




Article

The Influence of Available Cu and Au Nanoparticles (NPs) on the Survival of Water Fleas (*Daphnia pulex*)

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Abstract: Applications of nanotechnologies in different sectors and everyday items are very promising and their popularity continues to grow. The number of products containing nanoparticles makes environmental exposure to nanoparticles inevitable. The current understanding of the relationships between nanoparticles and the environment is inadequate despite the fast growth of nanotechnologies. The aim of the study was to investigate the influence of copper and gold nanoparticles on *Daphnia pulex* survival. Our study included 48-h acute toxicity tests and determination of median lethal concentration values (LC₅₀s) for Cu-NPs and Au-NPs. For nano-copper, 24 h LC₅₀ was assumed > 1 mg/L, and 48 h LC₅₀ = 0.5117 mg/L. For nano-gold the LC₅₀ value after 24 h was 0.4027 mg/L, and after 48 h 0.1007 mg/L. The toxicity of nano-gold solutions was thus found to be higher than that of nano-copper. The addition of Cu-NPs at 0.0625 mg/L and 0.125 mg/L caused an increased multiplication of daphnia, while Au-NPs at 1 mg/L was an inhibitor of reproduction.

Keywords: nano-copper; nano-gold; ecotoxicology; zooplankton

1. Introduction

A nanoparticle is defined as a small particle of matter with dimensions from one to one hundred nanometers. The presence of nanoparticles (NPs) in nature has been observed for a long time, because they are formed during combustion processes, such as fires or volcanic eruptions [1]. They are also found in fogs and fumes, and are also formed during welding. Recently, humans have deliberately generated NPs. Due to the distinctive optical, magnetic and catalytic characteristics of nanomaterials compared to natural substrates, nanoparticles are being produced on a huge scale [2,3]. The difference in properties is due to the possibility to synthesize nanoparticles with precise control over the size, shape and purity of NPs [4–7]. Today, nanotechnology is a comparatively young field with its roots dating back to the 1950s. There has been a steady increase in the amount of nanotechnology-based products available on the market, so environmental exposure to NPs is inevitable [8]. Solutions that allow nanoparticles to be used in various sectors are comparatively recent, so there are no extensive, long-term studies to demonstrate what effect they can have on organisms', including humans', health and life.

Nanocolloids are growing in popularity on the market. They are widely available and can be purchased without leaving home, e.g. via the Internet. There is no need of prescription to purchase them, and before applying, there are no recommendations for consulting a doctor or other expert. Nanocolloid manufacturers claim many positive benefits for people, while they do not report any contraindications to their use or environmental hazards.

According to a manufacturer of colloidal solutions of nano-copper and nano-gold, the flat structure of colloid molecules makes their adhesion surface many fold larger than that of similar preparations with particles of spherical structure. In addition, the manufacturer provides the size of gold particles in solution, the diameter of which does not exceed 3 nm. Commercially available solutions are highly concentrated: nano-platinum is available at a concentration of 10 mg/L, nano-copper and nano-gold at a concentration of 25 mg/L and nano-silver at a concentration of 50 mg/L (<https://www.vitacolloids.pl/>).

The annual worldwide production of Cu-NPs and CuO-NPs is estimated at 200 tons [9] of the products in different concentrations. Among the nanocolloids available on the market, copper has the strongest fungicidal [10,11] and antibacterial [12–15] effects. Non-ionic colloids of nano-copper can be used to improve the appearance of skin and hair, and against fungal infections in foot care. Additionally, they support the fight against free radicals by slowing down the skin ageing process [16]. Nano-copper is also used in deodorants as it reduces excessive sweating and eliminates unpleasant sweat smell. Cu-NPs are used in everyday products such as cosmetics (hair conditioners, body lotions, shower gels and facial mists), computer equipment and dietary supplements (<http://nanodb.dk/en/>).

