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A Sex-Specific Computational Framework for Ecological Risk Characterisation of Contaminants

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We use this protocol and it's working



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Disclaimer

The protocol provided here is intended for educational purposes only and should be conducted in appropriate laboratory settings. The developers of this protocol are not liable for any damages or consequences arising from its use. Users should ensure compliance with relevant safety regulations and ethical guidelines when conducting experiments involving live organisms.

Abstract

Ecological risk assessments often rely on pooled-sex toxicity data, which can obscure critical differences in susceptibility between males and females. This protocol outlines a standardised, multi-tiered computational framework for determining Hazard Quotients (HQs), Toxic Units (TUs), and Acute Risk Quotients (RQs). While validated here using Polycyclic Aromatic Hydrocarbons (PAHs) in the amphipod *Parhyale hawaiiensis*, this methodology is contaminant-agnostic. It is designed for application to any environmental pollutant (e.g., pesticides, pharmaceuticals) where sex-specific toxicity endpoints (LC50, EC50, EC10) and environmental monitoring data are available.

Materials

To execute this protocol, the following datasets are required:

a. Biological Endpoints (Sex-Specific):

- LC50: Lethal Concentration killing 50% of the population (Acute).
- EC50: Effective Concentration affecting 50% of the population (Acute/Sublethal, e.g., immobility).
- EC10: Effective Concentration affecting 10% of the population (Threshold for sublethal effects).

b. Environmental Concentrations:

- Monitoring data from the ecosystem of interest.

1. Introduction and Scope

- 1 This framework moves beyond simple lethality metrics to provide a nuanced view of ecological risk. By integrating three distinct risk indices: TUs for sublethal pressure, HQs for conservative safety limits, and RQs for peak acute risks. Researchers can build a comprehensive risk profile.

Applicability: This protocol applies to hydrophobic organic contaminants, emerging contaminants, and heavy metals.

Key Innovation: The separate calculation of risk indices for males and females allows for the detection of sex-biased risks that standard protocols miss.

2. Data Requirements

- 2 To execute this protocol, the following datasets are required:

- a. **Biological Endpoints (Sex-Specific):**

LC50: Lethal Concentration killing 50% of the population (Acute).

EC50: Effective Concentration affecting 50% of the population (Acute/Sublethal, e.g., immobility).

EC10: Effective Concentration affecting 10% of the population (Threshold for sublethal effects).

- b. **Environmental Concentrations:**

Monitoring data from the ecosystem of interest.

3. Procedure: Establishing Environmental Scenarios

- 3 Rationale: Risk is not static. To capture the variability of exposure in aquatic ecosystems, three exposure scenarios must be established for the contaminant of interest.

- 3.1 Data Curation: Compile reported environmental concentrations ($\mu\text{g/L}$) from literature or field sampling within the organism's distribution range. Convert all concentrations to $\mu\text{g L}^{-1}$ and exclude records lacking adequate metadata (sampling depth, matrix type, analytical method detection limits).

- 3.2 Scenario Calculation: For each contaminant, calculate the following:

- 3.3 Median Concentration: Represents the central tendency (typical background exposure).

- 3.4 90th Percentile: Represents the upper boundary of routine exposure.

- 3.5 **Maximum Concentration:** Represents the worst-case scenario (e.g., spills, point-source discharge).
This tiered approach aligns with probabilistic environmental risk assessment frameworks (e.g., EFSA, 2021; OECD, 2019; EPA, 2024), enabling researchers to distinguish between chronic, low-level risks and acute, high-level hazards.

4. Procedure: Hazard Quotient (HQ) Characterisation

- 4 **Rationale:** The HQ provides a conservative estimate of safety by comparing environmental levels to a 'Predicted No-Effect Concentration' (PNEC).
- 4.1 **Determine the Assessment Factor (AF):** Select an AF based on data availability. For acute toxicity data (LC₅₀), use 1000 to account for lab-to-field extrapolation and interspecies variability.
- 4.2 **Calculate PNEC:**

$$\text{PNEC} = \frac{\text{LC}_{50}}{\text{AF}}$$

Assessment factor = 1000 (default for single-species acute dataset).

- 4.3 **Calculate HQ:** Compute the HQ for the Median, 90th Percentile, and Maximum environmental scenarios:

$$\text{HQ} = \frac{\text{Environmental concentration}}{\text{PNEC}}$$

- 4.4 **Risk Classification:** Interpret the results using the standardised thresholds below:

HQ Value	Hazard Classification	Interpretation
< 0.1	No Hazard Exists	Concentration is well below safety limits.
0.1 - 1.0	Low Hazard	Approaches the safety threshold.
1.1 - 10	Moderate Hazard	Exceeds the conservative safety limit.
> 10	High Hazard	Indicates a high probability of adverse effects.

Case Study Validation: In *P. hawaiiensis*, Phenanthrene presented a 'High' hazard rating (HQ > 10) at maximum concentrations, whereas Naphthalene remained in the 'Low/Moderate' range, highlighting the need for compound-specific management.

