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Evaluation of the Inhibitory Effect of Nano Silver on Aquatic Organisms

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Abstract: Nano silver has been widely used in various consumer products in recent years because of its excellent antibacterial properties. However, nano silver will inevitably enter the water during the production and consumption of related products. Many literatures have studied the aquatic toxicity of nano silver, but the research mainly focuses on the toxic effects of water exposure. There are few studies on the dietary exposure toxicity of nano silver. The aquatic toxicity results and mechanism are not clear, which is not conducive to the environmental risk assessment of nano silver. In this paper, by studying the effects of nano silver on the growth, reproduction, bioaccumulation and other indicators of Daphnia magna under the water exposure (nano silver), combined with the blank control experiment, the toxic mechanism of nano silver on Daphnia magna was analyzed, so as to provide more accurate toxicity data for the environmental water risk assessment of nano silver.

1. Introduction

Compared with ordinary silver, nano silver (AGNPS) have larger active surface area, better porosity and excellent antibacterial ability. At present, they have been widely used in medical treatment, food, electronics, textiles, water purification, baby bottles, toys, toothbrushes, toothpastes, air purifiers and other fields [1]. The database lists 1628 products containing nano materials on the market, of which 383 products contain nano silver, ranking first in the number of nano materials. The wide use of nano silver has led to the inevitable discharge of more and more nano silver into the environmental water during the whole life cycle of production, use and waste, which has an impact on aquatic organisms [2]. Some studies have predicted the environmental concentration of nano silver in Europe and the United States using probabilistic statistical methods, but there is a lack of experimental data to confirm the concentration simulated by these models [3]. In order to accurately assess the environmental water risk of nanoparticles, the study on the aquatic biological toxicity and mechanism of silver nanoparticles has become a hot topic at home and abroad.

Among many tested aquatic organisms such as algae, Daphnia magna and fish, Daphnia magna is considered as one of the ideal model organisms to study the aquatic toxicological effects of nano silver [4]. Therefore, studying the toxicity of nano silver to Daphnia magna has important theoretical and practical significance for the environmental risk assessment of nano silver and other nano products. After entering the water environment, nano silver can enter the Daphnia magna through different ways such as body surface penetration, drinking water and feeding, and can also cause toxicity by changing the composition of Daphnia magna culture solution. At present, the toxic effect of nano silver on Daphnia magna is mainly studied by water exposure, that is, the toxic effect and mechanism of nano silver on Daphnia magna are studied after it is directly exposed to water [5]. The acute toxicity results, chronic toxicity results and bioaccumulation results of nano silver to Daphnia magna exposed to water in recent years are summarized. There are many factors affecting the toxicity of nano silver to Daphnia magna. Some studies have found that the toxicity of nano silver is related to the particle size of nano silver. Scholars have also proved that the toxicity of silver nanoparticles depends on its size. Hoheisel et al believed that the toxicity of different nano silver increased with the reduction of particle size (i.e., the half lethal concentration decreased), and the average half lethal concentration of nano silver increased from 27 μ G/l (50 nm) to 7.0 μ g/L(10 nm) Another related study also showed that the toxicity of silver nanoparticles with smaller particle size was significantly greater than that of silver nanoparticles with larger particle size [6]. Some studies have found that the toxicity of silver nanoparticles is related to the properties of silver nanoparticles. Park et al. Used two types of nano silver and compared their toxicity to Daphnia magna, proving that they were toxic to experimental organisms. The results showed that the toxicity of fractionated nano silver to Daphnia magna (48h-ec50 = 13.8 μ G/l) than colloidal nano silver (48h-ec50 = 6.1 μ G/l) is about 2 times lower [7-8]. Other studies show that the toxicity of silver nanoparticles mainly depends on the properties of the coating agent on the surface of silver nanoparticles. Environmental conditions (such as pH value, ionic strength and coexisting substances) can also change the toxic effects of silver nanoparticles on aquatic organisms. One study showed that low pH ($pH < 8$) was favorable for the adsorption of positively charged silver nanoparticles on the surface of algae. In conclusion, the research on the toxicity of nano silver to Daphnia magna has made some progress in water exposure toxicity, and some toxicity data have been obtained, which provides a theoretical basis for scientific assessment of the environmental risk of nano silver [9]. However, some research results have problems such as large differences and inconsistent conclusions, and the toxicity evaluation methods lack uniformity and comparability, which may be due to the single exposure route and complex interference factors [10]. To better study the toxicity mechanism of nano silver to Daphnia magna, we can compare the toxicity of nano silver to Daphnia magna under different exposure routes.