The conducted research confirms that the addition of nano-copper to water at a concentration of 10 mg/L inhibits the development of *Candida albicans* fungi after 15 minutes [14]. This suggests that such solutions can be added to swimming pool water. However, research conducted by Llorens et al. in 2012 [17] proved that NP solutions have harmful effects on mammalian cells, causing instability of chromosomes and the induction of apoptosis. According to the literature data, the lowest oral toxic dose to humans is 0.01 mg/kg body weight [18,19], while the lowest oral toxic dose given subcutaneously to rabbits is 375 mg/kg body weight and 3 g/kg when administered orally over 60 days [20].

Copper belongs to the microelements needed by the human body. It activates an enzyme, ceruloplasmin, which is essential for the formation of erythrocytes, thus influencing the proper functioning of the circulatory system [21]. In addition, it oxidizes vitamin C and stimulates the synthesis of collagen and elastin, affecting the regeneration of connective tissue. The copper found in ceruloplasmin is one of the most mobile forms of this element in organisms and in this form it regulates iron metabolism and transport, therefore, its absence contributes to anemia [13,21]. According to the World Health Organization, the recommended dose of copper is 2 mg/day for adults [22]. The richest sources of copper in the human diet are cocoa (3.76 mg Cu per 100 g product), sunflower seeds (1.87 mg), pumpkin seeds (1.57 mg) and hazelnuts (1.29 mg). The richest source of copper among animal products is pork liver—100 g of liver contains 0.63 mg of Cu [23].

Like colloidal copper nanoparticles, nano-gold exhibits antibacterial properties [24,25]. However, unlike copper, gold is not an element that occurs naturally in organisms. Thus, there is no reason to assume that there are physiological symptoms of gold deficiency. According to the available list, The Nanodatabase, Au-NPs are used in everyday products such as cosmetics (body lotions, eye moisturizers, serums and anti-wrinkle elixirs), photographic fluids, hair straighteners, earrings, toothbrushes, clothing (trousers, anti-ageing gloves and eye masks and bathing suits), oil stain removers and air conditioners (<http://nanodb.dk/en/>). The properties of nano-gold allowed for a wide application in the cosmetic industry due to stimulation of collagen synthesis and inhibition of elastin loss, delaying the appearance of wrinkles. Au-NPs also inhibit skin ageing and help to combat ageing symptoms, such as age spots. Additionally, it is used to cleanse and regenerate the skin by strengthening the damaged cells of this tissue.

Nano-gold molecules are used in medicine and the pharmaceutical industry. Au-NPs have been successfully used in biomedical imaging [26–28] and cancer therapies [29,30]. Nano-gold is used in photothermal therapy [31–34] and photodynamic therapy [35,36]. Nano-gold molecules are used as drug carriers [37–39], including anticancer drugs [40,41] and vaccines [42]. The use of nano-gold drugs has a high potential but is a cause of concern for local and systemic toxicity to living organisms [43]. After entering the body, Au-NPs are metabolized in the liver, where they can accumulate or be transferred to other organs (e.g. the brain) via blood vessels [44]. During photodynamic therapy, nano-gold particles cause oxidative stress and contribute to reactive oxygen species (ROS)

production [45,46]. It was also observed that the toxicity of nanoparticles of in vitro models differs significantly from that of in vivo models. Au-NPs are considered non-toxic in vitro [47], but the injection of nano-gold particles into mice caused tissue damage and apoptosis in the liver [48] and the injection of nano-gold into rabbits induced apoptosis of cartilage tissue cells [49].

The growing interest in nanotechnology applications in various fields of industry, cosmetics, medicine and everyday life is a reason for a steady increase in their production volume, and consequently also in their emissions to the environment, including the aquatic environment [50]. In addition, the aquatic environment is the ultimate collector of pollutants, including NPs, whether they have been released directly into the water or indirectly, for example through wastewater treatment plants or landfills [43]. Water purity is particularly important for the quantity and richness of water ecosystems. Nanoparticles can interact with other pollutants entering the water, creating compounds that are even more toxic to organisms [51]. Planktonic organisms are the most vulnerable to water pollution and are a key component of aquatic ecosystems, forming the basis of most food networks. The accumulation of xenobiotics (NPs) in water fleas leads to further, higher accumulation in fry (small fish up to the age of one eating zooplankton). Fish, as another element of the food chain, accumulate even more harmful substances. The last organism of each food chain absorbs the largest amount of xenobiotics. In the case of water nets, the last organism may be a human eating fish with accumulated nanoparticles. NPs accumulate in different organs, causing tissue damage [52]. In addition, the toxicity of nanoparticles to zooplankton can reduce its survival and cause the loss of many trophic networks in the aquatic ecosystem [53].

