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# A Practical Meta-analytic Framework for Integrating Diverse Biological Endpoints in Pediatric Environmental Health Research

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# Abstract

Environmental health studies of pesticide exposure in children often report outcomes using different effect measures, laboratory methods, and statistical formats, making quantitative synthesis difficult. To address these challenges, we developed a structured meta-analytic framework designed for studies of prenatal and early-life pesticide exposure. The framework covers study selection using a PECO structure, customized data extraction, conversion of diverse effect sizes, estimation of missing dispersion measures from incomplete summaries, and harmonization of outcomes across immunological, genotoxic, and epigenetic domains. It includes procedures for pooling correlation coefficients, odds ratios, standardized mean differences, gene expression fold changes, and DNA methylation measures, as well as strategies for handling multiple correlated outcomes within studies. We also adapted quality assessment and heterogeneity interpretation tools for environmental epidemiology and incorporated sensitivity analyses for imputed statistics and alternative synthesis methods. The framework was applied to 45 studies examining pesticide-associated immunotoxicity and genotoxicity in children under 5 years of age. Despite substantial variation in exposure assessment, biological endpoints, and reporting style, the approach supported quantitative synthesis and biologically meaningful subgroup analyses. This framework may improve the rigor, transparency, and reproducibility of meta-analyses in pediatric environmental health research and may be adapted to other complex exposure-outcome settings.


# 1. Introduction

Children and pregnant women face heightened vulnerability to pesticide toxicity due to several physiological factors. Developing organ systems, including the immune system, and genetic regulatory mechanisms, demonstrate increased sensitivity to environmental toxicants during critical windows of maturation. Additionally, children's higher metabolic rates and greater chemical intake per kilogram of body weight amplify their exposure burden compared to adults (1, 2). The prenatal period and first years of life represent particularly sensitive timeframes when environmental exposures can fundamentally alter immune system development and genomic integrity, potentially resulting in long-term health consequences (3, 4).

Current scientific literature reveals important gaps in our understanding of pesticide-induced toxicity across multiple biological systems in young populations. While numerous individual investigations have documented associations between pesticide contact and altered biological markers in children, the field lacks a comprehensive quantitative synthesis to establish the overall effect magnitude and consistency across studies. Previous systematic reviews have not adequately addressed the critical developmental windows of prenatal and early postnatal life when biological programming is most susceptible to disruption. Furthermore, substantial variation in how researchers assess exposure, define study populations, and measure health outcomes has prevented clear identification of which biological pathways are most reliably impacted by pesticide contact. The range of observed effects spans from immunosuppression—characterized by diminished lymphocyte counts and increased infection vulnerability—to pro-inflammatory and allergic responses, including elevated cytokine production and

enhanced sensitization patterns, as well as genotoxic effects including DNA damage, cytogenetic alterations, epigenetic modifications, and dysregulated gene expression (5).

Meta-analyses of environmental health studies, particularly those examining pesticide effects on biological system development, present unique methodological challenges not typically encountered in clinical research. Unlike randomized controlled trials with standardized outcome measures and consistent statistical reporting, environmental epidemiological studies exhibit substantial heterogeneity in exposure assessment methods, outcome definitions, and statistical approaches. The assessment is particularly complex due to diverse pesticide classes with distinct mechanisms of action, multiple biological parameters measured across different developmental stages, varied exposure assessment methods ranging from self-report to biomarker analysis, heterogeneous outcome measures including immunological markers, DNA damage indicators (comet assay parameters, micronuclei frequency), gene expression levels, and DNA methylation profiles and inconsistent statistical reporting formats including correlations, odds ratios, and mean differences. Here, we describe a comprehensive methodological framework addressing common challenges in data extraction, statistical synthesis, and heterogeneity assessment. The framework was developed and applied in our systematic reviews examining genotoxic and immunotoxic effects of prenatal and early childhood pesticide exposure in children under 5 years of age (6, 7).

## 2. Methods

# 2.1 Search Strategy and Study Selection Framework

We firstly developed a structured Population, Exposure, Comparator, Outcome (PECO) framework. The population included pregnant mothers (if placental transfer or tissue was investigated) and children aged 0–5 years. Exposure included any pesticide exposure measured through biomonitoring, environmental monitoring, or validated questionnaires. The comparator group consisted of unexposed or low-exposed reference groups. Outcomes included quantifiable biological parameters including genotoxic markers (DNA damage indicators, cytogenetic abnormalities, DNA methylation changes, gene expression alterations), hematological indices, cytokine levels, immunoglobulin concentrations, and functional immune assays.

