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Acute Toxic effects of Disposable Personal Hygiene Products on *Daphnia magna*

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Tek Kullanımlık Kişisel Hijyen Ürünlerinin *Daphnia magna* Üzerindeki Akut Toksik Etkileri

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Abstract

Aquatic ecosystems are increasingly exposed to pollution factors due to increasing human populations and technological developments in industrial production. Covid-19 pandemic has led to a notable increase in demand for personal hygiene products, which has consequently resulted in a significant rise in production levels in recent years. The use of disposable wet wipes has gained momentum with this trend. These versatile products have a wide range of applications, primarily in surface cleaning and personal hygiene. However, detailed information on the chemical compositions of these wipes and their effects on aquatic organisms is lacking. Existing toxicological studies have focused on the effects of a single toxic substance. Research on the combined effects is very limited. This study aimed to assess the acute toxicity of two wet wipe brands, WWA and WWB, on *Daphnia magna*. Each product was tested at 6 different concentrations: 100 ppm, 500 ppm, 1000 ppm, 2000 ppm, 4000 ppm and 8000 ppm. EC_{50} values for both brands were determined by probit analysis. The EC_{50} values for 24 and 48 h for WWA were 1259 ppm and 794 ppm, whereas the EC_{50} values for 24 and 48 h for WWB were 537 ppm. WWB was more toxic at lower concentrations according to the probit analysis results. To ensure the protection of aquatic ecosystems, the potential pathways by which these chemicals may contaminate aquatic systems must be identified and their levels in water should be monitored on a regular basis.

Keywords *Daphnia magna; Acute toxicity; Wet wipe; Freshwater; Pollutant.*

Öz

Teknolojinin hızla ilerlemesi ve artan insan nüfusu, sucul ekosistemlerin daha fazla kirletici madde ile kontaminasyonuna yol açmaktadır. Covid-19 pandemisi, kişisel hijyen ürünlerine yönelik talepte kayda değer bir artışa yol açmış ve bu da son yıllarda üretim seviyelerinde önemli bir artışa neden olmuştur. Bu eğilim ile birlikte, tek kullanımlık ıslak mendillerin kullanımı da ivme kazanmıştır. Bu çok yönlü ürünler, başta yüzey temizliği ve kişisel hijyen olmak üzere geniş bir uygulama yelpazesine sahiptir. Ancak, bu mendillerin kimyasal bileşimleri ve suda yaşayan organizmalar üzerindeki etkileri hakkında ayrıntılı bilgi bulunmamaktadır. Mevcut toksikolojik çalışmalar tek bir toksik maddenin etkilerine odaklanmıştır. Birleşik etkiler üzerine yapılan araştırmalar çok sınırlıdır. Bu çalışmanın amacı, iki ıslak mendil markası olan WWA ve WWB'nin *Daphnia magna* üzerindeki akut toksisitesini değerlendirmektir. Her ürün 6 farklı konsantrasyonda test edilmiştir: 100 ppm, 500 ppm, 1000 ppm, 2000 ppm, 4000 ppm ve 8000 ppm. Her iki marka için EC_{50} değerleri probit analizi ile belirlenmiştir. WWA için 24 ve 48 saat için EC_{50} değerleri 1259 ppm ve 794 ppm iken, WWB için 24 ve 48 saat için EC_{50} değerleri 537 ppm'dir. Probit analiz sonuçlarına göre WWB daha düşük konsantrasyonlarda daha toksiktir. Sucul ekosistemlerin korunmasını sağlamak için, bu kimyasalların sucul sistemleri kirletebileceği potansiyel yollar belirlenmeli ve sudaki seviyeleri düzenli olarak izlenmelidir.

Anahtar Kelimeler *Daphnia magna; Akut toksisiste; Islak mendil; Tatlısu, Kirletici.*

1. Introduction

Over the last century, many ecosystems, including aquatic ecosystems, have been adversely affected by environmental pollutants (Wang *et al.* 2021; Dewey *et al.* 2022). In recent years, due to the impact of the Covid-19 pandemic, the demand for personal hygiene products to protect against bacterial and viral diseases has increased (Steinemann *et al*. 2021). This trend has also led to increased demand for disposable personal care products, thereby increasing the supply of such products. Wet wipes (WW) will continue to be a popular consumer product, with a production volume of 1.36 million tonnes just in 2020 (Metcalf *et al*. 2024). These pollutants can affect both biota and habitat characteristics in ecosystems, depending on their physical and chemical properties (Zicarelli *et al*. 2022).

