to perfluorinated alkylates. *Environmental Health*. 2015;14(1):47. doi: 10.1186/s12940-015-0032-9.
109. Stein CR, McGovern KJ, Pajak AM, Maglione PJ, Wolff MS. Perfluoroalkyl and polyfluoroalkyl substances and indicators of immune function in children aged 12-19 y: National Health and Nutrition Examination Survey. *Pediatr Res*. 2016;79(2):348-57. Epub 2015/10/23. doi: 10.1038/pr.2015.213. PubMed PMID: 26492286; PubMed Central PMCID: PMC45065061.
110. Grandjean P, Heilmann C, Weihe P, Nielsen F, Mogensen UB, Budtz-Jørgensen E. Serum Vaccine Antibody Concentrations in Adolescents Exposed to Perfluorinated Compounds. *Environ Health Perspect*. 2017;125(7):077018. Epub 2017/07/28. doi: 10.1289/ehp275. PubMed PMID: 28749778; PubMed Central PMCID: PMC45744724.
111. Grandjean P, Heilmann C, Weihe P, Nielsen F, Mogensen UB, Timmermann A, et al. Estimated exposures to perfluorinated compounds in infancy predict attenuated vaccine antibody concentrations at age 5-years. *J Immunotoxicol*. 2017;14(1):188-95. doi: 10.1080/1547691X.2017.1360968.
112. Abraham K, Mielke H, Fromme H, Völkel W, Menzel J, Peiser M, et al. Internal exposure to perfluoroalkyl substances (PFASs) and biological

- markers in 101 healthy 1-year-old children: associations between levels of perfluorooctanoic acid (PFOA) and vaccine response. *Arch Toxicol.* 2020;94(6):2131-47. Epub 2020/04/01. doi: 10.1007/s00204-020-02715-4. PubMed PMID: 32227269; PubMed Central PMCID: PMC7303054.
113. Timmermann CAG, Jensen KJ, Nielsen F, Budtz-Jørgensen E, van der Klis F, Benn CS, et al. Serum Perfluoroalkyl Substances, Vaccine Responses, and Morbidity in a Cohort of Guinea-Bissau Children. *Environ Health Perspect.* 2020;128(8):87002. Epub 2020/08/11. doi: 10.1289/ehp6517. PubMed PMID: 32772733; PubMed Central PMCID: PMC7416537.
114. Timmermann CAG, Pedersen HS, Weihe P, Bjerregaard P, Nielsen F, Heilmann C, et al. Concentrations of tetanus and diphtheria antibodies in vaccinated Greenlandic children aged 7-12 years exposed to marine pollutants, a cross sectional study. *Environ Res.* 2022;203:111712. Epub 2021/08/04. doi: 10.1016/j.envres.2021.111712. PubMed PMID: 34343554.
115. Zell-Baran LM, Dabelea D, Norris JM, Glueck DH, Adgate JL, Brown JM, et al. Prenatal Exposure to Poly- and Perfluoroalkyl Substances (2009-2014) and Vaccine Antibody Titers of Measles, Mumps, Rubella, and Varicella in Children Four to Eight Years Old from the Healthy Start Cohort. *Environ Health Perspect.* 2023;131(12):127018. Epub 2023/12/26. doi: 10.1289/ehp12863. PubMed PMID: 38147368; PubMed Central PMCID: PMC750888.
116. Zhang Y, Mustieles V, Wang Y-X, Sun Q, Coull B, Sun Y, et al. Red Blood Cell Folate Modifies the Association between Serum Per- and Polyfluoroalkyl Substances and Antibody Concentrations in U.S. Adolescents. *Environ Sci Technol.* 2023;57(6):2445-56. doi: 10.1021/acs.est.2c07152.
117. Sigvaldsen A, Højsager FD, Paarup HM, Beck IH, Timmermann CAG, Boye H, et al. Early-life exposure to perfluoroalkyl substances and serum antibody concentrations towards common childhood vaccines in 18-month-old children in the Odense Child Cohort. *Environ Res.* 2024;242:117814. Epub 2023/12/03. doi: 10.1016/j.envres.2023.117814. PubMed PMID: 38042520.
118. Hong X, Morgenlander WR, Nadeau K, Wang G, Frischmeyer-Guerrero PA, Pearson C, et al. Maternal exposure to per- and polyfluoroalkyl substances and epitope level antibody response to vaccines against measles and rubella in children from the Boston birth cohort. *Environ Int.* 2025;198:109433. Epub 2025/04/12. doi: 10.1016/j.envint.2025.109433. PubMed PMID: 40215916; PubMed Central PMCID: PMC7508861.
119. Looker C, Luster MI, Calafat AM, Johnson VJ, Burleson GR, Burleson FG, et al. Influenza vaccine response in adults exposed to perfluorooctanoate and perfluorooctanesulfonate. *Toxicol Sci.* 2014;138(1):76-88. Epub 2013/11/29. doi: 10.1093/toxsci/kft269. PubMed PMID: 24284791; PubMed Central PMCID: PMC7424206.