The wide application of nano silver leads to the inevitable release of nano silver into various wastewater, which may cause serious harm to the aquatic ecosystem. Its toxic effect on the typical aquatic test organism Daphnia magna has become a research hotspot. Nano silver may be toxic to Daphnia magna through different ways in water environment. The toxic pathways of nano silver exposed to water are diverse, the toxic factors are complex, the toxicology is not completely clear, and even the research conclusions are inconsistent. In this paper, the dietary toxicity of nano silver to Daphnia magna was studied through Chlorella and liposome encapsulation technology. The liposome encapsulated nano silver can effectively avoid the indirect toxicity caused by the interference of nano silver on the growth environment of Daphnia magna under the conventional dietary exposure mode. By studying the influence of nano silver on the growth, reproduction and other indicators of Daphnia magna under the water exposure path, and combining the blank control experiment, the mechanism of nano silver toxicity to Daphnia magna was analyzed, So as to provide a more accurate reference value for the quality target of nano silver for the environmental water risk assessment of nano silver, and provide new ideas and methods for the aquatic toxicology research of other nano particles.

2. Overview of Related Concepts

2.1. Nano Silver

As a new material, nano silver is widely used as a high-quality substitute for antibiotics. Before, when the level of science and technology was low, silver was mainly used as an antibacterial substance, even before penicillin, chloramphenicol and other antibiotics. About 6000 years ago, the ancient Greek and Roman Empire first used silver as an anti-bacterial agent. At that time, the main function of silver was medical sterilization and wound disinfection. China's silver culture is brilliant. At present, simple silver products unearthed in the Shang and Xia dynasties have been discovered by means of Archaeology and other means. After investigation, there have been relatively complex silver utensils in the spring and Autumn period of China. At that time, in addition to the monetary value of silver products, the silver bowls, silver chopsticks and other utensils made of silver products can also be used for daily sterilization [11]. There are also many silver containing drugs in modern medicine. The antibacterial effect of the silver containing part comes from the interaction of silver ions with important enzymes and sulfhydryl groups in proteins, resulting in cell death. In recent decades, with the emergence of various super bacteria carrying antibiotic resistance, people urgently need a new class of highly effective antibacterial substances. With the development of nanotechnology, silver or other common silver compounds are gradually replaced by nano silver. As a new nano consumer product, it has more efficient disinfection and sterilization ability and quickly occupies the market [12].

Nano silver has spectral antibacterial ability, which can inhibit the propagation of Escherichia coli, Chlamydia trachomatis and other pathogenic bacteria without further drug resistance. It is widely used in the fields of antibiotic substitutes, building consumables, environmental protection and food packaging. According to relevant reports, the global use of nano silver can account for about 50% of the total use of nano materials, and about 400 nano silver is used in various industries every year [13]. The number of daily used nano silver consumer products has increased sharply, which will inevitably lead to the discharge of some nano particles into the natural environment during the production, use and waste process, which will lead to the contact of economic animals, plants and humans with nano silver through skin contact, food intake and other ways, and large-scale contact will inevitably lead to toxic stress on organisms [14]. According to relevant reports, some daily necessities contacted by human beings for daily needs contain nano silver particles, such as some baby supplies, sprays, electronic products, etc. when exposed to these articles for a long time, the absorbed nano silver particles will cause indelible damage to the body's liver, kidney and other metabolic organs. Although nano silver may cause greater ecological toxicity than metallic silver in some cases, nano silver particles can enter the water environment through various ways and have potential adverse effects on the marine ecological environment [15], there are few reports on the marine ecological toxicity of nano silver at present, and it is impossible to conduct dose analysis on the marine nano silver pollution, and the relevant toxicity mechanism is still unknown, There is an urgent need to study the mechanism of its toxicity.

