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Peer Review

Review of: "Mixture Toxicity: Evidence from Experimental Studies on Concurrent Chemical Exposures"

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1. General Assessment

This manuscript addresses a critical and pervasive issue in modern toxicology: the inadequacy of single-chemical risk assessment models in a world of complex, concurrent exposures. The author successfully synthesizes a wide range of evidence - spanning mammalian toxicology, aquatic ecotoxicology, and human epidemiology - to argue that "cocktail effects" (synergism and antagonism) are frequent and often unpredictable using standard additivity models.

The inclusion of "non-chemical stressors" (specifically maternal stress) as a modulating factor in chemical toxicity is a significant strength of this paper, adding a layer of ecological and physiological realism often missing from similar reviews.

2. Scientific Strengths

- **Integration of Non-Chemical Stressors:** The review meaningfully expands the definition of "mixture" to include physiological stressors. The evidence presented on how maternal restraint stress exacerbates the toxicity of metals (mercury, arsenic) and PFAS is compelling and highlights a major gap in current regulatory frameworks.
- **Cross-Species Evidence:** The manuscript does an excellent job of triangulating data. It connects mechanistic findings in *Daphnia magna* (aquatic models) with developmental outcomes in rodents and corroborates these with human epidemiological data on liver and kidney function. This "weight of evidence" approach strengthens the conclusion that mixture effects are a biological universal rather than a species-specific artifact.

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- **Critique of Theoretical Models:** The author provides a nuanced critique of the Concentration Addition (CA) and Independent Action (IA) models. The discussion on how "ecotoxicological mode of action" may be a better predictor than "pharmacological mode of action" for certain life-history traits in aquatic organisms is a sophisticated insight that adds theoretical depth.

3. Major Substantive Critiques

A. Discrepancy Between "Systematic" Methods and Narrative Output

The "Methods (Search Strategy)" section describes a "meticulously compiled" and "systematic search" using specific Boolean operators and inclusion criteria. However, the resulting manuscript lacks the standard components of a Systematic Review.

- **Issue:** There is no reporting of the number of studies screened vs. included (e.g., a PRISMA flow diagram), no assessment of the "risk of bias" in the included studies, and no quantitative meta-analysis of the interaction magnitudes.
- **Recommendation:** The author should either reclassify this as a "Narrative Review informed by a systematic search" or add the rigorous quantitative reporting required for a true Systematic Review. As it stands, the "Methods" section promises a level of analytical rigor that the "Results" section does not deliver.

B. Heavy Reliance on Self-Citation in Mammalian Section

While the author is evidently a leading expert in the field, the section "Experiments on Mammals" relies disproportionately on research from the author's own laboratory (referencing *Sánchez et al.*, *Bellés et al.*, *Colomina et al.*, *Torrente et al.*, *Fuentes et al.*).

Issue: By focusing so heavily on one research group's output, the review risks appearing insular. It implies that these synergistic effects might be unique to specific experimental protocols used by that lab.

Recommendation: To demonstrate global consensus, the author must incorporate more mammalian studies from independent laboratories. The "Aquatic Organisms" section is much more balanced in this regard; the mammalian section should match that breadth.

C. Generic "Future Directions"

The conclusion advocates for "mechanistically-informed frameworks" and "high-throughput omics technologies". While correct, these recommendations are standard in the field and lack specific application to the *mixture* problem discussed.

Recommendation: The manuscript would be significantly strengthened by a concrete example or case study. For instance, explaining *how* an Adverse Outcome Pathway (AOP) was used to predict a synergistic interaction that a traditional bioassay missed would move the recommendation from theoretical to practical.

4. Specific Comments on Content

- **Neurotoxicity:** The section on neurotoxicity is particularly effective. The discussion of "silent" neurotoxicity - where low doses of individual chemicals (below No Observed Adverse Effect Levels, or NOAELs) combine to cause measurable deficits - is a critical point for public health protection.
- **PBTK Modeling:** The mention of Physiologically-Based Toxicokinetic (PBTK) modeling is important. However, the text lists it as a tool without explaining *why* it helps with mixtures specifically (e.g., modeling how one chemical inhibits the metabolic clearance of another). Expanding on the mechanism of *metabolic competition* would add valuable mechanistic detail.
- **Recommendation:** To demonstrate global consensus, the author must incorporate more mammalian studies from independent laboratories. The "Aquatic Organisms" section is much more balanced in this regard; the mammalian section should match that breadth.

5. Recommendation

Decision: Major Revisions Required

The manuscript provides a valuable synthesis of the failure of additivity models and the importance of stress as a co-factor in toxicity. However, to be considered a definitive review of the field, the author must:

1. Align the methodology description with the actual output (Narrative vs. Systematic).
2. Diversify the references in the mammalian section to reduce self-citation bias.
3. Provide more concrete examples of how new technologies (omics, AOPs) are solving the specific problems identified.

Declarations

Potential competing interests: No potential competing interests to declare.