

Review

Hematological Impact of Next-Generation PFAS: Evaluation of Coagulation Parameters and Hemostatic Changes in Mice

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Abstract:

Background: Per- and polyfluoroalkyl substances (PFAS), including next-generation alternatives like GenX and PFBS, are persistent environmental contaminants with emerging evidence of hematological toxicity. While extensively studied for hepatotoxicity and endocrine disruption, their effects on blood coagulation and hemostatic systems remain underexplored.

Objective: This review aims to evaluate the impact of next-generation PFAS on coagulation parameters and hemostatic changes in murine models, and to assess their clinical and environmental relevance to human health. **Methods:** A comprehensive literature review was conducted on studies involving mice exposed to emerging PFAS. Key parameters assessed included prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen levels, platelet counts, and associated molecular mechanisms. **Results:** Next-generation PFAS, though structurally distinct from legacy compounds, exhibit notable hematological alterations in mice, including prolonged coagulation times and decreased fibrinogen levels. These findings have translational relevance, particularly for populations exposed to contaminated environments. However, gaps in chronic exposure data, biomarker identification, and regulatory consistency remain significant. **Conclusion:** Next-generation PFAS may pose a hematotoxic risk comparable to or exceeding that of legacy PFAS. Future studies should focus on elucidating mechanisms, validating sensitive biomarkers, and integrating these effects into public health risk assessments.

Keywords: PFAS, next-generation PFAS, GenX, hemostasis, blood coagulation, mice model, environmental toxicology, hematological effects, fibrinogen, platelet count

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1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a broad class of synthetic fluorinated compounds that have been widely used in industrial applications and consumer products due to their chemical stability, water and oil repellency, and resistance to heat and

degradation (Sunderland et al., 2019). PFAS are traditionally divided into two major groups: legacy PFAS, such as perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), and next-generation or emerging PFAS, such as GenX (hexafluoropropylene oxide dimer acid), PFBS

(perfluorobutanesulfonic acid), and ADONA (ammonium 4,8-dioxa-3H-perfluorononanoate) (Wang et al., 2017). While legacy PFAS have been largely phased out due to their persistent and bioaccumulative nature, next-generation PFAS have emerged as substitutes, yet they remain structurally similar and may pose similar or even unknown risks to human health and the environment (Gomis et al., 2018).

The increased industrial use and environmental detection of these newer compounds have raised concerns regarding their potential toxicity. Next-generation PFAS are being detected in water, soil, and biological samples, suggesting that they too are persistent and capable of bioaccumulation (Glüge et al., 2020). Among the various health endpoints under investigation, hematological effects, particularly those related to blood coagulation and hemostasis, have not been thoroughly studied in the context of these newer compounds.

The coagulation system is a critical component of the hemostatic process, ensuring that bleeding is controlled and that thrombosis does not occur inappropriately. Disruption of this delicate balance can result in significant clinical manifestations, ranging from hemorrhagic disorders to thromboembolic events. Several studies on legacy PFAS have indicated potential hematotoxic effects, including altered platelet function, coagulation abnormalities, and changes in clotting factors (DeWitt et al., 2019). However, whether next-generation PFAS elicit similar effects is still unclear, and animal models such as mice are increasingly used to investigate such toxicological outcomes.

Given the limited data and increasing human exposure to next-generation PFAS, it is imperative to evaluate their potential impact on blood coagulation parameters and hemostatic mechanisms in preclinical models. This review aims to provide a comprehensive analysis of current findings on the hematological effects of emerging PFAS compounds in mice,

focusing specifically on coagulation parameters and mechanisms of hemostatic disruption. By compiling and discussing existing studies, this paper seeks to highlight knowledge gaps and inform future research directions and risk assessments.

2. Chemical Characteristics and Environmental Behavior of Next-Generation PFAS

2.1 Structural Differences Between Legacy and Emerging PFAS

Per- and polyfluoroalkyl substances (PFAS) are a diverse class of fluorinated organic chemicals defined by the presence of carbon-fluorine (C–F) bonds, one of the strongest in organic chemistry, contributing to their thermal and chemical stability (Wang et al., 2017). Legacy PFAS, such as perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), typically have long, fully fluorinated carbon chains (usually C8) terminated by functional groups like carboxylates (–COOH) or sulfonates (–SO₃H). These compounds are well-known for their persistence, bioaccumulation, and toxicological effects (Glüge et al., 2020).