2.2. Water Environmental Behavior of Nano Silver

With the development of nanotechnology, nano silver, as one of the typical nano materials, has increased the application and usage of synthetic nano silver related consumer goods: during the production and use of daily necessities such as textile washing, toothbrush, toothpaste, cosmetics, etc., the nano silver particles contained will be washed and enter the wastewater environment [16]. The waste and incineration of decoration materials (paints) and electrical parts will also emit a large amount of nanoparticles to the environment. The leaching solution of the vertical plate outside the model house with nano silver on the surface of the study was washed by rain. After the analysis of the electron microscope, it was found that more than 30% of the nano silver particles would be released to the natural environment. Even if the domestic sewage containing nano silver particles was treated by the sewage treatment plant, some nano silver particles could not be completely removed and migrated in the natural environment. In addition to the physical, chemical and biological methods of artificial synthesis of silver nanoparticles, natural disasters such as volcanic eruptions, earthquakes and fires can also cause the inevitable discharge of silver nanoparticles into the ecological environment. The marine conditions are complex. After the discharged nano silver particles enter the sea, the toxicity of nano silver cannot be quantified, and its environmental behavior and biological toxicity mechanism have not been thoroughly studied [17]. Due to the existence of multiple exposure routes, silver nanoparticles are widely distributed in natural water. The aggregation and sedimentation behavior in water is one of the important factors causing environmental risks. Relevant studies show that nanoparticles are limited by their own properties, stable in morphology and structure, not easy to degrade, relatively stable in freshwater environment, and extremely unstable in seawater with complex components. Nanoparticles in seawater will interact with ions, organisms and organisms in the water body through van der Waals force, electrostatic force and other forces, resulting in dispersion or agglomeration, thus causing changes in their morphology and properties. At the same time, salinity, temperature, ionic strength, pH and light intensity in natural water will also affect the morphology and properties of nano silver to a certain extent [18].

2.3. Relevant Formulas

The intrinsic growth rate, also known as the maximum instantaneous growth rate, is one of the characteristics of the species and the internal reason affecting the population. It not only takes into account the birth rate and mortality of animals, but also the age structure, spawning capacity, development rate and other factors of the population, which can sensitively reflect the subtle changes of the environment. The intrinsic growth rate is an integral formula that integrates the survival period, survival rate and reproduction of Daphnia magna, and reflects the reproductive changes of Daphnia magna. The intrinsic growth rate of Daphnia magna is calculated as follows:

$$r = \frac{\ln R_0}{T} \tag{1}$$

$$R_0 = \sum l_x m_x \tag{2}$$

$$T = \frac{\sum x l_x m_x}{\sum l_x m_x} \tag{3}$$

Where LX is the survival rate of each Daphnia magna on day x, MX is the number of eggs laid by each female Daphnia on day x.

The bioaccumulation under water exposure was expressed by BCF, and the bioaccumulation under dietary exposure was expressed by BMF. BCF and BMF are calculated as follows:

$$BCF = C_f / C_w \tag{4}$$

CF = concentration in Daphnia magna(μ g/g)
CW = concentration in water(μ g/L)

$$BMF = C_f / C_{food} \tag{5}$$

CF = concentration in Daphnia magna(μ g/g)
Cfood = concentration in food(μ g/L)

3. Experimental Design and Method of Toxic Effect of Nano Silver on Daphnia Magna

Water exposure toxicity is the toxic effect caused by the direct contact between pollutants and aquatic organisms. In the aquatic ecotoxicological study of nanoparticles, it is of great significance to study the water exposure toxicity effects of silver nanoparticles on Daphnia magna to measure and understand the water exposure toxicity effects of other nanoparticles on aquatic organisms. Therefore, this chapter focuses on the acute and chronic toxicity of citric acid coated nano silver to Daphnia magna. The concentration of subchronic toxicity was determined through acute toxicity, and the toxicity effect of nano silver on Daphnia magna was preliminarily discussed through subchronic toxicity and bioaccumulation experiments.

3.1. Acute Toxicity Test

Add potassium dichromate culture solution into a 100ml beaker, set blank control at the same time, set 3 parallel samples for each concentration, and put 5 young Daphnia in each sample. After 24 hours, count or count the number of young Daphnia whose activity is inhibited, and calculate its 24h-ec50. The 24h-ec50 of potassium dichromate is 0.5-1.2mg/l, which indicates that Daphnia magna can be used in formal experiments.