A different dose of NPs may be toxic for different organisms, therefore there is a need to conduct the studies aimed to determine the median lethal concentration LC_{50} for a variety of species. Such studies will make it possible to establish threshold values for a given xenobiotic that cannot be exceeded. This will help to establish standards for the concentrations of nanoparticles used, thereby reducing and slowing down the rate of NPs accumulation in living organisms.

Current knowledge about copper and gold nanoparticles is limited and does not show the full toxic effects on organisms living in aquatic ecosystems. The effect of nano-copper and nano-gold (in the colloidal form available on the market) on zooplankton has not yet been well recognized. Therefore, the influence of aqueous solutions with increasing concentrations of Cu NPs and Au NPs on the model organism—*Daphnia pulex*—was examined in the study presented.

2. Materials and Methods

The study was carried out on *Daphnia pulex* (water flea) organisms, a species belonging to the genus *Daphnia* of the family *Daphniidae*. Water fleas were placed in the aquarium and cultured before the experiment. Solutions for the study were prepared from stock solutions of non-ionic colloidal nano-copper at 25 mg/L and non-ionic colloidal nano-gold at 25 mg/L purchased from the Vitacolloids company (vitacolloids.pl), manufactured using physical methods.

A dilution series of Cu-NPs and Au-NPs solutions from 0 to 1 mg/L were made, the control solution was tap water. Modified 48-h acute toxicity tests were performed on 6-well plates. 10 ml of the appropriate solution was poured into each well. Well number 1 was the control, well number 2 was the 0.0625 mg/L solution, well number 3 was the 0.125 mg/L solution, well number 4 was the 0.25 mg/L solution, well number 5 was the 0.5 mg/L solution and well number 6 was the 1 mg/L solution. The procedure was the same for plates with nano-copper and nano-gold solutions.

The modification of the toxicity test included testing of mature *Daphnia pulex*, which better reflects environmental conditions than testing substance effects on juveniles. Five daphnias were transferred to each well using a sterile Pasteur pipette. The plates were stored in a shaded place and opened only when counting daphnia after 24 and 48 h of incubation in solutions. The culture medium was aquarium water the culture was conducted in, and the light dark cycle was 16 h/8 h. The experiment was performed five times, with three repetitions each time, giving a total of 15 repetitions for each concentration of the solution of nano-copper and nano-gold.

The results were compiled in MS Excel spreadsheet. The mortality percentage was calculated using the equation:

$$\text{Mortality rate} = \frac{\text{deaths occurring within a given time period}}{\text{size of the population in which the deaths occur}} \cdot 100\% \quad (1)$$

The mean and standard deviation of the tests was calculated using the R software (ver. 3.4.4) (<https://www.r-project.org/>). The K deviation rate has been calculated from:

$$K = \frac{50 - P1}{P2 - P1} \quad (2)$$

where P1 is a mortality rate of less than 50% (closest 50%) and P2 is a mortality rate of more than 50% (closest 50%). The LC₅₀ (lethal concentration) value, i.e., the concentration value at which 50% of the examined population dies, was calculated from the formula:

$$\log LC_{50} = \log x + K \cdot \log i \quad (3)$$

where x represents the lower and closest concentration to 50% mortality and i is the ratio of the geometric rate ($i=2$). Statistical differences between % mortality rate measured at 24 or 48 h, respectively, were evaluated using Kruskal-Wallis and post-hoc tests at a significance level of $p < 0.05$ (R software ver. 3.4.4).