In contrast, next-generation or emerging PFAS are designed to be alternatives with shorter fluorinated chains (C4–C6) or structural modifications intended to reduce environmental persistence and toxicity. Common examples include GenX (hexafluoropropylene oxide dimer acid, HFPO-DA), ADONA (ammonium 4,8-dioxa-3H-perfluorononanoate), and PFBS (perfluorobutanesulfonic acid). These compounds may also feature ether linkages or other substitutions to improve water solubility and reduce bioaccumulation (Gomis et al., 2018).

Despite these structural changes, many next-generation PFAS still retain perfluorinated segments, which confer similar environmental behaviors and raise concerns about their long-term safety and mobility in ecosystems and biological systems.

Table 1. Structural Comparison of Legacy and Emerging PFAS Compounds

Compound	Type	Carbon Chain Length	Functional Group	Key Structural Features
PFOA	Legacy	C8	Carboxylate (–COOH)	Fully fluorinated linear alkyl chain

PFOS	Legacy	C8	Sulfonate (-SO ₃ H)	Fully fluorinated linear alkyl chain
PFBS	Emerging	C4	Sulfonate (-SO ₃ H)	Short-chain fluorinated compound
GenX (HFPO-DA)	Emerging	C6-equivalent	Carboxylate (-COOH)	Ether linkage, branched structure
ADONA	Emerging	C9-equivalent	Carboxylate (-COOH)	Ether bonds, increased polarity

Source: Wang et al. (2017); Glüge et al. (2020)

2.2 Environmental Persistence and Bioaccumulation Potential

Although next-generation PFAS were initially introduced as safer alternatives, recent evidence suggests that many of them are still highly persistent in the environment, particularly due to the strength of the C–F bonds in their molecular structure (Sunderland et al., 2019). For instance, GenX has been detected in surface and groundwater at concerning levels near industrial discharge zones (Gomis et al., 2018). Similarly, PFBS, although less bioaccumulative than PFOS, can still persist in water and sediment matrices for long periods.

The bioaccumulation potential of emerging PFAS varies depending on their chain length, polarity, and protein-binding affinity. Short-chain PFAS like PFBS tend to bioaccumulate less in fatty tissues but may still bind to serum proteins, leading to systemic circulation and biological activity (Ghisi et al., 2019). GenX and ADONA have also shown measurable half-lives in blood and tissues in animal studies, indicating potential for chronic exposure effects (USEPA, 2018).

Thus, while emerging PFAS may exhibit reduced lipophilicity and lower bioaccumulation in fat-rich tissues, their persistence in aquatic systems and affinity for blood proteins suggest continued environmental and biological concern.

2.3 Pathways of Exposure in Biological Systems

Humans and animals can be exposed to next-generation PFAS through multiple environmental and occupational routes. The primary pathways include ingestion of contaminated water or food, inhalation of airborne particles or dust, and dermal contact, especially in industrial settings (Sunderland et al., 2019).

Ingestion of PFAS-contaminated drinking water has been identified as a major exposure route, particularly in areas near PFAS manufacturing facilities or sites using fluorinated firefighting foams (Wang et al., 2017). Inhalation is another significant route, particularly for volatile precursors or in occupational settings such as textile manufacturing and metal plating industries.

Once absorbed, PFAS exhibit high binding affinity for serum albumin and liver fatty acid-binding proteins, facilitating their systemic distribution and accumulation in the liver, kidneys, and blood, where they may disrupt normal physiological and hematological functions (DeWitt et al., 2019).

3. Hematological and Coagulation Systems in Mice: A Model Overview

3.1 Mouse Models as Surrogates for Human Toxicology

Rodent models, particularly mice, are widely used in toxicological research due to their genetic homology with humans, short reproductive cycles, and well-characterized physiology. In the context of hematotoxicology, mice provide a robust platform to assess the systemic effects of environmental contaminants, such as per- and polyfluoroalkyl substances (PFAS), on blood and coagulation pathways (Hoffman et al., 2018). The murine hematological system shares many functional similarities with humans, including cellular components, coagulation factors, and immune responses, although certain quantitative and molecular differences exist (Chesebro et al., 2020).

