

Methods and Applications

Environmental Mixture–Health Associations: Current Analytical Practice and Reporting Recommendations

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ABSTRACT

Introduction: Analytical decisions for environmental mixture exposure remain under-standardized. The aim was to outline current analytical practice across studies and propose a reporting checklist.

Methods: An empirical methodological review was conducted for original environmental mixture-exposure studies using Bayesian kernel machine regression (BKMR), weighted quantile sum (WQS), or quantile-based g-computation (QGC), published in four high-impact journals between 2021 and 2025 for exposure types, sample size, and statistical methods. A three-stage analytical framework was outlined. Summaries were analyzed using R software, and descriptive statistics were used to characterize methodological usage and preferences.

Results: Of the 97 studies, the median sample size was 729 [interquartile range (IQR): 396–1,992], and the median number of exposures analyzed was 9 (IQR: 6–15). Most studies focused on internal exposures 86/97 (88.7%); 58/86 (67.4%) reported an explicit detection-rate threshold. Feature reduction was performed in 25/97 studies (25.8%). BKMR, WQS, and QGC were used in 78/97 (80.4%), 35/97 (36.1%), and 30/97 (30.9%) studies, respectively. Overall, 41/97 studies (42.3%) used at least two methods. Post-hoc analyses were common, with 87/97 (89.7%) studies assessing variable importance, 78/97 (80.4%) examining interactions, and 87/97 (89.7%) assessing nonlinearity. Common reporting gaps included incomplete model-parameter specification, insufficient documentation of preprocessing decisions, and limited justification for mixture-method selection.

Conclusion: Among published studies, analytical choices were heterogeneous, and key reporting details were incomplete. Justification of method choice, transparent documentation of preprocessing decisions, and standardized reporting are required to improve reproducibility and interpretability. The checklist

supports planning and reporting environmental mixture analyses, and provides transparency in analytical decisions, model specifications, and post-hoc interpretations.

The exposome concept was introduced to capture diverse life-course exposures, including environmental, psychosocial, physical, and lifestyle factors, and their health consequences (1–2) and has received increasing attention in public-health research in recent years (3–5). Exposomics typically examines the health effects of multiple simultaneous exposures, usually involving at least three independent chemicals or chemical groups (6). Environmental epidemiologists have largely been guided by the “one-exposure–one-disease” paradigm (7–9). Although conventional regression can adjust for co-pollutants or include prespecified interactions, it may not capture complex correlations, nonlinearities, and joint effects within real-world mixtures (10).

The U.S. National Institute of Environmental Health Sciences has prioritized mixed-exposure research through workshops held in 2011 and 2015 (11–12). In 2017, the institute launched the *Powering Research through Innovative Methods for Mixtures in Epidemiology* program, to accelerate methodological innovations for mixed-exposure analyses (13). Dedicated statistical techniques were developed, including weighted quantile sum (WQS) regression in 2013 (14), Bayesian kernel machine regression (BKMR) in 2015 (10), and quantile-based g-computation (QGC) in 2020 (15). User-friendly R packages (e.g., *gWQS*, *bkmr*, and *qgcomp*) have promoted their application (15–17).

Despite methodological advances, real-world mixture analyses remain challenging because researchers must handle high-dimensional exposure data, correlated pollutants, nonlinear exposure–response (ER) relationships, interactions, and multiple

competing statistical methods (18–19). This often leads to analytical strategies being chosen based on precedent or personal preference. Such choices can introduce bias, compromise reproducibility (20), and mislead conclusions. Because exposome research is fundamentally rooted in observational-study designs, Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) provides an important general framework for reporting (21). However, publications often omit these details. Advanced methods such as WQS, BKMR, and QGC require transparent reporting of model selection, parameter settings, variable reduction strategies, and interpretation (19). However, these critical details are often omitted from publications. Such incomplete reporting can limit reproducibility and interpretation, underscoring the need for practical guidance to standardize the analysis and reporting of environmental mixture studies.

To address these challenges, an empirical methodological review was conducted for recent environmental mixture studies from leading journals. Current analytical and reporting practices were summarized and organized into a three-stage framework covering data preparation, association analysis, and post-hoc interpretation, and a reporting checklist was developed to complement STROBE. The aim was to provide practical guidance for researchers to plan, conduct, and report mixture analyses more transparently and consistently.

METHODS

Literature Review

Firstly, a review was performed for analytical approaches used in environmental mixture-exposure studies published in four high-impact journals: Environmental Health Perspectives (EHP), Journal of Hazardous Materials (JHM), Environment International (EI), and Environmental Science & Technology (EST), selected because they are widely recognized as leading outlets in environmental health, exposure science, and epidemiology. Search keywords included "environmental pollutants," "mixed exposure analysis," "BKMR," "WQS," and "QGC." Studies published from 2021 to 2025 were included (Supplementary Material, available at <https://weekly.chinacdc.cn/>). Reviews, comments, and methodological studies were excluded. Supplementary Material provides more details regarding the literature search.

Analytical Framework in Current Literature

The analytical framework comprised three main stages. 1) Data preparation, referring to analytical steps conducted before fitting mixture models, including exposure screening based on detection rate, handling of values below the limit of detection (LOD) (22–23), transformation or standardization of exposure variables, correlation assessment, and feature reduction (24–25). 2) Association analysis, which is the core stage of environmental mixture research and may include both single-exposure models (exposome-wide association studies) (26), and mixture methods such as WQS, QGC, and BKMR. Single-exposure models assess each exposure separately, whereas mixture methods are more appropriate when exposures are correlated and the research question concerns joint or overall mixture effects. Notably, BKMR emerged as the most widely used method, appearing in over 200 studies since 2022 (Figure 1). 3) Post-hoc analysis, which further characterizes mixture findings by identifying influential components (27) and examining whether associations vary through nonlinear ER patterns (28) or interactions (29). These analyses clarify individual contributions and overall mixture complexity. Supplementary Material provides further details.

RESULTS

In the current analysis, 97 studies were included, comprising 19, 22, 39, and 17 from EHP, JHM, EI, and EST, respectively. Fifty-five studies had a sample size below 1,000, while only 11 studies had a sample size exceeding 5,000. Sample sizes varied considerably across studies, ranging from 76 to over 54.5 million (median: 729; interquartile range [IQR]: 396–1,992). Studies were categorized as examining external exposures (e.g., O₃, NO₂, PM_{2.5}, and its components) or internal exposures (e.g., per- and polyfluoroalkyl substances (PFAS), persistent organic pollutants (POPs), organochlorine pesticides (OCPs), and endocrine-disrupting chemicals (EDCs) measured in blood or urine). All included studies employed mixed exposure association methods, and the findings indicate that these methods are predominantly applied in internal exposure research, accounting for 86 of the 97 studies (88.7%), with generally similar proportions observed across the four journals (Table 1).