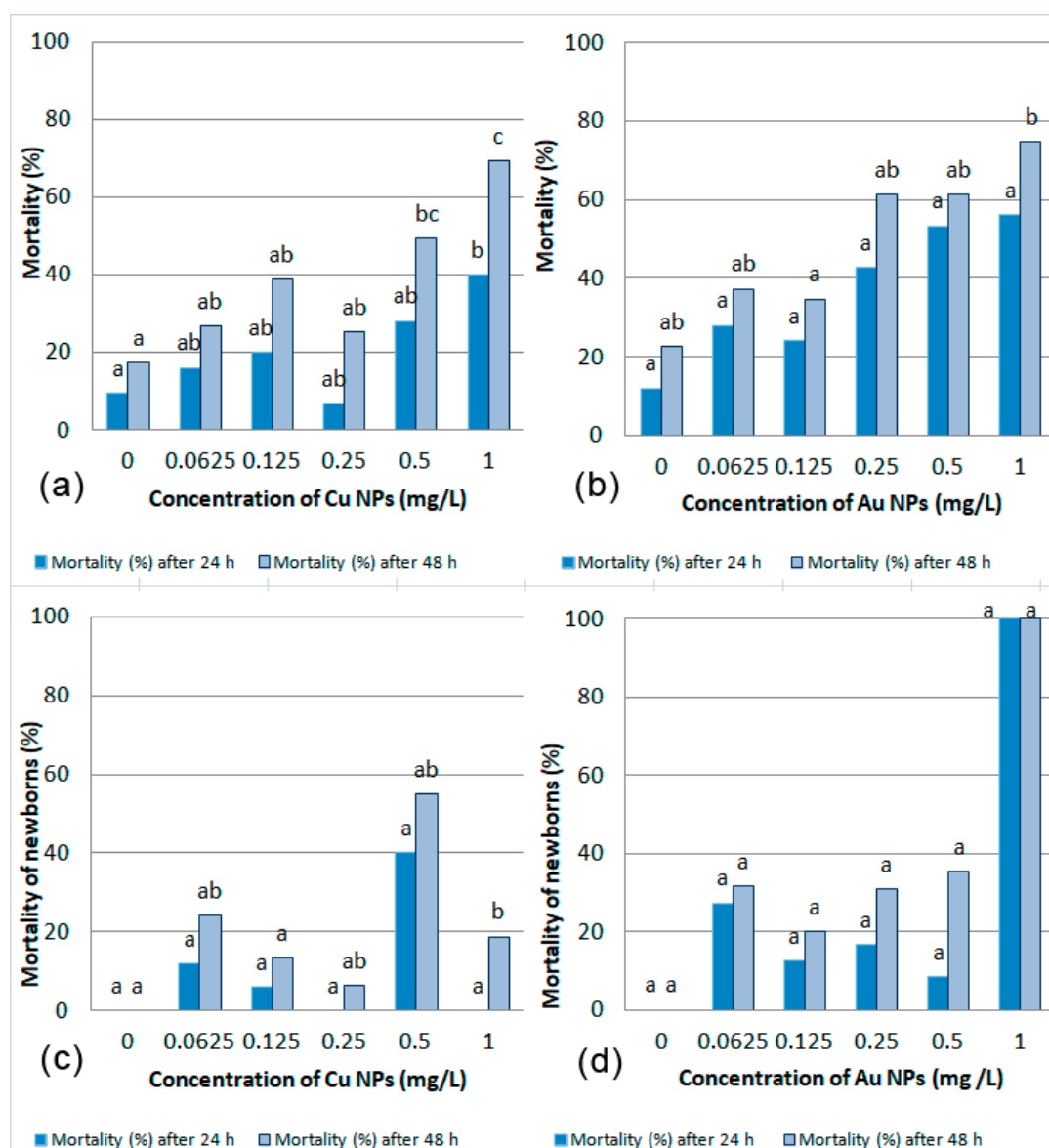
3. Results

The observed change of daphnia number as a result of 24- and 48-h incubation in solutions with different concentrations of copper and gold nanoparticles is presented in Table 1.

Table 1. Effects of different concentrations of NPs solution on survival of *Daphnia pulex*.

