ACS Partner Journal

ENVIRONMENT & HEALTH

Open Access

pubs.acs.org/EnvHealth

Viewpoint

Nontargeted Toxicological/Chemical Analysis in Complex Mixtures for Risk Assessment and Key Driver Discovery

Chunzhen Shi, Xiaoxi Yang, Jifu Liu, Haijiang Tian, Jie Gao, Yuhao Yan, Guotao Peng, Hua Qin, Meilin Lv, Yanna Liu, Runzeng Liu, Ningbo Geng,* Zongwei Cai, Guangbo Qu,* Jiping Chen, and Guibin Jiang

Cite This: *Environ. Health* 2025, 3, 701−704

Read Online

ACCESS | Metrics & More | Article Recommendations

Exposure to complex environmental mixtures leads to dynamic and evolving exposomes under different environmental conditions, presenting significant challenges to humans and other organisms. Therefore, on the demand of risk management of complex mixture exposure, the determination of the overall toxicity and the key components that drive the observed toxic effects is the prerequisite.

Typically, targeted toxicological assays are useful to evaluate the toxicity of extracted mixtures from environmental samples, especially when prior knowledge on chemical components and their potential modes of actions are known. However, real environmental samples are typically complex mixtures containing hundreds to thousands of unexpected or unknown chemicals. The frequently used toxicological assays based on targeted effects with the designed specific targets may neglect the important toxic effects and fail to capture the overall toxicity. Toxicological evaluation places high demands on a strategy without relying on specific hypotheses or predetermined toxicological targets. This call for the consideration of Non-Targeted Toxicity Analysis (NTTA), which can probe biological responses in an unbiased way, uncovering frequent alterations of driver molecules involved in critical function and pathological processes that would otherwise be missed with targeted toxicity analysis. NTTA integrates artificial intelligent (AI)-driven high-throughput imaging, multiomics techniques, bioinformatics, and computational toxicology, and thus provides a broad holistic biological response.

The rapid development of new methodologies in the field of molecular biology and bioinformatics could support NTTA (Figure 1), which could evaluate the toxicity of mixtures without designed toxicological targets. First, machine learning-driven high-throughput imaging is a powerful tool that served as *in vitro* NTTA, allowing us to rapidly analyze large numbers of heterogeneous cellular images simultaneously. By probing subtle changes in cellular features (shape, size, intensity, behavior, etc.), NTTA can provide a more comprehensive understanding of the potential toxic effects of mixtures.[1] Furthermore, with spheroids, organoids, or organisms as a model, NTTA could also enhance our understanding of mixture toxicity.[2,3] For instance, a zebrafish behavioral profiling assay has been used to characterize the toxicity of pollutants,[2] demonstrating the considerable potential of NTTA in a mixture toxicity assessment.[3]

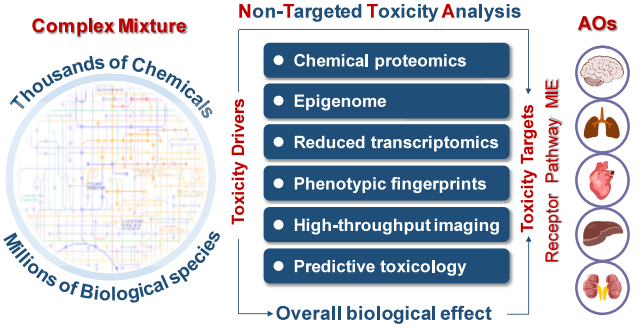

Figure 1. Workflow of NTTA for risk assessment of complex mixtures

Second, multiple layers of omics data (e.g., genomics, transcriptomics, proteomics, metabolomics, and epigenomics) allow researchers to study biological phenomena holistically, enabling the identification of biomarkers and exploration of underlying mechanism.[4] Integration of omic approaches has successfully unveiled the modes of actions of a given chemical under low-level exposure.[5,6] Reduced transcriptomic and dose-resolved proteomic approaches have been used for early determination of sensitive molecular responses.[7,8] Epigenomics analysis links mixture exposure to transgenerational effects through DNA/RNA/histone modifications, expanding our knowledge of the toxicity, adverse outcomes (AOs), and the toxicological mechanisms of mixtures.[9,10] Through fully mapping the molecular landscape and pathway-based toxicological interactions that occur within a living system, NTTA generates detailed phenotypic fingerprints and could be applied to describe AOs induced by mixtures.