Data-preparation and model-selection practices

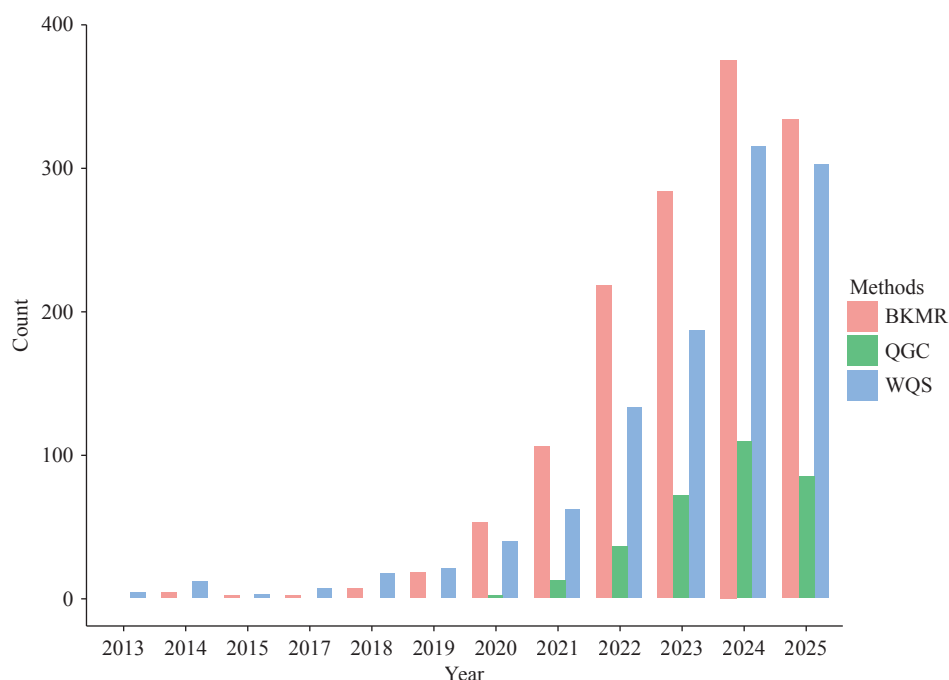


FIGURE 1. Publications using BKMR, WQS, and QGC from 2013 to 2025.

Abbreviation: BKMR=Bayesian kernel machine regression; WQS=weighted quantile sum; QGC=quantile-based g-computation.

varied substantially across studies. Among the 86 internal-exposure studies, 28 (32.6%) did not report a clear detection-rate exclusion threshold, whereas 58 (67.4%) applied a threshold of at least 50% for exposure inclusion. The number of exposures ranged from 3 to 98 (median: 9; IQR: 6–15), and 25 studies performed feature reduction, mainly with large initial exposure sets (Supplementary Material, Supplementary Tables S1–S4, available at <https://weekly.chinacdc.cn/>).

In the main association analyses, most studies (93/97, 95.9%) employed single-exposure–outcome association analysis, among which 26 (26.8%) applied false-discovery-rate correction to decrease false-positive findings. Regarding methods for mixed-exposure association analyses, BKMR was predominantly used, featuring in 78 studies (80.4%), owing to its versatility in capturing complex interactions and nonlinear relationships among exposures. Contrastingly, WQS and QGC were used in 35 (36.1%) and 30 (30.9%) studies, respectively. Notably, multimodel analyses were used in 41/97 studies (42.3%). The most common combinations were BKMR plus QGC (16/97, 16.5%) and BKMR plus WQS (15/97, 15.5%), followed by WQS plus QGC (5/97, 5.2%) and the use of all three methods (5/97, 5.2%). In stratified analyses, multimodel use was not restricted to

studies with larger sample sizes or greater numbers of pollutants, suggesting that multiple mixture methods were often used as a robustness or triangulation strategy (Table 1).

Patterns in the use of BKMR, WQS, and QGC were further examined in relation to sample size and the number of exposures (after feature reduction, if applied). BKMR remained the dominant method in studies with sample sizes below 2,000 and was also commonly used in studies with sample sizes between 2,000 and 6,000 (Figure 2A). In contrast, among studies with sample sizes exceeding 8,000, WQS and QGC were more frequently applied, with WQS becoming the most commonly used method in this subgroup. Regarding the number of exposures, BKMR was favored when fewer than five exposures were included, whereas WQS and QGC were more frequently applied when the number of exposures exceeded 18. For studies involving 5–18 exposures, no clear preference was observed, and the use of all three methods was similarly distributed (Figure 2B).

Post-hoc analyses were common, including variable importance assessment in 87 studies (89.7%), interaction assessment in 78 (80.4%), and nonlinearity assessment in 87 (89.7%). These analyses were most often conducted using the corresponding mixture method, with BKMR frequently used for variable

TABLE 1. Characteristics of included studies using mixed-exposure association approaches.

Characteristics	Overall (n=97)	EI (n=39)	EHP (n=19)	EST (n=17)	JHM (n=22)
Sample size, median (IQR)	729 (396, 1,992)	1,136 (377.0, 2,959.5)	943 (590, 1,192)	396 (326, 479)	810 (457.2, 2,380.2)
Sample size (%)					
≤200	10 (10.3)	5 (12.8)	1 (5.3)	2 (11.8)	2 (9.1)
201–500	28 (28.9)	9 (23.1)	3 (15.8)	11 (64.7)	5 (22.7)
501–1,000	17 (17.5)	5 (12.8)	6 (31.6)	0 (0.0)	6 (27.3)
1,001–2,000	18 (18.6)	7 (17.9)	6 (31.6)	3 (17.6)	2 (9.1)
2,001–5,000	13 (13.4)	6 (15.4)	2 (10.5)	0 (0.0)	5 (22.7)
>5,000	11 (11.3)	7 (17.9)	1 (5.3)	1 (5.9)	2 (9.1)
Study type (%)					
Internal	86 (88.7)	34 (87.2)	17 (89.5)	16 (94.1)	19 (86.4)
External	11 (11.3)	5 (12.8)	2 (10.5)	1 (5.9)	3 (13.6)
No. of exposures, median (IQR)	9 (6,15)	9 (6,13)	8 (6,15)	14 (10,17)	7 (6,12)
No. of exposures (%)					
≤5	15 (15.5)	6 (15.4)	4 (21.1)	2 (11.8)	3 (13.6)
6–10	45 (46.4)	20 (51.3)	8 (42.1)	4 (23.5)	13 (59.1)
11–15	14 (14.4)	6 (15.4)	3 (15.8)	3 (17.6)	2 (9.1)
16–20	14 (14.4)	3 (7.7)	3 (15.8)	4 (23.5)	4 (18.2)
>20	9 (9.3)	4 (10.3)	1 (5.3)	4 (23.5)	0 (0.0)
Feature reduction (%)					
Not performed	72 (74.2)	35 (89.7)	17 (89.5)	4 (23.5)	16 (72.7)
Performed	25 (25.8)	4 (10.3)	2 (10.5)	13 (76.5)	6 (27.3)
Single exposure analysis (%)					
Not performed	4 (4.1)	1 (2.6)	0 (0.0)	2 (11.8)	1 (4.5)
Performed	93 (95.9)	38 (97.4)	19 (100.0)	15 (88.2)	21 (95.5)
FDR (%)					
Not performed	71 (73.2)	29 (74.4)	14 (73.7)	12 (70.6)	16 (72.7)
Performed	26 (26.8)	10 (25.6)	5 (26.3)	5 (29.4)	6 (27.3)
BKMR (%)					
Not performed	19 (19.6)	10 (25.6)	3 (15.8)	3 (17.6)	3 (13.6)
Performed	78 (80.4)	29 (74.4)	16 (84.2)	14 (82.4)	19 (86.4)
WQS (%)					
Not performed	62 (63.9)	23 (59.0)	15 (78.9)	9 (52.9)	15 (68.2)
Performed	35 (36.1)	16 (41.0)	4 (21.1)	8 (47.1)	7 (31.8)
QGC (%)					
Not performed	67 (69.1)	23 (59.0)	15 (78.9)	14 (82.4)	15 (68.2)
Performed	30 (30.9)	16 (41.0)	4 (21.1)	3 (17.6)	7 (31.8)
BKMR & WQS (%)					
Not performed	82 (84.5)	32 (82.1)	18 (94.7)	13 (76.5)	19 (86.4)
Performed	15 (15.5)	7 (17.9)	1 (5.3)	4 (23.5)	3 (13.6)
BKMR & QGC (%)					
Not performed	81 (83.5)	33 (84.6)	15 (78.9)	15 (88.2)	18 (81.8)
Performed	16 (16.5)	6 (15.4)	4 (21.1)	2 (11.8)	4 (18.2)

