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Assessing the Combined Toxicity of Household Cleaning Chemicals on Freshwater Crustacean Communities

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Abstract: Domestic wastewater discharged from households contains surfactants, disinfectants, and oxidizers present in the water as mixtures of chemicals instead of individual compounds. This study is conducted to assess the toxicity of individual compounds and their mixtures on the freshwater crustaceans *Daphnia magna* and *Ceriodaphnia dubia* in laboratory-based experiments through parameters such as immobilization, reproduction, swimming behavior, and abundance tests. Effects of chemical mixtures were compared with the predictions of concentration addition (CA) and independent action (IA) models. Benzalkonium chloride was the most toxic compound with 48h EC50 of 0.12 mg/L for D. magna and 0.07 mg/L for *C. dubia,* with *C. dubia* being more sensitive to all chemicals tested. The SDS + BAC combination was additive in nature; whereas SDS + NaOCl showed antagonism, BAC + NaOCl and ternary mixture showed synergy with ternary mixture showing 74.5% immobilization as opposed to a CA value of 61.4%. Chronic ternary exposure reduced survival to 46%, offspring per female to 24.1, and community abundance by 68.7%. The results show that evaluations of a single substance underestimate the risk associated with household chemicals combined and highlight the need for an ecological risk assessment that takes mixtures into account.

Keywords: Household Chemicals; Combined Toxicity; Freshwater Crustaceans; Aquatic Pollution; Chemical Mixtures; Ecotoxicology; Ecological Risk.

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I. Introduction

1.1 Background of Household Chemical Pollution

The extensive use of household cleaning agents such as detergents, disinfectants, and surface cleaners has resulted in the increased discharge of chemical residues in domestic wastewater (Tiwari et al., 2021). These residues are inadequately treated and then enter the water bodies such as rivers and lakes, where the surfactants and disinfectants can be present at concentrations which have biological significance (Gray et al., 2022). This constant supply makes for continuous exposure to aquatic organisms. Freshwater crustaceans play an important role in the aquatic food chain since they feed on algae and bacteria and also are the source of food for fishes and other invertebrates (Maazouzi et al., 2016). As a result, damage to crustaceans may affect energy flow and lead to inefficient functioning of the ecosystem (de Castro-Català et al., 2016).

1.2 Environmental Concerns of Combined Chemical Exposure

Although the individual components of the cleaning products themselves are capable of affecting the survival, reproduction, and locomotion of the crustaceans at low doses, the simultaneous exposure to multiple substances that occur in the receiving water could result in a different effect than the sum of the individual effects (Di Poi et al., 2018). The interaction between the various chemicals could be additive, synergistic, or antagonistic, depending on the type of action and the nature of the chemicals (Rodney et al., 2013). The reduced survival and reproduction will have an effect on the population growth rate, destabilizing the population (Maazouzi et al., 2016). Without species-specific information, risk assessment based on individual compounds could greatly underestimate potential environmental risks (Köck et al., 2010).

1.3 Research Gap and Problem Statement

Most ecotoxicological experiments usually test the effects of single chemicals, but there is still a need to study the ecological implications of the mixture of household cleaning chemicals (Malev et al., 2022). There are only a few studies on the effect of mixtures on freshwater crustacean assemblages, which makes it hard to forecast their response when exposed to mixtures of chemicals (de Castro-Català et al., 2016). Previous investigations mostly concentrated on pesticide and pharmaceutical mixtures and did not investigate cleaning product mixtures (Rodney et al., 2013). This is the gap that will be filled with this research, through testing single and mixture exposure at species and community levels.

1.4 Research Objectives

The following are the five goals that this experiment aimed to achieve:

1. To study the individual toxicity of certain household chemicals on freshwater crustaceans.
2. To determine the toxicity of chemical mixtures within controlled exposure conditions.
3. To determine changes in the survival, reproduction, and movement behavior of the crustaceans.
4. To determine whether the interaction between the chemical mixtures is additive, synergistic, or antagonistic.
5. To study the ecological significance of chemical exposure in freshwater crustacean communities.

All the above-mentioned objectives help correlate toxicity with ecological effects on freshwater crustacean communities. This experiment will study the toxicity of household chemicals, the effects of these chemicals on freshwater crustaceans, and how to protect freshwater ecosystems.

This research report contains five main parts. Section 1 is about the introduction of the research, Section 2 describes the literature review, Section 3 outlines the methodology part, Section 4 discusses the results, and finally, Section 5 gives the conclusions.