3.2. Chronic Toxicity Test

The experiment was conducted in a 100ml beaker. In order to more accurately observe the growth changes of Daphnia magna exposed to water, only one 6-24h young Daphnia magna was placed in each beaker, and 10 parallel samples were set for each concentration. All experimental samples were put into a constant temperature incubator for chronic toxicity experiments to ensure the normal living conditions of Daphnia magna. During the experiment, a semi-static experimental system was used, and half of the experimental solution was changed every 24 hours during the experiment. Daphnia magna was exposed to nano silver solutions at concentrations of 8, 13, 19, 28, 43 μ G / L, fed with normal Chlorella. Observe the reproduction of Daphnia magna every day, and record the peeling, reproduction and death inhibition of Daphnia magna during the experiment.

3.3. Bioaccumulation Experiment

The bioaccumulation experiment was carried out for 14 days. At the beginning of the experiment, M7 culture solution was added to the beaker, nano silver was added, and 100 young Daphnia were added. The water was changed by 25% every day, and 2 × 105 / ml, fed 4 in the second week × 105 pieces / ml. after the first week, 50 Daphnia were collected and put into the clean M7 culture solution, and their body length was measured after 24 hours of purification. In the second week, 50 Daphnia were put into the M7 culture solution, and their body length was measured after 24 hours of purification. After that, all the samples were stored in the refrigerator. After the experiment, the samples were taken out and washed with 0.1mmol/l EDTA, and finally determined by AAS.

4. Analysis of Experimental Results

4.1. Analysis of Acute Toxicity Results

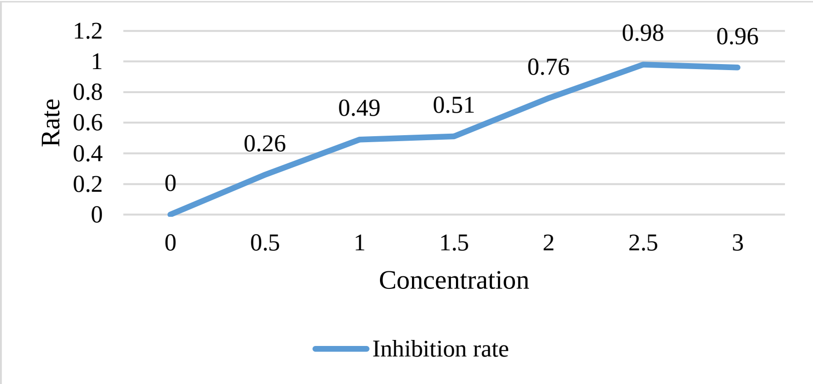

Figure 1. Inhibition rate of potassium dichromate on the activity of Daphnia magna

As shown in the figure, according to the pre experiment results, the concentration range set in the formal experiment is 0-65 μ g/L. The inhibition rate and mortality rate were calculated using Excel processing data, as shown in Figure 2. The 48h ECX and 48h LCX values were obtained by curve fitting of inhibition rate and mortality rate. According to the acute results of nano silver, it can be found that the acute toxicity of nano silver to Daphnia magna has a dose effect relationship. The fitting curve of 48h LCX is y = 62.51-45.84e - 0.009695x; The corresponding half inhibitory effect (48h-lc50) was 34.27 μ g/L.