# 2.2 Data Extraction Protocol

We developed custom Microsoft Excel templates incorporating specific data fields relevant to environmental health studies. Study characteristics included publication details, study design, geographic location and data collection timeframe, sample size and demographic characteristics. Exposure data included pesticide classes and specific compounds, exposure assessment methods, exposure timing, and quantitative exposure measures with units. Outcome data included specific biological parameters measured, analytical methods and laboratory techniques, units of measurement and detection limits, and statistical measures. Statistical data extraction focused on effect estimates

with confidence intervals, sample sizes for exposed and control groups, adjustment variables and confounders, and subgroup analyses by age, sex, or exposure level.

A critical challenge in environmental health meta-analyses involves extracting appropriate statistical measures from studies with diverse reporting formats. We developed a decision tree as displayed in Fig. 1 to systematically handle the diverse statistical reporting formats encountered in environmental health studies. We also developed specific protocols for common scenarios where standard deviations were not directly reported. When studies reported only geometric means with 95% confidence intervals, we approximated standard deviations using established methods. We calculated the standard error of the mean as (Upper CI - Lower CI) / (2 × 1.96), then approximated the standard deviation as SEM × √n (8). For pooled standard deviations across exposed and control groups, we used the established pooling formula. The specific conversion formulas and statistical assumptions for each scenario are detailed in Table 1.

For studies presenting only median values with interquartile ranges or box plots, we used established conversion methods. When Q1 and Q3 (IQR) were available and approximate normality was possible, we used the simple approximation SD ≈ (Q3 − Q1) / 1.349 (9); otherwise, we applied the more robust Wan et al. (2014) / Luo et al. (2018) estimators (9, 10) to obtain estimated means and SDs from median/IQR or median/min–max summaries. In studies where no numeric summaries were extractable, and only box plots or visual representations were available (e.g., comet assay tail length distributions), we approximated 95% CI ranges visually with Plot Digitizer to estimate SD only as a last resort when authors did not respond to data requests (11).

# 2.3 Meta-analytical Approach

We developed a systematic approach for handling diverse effect size measures commonly encountered in environmental toxicology studies. This approach included three major categories of outcomes: correlation-based associations, categorical outcomes with odds ratios, and continuous outcomes requiring standardized mean differences or specialized transformations.

# 2.3.1 Correlation Coefficients

For studies reporting Pearson coefficients between pesticide concentrations and biological parameters, we transformed each r to Fisher's z to stabilize variance and improve normality (12). The Fisher's z transformation is: $z = 0.5 * \ln((1 + r) / (1 - r))$. We then computed the variance of z as: $Var(z) = 1 / (n - 3)$ where n is the sample size. The standard error (SE) of z is the square root of this variance: $SE(z) = \sqrt{Var(z)}$.

However, when studies reported Spearman correlations, we treated Fisher-z pooling as an approximation (appropriate for moderate/large n) and explicitly noted those studies; sensitivity analyses excluding Spearman-only results were pre-planned (13). In the meta-analysis, we pooled the z-values using a random-effects model.

## 2.3.2 Odds Ratios and Relative Risk

For studies reporting a relative risk (RR), we converted it to an odds ratio (OR) using the formula: RR = OR / (1 - $p_0$ + $p_0$ * OR) where $p_0$ is the assumed baseline risk (14). This conversion was used only for unadjusted measures.

For studies that reported OR on a binary outcome, we first log-transformed the OR: ln(OR) = ln(OR). Next, we derived the standard error of ln(OR) from the 95% CI using: SE(ln(OR)) = (ln(OR_upper) - ln(OR_lower)) / (2 * 1.96) (15).

When adjusted odds ratios were available, we applied the same formula using the adjusted OR and its CI. These ln(OR) values and their standard errors were then entered into a separate meta-analysis using a random-effects model. We specified the between-study variance estimator ($\tau^2$) a priori and used REML (restricted maximum likelihood) as the primary estimator (16). Random-effects models is better than fixed-effects approaches in this regard because environmental studies exhibit substantial heterogeneity as explained in section 2.6.

