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Perspectives

From Evaluation to Practice: Bridging the Gap Between Air Pollution Health Risk Assessment and Policy-Making in China

Dongqun Xu^{1,*}

ABSTRACT

This article systematically reviews the significant progress China has made in the field of air pollution health risk assessment since 2013, including the establishment of the national monitoring network, the improvement of relevant laws and formulation of technical guidelines. The paper focuses on an in-depth discussion of the current core challenges: the disconnection between health risk and decision-making, the lack of a multi-sectoral coordination mechanism, the imperfection of the technical system (particularly for mixed exposures and emerging pollutants), and the novel risks posed by global climate change. Based on this analysis, we prospectively propose fundamental pathways to advance the field: 1) constructing a robust management mechanism and coordination framework; 2) promoting the integration of the full environmental health risk assessment process into the decision-making pipeline (an “assessment-management interaction” paradigm); and 3) strengthening interdisciplinary collaboration and leveraging innovative technologies to refine the technical assessment system.

A prolonged and extensive haze episode that engulfed China in January 2013 sparked widespread public concern about air pollution’s health impacts and prompted urgent government action. Conducting comprehensive health risk assessments can effectively guide air pollution control decision-making by establishing robust monitoring systems, conducting thorough investigations, and performing systematic risk assessment of air pollution and its health impacts. Following this pivotal event, China established the National Air Pollution (Haze) and Health Impact Monitoring Network in 2013 (1). Subsequently, both the revised Environmental Protection Law of the

People’s Republic of China in 2014 and the Basic Law on Medical and Health Care and Health Promotion of the People’s Republic of China promulgated in 2019 mandated that the state establish and continuously improve comprehensive environmental and health risk assessment systems. Regarding the development of technical frameworks for environmental health risk assessment, China has formulated several key guidelines: the “Technical Guidelines for Exposure Assessment of Environmental Pollutants”, “Technical Guidelines for Environmental Health Risk Assessment of Chemical Substances”, “Framework Guidelines for Technical Methods of Environmental Risk Assessment of Chemical Substances”, “Technical Specifications for Health Risk Assessment of Ambient Air Pollution”, “Technical Guidelines for Environmental and Health Exposure Assessment of Chemical Substances (Trial)” and “Technical Guidelines for Environmental and Health Risk Characterization of Chemical Substances (Trial).” These comprehensive initiatives have advanced health risk assessment capabilities for air pollution through the implementation of monitoring networks of air pollution and health impact, pilot projects of appropriate risk assessment technologies. In recent years, researchers have published a substantial body of literatures on air pollution health risk assessment. However, the integration of air pollution health risk assessment findings into air pollution control decision-making processes remains significantly inadequate.

The primary purpose of health risk assessment is to provide a scientific basis for air quality supervision and health promotion decision-making. However, a significant disconnect exists between available scientific data and the information needs of decision-makers. Current air pollution health risk assessments are not integrated into the decision-making process, resulting in assessment results that fail to inform air pollution control policies effectively. Decision-makers and stakeholders-including community groups,

environmental organizations, industry representatives, and consumers-remain disengaged from the risk assessment process. This lack of interaction between risk assessment and decision-making creates substantial barriers. Furthermore, assessment transparency remains insufficient, limiting decision-makers and stakeholders understanding and engagement. The health impacts of air pollution span multiple departments and disciplines, yet an effective multi-sector coordination mechanism or clearly defined operational and management framework has not been established. The technical system for air pollution health risk assessment remains incomplete, lacking methodologies for assessing mixed and cumulative exposure to multiple pollutants, as well as risk assessment guidelines for different health hazard outcomes. Additionally, risk assessment techniques for airborne pathogenic microorganisms are absent. These existing problems collectively hinder the effective utilization of risk assessment as a decision-making tool.

Conversely, as the number of chemical substances continues to increase exponentially (currently, the Chemical Abstracts Service (CAS) REGISTRY of the American Chemical Society has registered over 279 million chemical substances)(2), emerging pollutants will coexist with traditional pollutants for the foreseeable future. Global climate change has intensified the frequency of extreme weather and climate events in China, creating additional complexity. The superimposition of air pollution with extreme weather conditions, combined with the delayed and complex health effects, makes scientific and accurate assessment of cumulative air pollution health risks increasingly challenging. The diverse nature of emerging pollutants renders traditional assessment methods inadequate for effectively evaluating their exposure pathways and health effects. Critical gaps exist in epidemiological and toxicological data necessary for conducting comprehensive health risk assessments of emerging pollutants. These emerging challenges represent significant obstacles that the field must address moving forward.

Scientific and effective management is essential for advancing air pollution health risk assessment to support air pollution control decision-making. China urgently needs to establish and enhance both its management mechanisms and technical systems for air pollution health risk assessment. By developing management specifications for environmental health risk assessment, we can clarify management goals, principles, and requirements while strengthening

comprehensive process management of environmental health risk assessment. Air pollution demonstrates significant regional variations across the country. Related legislation has established the responsibilities of different governmental levels and departments, and local governments should implement hierarchical management of air pollution health risk assessment according to their legal responsibilities, national strategic priorities, management requirements, and regional air pollution challenges. Relevant departments must establish effective coordination mechanisms to achieve data resource sharing and information exchange.

Air pollution control measures should be grounded in risk assessment to optimize decision-making, thereby maximizing the utility of risk assessment (3). Drawing from the experience of the US Environmental Protection Agency, risk assessments should target specific problems and decisions to most effectively guide the decision-making process (4). During the early stages of health risk assessment, the interaction between risk assessment and management should be integrated into the risk assessment paradigm, establishing participation mechanisms for risk managers, decision-makers, and stakeholders in key assessment phases. Decision-makers, risk assessors, and other interested parties should participate in planning and scoping activities. At this stage, they collaboratively discuss the options under consideration and factors that may influence policies in the risk assessment, determine the assessment scope and appropriate evaluation level to meet decision-makers' needs, and establish the role of risk information in decision-making. During problem formulation, it is essential to establish an effective decision-maker participation mechanism involving discussions among risk management personnel, decision-makers, and risk assessors to develop detailed technical plans, conceptual models, and analysis frameworks for the assessment. At this stage, risk assessors must ensure that risk management personnel and decision-makers fully comprehend uncertainties and their implications (5). Risk assessors need to conduct dynamic evaluations, regularly update assessment results, and provide feedback to decision-makers, enabling informed decision-making through careful consideration while enhancing the transparency of environmental health risk assessments.

The technical system for assessing air pollution health risks requires continuous improvement through strengthened multidisciplinary cooperation and

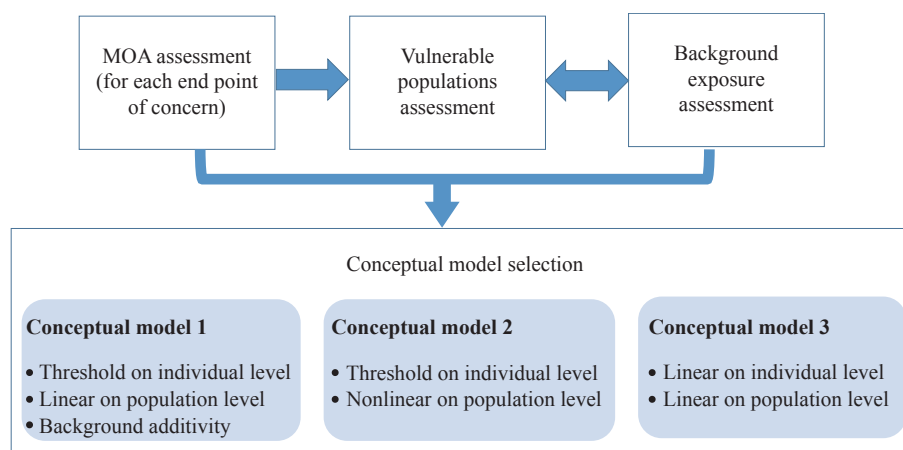


FIGURE 1. Three conceptual models in the unified framework.

innovative technology integration. Given the significant limitations in traditional carcinogenic and non-carcinogenic risk assessment methods — particularly regarding core concepts, uncertainty and variability treatment, and risk quantification — the “Conceptual Model in the Unified Framework (Figure 1)” was developed to assess exposure (dose)-response relationships more effectively (4).

Guidelines for exposure assessment should be developed that cover composite exposure, cumulative exposure, and different media and exposure scenarios. Guidelines for environmental health risk assessment that cover different populations (such as children, pregnant women, the elderly and other susceptible population) and different health outcomes should be developed. The combination of hazard information and toxicity data to describe and characterize the potential risks of emerging pollutants in the air should be expanded continuously by using quantitative structure-activity relationship (QSAR), adverse outcome path (AOP), computational toxicology, bio-informatics, and different machine learning models (6–9).

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Vital Surveillances

National Monitoring for Radioactivity in Drinking Water — China, 2012–2024

Liangliang Yin¹; Yuhan Xie¹; Yuxin Qian¹; Cen Shi¹; Yanqin Ji^{1,†}

ABSTRACT

Introduction: To establish baseline radioactivity levels and ensure the safety of drinking water quality in China, gross alpha and gross beta radioactivity levels in drinking water were surveyed from 2012 to 2024.

Methods: The surveillance was conducted through the national monitoring system for radioactivity in drinking water, organized by The National Institute for Radiological Protection (NIRP) during the period 2012–2024. Drinking water samples were collected and pretreated in accordance with a unified protocol, and radioactivity was determined using alpha/beta counting systems by local laboratories.

Results: From 2012 to 2024, over 11,000 drinking water samples were collected and analyzed across 29 provinces, including areas surrounding nuclear power plants. The mean concentration ranges of gross alpha and gross beta radioactivity levels in all regions and various water bodies were 0.01–0.17 Bq/L and 0.05–0.38 Bq/L, respectively, all of which are below the guidance values specified in the national standard GB 5749 (0.5 Bq/L for gross alpha and 1 Bq/L for gross beta). However, the gross alpha and gross beta activity levels in well water were higher than those in other water bodies. The results indicate that radioactivity in drinking water primarily originates from natural radionuclides.

Conclusions: Drinking water in China maintains normal background levels of radioactivity. Nuclear power plant operations do not seem to have an impact on surrounding water sources.

Drinking water safety is a critical health and development concern at the national, regional, and local levels. Although drinking water typically contributes minimally to overall radionuclide exposure under normal circumstances, radioactivity in drinking water remains an important consideration (1).

Radionuclides in drinking water can originate from both natural and human-made sources. Particularly significant for human radiation exposure from drinking water are naturally occurring radionuclides from the thorium and uranium decay series — such as radium-226, radium-228, polonium-210, lead-210, and radon — which are dissolved from rocks and soil. These radionuclides, typically present as dissolved ionic species, can infiltrate water sources through natural geological processes or anthropogenic activities, such as uranium mining and other extractive industries involving naturally occurring radioactive materials (2).

Natural radionuclide activity concentrations vary across regions, depending on local geology in drinking water. Global data on the levels of naturally occurring radionuclides in drinking water have been reviewed by UNSCEAR (2). In most cases, the activity concentrations are very low, making detailed analysis of specific radionuclides unnecessary for routine monitoring. Consequently, the most commonly used screening method is gross alpha and gross beta activity measurements (3–4). Regulatory frameworks for monitoring radioactivity in drinking water, along with corresponding guideline levels for gross alpha and gross beta activity, have been established in the European Union, the United States, Canada, and other countries (5–7). In China, gross alpha and gross beta are among the 43 regular indices listed in the Standards for Drinking Water Quality, with guidance values of 0.5 Bq/L for gross alpha and 1 Bq/L for gross beta (8), in line with WHO recommendations (1).

China's national radioactive monitoring data for drinking water largely predates 2000, with a gap in systematic monitoring during 2000–2011. To ensure drinking water safety under new circumstances of rapid nuclear energy development, monitoring was reinitiated in 2012. The National Institute for Radiological Protection (NIRP), under the China CDC, was tasked with organizing nationwide radioactive monitoring of drinking water. From 2012 to 2024, in collaboration with provincial laboratories, over 11,000 drinking water samples were collected and

analyzed for gross alpha and gross beta activity. This effort underscores the commitment to safeguarding public health by maintaining drinking water quality within safe and acceptable limits. The data obtained contribute to establishing the baseline levels of natural radioactivity in China's drinking water supplies.

METHODS

In accordance with the sampling and distribution requirements outlined in the technical manuals for drinking water radiation monitoring issued by NIRP, provincial monitoring institutes conducted annual collection and analysis of drinking water samples. The investigation covered 29 provinces, including areas within 30 km of nuclear power plants. Sampling was conducted in both dry (spring/winter) and wet (summer/autumn) seasons. Samples were categorized by water source: finished water, tap water, well water, and reservoir water. This systematic approach ensured comprehensive monitoring and assessment across diverse sources and conditions.

Sample collection and preservation strictly followed national standards (9). To ensure sample representativeness and prevent contamination, water was allowed to flow continuously for a short period before collection (10). Pretreatment steps, such as acidification and/or filtration, were performed to minimize potential interferences affecting radioactivity measurements. Samples were collected in pre-cleaned polyethylene containers and acidified with dilute nitric acid to a final concentration of 2% to maintain sample homogeneity and prevent the adsorption of radionuclides on container walls.

The analysis procedures followed national standards

(11). For each sample, one liter of acidified water was taken in a beaker and heated to sub-boiling on an electric heating plate. When reduced to less than 50 mL, the samples were transferred to an evaporating dish. One milliliter of sulfuric acid was added, and the mixture was heated until a residue was produced. The residue was placed in a muffle furnace at 350 °C for 1 hour to prepare measurement sources.

Gross alpha and beta radioactivity were measured using alpha/beta counting systems. Low-background multi-detector alpha/beta counters with different models were used as counting instruments. The most widely used measurement techniques are proportional counting and solid-state scintillation counting, while some laboratories employ semiconductor silicon detectors and grid ionization chambers. For alpha counting efficiency calibration, the most commonly used radionuclide is ^{241}Am , though a few laboratories utilize natural uranium. For beta counting efficiency calibration, ^{40}K (as potassium chloride) is typically employed.

To ensure data quality, all instruments were verified by the national metrology department. All participating laboratories participated in annual inter-comparison exercises organized by NIRP (3). NIRP also drafted annual monitoring manuals, conducted training, and provided on-site guidance to ensure consistent, high-quality monitoring practices nationwide.

RESULTS

From 2012 to 2024, over 11,000 drinking water samples were collected and analyzed. Figure 1 shows the activity concentrations of gross alpha and gross beta

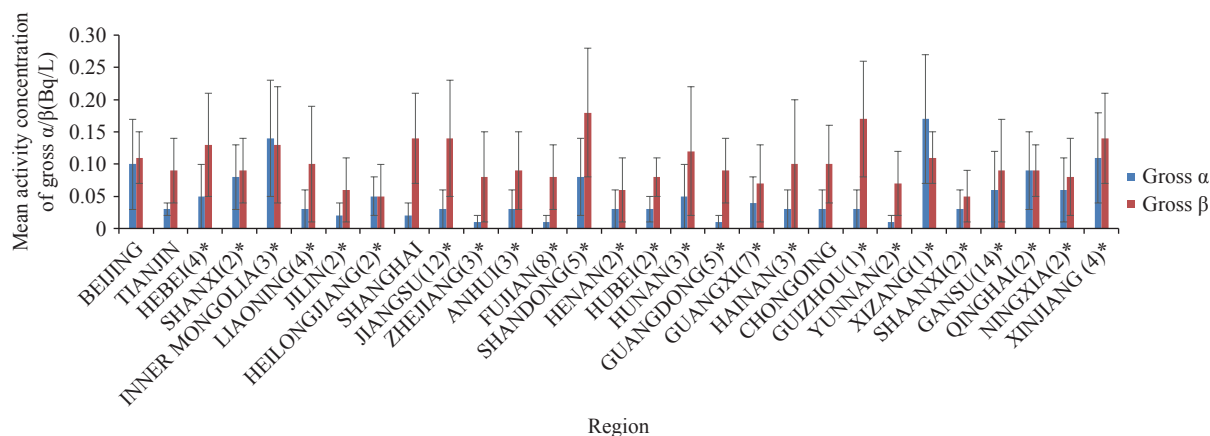


FIGURE 1. Concentrations of gross alpha and gross beta activity in finished water across different regions. Note: * number of cities covered by the data.

in finished water bodies across different regions. The mean gross alpha activity concentration ranged from 0.01 Bq/L to 0.17 Bq/L, while the gross beta activity concentration ranged from 0.05 Bq/L to 0.18 Bq/L. Although some regional variation was observed, all values remained below the national guideline levels.