Third, some emerging techniques, such as proteome-scale mapping of the chemical-protein interactions, take great advantage in identifying molecular initiating events of pollutants in mixtures. For example, disinfection byproducts preferentially targeted redox proteins with thiols to induce oxidative stress.[11]

Received: February 25, 2025
Accepted: March 16, 2025
Published: March 27, 2025

https://doi.org/10.1021/envhealth.5c00052
Environ. Health 2025, 3, 701−704

Moreover, machine learning-driven phenotypic discernment, multiomics integration, and multiscale predictive modeling can benefit the causal links between modes of actions and phenotypes.[12] By integrating *in vivo* and *in vitro* toxicity databases with a deep learning model, the 15 most relevant toxicities for complex plastic additives were identified.[13] A data-driven, hypothesis-free approach was developed to reduce the complexity of real-world mixture toxicity and characterize biomolecular effects, enabling the discovery of new pathways linked to undiscovered potential hazards.[14] Therefore, armed with multiomics, NTTA can facilitate mechanistic insights of observed AOs or predict the potential AOs in a mixture.

After a sufficient understanding of the possible toxicity of a given mixture, chemical analysis is required to pinpoint the key drivers in the mixture. Through the characterization of chemical "fingerprinting" of complex mixtures, Non-Targeted Chemical Analysis (NTCA) allows for discovering emerging unknown structures, largely expanding the chemical candidates. In general, for a complex mixture, NTCA facilitates the possibility of fully understanding the chemical exposome, while NTTA aims to obtain the key targets or pathways from thousands of end points. The combination of NTTA with NTCA can guide the subsequent targeted toxicity assays and chemical analysis to further sophisticatedly identify the explainable toxic drivers in the mixture.

The integration of NTTA and NTCA and associated techniques offers an innovative, powerful approach to assess the full spectrum of biological alterations, to detect unexpected toxicological responses, unknown emerging toxic chemical structures, and the resulting combined effects of mixtures. While this strategy has the potential to significantly enhance our understanding of the risks posed by complex chemical mixtures and emerging pollutants, there are several challenges that still need to be addressed. (1) The data generated from NTTA and NTCA are inherently complex and multidimensional, requiring advanced data processing tools, bioinformatics, and machine learning techniques for interpretation. It is difficult to draw straightforward conclusions without sufficient support of computational power; (2) Ensuring that results from NTTA and NTCA across different laboratories, platforms, and experimental conditions are reproducible, which is essential for building trust in these assays. Standardized methods and protocols, advanced computational tools and platforms, open-source databases, and data visualization are urgently needed to provide universal guidelines for NTTA; (3) Complex mixtures often involve chemicals with low concentrations, making it difficult to identify a single compound as the main toxic driver. The description of synergistic or antagonistic effects of these mixtures is critical and difficult for developing accurate risk assessments; (4) Nontargeted approaches may face challenges in gaining regulatory acceptance, especially when it fails to link with specific risk thresholds or established toxicological end points. Therefore, critical findings from nontargeted toxicity analyses will need to be validated by independent targeted analyses; in addition, benefits of the nontargeted strategy in risk assessment and management ought to be demonstrated prior to its findings being incorporated into new environmental policies.

AUTHOR INFORMATION

Corresponding Authors

Ningbo Geng − *Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China;* orcid.org/0000-0002-0809-7463; Email: gengningbo@dicp.ac.cn

Guangbo Qu − *School of Environment, Hangzhou Institute for Advanced Study, University of Chinese Academy of Sciences, Hangzhou 310024, China; State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China; Institute of Environment and Health, Jianghan University, Wuhan 430056, China; University of Chinese Academy of Sciences, Beijing 100049, China;* orcid.org/0000-0002-5220-7009; Email: gbqu@rcees.ac.cn

Authors

Chunzhen Shi − *School of Environment, Hangzhou Institute for Advanced Study, University of Chinese Academy of Sciences, Hangzhou 310024, China; State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China; Department of Environmental Science and Engineering, Beijing Technology and Business University, Beijing 100048, China;* orcid.org/0000-0002-7638-1431