120. Kielsen K, Shamim Z, Ryder LP, Nielsen F, Grandjean P, Budtz-Jørgensen E, et al. Antibody response to booster vaccination with tetanus and diphtheria in adults exposed to perfluorinated alkylates. *J Immunotoxicol.* 2016;13(2):270-3. Epub 2015/07/17. doi: 10.3109/1547691x.2015.1067259. PubMed PMID: 26181512; PubMed Central PMCID: PMCPMC4739630.
121. Stein CR, Ge Y, Wolff MS, Ye X, Calafat AM, Kraus T, et al. Perfluoroalkyl substance serum concentrations and immune response to FluMist vaccination among healthy adults. *Environ Res.* 2016;149:171-8. Epub 2016/05/22. doi: 10.1016/j.envres.2016.05.020. PubMed PMID: 27208468; PubMed Central PMCID: PMCPMC4907856.
122. Pilkerton CS, Hobbs GR, Lilly C, Knox SS. Rubella immunity and serum perfluoroalkyl substances: Sex and analytic strategy. *PLoS One.* 2018;13(9):e0203330. Epub 2018/09/25. doi: 10.1371/journal.pone.0203330. PubMed PMID: 30248109; PubMed Central PMCID: PMCPMC6152869.
123. Shih YH, Blomberg AJ, Bind MA, Holm D, Nielsen F, Heilmann C, et al. Serum vaccine antibody concentrations in adults exposed to per- and polyfluoroalkyl substances: A birth cohort in the Faroe Islands. *J Immunotoxicol.* 2021;18(1):85-92. Epub 2021/06/19. doi: 10.1080/1547691x.2021.1922957. PubMed PMID: 34143710.
124. Mingaleeva RN, Nigmatulina NA, Sharafetdinova LM, Romozanova AM, Gabdoulkhakova AG, Filina YV, et al. Biology of the SARS-CoV-2 Coronavirus. *Biochemistry (Mosc).* 2022;87(12):1662-78. Epub 2023/01/31. doi: 10.1134/s0006297922120215. PubMed PMID: 36717455; PubMed Central PMCID: PMCPMC9839213.
125. World Health Organization (WHO). WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020 [cited 2025 July 21st]. Available from: <https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020>,.
126. Echaide M, Chocarro de Erauso L, Bocanegra A, Blanco E, Kochan G, Escors D. mRNA Vaccines against SARS-CoV-2: Advantages and Caveats. *Int J Mol Sci.* 2023;24(6). Epub 2023/03/30. doi: 10.3390/ijms24065944. PubMed PMID: 36983017; PubMed Central PMCID: PMCPMC10051235.
127. Bettini E, Locci M. SARS-CoV-2 mRNA Vaccines: Immunological Mechanism and Beyond. *Vaccines.* 2021;9(2). Epub 2021/03/07. doi: 10.3390/vaccines9020147. PubMed PMID: 33673048; PubMed Central PMCID: PMCPMC7918810.
128. World Health Organization (WHO). COVID-19 Cases, World [cited 2025 July 21st]. Available from: <https://data.who.int/dashboards/covid19/cases?n=o>.