Figure 2 presents the gross alpha and gross beta radioactivity levels in four types of water bodies during the dry and wet seasons. The interquartile range (25%–75%) analysis revealed minimal seasonal variations in the gross alpha and gross beta activity concentrations of each water body type. Two independent sample *t*-tests revealed no statistically significant differences ($\alpha=0.05$) in both gross alpha and gross beta activity concentrations between the dry and wet seasons for any water body, except for well water, which showed a statistically significant difference ($\alpha=0.05$) in mean gross alpha radioactivity concentration between the dry and wet seasons.

Additionally, the gross alpha and gross beta radioactivity levels were higher in well water, as natural radionuclides are more commonly found in drinking water derived from groundwater sources than surface water (2).

Figure 3 presents the mean gross alpha and gross beta radioactivity concentrations in various water bodies during 2018–2024. In finished water, the gross alpha range was 0.03–0.04 Bq/L, and the gross beta range was 0.09–0.10 Bq/L. In tap water, the gross alpha range was 0.03–0.04 Bq/L, and the gross beta range was 0.11–0.13 Bq/L. In reservoir water, the gross alpha range was 0.02–0.03 Bq/L, and the gross beta range was 0.08–0.12 Bq/L. In well water, the gross alpha range was 0.09–0.17 Bq/L, and the gross beta range was 0.21–0.38 Bq/L. The *t*-tests comparing well water with the other 3 water types (finished water, tap water, and reservoir water) showed that the radioactivity concentration in well water (mean=

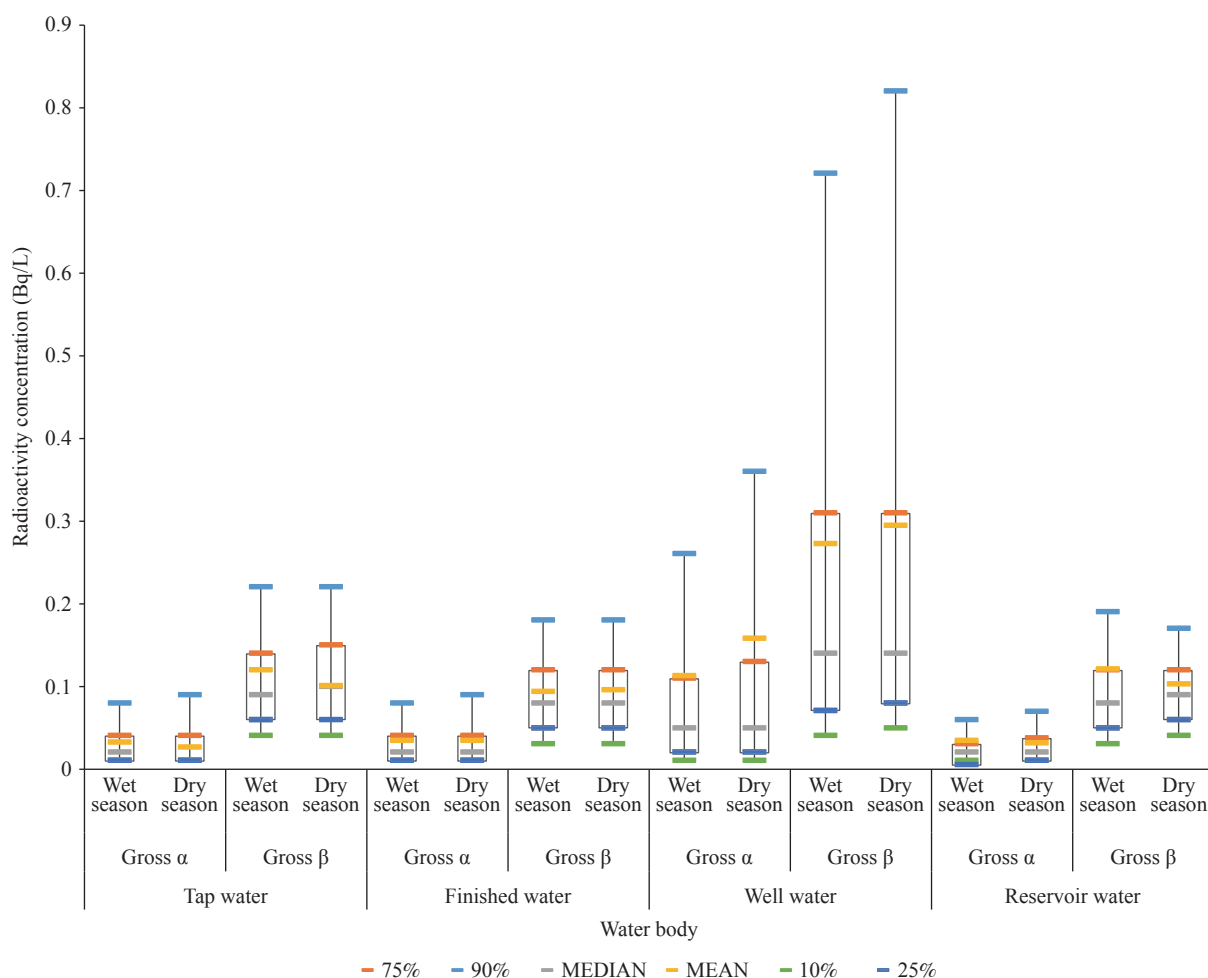


FIGURE 2. Gross alpha and gross beta activity concentrations in drinking water during the wet and dry seasons (2012–2024).

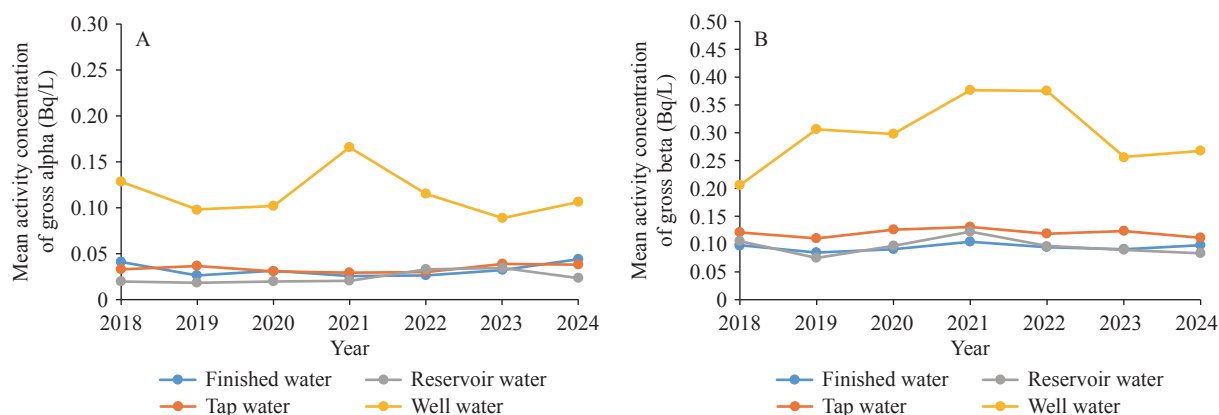


FIGURE 3. Gross alpha and gross beta activity concentrations in drinking water during 2018–2024. (A) Gross alpha; (B) Gross beta.

TABLE 1. Gross alpha and gross beta concentrations in drinking water across the nation and around nuclear power plants during 2012–2024.

Water body type	No. of samples	National regions (excluding areas near nuclear power plants)				No. of samples	Near nuclear power plants			
		Gross alpha		Gross beta			Gross alpha		Gross beta	
		Mean	Range (P ₁₀ , P ₉₀)*	Mean	Range (P ₁₀ , P ₉₀)*		Mean	Range (P ₁₀ , P ₉₀)*	Mean	Range (P ₁₀ , P ₉₀)*
Reservoir water	216	0.03	<0.01–0.07	0.11	0.03–0.23	360	0.02	<0.01–0.06	0.10	0.04–0.15
Finished water	3,733	0.04	<0.01–0.10	0.09	0.01–0.18	1,477	0.02	<0.01–0.05	0.10	0.04–0.18
Tap water	624	0.04	<0.01–0.11	0.11	0.04–0.22	2,549	0.03	<0.01–0.08	0.12	0.04–0.22
Well water	203	0.09	<0.01–0.19	0.17	0.04–0.35	812	0.14	<0.01–0.34	0.31	0.05–0.81

* This represents the data between the 10th and 90th percentiles.

0.12 Bq/L) was significantly higher than that in other water types ($\alpha = 0.05$).

Table 1 presents the gross alpha and gross beta radioactivity concentrations in drinking water nationwide and around nuclear power plants. The Shapiro-Wilk test showed a non-normal distribution of data ($P < 0.05$). To evaluate the overall distribution level of the monitoring data, the data range between the 10th and 90th percentiles is provided. The gross alpha and gross beta concentrations in reservoir water, finished water, and tap water were consistent between the two regions. Although gross beta concentrations in finished water and tap water around nuclear power plants were slightly higher than the national levels, the differences were not statistically significant ($\alpha = 0.05$). A statistically significant difference ($\alpha = 0.05$) was observed in gross alpha and beta concentrations in well water between the 2 regions. However, the difference is not considered practically significant. Certain individual monitoring points, mainly those involving well water, exhibited relatively high levels of gross alpha (> 0.5 Bq/L) or gross beta (> 1 Bq/L) radioactivity. For samples with gross beta exceeding 1

Bq/L, the result included the contribution of ^{40}K . However, after subtracting the contribution of ^{40}K , the gross beta in these samples did not exceed 1 Bq/L.

DISCUSSION

The radioactivity levels in drinking water are an important factor for assessing drinking water safety and public health risks. Nationwide surveys of the radioactivity levels in drinking water have been conducted by the Health Department since the 1960s. According to previous reports (12), the highest levels of radioactivity in tap water were observed in the 1960s, attributed to atmospheric nuclear tests; levels subsequently declined and eventually stabilized. However, systematic monitoring was lacking between 2000 and 2011. Nationwide monitoring of drinking water radioactivity was reinitiated in 2012, providing valuable data on the natural background levels of radioactivity in China's drinking water. This study presents the national survey results from 2012 to 2024, including monitoring data from areas surrounding nuclear power plants.

In the initial phase of this survey, monitoring focused primarily on nuclear power plant provinces, expanding nationwide in 2018. The results indicate that radioactivity levels in drinking water consistently fluctuate within background ranges (12–13).

The results indicate no significant difference in radioactivity levels between drinking water samples collected near nuclear power plants and those from other regions, except for slightly higher levels in well water, which are not considered practically significant. This is because whenever well water samples exceed the standard, continuous sampling and monitoring are implemented at those points.

The radioactivity levels vary across provinces, as the activity concentrations of natural radionuclides – particularly in groundwater – can vary significantly due to geological processes (1).

Among the monitored samples, certain individual monitoring points, primarily those involving well water and other underground sources, exhibited relatively high levels of gross alpha radioactivity (>0.5 Bq/L). These points have been monitored continuously for several years, with their radioactivity levels remaining stable. Radionuclide analysis confirmed that the primary contributors are natural uranium or radium-226. No industrial activities were found near these points that could introduce radioactive contamination. Because these locations are in mineral-rich regions of China, the elevated levels are inferred to result from geological formations.

Additionally, gross beta measurements include contributions from potassium-40, a naturally occurring beta emitter present in a fixed ratio to stable potassium. When the national standard guideline level of 1 Bq/L for gross beta is exceeded, the contribution of potassium-40 should be subtracted following a separate determination of total potassium (1–2). Monitoring data showed that gross beta activity concentrations – after subtracting potassium-40 – remained consistently below 1 Bq/L.

Natural radionuclides such as uranium and thorium decay series elements and potassium-40, which are commonly found in rocks and soils at low concentrations, may leach into groundwater. Therefore, they are more commonly found in drinking water derived from groundwater sources than surface water (1).

Based on the survey results, combined with drinking water consumption data (2) and dose coefficients given by the United Nations Scientific Committee on the Effects of Atomic Radiation UNSCEAR (14), the

annual effective dose from drinking water can be estimated. Generally, if the gross alpha activity concentration is below 0.5 Bq/L and gross beta below 1 Bq/L, no further action is required, as the annual dose from ingesting such water will not exceed 0.1 mSv, representing a very low health risk (1–2). However, to assess public radiation exposure in China, additional analyses of specific radionuclides – such as uranium, radium-226/228, and lead-210 – are required.

The monitoring points in this survey were distributed nationwide, providing preliminary baseline data on the radioactivity levels in drinking water. However, the data primarily covered urban and rural water sources from major cities (including areas near nuclear power plants), while smaller cities were excluded. This limits the detailed understanding of regional variations in radioactivity, necessitating additional monitoring points for more refined analysis.

Survey results demonstrate that China's drinking water poses an extremely low radioactivity risk, with minimal health implications for the public through water consumption. As a critical component of water safety assurance, long-term radioactivity monitoring is critical to enable timely water quality assessments, early warnings, and effective emergency response. It is imperative to enhance radionuclide-specific analyses and conduct comprehensive risk assessments in sensitive areas, particularly in the vicinity of nuclear facilities and associated mining operations.

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Methods and Applications

Wastewater-Based Monitoring of Dengue Fever at Community Level — Guangzhou City, Guangdong Province, China, May 2024

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ABSTRACT

Introduction: Traditional dengue surveillance operates reactively, frequently lagging behind viral transmission patterns and thereby impeding timely public health responses. Wastewater-based epidemiology (WBE) presents significant potential for proactive early warning systems. This study sought to implement and validate the first community-level WBE system for dengue during an active outbreak, evaluating its capacity to detect cryptic transmission and provide actionable intelligence for public health interventions.

Methods: During a dengue virus serotype 1 (DENV-1) outbreak, we collected 618 wastewater grab samples from manholes within a 200-m radius of 8 reported cases, along with matched patient serum and urine samples. We systematically compared magnetic bead and polyethylene glycol (PEG) concentration methods for viral recovery efficiency. DENV-1 ribonucleic acid (RNA) was detected and quantified using reverse transcription quantitative polymerase chain reaction (RT-qPCR). Positive samples underwent genomic sequencing and phylogenetic analysis to confirm environmental signals and determine viral lineages.

Results: The magnetic bead method demonstrated superior performance with a limit of detection of 10 copies/mL and was selected based on its higher recovery efficiency (59.7%). We successfully detected DENV-1 in 14 of 618 wastewater samples tested. Critically, a positive wastewater signal from one residential building preceded the clinical diagnosis of a new case within that same location by several hours. For a single patient, we successfully generated matched viral genomic sequences from serum, urine, and wastewater samples, providing definitive validation of the environmental signal's authenticity.

Conclusions: Community-level wastewater

surveillance represents a powerful and effective tool for dengue control programs. This approach provides actionable early warnings by detecting cryptic viral transmission before cases receive clinical identification. Such capabilities enable public health authorities to deploy preemptive, geographically-targeted interventions, including vector control measures, fundamentally improving both the speed and precision of outbreak responses while helping to mitigate disease spread.