## 2.3.3 Standardized Mean Differences for Continuous Outcomes

For continuous outcome measures (e.g., DNA damage parameters, immune cell counts), we calculated standardized mean differences (SMD) using: SMD = (Mean_exposed - Mean_control) / SD_pooled, where the pooled standard deviation was calculated as: SD_pooled = √[((n_exposed − 1) × SD_exposed² + (n_control − 1) × SD_control²) / (n_exposed + n_control − 2)] (17). When computing standardized mean differences (SMDs), we used Hedges' g (small-sample correction) rather than raw Cohen's d, and applied the correction especially when group sizes were small (18).

The standard error of the SMD was determined using: SE_SMD = √[(n_exposed + n_control) / (n_exposed × n_control) + SMD² / (2(n_exposed + n_control − 2))] (19). This approach was particularly essential for synthesizing genotoxicity studies reporting DNA damage parameters (tail length, tail moment) and immunotoxicity studies with continuous markers measured on different scales.

## 2.3.4 Gene Expression Meta-Analysis

For gene expression studies reporting fold changes (FC), we standardized all values to $\log_2$ fold change for consistency: $\text{Log}_2\text{FC} = \log_2(\text{FC})$ (20).

For genes reported as "X-fold lower" in expression, we calculated the reciprocal (1/X) before log transformation to maintain directional consistency (21). Standard errors (SE) for each effect size were calculated using the reported p-values and degrees of freedom (df) according to:

SE = |ln(fold change)| / t-critical, where t-critical represents the critical t-value corresponding to the reported p-value and study-specific degrees of freedom (22, 23). For studies reporting sample sizes, df

was calculated as total sample size − 2.

To address biological heterogeneity in gene function, we grouped genes into functional pathways (inflammatory response, oxidative stress response, DNA damage/repair, cell signaling/metabolism). For pathway-level analysis, we calculated weighted averages of $\log_2$ fold changes within each pathway and study using inverse variance weighting. The pooled standard error for pathway-level effects was calculated as: $SE_pooled = 1 / \sqrt{(\sum(1/SE^2))}$, where SE represents the standard error of individual gene effect sizes within each pathway (8). This pathway-based approach reduced the dimensionality of gene expression data while preserving biological interpretability.

# 2.3.5 DNA Methylation Data Standardization

DNA methylation studies presented unique challenges due to diverse reporting formats including beta coefficients (percentage methylation change), odds ratios, and log fold changes from methylation arrays. We standardized all effect measures to Cohen's d values to facilitate comparison across studies.

For studies reporting beta coefficients (β) representing percentage methylation change: $d = \beta / SD$, where SD represents the standard deviation of methylation levels for the specific gene or region (24). When SD values were not directly reported, we estimated them from the reported mean methylation levels and ranges or imputed from similar studies when methodologically justified.

For studies reporting odds ratios for methylation changes: $d = \ln(OR) \times \sqrt{3} / \pi$, where the factor $\sqrt{3}/\pi$ (approximately 0.5513) converts the log odds scale to an approximate standardized mean difference (25).

For studies reporting log fold changes (logFC) in M-values from methylation arrays: $d = logFC / SD$, where SD was estimated from reported distributions of M-values (26). Standard errors for the converted Cohen's d values were calculated from the reported confidence intervals using: SE = (Upper CI - Lower CI) / (2 × 1.96). These SE values were then used to calculate variance ($var_d = SE^2$) for inverse variance weighting in the meta-analysis (18). Given the heterogeneity in gene functions and methylation contexts, we conducted subgroup analyses by sample type (blood, placenta, cord blood), gene function (e.g., imprinted genes, metabolic regulation genes), and pesticide class to explore sources of between-study heterogeneity.

Table 1

**Statistical Conversion Formulas for Environmental Health Meta-analyses.** This table presents standardized approaches for converting diverse statistical measures commonly encountered in environmental toxicology studies (including immunotoxicity, genotoxicity, gene expression, and epigenetic studies) to formats suitable for meta-analysis, including required assumptions and methodological considerations for each conversion type.