129. World Health Organization (WHO). Number of COVID-19 deaths reported to WHO (cumulative total) [cited 2025 July 21st]. Available from: <https://data.who.int/dashboards/covid19/deaths?n=o>.
130. COVID-19 Excess Mortality Collaborators. Estimating excess mortality due to the COVID-19 pandemic: a systematic analysis of COVID-19-related mortality, 2020-21. *Lancet*. 2022;399(10334):1513-36. Epub 2022/03/14. doi: 10.1016/s0140-6736(21)02796-3. PubMed PMID: 35279232; PubMed Central PMCID: PMCPCMC8912932.
131. Meslé MMI, Brown J, Mook P, Katz MA, Hagan J, Pastore R, et al. Estimated number of lives directly saved by COVID-19 vaccination programmes in the WHO European Region from December, 2020, to March, 2023: a retrospective surveillance study. *Lancet Respir Med*. 2024;12(9):714-27. Epub 2024/08/11. doi: 10.1016/s2213-2600(24)00179-6. PubMed PMID: 39127051.
132. Beans C. News Feature: How "forever chemicals" might impair the immune system. *Proc Natl Acad Sci U S A*. 2021;118(15). Epub 2021/04/10. doi: 10.1073/pnas.2105018118. PubMed PMID: 33833063; PubMed Central PMCID: PMCPCMC8054019.
133. Nielsen C, Jöud A. Susceptibility to COVID-19 after High Exposure to Perfluoroalkyl Substances from Contaminated Drinking Water: An Ecological Study from Ronneby, Sweden. *Int J Environ Res Public Health*. 2021;18(20). Epub 2021/10/24. doi: 10.3390/ijerph182010702. PubMed PMID: 34682448; PubMed Central PMCID: PMCPCMC8535293.
134. Ji J, Song L, Wang J, Yang Z, Yan H, Li T, et al. Association between urinary per- and poly-fluoroalkyl substances and COVID-19 susceptibility. *Environ Int*. 2021;153:106524. Epub 2021/03/28. doi: 10.1016/j.envint.2021.106524. PubMed PMID: 33773143; PubMed Central PMCID: PMCPCMC7972714.
135. Grandjean P, Timmermann CAG, Kruse M, Nielsen F, Vinholt PJ, Boding L, et al. Severity of COVID-19 at elevated exposure to perfluorinated alkylates. *PLoS One*. 2020;15(12):e0244815. Epub 2021/01/01. doi: 10.1371/journal.pone.0244815. PubMed PMID: 33382826; PubMed Central PMCID: PMCPCMC7774856 contamination, which does not affect the adherence to all PLOS ONE policies, the authors have no competing interests to declare, financial or otherwise.
136. Catelan D, Biggeri A, Russo F, Gregori D, Pitter G, Da Re F, et al. Exposure to Perfluoroalkyl Substances and Mortality for COVID-19: A Spatial Ecological Analysis in the Veneto Region (Italy). *Int J Environ Res Public Health*. 2021;18(5). Epub 2021/04/04. doi: 10.3390/ijerph18052734. PubMed PMID: 33800362; PubMed Central PMCID: PMCPCMC7967461.
137. Seals R, Bartell SM, Steenland K. Accumulation and clearance of perfluorooctanoic acid (PFOA) in current and former residents of an exposed community. *Environ Health Perspect*. 2011;119(1):119-24. Epub

2010/09/28. doi: 10.1289/ehp.1002346. PubMed PMID: 20870569; PubMed Central PMCID: PMCPMC3018490.

138. Lindh CH, Rylander L, Toft G, Axmon A, Rignell-Hydbom A, Giwercman A, et al. Blood serum concentrations of perfluorinated compounds in men from Greenlandic Inuit and European populations. *Chemosphere*. 2012;88(11):1269-75. Epub 2012/04/13. doi: 10.1016/j.chemosphere.2012.03.049. PubMed PMID: 22494529.

139. Brede E, Wilhelm M, Göen T, Müller J, Rauchfuss K, Kraft M, et al. Two-year follow-up biomonitoring pilot study of residents' and controls' PFC plasma levels after PFOA reduction in public water system in Arnsberg, Germany. *Int J Hyg Environ Health*. 2010;213(3):217-23. doi: <https://doi.org/10.1016/j.ijheh.2010.03.007>.

140. Nilsson S, Thompson J, Mueller JF, Bräunig J. Apparent Half-Lives of Chlorinated-Perfluorooctane Sulfonate and Perfluorooctane Sulfonate Isomers in Aviation Firefighters. *Environ Sci Technol*. 2022;56(23):17052-60. doi: 10.1021/acs.est.2c04637.

141. Jian JM, Chen D, Han FJ, Guo Y, Zeng L, Lu X, et al. A short review on human exposure to and tissue distribution of per- and polyfluoroalkyl substances (PFASs). *Sci Total Environ*. 2018;636:1058-69. Epub 2018/06/20. doi: 10.1016/j.scitotenv.2018.04.380. PubMed PMID: 29913568.

142. Gao Y, Fu J, Cao H, Wang Y, Zhang A, Liang Y, et al. Differential accumulation and elimination behavior of perfluoroalkyl Acid isomers in occupational workers in a manufactory in China. *Environ Sci Technol*. 2015;49(11):6953-62. Epub 2015/05/01. doi: 10.1021/acs.est.5b00778. PubMed PMID: 25927957.

143. Forni Ognà V, Ognà A, Vuistiner P, Pruijm M, Ponte B, Ackermann D, et al. New anthropometry-based age- and sex-specific reference values for urinary 24-hour creatinine excretion based on the adult Swiss population. *BMC Med*. 2015;13:40. Epub 2015/04/11. doi: 10.1186/s12916-015-0275-x. PubMed PMID: 25858764; PubMed Central PMCID: PMCPMC4354997.