Dengue fever is an acute mosquito-borne infectious disease caused by any of the four serotypes of dengue viruses (dengue virus serotypes 1 to 4), a single-stranded ribonucleic acid (RNA) virus. The disease is endemic in over 132 countries, with an estimated 96 million symptomatic infections and 40,000 deaths annually (1). Traditional dengue surveillance relies primarily on passive case detection, which involves identifying dengue virus (DENV), its components, or antibodies against DENV in blood samples from symptomatic individuals. However, this approach is inherently limited by diagnostic delays, leading to a lag in outbreak detection and response. Moreover, a significant proportion of infected individuals do not seek medical attention or choose not to report their illness, contributing to the underestimation of actual case numbers. Serum epidemiological studies indicate that 4% to 92% of dengue infections are asymptomatic (2), however, these individuals may still contribute to viral transmission. The inability to detect asymptomatic infections not only hampers early warning and rapid intervention but also prevents accurate assessment of infection prevalence. Given these limitations, there is an urgent need for a more efficient and comprehensive surveillance strategy to enable early detection, timely outbreak control, and

effective dengue prevention.

Wastewater-based epidemiology (WBE) was initially developed to assess the prevalence of drug use in communities and has since been applied to the surveillance of various pathogens, including viruses (3). During the coronavirus disease 2019 (COVID-19) pandemic, WBE became widely adopted across multiple countries for monitoring severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) levels in wastewater, providing critical insights into community transmission dynamics (4). Compared to traditional surveillance methods, WBE offers several advantages, including near real-time monitoring of large populations at relatively low cost (5). Importantly, as asymptomatic individuals also excrete pathogens in urine and feces, this approach captures both symptomatic and asymptomatic infections, allowing for a more comprehensive estimation of disease prevalence. Additionally, its applicability at the community level enables early outbreak detection, rapid response, and more effective epidemic control.

Previous studies have confirmed the presence of DENV nucleic acids in the saliva and urine of infected individuals (6), suggesting the feasibility of wastewater-based dengue surveillance. However, only two studies to date have reported wastewater detection of DENV. Wolfe et al. (7) analyzed weekly wastewater solids from three wastewater treatment plants (WWTPs) in the United States and consistently detected dengue virus serotype 3 (DENV-3) RNA. Their findings suggested that wastewater-based detection of DENV RNA was possible with as few as five laboratory-confirmed dengue cases per million people. Monteiro et al. (8) investigated the presence of DENV RNA in wastewater samples from 11 WWTPs in Portugal, identifying two seasonal peaks in viral prevalence and load (summer and winter). While these studies demonstrated the feasibility of wastewater-based DENV surveillance, both were conducted at the WWTP level, limiting their ability to assess the sensitivity and timeliness of detection. Consequently, this approach may not be suitable for epidemic monitoring in low-prevalence areas, where viral concentrations in wastewater are likely to fall below detectable thresholds. Furthermore, neither study performed sequencing to distinguish DENV serotypes, leaving gaps in the phylogenetic characterization of circulating strains.

In this study, following the diagnosis of the first locally acquired dengue case in Guangzhou in May

2024, wastewater monitoring and epidemiological surveys were conducted in parallel. Wastewater samples were collected near the patient's residence, and two concentration methods were compared, with the magnetic bead-based method selected for routine DENV-1 monitoring. To validate the wastewater surveillance findings, serum and urine samples from patients were concurrently analyzed via polymerase chain reaction (PCR) and sequencing. To our knowledge, this represents the first study to implement wastewater-based dengue surveillance at the community level immediately following the emergence of local cases. Notably, this approach facilitated the identification of a previously undetected case based on wastewater signals. Whole-genome sequencing of DENV-1 from wastewater was successfully obtained, further demonstrating the potential of wastewater surveillance for early dengue detection and outbreak prevention.

METHODS

Serum and urine samples from confirmed cases (Supplementary Material, available at <https://weekly.chinacdc.cn/>) were obtained from hospitals or district CDCs in Guangzhou. On the day of diagnosis, 1 mL of serum was collected from each case. Subsequently, a total of 36 midstream urine samples (50 mL each) were collected from three cases. Given the urgent need for epidemic response, grab wastewater samples were collected as a practical alternative to 24-hour composite sampling. In total, 618 grab samples were obtained from wastewater manholes within 200 m of all confirmed case residences — a distance corresponding to the typical activity range of *Aedes* mosquitoes (Figure 1). Additionally, 24-hour composite influent samples were collected from WWTPs to enable a comprehensive assessment of detection sensitivity. Each wastewater sample volume was 500 mL. Wastewater surveillance encompassed all of Guangzhou during the initial wave of local dengue fever outbreaks, with sampling conducted at 115 distinct sites. All samples were transported at 4 °C to the laboratory for analysis within 24 hours.

Serum RNA was extracted using the magnetic bead virus nucleic acid extraction kit (Jiangsu Biopurfectus Technologies Co., Ltd, Taizhou, Jiangsu, China). A 2 mL-aliquot of the urine sample was used for one-step virus concentration and RNA extraction using a magnetic bead-based biological sample virus

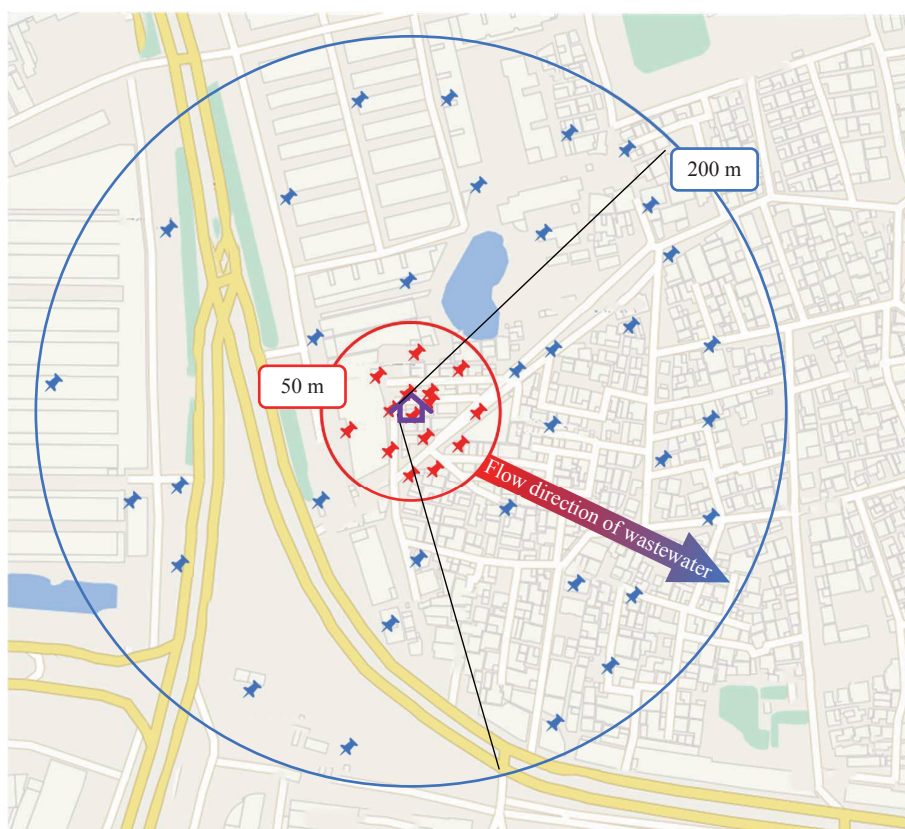


FIGURE 1. A schematic layout of sampling sites from wastewater manholes within 200 m of the residence of the first locally acquired dengue case (Case 1).

concentration kit (Suzhou Advanced Molecular Diagnostics Co., Ltd, Suzhou, Jiangsu, China).

Two concentration methods, polyethylene glycol precipitation and the magnetic bead-based method, were systematically compared in terms of limit of detection (LOD), PCR inhibition, and recovery efficiency (Supplementary Material). Primers and probes used are shown in Supplementary Table S1 (available at <https://weekly.chinacdc.cn/>). The method demonstrating superior performance was selected for wastewater sample concentration.

To further confirm the type of DENV infection in patients, reverse transcription PCR (RT-PCR) of serum RNA was conducted on four serotypes. The RNA concentration of DENV-1 in wastewater and urine samples was obtained by RT-qPCR. Sanger and whole-genome sequencing of DENV-1 was performed in positive serum, urine, and wastewater samples. Sequence analysis and visualization were carried out. The detailed descriptions are shown in the Supplementary Material.

For DENV-1 detection in wastewater samples, spiked murine hepatitis virus (MHV) served as the

sample processing control (9). Following RNA extraction, the OneStep™ PCR Inhibitor Removal Kit (Zymo Research, CA, USA) was employed to eliminate potential inhibitors.

RESULTS

Genotype Branches of DENV-1 in Serum Samples

DENV-1 target sequences were successfully obtained from serum samples of 6 out of 8 cases, with GenBank (National Center for Biotechnology Information genetic sequence database) accession numbers PQ326420-PQ326425. Phylogenetic tree analysis revealed that the DENV infections in 2 cases belonged to DENV-1 genotype I (branch 1 and branch 2), while the DENV infections in 4 cases belonged to DENV-1 genotype III (branch 3) (Figure 2). Through online Nucleotide Basic Local Alignment Search Tool (BLAST) analysis, branches 1, 2, and 3 showed the closest similarity to KY057370 (isolated in 2012, Indonesia), OQ678061 (isolated in 2019, Cambodia), and MZ312929 (isolated in 2018, India), respectively.

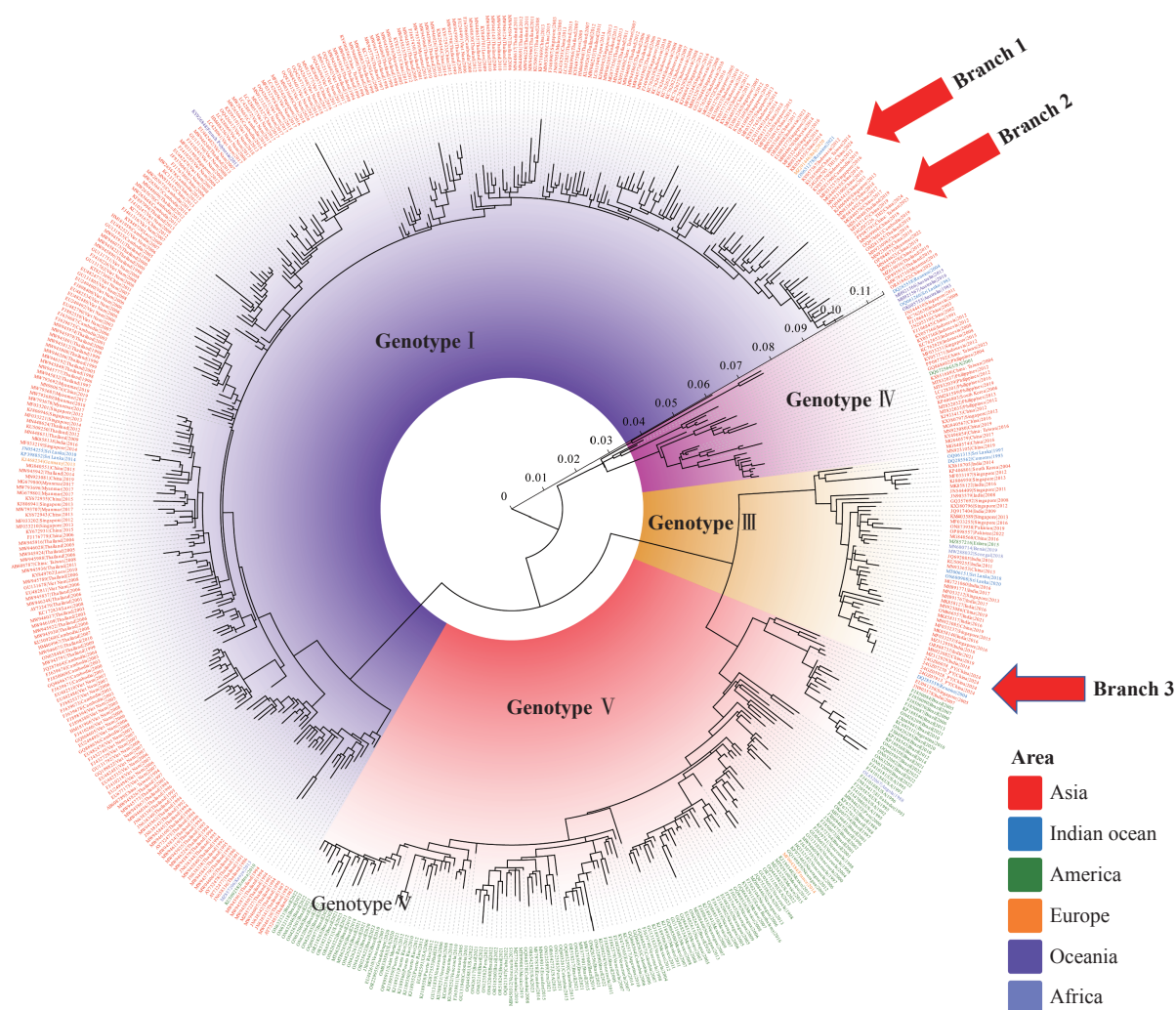


FIGURE 2. The phylogenetic tree of dengue virus serotype 1 (DENV-1) branches in serum samples.

These sequences demonstrated close phylogenetic relationships with Southeast Asian strains, consistent with genomic analysis results from previous dengue cases in Guangzhou (10).

RNA Concentration of DENV-1 in Urine Samples

Urine samples demonstrated a 100% positivity rate for DENV-1, with RNA concentrations ranging from 7.0 to 50,065.5 copies/mL, consistent with previously reported values (10^2 to 10^4 copies/mL) (6). Prior studies (6,11–12) indicate that the primary source of detectable DENV RNA in wastewater originates from patient urine, whereas SARS-CoV-2 RNA predominantly derives from fecal shedding, with reported concentrations ranging from 10^2 to 10^8 copies/mL (13). Consequently, the substantially lower concentration of DENV in wastewater compared to

SARS-CoV-2 presents a significant analytical challenge for wastewater-based surveillance of dengue fever.

RNA Concentration and Sequence Distribution of DENV-1 in Wastewater Samples

Both concentration methods demonstrated a LOD of 10 copies/mL (Supplementary Table S2, available at <https://weekly.chinacdc.cn/>), with no evidence of PCR inhibition (Supplementary Table S3, available at <https://weekly.chinacdc.cn/>). However, the magnetic bead-based method exhibited superior recovery efficiency (59.7%) compared to polyethylene glycol precipitation (50.3%) (Supplementary Table S4, available at <https://weekly.chinacdc.cn/>). Given its enhanced recovery rate and automation potential, the magnetic bead-based method was selected for routine wastewater monitoring. In total, 14 wastewater samples

tested positive for DENV-1, yielding a positivity rate of 2.3%. All positive samples were collected from wastewater manholes within 50 m (straight-line distance) downstream of patients' residences during the initial 5 days of sampling. Sanger sequencing results of the RT-qPCR products from positive wastewater samples further confirmed the detection of DENV-1 sequences. The sequence alignment coverage reached 100.0%, with similarity ranging from 98.2% to 100.0%. RNA concentrations ranged from 0.7 to 97.5 copies/mL. These values were slightly lower than those reported in WWTPs (31–450 copies/mL) (8), highlighting potential differences in sampling environments and viral persistence.