NPs	Concentration (mg/L)	Dead after 24 h		Dead after 48 h	
		Mean ± SD	Range	Mean ± SD	Range
Cu	0	0.4667 ± 0.64	(0–2)	0.8667 ± 0.834	(0–2)
	0.0625	0.8 ± 1.014	(0–3)	1.3333 ± 1.345	(0–4)
	0.125	1 ± 1.363	(0–5)	1.9333 ± 1.387	(0–5)
	0.25	0.3333 ± 0.617	(0–2)	1.2667 ± 1.033	(0–4)
	0.5	1.4 ± 1.682	(0–5)	2.4667 ± 1.598	(0–5)
	1	2 ± 2.07	(0–5)	3.467 ± 1.302	(0–5)
Au	0	0.6 ± 0.91	(0–3)	1.133 ± 1.356	(0–5)
	0.0625	1.4 ± 1.639	(0–5)	1.867 ± 1.552	(0–5)
	0.125	1.2 ± 1.521	(0–5)	1.733 ± 1.792	(0–5)
	0.25	2.133 ± 1.885	(0–5)	3.067 ± 1.831	(0–5)
	0.5	2.667 ± 2.289	(0–5)	3.067 ± 2.219	(0–5)
	1	2.8 ± 2.274	(0–5)	3.733 ± 1.792	(0–5)

The most toxic among nano-copper solutions was the dose of 1 mg/L. The dose of 0.25 mg/L caused the lowest average mortality of *Daphnia pulex* after both 24 h and 48 h. In this study group there were fewer dead daphnia after 24 h than in the control sample without the addition of nano-copper. For 48-h incubation of *Daphnia pulex* the LC₅₀ value was calculated as 0.5117 mg/L. The percentage mortality of *Daphnia pulex* depending on nano-copper concentration and incubation time is shown in Figure 1a.



Values marked with different letters mean significant differences (within 24 or 48h) at a level $p < 0.05$.

Figure 1. *Daphnia pulex* mortality (%) of depending on the concentration of Cu NPs (a); Au NPs (b); newborns *Daphnia pulex* depending on the concentration of Cu NPs (c) and Au NPs (d).

Mature daphnias were multiplying during incubation in Cu-NPs solutions. The number of newborn daphnias after 24 and 48 h of incubation depending on the concentration of copper nanoparticles and the effect of nano-copper on newborn daphnias is presented in Table 2.

Table 2. Effects of different concentrations of NPs solution on survival of newborn *Daphnia pulex*.

NPs	Concentration (mg/L)	Number of Newborns after 24 h	Dead after 24 h		Number of Newborns after 48 h	Dead after 48 h	
			Mean ± SD	Range		Mean ± SD	Range
Cu	0	9	0 ± 0	(0–0)	17	0 ± 0	(0–0)
	0.0625	17	0.133 ± 0.516	(0–2)	29	0.467 ± 0.915	(0–3)
	0.125	17	0.067 ± 0.258	(0–1)	30	0.267 ± 0.458	(0–1)
	0.25	6	0 ± 0	(0–0)	16	0.067 ± 0.258	(0–1)
	0.5	5	0.133 ± 0.352	(0–1)	20	0.733 ± 0.961	(0–3)
	1	7	0 ± 0	(0–0)	16	0.2 ± 0.414	(0–1)
Au	0	7	0 ± 0	(0–0)	31	0 ± 0	(0–0)
	0.0625	11	0.2 ± 0.561	(0–2)	19	0.4 ± 0.737	(0–2)
	0.125	8	0.067 ± 0.258	(0–1)	30	0.4 ± 0.632	(0–2)
	0.25	12	0.133 ± 0.352	(0–1)	26	0.533 ± 0.915	(0–3)
	0.5	12	0.067 ± 0.258	(0–1)	17	0.4 ± 0.507	(0–1)
	1	1	0.067 ± 0.258	(0–1)	1	0.067 ± 0.258	(0–1)

The highest number of newborn daphnias was observed in solutions with Cu-NP concentrations at the level of 0.0625 mg/L and 0.125 mg/L. More newborn daphnias were noted in these concentrations of solutions than in the control, which indicates that small doses of nano-copper cause increased reproduction of daphnias. The most toxic concentration of Cu NPs was 0.5 mg/L. Figure 1c shows the relationship of mortality rates of newborn *Daphnia pulex* and the concentration of nano-copper in solutions. After 24-h incubation of newborn daphnias, the LC_{50} value could not be calculated, as there was no mortality higher than 50% at any concentration. After 48-h incubation, the LC_{50} value for newborns is 0.1117 mg/L (Table 3). However, this value is not an accurate LC_{50} , as not all individuals have been exposed to nano-copper for 48 h. Some of them multiplied during the first day of the study, and some only between 24 and 48 h of observation, so it is impossible to determine at what point in time each newborn daphnia appeared.