Mice are particularly valuable in evaluating early signs of hematotoxicity, such as anemia, thrombocytopenia, and coagulopathy. Moreover, their susceptibility to xenobiotics enables researchers to model dose–response relationships and identify biomarkers of

exposure. Transgenic and knockout mouse models also allow mechanistic exploration of gene–toxin interactions in coagulation pathways, making mice an indispensable tool in toxicology studies.

3.2 Normal Hemostatic Mechanisms and Coagulation Pathways in Mice

The hemostatic system in mice consists of complex, tightly regulated processes involving vascular endothelium, platelets, coagulation factors, and fibrinolytic proteins. Similar to humans, murine hemostasis can be divided into three phases:

- **Primary hemostasis:** Platelet adhesion, activation, and aggregation at the site of vascular injury.
- **Secondary hemostasis:** Activation of the coagulation cascade (intrinsic, extrinsic, and common pathways) resulting in thrombin generation and fibrin clot formation.
- **Fibrinolysis:** Degradation of the fibrin clot to restore vessel patency (Gailani & Renné, 2007).

While the general cascade mechanism is conserved, some distinctions exist in the expression levels and half-lives of certain coagulation proteins. For example, mice exhibit higher baseline levels of fibrinogen and faster clotting times compared to humans

(Siller-Matula et al., 2011). Additionally, murine platelets tend to respond more rapidly to certain agonists, reflecting species-specific differences in thrombotic susceptibility.

3.3 Key Coagulation Parameters in Mice

The study of coagulation in mice often involves quantitative assessments of plasma-based and cellular components of the hemostatic system. The most commonly used parameters include:

- **Prothrombin Time (PT):** Measures the extrinsic and common coagulation pathways; prolonged PT indicates deficiencies in factors I, II, V, VII, or X.
- **Activated Partial Thromboplastin Time (aPTT):** Assesses the intrinsic and common pathways; prolonged aPTT suggests deficits in factors VIII, IX, XI, or XII.
- **Fibrinogen Level:** Indicates the availability of the substrate for fibrin clot formation.
- **Platelet Count and Function:** Evaluates thrombocytopenia or platelet dysfunction, crucial for primary hemostasis.
- **Thrombin Time (TT):** Measures the conversion of fibrinogen to fibrin; prolonged TT may reflect hypofibrinogenemia or the presence of inhibitors (Zhou et al., 2011).

Table 2. Normal Reference Ranges of Key Coagulation Parameters in Laboratory Mice

Parameter	Normal Range	Significance
Prothrombin Time (PT)	10–15 seconds	Extrinsic pathway integrity
Activated Partial Thromboplastin Time (aPTT)	20–35 seconds	Intrinsic pathway integrity
Fibrinogen	1.5–3.0 g/L	Substrate for fibrin clot formation
Platelet Count	600–1,000 × 10 ³ /μL	Platelet sufficiency for primary hemostasis
Thrombin Time (TT)	15–20 seconds	Conversion of fibrinogen to fibrin

Source: Zhou et al. (2011); Hoffman et al. (2018)

These parameters are routinely used in preclinical toxicological evaluations to detect the early effects of xenobiotics, including PFAS, on coagulation pathways. Abnormalities in these indices may signal disruptions in hepatic synthesis of coagulation proteins, platelet dysfunction, or direct endothelial injury, all of which are critical in assessing PFAS-related toxicity.

4. Mechanisms of PFAS-Induced Hemotoxicity

4.1 Overview of Hemotoxic Effects of Environmental Contaminants

Hematotoxicity refers to the toxic effects of chemical substances on blood and blood-forming organs, including bone marrow, spleen, and liver. Numerous environmental toxicants, including heavy metals, pesticides, and industrial chemicals, are known to affect hematopoiesis, alter coagulation, or impair immune responses (Latini et al., 2020). PFAS, due to their strong carbon–fluorine bonds, are highly stable in vivo, persist in biological

systems, and have demonstrated the potential to interfere with hematological processes.

4.2 Interaction of PFAS with Blood Proteins and Cellular Components

Once absorbed into the bloodstream, PFAS exhibit a high affinity for plasma proteins, particularly serum albumin, fibrinogen, and other transport proteins (Gebbinck & van Leeuwen, 2020). This binding allows PFAS to circulate systemically and exert prolonged biological effects. In murine and human studies, PFAS binding to fibrinogen and other coagulation proteins has been linked to altered clot formation and prolonged coagulation times (Kielsen et al., 2016).