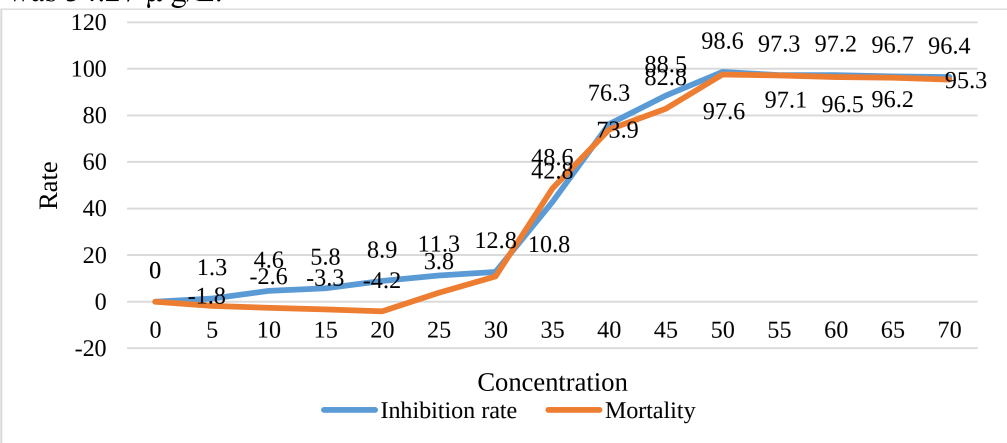

Figure 2. Mortality rate and inhibition rate of AgNPs to Daphnia magna

4.2. Analysis of Chronic Toxic Effects

According to the results of acute experiments, the concentrations of chronic experiments were designed as 0, 0.75, 1.5, 3, 6, 12, 28 and 43 μ g/L During the 21 day chronic experiment, no death occurred in the blank control group, which met the requirements of oecd211 experiment, and the data were reliable. Using SPSS 21 one way ANOVA was performed. If the difference was significant, LSD method was used for multiple comparison. The results of 21-d chronic experiment are shown in Table 1. In the 21-d chronic experiment, the concentrations were between 28 and 43 μ G / L because the mortality rate is too high to be counted, the total number of Daphnia magna under 21-d water exposure is shown in Figure 3.

Table 1. 21-d chronic toxicity of AgNPs to Daphnia magna

Concentration	Molting times	Total output	First birth time	Number of first births	Intrinsic growth rate	Mortality
Blank control	8.8	46.6	11.2	7.67	0.23	0
0.75	8.8	49.6	10.8	7.5	0.23	0
1.5	8.8	46.8	11	8.17	0.23	0
3	8.4	44.8	11	10.5	0.24	0
6	8.4	42.8	11	8	0.24	0
12	8	38	11.6	8	0.24	0