Continued

Characteristics	Overall (n=97)	EI (n=39)	EHP (n=19)	EST (n=17)	JHM (n=22)
WQS & QGC (%)					
Not performed	92 (94.8)	36 (92.3)	19 (100.0)	17 (100.0)	20 (90.9)
Performed	5 (5.2)	3 (7.7)	0 (0.0)	0 (0.0)	2 (9.1)
WQS & QGC & BKMR (%)					
Not performed	92 (94.8)	36 (92.3)	19 (100.0)	16 (94.1)	21 (95.5)
Performed	5 (5.2)	3 (7.7)	0 (0.0)	1 (5.9)	1 (4.5)
Variable Importance (%)					
Not performed	10 (10.3)	6 (15.4)	1 (5.3)	0 (0.0)	3 (13.6)
Performed	87 (89.7)	33 (84.6)	18 (94.7)	17 (100.0)	19 (86.4)
Interaction (%)					
Not performed	19 (19.6)	7 (17.9)	3 (15.8)	4 (23.5)	5 (22.7)
Performed	78 (80.4)	32 (82.1)	16 (84.2)	13 (76.5)	17 (77.3)
Nonlinear (%)					
Not performed	10 (10.3)	7 (17.9)	3 (15.8)	0 (0.0)	0 (0.0)
Performed	87 (89.7)	32 (82.1)	16 (84.2)	17 (100.0)	22 (100.0)

Abbreviation: No.=number; EHP=Environmental Health Perspectives; EST=Environmental Science & Technology; JHM=Journal of Hazardous Materials; EI=Environment International; FDR=false discovery rate; BKMR=Bayesian Kernel Machine Regression; WQS=Weighted Quantile Sum; QGC=quantile-based g-computation.

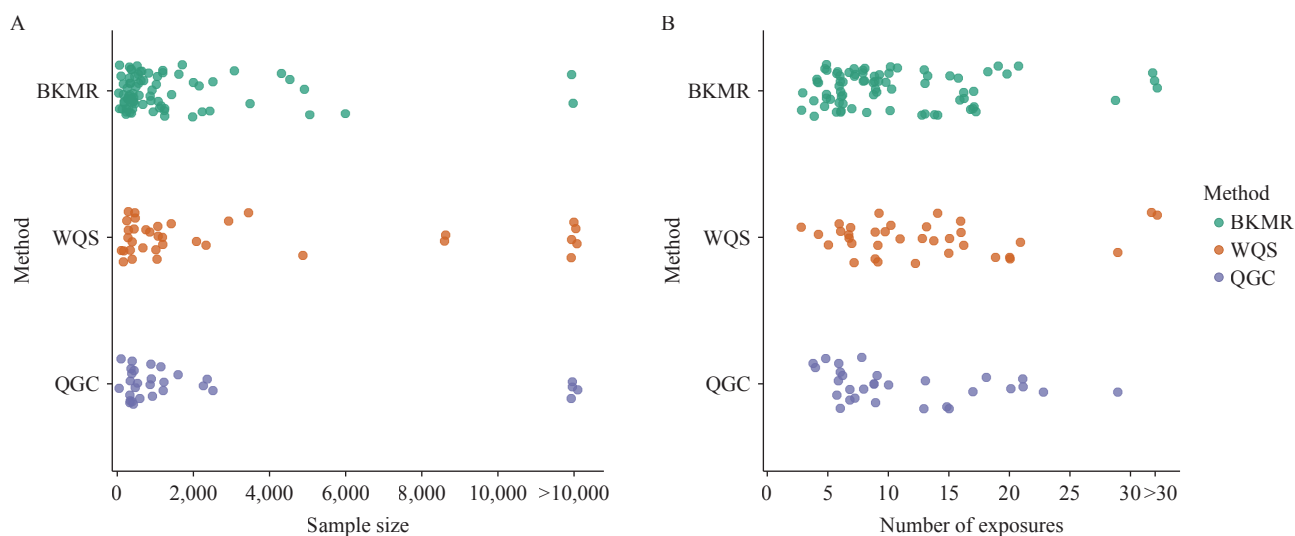


FIGURE 2. Motives for choosing BKMR, WQS, and QGC in relation to (A) sample size and (B) number of exposures. Abbreviation: BKMR=Bayesian kernel machine regression; WQS=weighted quantile sum; QGC=quantile-based g-computation.

importance, interaction, and nonlinearity assessments, whereas restricted cubic splines were mainly used for single-exposure nonlinearity analyses ([Supplementary Material, Supplementary Table S1–S3](#)). Notably, one study used the Relative Excess Risk due to Interaction to enhance the practical interpretability of interaction effects ([30](#)), and three studies used QGC to investigate nonlinear associations ([31–33](#)).

DISCUSSION

Large-scale environmental health data and advances in mixture methods have created new opportunities for exposome research ([34](#)); however, the lack of mixture-specific reporting guidance limits transparency and reproducibility ([35](#)). Based on studies published in leading journals, current analytical practices were organized into three stages, namely data preparation,

association analysis, and post-hoc evaluation, and used this structure to develop practical reporting recommendations.

Principal Findings and Methodological Implications

Most (86/97, 88.7%) included studies focused on internal exposure biomarkers, reflecting both their biological relevance and the analytical challenges posed by correlated biomarker data. These studies require careful preprocessing as detection-rate thresholds, LOD handling (36), and feature reduction may influence results when missingness or exposure dimensionality is high (37). Contrastingly, external-exposure studies often involved very large populations but fewer exposures, increasing computational burdens. These differences highlight the need to tailor preprocessing and model-selection strategies to study design, exposure dimension, and data structure.

Method selection should be guided by the research question and data structure. WQS, QGC, and BKMR are designed for mixture analyses, but they differ in assumptions, interpretability, and computational burden: WQS is efficient and interpretable, QGC allows mixed effect directions, and BKMR flexibly models nonlinear and interactive relationships but is computationally intensive (6,19,24–25). Single-pollutant models may identify priority pollutants, but mixture methods should play a central role rather than being used only as supplementary analyses (38–40). Multimodel analyses may strengthen interpretations when findings are consistent; discrepant results should be reported transparently and interpreted according to each method's assumptions and target estimand (41–44).

Post-hoc analyses were frequently used to interpret mixture findings, including variable importance (89.7%), interaction (80.4%), and nonlinearity (89.7%) assessments. Therefore, recent mixture studies have increasingly aimed not only to estimate overall mixture effects but also to identify influential components and characterize complex risk patterns, including potential nonlinear and interactive relationships (45).

Reporting Gaps and the Need for STROBE Extension

As exposome research is largely observational, analytical decisions should be reported transparently to evaluate validity and reproducibility (46–47). Several

recurring reporting gaps were identified: incomplete documentation of detection-rate thresholds and other preprocessing decisions; limited justification for mixture-method selection; and insufficient reporting of key model specifications such as BKMR parameters, WQS bootstrap settings, and QGC assumptions. These gaps hinder assessment of model appropriateness, reproducibility, and interpretation of discrepant findings (48). Similarly, a considerable number of studies relied on restricted cubic splines to assess nonlinearity, which is designed to model the nonlinear relationship of a single continuous variable (49–50). When analyzing multiple correlated pollutants, this approach may still misrepresent the true overall ER relationship.

Although STROBE provides essential guidance for observational studies (51), it does not address mixture-specific reporting needs, including exposure screening, LOD handling, feature reduction, method selection, model settings, multimodel interpretation, and post-hoc analyses. We therefore developed a reporting checklist distinguishing core and method-specific items for BKMR, WQS, QGC, and other approaches to improve reporting transparency and facilitate peer review (Supplementary Table S5, available at <https://weekly.chinacdc.cn/>).

Several limitations should be noted. First, because the analysis focused on studies published in several leading journals, the findings reflect analytical and reporting practices within these influential journals rather than the entire exposome literature. Second, the extraction was based on published reports; therefore, incompletely reported practices may have been underestimated. Finally, this was a descriptive methodological review; individual-level data were not re-analyzed, nor were simulations conducted to determine which practices produced the most valid estimates.