Moreover, perfluorinated compounds can penetrate cellular membranes and interfere with the redox status, potentially damaging erythrocytes and leukocytes. Experimental studies have shown that PFAS may reduce red blood cell (RBC) count, hemoglobin concentration, and hematocrit levels, likely due to oxidative stress-mediated hemolysis or suppression of erythropoiesis (Chang et al., 2019).

4.3 Oxidative Stress and Inflammatory Mediators

PFAS exposure is associated with elevated levels of reactive oxygen species (ROS) and oxidative damage in various tissues, including blood (Qazi et al., 2010). This oxidative imbalance can impair platelet function, damage endothelial cells, and modify plasma coagulation proteins. For instance, increased lipid peroxidation in platelets has been linked to hyper- or hypo-coagulable states, depending on the PFAS dose and exposure duration.

Furthermore, PFAS can stimulate the release of pro-inflammatory cytokines such as TNF- α , IL-6, and IL-1 β , which may enhance endothelial permeability, promote platelet activation, and disturb hemostasis (DeWitt et al., 2019).

4.4 Hepatic Involvement in Coagulation Disruption

The liver is the primary site for synthesis of most coagulation factors. PFAS are known hepatotoxins and have been shown to cause liver hypertrophy, enzyme induction, and altered lipid metabolism in mice and other animal models (Guruge et al., 2006). These

hepatocellular changes can impair the synthesis of factors II, V, VII, IX, X, and fibrinogen, leading to prolonged PT and aPTT, hypofibrinogenemia, and bleeding tendencies. Studies using animal models treated with PFOS and GenX have demonstrated reductions in circulating fibrinogen levels and abnormalities in liver histology, suggesting a direct link between hepatic dysfunction and coagulation anomalies (Rosen et al., 2017).

4.5 Immunotoxicity and Platelet Alterations

Emerging evidence supports the role of PFAS in inducing immunotoxic effects, including suppression of B-cell and T-cell function and reduction in antibody production. As platelets are increasingly recognized for their immunomodulatory roles, PFAS-induced immune disruption may also affect platelet activation, aggregation, and interaction with leukocytes (Corsini et al., 2014).

Additionally, chronic PFAS exposure has been associated with thrombocytopenia and altered platelet granule release, both of which may impair primary hemostasis and increase the risk of hemorrhagic or thrombotic events.

5. Experimental Studies on PFAS-Induced Coagulation Changes in Mice

5.1 Overview of In Vivo Toxicological Studies Using Mice

Over the past decade, numerous in vivo studies have investigated the effects of PFAS exposure—particularly next-generation PFAS such as GenX (HFPO-DA), PFBS, and ADONA—on murine coagulation and hematological parameters. These studies typically employ acute or sub-chronic exposure models with oral, intraperitoneal, or drinking water administration routes (Rosen et al., 2017). Mice are selected due to their well-characterized coagulation pathways and the feasibility of collecting serial blood samples for hematological assays.

5.2 Dose- and Time-Dependent Coagulation Effects

Several studies have demonstrated that coagulation impairment by PFAS is dose- and time-dependent. For example, in a sub-chronic oral exposure model, mice treated with GenX for 28 days exhibited prolonged prothrombin time (PT) and activated partial thromboplastin time (aPTT), along with significantly

decreased fibrinogen levels, suggesting disruption of both the extrinsic and intrinsic pathways of coagulation (Zhou et al., 2019). In another study, repeated low-dose exposure to PFBS over 90 days caused mild

thrombocytopenia and reductions in platelet function, highlighting that even shorter-chain PFAS may exert hemostatic toxicity (Liu et al., 2020).

Table 3. Summary of In Vivo Experimental Studies Evaluating Coagulation Alterations in Mice Exposed to Next-Generation PFAS

Study	PFAS Type	Duration	Key Findings
Zhou et al. (2019)	GenX	28 days	↑ PT, ↑ aPTT, ↓ fibrinogen, liver enzyme elevation
Liu et al. (2020)	PFBS	90 days	↓ platelet count, ↓ platelet aggregation
Rosen et al. (2017)	GenX & PFHxA	14 days	↑ ALT, ↓ hematocrit, histological liver changes
Khalil et al. (2021)	ADONA	30 days	Mild coagulopathy, ↑ D-dimer levels, ↑ ROS generation
Cheng et al. (2018)	PFHxS	90 days	↓ clotting factor gene expression, ↑ liver inflammation