The chemicals used in WWs can be categorised as surfactants (e.g. bis-PEG/PPG, coco betaine, glyceryl stearate citrate), pH regulators, preservatives (benzoic acid, phenoxyethanol, potassium sorbate, citric acid) and skin conditioners (glycerine, butoxy PEG-4 PG-amodimethicone) (Rodriguez *et al*. 2020). According to a previous study, 132 different substances were detected in WWs of 54 different brands examined, while the number

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of ingredients in an average brand was determined as 11.9 (Aschenbeck and Warshaw, 2017). Additionally, various chemicals or plant extracts can be used as additives for odour diversity. Products manufactured for different purposes, such as surface cleaners, baby care or general personal hygiene, contain different chemical ingredients.

WWs, which are generally single-use materials, are either sent directly to landfills or to wastewater treatment plants with wastewater. Those that do not dissolve in wastewater treatment plants disrupt the operation of infrastructure, causing equipment failure and significant additional costs (Cheoafă *et al*. 2022, Metcalf et al. 2024). In addition, WWs, especially those made from synthetic plastic derivatives, cause a large amount of fiber-like microplastic particles to be released into the environment; many studies show that fiber-like microplastic particles are plastic derivatives that are observed in aquatic ecosystems, and there are studies that indicate their negative effects on aquatic ecosystem organisms (McCoy *et al.* 2020). It is known that a single WW causes the formation of 693-1066 p/sheet of fiber particles in aquatic ecosystems (Hu *et al*. 2022). Some countries have banned the production of WWs containing plastic (Metcalf *et al*. 2024) (URL-2).

The results of several studies on human health indicate that the chemicals used in WWs may cause irritation and allergic reactions in the human body (Faraz and Seely 2024, Aschenbeck and Warshaw 2017). It has also been demonstrated that bacteria can survive on WWs that are released into the natural environment as waste (Metcalf *et al*. 2024). Although there are studies on the effects of WWs on human health in general, the effect of chemicals released from these products on aquatic ecosystem biota is not yet well known. This study aimed to determine the acute toxic effect on *Daphnia magna* of a combination of ingredients of two different brands of disposable WWs produced for infants and general hygiene.

2. Materials and Methods

Daphnia are known as key species in aquatic ecosystems and have been widely used in aquatic ecotoxicity studies (Seda and Petrusek 2011; He *et al*. 2023; Pikuda et al. 2023). *Daphnia magna* is a model organism for aquatic toxicity studies (Reynolds, 2011). In addition to its significant role in the ecosystem, *Daphnia* has been utilized extensively in aquatic system studies due to its parthenogenetic reproduction, short life cycle, ease of cultivation in laboratory conditions, and sensitivity to environmental factors (Seda and Petrusek 2011; Bownik 2017; Mishra, 2024).

Adult *D. magna* colonies were obtained from Middle East Technical University, Limnology Laboratory. They were kept in the laboratory for about 4 months at 16:8 hours (light : dark) period 20 ± 1 °C. The water was renewed twice a week. Cultured daphnids were fed with a suspension of the *Chlorella vulgaris* and yeast daily. *D. magna* were selected from neonates and 5 neonates (age < 24 h) were used for each experimental concentration. During the experiment, neonates were not fed and the experimental medium was not ventilated.

The WWs that were used in the study were obtained from commonly used local markets. One of them is described as suitable for baby hygiene, while the other product is considered convenient for general hygiene use in adults. The brand names were anonymised and are referenced as WWA and WWB, respectively. The additives to be used in the experiment were obtained by pressing the wipes and 6 different concentrations were tested for each of them. For each product 100 ppm, 500 ppm, 1000 ppm, 2000 ppm, 4000 ppm and 8000 ppm (Figure 1). The concentrations to be used were determined according to the results of previous experiments. The total volume of each beaker was adjusted to 50 ml. All experiments were conducted in triplicate.