Environmental mixture studies have made substantial methodological progress; however, greater standardization and transparency are still needed. The empirical review of studies published in several leading journals summarizes current analytical practices and provides practical reporting recommendations for researchers conducting mixture analyses. Because no single method can address all mixture-related questions, the method selection should be guided by the research objective, data structure, and target estimand. Researchers should justify mixture-method selection, report preprocessing, and model-specification decisions; interpret multimodel findings transparently; and use reporting guidance such as the

checklist proposed here to improve reproducibility, interpretability, and peer review efficiency.

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REFERENCES

- Vermeulen R, Schymanski EL, Barabási A, Miller GW. The exposome and health: where chemistry meets biology. *Science* 2020;367(6476):392 – 6. <https://doi.org/10.1126/science.aay3164>.
- Cui YX, Balshaw DM, Kwok RK, Thompson CL, Collman GW, Birnbaum LS. The exposome: embracing the complexity for discovery in environmental health. *Environ Health Perspect* 2016;124(8):A137 – 40. <https://doi.org/10.1289/EHP412>.
- Münzel T, Sørensen M, Hahad O, Nieuwenhuijsen M, Daiber A. The contribution of the exposome to the burden of cardiovascular disease. *Nat Rev Cardiol* 2023;20(10):651 – 9. <https://doi.org/10.1038/s41569-023-00873-3>.
- Barouki R, Samson M, Blanc EB, Colombo M, Zucman-Rossi J, Lazaridis KN, et al. The exposome and liver disease - how environmental factors affect liver health. *J Hepatol* 2023;79(2):492 – 505. <https://doi.org/10.1016/j.jhep.2023.02.034>.
- Beulens JWJ, Pinho MGM, Abreu TC, den Braver NR, Lam TM, Huss A, et al. Environmental risk factors of type 2 diabetes—an exposome approach. *Diabetologia* 2022;65(2):263 – 74. <https://doi.org/10.1007/s00125-021-05618-w>.
- Gibson EA, Goldsmith J, Kioumourtzoglou MA. Complex mixtures, complex analyses: an emphasis on interpretable results. *Curr Environ Health Rep* 2019;6(2):53 – 61. <https://doi.org/10.1007/s40572-019-00229-5>.
- Braun JM, Gennings C, Hauser R, Webster TF. What can epidemiological studies tell us about the impact of chemical mixtures on human health? *Environ Health Perspect* 2016;124(1):A6-9. <http://dx.doi.org/10.1289/ehp.1510569>.
- Zhu GM, Wen YX, Cao KX, He SM, Wang T. A review of common statistical methods for dealing with multiple pollutant mixtures and multiple exposures. *Front Public Health* 2024;12:1377685. <https://doi.org/10.3389/fpubh.2024.1377685>.
- Dominici F, Peng RD, Barr CD, Bell ML. Protecting human health from air pollution: shifting from a single-pollutant to a multipollutant approach. *Epidemiology* 2010;21(2):187 – 94. <https://doi.org/10.1097/EDE.0b013e3181cc86e8>.
- Bobb JF, Valeri L, Claus Henn B, Christiani DC, Wright RO, Mazumdar M, et al. Bayesian kernel machine regression for estimating the health effects of multi-pollutant mixtures. *Biostatistics* 2015;16(3):493 – 508. <https://doi.org/10.1093/biostatistics/kxu058>.
- Taylor KW, Joubert BR, Braun JM, Dilworth C, Gennings C, Hauser R, et al. Statistical approaches for assessing health effects of environmental chemical mixtures in epidemiology: lessons from an innovative workshop. *Environ Health Perspect* 2016;124(12):A227 – 9. <https://doi.org/10.1289/EHP547>.
- Carlin DJ, Rider CV, Woychik R, Birnbaum LS. Unraveling the health effects of environmental mixtures: an NIEHS priority. *Environ Health Perspect* 2013;121(1):A6 – 8. <https://doi.org/10.1289/ehp.1206182>.
- Joubert BR, Kioumourtzoglou MA, Chamberlain T, Chen HY, Gennings C, Turyk ME, et al. Powering research through innovative methods for mixtures in epidemiology (PRIME) program: novel and expanded statistical methods. *Int J Environ Res Public Health* 2022;19(3):1378. <https://doi.org/10.3390/ijerph19031378>.
- Carrico C, Gennings C, Wheeler DC, Factor-Litvak P. Characterization of weighted quantile sum regression for highly correlated data in a risk analysis setting. *J Agric Biol Environ Stat* 2015;20(1):100 – 20. <https://doi.org/10.1007/s13253-014-0180-3>.
- Keil AP, Buckley JP, O'Brien KM, Ferguson KK, Zhao SS, White AJ. A quantile-based g-computation approach to addressing the effects of exposure mixtures. *Environ Health Perspect* 2020;128(4):047004. <https://doi.org/10.1289/EHP5838>.
- Czarnota J, Gennings C, Wheeler DC. Assessment of weighted quantile sum regression for modeling chemical mixtures and cancer risk. *Cancer Inform* 2015;14(S2):159 – 71. <https://doi.org/10.4137/CIN.S17295>.
- Bobb JF, Claus Henn B, Valeri L, Coull BA. Statistical software for analyzing the health effects of multiple concurrent exposures via Bayesian kernel machine regression. *Environ Health* 2018;17(1):67. <https://doi.org/10.1186/s12940-018-0413-y>.
- Maitre L, Guimbaud JB, Warembourg C, Güil-Oumrait N, Petrone PM, Chadeau-Hyam M, et al. State-of-the-art methods for exposure-health studies: results from the exposome data challenge event. *Environ Int* 2022;168:107422. <https://doi.org/10.1016/j.envint.2022.107422>.
- Yu LL, Liu W, Wang X, Ye Z, Tan QY, Qiu WH, et al. A review of practical statistical methods used in epidemiological studies to estimate the health effects of multi-pollutant mixture. *Environ Pollut* 2022;306:119356. <https://doi.org/10.1016/j.envpol.2022.119356>.
- Macleod M. Improving the reproducibility and integrity of research: what can different stakeholders contribute? *BMC Res Notes* 2022;15(1):146. <http://dx.doi.org/10.1186/s13104-022-06030-2>.
- Skrivankova VW, Richmond RC, Woolf BAR, Yarmolinsky J, Davies NM, Swanson SA, et al. Strengthening the reporting of observational studies in epidemiology using Mendelian randomization: the STROBE-MR statement. *JAMA* 2021;326(16):1614 – 21. <https://doi.org/10.1001/jama.2021.18236>.
- Nie L, Chu HT, Liu CL, Cole SR, Vexler A, Schisterman EF. Linear regression with an independent variable subject to a detection limit. *Epidemiology* 2010;21 Suppl 4(Suppl 4):S17-24. <http://dx.doi.org/10.1097/EDE.0b013e3181ce97d8>.
- Schisterman EF, Vexler A, Whitcomb BW, Liu AY. The limitations due to exposure detection limits for regression models. *Am J Epidemiol* 2006;163(4):374 – 83. <https://doi.org/10.1093/aje/kwj039>.
- Pan SD, Li ZJ, Rubbo B, Quon-Chow V, Chen JC, Baumert BO, et al. Applications of mixture methods in epidemiological studies investigating the health impact of persistent organic pollutants exposures: a scoping review. *J Expo Sci Environ Epidemiol* 2025;35(4):522 – 34. <https://doi.org/10.1038/s41370-024-00717-3>.
- Lazarevic N, Barnett AG, Sly PD, Knibbs LD. Statistical methodology in studies of prenatal exposure to mixtures of endocrine-disrupting chemicals: a review of existing approaches and new alternatives. *Environ Health Perspect* 2019;127(2):026001. <https://doi.org/10.1289/EHP2207>.
- Chung MK, House JS, Akhtari FS, Makris KC, Langston MA, Islam KT, et al. Decoding the exposome: data science methodologies and implications in exposome-wide association studies (ExWASs).