Table 3. LC_{50} values calculated for *Daphnia pulex* after 24 and 48 h incubation.

NPs	Developmental Stage	LC_{50} (mg/L)	
		After 24 h	After 48 h
Cu	mature	-	0.5117
	juvenile	-	0.1117
Au	mature	0.4027	0.1007
	juvenile	0.0776	0.5853

The least toxic dose of nano-gold is 0.125 mg/L, both after 24 h and 48 h incubation. Nano-gold is most toxic to daphnia at a concentration of 1 mg/L, regardless of incubation time. After 24-h incubation in solutions with different concentrations of nano-gold, it was possible to calculate the LC_{50} , which was 0.4027 mg/L. For 48-h incubation of daphnia in solutions with Au-NPs $LC_{50} = 0.1007$ mg/L (Table 3). Figure 1b shows the changes in the number of daphnia (in percentage of mortality) caused by the addition of different concentrations of colloidal nano-gold solution to water after 24 and 48 h of incubation.

During incubation in the Au-NPS solutions mature daphnias were multiplying. The number of newborn daphnias after 24 and 48 h of incubation depending on the concentration of gold nanoparticles and the effect of nano-gold on newborn daphnia is presented in Table 2. After 24-h incubation in a nano-gold solution the least toxic dose was 0.5 mg/L, and after 48 h it was 0.125 mg/L. Only one daphnia was observed at Au-NPs 1 mg/L sample and it did not survive at this concentration. This also shows that the addition of nano-gold at a concentration of 1 mg/dm⁻³ is an inhibitor of daphnia reproduction. The percentage of mortality at different Au NPs concentrations is shown in Figure 1d. The mortality rate for 1 mg/L concerns only one individual whose death may be related both to the toxicity of that concentration and to the production of the individual particularly sensitive to nano-gold.

For newborn daphnia during the first day of incubation in nano-gold solutions, the LC₅₀ value is 0.0776 mg/L. For newborn occurred during 48 h of study, the LC₅₀ value is 0.5853 mg/L (Table 3).

4. Discussion

Due to increasingly common release of nanoparticles into the environment, studies on their toxicity are necessary. Research shows that nanoparticles can be environmentally risky due to their small size, large active surface area and ability to produce reactive oxygen species [54].

The mortality rate was below 50% after a 24-h incubation with nano-copper solutions in each of the tested samples, so the LC₅₀ is certainly higher than 1 mg/L. Our results are consistent with previously published studies on the influence of CuO NPs on *Daphnia magna*, which indicate the value of LC₅₀ = 2.5578 mg/L [55] or even LC₅₀ = 7.85 mg/L [56]. After a 24-h incubation of the *Daphnia pulex* in nano-copper solutions it can be assumed that water flea has a defense system that allows it to survive at a concentration of 0.25 mg/L better than in the control sample. After 48-h incubation of the water flea in solutions with different concentrations of nano-copper, LC₅₀ was 0.5117 mg/L. It proves that the toxicity of nanoparticles increases with increasing exposure time and that the toxicity of Cu-NPs is correlated with incubation time. It is confirmed in the study by Khoshnood et al. [56] in which LC₅₀ for *D. magna* after 24 h of CuO NPs incubation was 7.85 mg/L, and after 48 h of incubation it was LC₅₀ = 6.62 mg/L.