Xiaoxi Yang − *State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China;* orcid.org/0000-0002-5381-146X

Jifu Liu − *School of Environment, Hangzhou Institute for Advanced Study, University of Chinese Academy of Sciences, Hangzhou 310024, China; State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China; University of Chinese Academy of Sciences, Beijing 100049, China;* orcid.org/0009-0000-0563-7869

Haijiang Tian − *State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China;* orcid.org/0000-0001-8686-2506

Jie Gao − *State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China*

Yuhao Yan − *State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China*

Guotao Peng − *College of Environmental Science and Engineering, Key Laboratory of Yangtze River Water Environment, Tongji University, Shanghai 200092, China;* orcid.org/0000-0001-7169-9961

Hua Qin − *Research Center for Analytical Sciences, Department of Chemistry, College of Sciences, Northeastern University, Shenyang 110819, China; State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China*

Meilin Lv − *Research Center for Analytical Sciences, Department of Chemistry, College of Sciences, Northeastern University, Shenyang 110819, China; State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center*

https://doi.org/10.1021/envhealth.5c00052
Environ. Health 2025, 3, 701−704

for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

Yanna Liu − *State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China;* orcid.org/0000-0001-7893-9876

Runzeng Liu − *Shandong Key Laboratory of Environmental Processes and Health, School of Environmental Science and Engineering, Shandong University, Qingdao 266237, China; State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China;* orcid.org/0000-0002-0219-8052

Zongwei Cai − *State Key Laboratory of Environmental and Biological Analysis, Department of Chemistry, Hong Kong Baptist University, Kowloon 999077 Hong Kong SAR, China;* orcid.org/0000-0002-8724-7684

Jiping Chen − *Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China;* orcid.org/0000-0003-0553-7811

Guibin Jiang − *School of Environment, Hangzhou Institute for Advanced Study, University of Chinese Academy of Sciences, Hangzhou 310024, China; State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China; Institute of Environment and Health, Jianghan University, Wuhan 430056, China; University of Chinese Academy of Sciences, Beijing 100049, China;* orcid.org/0000-0002-6335-3917

Complete contact information is available at: https://pubs.acs.org/10.1021/envhealth.5c00052

Notes

The authors declare no competing financial interest.

Biographies

Ningbo Geng is an associate professor in Dalian Institute of Chemical Physics (DICP), Chinese Academy of Sciences (CAS). Her research focuses on developing advanced high-throughput analytical methodologies to investigate the environmental behavior, toxicological effects, and health risks of chemical pollutants.

Guangbo Qu is a Professor at the Research Center for Eco-Environmental Sciences (RCEES), Chinese Academy of Sciences (CAS). His major research interests are screening bioactive chemicals through the combination of high-throughput biological evaluation and nontarget screening with high-resolution mass spectrometry.

■ ACKNOWLEDGMENTS

This work was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (XDB0750300) and the National Natural Science Foundation of China (22325606, 22241604, 52170106).

■ REFERENCES

(1) Moshkov, N.; Bornholdt, M.; Benoit, S.; Smith, M.; McQuin, C.; Goodman, A.; Senft, R. A.; Han, Y.; Babadi, M.; Horvath, P.; Cimini, B. A.; Carpenter, A. E.; Singh, S.; Caicedo, J. C. Learning representations for image-based profiling of perturbations. *Nat. Commun.* **2024**, *15* (1), 1594.

(2) Wang, N.; Dong, G.; Qiao, R.; Yin, X.; Lin, S. Bringing Artificial Intelligence (AI) into Environmental Toxicology Studies: A Perspective of AI-Enabled Zebrafish High-Throughput Screening. *Environ. Sci. Technol.* **2024**, *58* (22), 9487−9499.

(3) Zhang, K.; Liang, J.; Brun, N. R.; Zhao, Y.; Werdich, A. A. Rapid Zebrafish Behavioral Profiling Assay Accelerates the Identification of Environmental Neurodevelopmental Toxicants. *Environ. Sci. Technol.* **2021**, *55* (3), 1919−1929.