II. Literature Review

2.1 Household Cleaning Chemicals in Aquatic Environments

Detergents, surfactants, and disinfectants are some of the most commonly observed organic pollutants found in wastewater effluents and recipient waterways (Tiwari et al., 2021). This happens according to their usage rates, treatment effectiveness, and the dilution factor of the rivers (Köck et al., 2010). Surfactants can cause membrane disruption, whereas the quaternary ammonium disinfectants and oxidizers cause damage to cell integrity and respiration sites. The experiments performed to assess the differences between conventional and green products in terms of grass shrimp and Daphnia magna revealed that the labeling does not necessarily mean less toxicity to aquatic organisms (Gray et al., 2022). This evidence suggests that product contents, not labels, should be used to assess environmental hazards.

2.2 Toxicological Responses of Freshwater Crustaceans

Freshwater crustaceans like *Daphnia magna* and *Ceriodaphnia dubia* are commonly used in tests since they are highly sensitive, abundant, and easy to rear in the laboratory (Maazouzi et al., 2016). House product exposure has been shown to result in mortality and immobilization in D. magna Tiwari et al., (2021). The sub-lethal effects of chemical exposure include reduced reproduction, growth, and swimming behavior at sub-lethal concentrations (Gray et al., 2022). The sensitivity varies among crustaceans and even within ecological groups, hence the need to avoid extrapolating findings across various species (Maazouzi et al., 2016). Behavioral endpoints are highly useful since they react quickly to stress (Malev et al., 2022).

2.3 Combined Toxicity and Chemical Interaction Models

Mixture toxicities are usually predicted using concentration addition (CA), which is based on the assumption of similarity of mode of action, and independent action (IA), which is based on dissimilarity of

mode of action (Rodney et al., 2013). Effects above the predictions are termed synergisms, while those below the predictions are termed antagonisms. Interactions in binary and tertiary mixtures of emerging pollutants have been observed to be dependent on the nature and ratios of mixture compositions (Di Poi et al., 2018). There is a need for confidence intervals when interpreting the results, as deviations from additivity can be attributed to experimental errors. Reactive compounds like hypochlorite complicate interpretations further.

2.4 Research Gap and Study Contribution

While prior studies have identified the toxicity of individual products and the approach of mixture modeling, controlled comparisons between individual substances and mixtures have rarely been performed for ingredients of cleaning products (Diepens et al., 2014). Combining the species-level and community-level indices provides enhanced ecological significance, as shown through techniques that link toxicity assays with community information (de Castro-Català et al., 2016). Concepts in species sensitivity can be used to extrapolate experimental findings to ecological communities (Belanger et al., 2016). This study provides an example of a controlled comparison of SDS, BAC, and NaOCl, using CA and IA models and multiple crustacean endpoints.