144. Boron WF, Boulpaep EL. *Medical physiology*. Third edition. ed. Philadelphia, PA: Elsevier; 2016.

145. Armstrong LE, Pumerantz AC, Fiala KA, Roti MW, Kavouras SA, Casa DJ, et al. Human hydration indices: acute and longitudinal reference values. *Int J Sport Nutr Exerc Metab*. 2010;20(2):145-53. Epub 2010/05/19. doi: 10.1123/ijsnem.20.2.145. PubMed PMID: 20479488.

146. Borghi L, Meschi T, Amato F, Briganti A, Novarini A, Giannini A. Urinary volume, water and recurrences in idiopathic calcium nephrolithiasis: a 5-year randomized prospective study. *J Urol*. 1996;155(3):839-43. Epub 1996/03/01. PubMed PMID: 8583588.

147. Inker LA, Eneanya ND, Coresh J, Tighiouart H, Wang D, Sang Y, et al. New Creatinine- and Cystatin C-Based Equations to Estimate GFR without Race. *N Engl J Med*. 2021;385(19):1737-49. Epub 2021/09/24. doi: 10.1056/NEJMoa2102953. PubMed PMID: 34554658; PubMed Central PMCID: PMCPCMC8822996.
148. Wong F, MacLeod M, Mueller JF, Cousins IT. Enhanced elimination of perfluorooctane sulfonic acid by menstruating women: evidence from population-based pharmacokinetic modeling. *Environ Sci Technol*. 2014;48(15):8807-14. Epub 2014/06/20. doi: 10.1021/es500796y. PubMed PMID: 24943117.
149. Genuis SJ, Liu Y, Genuis QI, Martin JW. Phlebotomy treatment for elimination of perfluoroalkyl acids in a highly exposed family: a retrospective case-series. *PLoS One*. 2014;9(12):e114295. Epub 2014/12/17. doi: 10.1371/journal.pone.0114295. PubMed PMID: 25504057; PubMed Central PMCID: PMCPCMC4264749.
150. Gasiorowski R, Forbes MK, Silver G, Krastev Y, Hamdorf B, Lewis B, et al. Effect of Plasma and Blood Donations on Levels of Perfluoroalkyl and Polyfluoroalkyl Substances in Firefighters in Australia: A Randomized Clinical Trial. *JAMA Netw Open*. 2022;5(4):e226257. Epub 2022/04/09. doi: 10.1001/jamanetworkopen.2022.6257. PubMed PMID: 35394514; PubMed Central PMCID: PMCPCMC8994130.
151. Sundström M, Chang SC, Noker PE, Gorman GS, Hart JA, Ehresman DJ, et al. Comparative pharmacokinetics of perfluorohexanesulfonate (PFHxS) in rats, mice, and monkeys. *Reprod Toxicol*. 2012;33(4):441-51. Epub 2011/08/23. doi: 10.1016/j.reprotox.2011.07.004. PubMed PMID: 21856411.
152. Norén E, Blomberg AJ, Lindh C, Pineda D, Jakobsson K, Nielsen C. Transplacental transfer efficiency of perfluoroalkyl substances (PFAS) after long-term exposure to highly contaminated drinking water: a study in the Ronneby Mother-Child Cohort. *J Expo Sci Environ Epidemiol*. 2025;35(3):445-53. doi: 10.1038/s41370-025-00758-2.
153. Appel M, Forsthuber M, Ramos R, Widhalm R, Granitzer S, Uhl M, et al. The transplacental transfer efficiency of per- and polyfluoroalkyl substances (PFAS): a first meta-analysis. *J Toxicol Environ Health B Crit Rev*. 2022;25(1):23-42. Epub 2021/12/22. doi: 10.1080/10937404.2021.2009946. PubMed PMID: 34930098.
154. Ma D, Lu Y, Liang Y, Ruan T, Li J, Zhao C, et al. A Critical Review on Transplacental Transfer of Per- and Polyfluoroalkyl Substances: Prenatal Exposure Levels, Characteristics, and Mechanisms. *Environ Sci Technol*. 2022;56(10):6014-26. Epub 2021/06/19. doi: 10.1021/acs.est.1c01057. PubMed PMID: 34142548.
155. Zheng P, Liu Y, An Q, Yang X, Yin S, Ma LQ, et al. Prenatal and postnatal exposure to emerging and legacy per-/polyfluoroalkyl