| Scenario | Available Data | Conversion Formula | Notes/Assumptions |
|---|---|---|---|
| **Standard Deviation from Confidence Intervals** | Geometric mean with 95% CI | SE = (Upper CI - Lower CI) / (2 × 1.96)<br><br>SD = SE × √n | Assumes normal distribution on log scale |
| **SD from Interquartile Range** | Median, Q1, Q3 | SD ≈ (Q3 - Q1) / 1.349<br><br>For non-normal: Use Wan et al. (2014) / Luo et al. (2018) estimators | 1.349 factor assumes normality; use robust estimators otherwise |
| **Pooled Standard Deviation** | $SD_1$, $SD_2$, $n_1$, $n_2$ | $SD_{pooled} = \sqrt{[((n_1-1)SD_1^2 + (n_2-1)SD_2^2) / (n_1+n_2-2)]}$ | For two-group comparisons |
| **Pearson to Fisher's z** | Pearson correlation (r) | $z = 0.5 \times \ln[(1 + r)/(1-r)]$<br><br>$Var(z) = 1/(n-3)$<br><br>$SE(z) = \sqrt{[1/(n-3)]}$ | Stabilizes variance; back-transform for interpretation |
| **Spearman to Fisher's z** | Spearman correlation (ρ) | $z \approx 0.5 \times \ln[(1 + \rho)/(1-\rho)]$<br><br>Approximation for large n | Requires n ≥ 10; conduct sensitivity analysis |
| **Log Odds Ratio** | OR with 95% CI | ln(OR) = ln(OR)<br><br>SE[ln(OR)] = [ln(OR_upper) - ln(OR_lower)] / (2 × 1.96) | Use for both adjusted and unadjusted ORs |
| **Relative Risk to Odds Ratio** | RR, baseline risk ($p_0$) | $OR = RR \times (1-p_0) / (1 - RR \times p_0)$<br><br>Rearranged: $RR = OR / (1 - p_0 + p_0 \times OR)$ | Only for unadjusted measures; requires baseline risk estimate |
| **Standardized Mean Difference** | $Mean_1$, $Mean_2$, SD_pooled, n | Hedges' $g = (Mean_1 - Mean_2) / SD_{pooled} \times J$ | J is small-sample correction factor. Essential for genotoxicity studies with continuous outcomes |

Note: All formulas assume appropriate data distributions and sufficient sample sizes. When assumptions are violated, conduct sensitivity analyses. For gene expression and DNA methylation data, consider biological heterogeneity through pathway-based grouping and functional subgroup analyses.

| Scenario | Available Data | Conversion Formula | Notes/Assumptions |
|---|---|---|---|
| | | $J = 1-3/(4(n_1+n_2-2)-1)$<br><br>$SE_SMD = \sqrt{[(n_1 + n_2)/(n_1 \times n_2) + SMD^2/(2(n_1 + n_2 - 2))]}$ | |
| **Gene Expression (Fold Change to $Log_2$)** | Fold change (FC), p-value, df | $Log_2FC = log_2(FC)$<br><br>For X-fold lower: $Log_2FC = log_2(1/X)$<br><br>$SE = \lvert ln(FC) \rvert / t\text{-critical}$<br><br>where $df = n - 2$ | Calculate t-critical from p-value and df. Group genes by biological pathway for meta-analysis |
| **Gene Expression (Pathway Aggregation)** | Multiple genes per pathway with $Log_2FC$ and SE | Weighted average: $\Sigma(w_i \times Log_2FC_i) / \Sigma w_i$<br><br>where $w_i = 1/SE_i^2$<br><br>Pooled SE = $1/\sqrt{(\Sigma(1/SE^2))}$ | Reduces dimensionality while preserving biological meaning. Apply to inflammatory, oxidative stress, DNA repair pathways |
| **DNA Methylation (Beta to Cohen's d)** | β coefficient (%), SD of methylation | $d = \beta / SD$ | SD from reported methylation distributions or estimated from similar studies |
| **DNA Methylation (OR to Cohen's d)** | OR for methylation change | $d = \ln(OR) \times \sqrt{3}/\pi$<br><br>($\sqrt{3}/\pi \approx 0.5513$) | Converts log odds scale to standardized mean difference |
| **DNA Methylation (logFC to Cohen's d)** | logFC from methylation arrays (M-values), SD | $d = logFC / SD$ | SD estimated from M-value distributions. Subgroup by sample type and gene function |
| Note: All formulas assume appropriate data distributions and sufficient sample sizes. When assumptions are violated, conduct sensitivity analyses. For gene expression and DNA methylation data, consider biological heterogeneity through pathway-based grouping and functional subgroup analyses. | | | |

# 2.3.6 Handling Multiple Correlated Effect Sizes

To address multiple correlated effect sizes reported within the same study (for example, several immune parameters or multiple pesticides measured in the same cohort, or multiple genes or DNA methylation sites), we pre-specified one of the following approaches depending on data availability: (i) select a single effect per study per outcome domain (pre-specified selection rules), or (ii) conduct multilevel/multivariate meta-analyses or apply robust variance estimation (RVE) to account for within-study dependence (27). For gene expression and DNA methylation studies with multiple related outcomes, we preferentially used pathway-level aggregation or functional grouping before meta-analysis to reduce dependency. Sensitivity analyses compared results across these approaches.