- Exposome 2024;4(1):osae001. <https://doi.org/10.1093/exposome/osae001>.
27. Gibson EA, Nunez Y, Abuawad A, Zota AR, Renzetti S, Devick KL, et al. An overview of methods to address distinct research questions on environmental mixtures: an application to persistent organic pollutants and leukocyte telomere length. *Environ Health* 2019;18(1):76. <https://doi.org/10.1186/s12940-019-0515-1>.
 28. Xu HB, Chen C, Lu ZX, Nie ZQ. *rescvi*: an R package for visualization of restricted cubic spline. *Med Res* 2025;1(2):201 – 6. <https://doi.org/10.1002/mdr2.70015>.
 29. Knol MJ, VanderWeele TJ. Recommendations for presenting analyses of effect modification and interaction. *Int J Epidemiol* 2012;41(2):514 – 20. <https://doi.org/10.1093/ije/dyr218>.
 30. Yang Z, Liu HB, Wei JM, Liu RF, Zhang JY, Sun MQ, et al. Bisphenol mixtures, metal mixtures and type 2 diabetes mellitus: insights from metabolite profiling. *Environ Int* 2024;190:108921. <https://doi.org/10.1016/j.envint.2024.108921>.
 31. Zhan WQ, Qiu W, Ao Y, Zhou W, Sun Y, Zhao H, et al. Environmental exposure to emerging alternatives of per- and polyfluoroalkyl substances and polycystic ovarian syndrome in women diagnosed with infertility: a mixture analysis. *Environ Health Perspect* 2023;131(5):057001. <https://doi.org/10.1289/EHP11814>.
 32. Huang Y, Fang F, Chen Y, He XQ, Chen Q, Wang H, et al. Prenatal exposure to per- and polyfluoroalkyl substances and infant sleep disturbance: a prospective cohort study. *Environ Int* 2023;178:108070. <https://doi.org/10.1016/j.envint.2023.108070>.
 33. Fu JF, Yao Y, Huang ZH, Huang JY, Zhang D, Li XY, et al. Prenatal exposure to benzophenone-type UV filters and the associations with neonatal birth outcomes and maternal health in south China. *Environ Int* 2024;189:108797. <https://doi.org/10.1016/j.envint.2024.108797>.
 34. Stingone JA, Buck Louis GM, Nakayama SF, Vermeulen RCH, Kwok RK, Cui YX, et al. Toward greater implementation of the exposome research paradigm within environmental epidemiology. *Annu Rev Public Health* 2017;38:315 – 27. <https://doi.org/10.1146/annurev-publhealth-082516-012750>.
 35. Ioannidis JPA. Why most published research findings are false. *PLoS Med* 2005;2(8):e124. <https://doi.org/10.1371/journal.pmed.0020124>.
 36. Lee M, Saha A, Sundaram R, Albert PS, Zhao SS. Accommodating detection limits of multiple exposures in environmental mixture analyses: an overview of statistical approaches. *Environ Health* 2024;23(1):48. <https://doi.org/10.1186/s12940-024-01088-w>.
 37. Hamra GB, Buckley JP. Environmental exposure mixtures: questions and methods to address them. *Curr Epidemiol Rep* 2018;5(2):160 – 5. <https://doi.org/10.1007/s40471-018-0145-0>.
 38. Khan S, Ouidir M, Lemaitre N, Jovanovic N, Bayat S, Lyon-Caen S, et al. PFAS exposure during pregnancy: Implications for placental health and functioning. *Environ Int* 2025;197:109308. <https://doi.org/10.1016/j.envint.2025.109308>.
 39. Wang YQ, Aimuzi R, Nian M, Zhang Y, Luo K, Zhang J. Perfluoroalkyl substances and sex hormones in postmenopausal women: NHANES 2013-2016. *Environ Int* 2021;149:106408. <https://doi.org/10.1016/j.envint.2021.106408>.
 40. Zhang YH, Zhang ZL, Zhou FY, Xu GC, Huang WC, Wang MY, et al. Association between endocrine disrupting chemicals exposure and the risk of all-cause mortality in individuals with diabetes mellitus or its complications: a prospective cohort study. *Environ Int* 2025;200:109556. <https://doi.org/10.1016/j.envint.2025.109556>.
 41. Wen Y, Wang YBN, Chen RJ, Guo Y, Pu JL, Li JW, et al. Association between exposure to a mixture of organochlorine pesticides and hyperuricemia in U. S. adults: a comparison of four statistical models. *Eco Environ Health* 2024;3(2):192 – 201. <https://doi.org/10.1016/j.eehl.2024.02.005>.
 42. Liu W, Yu LL, Ye Z, Wang X, Qiu WH, Tan QY, et al. Assessment for the associations of twenty-three metal(loid)s exposures with early cardiovascular damage among Chinese urban adults with five statistical methods: insight into assessing health effect of multipollutant exposure. *Chemosphere* 2022;307(Pt 2):135969. <http://dx.doi.org/10.1016/j.chemosphere.2022.135969>.
 43. Yu T, Zhang DY, Li ZL, Zhai MX, Lin WL, Ren SW, et al. Evaluating the association between exposure to multiple metals and adverse pregnancy outcomes based on a multi-step statistical analysis strategy. *Ecotoxicol Environ Saf* 2025;303:118768. <https://doi.org/10.1016/j.ecoenv.2025.118768>.
 44. Puvvula J, Hwang WT, McCandless L, Xie CC, Braun JM, Vuong AM, et al. Gestational exposure to environmental chemical mixtures and cognitive abilities in children: a pooled analysis of two North American birth cohorts. *Environ Int* 2025;196:109298. <https://doi.org/10.1016/j.envint.2025.109298>.
 45. Sun ZC, Tao YB, Li S, Ferguson KK, Meeker JD, Park SK, et al. Statistical strategies for constructing health risk models with multiple pollutants and their interactions: possible choices and comparisons. *Environ Health* 2013;12(1):85. <https://doi.org/10.1186/1476-069X-12-85>.
 46. Shepherd DA, Amor DJ, Moreno-Betancur M. Statistical analysis of observational studies in disability research. *Dev Med Child Neurol* 2024;66(11):1408 – 18. <https://doi.org/10.1111/dmcn.15948>.
 47. Carmona-Bayonas A, Jimenez-Fonseca P, Fernández-Somoano A, Álvarez-Manceño F, Castañón E, Custodio A, et al. Top ten errors of statistical analysis in observational studies for cancer research. *Clin Transl Oncol* 2018;20(8):954 – 65. <https://doi.org/10.1007/s12094-017-1817-9>.
 48. Sterrantino AF. Observational studies: practical tips for avoiding common statistical pitfalls. *Lancet Reg Health Southeast Asia* 2024;25:100415. <https://doi.org/10.1016/j.lansea.2024.100415>.
 49. Desquilbet L, Mariotti F. Dose-response analyses using restricted cubic spline functions in public health research. *Stat Med* 2010;29(9):1037 – 57. <https://doi.org/10.1002/sim.3841>.
 50. Perperoglou A, Sauerbrei W, Abrahamowicz M, Schmid M. A review of spline function procedures in R. *BMC Med Res Methodol* 2019;19(1):46. <https://doi.org/10.1186/s12874-019-0666-3>.
 51. Lachat C, Hawwash D, Ocké MC, Berg C, Forsum E, Hörnell A, et al. Strengthening the reporting of observational studies in epidemiology—nutritional epidemiology (STROBE-nut): an extension of the STROBE statement. *PLoS Med* 2016;13(6):e1002036. <https://doi.org/10.1371/journal.pmed.1002036>.
 52. Billionnet C, Sherrill D, Annesi-Maesano I. Estimating the health effects of exposure to multi-pollutant mixture. *Ann Epidemiol* 2012;22(2):126 – 41. <https://doi.org/10.1016/j.annepidem.2011.11.004>.

