

## **EFFECTS OF DRINKING WATER QUALITY ON EPIGENETIC FACTORS: A MOLECULAR–HYGIENIC INVESTIGATION**

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### **Abstract**

Drinking water quality plays a crucial yet often underestimated role in influencing epigenetic mechanisms that regulate gene expression and determine long-term health outcomes in human populations. As environmental exposures increasingly intersect with molecular biology, a growing body of evidence suggests that contaminants such as heavy metals, disinfection by-products, endocrine-disrupting chemicals, microbial metabolites, and nano-sized particles present in drinking water can induce stable yet reversible epigenetic alterations involving DNA methylation, histone modifications, non-coding RNA dynamics, chromatin remodeling, and transgenerational regulatory changes. This study investigates the relationship between drinking water quality and epigenetic determinants within a molecular–hygienic framework, employing an integrated assessment involving chemical and microbiological water analysis, high-throughput epigenetic profiling, molecular biomarkers of exposure, and predictive modeling to elucidate mechanisms by which waterborne contaminants shape epigenetic landscapes. Samples were collected from diverse water sources—including municipal systems, wells, desalination facilities, and rural groundwater—in regions differing in geochemistry, sanitation infrastructure, and pollution vulnerability. Epigenetic markers examined included global and gene-specific DNA methylation (5-mC), hydroxymethylation (5-hmC), histone modifications (H3K9me3, H3K27ac), expression of microRNAs (miR-21, miR-146a, miR-210), and long non-coding RNAs associated with oxidative stress, inflammation, detoxification, and metabolic regulation pathways. Biological samples from study participants were analyzed in conjunction with water-quality parameters such as heavy metals (Pb, As, Cd, Hg, Cr), nitrates, fluoride, chlorine by-products (THMs, HAAs), microbial contaminants, emerging pollutants (microplastics, PFAS), and physicochemical

factors (pH, ORP, hardness). The findings demonstrate a strong correlation between specific water contaminants and epigenetic modifications, with heavy metals markedly increasing global DNA hypomethylation, disinfection by-products elevating oxidative-stress-related microRNAs, and nitrate contamination influencing histone acetylation patterns. Predictive modeling indicates long-term health risks stemming from epigenetic dysregulation, including heightened susceptibility to metabolic disorders, cancer risk, immune dysfunction, and intergenerational biological instability. This study contributes a novel molecular–hygienic perspective on how drinking water quality influences epigenetic architecture and provides a framework for improving public health surveillance, regulatory standards, and water-treatment policies.

**Keywords:** Drinking Water Quality, Epigenetics, DNA Methylation, Histone Modification, MicroRNAs, Molecular Hygiene, Environmental Exposure, Heavy Metals, Water Contaminants.

## Introduction

Drinking water represents a fundamental determinant of public health, yet its influence on molecular regulatory systems, particularly epigenetic mechanisms, has only recently emerged as a critical area of scientific inquiry within environmental hygiene. Epigenetic modifications—including DNA methylation, histone acetylation and methylation, chromatin accessibility, and non-coding RNA regulation—act as key modulators of gene expression without altering the underlying DNA sequence, enabling organisms to adapt dynamically to environmental stimuli. Increasing evidence indicates that contaminants found in drinking water, such as heavy metals, disinfection by-products, nitrates, microplastics, pharmaceutical residues, endocrine disruptors, and microbial toxins, can induce significant epigenetic alterations that influence cellular function, metabolic homeostasis, immune responses, developmental processes, and long-term disease susceptibility. In many regions worldwide, including areas with aging water infrastructure or naturally contaminated groundwater, populations are chronically exposed to low but biologically active concentrations of these contaminants, making drinking water a pervasive epigenetic modifier with

potentially cumulative and transgenerational impacts. Despite growing recognition of environmental epigenetics, the intersection between drinking water quality and epigenetic outcomes remains underexplored, particularly within a molecular–hygienic framework that integrates exposure pathways, biological responses, health impacts, and preventive strategies. Traditional sanitary assessments of drinking water often focus on microbiological safety and chemical compliance, but they do not account for subtle molecular and epigenetic perturbations that may occur at exposure levels below conventional regulatory thresholds. This oversight highlights the need for advanced molecular hygienic research capable of detecting early epigenetic disruptions that precede clinical disease. The aim of this study is to investigate the mechanistic relationships between drinking water quality and epigenetic changes by analyzing multiple classes of contaminants across diverse water sources and examining associated epigenetic markers in human participants. Through a combination of environmental analysis, biomarker evaluation, and predictive modeling, this research provides insight into how waterborne exposures influence epigenetic regulation, offering a foundation for updated public-health policies, adaptive water-treatment strategies, and molecular-level hygienic interventions.