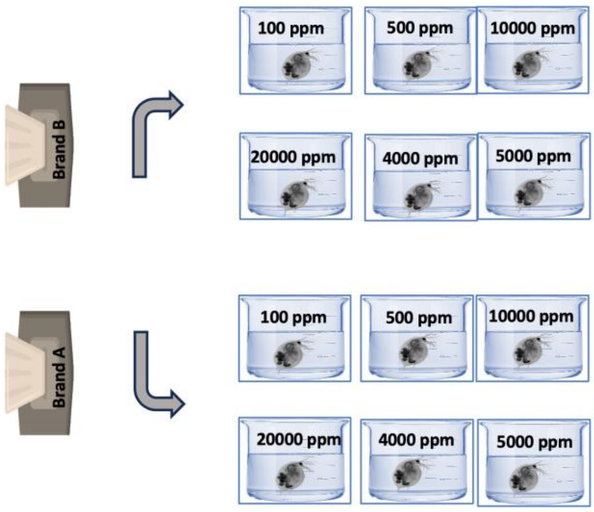

Figure 1: Experimental set up.

The acute toxicity test was performed according to the OECD guideline *D. magna* Acute Immobilisation Test (OECD, 2004). Daphnids were examined at 1, 2, 4, 8, 12, 24 and 48 hours. Animals that are not able to swim within 15 seconds after gentle agitation of the test vessel are defined as immobile (OECD, 2004). The data was subjected to a probit analysis to calculate EC_{50} values for 24 and 48 hours (Finney, 1952, 1964). The calculations were performed using Excel software. In the chemical-free media, which was carried out concurrently with the experiments, no immobilisation was observed in the control daphnid groups.

3. Results and Discussions

The results of the experiments showed that two different brands had adverse effects on *D. magna* above certain concentrations. The acute toxicity test results for two different companies' products are shown in Table 1. Looking at the 24-hour test results for WWA, the percentages of immobility from the lowest concentration to the highest concentration at the end of 24 hours were 0%, 46.7%, 20%, 40%, 73.3% and 100% (Figure 1). Considering the 48-hour results for the same brand, the difference was observed at 40 % and 100 % immobility at 1000 ppm and 4000 ppm, respectively. For WWB, there was no difference between the 24- and 48-hours results (Figure 2).

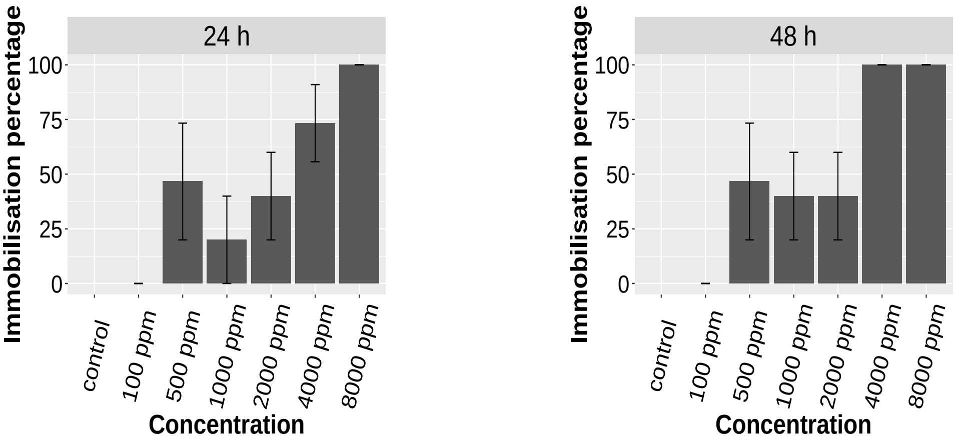

Figure 1: Immobilisation effects of different concentrations of WWA on *D. magna.*