SUPPLEMENTARY MATERIALS

METHODS

Literature Search and Data Extraction

PubMed was searched for original studies published between July 2021 and July 2025 in the four selected journals. The search combined mixture-method terms with journal restrictions. For example, the search syntax for Environmental Science & Technology was:

((bkmr OR qgc OR wqs) AND ("Environmental Science & Technology"[Journal]) AND ("2021/7"[Date - Publication] : "2025/7"[Date - Publication])).

The same syntax was applied to Environmental Health Perspectives, Environment International, and Journal of Hazardous Materials by replacing the journal name.

The design characteristics (year of publication, outcome, type of exposure, covariates, and sample size) and the statistical methodologies (including data management, association, interaction, non-linear analysis, and variable importance) were extracted. Each study was treated as the unit of analysis. For studies that used multiple mixture methods, each method was recorded separately using binary indicators for Bayesian Kernel Machine Regression (BKMR), Weighted Quantile Sum (WQS), and quantile-based g-computation. Multimodel use was defined as the use of at least two of the three main mixture methods.

The extracted results were further double-checked, and any identified discrepancies were resolved through discussion until a consensus was reached. Categorical variables are presented as frequencies and percentages. Continuous variables are presented as medians and interquartile ranges (IQRs). Statistical analyses were performed using R (version 4.5.0; R Foundation for Statistical Computing, Vienna, Austria).

Analytical Framework in Current Literature

Data Preparation: In this study, data preprocessing referred to analytical steps conducted before fitting mixture models, including exposure screening based on detection rate, handling of values below the limit of detection (LOD), transformation or standardization of exposure variables, correlation assessment, and feature reduction (1–2). Of note, although both the exclusion of exposures with low detection rates and feature reduction result in a reduced set of exposure variables, they serve distinct purposes. The former is used to identify which exposures are qualified for analysis, as those with very low detection rates may not be analytically reliable; the latter aims to reduce dimensionality among the qualified exposures owing to the limited samples or computation burdens.

Association Analysis: This constitutes the core of the analytical process. Based on the review, most studies employed multiple methods to examine the associations between exposures and health outcomes. The most common approach is single-exposure association analysis, sometimes referred to as exposome-wide association studies (3), which assesses the relationship between each exposure and the outcome under the assumption of independence among exposures. It is then advisable to conduct a correlation analysis among the exposures to evaluate whether this independence assumption holds. When strong correlations are found, multi-exposure analysis methods, including WQS regression, QGC, and BKMR are recommended. In fact, the application of WQS, QGC, and BKMR increased significantly after they were developed. Notably, BKMR emerged as the most widely used method, appearing in over 200 studies since 2022 (Figure 1).

In summary, WQS estimates a weighted index of exposure and tests its association with the outcome, offering interpretability but assuming directional homogeneity; that is, all exposures act in the same direction. QGC, a more flexible extension of WQS, relaxes this assumption and allows for mixed directional effects, although it is fundamentally a generalized linear model and computationally efficient. BKMR uses a kernel function to capture complex effects within the mixture in a Bayesian framework, offering high flexibility and the ability to estimate the overall mixture effect and individual exposure–response (ER) functions. However, BKMR is computationally intensive, particularly with a large sample size.

Further Analyses: This stage involves more in-depth post-hoc examinations to elucidate complex relationships within the mixture. The first step is to identify the most important components within the mixture, which is

achieved by calculating the variable importance (2,4,5). Researchers often examine potential interactions among exposures by assessing both the direction and magnitude of these interactive effects on health outcomes. Furthermore, this phase frequently incorporates investigations into non-linear ER relationships, which may reveal threshold effects or other complex patterns that are not captured by linear models. Collectively, these analyses provide a more comprehensive understanding of the overall effects of the mixture and the roles of its components.

1) Variable Importance: WQS, QGC and BKMR can be used to evaluate the importance of exposure. In both WQS and QGC, the relative importance of each exposure was assessed using weights assigned to individual components. In BKMR, variable importance is evaluated using posterior inclusion probability (PIP). A notable advantage of BKMR is its ability to support hierarchical or grouped variables. This allows exposures to be categorized into predefined groups prior to analysis, enabling the simultaneous estimation of both group- and within-group exposure-specific importance.

2) Non-linear Effect: The most common approach for investigating the potential non-linear ER curve for single-exposure analyses is to apply restricted cubic splines (RCS). The key parameter of RCS is the number of knots, with default values typically ranging from three to five, depending on the sample size and R packages used (6). It is recommended to explicitly specify the number and location of knots in the analysis and conduct sensitivity analyses using different knots to verify that the non-linear ER shapes remain robust.

3) Interaction Effect: An interaction effect occurs when the effect of one exposure on the outcome varies across different levels of another independent variable. In other words, the association between an exposure and an outcome is modified by a third variable—the modifier or effect modifier. Specifically, interaction effects can be categorized into multiplicative and additive interactions. Multiplicative interactions are commonly evaluated by including a product term in a regression model. Alternatively, stratified (subgroup) analysis can be performed to assess whether the exposure effect differs significantly across subgroups. Additive interactions, on the other hand, exist when the combined effect of two exposures deviates from the sum of their individual effects, and it is assessed using metrics such as the Relative Excess Risk due to Interaction. Additive interaction is especially important in public health and epidemiological research, as it helps determine whether combined interventions targeting multiple risk factors may produce greater benefits than those targeting each factor individually (7).

Mixture Association Analysis Methods:

WQS regression: The WQS formula is:

$$g(\mu) = \beta_0 + \beta_1 \left(\sum_{i=1}^m w_i q_i \right) + \mathbf{z}' \boldsymbol{\varphi}$$

$$\text{WQS} = \sum_{j=1}^m \bar{w}_j q_j$$

where m exposure components are transformed into quartiles ($q_i = 0, 1, 2, 3$), w_i denotes component weights, $\bar{w}_j$ is the average weight after bootstrap sampling, β_0 is the intercept, β_1 represents the regression coefficient for the WQS (interpretable as the outcome change per quartile increase in the overall mixture exposure), $\mathbf{z}$ is the covariate matrix, and $\boldsymbol{\varphi}$ contains covariate coefficients. The link function $g(\cdot)$ accommodates various generalized linear model (GLM) specifications. The WQS model mitigates overfitting and multicollinearity by aggregating mixture effects, but it has several limitations: the restriction of unidirectional effects, need for predefined effect directionality, and inability to evaluate nonlinearity or interactions among the mixture components.

QGC: To address these limitations, researchers developed the QGC, a method that allows mixture components to have both negative and positive directions. Like WQS regression, QGC combines multiple exposures into a single index; however, it does not assume directional homogeneity, offering greater flexibility in real-world scenarios where exposures may have opposing effects on the outcome. Furthermore, QGC supports the modeling of non-linear ER relationships and interaction effects among mixture components, enhancing its ability to capture complex toxicological dynamics. Computationally, QGC is highly efficient and requires relatively limited resources, making it particularly advantageous for large-scale studies where traditional methods would impose a substantial computational burden.

In the QGC framework, the coefficient ψ represents the g -computation estimator derived from a marginal

structural model. It quantifies the change in the outcome associated with a simultaneous one-quantile increase in all exposures. The analytical procedure involves three key steps: 1) transformation of exposures into quantiles, 2) specification of a GLM for the outcome as a function of the exposure quantiles, and 3) estimation of the mixture effect and optional component weights. For statistical robustness, both QGC and WQS typically employ bootstrapping to estimate standard errors and confidence intervals. However, unlike WQS, which requires data splitting and repeated bootstrapping for weight estimation, QGC estimates the joint effect directly in a single GLM model, thereby significantly reducing the computational burden.