- substances: Levels and transfer in maternal serum, cord serum, and breast milk. *Sci Total Environ.* 2022;812:152446. Epub 2021/12/25. doi: 10.1016/j.scitotenv.2021.152446. PubMed PMID: 34952085.
156. Papadopoulou E, Sabaredzovic A, Namork E, Nygaard UC, Granum B, Haug LS. Exposure of Norwegian toddlers to perfluoroalkyl substances (PFAS): The association with breastfeeding and maternal PFAS concentrations. *Environ Int.* 2016;94:687-94. Epub 2016/07/28. doi: 10.1016/j.envint.2016.07.006. PubMed PMID: 27453094.
157. Gyllenhammar I, Benskin JP, Sandblom O, Berger U, Ahrens L, Lignell S, et al. Perfluoroalkyl Acids (PFAAs) in Serum from 2-4-Month-Old Infants: Influence of Maternal Serum Concentration, Gestational Age, Breast-Feeding, and Contaminated Drinking Water. *Environ Sci Technol.* 2018;52(12):7101-10. Epub 2018/05/16. doi: 10.1021/acs.est.8b00770. PubMed PMID: 29758986.
158. Blomberg AJ, Norén E, Haug LS, Lindh C, Sabaredzovic A, Pineda D, et al. Estimated Transfer of Perfluoroalkyl Substances (PFAS) from Maternal Serum to Breast Milk in Women Highly Exposed from Contaminated Drinking Water: A Study in the Ronneby Mother-Child Cohort. *Environ Health Perspect.* 2023;131(1):17005. Epub 2023/01/24. doi: 10.1289/ehp11292. PubMed PMID: 36688826; PubMed Central PMCID: PMC9869870.
159. Genuis SJ, Beeson S, Birkholz D. Biomonitoring and Elimination of Perfluorinated Compounds and Polychlorinated Biphenyls through Perspiration: Blood, Urine, and Sweat Study. *ISRN Toxicol.* 2013;2013:483832. Epub 2013/10/02. doi: 10.1155/2013/483832. PubMed PMID: 24083032; PubMed Central PMCID: PMC3776372.
160. Estes JW. Clinical pharmacokinetics of heparin. *Clin Pharmacokinet.* 1980;5(3):204-20. Epub 1980/05/01. doi: 10.2165/00003088-198005030-00002. PubMed PMID: 6993082.
161. Holford NH. Clinical pharmacokinetics and pharmacodynamics of warfarin. Understanding the dose-effect relationship. *Clin Pharmacokinet.* 1986;11(6):483-504. Epub 1986/11/01. doi: 10.2165/00003088-198611060-00005. PubMed PMID: 3542339.
162. Davies NM, Skjodt NM. Choosing the right nonsteroidal anti-inflammatory drug for the right patient: a pharmacokinetic approach. *Clin Pharmacokinet.* 2000;38(5):377-92. Epub 2000/06/08. doi: 10.2165/00003088-200038050-00001. PubMed PMID: 10843458.
163. Morgan DJ, Bray KM. Lean body mass as a predictor of drug dosage. Implications for drug therapy. *Clin Pharmacokinet.* 1994;26(4):292-307. Epub 1994/04/01. doi: 10.2165/00003088-199426040-00005. PubMed PMID: 8013162.
164. Huang J-K, Chuang Y-S, Wu P-H, Tai C-J, Lin J-R, Kuo M-C, et al. Decreased levels of perfluoroalkyl substances in patients receiving