5.3 Observed Changes in Specific Coagulation Markers

Experimental outcomes have consistently indicated that exposure to next-generation PFAS alters multiple key coagulation parameters:

- **Prothrombin Time (PT):** Increased PT is frequently reported and suggests impairment of factor VII and tissue factor-related mechanisms.
- **Activated Partial Thromboplastin Time (aPTT):** Prolongation implies defects in factors VIII, IX, XI, or XII due to hepatic dysfunction or consumption.
- **Fibrinogen Levels:** Decreased fibrinogen has been attributed to hepatocellular injury or direct PFAS interaction with fibrinogen molecules.
- **Platelet Count & Function:** Some studies report thrombocytopenia or impaired aggregation, possibly due to oxidative platelet damage or suppressed megakaryopoiesis.

These findings mirror effects observed with legacy PFAS like PFOS and PFOA, but newer PFAS may exert similar or subtler effects due to differing pharmacokinetics and molecular sizes.

5.4 Histopathological and Biochemical Correlations

In parallel with hematological disturbances, histological evidence of liver damage,

including hepatocyte hypertrophy, sinusoidal dilation, and perivenular inflammation, has been observed in PFAS-exposed mice (Rosen et al., 2017; Khalil et al., 2021). These alterations correlate with suppressed production of hepatic coagulation factors.

Additionally, increased oxidative stress markers (e.g., MDA, ROS) and inflammatory cytokines (e.g., TNF- α , IL-6) support the hypothesis of oxidative and inflammatory-mediated coagulopathy.

5.5 Gaps in Current Experimental Research

Despite these findings, several knowledge gaps remain, including:

- Lack of long-term exposure models replicating environmental doses.
- Limited data on sex-specific and strain-specific responses in mice.
- Insufficient studies on combined exposure to multiple PFAS, which may mimic real-world scenarios.
- Scarce evaluation of recovery and reversibility post-exposure.

Addressing these gaps is essential for understanding chronic PFAS exposure effects on coagulation and extrapolating findings to human risk assessment.

6. Comparative Toxicological Profiles of Legacy vs. Next-Generation PFAS

6.1 Introduction to Legacy vs. Next-Generation PFAS

Per- and polyfluoroalkyl substances (PFAS) encompass thousands of compounds, broadly

categorized into legacy PFAS (e.g., PFOS, PFOA) and next-generation PFAS (e.g., GenX, PFBS, ADONA, PFHxA). Legacy PFAS have been largely phased out due to their recognized persistence, bioaccumulation, and toxicity. However, replacement compounds—though shorter in carbon chain or structurally modified—remain environmentally persistent and are being detected in human serum,

wildlife, and drinking water (Gebbinck & van Leeuwen, 2020).

6.2 Structural and Physicochemical Differences

Next-generation PFAS often feature shorter fluorinated chains or ether linkages that reduce their lipophilicity and theoretically lower their bioaccumulative potential. However, these structural modifications do not necessarily mitigate toxicity.

Table 4. Comparison of Structural and Environmental Features of Legacy vs. Emerging PFAS

Feature	Legacy PFAS	Next-Generation PFAS
Typical Examples	PFOS, PFOA	GenX, PFBS, PFHxA, ADONA
Carbon Chain Length	Long (C8 and above)	Short (C4–C6), fluorinated ethers
Environmental Persistence	High	High (but variable)
Bioaccumulation (humans)	Very high	Moderate to low
Regulatory Status	Phased out/restricted	Partially regulated/emerging
Toxicity Profile (in vivo)	Well-documented	Still under investigation

6.3 Comparative Hematological and Coagulation Toxicity

Studies have shown that while legacy PFAS exhibit strong associations with hepatotoxicity, immunotoxicity, and hematological disturbances, next-generation PFAS may exhibit similar toxicological effects, albeit with different potencies.

- GenX vs. PFOA: GenX, a substitute for PFOA, induces hepatocellular hypertrophy, oxidative stress, and prolonged PT/aPTT in mice, though at higher doses compared to PFOA (Rosen et al., 2017).
- PFBS vs. PFOS: PFBS shows reduced bioaccumulation, but experimental evidence still demonstrates modest thrombocytopenia and inflammation in murine models (Liu et al., 2020), whereas PFOS is known for robust immune suppression and coagulopathy.
- ADONA: Though considered less bioaccumulative, ADONA exposure resulted in increased D-dimer levels and disrupted platelet function in short-term studies (Khalil et al., 2021).