5. Procedure: Toxic Unit (TU) Analysis

5 Rationale: TUs are valuable for assessing the threshold of sublethal effects. Because TUs are additive, this metric is the gold standard for assessing mixture toxicity (e.g., sum of TUs for all contaminants in a sample). TUs quantify the magnitude of sublethal toxic stress under different exposure conditions, enabling a threshold-based assessment of sublethal effects, as advocated in the oil toxicity testing frameworks (Parkerton et al., 2023).

5.1 Select Endpoint: Use the EC10 value, which represents the onset of sublethal stress.

5.2 **Calculate TU:**

$$TU = \frac{\text{Environmental concentration}}{EC_{10}}$$

5.3 Implementation: Calculate TUs for each sex and PAH under: Median concentration, 90th percentile concentration, and Maximum concentration

5.4 Interpretation:

- TU < 0.01: negligible sublethal risk

- $0.01 \leq TU \leq 0.1$: low but measurable sublethal stress
- $TU < 1$: The environmental concentration is below the threshold for initiating sublethal effects.
- $TU > 1$: The environmental concentration exceeds the 10% effect level, suggesting sublethal physiological stress is likely occurring.

6. Procedure: Acute Risk Quotient (RQ) Determination

6 Rationale: The RQ assesses the immediate, acute risk posed by peak pollution events. It is less conservative than the HQ but more indicative of immediate population-level impact.

6.1 Select Exposure Scenario: Use the Peak (Maximum) Reported Concentration to model the worst-case event.

6.2 Select Endpoint: Use the EC₅₀ (Effective Concentration for 50% of the population).

6.3 **Calculate RQ:**

$$RQ = \frac{\text{Peak environmental concentration}}{EC_{50}}$$

6.4 Interpretation Thresholds

- $RQ < 0.1$: negligible acute risk
- $0.1\text{--}0.5$: low acute risk
- ≥ 0.5 : significant acute risk (EPA, 2024; Singh et al., 2023).

This sex-specific calculation is essential given observed differences in amphipod PAH sensitivity.

7. Procedure: Sex-Specific Data Analysis

7 Rationale: This is the critical analytical step often missed in standard protocols.

- 7.1 Segregate Data: Perform calculations for Steps 4, 5, and 6 separately for the Male and Female datasets.
- 7.2 Calculate Divergence: Compare the TUs, HQs and RQs between sexes.
- 7.3 Validation: In the validation dataset using PAHs, female *P. hawaiiensis* exhibited an RQ of 11.89 for Phenanthrene, compared to 7.29 for males. *Implication*: A risk assessment based solely on male data (or pooled data) would underestimate the risk to the reproductive female population by nearly 40%.

8. Data Management 6 Reporting

- 8 Automation: For datasets involving multiple contaminants, it is recommended to use statistical software (R or Python) to automate these calculations to prevent manual error.
- 8.1 Visualisation: Present data in a comparative table that aligns molecular weight, LogKow, and risk indices to visualise trends (e.g., increasing hydrophobicity correlating with increased risk).

Conclusion:

The TU–HQ–RQ workflow described above is intentionally generic and may be applied to a broad range of aquatic toxicants beyond PAHs, provided toxicant-specific properties and toxicological endpoints are accounted for during problem formulation. This tiered, percentile-based exposure strategy and the three-metric hazard characterisation (TUs from sublethal thresholds; HQs from PNECs; RQs from acute benchmarks) align with established international guidance for chemical risk assessment and are therefore appropriate for pesticides, pharmaceuticals, polar organics, metals, and other contaminants subject to routine environmental monitoring.

Practical caveats apply: for ionisable or strongly polar compounds, adjust exposure metrics to account for differing bioavailability and use appropriate effect metrics (e.g. chronic NOEC/EC10 derived from water-column assays or internal dose metrics where available). For metals, incorporate speciation and water chemistry (pH, hardness, dissolved organic carbon) into exposure conversion or apply bioavailability models (e.g. the Biotic Ligand Model) before calculating TUs and HQs. For chemicals with chronic, low-dose modes of action (endocrine disruptors, many pharmaceuticals), derive PNECs from chronic endpoints with reduced assessment factors rather than acute LC50 values. When multiple compounds co-occur, consider dose-addition approaches or mixture assessment groups following guidance on grouping and combined exposure.

Protocol references

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Implementation: Calculate TUs for each sex and PAH under: Median concentration, 90th percentile concentration, and Maximum concentration

Interpretation:

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Select Exposure Scenario: Use the Peak (Maximum) Reported Concentration to model the worst-case event.

Select Endpoint: Use the EC50 (Effective Concentration for 50% of the population).

Calculate RQ:

$$RQ = \left(\frac{\text{Peak environmental concentration}}{\text{EC}_{50}} \right)$$

Interpretation Thresholds

- RQ 3c 0.1: negligible acute risk
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