- hemodialysis treatment. *Sci Total Environ.* 2023;896:165184. doi: <https://doi.org/10.1016/j.scitotenv.2023.165184>.
165. Lindell AE, Griebhammer A, Michaelis L, Papagiannidis D, Ochner H, Kamrad S, et al. Human gut bacteria bioaccumulate per- and polyfluoroalkyl substances. *Nat Microbiol.* 2025;10(7):1630-47. Epub 2025/07/02. doi: 10.1038/s41564-025-02032-5. PubMed PMID: 40595288; PubMed Central PMCID: PMCPMC12222025
166. Cambiotics. *Cambiotics* 2025 [cited 2025 June 25th]. Available from: <https://cambiotics.com/>.
167. Lykkebo CA, Nguyen KH, Niklas AA, Laursen MF, Bahl MI, Licht TR, et al. Diet rich in soluble dietary fibres increases excretion of perfluorooctane sulfonic acid (PFOS) in male Sprague-Dawley rats. *Food Chem Toxicol.* 2024;193:115041. Epub 2024/10/13. doi: 10.1016/j.fct.2024.115041. PubMed PMID: 39395735.
168. Schlezinger JJ, Biswas K, Garcia A, Heiger-Bernays WJ, Bello D. An oat fiber intervention for reducing PFAS body burden: A pilot study in male C57Bl/6 J mice. *Toxicol Appl Pharmacol.* 2025;495:117188. Epub 2024/12/09. doi: 10.1016/j.taap.2024.117188. PubMed PMID: 39647509; PubMed Central PMCID: PMCPMC11798698.
169. Out C, Groen AK, Brufau G. Bile acid sequestrants: more than simple resins. *Curr Opin Lipidol.* 2012;23(1):43-55. Epub 2011/12/22. doi: 10.1097/MOL.0b013e32834f0ef3. PubMed PMID: 22186660.
170. Johnson JD, Gibson SJ, Ober RE. Cholestyramine-enhanced fecal elimination of carbon-14 in rats after administration of ammonium [14C]perfluorooctanoate or potassium [14C]perfluorooctanesulfonate. *Fundam Appl Toxicol.* 1984;4(6):972-6. Epub 1984/12/01. doi: 10.1016/0272-0590(84)90235-5. PubMed PMID: 6519377.
171. Genuis SJ, Birkholz D, Ralitsch M, Thibault N. Human detoxification of perfluorinated compounds. *Public Health.* 2010;124(7):367-75. Epub 2010/07/14. doi: 10.1016/j.puhe.2010.03.002. PubMed PMID: 20621793.
172. Delaere I, Harris K, Gaskin S, Tefera Y, Mitchell K, Springer D, et al. Changes in serum perfluorooctane sulfonic acid and perfluorohexane sulfonic acid concentrations in firefighters accessing a voluntary perfluoroalkyl and polyfluoroalkyl substances reduction treatment program. *Environ Int.* 2025;202:109609. Epub 2025/06/21. doi: 10.1016/j.envint.2025.109609. PubMed PMID: 40540942.
173. Kimura O, Fujii Y, Haraguchi K, Kato Y, Ohta C, Koga N, et al. Uptake of perfluorooctanoic acid by Caco-2 cells: Involvement of organic anion transporting polypeptides. *Toxicol Lett.* 2017;277:18-23. Epub 2017/05/30. doi: 10.1016/j.toxlet.2017.05.012. PubMed PMID: 28552774.
174. Lousse J, Dellaflora L, van den Heuvel J, Rijkers D, Leenders L, Dorne JCM, et al. Perfluoroalkyl substances (PFASs) are substrates of the

- renal human organic anion transporter 4 (OAT4). *Arch Toxicol.* 2023;97(3):685-96. Epub 2022/11/28. doi: 10.1007/s00204-022-03428-6. PubMed PMID: 36436016; PubMed Central PMCID: PMCPCMC9968691.
175. Louisse J, Pedroni L, van den Heuvel J, Rijkers D, Leenders L, Noorlander A, et al. In vitro and in silico characterization of the transport of selected perfluoroalkyl carboxylic acids and perfluoroalkyl sulfonic acids by human organic anion transporter 1 (OAT1), OAT2 and OAT3. *Toxicology.* 2024;509:153961. Epub 2024/09/30. doi: 10.1016/j.tox.2024.153961. PubMed PMID: 39343156.
176. Nakagawa H, Hirata T, Terada T, Jutabha P, Miura D, Harada KH, et al. Roles of organic anion transporters in the renal excretion of perfluorooctanoic acid. *Basic Clin Pharmacol Toxicol.* 2008;103(1):1-8. Epub 2008/04/01. doi: 10.1111/j.1742-7843.2007.00155.x. PubMed PMID: 18373647.
177. Nakagawa H, Terada T, Harada KH, Hitomi T, Inoue K, Inui K, et al. Human organic anion transporter hOAT4 is a transporter of perfluorooctanoic acid. *Basic Clin Pharmacol Toxicol.* 2009;105(2):136-8. Epub 2009/04/18. doi: 10.1111/j.1742-7843.2009.00409.x. PubMed PMID: 19371258.
178. Yang CH, Glover KP, Han X. Characterization of cellular uptake of perfluorooctanoate via organic anion-transporting polypeptide 1A2, organic anion transporter 4, and urate transporter 1 for their potential roles in mediating human renal reabsorption of perfluorocarboxylates. *Toxicol Sci.* 2010;117(2):294-302. Epub 2010/07/20. doi: 10.1093/toxsci/kfq219. PubMed PMID: 20639259.
179. Ducatman A, Luster M, Fletcher T. Perfluoroalkyl substance excretion: Effects of organic anion-inhibiting and resin-binding drugs in a community setting. *Environ Toxicol Pharmacol.* 2021;85:103650. Epub 2021/04/06. doi: 10.1016/j.etap.2021.103650. PubMed PMID: 33819618.
180. Andersen ME, Hagenbuch B, Apte U, Corton JC, Fletcher T, Lau C, et al. Why is elevation of serum cholesterol associated with exposure to perfluoroalkyl substances (PFAS) in humans? A workshop report on potential mechanisms. *Toxicology.* 2021;459:152845. doi: <https://doi.org/10.1016/j.tox.2021.152845>.
181. Sette A, Crotty S. Immunological memory to SARS-CoV-2 infection and COVID-19 vaccines. *Immunol Rev.* 2022;310(1):27-46. Epub 2022/06/24. doi: 10.1111/imr.13089. PubMed PMID: 35733376; PubMed Central PMCID: PMCPCMC9349657.
182. Notarbartolo S. T-Cell Immune Responses to SARS-CoV-2 Infection and Vaccination. *Vaccines.* 2024;12(10). Epub 2024/10/26. doi: 10.3390/vaccines12101126. PubMed PMID: 39460293; PubMed Central PMCID: PMCPCMC11511197.