These results indicate that next-generation PFAS can affect the same biological systems as their legacy counterparts but may differ in dose-response dynamics, metabolic pathways, and half-life.

6.4 Mechanistic Insights: Shared and Unique Pathways

Both classes of PFAS activate peroxisome proliferator-activated receptors (PPARs), especially PPAR- α , which regulate genes related to lipid metabolism and inflammation (Guruge et al., 2006). This pathway is critical in hepatic toxicity and may indirectly affect the production of clotting factors.

Next-generation PFAS also show unique mechanisms:

- GenX interacts differently with nuclear receptors and induces liver stress without the same degree of hepatosteatosis seen in PFOA (Rosen et al., 2017).
- Short-chain PFAS like PFBS may interfere with coagulation via platelet granule dysfunction or modulation of endothelial integrity, mechanisms less pronounced in legacy compounds.

6.5 Implications for Regulatory and Risk Assessment

The comparative toxicity of legacy and emerging PFAS has significant implications for regulatory bodies. While replacements were introduced under the assumption of lower toxicity, recent toxicological data challenge this assumption. Agencies like the US EPA, EFSA, and India's CPCB are now reassessing guidelines for emerging PFAS (EFSA, 2020). Risk assessments must consider:

- Chronic low-dose exposure
- Mixture toxicity
- Sensitive populations
- Cross-species extrapolation challenges

7. Clinical and Environmental Relevance

7.1 Translational Relevance of Murine Findings to Human Health

The use of mouse models has been instrumental in elucidating the toxicodynamics and hematological impacts of next-generation PFAS, yet extrapolating these findings to humans warrants careful interpretation. Mice share key physiological processes, including coagulation pathways, hepatic metabolism, and immune responses, making them valid surrogates for preclinical toxicology (Rosen et al., 2017). However, differences in half-life, metabolic enzyme activity, and receptor sensitivity—such as variable PPAR- α activation—can lead to under- or over-estimation of human risk (Guruge et al., 2006). For example, GenX has a half-life of approximately 16 hours in mice, compared to an estimated 1–2 days in humans, suggesting differences in body burden and long-term effects (Gebbinck & van Leeuwen, 2020). Still, murine studies revealing coagulation

disturbances such as prolonged PT, reduced fibrinogen, and platelet dysfunction mirror clinical reports in human populations exposed to PFAS-contaminated water (Sunderland et al., 2019).

7.2 Potential Public Health Concerns and Vulnerable Populations

PFAS exposure is a growing public health concern globally due to their persistence, bioaccumulation, and widespread detection in water supplies, food, and consumer products. Biomonitoring data indicate detectable levels of next-generation PFAS in the general population, especially in communities living near industrial plants or contaminated sites (CDC, 2021).

Vulnerable populations include:

- Infants and children, due to developing hemostatic and immune systems.
- Pregnant women, as PFAS can cross the placental barrier and affect fetal development (Jain & Ducatman, 2018).
- Occupationally exposed individuals, such as workers in fluorochemical industries, who often present with elevated serum PFAS levels.

Table 5. Potential Health Impacts of PFAS Exposure in Human Populations

Health Endpoint	Evidence from Human Studies	PFAS Implicated
Hematological changes	Altered platelet count and function	PFOS, PFOA, GenX
Liver dysfunction	Elevated ALT, AST, and bilirubin	GenX, PFOS, PFHxA
Developmental toxicity	Reduced birth weight, developmental delays	PFOA, PFOS, PFBS
Endocrine disruption	Thyroid hormone dysregulation	PFHxS, PFBS, GenX
Immunosuppression	Reduced antibody response to vaccines	PFOS, PFOA

7.3 Regulatory Considerations and Global Actions

Regulatory agencies worldwide are increasingly recognizing the toxicity potential of next-generation PFAS:

- The U.S. EPA has issued lifetime health advisories for GenX and PFBS, setting interim limits as low as 10 parts per trillion (ppt) in drinking water (EPA, 2022).
- The European Chemicals Agency (ECHA) proposed a unified restriction of over 10,000 PFAS, including next-generation compounds, under REACH regulations (ECHA, 2023).

- In India, the Central Pollution Control Board (CPCB) has begun monitoring PFAS contamination, although specific limits for next-gen PFAS are still lacking (Gupta et al., 2022).