Whole-genome sequencing revealed that the read counts of the obtained DENV-1 sequences from wastewater, urine, and serum samples from the same

patient were 6,311/559,029 (1.1%), 206,362/341,880 (60.4%), and 1,573,040/1,590,306 (98.9%), respectively (Figure 3). These results indicate that the degradation of DENV-1 sequences in wastewater is substantially greater than in serum and urine samples. This finding suggests that wastewater-based monitoring and whole-genome sequencing of dengue fever present greater technical challenges compared to clinical specimens.

DISCUSSION

Ongoing dengue control efforts have successfully maintained low case numbers in Guangzhou. Between 2010 and 2019, annual dengue incidence fluctuated from 58 to 1,925 cases, with a significant outbreak in 2014 resulting in 38,036 reported cases (10). In recent

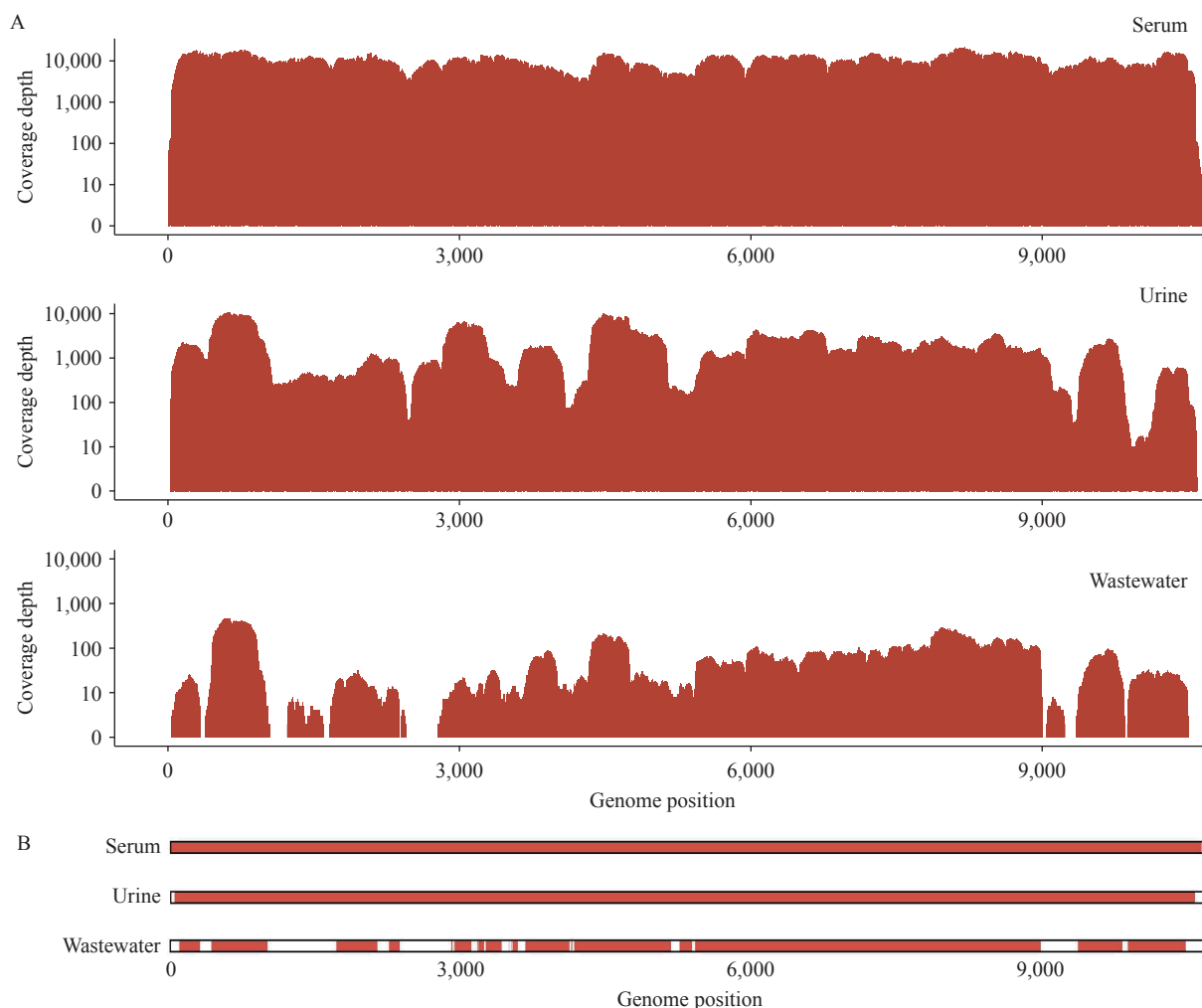


FIGURE 3. Distribution of dengue virus serotype 1 (DENV-1) sequences in serum, urine, and wastewater samples based on whole-genome sequencing. (A) The distribution and coverage depth of all sequences; (B) The distribution of sequences with a coverage depth of no less than 10.

years, dengue cases have remained consistently low, with only 9–37 cases reported annually from 2020 to 2022. Dengue virus RNA concentrations in urine, as documented in this and previous studies, range from 10^1 to 10^4 copies/mL (6). Assuming an average daily urine output of 2 L per person, daily dengue virus shedding is estimated at 2×10^4 to 2×10^7 copies per individual, approximately 100 times lower than SARS-CoV-2 shedding [1.27×10^6 to 1.04×10^8 copies/(day-person)] (12). The combination of low dengue prevalence and substantially reduced viral shedding creates considerable challenges for wastewater-based surveillance. Unlike SARS-CoV-2, which remains detectable in wastewater from large catchments even with minimal case numbers, dengue virus was only detectable within a 50 m radius of infected residences, with WWTP samples falling below

detection limits. Under these conditions of low case numbers and limited viral shedding, wastewater-based surveillance at WWTPs provides insufficient sensitivity for effective outbreak detection. Instead, robust dengue surveillance requires targeted sampling at the community level, with strategic focus on locations proximal to identified cases.

Despite these challenges, our findings demonstrate the significant potential of wastewater-based surveillance as a complementary tool for community-level dengue monitoring. Notably, all positive wastewater samples in this study correlated precisely with epidemiological survey results, with no false-positive detections observed. More importantly, wastewater surveillance demonstrates promise for early detection of asymptomatic or pre-symptomatic infections, enabling timely interventions. The first

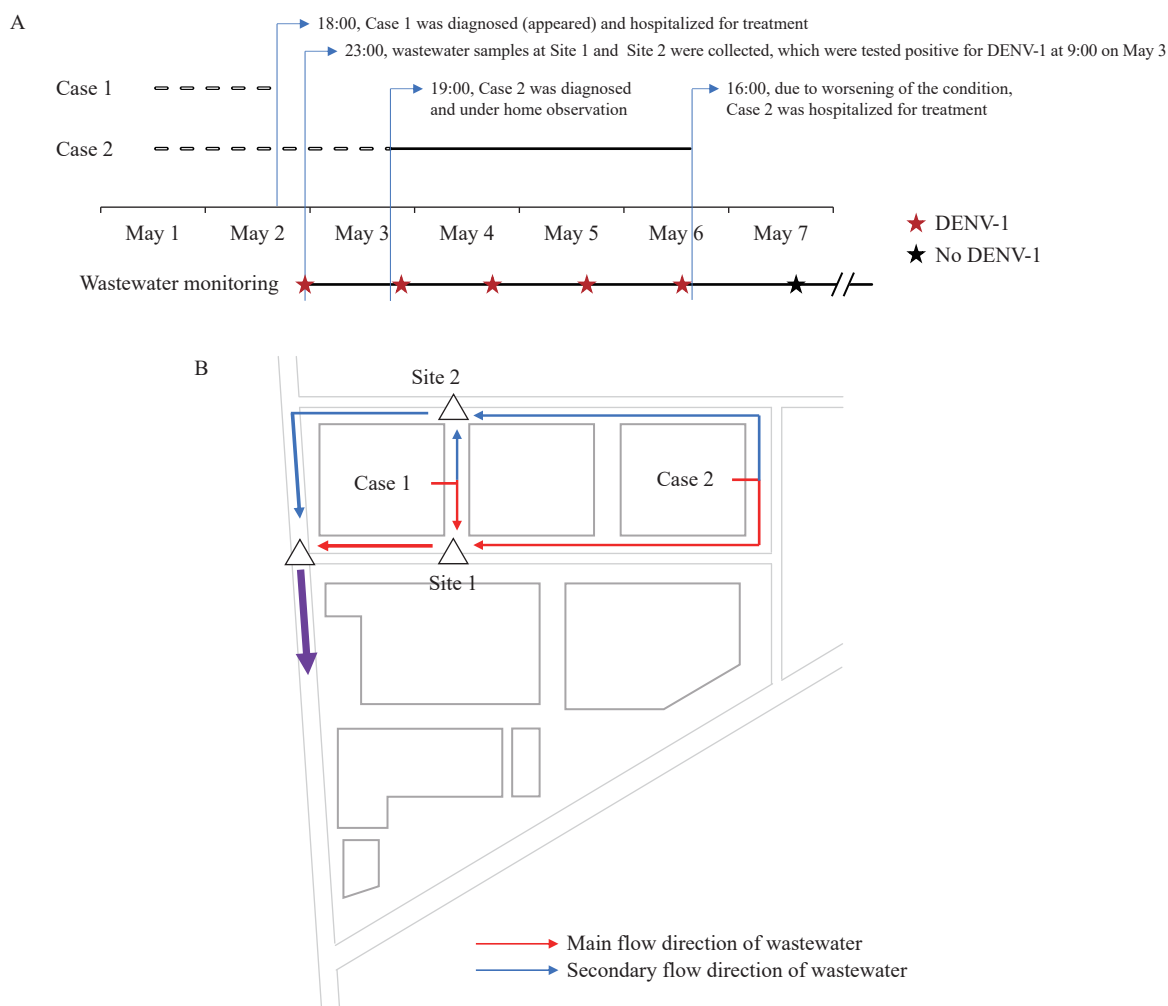


FIGURE 4. Schematic diagram of wastewater surveillance identifying Case 2 prior to clinical diagnosis. (A) Timeline of clinical confirmation/hospitalization for Case 1 and Case 2, and corresponding positive wastewater signals. (B) Relative geographical locations of Case 1 and Case 2 residences, and case-origin attribution of positive wastewater samples. Abbreviation: DENV-1=dengue virus serotype 1.

locally acquired dengue case was clinically diagnosed and hospitalized at 18:00 on May 2. Later that evening at 23:00, wastewater monitoring and epidemiological surveys were initiated (Figure 4). Remarkably, despite the hospitalization of this initial case, positive wastewater signals persisted at Site 1 and Site 2 from May 2 to May 6. This unexpected persistence prompted further investigation. Since grab wastewater samples were collected from manholes beneath the patient's building rather than from wastewater storage tanks, we excluded the possibility that the detected viral signals originated from Case 1. Subsequent epidemiological investigations identified a second infected individual residing in the same building at 19:00 on May 3. This patient remained at home under observation for three days before hospitalization on May 6. The spatiotemporal correlation between wastewater viral concentrations and confirmed cases strongly indicates that the wastewater signals at Site 1 and Site 2 were attributable to Case 2. These findings underscore the utility of wastewater surveillance in identifying undetected transmission chains. Furthermore, trends in wastewater viral levels provide valuable insights into the temporal dynamics of outbreaks, offering a complementary tool for real-time epidemiological monitoring and early intervention strategies.

Although wastewater monitoring for dengue frequently yields negative results, these findings provide valuable insights for tracking epidemic trends. For instance, wastewater surveillance continued for nine days following the hospitalization of Case 2, during which all wastewater samples collected near the residence tested negative — consistent with the absence of further infections. A similar pattern was observed for other patients, as no additional cases emerged during their hospitalization, aligning with the lack of detectable DENV-1 in wastewater samples collected around their residences. These results highlight the potential of wastewater surveillance as a valuable tool for assessing the ongoing risk of dengue transmission and determining whether continued prevention and control measures are necessary in a given area.

While this study successfully demonstrated the potential of wastewater-based surveillance for early detection and outbreak monitoring, several limitations warrant consideration in future research. First, the study relied on grab sampling, which may have reduced detection sensitivity. Given the generally lower

concentrations of DENV compared to SARS-CoV-2, employing larger-volume samples, 24-hours composite sampling, or solid-phase extraction methods (which enhance concentration factors) could improve detection sensitivity (3,8,14–15). Enhanced sensitivity would extend the effective monitoring range, increasing the likelihood of detecting infections at a given density of sampling sites. Second, sequence analysis of DENV-1 ribonucleic acid (RNA) from urine and wastewater samples revealed significant viral degradation in wastewater. As samples were collected in close proximity to infected residences, degradation is unlikely to have occurred within the sewer system. Instead, it is more plausible that RNA degradation resulted from suboptimal conditions during sample transport and storage prior to analysis. Future studies should focus on optimizing these processes to minimize degradation, potentially through the use of stabilizing agents or rapid processing techniques to preserve viral RNA integrity. Third, this study was conducted during a period of sporadic dengue cases in Guangzhou, whereas dengue outbreaks typically peak in October and November. Ongoing surveillance efforts aim to assess the correlation between wastewater detection rates at WWTP inlets and the number of clinically diagnosed cases within corresponding catchment areas. This extended monitoring will provide critical insights into the feasibility of wastewater-based surveillance for outbreak prediction at a larger scale.

Conflicts of interest: No conflicts of interest.

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SUPPLEMENTARY MATERIAL

Case Information

In May 2024, through systematic epidemic surveillance and comprehensive record analysis, a total of 8 cases from 5 distinct epidemic locations were identified during the initial wave of local dengue fever outbreaks in Guangzhou. All cases were confirmed to be infected with DENV-1 through blood sample analysis conducted by hospitals or district CDCs. The diagnostic methodology adhered to the latest dengue fever diagnostic standard (1). All diagnostic results included positive detection based on DENV-1 nucleic acid identification. Case numbers were assigned chronologically according to the date of discovery. All 8 patients represented locally acquired dengue fever cases.

Concentration Methods

PEG precipitation method. A 50 mL wastewater sample was centrifuged at 4 °C and 2,000 *g* for 2 minutes. From the resulting supernatant, 40 mL was mixed with 4.0±0.1 g of polyethylene glycol (PEG) and 0.80±0.01 g of sodium chloride, then shaken at 4 °C and 150 r/min for 120 minutes before centrifugation at 4 °C and 4,800 × *g* for 45 minutes. After removing the supernatant, the remaining 1 mL was transferred to a 1.5 mL tube and centrifuged at 4 °C and 20,000 × *g* for 8 minutes. The supernatant was discarded, leaving 500 µL of concentrate, of which 200 µL was used for nucleic acid extraction using the Tianlong method. Specifically, the concentrated virus solution underwent extraction using Nucleic acid extraction or purification reagents T183 with the Nucleic Acid Extractor GeneRotex 96 (Xi'an Tianlong Technology Co., Ltd, Xian, Shaanxi, China). This process yielded 80 µL of nucleic acid solution for PCR analysis.

Magnetic bead-based method. One-step virus concentration and nucleic acid extraction for wastewater samples were performed using a magnetic bead wastewater virus concentration kit combined with a wastewater molecular concentrator (WMC-24C, Suzhou Advanced Molecular Diagnostics Co., Ltd, Suzhou, Jiangsu, China). A 15 mL wastewater sample was centrifuged at 4 °C and 2,500 *g* for 10 minutes. The supernatant, enhancer, and eluent were then added according to the protocol described by Xu et al. (2). The instrument automatically executed sequential steps including nucleic acid fragment release from wastewater using lysis buffer, nucleic acid adsorption by magnetic beads, nucleic acid washing, and final elution. This automated process yielded 80 µL of nucleic acid solution suitable for PCR analysis.