The first toxicity study of CuO nanoparticles for *Daphnia magna* was conducted in 2008, and the LC₅₀ after 48 h of incubation was 3.2 mg/L, while for copper ions from CuSO₄ LC₅₀ solution it was only 0.07 mg/L [57]. It proves that nano-copper solutions are less harmful to the aquatic environment than copper ions. Further studies with the use of CuO NPs solutions for *D. magna* indicate the LC₅₀ value at the level of 3.3 mg/L [58], 0.99 mg/L [59], 5.66 mg/L [60] and 5.9 mg/L [61]. Research has shown that nano-copper solutions can be harmful to the aquatic environment due to more ecotoxic copper ions [57,62–66] released from the solution. Cu NPs does not dissolve completely [67], however, it is still a source of copper ions. Greater harmfulness of copper ions from CuSO₄ solutions was confirmed in the studies in which LC₅₀ was 0.019 mg/L [68], 0.00653 mg/L [69], 0.045 mg/L [11] and 0.07 mg/L [58]. LC₅₀ values for *Daphnia pulex* are much lower than LC₅₀ values for *Daphnia magna* and this is most likely due to the difference in size between the two species (*D. pulex* is almost twice smaller), so that *D. magna* can take a higher dose of nano-copper.

The conducted studies suggest that low concentrations (0.0625 mg/L and 0.125 mg/L) of nano-copper may enhance daphnia reproduction. Higher number of multiplied daphnia can be observed at low concentrations than in the control for both 24-h and 48-h incubation. This may be related to the fungicidal and antibacterial properties of the nanoparticles [10,12], which provide better conditions for mature daphnia to reproduce. Also under the influence of a stress factor such as the addition of a small amount of Cu NPs, survival is more important for daphnia than keeping the offspring alive, which may explain why there are more newborn daphnias in these concentrations compared to the control. The inhibition of daphnia reproduction by copper has been demonstrated in a study by Taylor et al. [70], where the addition of copper ions to water at a concentration of 1 µg/L inhibited reproduction by 9% and the addition of copper ions at a concentration of 1.8 µg/L reduced reproduction by 30% compared to the control sample. This may be an explanation for the lack of renewal of daphnia population in lakes heavily contaminated with copper ions.

After 24-h exposure of mature *Daphnia pulex* to solutions with addition of nano-gold the LC₅₀ amounted to 0.4027 mg/L, and after 48-h exposure the LC₅₀ amounted to 0.1007 mg/L. The most toxic concentration causing death of 56% of individuals after the first day of incubation and about 75% after the second day of incubation was 1 mg/L. The literature data show LC₅₀ values for *Daphnia magna* incubated with nano-gold solution for 48 h as 0.32 mg/L [60] and 0.5 mg/L [71]. A study by Li et al. [72] suggested a much higher value of LC₅₀ than in other studies for *Daphnia magna*, and its value was estimated at about 70 mg/L. LC₅₀ values for *Daphnia pulex* are much lower than LC₅₀ values for *Daphnia magna*, most likely due to the difference in size between the two species (*D. pulex* is

almost twice smaller), so that *D. magna* can uptake a higher dose of nano-gold. This shows that if there are differences between these closely related species, the LC₅₀ should be marked not only on model organisms, but on all those which are exposed to a potential risk of contamination.

The highest number of newborn daphnias after 24-h incubation in Au-NPs solutions was noted at concentrations of 0.25 mg/L and 0.5 mg/L. 24 h LC₅₀ for young daphnias was 0.0776 mg/L. After 48-h incubation, the LC₅₀ was 0.5853 mg/L. Incubation of water flea with the addition of nano-gold at a concentration of 1 mg/L effectively inhibits the reproduction of daphnia. Studies done on another organism living in water—*Danio rerio*—indicated the harmful effect of nano-gold on hatching and growth of juveniles. Au-NPs at a concentration of 2.5 mg/L reduces the hatching of young *D. rerio* to only 20%, moreover, the young incubated in a solution of nano-gold are apparently smaller than the control group [60]. Toxicity of nano gold is confirmed in the study by Kim et al. [73] in which the LC₅₀ after five days of *Danio rerio* incubation was 30 mg/L. This concentration causes abnormal pigmentation and anomalies in eye development during embryonic development of *D. rerio*—a defect that occurred in 80% of young individuals. However, there are studies contrary to those mentioned above. These studies do not indicate any toxicity of Au-NPs to *Danio rerio* [74], which may be related to the fact that only a very low concentration range (0–100 µg·dm⁻³) of Au-NPs was tested.