## 2.4 Publication Bias Assessment

Publication bias was evaluated visually with funnel plots and with regression-based tests (e.g., Egger's test) when there were sufficient studies. Asymmetry interpretation considered mechanistic asymmetry where different pesticide classes may show directionally opposite effects, methodological asymmetry where varied exposure assessment methods may create apparent bias, and true publication bias from selective reporting of significant results. Sensitivity analyses were conducted when outliers or asymmetry were detected in funnel plots by excluding studies with approximated standard deviations or very small sample sizes. Any funnel-plot-based sensitivity analyses (e.g., trim-and-fill), given the limited number of studies typically available in environmental health meta-analyses, were interpreted cautiously and considered exploratory (28) taking into consideration genuine mechanistic differences between exposures, methodological diversity across studies, and limited statistical power for bias detection.

## 2.5 Statistical Software Implementation

We used IBM SPSS version 29 with specific meta-analysis extensions for different effect size types. Forest plots were generated for each meta-analysis showing individual study effect estimates with confidence intervals, study weights, overall pooled estimate with confidence interval, and $I^2$ statistic for heterogeneity assessment (29). For correlation meta-analysis, we used Fisher's z as the effect size with its standard error. For odds ratio meta-analysis, we used the natural logarithm of the odds ratio as the effect size with its standard error. For mean differences, we used standardized mean differences with their standard errors when converting studies with different units or scales. All analyses employed random-effects models with inverse variance weighting to account for cross-study heterogeneity (30). Where specific estimators or inferential options (e.g., REML with Hartung–Knapp, multilevel random-effects, or robust variance estimation) were required and not available in SPSS, we performed confirmatory/sensitivity analyses in R using the metafor and robumeta packages (16, 31).

## 2.6 Quality Assessment Adaptation

We adapted the Newcastle-Ottawa Scale for environmental health studies following our established framework (6, 7). For cohort studies, selection assessment evaluated representativeness of exposed cohort, selection of non-exposed cohort, ascertainment of exposure, and demonstration of outcome not present at start. Comparability assessment evaluated cohort comparability based on study design/analysis and control for important confounding factors. Outcome assessment considered assessment methods, sufficient follow-up length, and adequacy of follow-up.

For cross-sectional studies, we developed a modified assessment tool based on NOS principles. Selection domain evaluated sample representativeness and non-response rate. Data domain assessed exposure measurement accuracy, outcome measurement accuracy, and comparability of exposure and outcome measures across the study design. Rather than traditional "outcome" assessment, cross-sectional studies were evaluated on data quality and analytical rigor.

Case-control studies followed similar domains with specific emphasis on case definition validity, control selection appropriateness, and comparability of exposure ascertainment methods between cases and controls.

## 2.7 Heterogeneity Assessment and Interpretation

Given the unique challenges in environmental health research—including diverse pesticide classes, varying biological parameters, different exposure timings, and methodological differences across studies—we developed a structured framework for interpreting and managing heterogeneity using Cochran's Q and $I^2$ statistics. Our approach provides specific guidance for each level of heterogeneity, from low ($I^2$ = 0–25%) to considerable ($I^2$ = 75–100%), with corresponding recommended actions and reporting standards detailed in Table 2. This framework addresses not only statistical heterogeneity but also the mechanistic, temporal, measurement, and population-based sources of variation commonly encountered in environmental health meta-analyses.