Analytical Methods

Limit of detection (LOD) test. Different volumes of inactivated DENV-1 were introduced into DENV-1-free wastewater samples to achieve final concentrations of 0 copies/mL (negative control), 5 copies/mL, 10 copies/mL, 50 copies/mL, 100 copies/mL, and 250 copies/mL. Three parallel samples were prepared for each concentration level. Wastewater samples underwent concentration and extraction using both methods described above, followed by RT-qPCR detection for DENV-1. Each sample was amplified in triplicate. The concentration producing at least 95% positive replicates was designated as the LOD for the RT-qPCR assay (3).

PCR inhibition test. DENV-1-free wastewater samples underwent concentration and extraction using both methods described above. Subsequently, 40 µL of nucleic acid solution was spiked with 10 µL of 2.6×10⁶ copies/mL DENV-1 plasmid for RT-qPCR analysis. Parallel testing was conducted using nuclease-free water instead of nucleic acid solution as a control. Each sample was amplified in triplicate. Samples were considered to contain PCR inhibitors if the mean quantification cycle (C_q) value exceeded the reference C_q value for nuclease-free water by >2 cycles (4).

Recovery efficiency test. Various ribonucleic acid (RNA) concentrations of pepper mild mottle virus (PMMoV) were employed to calculate recovery efficiency. DENV-1-free wastewater samples underwent concentration and extraction using both methods described above. Additionally, 200 µL of wastewater samples were directly extracted for nucleic acid using the Tianlong method as the reference standard. RT-qPCR detection of PMMoV was performed on nucleic acid extracts using the Hieff Unicon® V Universal Multiplex One Step RT-qPCR Probe Kit (Yisheng Biotechnology (Shanghai) Co., Ltd, Shanghai, China). Primer and probe sequences are detailed in Supplementary Table S1. Each sample was amplified in triplicate. PMMoV recovery efficiency was calculated based

SUPPLEMENTARY TABLE S1. Primer and probe sequences for polymerase chain reaction (PCR) amplification.

Amplification method	Virus	Primer or probe	Sequence (5'–3')	Genomic target	Amplicon size (bp)	References
RT-PCR	DENV-1	Forward primer	TGAGACACCCAGGATTCACGG	membrane	1,782	this study
		Reverse primer	TRGCTGATCGAATTCCACACAC	glycoprotein M (partial) + envelope protein E (complete) + nonstructural protein NS1 (partial)		
	DENV-2	Forward primer	GACACGAACYGAAACATGGATGTC		1,696	
		Reverse primer	CCAGCTCACAACRCAACCAC			
	DENV-3	Forward primer	CAAGTCGAGAAGGTAGAGACATGG		1,847	
		Reverse primer	AGYTCATTGGCTATTTGYTTCCAC			
	DENV-4	Forward primer	CCCATCYACGGAATGCGATG		1,592	
		Reverse primer	ACTGTTCTGTCCAAGTGTGCAC			
RT-qPCR	DENV-1	Forward primer	CAAAAGGAAGTCGYGCAATA	RNA-dependent RNA polymerase NS5 (partial)	112	(7-8)
		Reverse primer	CTGAGTGAATTCTCTCTGCTRAAC			
		Probe	6-FAM-CATGTGGYTGGGAGCRCGC-BHQ1			
	PMMoV	Forward primer	GAGTGGTTTGACCTTAACGTTTGA	replicase protein (partial)	68	(9-10)
		Reverse primer	TTGTCGGTTGCAATGCAAGT			
		Probe	CY5-CCTACCGAAGCAAATG-MGB			
	MHV	Forward primer	GGAACTTCTCGTTGGGCATTATACT	membrane protein (partial)	108	(11)
		Reverse primer	ACCACAAGATTATCATTTTCACAACATA			
		Probe	CY5-ACATGCTACGGCTCGTGTAAACCGAACTGT-BHQ3			

Abbreviation: DENV-1=dengue virus serotype 1; DENV-2=dengue virus serotype 2; DENV-3=dengue virus serotype 3; DENV-4=dengue virus serotype 4; PMMoV=pepper mild mottle virus; MHV=murine hepatitis virus; Y=C/T; R=A/G.

SUPPLEMENTARY TABLE S2. Detection sensitivity of two concentration methods for dengue virus serotype 1 (DENV-1) in wastewater samples by reverse transcription quantitative polymerase chain reaction (RT-qPCR).

Concentration method	5 copies/mL	10 copies/mL	50 copies/mL	100 copies/mL	250 copies/mL
Magnetic bead-based method	6/9 (66.7)	9/9 (100.0)	9/9 (100.0)	9/9 (100.0)	9/9 (100.0)
Polyethylene glycol precipitation method	8/9 (88.9)	9/9 (100.0)	9/9 (100.0)	9/9 (100.0)	9/9 (100.0)

Note: Data are presented as positive/total replicates (positivity rate, %). Example: 6/9 (66.7)=6 positives out of 9 replicates (66.7%).

SUPPLEMENTARY TABLE S3. The quantification cycle (Cq) value of two concentration methods for dengue virus serotype 1 (DENV-1) in polymerase chain reaction (PCR) inhibition test.

Concentration method	Cq value		
	Range	Mean	ΔCq
Magnetic bead-based method	30.03–30.15	30.09	0.62
PEG precipitation method	29.82–30.90	30.34	0.37
Reference	29.82–31.30	30.71	–

Note: “–” means that the data is not applicable.

SUPPLEMENTARY TABLE S4. The mean ribonucleic acid (RNA) concentration and recovery efficiency of two concentration methods for pepper mild mottle virus (PMMoV) in wastewater samples by reverse transcription polymerase chain reaction (RT-qPCR).

Concentration method	Mean ribonucleic acid (RNA) concentration ($\times 10^6$ copies/mL)	Recovery efficiency (%)
Magnetic bead-based method	5.99±0.15	59.7
PEG precipitation method	5.05±0.07	50.3
Reference	10.04±1.85	–

Note: “–” means that the data is not applicable.

on the ratio of detected mean viral concentration in concentrated samples versus unconcentrated samples, as determined by RT-qPCR analysis.

RT-PCR for DENV Serotypes

One-step RT-PCR of serum RNA was performed using the C1000 Touch™ Thermal Cycler (Bio-Rad, CA, USA) to confirm the DENV serotype. The 50 µL RT-PCR cocktails were prepared using PrimeScript™ II High Fidelity One Step RT-PCR Kit (Takara, Shiga, Japan), with primer sequences shown in Supplementary Table S1. The RT-PCR cycling conditions were as follows: reverse transcription for 30 minutes at 45 °C, an initial denaturation for 2 minutes at 94 °C, followed by 40 cycles consisting of amplification for 15 s at 98 °C, 15 s at 50 °C and 30 s at 68 °C, and a final extension for 5 min at 68 °C. The amplicon length was analyzed using QIAxcel System (QIAGEN, Hilden, Germany).

RT-qPCR for DENV-1

One-step RT-qPCR assays for DENV-1 concentration of urine and wastewater samples were performed on the Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific, MA, USA). The 20 µL RT-qPCR cocktails contained 10 µL of 2× One Step RT-qPCR Probe Buffer IV and 2 µL of One Step Probe Enzyme Mix IV (ABclonal Technology, Wuhan, Hubei, China), 0.5 µL of 10 µM each primer and probe (Sangon Biotech, Shanghai, China), 5 µL of RNA, and 1.5 µL of nuclease-free water. The sequences of primers and probe are shown in Supplementary Table S1. The target fragment is the same as that used for blood sample diagnosis by RT-qPCR of nucleic acid in the above dengue fever diagnostic standard (1). The RT-qPCR cycling conditions were as follows: UDG reaction for 5 min at 25 °C, reverse transcription for 5 min at 50 °C, an initial denaturation for 3 min at 95 °C, followed by 45 cycles consisting of amplification for 15 s at 95 °C and 31 s at 55 °C. Nuclease-free water was used as the negative amplification control. For the standard curve, 4.5×10^9 copies/mL of DENV-1 RNA standard solution (BNCC, Xinyang, Henan, China) was sequentially diluted 10 fold to 4.5×10^8 to 4.5×10^1 copies/mL, and each concentration gradient were amplified in sextuplicate.

A negative wastewater and a positive sample (200 µL of 4.5×10^5 copies/mL of inactivated DENV-1) were included as the negative and positive extraction control, respectively. The two samples were simultaneously subjected to RNA extraction and RT-PCR to check for contamination during the experimental process. Each sample was amplified in duplicate. If a wastewater sample was tested positive, other commercial kits would be used for rechecking.

Sequencing

The positive RT-PCR and RT-qPCR products were submitted to Guangzhou Tianyi Huiyuan Gene Technology Co., Ltd (Guangzhou, Guangdong, China), and Sangon Biotech (Shanghai, China) Co., Ltd. for Sanger sequencing, respectively. One serum sample (from Case 4), one positive urine sample (from Case 4), and one positive wastewater sample (from the site around residence of Case 4) were selected for whole-genome sequencing, following a procedure similar to that of Su et al. (5). Reverse transcription was performed on their RNA solution using SuperScript™ IV VIL0™ Master Mix (Invitrogen, MA, USA) to obtain complementary DNA (cDNA). A multiplex PCR method with multiple primer combinations was used to perform targeted amplification of the entire DENV genome on cDNA (6). After purification, quantification, and normalization of the amplification products, Nextera XT DNA Library Preparation Kit (Illumina, CA, USA) was used to construct a sequencing library. After purification, quantification, normalization, and mixing of the library, the concentration of the mixture for sequencing was 1.2–1.5 pM. The MiniSeq™ Mid Output Kit (Illumina, CA, USA) was used on the Miniseq sequencing platform for paired-end 150 cycles of sequencing.

Sequence Analysis and Visualization

Using the online alignment software Nucleotide Basic Local Alignment Search Tool (BLAST) from the National Center for Biotechnology Information, the sequences obtained by Sanger sequencing were aligned with all sequences from nucleotide databases to identify aligned species. Additionally, for the Sanger sequences of positive RT-PCR

products, IQtree software (v2.3.6, <https://iqtree.github.io/about>) was used to construct the phylogenetic tree based on the Maximum Likelihood method and calculate branch length based on Bayes method. The beautification and display of phylogenetic tree were completed through ChiPlot software.

CLC Genomics Workbench (QIAGEN, Hilden, Germany) was used for quality control, trimming, and mapping of whole-genome sequencing downstream data (5). The genomes submitted under GenBank (genetic sequence database) accession numbers NC_001477 (DENV-1), NC_001474 (DENV-2), NC_001475 (DENV-3), and NC_002640 (DENV-4) were used as the reference sequences for initial mapping. The consensus sequence for the initial mapping was generated with a coverage depth of no less than 10, which was then used as a reference sequence for the second mapping and correction to generate the final consensus sequence. Statistical analysis and data visualization were conducted using Hiplot (<https://hiplot.org>).

Research Ethics

This study received approval from the Ethics Committee of the Guangzhou Center for Disease Control and Prevention (CDC) under protocols GZCDC-ECHR-2022P0044 and GZCDC-ECHR-2023P0009. Written informed consent was obtained from all patients for surveillance activities and data collection related to disease control and subsequent analysis. All personally identifiable information in this study was pseudonymized to protect participant privacy.

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Review

Characteristics of Spatial Distribution, Health Risk Assessment, and Regulation of PFAS in Global Drinking Water

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ABSTRACT

This study systematically evaluated the spatial distribution, health risks, and regulation of per- and polyfluoroalkyl substances (PFAS) in global drinking water using the PubMed and Web of Science databases (January 1, 2000 to February 25, 2025). Among the 122 studies reviewed, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) received the greatest research attention (detected in 102 and 100 studies, respectively) and showed the highest detection rates (64.69% and 60.72%, respectively). Several other compounds, including perfluorooctane sulfonamide, perfluorobutanesulfonamide, and perfluoropropane sulfonate, also exhibited high detection rates but remain underregulated, underscoring the need for further research and regulatory oversight. The three countries with the highest concentrations of PFAS were the Republic of Korea, the United States, and China. Risk assessments indicated that perfluorohexanoic acid, perfluorobutanoic acid, and perfluorobutanesulfonic acid posed negligible health risks, while perfluorohexane sulfonic acid (PFHxS), PFOA, PFOS, and perfluorononanoic acid (PFNA) showed descending levels of health risk (PFHxS > PFOA > PFOS > PFNA). Regulatory approaches are shifting from compound-specific standards to integrated mixture-based frameworks, reinforced by progressively stringent limits.

Per- and polyfluoroalkyl substances (PFAS) are widely used in food packaging, textiles, firefighting, and other industries (1–2). These compounds migrate through environmental media and pose health risks (3–5). Conventional water treatment processes fail to remove PFAS from environmental water sources, making drinking water a major human exposure pathway (6). In China, the *Standards for Drinking Water Quality* (GB5749-2022) established limits for perfluorooctanoic acid (PFOA) and perfluorooctane

sulfonic acid (PFOS) at 80 ng/L and 40 ng/L, respectively (7–8). In contrast, the U.S. Environmental Protection Agency (EPA) set stricter limits of 4 ng/L for both compounds in its 2024 National Primary Drinking Water Regulation, while Denmark imposed a combined limit of 2 ng/L for four PFAS [(PFOA, PFOS, perfluorononanoic acid (PFNA), and perfluorohexane sulfonic acid [PFHxS]) in 2023 – significantly lower than China's standards. Since PFAS have not yet been routinely monitored in China's drinking water surveillance system, existing research remains limited to project-based studies with insufficient national-level data. Most existing reviews provide qualitative summaries of single countries or specific PFAS, lacking quantitative assessments (9–10). This study systematically quantifies the global spatial distribution, health risks, and regulations of PFAS in drinking water, providing critical evidence to strengthen China's regulatory framework for PFAS management.

METHOD

Literature Screening and Data Collection

We systematically reviewed original studies (January 1, 2000 to February 25, 2025) on PFAS in drinking water from PubMed and Web of Science using keywords including “PFAS” with “drinking water” or related terms. Studies were eligible if they provided original or summary data on PFAS concentrations in drinking water. Exclusion criteria were: 1) reporting only total PFAS without compound-specific concentrations, 2) omitting detection/quantitation limits while including non-detectable/non-quantifiable values, or 3) lacking both raw measurements and adequate summary statistics (defined as requiring either mean ± standard deviation or two or more percentiles). The review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (11). Data extracted included country, sampling date, sample size, target PFAS compounds, and concentrations. PFAS concentrations were aggregated nationally by compound, assuming a log-

normal distribution.

Health Risk Assessment

Risk assessment followed the U.S. EPA's environmental health risk assessment framework (12) and the *Technical Guide for Environmental Health Risk Assessment of Chemical Exposure* (WS/T 777-2021) (13) through four steps:

Hazard identification. Evaluate potential harm of stressors to humans and ecosystems.

Dose-response assessment. Assess non-carcinogenic risks by quantifying exposure–effect relationships using Formula (1). The reference dose [RfD , mg/(kg·d)] was derived from the U.S. Risk Assessment Information System (RAIS) (<https://rais.ornl.gov/>). The No Observed Adverse Effect Level [NOAEL, mg/(kg·d)] was used when available; otherwise, the Lowest Observed Adverse Effect Level (LOAEL) was applied. Uncertainty factors (UF_i) were incorporated.

Exposure assessment. Determine frequency, timing, and levels of contact with the stressor using Formula (2): ADD , average daily dose [mg/(kg·d)]; c , PFAS concentration (mg/L); IR , daily water intake (L/d). EF , exposure frequency (365 d/a); ED , exposure duration (1); BW , body weight (kg); AT , averaging time (d; calculated as $EF \times ED$ for chronic effects). We calculated the population exposure parameter $BW \sim (59.96, 4.16)$, $\ln(IR) \sim N(6.50, 0.82)$ based on age-stratified and general population data from the U.S. EPA *Exposure Factors Handbook*, assuming normal and log-normal distributions, respectively (14).