BKMR: BKMR is a Bayesian method that models complex exposure–response relationships using kernel-based techniques. The BKMR model is given below:

$$Y_i = h(\mathbf{z}_i) + \mathbf{x}_i^T \boldsymbol{\beta} + \epsilon_i$$

where Y_i denotes the health outcome, $\mathbf{z}_i = (z_{i1}, \dots, z_{iM})^T$ represents a vector of M exposure variables, and $\mathbf{x}_i$ is the matrix of potential covariates (assuming a linear relationship between covariates and outcomes). The term $\boldsymbol{\beta}$ denotes the regression coefficients, and $h(\cdot)$ is the high-dimensional ER function to be estimated, which may incorporate nonlinearities or interactions among the exposure variables. The core assumption is that individuals with similar exposure characteristics may share similar ER functions $h(\cdot)$. To ensure robust model fitting, a substantial number of Markov chain Monte Carlo iterations is required (for example, *iter* $\geq 10,000$).

Notably, BKMR is a flexible, semiparametric method to understand: 1) the overall mixture effect: The change in the outcome when all exposures are fixed at a certain percentile (e.g., 25th percentile) compared to when all of the factors are fixed at their medians; 2) the univariate effect: the change in the outcome associated with increasing a single exposure from a low (e.g., 25th) to a high (e.g., 75th) percentile, while all other exposures are held constant at a fixed level (e.g., their medians) and covariates held constant; 3) bivariate ER: the pairwise dose–response relationship for each exposure on the outcome at varying levels of a second exposure, when remaining exposures are fixed at their medians.

Relation to Existing Literature

Several studies have reviewed statistical methods for assessing the health effects of environmental mixtures, often focusing on specific classes of chemicals. For instance, Pan *et al.* and Gibson *et al.* summarized methods for investigating persistent organic pollutants (POPs) (1,5), while Lazarevic *et al.* focused on endocrine-disrupting chemicals (2), and Billionnet *et al.* examined air pollution (8). All the reviews reported the most prevalent exposure components and health outcomes within their respective domains. Additionally, Pan *et al.* identified 23 distinct methods for estimating the overall effect of POP mixtures, highlighting that BKMR and its combination with WQS were the most frequently used approaches. The study by Gibson *et al.* compared supervised methods (e.g. WQS and BKMR) to unsupervised methods (clustering, principal component analysis, and exploratory-factor analysis). Meanwhile, Lazarevic *et al.* and Billionnet *et al.* detailed the strengths and limitations of available approaches, particularly in feature reduction and association analyses, and provided guidance on their software implementation. For researchers seeking detailed instructions on method applications using R software, a recent comprehensive review offers step-by-step workflows and R codes (9).

In contrast, the present study specially focused on the application of three specially proposed methods for analyzing mixed-exposure associations and adopted a broader exposome perspective, incorporating both internal and external chemical exposures in terms of study scope. The present study also differed in terms of research objectives and methodological emphasis, thus contributing different insights to the field. The analytical process was summarized into three distinct stages: data preparation, association analysis, and post-hoc analysis. Importantly, the present study emphasizes that both the selection of analytical methods and the detailed reporting of these methods (alongside related findings) are critical to rigorous research. To address these unmet demands, this study developed a structured analytical framework and a practical checklist aimed at improving both the efficiency of peer review and the overall quality of publications in this field. The proposed three-stage framework should be interpreted as a synthesis and organization of existing analytical practices observed in the reviewed studies, rather than as a novel statistical method. Its purpose is to provide a practical structure for researchers to plan, conduct, and report environmental mixture analyses more transparently and consistently.

SUPPLEMENTARY TABLE S1. Current analytical practices in environmental mixture studies a reporting of detection-rate threshold in internal exposure studies.

Detection-rate threshold (%)	Overall (n=86)	EI (n=34)	EHP (n=17)	EST (n=16)	JHM (n=19)
Not reported	28 (32.6)	10 (29.4)	4 (23.5)	8 (50.0)	6 (31.6)
0.5–0.7	25 (29.1)	17 (50.0)	3 (17.6)	2 (12.5)	3 (15.8)
0.7–0.8	14 (16.3)	4 (11.8)	3 (17.6)	2 (12.5)	5 (26.3)
0.8–0.9	7 (8.1)	0 (0.0)	4 (23.5)	1 (6.2)	2 (10.5)
≥0.9	12 (14.0)	3 (8.8)	3 (17.6)	3 (18.8)	3 (15.8)

SUPPLEMENTARY TABLE S2. Comparison of methods used for analyzing interaction effects in mixed-exposure studies.

Method	Overall (n=78)	EI (n=32)	EHP (n=16)	EST (n=13)	JHM (n=17)
BKMR (%)					
Not performed	27 (34.6)	17 (53.1)	2 (12.5)	3 (23.1)	5 (29.4)
Performed	51 (65.4)	15 (46.9)	14 (87.5)	10 (76.9)	12 (70.6)
Interaction term (%)					
Not performed	43 (55.1)	13 (40.6)	13 (81.2)	9 (69.2)	8 (47.1)
Performed	35 (44.9)	19 (59.4)	3 (18.8)	4 (30.8)	9 (52.9)

SUPPLEMENTARY TABLE S3. Comparison of methods used for nonlinear associations in mixed-exposure studies.

Method	Overall (n=87)	EI (n=32)	EHP (n=16)	EST (n=17)	JHM (n=22)
BKMR (%)					
Not performed	18 (20.7)	9 (28.1)	1 (6.2)	4 (23.5)	4 (18.2)
Performed	69 (79.3)	23 (71.9)	15 (93.8)	13 (76.5)	18 (81.8)
RCS (%)					
Not performed	57 (65.5)	19 (59.4)	11 (68.8)	14 (82.4)	13 (59.1)
Performed	30 (34.5)	13 (40.6)	5 (31.2)	3 (17.6)	9 (40.9)

Abbreviations: EHP=Environmental Health Perspectives; EST=Environmental Science & Technology; JHM=Journal of Hazardous Materials; EI=Environment International; BKMR=Bayesian Kernel Machine Regression; WQS=Weighted Quantile Sum; QGC=quantile-based g-computation; RCS=Restricted Cubic Spline.

SUPPLEMENTARY TABLE S4. Comparison of feature reduction methods used in different initial numbers of exposure.

Feature Reduction (%)	Initial number of exposures	
	≤10 (n=49)	>10 (n=48)
Not performed	48 (98.0)	24 (50.0)
Performed	1 (2.0)	24 (50.0)

Analytical Framework and Reporting Checklist for Environmental Mixture Health Effects Studies

Primary Objective (Required): (For example:) To systematically evaluate the complex relationships between multiple environmental exposures (mixtures) and a health outcome.

Secondary Objectives (Optional): 1) To identify the most important risky exposure(s), 2) explore the potential non-linear associations, and 3) explore the potential interaction effects.

Three-stage Analytical Framework: 1) Data Preparation: Data preprocessing steps conducted before model fitting, including exposure screening, handling values below the LOD, transformation or standardization, correlation assessment, and confirmation of the final analytical dataset. 2) Association Analysis: The core analytical phase, including single-exposure models, assessment of exposure correlations, and formal mixture models selected according to the research question and data structure. 3) Further Analyses: Post-hoc and sensitivity analyses used to interpret mixture findings, including identification of important components, assessment of nonlinearity or interaction, and robustness checks when appropriate.

Analytical Procedure Checklist: In the checklist below, core items apply broadly to environmental mixture analyses, whereas method-specific items apply to particular approaches such as BKMR, WQS, or QGC.

SUPPLEMENTARY TABLE S5. Analytical framework and reporting checklist for environmental mixture health effects studies.