III. Materials and Methods

3.1 Study Design and Experimental Framework

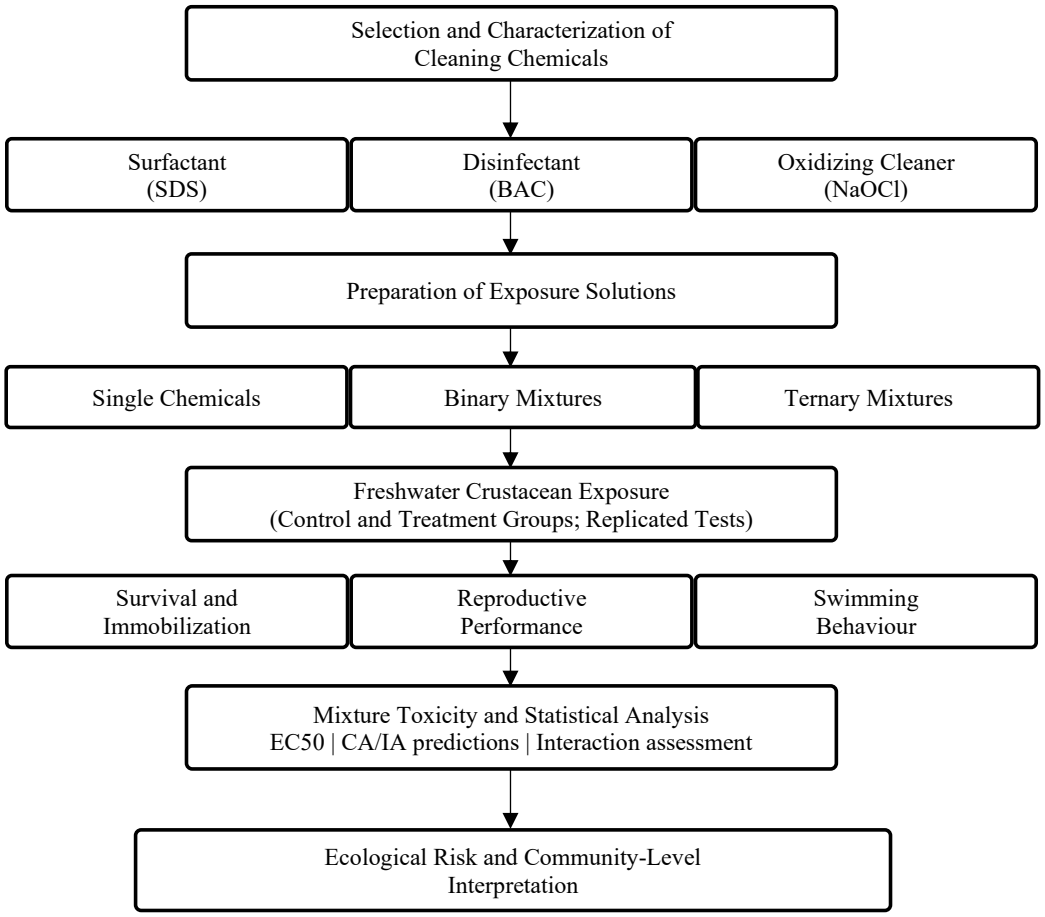


Figure 1: Experimental Framework for Assessing Individual and Combined Household Cleaning Chemical Toxicity in Freshwater Crustaceans

An ecotoxicological study was conducted in a laboratory setting using a combination of chemicals in contrast to chemicals individually, as demonstrated in figure 1. The selection of chemicals included three cleaning product components: the anionic surfactant sodium dodecyl sulfate (SDS), the disinfectant benzalkonium chloride (BAC), and the oxidizing agent sodium hypochlorite (NaOCl). This is a representation of some selected components of the products rather than the products themselves. These stock solutions were prepared in the reconstituted freshwater system, and their concentrations were analyzed where possible, especially for the reactive hypochlorite.

3.2 Selection and Maintenance of Freshwater Crustaceans

Both Daphnia magna and Ceriodaphnia dubia were chosen to serve as typical test organisms of freshwater crustaceans. Cultivation was performed in laboratory conditions using temperature control, a 16/8 h light/dark regime, and regular algae feed. Temperature, dissolved oxygen, pH, and water hardness were determined at the beginning and the end of each experiment. All tests were carried out in accordance with guidelines for OECD toxicity testing. In addition to the single-species experiments, a multispecies microcosm with known species numbers was prepared to assess the community-level responses.

3.3 Preparation of Chemical Mixtures and Exposure Conditions

The experimental group was constituted of an untreated control, three single chemical treatments, three binary chemical treatments, and one ternary chemical treatment. The suitable concentration ranges for each chemical were found through preliminary range-finding tests. These chemicals were used in a predetermined ratio with respect to the effect concentrations of each chemical constituent to be able to compare the experimental findings with model predictions. Temperature, light intensity, and medium type were maintained the same for all treatments. Four biological replicates were taken at each concentration, with five organisms per replicate.

3.4 Measurement of Toxicity Indicators

Four response categories were quantified. Survival (%) and immobilization (%) were quantified at 24 and 48 hours. Reproduction was quantified as offspring per female for a 21-day chronic test. Swimming behavior was quantified using video tracking, and reduction was determined relative to controls. Abundance and composition of species were quantified in microcosms at the conclusion of treatment. Immobilization was tested acutely and reproduction was tested chronically, each using the proper exposure duration to ensure that these responses were not confounded by acute lethality.

3.5 Combined Toxicity and Interaction Assessment

Models such as concentration addition and independent action have been utilized for determining the toxicity of the mixture. The sum of toxic units is one when using the concentration addition approach:

$$\sum_{i=1}^{n} \frac{C_i}{EC_{x,i}} = 1 \qquad (1)$$

Equation (1), where C stands for the concentration of chemical i in the mixture, and EC represents the effect concentration of chemical i at response level x. For independent action, the combined fractional response would be:

$$E_{mix} = 1 - \prod_{i=1}^{n}(1 - E_i) \qquad (2)$$

Equation (2) where E is the fractional response due to each chemical individually. Mixture effects were then compared to the predicted effect to determine deviations from additivity.

3.6 Statistical Analysis

A log-logistic dose-response relationship model was used to estimate EC50s with 95% confidence intervals. Survival, reproduction, and behavior responses were analyzed with either generalized linear or mixed-effects models (with replicate as a random effect). Treatment differences were determined through

multiple comparison tests, including Tukey or Dunnett. Deviations of observed interaction from model prediction were calculated along with their uncertainty levels, so that only interaction with confidence interval that did not overlap with the model prediction could be considered either synergism or antagonism. Significance level was $p < 0.05$.