Risk characterization. Calculate the hazard quotient (HQ , unitless), with $HQ \geq 1$ indicating potential health risk (acceptable or low if <1).

$$RfD = \frac{NOAEL}{\prod_{i=1}^n UF_i} \quad (1)$$

$$ADD = \frac{c \times IR \times EF \times ED}{BW \times AT} \quad (2)$$

$$HQ = \frac{ADD}{RfD} \quad (3)$$

We performed 10,000 Monte Carlo simulations to estimate HQ values at the 50th and 95th percentiles using probabilistic risk quotient methodology.

RESULTS

Literature Screening and PFAS Detection Profiles

A total of 122 studies from 37 countries across six

continents were included by searching the PubMed and Web of Science databases (Figure 1). Among 5,600 water samples analyzed, 102 PFAS compounds were detected (Supplementary Table S1, available at <https://weekly.chinacdc.cn/>). Figure 2A classifies PFAS into high-concern (>20 studies) and low-concern (≤ 20 studies) compounds with $\geq 30\%$ detection rates. PFOA and PFOS received the highest research attention (102 and 100 studies, respectively) and showed the highest detection frequencies (64.69% and 60.72%) (Figure 2A).

Spatial Distribution of PFAS in Drinking Water

The study areas were categorized into background contamination zones (104 studies) and point-source zones (18 studies, including contamination from fluorochemical plants, firefighting training areas, paper, textile, and leather industries, or oil and gas-producing regions). Contamination patterns were characterized by nine high-priority PFAS detected in both categories: PFOA, PFOS, PFHxS, PFNA, perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluorobutanesulfonic acid (PFBS), and perfluoroheptanoic acid (PFHpA).

In background contamination zones, research has primarily focused on Asia (particularly China), North America (notably the United States), and parts of Europe. Sixteen countries provided complete concentration data for all nine PFAS (Figure 2B), with the highest levels in the Republic of Korea (26.20 ng/L), the United States (14.34 ng/L), China (13.43 ng/L), and France (13.21 ng/L). In China, the compositional profile was PFBA (67.27%) > PFOA (15.20%) > PFPeA (5.23%) > PFOS (4.26%) (Figure 2B).

In point-source zones, peak geometric mean concentrations were observed in Japan (PFOA, 855.62 ng/L; PFHxA, 46.50 ng/L; PFHpA, 13.52 ng/L; PFNA, 8.39 ng/L), Ghana (PFOS, 86.33 ng/L), China (PFBA, 27.81 ng/L; PFPeA, 3.77 ng/L; PFBS, 7.41 ng/L), and Sweden (PFHxS, 12.24 ng/L). PFOA dominated compositional profiles in China (40.77%) and Pakistan (69.37%), while PFBS was dominant in the United States (18.01%) and the Netherlands (23.26%) (Figure 2B).

China, the Netherlands, the United States, and Burkina Faso reported all nine high-priority PFAS in both background and point-source zones. The mean

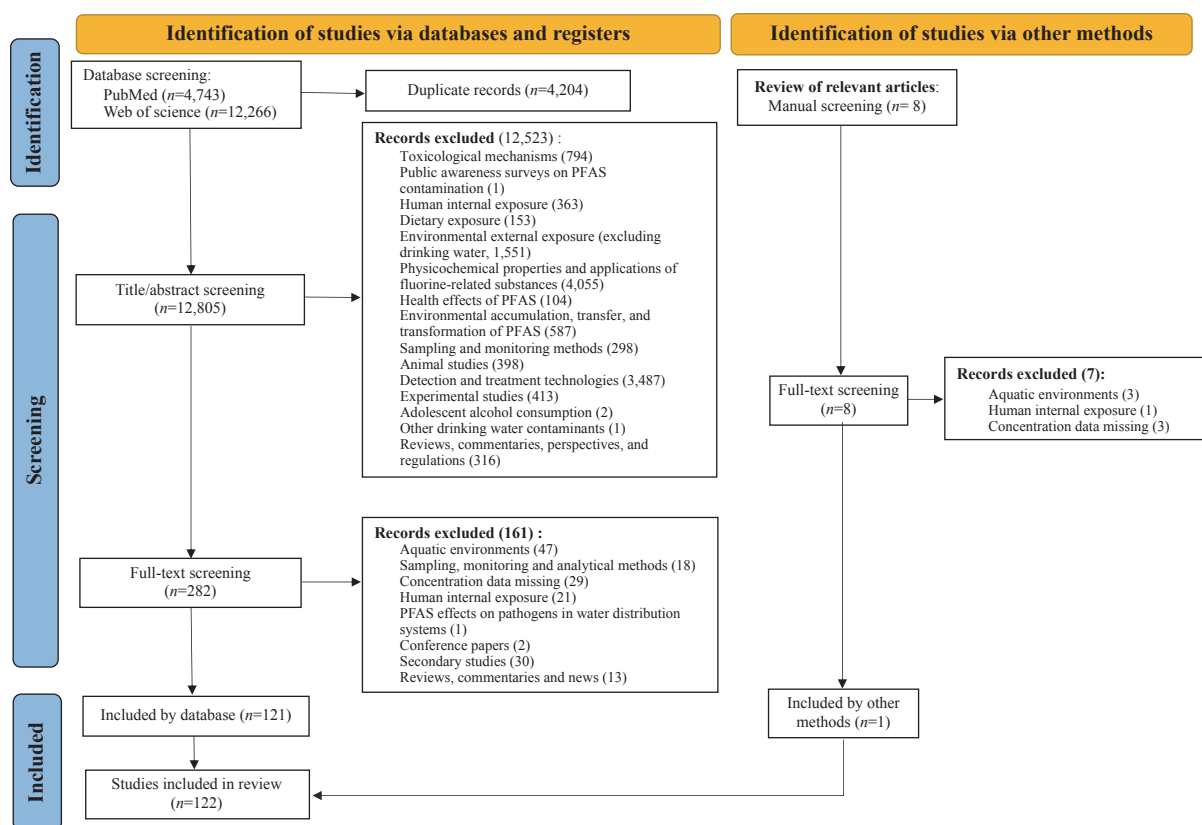


FIGURE 1. Literature screening.

(range) total concentrations of these nine PFAS across these four countries were 13.25 (1.74–29.20) ng/L in background zones and 30.11 (5.46–83.66) ng/L in point-source zones. As shown in Figure 2B, PFOA, PFBA, and PFBS were dominant in point-source zones, whereas PFBA predominated in background zones.

Health Risk Assessment

The HQ values for PFHxA, PFBS, and PFBA were below 1, indicating acceptable health risks. For PFHxS, PFOA, PFOS, and PFNA, the HQ P_{50} values were 10.30, 0.33, 0.07, and 0.001, respectively, while the HQ P_{95} values were 698.72, 9.58, 3.30, and 0.03, respectively. The contribution to overall human health risk ranked as follows: PFHxS (80.63%), PFOA (28.01%), PFOS (12.95%), and PFNA (0.07%) (Figure 2C).

PFAS Regulations in Drinking Water by Different Country/Region

The World Health Organization (WHO) recommends localized standards based on actual needs and resources, with regular reviews and timely updates

(15). Analysis of regulatory frameworks in several countries (Supplementary Table S2, available at <https://weekly.chinacdc.cn/>) revealed two major trends: First, PFOA and PFOS remain the primary targets of regulation, with increasingly stringent limits reflecting scientific consensus on their risks even at very low concentrations. Second, regulation is shifting from single-compound limits to combined PFAS limits, broadening the scope of oversight.

DISCUSSION

Research on PFAS exposure in drinking water is concentrated in the United States, China, and parts of the European Union, with limited studies in most developing countries due to technological, infrastructural, or funding constraints (16). We identified 102 PFAS in drinking water, with significant disparities in research output across compounds (Figure 2A). These differences may reflect variations in usage, environmental persistence, and toxicity. Demand for data on PFAS exposure, toxicity, and population health effects has driven advances in testing technology, which, in turn, facilitates further research. This feedback loop reinforces focus on high-priority

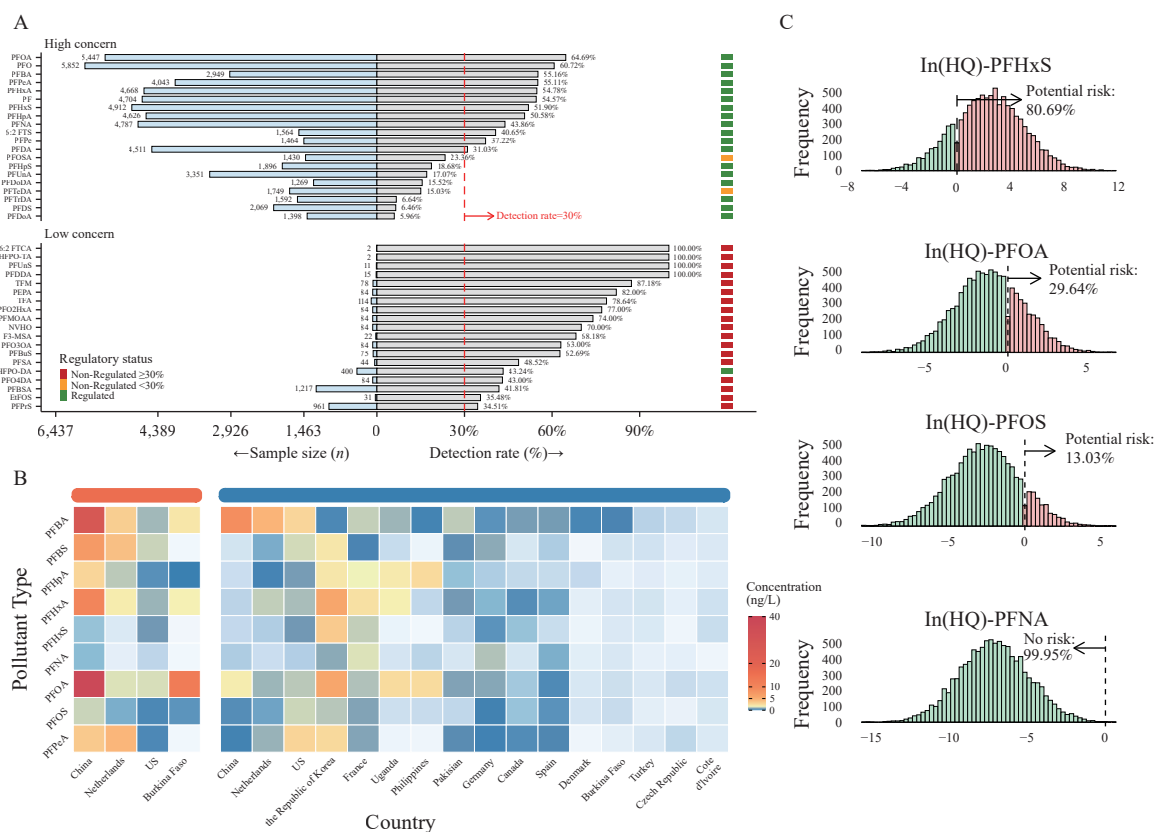


FIGURE 2. Characteristics of spatial distribution and risk assessment of PFAS. (A) Detection rates and regulatory status of PFAS; (B) Exposure in point-source pollution and background pollution; (C) Risk assessment.

Abbreviations: PFTeDA=perfluorotetradecanoic acid; 8:2 FTCA=8:2 fluorotelomer carboxylic acid; HFPO-TA=hexafluoropropylene oxide trimer acid; PFUnS=perfluoroundecanesulfonic acid; PFDDA=perfluorododecanedioic acid; TFMS=trifluoromethanesulfonic acid; PEPA=perfluorinated ether phosphonic acid; TFA=trifluoroacetic acid; PFO2HxA=perfluoro(3,5-dioxahexanoic) acid; PFMOAA=perfluoro-2-methoxyacetic acid; NVHOS=1,1,2,2-tetrafluoro-2-(1,2,2,2-tetrafluoroethoxy) ethane sulfonate; F3-MSA=trifluoromethane sulfonic acid; PFO3OA=perfluoro(3,5,7-trioxaoctanoic) acid; PFBuS=perfluorobutanesulfonic acid; PFO4DA=perfluoro(3,5,7,9-butoxadecanoic) acid; EtFOSE=N-Ethylperfluorooctane sulfonamidoethanol; PFPrS=perfluoropropanesulfonic acid.

PFAS while potentially neglecting others. Notably, low-priority PFAS such as hexafluoropropylene oxide dimer acid, perfluorobutanesulfonamide, and Perfluoropropanesulfonate — detected in $\geq 30\%$ of samples ($n \geq 400$) but currently unregulated (Figure 2A) — require urgent investigation.

Our risk assessment indicates negligible health risks from PFHxA, PFBS, and PFBA, but highlights potential hazards from PFHxS, PFOA, PFOS, and PFNA, ranked as PFHxS > PFOA > PFOS > PFNA. These findings align with previous studies by Thomaidi et al. (10) and Li et al. (17), which identified PFOA and PFOS as significant contributors to global and Chinese drinking water risks. The RfDs used in this study integrate comprehensive toxicological data: PFOA at 3×10^{-8} ng/L (pediatric vaccine response, birth weight, adult cholesterol), PFOS at 1×10^{-7} ng/L (immune, developmental, cardiovascular, and hepatic

effects), PFHxS at 4×10^{-8} ng/L (immunotoxic and thyroid effects), and PFNA at 2×10^{-9} ng/L (immunotoxic and developmental effects). These precautionary thresholds underscore the need for cautious interpretation of risk estimates.

As toxicological and epidemiological evidence grows, regulatory standards for PFAS in drinking water are becoming more stringent worldwide. However, current Chinese standards for PFOA and PFOS — based solely on developmental endpoints such as reduced osteogenesis and altered puberty in juvenile rodents (18–19) — remain comparatively lenient. In contrast, the U.S. EPA's 2024 *Primary Drinking Water Regulations (PDWR)* set a maximum containment level of 4 ng/L for both PFOA and PFOS, based on RfD values (3×10^{-8} ng/L for PFOA and 1×10^{-7} ng/L for PFOS) derived from multiple endpoints, including immunotoxicity, developmental, hepatic, and

cardiovascular effects (20–21). In China, PFOA and PFOS are currently only reference indicators in GB5749-2022 and are not included in routine national monitoring. Most PFAS data derive from small-scale studies, limiting representativeness. Enhancing local exposure data, advancing mechanistic toxicology, and adopting a risk-based, multi-endpoint dose–response approach similar to the U.S. EPA’s framework are essential to support phased standard updates.

This study has limitations. First, variability in the PFAS compounds analyzed across studies limits global comparability of total PFAS exposure. Moreover, emerging contaminant surveys often target suspected contamination zones — even when classified as background — potentially inflating exposure estimates. Second, reliance on self-reported point-source contamination data from primary literature means unreported contamination cannot be excluded. Third, uniform assumptions applied across populations ignore physiological and lifestyle differences due to a lack of region-specific toxicity and exposure data. Finally, heterogeneity in sampling, pretreatment, analytical methods, and quality control across the 122 studies likely contributes to variability (22). Thus, results should be interpreted with caution.

Drinking water safety has become an urgent global health concern (23). Despite these limitations, our findings offer meaningful insights for PFAS management: First, stricter regulatory limits for PFOA and PFOS are needed, incorporating multi-system toxicity endpoints, population-specific exposure factors, technical feasibility, and cost considerations, alongside enhanced monitoring in point-source areas. Second, regulatory expansion to include PFHxS and PFNA, either as individual limits or under a combined standard, should be considered. Implementation of these recommendations requires more comprehensive, targeted exposure assessments and health risk studies. Furthermore, while our analysis focuses on drinking water as an exposure pathway to inform PFAS standards, future high-quality research should address combined risks from diet, inhalation, and dermal contact.