No.	Task description	Key considerations	Reporting recommendation	Applicability
Stage 1: Data Preparation				
1.1	Exclusion of exposures with low detection frequencies	Determine and justify the minimum detection-frequency threshold used to include exposures in the mixture. Very low detection frequencies (e.g., <50%) generally limit interpretability; thresholds of 70% or 80% are commonly used but should be justified according to the study context.	Report the threshold, number of exposures before and after exclusion, and rationale for the selected threshold.	Core item: broadly applicable to all mixture analyses.
1.2	Handling of values below the limit of detection (LOD) and missing values	Describe how values below the limit of detection and other missing values are handled. Common approaches include substitution using LOD/2 or $LOD/\sqrt{2}$, multiple imputation, or model-based methods.	Specify the approach used for values below the LOD and missing data, and consider sensitivity analyses using alternative approaches.	Core item: broadly applicable to all mixture analyses.
1.3	Variable transformation	Assess exposure distributions and apply transformation if needed, such as log transformation or square-root transformation, to reduce skewness or improve model fit.	Report whether raw or transformed exposure variables were used, and describe the transformation applied to each exposure.	Core item: broadly applicable to all mixture analyses.
1.4	Standardization or scaling	Scale or standardize exposure variables when required by the selected method or needed for comparability across pollutants. WQS and QGC typically use quantile-based exposure scores, whereas BKMR and regression-based models often require careful scaling or standardization.	Report whether exposures were standardized, scaled, or converted to quantiles, and describe the specific procedure used.	Core item, with method-dependent scaling requirements.
1.5	Feature reduction or exposure selection	Consider feature reduction or exposure selection when the number of exposures is large, when collinearity is substantial, or when the scientific question requires a focused mixture. This step may not be necessary for a small number of prespecified exposures.	If feature reduction or exposure selection is performed, report the rationale, method, and number of exposures retained.	Conditional item: mainly relevant for high-dimensional mixtures or highly correlated exposure sets.
1.6	Final analytical dataset confirmation	Prepare the dataset used for formal analyses after preprocessing decisions have been applied.	Report the final sample size, the final number of exposures included in the mixture analysis, and any exclusions from the analytical sample.	Core item: broadly applicable to all mixture analyses.
Stage 2: Association Analysis				
2.1	Single-exposure analysis	Fit separate regression models for individual exposures when relevant for comparison, screening, or interpretation. These models should be interpreted cautiously because they do not directly estimate the joint effect of correlated exposures.	Report model specification, covariate adjustment, raw p-values, and, if multiple testing correction is used, q-values and the significance threshold.	Contextual item: useful for comparison or preliminary assessment, but not a mixture method.
2.2	Correlation analysis among exposures	Assess correlations or other dependence structures among exposures before mixture modeling. Spearman correlation is commonly used when distributions are skewed or monotonic but not necessarily linear.	Report the correlation metric used and present the exposure-correlation structure (for example, using a heatmap or correlation matrix).	Core item: broadly applicable to all mixture analyses.
2.3	Multi-exposure mixture analysis	Select a mixture method according to the research question, exposure structure, sample size, and method assumptions. Common approaches include BKMR, WQS, and QGC.	Report the mixture method, software or R package, version if available, and key method-specific parameters, such as MCMC iterations for BKMR, bootstrap samples and directionality for WQS, and quantile construction for QGC.	Core mixture item: method-specific parameters should be reported for BKMR, WQS, QGC, or other approaches.

Continued

No.	Task description	Key considerations	Reporting recommendation	Applicability
Stage 3: Further Analyses				
3.1	Identification of important components	Use method-specific metrics to identify exposures that contribute most to the mixture association, such as weights in WQS, posterior inclusion probabilities in BKMR, and positive or negative partial effects in QGC.	Report whether component importance was evaluated, which metric was used, and how the metric was interpreted.	Method-specific item: WQS weights, BKMR PIPs, QGC positive/negative partial effects, or analogous metrics.
3.2	Assessment of interaction effects	Assess potential interactions among mixture components when supported by the research question, sample size, and selected method. For example, BKMR can be used to explore interaction patterns between exposures.	Report whether interactions were examined, how they were assessed, and whether the analysis was prespecified or exploratory.	Method-specific or optional item: particularly relevant for BKMR or models with explicit interaction terms.
3.3	Assessment of non-linear relationships	Examine non-linear associations for the overall mixture or individual exposures when the selected method allows such interpretation, such as BKMR exposure-response functions or spline-based models.	Report whether nonlinearity was examined, the method used, and how non-linear patterns were interpreted.	Method-specific or optional item: particularly relevant for BKMR response functions, splines, or other non-linear models.
3.4	Sensitivity and robustness checks	Evaluate the robustness of key findings using analyses such as alternative covariate adjustment sets, alternative LOD or missing-data handling methods, different exposure-selection thresholds, or alternative mixture methods.	Report the sensitivity analyses performed and whether the main conclusions were materially changed.	Core item: broadly applicable when sensitivity analyses are feasible.

Abbreviations: BKMR=Bayesian Kernel Machine Regression; WQS=Weighted Quantile Sum; QGC=quantile-based g-computation.

Instructions for Use

Sequential Execution: It is recommended to sequentially proceed through Stages 1, 2, and 3.

Method Selection: The choice of specific methods should be justified based on the data characteristics, research question, target estimand, and assumptions of the selected mixture method.

Items in Stage 3 should be applied according to the research question, sample size, model capability, and interpretability, rather than treated as mandatory analyses for every study.

Documentation: Use the "Reporting Recommendation" and "Applicability" columns to report key decisions, methods, assumptions, and parameters. This is critical for ensuring reproducibility and transparency.

NA Option: If a step is not applicable to your study (e.g., Feature Reduction for Step 1.5), mark it as "N/A" and provide a brief reason.

REFERENCES

- Pan S, Li Z, Rubbo B et al. Applications of mixture methods in epidemiological studies investigating the health impact of persistent organic pollutants exposures: a scoping review. *J Expo Sci Environ Epidemiol* 2025;35:522 – 534. <https://doi.org/10.1038/s41370-024-00717-3>.
- Lazarevic N, Barnett AG, Sly PD, Knibbs LD. Statistical Methodology in Studies of Prenatal Exposure to Mixtures of Endocrine-Disrupting Chemicals: A Review of Existing Approaches and New Alternatives. *Environ Health Perspect* 2019;127:26001. <https://doi.org/10.1016/j.envint.2022.107280>.
- Chung MK, House JS, Akhtari FS et al. Decoding the exposome: data science methodologies and implications in exposome-wide association studies (ExWASs). *Exposome* 2024;4:osae001. <https://doi.org/10.1093/exposome/osae001>.
- Gibson EA, Goldsmith J, Kioumourtoglou M. Complex Mixtures, Complex Analyses: an Emphasis on Interpretable Results. *Curr Environ Health Rep* 2019;6:53 – 61. <https://doi.org/10.1007/s40572-019-00229-5>.
- Gibson EA, Nunez Y, Abuawad A et al. An overview of methods to address distinct research questions on environmental mixtures: an application to persistent organic pollutants and leukocyte telomere length. *Environ Health* 2019;18:76. <https://doi.org/10.1186/s12940-019-0515-1>.
- Xu H, Chen C, Lu ZX, Nie Z. rccsci: An R Package for Visualization of Restricted Cubic Spline. *Med Research* 2025;00:1 – 6. <https://doi.org/10.1002/mdr2.70015>.
- Knol MJ, VanderWeele TJ. Recommendations for presenting analyses of effect modification and interaction. *Int J Epidemiol* 2012;41:514 – 520. <https://doi.org/10.1093/ije/dyr218>.
- Billionnet C, Sherrill D, Annesi-Maesano I. Estimating the health effects of exposure to multi-pollutant mixture. *Ann Epidemiol* 2012;22:126 – 141. <https://doi.org/10.1016/j.annepidem.2011.11.004>.
- Zhu G, Wen Y, Cao K et al. A review of common statistical methods for dealing with multiple pollutant mixtures and multiple exposures. *Front Public Health* 2024;12:1377685. <https://doi.org/10.3389/fpubh.2024.1377685>.