Table 2

**Framework for Environmental Health Meta-analyses.** This framework provides structured guidance for interpreting $I^2$ statistics, identifying likely sources of heterogeneity (including biological pathway differences, exposure timing variations, outcome measurement heterogeneity), implementing appropriate analytical strategies, and reporting results based on the level of between-study variation observed in environmental health meta-analyses.

| $I^2$ Range | Interpretation | Likely Sources | Recommended Actions | Reporting Guidance |
|---|---|---|---|---|
| **0–25%** | Low heterogeneity | - Similar pesticide classes<br>- Consistent exposure assessment<br>- Homogeneous populations<br>- Similar outcome measures<br>- Comparable biological pathways | - Proceed with pooled estimate<br>- Report combined effect<br>- Check for outliers<br>- Consider fixed-effects as sensitivity | "Studies showed consistent effects across populations"<br>- Report: Pooled estimate, CI, p-value |
| **25–50%** | Moderate heterogeneity | - Mixed exposure assessment methods<br>- Different study designs<br>- Varying adjustment factors<br>- Different exposure windows<br>- Mixed biological endpoints (immune vs. genotoxic) | - Explore methodological differences<br>- Conduct pre-planned subgroups<br>- Meta-regression if $n \geq 10$<br>- Sensitivity analyses | "Moderate heterogeneity observed"<br>- Describe explored sources<br>- Report subgroup results |
| **50–75%** | Substantial heterogeneity | - Different pesticide classes<br>- Diverse biological parameters (cytokines vs. DNA damage vs. methylation)<br>- Varied age groups<br>- Geographic/genetic differences<br>- Exposure timing variations<br>- Different analytical platforms (gene expression, epigenetic assays) | - Mandatory subgroup analysis<br>- Stratify by pesticide class<br>- Separate by outcome type<br>- Separate by biological pathway<br>- Consider prediction intervals<br>- Robust variance estimation | "Substantial heterogeneity necessitated subgroup analyses"<br>- Present stratified results<br>- Discuss clinical implications |
| **75–100%** | Considerable heterogeneity | - Fundamentally different exposures | - Question pooling appropriateness | "High heterogeneity precluded |

**Note**: Cochran's Q test should supplement $I^2$ interpretation. Consider $\tau^2$ (between-study variance) for absolute heterogeneity assessment.

| $I^2$ Range | Interpretation | Likely Sources | Recommended Actions | Reporting Guidance |
|---|---|---|---|---|
| | | - Opposing biological mechanisms (immunosuppression vs. inflammation; hypermethylation vs. hypomethylation)<br><br>- Major methodological differences<br><br>- Publication bias<br><br>- Small study effects<br><br>- Mixed outcome domains | - Consider narrative synthesis<br><br>- Report ranges of effects<br><br>- Extensive subgroup analyses<br><br>- Individual study discussion<br><br>- Check for errors in data extraction and/or conversions | meaningful pooling"<br><br>- Provide narrative synthesis<br><br>- Display forest plot without pooled estimate<br><br>- Discuss patterns and outliers |
| **Additional Considerations for Environmental Health Studies**: | | | | |
| **Mechanistic heterogeneity** | | Different pesticides may have opposing effects (e.g., immunosuppression vs. pro-inflammatory). | | |
| **Temporal heterogeneity** | | Effects may vary by exposure timing (prenatal vs. postnatal) and duration. | | |
| **Measurement heterogeneity** | | Biomarker vs. questionnaire assessment may yield different effect magnitudes. Different gene expression platforms (qPCR vs. microarray) may produce varying fold changes | | |
| **Biological pathway heterogeneity** | | Different genes within a pathway may respond differently. Immune outcomes vs. genotoxic outcomes vs. epigenetic outcomes represent distinct biological mechanisms | | |
| **Population heterogeneity** | | Genetic polymorphisms (e.g., PON1, GSTT1) and nutritional status may modify pesticide effects across populations | | |
| **Outcome heterogeneity** | | Combining diverse endpoints (WBC counts, cytokines, DNA damage, gene expression, methylation) increases heterogeneity despite biological relationships | | |
| **Note**: Cochran's Q test should supplement $I^2$ interpretation. Consider $\tau^2$ (between-study variance) for absolute heterogeneity assessment. | | | | |

# 3. Validation Results

# 3.1 Framework Application

This methodological framework was successfully applied to synthesize evidence from 45 studies examining pesticide-associated genotoxicity and immunotoxicity in children (6, 7). The approach enabled successful meta-analysis of diverse biological endpoints including 12 immune parameters, DNA damage indicators (comet assay, micronuclei), gene expression across multiple pathways, and DNA methylation at various genomic loci, quantitative synthesis despite diverse statistical reporting formats,

meaningful subgroup analyses by pesticide class and biological parameter type, and robust heterogeneity assessment and interpretation.