(4) Liu, S.; Wang, W.; Zhao, Y.; Li, L.; Zhang, W.; Ji, X.; Yang, D.; Chen, Y.; Guo, X.; Deng, F. Indoor Environment and Health Effects: Protocol of an Exploratory Panel Study among Young Adults in China (China IEHE Study). *Environ. Health (Wash)* **2025**, *3* (1), 26−39.

(5) Chen, Q.; Lian, X.; An, J.; Geng, N.; Zhang, H.; Challis, J. K.; Luo, Y.; Liu, Y.; Su, G.; Xie, Y.; Li, Y.; Liu, Z.; Shen, Y.; Giesy, J. P.; Gong, Y. Life Cycle Exposure to Environmentally Relevant Concentrations of Diphenyl Phosphate (DPhP) Inhibits Growth and Energy Metabolism of Zebrafish in a Sex-Specific Manner. *Environ. Sci. Technol.* **2021**, *55* (19), 13122−13131.

(6) Li, Z.; Xian, H.; Ren, X.; Ye, R.; Zhong, Y.; Huang, Y.; Liang, B.; Deng, Y.; Dai, M.; Guo, J.; Tang, S.; Pan, J.; Feng, Y.; Bai, R.; Chen, X.; Ichihara, S.; Ichihara, G.; Chen, D.; Yang, X.; Huang, Z. Insights into Triclosan-Induced Endocrine Disruption: Evidence from the National Health and Nutrition Examination Survey and Zebrafish Models. *Environ. Health (Wash)* **2024**, *2* (7), 424−440.

(7) Dai, J. Reduced Transcriptomic Approach for Screening and Prediction of Chemical Toxicity. *Chem. Res. Toxicol.* **2018**, *31* (7), 532−533.

(8) Eckert, S.; Berner, N.; Kramer, K.; Schneider, A.; Muller, J.; Lechner, S.; Brajkovic, S.; Sakhteman, A.; Graetz, C.; Fackler, J.; Dudek, M.; Pfaffl, M. W.; Knolle, P.; Wilhelm, S.; Kuster, B. Decrypting the molecular basis of cellular drug phenotypes by dose-resolved expression proteomics. *Nat. Biotechnol.* **2024**, 1−10.

(9) Wu, H.; Eckhardt, C. M.; Baccarelli, A. A. Molecular mechanisms of environmental exposures and human disease. *Nat. Rev. Genet* **2023**, *24* (5), 332−344.
(10) Zheng, R.; Du, M.; Tian, M.; Zhu, Z.; Wei, C.; Chu, H.; Gan, C.; Liang, J.; Xue, R.; Gao, F.; Mao, Z.; Wang, M.; Zhang, Z. Fine Particulate Matter Induces Childhood Asthma Attacks via Extracellular Vesicle-Packaged Let-7i-5p-Mediated Modulation of the MAPK Signaling Pathway. *Adv. Sci.* **2022**, *9* (3), No. e2102460.
(11) Hall, D. R.; Yeung, K.; Peng, H. Monohaloacetic Acids and Monohaloacetamides Attack Distinct Cellular Proteome Thiols. *Environ. Sci. Technol.* **2020**, *54* (23), 15191−15201.
(12) Han, P.; Li, X.; Yang, J.; Zhang, Y.; Chen, J. Advancing Toxicity Predictions: A Review on in Vitro to in Vivo Extrapolation in Next-Generation Risk Assessment. *Environ. Health (Wash)* **2024**, *2* (7), 499−513.
(13) Jeong, J.; Choi, J. Development of AOP relevant to microplastics based on toxicity mechanisms of chemical additives using ToxCast and deep learning models combined approach. *Environ. Int.* **2020**, *137*, 105557.
(14) Li, X.; Zhou, J.; Bai, Y.; Qiao, M.; Xiong, W.; Schulze, T.; Krauss, M.; Williams, T. D.; Brown, B.; Orsini, L.; Guo, L. H.; Colbourne, J. K. Bioactivity Profiling of Chemical Mixtures for Hazard Characterization. *Environ. Sci. Technol.* **2025**, *59* (1), 291−301.

https://doi.org/10.1021/envhealth.5c00052
Environ. Health 2025, 3, 701−704