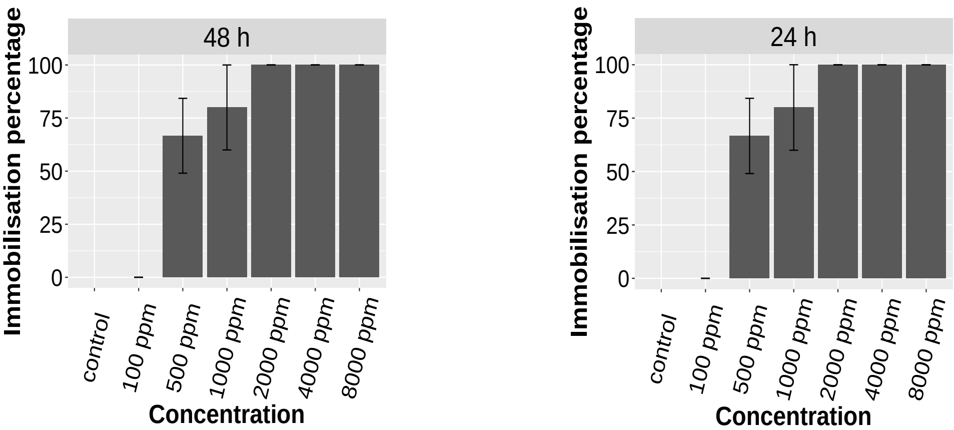

Figure 2: Immobilisation effects of different concentrations of WWB on *D. magna.*

The immobility rates were 0%, 66.7%, 80%, 100%, 100%, 100% and 100% according to the concentration increase. The regression equations for the probit analysis are as shown in Table 3. The EC_{50} values for 24 and 48 h for WWA were 1259 and 794, whereas the EC_{50} values for 24 and 48 h for WWB were 537. WWB was more toxic at lower concentrations according to the probit results.

The common chemicals used in both products are aqua, phenoxyethanol, perfume, benzoic acid, dehydroacetic acid, glycerine, *Chamomilla recucita* flower extract and citric acid (Table 2). Phenoxyethanol, one of these chemicals, is generally known to have an inhibitory effect on gram-positive and gram-negative bacteria and is commonly used as a preservative in cosmetic products, although it is known not to be irritating to human skin, the safe level of use should not exceed 1 % (Dréno *et al*. 2019.) (Table 2).

Table 1. Percentages of immobilisation of *D. magna* at different concentrations of WWA and WWB.

	WWA		WWB	
	% immobility			
Cont. (ppm)	**24h**	**48h**	**24h**	**48h**
100	0	0	0	0
500	46,7	46,7	66,7	66,7
1000	20	40	80	80
2000	40	40	100	100
4000	73,3	100	100	100
8000	100	100	100	100

Due to a lack of information on the percentages in both products, it is not possible to make a statement in this regard. The toxic effect of phenoxyethanol on *D. magna* was shown to be limited in a former study (Tamura *et al*. 2013). Previous studies on benzoic acid have shown that the number and position of phenolic hydroxyl groups are important determinants of toxicity (Kamaya et al. 2005). However, there is no detailed explanation on the product label about the chemical form of the benzoic acid that is used in the products. Dehydroacetic acid is used as a preservative and fungicide, but there are no detailed studies on its toxic effects on organisms in the aquatic ecosystem. Nevertheless, it has been included in Annex V of Regulation 1223/2009. It is limited to 0.6% in finished products and banned in aerosols (URL-3). Citric acid is naturally and industrially produced and widely used in pesticides, food, beverages and cosmetics. It is highly soluble in the aquatic environment and has been produced in increasing quantities in recent years Ciriminna *et al*. 2017). According to the EPA data, there are no warnings for adverse effects, but it is recommended that it be stated as a warning on the product that it is a severe eye irritant and a moderate skin irritant (EPA 2009).

Table 2: The ingredients of two products.