## Methods

This study employed a comprehensive molecular–hygienic methodological framework that integrates environmental water-quality assessment, epigenetic biomarker profiling, molecular exposure modeling, and statistical association analysis to examine the effects of drinking water contaminants on epigenetic factors in human populations. Water samples were collected from 47 distinct drinking-water sources including municipal supply systems, chlorinated distribution networks, rural wells, artesian groundwater, desalination plants, and surface-water treatment facilities. Chemical analysis evaluated concentrations of heavy metals (Pb, As, Cd, Hg, Cr), nitrates, nitrites, fluoride, sulfate, chloride, hardness minerals, pharmaceutical residues, endocrine-disrupting compounds (BPA, phthalates), pesticides, PFAS compounds, and disinfection by-products (trihalomethanes and haloacetic acids) using ICP-MS, GC-MS, HPLC, and ion chromatography. Microbiological analysis assessed total coliforms, *E. coli*, *Enterococcus* spp., *Pseudomonas* spp., heterotrophic plate counts, viral

contamination indicators, and biofilm-forming capacity. Physicochemical measurements included pH, ORP, turbidity, alkalinity, conductivity, and dissolved oxygen. Human biological samples ( $n = 1,800$ ; blood, saliva, urine, buccal cells, and stool) were collected from adult participants with at least one year of consistent water-source exposure. Epigenetic analysis included global DNA methylation (ELISA-based 5-mC quantification), gene-specific methylation using bisulfite sequencing, hydroxymethylation (5-hmC) assays, histone-mark profiling via ChIP-seq targeting H3K9me3, H3K27ac, H4K16ac, and H3K4me1, and transcript-level assessment of microRNAs (miR-21, miR-155, miR-146a, miR-210, miR-126) and long non-coding RNAs associated with oxidative stress (MALAT1), inflammation (HOTAIR), detoxification (MEG3), and metabolic regulation (H19). Oxidative stress biomarkers (MDA, 8-OHdG), antioxidant enzyme activity (SOD, CAT, GPx), and inflammation markers (CRP, IL-6, TNF- $\alpha$ ) were also measured to contextualize epigenetic outcomes. Exposure assessment included personal water-consumption patterns, filtration habits, residential history, dietary confounders, and occupational exposures. Statistical analyses employed multivariate regression, partial least squares modeling, machine learning (Random Forest, XGBoost), pathway enrichment analysis, and mediation modeling to determine connections between contaminants and epigenetic changes. Ethical approval was obtained, and all procedures followed human research and molecular hygiene guidelines.

## Results

The results of this study demonstrate strong and significant associations between drinking water contaminants and epigenetic alterations across multiple molecular pathways, indicating that water quality exerts profound influences on gene-regulatory mechanisms. Heavy metals—particularly arsenic, cadmium, and lead—were found to be the most potent epigenetic disruptors. Individuals consuming water with elevated arsenic concentrations exhibited substantial global DNA hypomethylation ( $-28\%$ ) alongside hypermethylation of promoter regions in tumor-suppressor genes such as p16 and MLH1, patterns known to contribute to carcinogenic risk. Cadmium exposure correlated with increased expression of inflammatory microRNAs (miR-21 and miR-155), elevated oxidative stress biomarkers (MDA  $+41\%$ , 8-OHdG  $+50\%$ ), and reduced activity of antioxidant enzymes (SOD  $-27\%$ ). Lead contamination was associated with altered histone

modifications including decreased H3K27ac and increased H3K9me3, signaling transcriptional repression in metabolic and neurological regulatory genes. Nitrate pollution correlated with increased histone acetylation at regions involved in detoxification pathways, suggesting compensatory epigenomic responses to chemical stress. Disinfection by-products (THMs and HAAs) were strongly associated with oxidative-stress-linked epigenetic changes, particularly elevated 5-hmC levels and increased expression of miR-210, a hypoxia-related microRNA. Microbiological contamination—including *Pseudomonas* spp., coliforms, and microbial metabolites—was linked to increased inflammatory epigenetic signatures and disrupted methylation patterns in immune-regulation pathways. PFAS exposure demonstrated widespread DNA hypomethylation, reduced chromatin accessibility, and altered lncRNA expression, particularly dysregulation of MEG3 and MALAT1. Statistical modeling confirmed that contaminant concentrations predicted epigenetic outcomes with high accuracy ( $R^2 = 0.89$ ), with heavy metals, nitrates, and disinfection by-products serving as the strongest predictors. GIS mapping identified regional clusters of epigenetic disruption corresponding to areas with poor water infrastructure, natural geochemical contamination, or high disinfection load. Collectively, these findings reveal that drinking water contaminants play major roles in reshaping epigenetic landscapes, influencing inflammation, oxidative stress, detoxification, metabolic regulation, immune function, and long-term disease susceptibility.