## 3.2 Key Methodological Outcomes

Data extraction using our protocols achieved a near-complete success rate, with all included studies yielding extractable quantitative data. Meta-analysis feasibility was demonstrated with multiple meta-analyses completed with two or more studies per outcome across immunological, genotoxic, and epigenetic endpoints. Heterogeneity management showed $I^2$ values ranging from 0% to 99%, with successful subgroup analyses reducing heterogeneity in 8 of 12 analyses. Quality assessment reliability achieved inter-rater agreement $\kappa = 0.84$ for adapted quality assessment tools.

## 3.3 Methodological Insights

Our methods for estimating missing standard deviations from geometric means and confidence intervals proved essential for including key studies. Specialized conversion techniques for gene expression (fold change to $\log_2$FC) and DNA methylation data (diverse formats to Cohen's d) enabled synthesis across heterogeneous reporting formats that would otherwise have been incomparable. Pre-planned subgroup analyses by pesticide class and biological pathway substantially reduced heterogeneity and revealed mechanistically meaningful patterns. High heterogeneity across diverse biological outcomes validated our priori decision to use random-effects models. Modified quality assessment tools were found suitable to assess the quality of environmental health studies. Pathway-based aggregation of gene expression data and functional grouping of DNA methylation results proved to be valuable for managing within-study dependencies and enhancing biological interpretability.

# 4. Discussion

This methodological system addresses several fundamental obstacles in environmental health meta-analyses. By providing a systematic strategy for managing correlations, odds ratios, and mean differences, as well as specialized techniques for gene expression and epigenetic data, it facilitates synthesis across varied study designs and statistical approaches. The protocols for estimating standard deviations from geometric means and confidence intervals substantially expand the number of studies eligible for quantitative integration. The standardization procedures for converting diverse DNA methylation measures (beta coefficients, odds ratios, log fold changes) to comparable effect sizes enable synthesis across studies using different analytical platforms and reporting conventions. Pre-planned subgroup evaluations by exposure type, biological pathway, and outcome category deliver mechanistic insights while addressing statistical heterogeneity. Modified quality evaluation instruments better reflect the specific methodological considerations relevant to environmental epidemiological investigations.

Besides the advantages, several limitations and considerations must also be recognized. While our techniques for estimating absent statistical parameters are well-established, they introduce additional uncertainty that should be considered in sensitivity evaluations. Conversions of DNA methylation effect

measures and gene expression data to standardized formats require assumptions about underlying distributions and may not fully capture the complexity of epigenetic regulation or transcriptional responses. Environmental health meta-analyses often incorporate fewer studies than clinical meta-analyses, limiting statistical power and publication bias assessment capabilities, mainly because environmental exposures often involve complex mixtures and multiple pathways (32), creating heterogeneity that may not be fully captured by standard meta-analytical approaches. In addition, environmental health effects may vary significantly by exposure timing and duration, requiring careful consideration of temporal factors in subgroup evaluations. Biological heterogeneity across outcome types (immunological versus genotoxic versus epigenetic) necessitates careful interpretation of pooled estimates and emphasizes the importance of mechanism-specific subgroup analyses.

This methodological system is readily adaptable to other environmental health systematic reviews, particularly those examining chemical exposures and developmental outcomes, environmental toxicants and and multiple biological system effects, pollutant exposures and respiratory health, and endocrine disruptor effects on child development. For different applications, we recommend modifying the PECO framework for specific exposure-outcome relationships, adapting quality evaluation instruments for relevant study designs, developing exposure-specific subgroup analysis plans, and considering mechanism-specific heterogeneity sources.

The framework's effective implementation to pesticide toxicity research demonstrates its value for synthesizing complex environmental health evidence. The methodological principles may help standardize meta-analytical approaches in this important but methodologically challenging field. Future refinements might include development of automated data extraction tools for environmental health studies and expanded guidance for mixture exposure analyses. As environmental health research continues to grow in importance, standardized meta-analytical approaches will be essential for translating research evidence into effective public health policies.

# 5. Conclusions

This comprehensive meta-analytical system provides a standardized approach for conducting systematic reviews and meta-analyses in environmental toxicology including immunotoxic, genotoxic, and epigenetic endpoints. The methodology successfully addresses common challenges including diverse statistical presentation formats, absent parameters, heterogeneous biological outcome measures across different mechanistic domains, and substantial heterogeneity. The methodological principles applied in this framework are adaptable to other environmental health topics requiring synthesis of evidence across multiple biological systems and diverse analytical platforms.