Conflicts of interest: No conflicts of interest.

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE S1. PFAS detected in drinking water.

No.	PFAS	Abbreviation	Study	Sample size	Detection rate (%)	Mean±SD/median (range) (ng/L)*	Regulation
Perfluoroalkyl carboxylic acids (PFCAs)							
1	Perfluorooctanoic acid	PFOA	102	5,447	64.69	1.15±8.16	Yes
2	Perfluorononanoic acid	PFNA	85	4,787	43.86	0.24±6.89	Yes
3	Perfluorohexanoic acid	PFHxA	81	4,668	54.78	0.83±13.54	Yes
4	Perfluoroheptanoic acid	PFHpA	81	4,626	50.58	0.49±12.61	Yes
5	Perfluorodecanoic acid	PFDA	78	4,511	31.03	0.21±5.66	Yes
6	Perfluoroundecanoic acid	PFUnA	69	3,351	17.07	0.11±8.22	No
7	Perfluoropentanoic acid	PFPeA	63	4,043	55.11	1.04±9.86	Yes
8	Perfluorobutanoic acid	PFBA	61	2,949	55.16	1.87±10.06	Yes
9	Perfluorododecanoic acid	PFDoDA	33	1,269	15.52	0.12±6.68	Yes
10	Perfluorotridecanoic acid	PFTTrDA	29	1,592	6.64	0.03±7.62	Yes
11	Perfluorotetradecanoic acid	PFTeDA	26	1,749	15.03	0.07±6.52	No
12	Perfluorododecanoic acid	PFDoA	22	1,398	5.96	0.10±6.85	Yes
13	Perfluorohexadecanoic acid	PFHxDA	14	1,119	9.26	0.03±6.93	No
14	Perfluoropropionic acid	PFPrA	13	851	20.14	1.25±10.76	No
15	Perfluorooctadecanoic acid	PFODA	10	454	15.85	0.07±4.55	No
16	Perfluorotetradecanoic acid	PFTDA	3	58	3.45	0.06±3.07	No
17	Perfluorodecanoic acid	PFDPA	2	97	21.65	1.06±3.53	No
18	Perfluorooctylphosphonic acid	PFOPA	2	97	18.56	0.002±52.63	No
19	Perfluorotetradecanoic acid	PFTA	2	116	8.53	1.79±2.87	No
20	Perfluorohexylphosphonic acid	PFHxPA	2	97	15.46	0.30±1.32	No
21	Perfluorotetradecanoic acid	PFTeA	2	186	3.76	2.39±1.88	No
22	Perfluoro (4-methoxybutanoic) acid	PFMBA	2	53	1.89	NA [†]	Yes
23	Perfluoro-3-methoxypropanoic acid	PFMPA	2	53	1.89	NA	No
24	Perfluorododecanoic acid	PFDDA	1	15	100.00	0.1 (0.069, 0.85)	No
Perfluoroalkyl sulfonic acids (PFSAAs)							
25	Perfluorooctane sulfonic acid	PFOS	100	5,852	60.72	0.97±11.60	Yes
26	Perfluorohexane sulfonic acid	PFHxS	87	4,912	51.90	0.62±16.14	Yes
27	Perfluorobutanesulfonic acid	PFBS	78	4,704	54.57	0.52±16.23	Yes
28	Perfluorodecane sulfonic acid	PFDS	39	2,069	6.46	0.13±4.60	Yes
29	Perfluoroheptane sulfonic acid	PFHpS	28	1,896	18.68	0.09±7.73	Yes
30	Perfluoropentanesulfonic acid	PFPeS	16	1,464	37.22	0.11±12.00	Yes
31	Perfluorononanesulfonic acid	PFNS	15	1,078	2.69	0.01±7.50	Yes
32	Perfluoropropanesulfonate	PFPrS	12	961	34.51	0.05±2.85	No
33	Perfluorobutanesulfonamide	PFBSA	9	1,217	41.81	0.001±0.25	No
34	Perfluorohexanesulfonic acid	PFHxSA	9	1,224	9.95	0.05±3.37	No
35	Perfluorobutanesulfonic acid	PFBS	5	75	62.69	0.24±7.83	No
36	Perfluorododecane sulfonic acid	PFDoS	4	512	2.15	0.09±1.91	Yes
37	Trifluoromethanesulfonic acid	TFMS	3	78	87.18	5.53±15.62	No
38	Perfluoroethanesulfonic acid	PFETs	3	528	2.81	0.01±2.36	No

Continued

No.	PFAS	Abbreviation	Study	Sample size	Detection rate (%)	Mean±SD/median (range) (ng/L)*	Regulation
39	Perfluoropropanesulfonic acid	PFPS	2	82	9.30	0.004±0.32	No
40	Tetrahydroperfluorooctanesulfonic acid	THPFOS	2	48	10.42	0.28±2.52	No
41	Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	2	71	8.45	0.15±2.35	Yes
42	Perfluoroundecane sulfonic acid	PFUnS	1	11	100.00	NA	No
43	Perfluoroalkane sulfonic acid	PFSA	1	44	48.52	4 (1, 32)	No
44	Fluoropentyl Sulfonamide	FPeSA	1	463	21.80	NA (0.003, 0.46)	No
45	Fluoropropyl Sulfonamide	FPrSA	1	463	18.60	NA (0.002, 0.07)	No
46	Hydrogen-substituted Undecafluorooctane Sulfonate	H-U-PFOS	1	463	1.90	NA (0.010-0.20)	No
47	Perfluoromethylcyclohexanesulfonic acid	PFMeCHS	1	463	14.00	NA (0.006, 0.3)	No
48	Perfluoromethylcyclopentanesulfonic acid	PFMeCPeS	1	463	12.70	NA (0.003, 0.9)	No
Polyfluoroalkyl ether carboxylic acids (PFECs)							
49	Hexafluoropropylene oxide dimer acid	HFPO-DA	12	400	43.24	0.10±5.25	Yes
50	Hexafluoropropylene oxide trimer acid	HFPO-TA	1	2	100.00	NA (50, 87.1)	No
51	Perfluoro-2-ethoxypropanoic acid	PEPA	1	84	82.00	81 (NA, NA)	No
52	Perfluoro-2-methoxyacetic acid	PFMOAA	1	84	74.00	43 (NA, NA)	No
53	Perfluoro(3,5-dioxahexanoic) acid	PFO2HxA	1	84	77.00	107 (NA, NA)	No
54	Perfluoro(3,5,7-trioxaoctanoic) acid	PFO3OA	1	84	63.00	8 (NA, NA)	No
55	Perfluoro-3,6-dioxaheptanoic acid	PFHO-DA	1	18	4.28	0.4 (0.03, 9.83)	No
56	Perfluoro(3,5,7,9-butaodecanoic) acid	PFO4DA	1	84	43.00	NA	No
57	2,2,3,3-tetrafluoro-3-((1,1,1,2,3,3-hexafluoro-3-(1,2,2,2-tetrafluoroethoxy)propan-2-yl)oxy)propanoic acid	Hydro-EVE	1	84	21.00	NA	No
58	Perfluoro(3,5,7,9,11-pentaoxadodecanoic) acid	PFO5DoA	1	84	7.00	NA	No
Polyfluoroalkyl ether sulfonic acids (PFESAs)							
59	6:2 Chlorinated polyfluoroalkyl ether sulfonic acid	6:2 Cl-PFESA	13	398	5.78	0.06±3.74	No
60	8:2 Chlorinated polyfluoroalkyl ether sulfonic acid	8:2 Cl-PFESA	8	322	2.48	0.08±5.76	No
61	4:2 Chlorinated polyfluoroalkyl ether sulfonic acid	4:2 Cl-PFESA	2	6	16.67	0.005±1	No
62	2-[1-[Difluoro(1,2,2,2-tetrafluoroethoxy)methyl]-1,2,2,2-tetrafluoroethoxy]-1,1,2,2-tetrafluoroethanesulfonic acid	Nafion byproduct 2	1	84	73.00	14 (NA, NA)	No
63	1,1,2,2-tetrafluoro-2-(1,2,2,2-tetrafluoro-ethoxy)ethane sulfonate	NVHOS	1	84	70.00	3 (NA, NA)	No
Perfluoroalkyl Sulfonamides							
64	Perfluorooctanesulfonamide	PFOSA	23	1,430	23.36	0.23±2.89	Yes
65	N-Ethyl perfluorooctane sulfonamidoacetate	EtFOSAA	9	817	2.69	0.13±4.39	No
66	N-Methyl perfluorooctane sulfonamidoacetate	MeFOSAA	8	767	2.59	0.17±1.77	No
67	N-Methyl perfluorooctane sulfonamidoacetic acid	MeFOSA	5	695	4.44	0.01±3.33	No
68	N-Ethyl perfluorooctane sulfonamide	EtFOSA	4	575	2.24	0.04±2.53	No
69	N-Ethyl perfluorooctane sulfonamidoethanol	EtFOSE	2	31	35.48	0.03±2.07	No
70	N-Methyl perfluorooctane sulfonamidoethanol	MeFOSE	2	31	22.58	0.02±3.31	No
71	N-Substituted Hydroxy-Oxy-Perfluoroalkylamidoalkyl Phosphonate – Fluorohexyl Sulfonamide Hydroxy-Oxy-Propyl Sulfonate	N-SHOPAmP-FHxSAHOPS	1	463	1.10	NA (0.003, 0.18)	No
72	N-Substituted Perfluoroalkylamidoalkyl Phosphonate – Fluorohexyl Sulfonamide	N-SPAmP-FHxSA	1	463	1.30	NA (0.005, 0.11)	No
73	N-Substituted Perfluoroalkylamidoalkyl Phosphonate – Fluorohexyl Sulfonamide Acetic Acid	N-SPAmP-FHxSAA	1	463	0.60	NA (0.003, 0.07)	No
74	N-Substituted Perfluoroalkylamidoalkyl Phosphonate – Fluoropentyl Sulfonamide	N-SPAmP-FPeSA	1	463	0.90	NA (0.007, 0.20)	No

Continued

No.	PFAS	Abbreviation	Study	Sample size	Detection rate (%)	Mean±SD/median (range) (ng/L)*	Regulation
75	N-Substituted Perfluoroalkylamidoalkyl Phosphonate – Fluoropentyl Sulfonamide Alkyl Phosphonate Sulfonate	N-SPAmP-FPeSAPS	1	463	0.90	NA (0.005, 0.04)	No
76	N-Substituted Phosphonoalkyl Hydroxyalkyl Polyfluoroalkyl Amide	NSPHAPA	1	463	4.30	NA (0.003, 0.64)	No
77	Perfluorohexanesulfonamide sulfate	PFHxSAmS	1	463	0.40	NA (ND, 0.02)	No
78	Perfluorooctane sulfonamide quaternary ammonium salt	PFOSAmS	1	463	0.40	NA (0.015, 0.02)	No
Fluorotelomer sulfonic acids (FTS)							
79	1H, 1H, 2H, 2H-Perfluorooctane sulfonic acid	6:2 FTS	18	1,564	40.41	0.61±10.31	Yes
80	4:2 Fluorotelomer sulfonic acid	4:2 FTS	12	912	5.80	0.02±5.26	Yes
81	8:2 Fluorotelomer sulfonic acid	8:2 FTS	10	1,254	7.08	0.33±4.47	Yes
82	10:2 Fluorotelomer sulfonic acid	10:2 FTS	2	481	0.10	0.007±1.66	No
83	6:2 Fluorotelomer carboxylic acid	6:2 FTCA	1	2	100.00	NA(0.915, 1.31)	No
84	4:2 Fluorotelomer iodinated sulfonate	4:2 FIS	1	448	26.00	0.60 (0.12, 2.10)	No
85	5:1:2 Fluorotelomer betaine	5:1:2 FtB	1	463	9.90	NA (0.023, 2.70)	No
86	5:3 Fluorotelomer carboxylate	5:3 acid	1	463	0.60	NA (0.074, 0.15)	No
87	5:3 Fluorotelomer betaine	5:3 FtB	1	463	3.50	NA (0.012, 0.58)	No
88	6:2 Fluorotelomer sulfonamidopropyl betaine	6:2 FTAB	1	463	5.40	NA (0.021, 2.10)	No
89	6:2 Fluorotelomer Sulfonamide Oxide Propionic Acid	6:2 FTSO2PA	1	463	0.60	NA (0.036, 0.06)	No
90	6:2 Fluorotelomer Sulfonamide Alkyl Sulfonate	6:2-FTSAS	1	463	0.40	NA (0.018, 0.06)	No
91	6:2 Fluorotelomer Sulfonamide Alkyl Sulfone Sulfonate	6:2-FTSAS-sulfone	1	463	8.00	NA (0.010, 15.00)	No
92	6:2 Fluorotelomer Sulfonamide Alkyl Sulfoxide Sulfonate	6:2-FTSAS-sulfoxide	1	463	0.90	NA (0.024, 14.00)	No
93	7:1:2 Fluorotelomer Betaine	7:1:2 FtB	1	463	0.90	NA (0.091, 0.84)	No
94	7:3 Fluorotelomer Betaine	7:3 FtB	1	463	0.40	NA (0.096, 0.10)	No
95	Hydroxy-4:2 Fluorotelomer Sulfonate	HO-4:2-FtS	1	463	1.30	NA (0.014, 0.17)	No
96	Hydroxy-5:2 Fluorotelomer Sulfonate	HO-5:2-FtS	1	463	1.50	NA (0.016, 0.04)	No
97	6:2 Fluorotelomer ω-Hydroxyalkyl Sulfonate	HO-6:2-FtS	1	463	0.40	NA (0.075, 0.09)	No
Polyfluoroalkyl cyclic compounds							
98	Potassium perfluoro(4-ethylcyclohexane)sulfonate	PFECHS	5	566	33.18	0.13±3.48	Yes
99	Sodium perfluoro-3,5-dioxahexanoate	NaDONA	4	612	7.34	0.01±18.58	No
100	Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	2	53	5.66	NA	Yes
Else							
101	Trifluoroacetic acid	TFA	3	114	78.64	65.78±2.72	No
102	Trifluoromethanesulfonamide	F3-MSA	1	22	68.18	32 (ND [§] , 165)	No

Abbreviation: SD=standard deviation.

* Report as Mean±SD when calculable; otherwise provide Median (Range);

† NA, non available;

§ ND, non detected.

SUPPLEMENTARY TABLE S2. PFAS limits in drinking water of selected countries/regions.