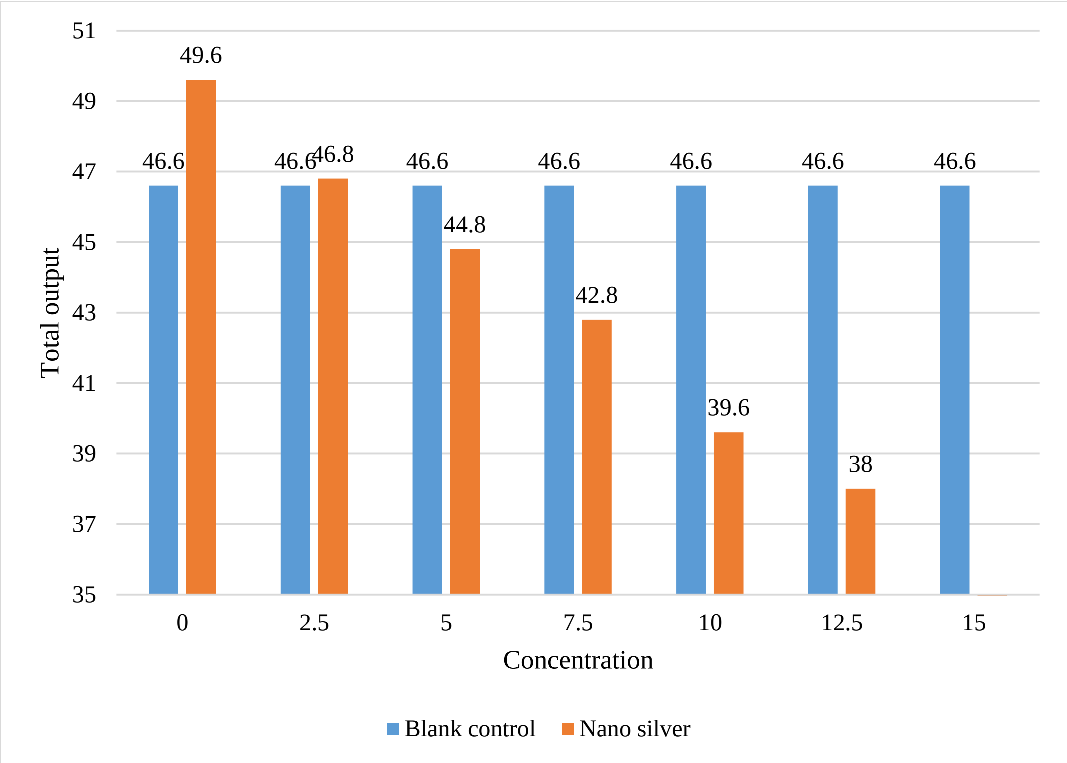

Figure 3. Total reproduction of Daphnia magna in 21-d AgNPs exposure

From the chronic data in Table 1, it can be concluded that the number of molting of Daphnia magna decreases with the increase of nano silver concentration, and the number of Daphnia magna in the first fetus is gradually delayed with the increase of nano silver concentration. There is no death of Daphnia magna in all concentrations. According to figure 3, the total number of Daphnia magna decreases gradually with the increase of nano silver concentration, and the lowest concentration is 0.75 μ The total number of Daphnia magna was slightly more than that of the blank control at the concentration of g/l μ G / L significantly inhibited the total number of Daphnia magna ($P < 0.05$), indicating that 12 μ G / L is the lowest observable effect concentration. The total number of Daphnia magna at 14-d was 12 μ G/l was the same as that of the blank control, while the concentration of Daphnia magna at 21-d was 1.5 μ G/l was the same as that of the blank control, indicating that with the increase of exposure time, nano silver will cause a large toxic effect on Daphnia magna.

4.3. Bioaccumulation Effect Analysis

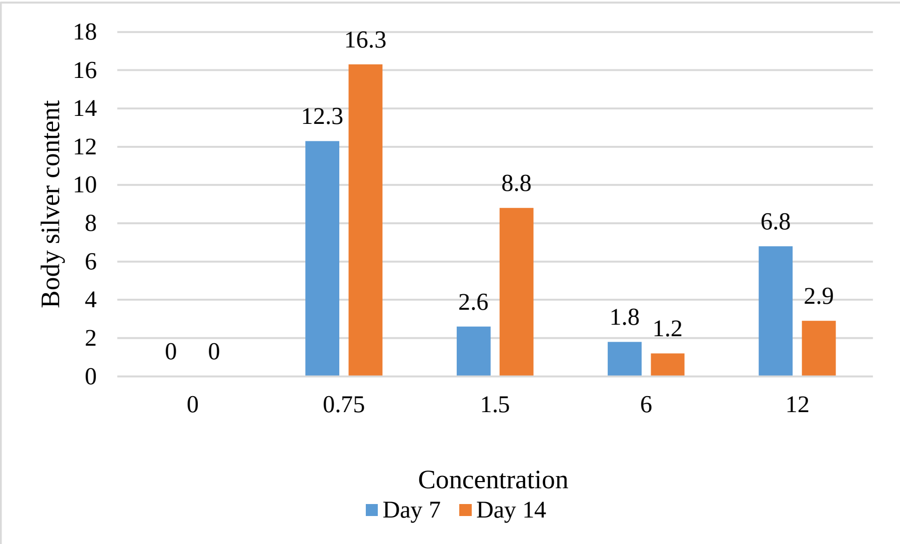

Figure 4. Bioaccumulation of AgNPs at different concentrations in Daphnia magna

This study measured the silver content in Daphnia magna on day 7 and day 14. The results are shown in Figure 4. The accumulated amount in Daphnia magna on day 7 and day 14 was 0.75 μ G / L; Comparing the silver content in Daphnia magna on day 7 and day 14, the silver content in the low concentration group increased on day 14, and the silver content in the high concentration group decreased.

5. Conclusion

In this paper, the acute toxicity, chronic toxicity and bioaccumulation of nano silver on Daphnia magna were studied, and the water toxicity effect of nano silver on Daphnia magna was explored. The conclusions were as follows: the accumulated amount in Daphnia magna decreased with the increase of concentration, and decreased with the increase of time when the concentration was high. It indicates that the higher concentration of nano silver may inhibit the feeding of Daphnia magna on nano silver. The water exposure toxicity data of nano silver to Daphnia magna obtained in the study want to comprehensively evaluate the toxicity of nano silver to Daphnia magna and provide data support for the water risk assessment of nano silver.

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Data Availability

Data sharing is not applicable to this article as no new data were created or analysed in this study.

Conflict of Interest

The author states that this article has no conflict of interest.

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