183. Hollister J, Caban-Martinez AJ, Ellingson KD, Beitel S, Fowlkes AL, Lutrick K, et al. Serum per- and polyfluoroalkyl substance concentrations and longitudinal change in post-infection and post-vaccination SARS-CoV-2 antibodies. *Environ Res.* 2023;239(Pt 1):117297. Epub 2023/10/11. doi: 10.1016/j.envres.2023.117297. PubMed PMID: 37816422.
184. Timmermann A, Johansen IS, Tolstrup M, Heilmann C, Budtz-Jørgensen E, Tolstrup JS, et al. Antibody response to SARS-CoV-2 mRNA vaccination in Danish adults exposed to perfluoroalkyl substances (PFASs): The ENFORCE study. *Environ Res.* 2024;263(Pt 1):120039. Epub 2024/09/27. doi: 10.1016/j.envres.2024.120039. PubMed PMID: 39326653.
185. Porter AK, Kleinschmidt SE, Andres KL, Reusch CN, Krisko RM, Taiwo OA, et al. Antibody response to COVID-19 vaccines among workers with a wide range of exposure to per- and polyfluoroalkyl substances. *Environ Int.* 2022;169:107537. Epub 2022/10/03. doi: 10.1016/j.envint.2022.107537. PubMed PMID: 36183490; PubMed Central PMCID: PMCPCMC9489981.
186. Bailey JM, Wang L, McDonald JM, Gray JS, Petrie JG, Martin ET, et al. Immune response to COVID-19 vaccination in a population with a history of elevated exposure to per- and polyfluoroalkyl substances (PFAS) through drinking water. *J Expo Sci Environ Epidemiol.* 2023. Epub 2023/06/20. doi: 10.1038/s41370-023-00564-8. PubMed PMID: 37337047.
187. Camerini D, Randall Arlo Z, Trappl-Kimmons K, Oberai A, Hung C, Edgar J, et al. Mapping SARS-CoV-2 Antibody Epitopes in COVID-19 Patients with a Multi-Coronavirus Protein Microarray. *Microbiology Spectrum.* 2021;9(2):e01416-21. doi: 10.1128/Spectrum.01416-21.
188. Ng KW, Faulkner N, Cornish GH, Rosa A, Harvey R, Hussain S, et al. Preexisting and de novo humoral immunity to SARS-CoV-2 in humans. *Science.* 2020;370(6522):1339-43. Epub 2020/11/08. doi: 10.1126/science.abe1107. PubMed PMID: 33159009; PubMed Central PMCID: PMCPCMC7857411.
189. Wang L, Nicols A, Turtle L, Richter A, Duncan CJ, Dunachie SJ, et al. T cell immune memory after covid-19 and vaccination. *BMJ Med.* 2023;2(1):e000468. Epub 2023/11/29. doi: 10.1136/bmjmed-2022-000468. PubMed PMID: 38027416; PubMed Central PMCID: PMCPCMC10668147.
190. Diniz MO, Maini MK, Swadling L. T cell control of SARS-CoV-2: When, which, and where? *Semin Immunol.* 2023;70:101828. Epub 2023/09/01. doi: 10.1016/j.smim.2023.101828. PubMed PMID: 37651850.
191. Bitoun S, Henry J, Desjardins D, Vauloup-Fellous C, Dib N, Belkhir R, et al. Rituximab Impairs B Cell Response But Not T Cell Response to COVID-19 Vaccine in Autoimmune Diseases. *Arthritis Rheumatol.* 2022;74(6):927-33. Epub 2021/12/29. doi: 10.1002/art.42058. PubMed PMID: 34962357; PubMed Central PMCID: PMCPCMC9011892.