IV. Results and Discussion

4.1 Individual Toxicity of Household Cleaning Chemicals

There were considerable variations between the acute toxicity of chemicals and organisms (Table 1). Among all tested compounds, BAC showed highest toxicity with 48 h EC50 values of 0.12 mg/L in D. magna and 0.07 mg/L in C. dubia, followed by NaOCl and then SDS. C. dubia appeared to be more sensitive to all chemicals with EC50 values 1.6-1.7 times lower than D. magna due to smaller body size and higher surface area/volume ratio. The order of toxicity of BAC>NaOCl>SDS corresponds to the high membrane-destroying effect of quaternary ammonium compounds.

Table 1: Acute Toxicity Responses of Freshwater Crustaceans Exposed to Individual Household Cleaning Chemicals

Chemical	Species	48-h EC50 (mg/L)	95% CI	Immobilization at selected dose (%)
SDS	*D. magna*	8.40	7.2–9.7	26
BAC	*D. magna*	0.12	0.09–0.15	41
NaOCl	*D. magna*	0.45	0.38–0.53	31
SDS	*C. dubia*	5.10	4.3–6.0	49
BAC	*C. dubia*	0.07	0.05–0.09	67
NaOCl	*C. dubia*	0.28	0.22–0.34	53

Selected doses: SDS 5.0 mg/L, BAC 0.10 mg/L, NaOCl 0.30 mg/L.

4.2 Combined Toxicity of Binary and Ternary Chemical Mixtures

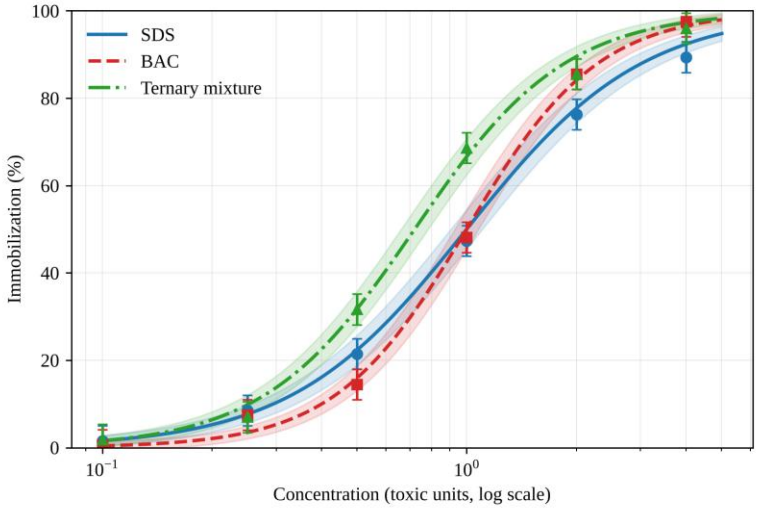


Figure 2: Concentration-Dependent Immobilization Responses of Freshwater Crustaceans Exposed to Individual Chemicals and the Ternary Mixture

The results of the mixture response were compared with the model predictions for table 2 and figure 2. SDS + BAC gave a result of 43.2% immobilization that was close to the CA prediction of 41.4%, implying an additive effect. The results for SDS + NaOCl were 29.6%, which was less than the CA prediction, showing antagonistic effects since the hypochlorite is likely to oxidize the surfactant. On the other hand, the combination of BAC + NaOCl resulted in 58.3% immobilization, while the ternary mixture gave 74.5%,

which exceeded CA and IA predictions and thus showed a synergistic effect. Immobilization increased with increasing concentration, and the ternary curve showed a trend of lower concentrations than those of any chemical alone.

Table 2: Comparison of Observed and Predicted Toxicity Responses Under Combined Chemical Exposure

Treatment	Observed Effect (%)	CA Prediction (%)	IA Prediction (%)	Interaction Classification
SDS + BAC	43.2 ± 3.1	41.4	27.8	Additive
SDS + NaOCl	29.6 ± 2.8	41.4	27.8	Antagonistic
BAC + NaOCl	58.3 ± 3.4	41.4	27.8	Synergistic
SDS + BAC + NaOCl	74.5 ± 3.9	61.4	38.6	Synergistic

Each component was applied at its EC15 for D. magna. Values are mean ± SD.

4.3 Effects on Reproduction, Behaviour and Community Structure

Increased chronic exposure impacts were noted as the complexity of the chemicals increased (Table 3 and figure 3). The control gave 96% survival with 84.2 offspring per female. In single chemicals, BAC induced the highest decrease in survival, with 74% survival and 52.3 offspring per female. In the case of ternary mixtures, there was 46% survival, 24.1 offspring per female, a 67.3% decrease in swimming activity, and a 68.7% decrease in community abundance. Sub-lethal reproductive impairment is an indicator of potential risk at the population level since reproduction decreased more than survival in all treatments.