PFAS	Value type class	Year	Country	Department/Institute	Guideline values (ng/L)	Value type	Legal effect	Source
PFOA	Health-technology-cost-based	2024	America	Environmental Protect Agency	4	MCL	Yes	https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations#PFAS
		2024	Australia	Department of Health	200	Proposed guideline value	No	https://www.nhmrc.gov.au/health-advice/environmental-health/water/PFAS-review?
		2022	China	National Health Commission of the People's Republic of China	80	Quality criteria	Yes	https://www.ndcpa.gov.cn/jbkzzx/c100201/common/content/content_1665979083259711488.html
		2021	America	New York	10	MCL	Yes	https://dec.ny.gov/environmental-protection/water/water-quality/standards-classifications
		2020	America	New Jersey, Department of Environmental Protection	14	MCL	Yes	https://nj.gov/health/ceohs/documents/pfas_drinking%20water.pdf
		2020	America	California	10	Health based advisory level	No	https://cpu.sjuku.top/https/77726476706e69737468656265737421e0e2438f69316b4330079bab/doi/10.1021/acsestwater.2c00387
		2019	America	New Hampshire	12	MCL	Yes	https://cpu.sjuku.top/https/77726476706e69737468656265737421e0e2438f69316b4330079bab/doi/10.1021/acsestwater.2c00387
		2018	Canada	Health Canada	200	MAC	Yes	https://gazette.gc.ca/rp-pr/p1/2018/2018-12-08/html/notice-avis-eng.html?
		2017	America	New Jersey, Department of Environmental Protection	14	MCL	Yes	https://dep.nj.gov/newsrel/17_0104/
		2015	Denmark	Environmental Protection Agency	100	Quality criteria	Yes	(1)
		2006	America	Minnesota, Department of Health	1,000	Advisory guideline	No	https://www.health.mn.gov/communities/environment/hazardous/topics/history.html#2022
	Health-based	2024	America	Environmental Protect Agency	0	MCLG	No	https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations#PFAS
		2024	America	Minnesota, Department of Health	0.0079	Health based value	No	https://www.health.mn.gov/communities/environment/hazardous/topics/history.html#2022
		2022	America	Environmental Protect Agency	0.004	Interim updated health advisory	No	https://www.epa.gov/system/files/documents/2022-06/drinking-water-ha-pfas-factsheet-communities.pdf?
		2020	America	Michigan	8	MCL	Yes	https://www.michigan.gov/-/media/Project/Websites/mdhhs/Folder4/Folder25/Folder3/Folder125/Folder2/Folder225/Folder1/Folder325/PFAS_-_Overview_of_Michigan_Values_FINAL.pdf
		2018	Australia	Department of Health	560	Health based guidance value	No	https://www.health.gov.au/sites/default/files/documents/2022/07/health-based-guidance-values-for-pfas-for-use-in-site-investigations-in-australia_0.pdf
		2017	America	Minnesota, Department of Health	35	Health based value	No	https://www.health.mn.gov/communities/environment/hazardous/topics/history.html#2022

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PFAS	Value type class	Year	Country	Department/Institute	Guideline values (ng/L)	Value type	Legal effect	Source
PFOA	Health-based	2016	America	Environmental Protect Agency	70	Provisional health advisory	No	https://www.epa.gov/sites/default/files/2016-06/documents/drinkingwaterhealthadvisories_pfoa_pfos_updated_5.31.17.pdf
		2014	Italy	National Institute of Health	500	Health based level	No	(1)
		2009	America	Environmental Protect Agency	400	Provisional health advisory	No	https://www.epa.gov/sites/default/files/2015-09/documents/pfoa-pfos-provisional.pdf
		2007	America	Minnesota, Department of Health	500	Health based value	No	https://www.health.mn.gov/communities/environment/hazardous/topics/history.html#2022
		2007	America	New Jersey, Department of Environmental Protection	40	Preliminary guidance level	No	https://dep.nj.gov/newsrel/17_0104/
		2002	America	Minnesota, Department of Health	7,000	Health based value	No	https://www.health.mn.gov/communities/environment/hazardous/topics/history.html#2022
PFOS	Health-technology-cost-based	2024	America	Environmental Protect Agency	4	MCL	Yes	https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations#PFAS
		2024	Australia	Department of Health	4	Proposed guideline value	No	https://www.nhmrc.gov.au/health-advice/environmental-health/water/PFAS-review?
		2022	China	National Health Commission of the People's Republic of China	40	Quality criteria	Yes	https://www.ndcpa.gov.cn/jbkzzx/c100201/common/content/content_1665979083259711488.html
		2021	America	New York	10	MCL	Yes	https://dec.ny.gov/environmental-protection/water/water-quality/standards-classifications
		2020	America	New Jersey, Department of Environmental Protection	13	MCL	Yes	https://nj.gov/health/ceohs/documents/pfas_drinking%21water.pdf
		2020	America	California	40	Health based advisory level	No	https://cpu.sjuku.top/https/77726476706e69737468656265737421e0e2438f69316b4330079bab/doi/10.1021/acsestwater.2c00387
		2019	America	New Hampshire	15	MCL	Yes	https://cpu.sjuku.top/https/77726476706e69737468656265737421e0e2438f69316b4330079bab/doi/10.1021/acsestwater.2c00387
		2018	Canada	Health Canada	600	MAC	Yes	https://gazette.gc.ca/rp-pr/p1/2018/2018-12-08/html/notice-avis-eng.html?
		2017	America	New Jersey, Department of Environmental Protection	13	MCL	Yes	https://dep.nj.gov/newsrel/17_0104/
		2015	Denmark	Environmental Protection Agency	100	Quality criteria	Yes	(1)
		2006	America	Minnesota, Department of Health	600	Advisory guideline	No	https://www.health.mn.gov/communities/environment/hazardous/topics/history.html#2022

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PFAS	Value type class	Year	Country	Department/Institute	Guideline values (ng/L)	Value type	Legal effect	Source
PFOS	Health-based	2024	America	Environmental Protect Agency	0	MCLG	No	https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations#PFAS
		2024	America	Minnesota, Department of Health	2.3	Health based value	No	https://www.health.mn.gov/communities/environment/hazardous/topics/history.html#2022
		2022	America	Environmental Protect Agency	0.02	Interim updated health advisory	No	https://www.epa.gov/system/files/documents/2022-06/drinking-water-ha-pfas-factsheet-communities.pdf?
		2020	America	Michigan	16	MCL	Yes	https://www.michigan.gov/-/media/Project/Websites/mdhhs/Folder4/Folder25/Folder3/Folder125/Folder2/Folder225/Folder1/Folder325/PFAS_-_Overview_of_Michigan_Values_FINAL.pdf
		2019	America	Minnesota, Department of Health	15	Health based value	No	https://www.health.mn.gov/communities/environment/hazardous/topics/history.html#2022
		2017	America	Minnesota, Department of Health	27	Health based value	No	https://www.health.mn.gov/communities/environment/hazardous/topics/history.html#2022
		2016	America	Environmental Protect Agency	70	Provisional health advisory	No	https://www.epa.gov/sites/default/files/2016-06/documents/drinkingwaterhealthadvisories_pfoa_pfos_updated_5.31.16.pdf
		2014	Italy	National Institute of Health	30	Health based level	No	(1)
		2009	America	Environmental Protect Agency	200	Provisional health advisory	No	https://www.epa.gov/sites/default/files/2015-10/documents/pfoa-pfos-provisional.pdf
		2007	America	Minnesota, Department of Health	300	Health based value	No	https://www.health.mn.gov/communities/environment/hazardous/topics/history.html#2022
		2002	America	Minnesota, Department of Health	1,000	Health based value	No	https://www.health.mn.gov/communities/environment/hazardous/topics/history.html#2022
PFBS	Health-technology-cost-based Health-based	2024	Australia	Department of Health	1,000	Proposed guideline value	No	https://www.nhmrc.gov.au/health-advice/environmental-health/water/PFAS-review?
		2022	America	Environmental Protect Agency	2,000	Final health advisory	No	https://www.epa.gov/system/files/documents/2022-06/drinking-water-ha-pfas-factsheet-communities.pdf?
		2022	America	Minnesota, Department of Health	100	Health based value	No	https://www.health.mn.gov/communities/environment/hazardous/topics/history.html#2022
		2020	America	Michigan	420	MCL	Yes	https://www.michigan.gov/-/media/Project/Websites/mdhhs/Folder4/Folder25/Folder3/Folder125/Folder2/Folder225/Folder1/Folder325/PFAS_-_Overview_of_Michigan_Values_FINAL.pdf
		2017	America	Minnesota, Department of Health	2,000	Health based value	No	https://www.health.mn.gov/communities/environment/hazardous/topics/history.html#2022
PFBA	Health-based	2017	America	Minnesota, Department of Health	7,000	Health based value	No	https://www.health.mn.gov/communities/environment/hazardous/topics/history.html#2022

Continued

PFAS	Value type class	Year	Country	Department/Institute	Guideline values (ng/L)	Value type	Legal effect	Source
PFHxS	Health-technology-cost-based	2024	America	Environmental Protect Agency	10	MCL	Yes	https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations#PFAS
		2024	Australia	Department of Health	30	Proposed guideline value	No	https://www.nhmrc.gov.au/health-advice/environmental-health/water/PFAS-review?
		2019	America	New Hampshire	18	MCL	Yes	https://cpu.sjuku.top/https/77726476706e69737468656265737421e0e2438f69316b4330079bab/doi/10.1021/acsestwater.2c00387
	Health-based	2020	America	Michigan	51	MCL	Yes	https://www.michigan.gov/-/media/Project/Websites/mdhhs/Folder4/Folder25/Folder3/Folder125/Folder2/Folder225/Folder1/Folder325/PFAS_-_Overview_of_Michigan_Values_FINAL.pdf
		2019	America	Minnesota, Department of Health	47	Health based value	No	https://www.health.mn.gov/communities/environment/hazardous/topics/history.html#2022
PFHxA	Health-based	2020	America	Michigan	400,000	MCL	Yes	https://www.michigan.gov/-/media/Project/Websites/mdhhs/Folder4/Folder25/Folder3/Folder125/Folder2/Folder225/Folder1/Folder325/PFAS_-_Overview_of_Michigan_Values_FINAL.pdf
PFNA	Health-technology-cost-based	2024	America	Environmental Protect Agency	10	MCL	Yes	https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations#PFAS
		2020	America	New Jersey, Department of Environmental Protection	13	MCL	Yes	https://nj.gov/health/ceohs/documents/pfas_drinking%22water.pdf
		2019	America	New Hampshire	11	MCL	Yes	https://cpu.sjuku.top/https/77726476706e69737468656265737421e0e2438f69316b4330079bab/doi/10.1021/acsestwater.2c00387
		2018	America	New Jersey, Department of Environmental Protection	13	MCL	Yes	https://www.eikonplanning.com/blog/pfas-regulatory-standards?
	Health-based	2020	America	Michigan	6	MCL	Yes	https://www.michigan.gov/-/media/Project/Websites/mdhhs/Folder4/Folder25/Folder3/Folder125/Folder2/Folder225/Folder1/Folder325/PFAS_-_Overview_of_Michigan_Values_FINAL.pdf
HFPO-DA	Health-technology-cost-based	2024	America	Environmental Protect Agency	10	MCL	Yes	https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations#PFAS
	Health-based	2022	America	Environmental Protect Agency	10	Final health advisory	No	https://www.epa.gov/system/files/documents/2022-06/drinking-water-ha-pfas-factsheet-communities.pdf?
		2020	America	Michigan	370	MCL	Yes	https://www.michigan.gov/-/media/Project/Websites/mdhhs/Folder4/Folder25/Folder3/Folder125/Folder2/Folder225/Folder1/Folder325/PFAS_-_Overview_of_Michigan_Values_FINAL.pdf

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PFAS	Value type class	Year	Country	Department/Institute	Guideline values (ng/L)	Value type	Legal effect	Source
PFOA+PFOS	Health-based	2020	Japan	Ministry of Health, Labour and Welfare	50	Provisional target value	No	https://jsdfe.org/topics/2-3_PFASto20policy%20Japan-221019.pdf
		2016	America	Environmental Protection Agency	70	Provisional health advisory	No	https://www.epa.gov/sites/default/files/2016-06/documents/drinkingwaterhealthadvisories_pfoa_pfos_updated_5.31.18.pdf
		2006	Germany	Ministry of Health	300	Health-based guidance value	No	https://www.umweltbundesamt.de/sites/default/files/medien/pdfs/pft-in-drinking-water.pdf
		2006	Germany	Ministry of Health	100	Health-based precautionary value	No	https://www.umweltbundesamt.de/sites/default/files/medien/pdfs/pft-in-drinking-water.pdf
PFHxS+PFNA+HPFO-DA+PFBS	Health-technology -cost-based	2024	America	Environmental Protection Agency	1 (unitless)	MCL	Yes	https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations#PFAS
PFOA + PFOS + PFNA + PFHxS	Health-technology -cost-based	2023	Denmark	Environmental Protection Agency	2	MCL	Yes	https://www.retsinformation.dk/eli/ta/2023/1023
PFOA+PFNA+PFHxS+PFOS	Health-technology -cost-based	2023	Germany	Ministry of Health	20	Limit value	Yes	https://www.gesetze-im-internet.de/englisch_trinkvw/englisch_trinkvw.html
PFOS + PFHxS	Health-based	2018	Australia	Department of Health	70	Health based guidance value	No	https://www.health.gov.au/sites/default/files/documents/2022/07/health-based-guidance-values-for-pfas-for-use-in-site-investigations-in-australia_1.pdf
PFAS (25)*	Health-technology -cost-based	2024	Canada	Health Canada	30	MCL	Yes	https://publications.gc.ca/collections/collection_2024/sc-hc/H144-132-2024-eng.pdf
PFAS (20)†	Health-technology -cost-based	2023	Germany	Ministry of Health	100	Limit value	Yes	https://www.gesetze-im-internet.de/englisch_trinkvw/englisch_trinkvw.html
PFAS Total§	Health-technology -cost-based	2020	European Union	European Commission	500	Drinking Water Directive	Yes	https://eurlex.europa.eu/eli/dir/2020/2184
Sum of PFAS¶	Health-technology -cost-based	2020	European Union	European Commission	100	Drinking Water Directive	Yes	https://eurlex.europa.eu/eli/dir/2020/2185

Abbreviation: MAC=maximum acceptable concentration; MCL=maximum contaminant level; MCLG=maximum contaminant level goal; PFPA=perfluoropentanoic acid; PFDA=perfluorodecanoic acid; PFUnDA=perfluoroundecanoic acid; PFDoDA=perfluorododecanoic acid; PFTrDA=perfluorotridecanoic acid; PFPS=perfluoropentane sulfonic acid; PFDS=perfluorodecane sulfonic acid; PFUnDS=perfluoroundecane sulfonic acid; PFDoS=perfluorododecane sulfonic acid; PFTrDS=perfluorotridecane sulfonic acid; PFPS=perfluoropentane sulfonic acid; PFHpS=perfluoroheptane sulfonic acid; PFPeS=perfluoropentanesulfonic acid; 6:2 FTS=1H,1H,2H,2H-Perfluorooctane sulfonic acid; PFMBa=perfluoro-4-methoxybutanoic acid; 8:2 FTS=1H,1H,2H,2H-perfluorodecane sulfonic acid; NFDHA=nonafluoro-3,6-dioxahexanoic acid; PFUnA=perfluoroundecanoic acid; HFPO-DA=hexafluoropropylene oxide dimer acid; 9Cl-PF3ONS=9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid; ADONA=4,8-dioxa-3H-perfluorononanoic acid; 11Cl-PF3OUdS=11-chloroeicosafuoro-3-oxaundecane-1-sulfonic acid; 4:2 FTS=1H,1H,2H,2H-perfluorohexane sulfonic acid; PFEESA=perfluoro (2-ethoxyethane) sulfonic acid.

* 25 PFAS: PFBA, PFNA, PFPeS, 6:2 FTS, PFMBa, PFPeA, PFDA, PFHxS, 8:2 FTS, NFDHA, PFHxA, PFUnA, PFHpS, HFPO-DA, 9Cl-PF3ONS, PFHpA, PFDoA, PFOS, ADONA, 11Cl-PF3OUdS, PFOA, PFBS, 4:2 FTS, PFMPA, PFEESA.

† 20 PFAS: PFBA, PFPA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFBS, PFPS, PFHxS, PFHpS, PFOS, PFNS, PFDS, PFUnDS, PFDoS, and PFTrDS.

§ PFAS Total, the totality of per- and polyfluoroalkyl substances;

¶ Sum of PFAS (20): PFBA, PFPA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFBS, PFPS, PFHxS, PFHpS, PFOS, PFNS, PFDS, PFUnDS, PFDoS, PFTrDS.

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