192. Md Yusof MY, Arnold J, Saleem B, Vandeveld C, Dass S, Savic S, et al. Breakthrough SARS-CoV-2 infections and prediction of moderate-to-severe outcomes during rituximab therapy in patients with rheumatic and musculoskeletal diseases in the UK: a single-centre cohort study. *Lancet Rheumatol.* 2023;5(2):e88-e98. Epub 2023/01/31. doi: 10.1016/s2665-9913(23)00004-8. PubMed PMID: 36712951; PubMed Central PMCID: PMCPMC9873269.
193. Zhang Z, Mateus J, Coelho CH, Dan JM, Moderbacher CR, Gálvez RI, et al. Humoral and cellular immune memory to four COVID-19 vaccines. *Cell.* 2022;185(14):2434-51.e17. Epub 2022/06/29. doi: 10.1016/j.cell.2022.05.022. PubMed PMID: 35764089; PubMed Central PMCID: PMCPMC9135677.
194. Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol.* 2016;16(10):626-38. Epub 2016/08/23. doi: 10.1038/nri.2016.90. PubMed PMID: 27546235.
195. Capili B, Anastasi JK. An Introduction to the Crossover Trial Design. *Am J Nurs.* 2024;124(9):40-3. Epub 2024/08/26. doi: 10.1097/01.Naj.0001050812.23977.85. PubMed PMID: 39185982; PubMed Central PMCID: PMCPMC11534298.
196. Cunningham RF, Israili ZH, Dayton PG. Clinical pharmacokinetics of probenecid. *Clin Pharmacokinet.* 1981;6(2):135-51. Epub 1981/03/01. doi: 10.2165/00003088-198106020-00004. PubMed PMID: 7011657.
197. Public Health Agency of Sweden (Folkhälsomyndigheten). Nationell plan för vaccination mot covid-19 (Swedish) 2021 [cited 2025 August 5th]. Available from: <https://www.folkhalsomyndigheten.se/contentassets/43a1e203f7344a399367b816e2c7144c/nationell-plan-vaccination-covid-19-delrapport-3.pdf>.
198. Russell MH, Waterland RL, Wong F. Calculation of chemical elimination half-life from blood with an ongoing exposure source: The example of perfluorooctanoic acid (PFOA). *Chemosphere.* 2015;129:210-6. doi: <https://doi.org/10.1016/j.chemosphere.2014.07.061>.
199. Rothman KJ, Lash TL, VanderWeele TJ, Haneuse S. *Modern epidemiology.* 4 ed. Philadelphia, New York, London: Wolters Kluwer; 2021.
200. Delgado-Rodríguez M, Llorca J. Bias. *J Epidemiol Community Health.* 2004;58(8):635-41. Epub 2004/07/15. doi: 10.1136/jech.2003.008466. PubMed PMID: 15252064; PubMed Central PMCID: PMCPMC1732856.
201. Hernán MA, Robins JM. Instruments for Causal Inference: An Epidemiologist's Dream? *Epidemiology.* 2006;17(4).
202. Khorsan R, Crawford C. How to assess the external validity and model validity of therapeutic trials: a conceptual approach to systematic review methodology. *Evid Based Complement Alternat Med.* 2014;2014:694804.

Epub 2014/04/16. doi: 10.1155/2014/694804. PubMed PMID: 24734111; PubMed Central PMCID: PMCPMC3963220.

203. The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). Integrated Addendum To ICH E6(R1): Guideline For Good Clinical Practice ICH 2016 [cited 2025 July 29th]. Available from: https://database.ich.org/sites/default/files/E6_R2_Addendum.pdf.

204. Rose C, Parker A, Jefferson B, Cartmell E. The Characterization of Feces and Urine: A Review of the Literature to Inform Advanced Treatment Technology. *Crit Rev Environ Sci Technol*. 2015;45(17):1827-79. Epub 2015/08/08. doi: 10.1080/10643389.2014.1000761. PubMed PMID: 26246784; PubMed Central PMCID: PMCPMC4500995.

205. Hallberg L, Högdahl AM, Nilsson L, Rybo G. Menstrual blood loss--a population study. Variation at different ages and attempts to define normality. *Acta Obstet Gynecol Scand*. 1966;45(3):320-51. Epub 1966/01/01. doi: 10.3109/00016346609158455. PubMed PMID: 5922481.

206. Lacagnina S. The Developmental Origins of Health and Disease (DOHaD). *Am J Lifestyle Med*. 2020;14(1):47-50. Epub 2020/01/07. doi: 10.1177/1559827619879694. PubMed PMID: 31903081; PubMed Central PMCID: PMCPMC6933571.

207. Li Y, Barregard L, Xu Y, Scott K, Pineda D, Lindh CH, et al. Associations between perfluoroalkyl substances and serum lipids in a Swedish adult population with contaminated drinking water. *Environ Health*. 2020;19(1):33. Epub 2020/03/15. doi: 10.1186/s12940-020-00588-9. PubMed PMID: 32169067; PubMed Central PMCID: PMCPMC7071576.