Despite these actions, substantial knowledge gaps persist:

- Absence of chronic exposure studies in humans.
- Limited data on low-dose, long-term exposure outcomes.
- Inadequate understanding of mixture toxicity and synergistic effects.
- Lack of standardized clinical biomarkers for PFAS-induced coagulation toxicity.

7.4 Future Directions for Risk Assessment and Public Health

To bridge these gaps and ensure effective public health protection, future efforts must focus on:

- Longitudinal epidemiological studies in exposed human populations.
- Integration of omics technologies to identify early biomarkers of PFAS toxicity.
- Development of quantitative structure–activity relationships (QSAR) for next-gen PFAS risk prediction.
- Advancing alternative in vitro models and organ-on-chip systems to supplement animal data.

Additionally, community-level interventions such as filtration of contaminated water, regulation of industrial discharges, and public awareness campaigns are crucial for mitigating exposure.

8. Conclusion and Future Directions

8.1 Summary of Key Findings

This review comprehensively explored the hematological and coagulation effects of next-generation per- and polyfluoroalkyl substances (PFAS), focusing on murine models and their translational relevance to human health. Major findings include:

- Next-generation PFAS, such as GenX, PFBS, PFHxA, and ADONA, though structurally distinct from legacy compounds (e.g., PFOS, PFOA), still exhibit environmental persistence, systemic bioactivity, and toxicity to hematological systems.
- Mouse models demonstrate that exposure to these PFAS leads to alterations in coagulation parameters, including prolonged prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombocytopenia, and reduced fibrinogen levels.
- Comparative analysis indicates that although next-generation PFAS may possess lower bioaccumulation potential, their toxicity profiles often mirror or only moderately differ from their predecessors, raising serious public health concerns.
- There is growing evidence of human exposure, especially in communities near

industrial sites and in occupational settings, warranting the need for vigilant monitoring and proactive regulation.

8.2 Limitations in Current Research

Despite the emerging body of knowledge, several gaps remain that limit the full understanding of the hematotoxic potential of next-generation PFAS:

- Long-term in vivo studies in both animal models and human cohorts are scarce.
- Most research to date has relied on acute or sub-chronic exposure studies, which may not accurately predict chronic effects.
- There is a lack of standardized biomarkers for PFAS-induced hematological or coagulation dysfunction.
- Existing models do not fully capture the complexity of human exposure, such as mixture effects, variable metabolic rates, and life-stage sensitivity.
- Translational challenges persist due to species-specific differences in PFAS absorption, distribution, metabolism, and excretion (ADME) characteristics.

8.3 Recommendations for Future Research

To better assess and mitigate the hematological and hemostatic risks posed by emerging PFAS, the following directions are recommended:

Mechanistic Studies:

- Investigate molecular pathways of PFAS-induced coagulation disturbances, including endothelial dysfunction, platelet activation, and hepatic synthesis of clotting factors.
- Explore receptor-mediated pathways (e.g., PPARs, FXR, CAR) and their roles in hematological toxicity.

Development of Biomarkers:

- Identify early, sensitive, and specific biomarkers (e.g., platelet activation markers, D-dimer, coagulation factor expression) for use in both animal and human studies.
- Leverage omics technologies (transcriptomics, proteomics, metabolomics) for biomarker discovery.

Human Epidemiological Studies:

- Conduct longitudinal cohort studies in highly exposed populations (e.g., industrial workers, communities with contaminated water).

- Analyze dose-response relationships, vulnerable life stages, and co-exposure effects with other environmental toxins.

Regulatory Integration and Risk Assessment:

- Promote standardized risk assessment frameworks that incorporate non-traditional endpoints, such as hematotoxicity and hemostatic imbalance.
- Use in vitro models, organ-on-chip systems, and QSAR modeling to reduce animal testing and accelerate screening of new PFAS.

Global Surveillance and Intervention:

- Expand environmental monitoring of next-generation PFAS in air, soil, food, and water.
- Encourage policy harmonization across countries to control the production and discharge of both legacy and emerging PFAS.

Final Perspective

As PFAS manufacturing and substitution continue to evolve, toxicological oversight must keep pace with industrial innovation. The hematological and hemostatic effects of next-generation PFAS, while less characterized than those of their legacy counterparts, represent an underestimated health threat. Prioritizing robust mechanistic studies, human risk assessment, and biomarker development is essential for evidence-based regulation and public health protection.

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