# Declarations

**Ethics approval and consent to participate**

Not applicable. This study is a methodological framework paper that does not involve human participants, human data, or human tissue.

**Consent for publication**

Not applicable.

**Availability of data and materials**

Data will be made available on request.

**Competing interests**

The authors declare that they have no competing interests.

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**Authors' contributions**

Moustafa Sherif and Aya Darwish contributed equally to conceptualization, methodology, formal analysis, data curation, software, investigation, and writing of the original draft. Aya Samy contributed to methodology, software, data curation, and writing of the original draft. Shimaa Sami contributed to investigation, data curation, and writing of the original draft. Balázs Ádám contributed to conceptualization, supervision, and review & editing of the manuscript. All authors read and approved the final manuscript.

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# Figures

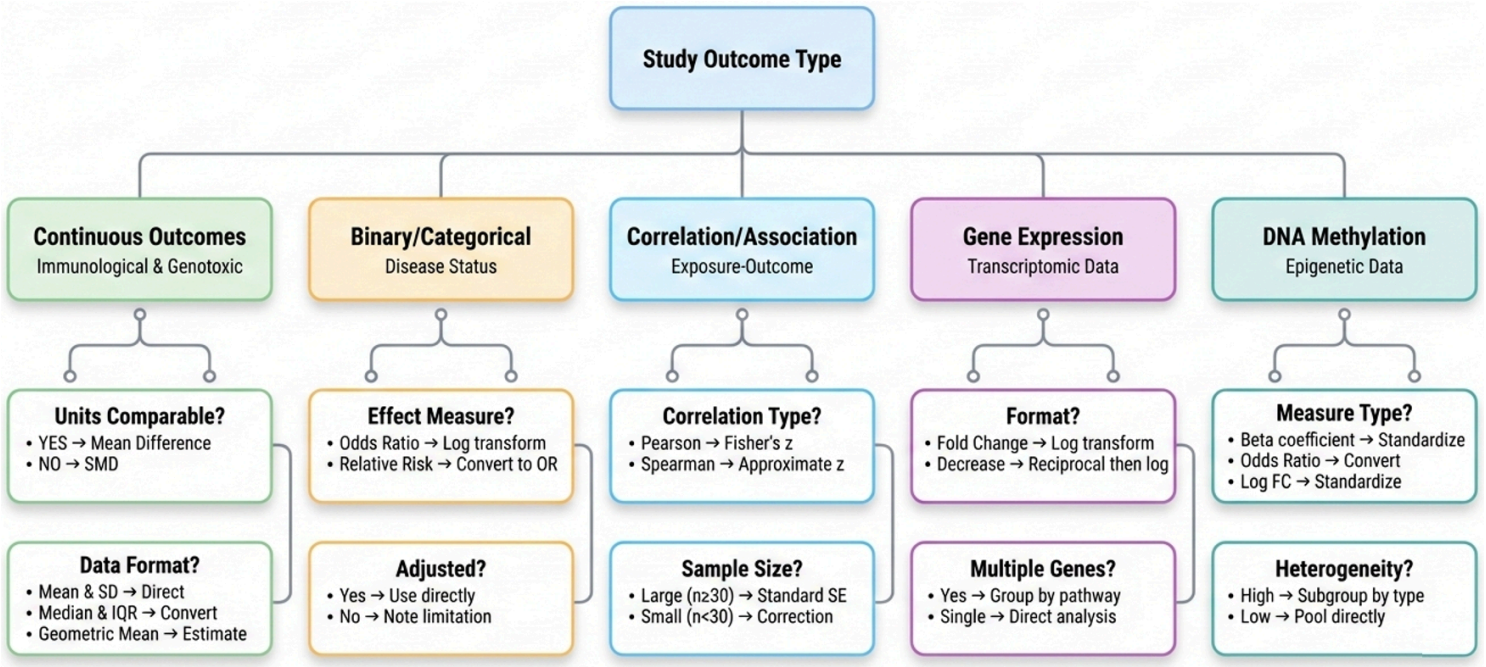



**Figure 1**

**Decision tree for effect size selection and standardization in environmental health meta-analysis.** The flowchart guides researchers through appropriate effect size calculations based on study outcome types (immunological, genotoxic, epigenetic) and available data formats to ensure consistent effect size calculation across diverse environmental epidemiological studies.