WWA	WWB
Aqua	Aqua
Phenoxiethanol	Phenoxiethanol
Parfum	Parfum
Benzoic acid	Benzoic acid
Glycerin	Glycerin
Dehydroacetic acid	Dehydroacetic acid
Chamomilla recutita flower extract	Chamomilla recutita flower extract
Citric acid	Citric acid
C12-15 PARETH -12	Butylene Glycol
	Sodium cocoamphoacetate
	Dimethicone

Dimethicone, C12-15 pareth-12 are only present in WWA, while sodium cocoamphoacetate, butylene glycol are only present in WWB. Dimethicone in WWA is a silicon-based chemical, but its effect on daphnids is still unclear, although no toxic effect has been observed at high concentrations (6-79%) in studies on different organisms (Raposo *et al*. 2013). C12-15 pareth-12 is widely used as an emulsifier and surfactant. According to the literature, polyethylene glycol (PEG)/polypropylene glycol ethers are used in cosmetics and have been found to be safe and non-irritating when formulated correctly. The results of the study on *D. magna* showed that the PEG did not have any acute toxic effects. The results of the study on *D. magna* have also shown that PEG does not have any acute toxic effects (Sönmez *et al*. 2020). Sodium cocoamphoacetate is used as a surfactant and is likely to cause allergic reactions, though it is classified as moderately toxic in aquatic systems (Raposo *et al*. 2013). Butylene glycol is an organic alcohol that is used as a solvent and a conditioning agent and known as safe. No toxic effects of butylene glycol on the skin and mucous membranes of humans have been observed, but eye irritation has been observed in experiments with rodents (Dionisio *et al*. 2018). *Chammomilla recutita* extract is common to both products. The plant is widely known for its antibacterial properties and its use in traditional medicine (Shikov *et al*. 2008; Lairikyengbam *et al*. 2024). While its use for cosmetic purposes is characterized as safe, its effect on *D. magna* is unknown (URL-1).

Table 3: Regression equations and EC_{50} values of two brands according to probit analysis results.

Hours	Sample	Equation for the regression analysis	R^2	EC_{50}
24	WWA	y = 3,4x + (-5,6)	0,8	125
48	WWA	y = 4,14 x + (-7,21)	0,8	794
24	WWB	y = 4,40x + (-7,07)	0,9	537
48	WWB	y = 4,40x + (-7,07)	0,9	537

The toxic effects of the chemicals used in both brands have generally been evaluated in terms of their effects on humans, and the combined effects of these compounds on *D. magna* have not been reported. However, the combined release of different substances into the environment may result in more toxic effects. In a recent study, the effects of nine different WW brands, including products from various countries, were investigated on *Lepidium sativum*. It was found that 78% of the WWs tested in the experimental results had toxic effects on the plant (Tkachuk and Zelena 2023). The EC_{50} values ranged from 4 to 85 mg/l and 15 to 166 mg/l in a study investigating the effects of 26 detergents and fabric softeners on *D. magna* (Pettersson *et al*. 2000). In another study, different types of household products, such as toilet cleaner, liquid hand washing, glassware washing liquid, detergent, shampoo, three types of cooking oils (Crude mustard oil, cooked mustard oil and refined oil) were purchased from the local market and selected as test chemicals and their effects on *D. magna* studied. Sequentially, the sensitivity of the organisms to the tested products was found in the order (from most toxic to least toxic): toilet cleaner = glass washing liquid > detergent > liquid hand wash > shampoo (Tiwari *et al*. 2021). However, it is not possible to compare the magnitude of the effect between different studies because the chemical compounds are not identical. It is important, however, to determine the combined effect as this is representative of the situation that remains in the natural environment.

4. Conclusion

In recent years, there has been a notable increase in literature examining the environmental effects of WWs, with a particular focus on microplastic pollution. However, studies on chemical effects are limited. Current study results shows that both brands have negative effects on *D. magna* at certain concentrations. However, further research is required to fully understand the effects of these chemicals separately and in combination. Considering that the global production of these personal care products is increasing every year, it is necessary to take precautions, eliminate the negative effects of this situation in disposal facilities and conduct regular monitoring studies in aquatic systems.

Declaration of Ethical Standards
The author declares that they comply with all ethical standards.

Credit Authorship Contribution Statement
Author: Resources, Conceptualization, Investigation, Methodology, Data curation, Writing – original draft, Visualization.

Declaration of Competing Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability
All data generated or analysed during this study are included in this published article.

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