Table 3: Chronic Toxicity and Ecological Responses of Freshwater Crustaceans Under Individual and Combined Chemical Exposure

Treatment	Survival (%)	Offspring per female	Swimming activity reduction (%)	Community abundance change (%)
Control	96	84.2	Reference	Reference
SDS	88	71.5	18.4	−12.6
BAC	74	52.3	36.7	−31.8
NaOCl	81	60.8	29.2	−24.3
Binary mixture	58	33.6	54.9	−52.4
Ternary mixture	46	24.1	67.3	−68.7

Binary mixture = BAC + NaOCl. Community abundance change was measured in the multispecies microcosm.

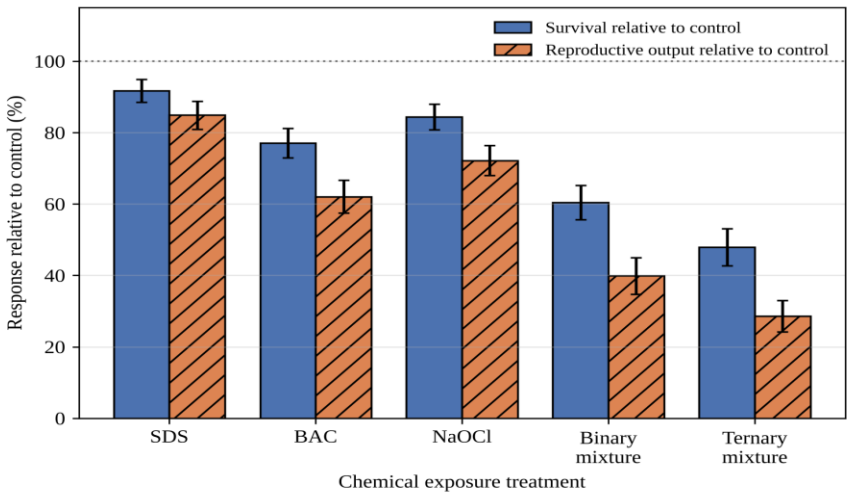


Figure 3: Comparative Effects of Individual and Combined Household Cleaning Chemicals on Crustacean Survival and Reproduction

4.4 Discussion of Combined Toxicity and Ecological Implications

Mechanisms of chemical interactions: The deviations from CA and IA models probably depend on different modes of action; the oxidation of SDS with NaOCl caused a reduction in toxicity, while membrane disruption by BAC might increase oxidative agent entry.

Species-specific sensitivity: The greater sensitivity of C. dubia indicates that the D. magna-based criteria do not cover the smaller cladocerans.

Consequences for the community: The 68.7% drop in microcosms' abundance is noted, while impacts on food webs, like reduced grazing of algae or fish prey availability, are hypothetical threats.

Environmental management considerations: better wastewater purification, household release control, monitoring, and consideration of mixture risks are required. Extrapolation to field conditions, chemical transformation, and concentration disparity are obstacles to practical application.

V. Conclusion

Cleaning compounds from domestic use contaminate freshwaters as mixtures, but the impact of their interactions is poorly studied. The most toxic compound was BAC, which showed 48-h EC50 equaling 0.12 mg/L in D. magna and 0.07 mg/L in C. dubia. The interaction between SDS and BAC was additive; SDS and NaOCl were antagonistic, while BAC and NaOCl and the whole mixture were synergistic. Chronic exposure to the ternary mixture led to a survival rate drop to 46% and an offspring number decrease to 24.1 per female, thus proving the lethal and sublethal damage caused by the joint exposure exceeds ingle-compound estimates. The interaction of compounds has significant implications for freshwater biodiversity conservation insofar as crustaceans ensure food web balance. Evaluations made based on a single compound do not necessarily reflect the full scale of hazards; moreover, the risks can be underestimated especially when reactive or membrane active compounds interact with each other. It means that chemical risk assessments should incorporate mixture tests using CA and IA criteria; meanwhile, wastewater treatment should concentrate on the joint discharging of surfactants, disinfectants, and oxidizers.

The present research was conducted at high laboratory concentrations above those found in the environment. In order to increase the ecological relevance of the results, further research should be conducted with more realistic mixture concentrations. It would also be better to test longer exposure periods, more crustacean species, and transformations of chemicals. In addition, there is a need for field validation of community interactions with a focus on mesocosm and in situ monitoring.

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