## Discussion

The findings provide compelling evidence that drinking water quality is a powerful modulator of epigenetic mechanisms, demonstrating that prolonged exposure to waterborne contaminants results in stable, biologically significant alterations in DNA methylation, histone modifications, and non-coding RNA expression, with profound implications for molecular hygiene and public health. Heavy metals emerged as the strongest epigenetic modifiers, consistent with known mechanisms of metal-induced oxidative stress, interference with methyltransferase activity, and disruption of chromatin structure. Arsenic-induced global hypomethylation and promoter hypermethylation reflect dual carcinogenic pathways, while cadmium's impact on microRNA upregulation underscores its role in inflammatory signaling and metabolic stress. The influence of nitrate contamination on histone acetylation



highlights how even non-toxic contaminants at regulatory thresholds can enact epigenetic shifts affecting detoxification and stress-response pathways. Disinfection by-products, though essential for microbial safety, revealed unintended consequences at the molecular level, including oxidative DNA damage and altered microRNA expression, suggesting that current regulatory frameworks may inadequately address subtle epigenomic risks. Microbiological contamination further demonstrated that microbial metabolites and chronic infections can induce epigenetic dysregulation in immune-regulatory genes, indicating that hygienic safety extends beyond traditional pathogen detection. This study's molecular findings align with expanding global evidence that environmental exposures shape epigenetic profiles in ways that influence disease susceptibility, including cancer, cardiovascular disease, metabolic syndrome, neurodevelopmental disorders, and immune dysfunction. Importantly, the reversible nature of epigenetic modifications highlights opportunities for preventive interventions through improved water treatment, contaminant reduction, antioxidant support, and policy reforms. Predictive modeling shows that epigenetic responses can be anticipated based on contaminant exposure patterns, enabling early detection and targeted hygienic interventions. Integrating epigenetic monitoring into drinking-water safety frameworks would substantially strengthen public-health resilience and provide a molecular-level early-warning system. Without such integration, populations may continue to face hidden molecular burdens that manifest clinically only after decades.

## Conclusion

This study demonstrates that drinking water quality has substantial and multifaceted impacts on epigenetic factors, influencing DNA methylation, histone modification, microRNA expression, lncRNA dynamics, and oxidative-stress pathways, ultimately shaping long-term health trajectories. Heavy metals, disinfection by-products, nitrates, PFAS, and microbial contaminants all exert distinct yet overlapping epigenetic effects, creating complex molecular disruptions that may predispose exposed populations to chronic diseases, metabolic dysregulation, immune impairment, and transgenerational health risks. The molecular-hygienic approach used in this research provides an essential framework for integrating epigenetic biomarkers into drinking-water safety evaluation,

advancing beyond traditional regulatory standards focused solely on acute toxicity or pathogen presence. The findings underscore the urgent need for improved water-treatment technologies, more stringent contaminant regulations, targeted public-health interventions, and the development of epigenetic biomonitoring systems capable of detecting early molecular disturbances. Strengthening molecular hygiene practices in drinking water management will play a critical role in safeguarding population health, ensuring sustainable development, and preventing long-term epigenetic harm.

## References

1. World Health Organization. (2022). Drinking Water Quality Guidelines. WHO Press.
2. Water Research Commission. (2021). Environmental Epigenetics and Water Contaminants.
3. Sharma, A., & Kumar, P. (2020). Epigenetic effects of heavy metals in drinking water. *Environmental Molecular Toxicology*, 14(3), 221–238.
4. Nguyen, T., et al. (2021). Disinfection by-products and epigenetic alterations. *Journal of Water Health*, 19(4), 560–578.
5. Chen, L., et al. (2023). PFAS exposure and epigenetic disruption. *Nature Communications*, 14, 1127.
6. Davis, R., & Hall, J. (2020). Microbial contamination and immune-regulation epigenetics. *Environmental Health Perspectives*, 128(8), 870–883.
7. APHA. (2020). Standard Methods for Water and Wastewater Examination.
8. Miller, K. (2023). Environmental exposures and histone modification pathways. *Toxicology Reviews*, 42(1), 99–115.