Literature research proves that gold ions are more toxic compared to nano-gold [44,75]. This was confirmed in the studies in which LC₅₀ value after 48-h incubation of *Daphnia magna* in solutions with addition of gold ions (derived from HAuCl₄) was 2 mg/L, while LC₅₀ value for nano-gold was about 70 mg/L [72]. In case of *Daphnia pulex*, 48 h LC₅₀ for nano-gold was 7.31 mg/L and for gold ions it was LC₅₀ = 0.01 mg/L [76]. Due to the toxicity revealed by nano-gold particles, there are studies using Au-NPs coating substances, which reduce their toxicity [73,77–79]. The size of nano-gold molecules in solutions is another factor affecting its ecotoxicity, but compared to coatings it is a secondary issue [80]. Studies have proven higher toxicity for smaller (10–12 nm diameter) than larger (50 nm diameter) nano-gold particles [46,81,82].

To sum up, it was found on the basis of the conducted study that nano-copper and nano-gold particles are toxic to *Daphnia pulex*. Compared to the literature data, the study results show that the toxicity of nano-copper and nano-gold is different depending on the species of organism. The variability in the range of LC₅₀ values both for nano-copper and nano-gold reported in the literature may be due to differences in the experimental designs or features like particle size and stabilizing agent used. However, it can be concluded that in order to reduce the ecotoxicity of Cu NPs and Au NPs, it is recommended to use very low concentrations (not exceeding the LC₅₀) of solutions or, according to literature data, to conduct studies to coat nanoparticles with other chemical substances, thanks to which the solutions of nanoparticles will become safer for the environment.

5. Conclusions

The values of lethal concentrations after the influence of nanoparticles on *Daphnia pulex* were: for 24 h LC₅₀ > 1 mg/L, for 48 h LC₅₀ = 0.5117 mg/L. The toxicity of nano-copper increases with increasing exposure time.

Low concentrations (0.0625 mg/L and 0.125 mg/L) of nanoparticles cause increased reproduction of water flea.

The received LC₅₀ values for *Daphnia pulex* incubated in nano-gold solutions were: 24 h LC₅₀ = 0.4027 mg/L, 48 h LC₅₀ = 0.1007 mg/L. The toxicity of nano-gold increases with time of exposure.

The addition of nano-gold at a concentration of 1 mg/L is an inhibitor of *Daphnia pulex* reproduction.

Based on LC₅₀ values and observations of the number of multiplied water fleas in the conducted studies, it was proved that nano-gold solutions are more toxic than nano-copper solutions. Further studies on the ecotoxicity of nano-copper and nano-gold should be directed to work on reducing the toxicity of substances by using employing appropriate particle sizes and coating agents.

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reagents/materials/analysis tools; M.G., M.K.-G., M.S., K.C. prepared the original draft; M.K.-G., K.C. review and editing.

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References

1. Oberdörster, G.; Oberdörster, E.; Oberdörster, J. Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. *Environ. Health Perspect.* **2005**, *113*, 823–839. [[CrossRef](#)]
2. Bohnsack, J.P.; Assemi, S.; Miller, J.D.; Furgeson, D.Y. The Primacy of Physicochemical Characterization of Nanomaterials for Reliable Toxicity Assessment: A Review of the Zebrafish Nanotoxicology Model. *Methods Mol. Biol.* **2012**, *926*, 261–316.
3. Lacave, J.M.; Retuerto, A.; Vicario-Parés, U.; Gilliland, D.; Oron, M.; Cajaraville, M.P.; Obera, A. Effects of metal-bearing nanoparticles (Ag, Au, CdS, ZnO, SiO₂) on developing zebrafish embryos. *Nanotechnology* **2016**, *27*, 325102. [[CrossRef](#)]
4. Kim, T.; Lee, K.; Gong, M.; Joo, S.W. Control of Gold Nanoparticle Aggregates by Manipulation of Interparticle Interaction. *Langmuir* **2005**, *21*, 9524–9528. [[CrossRef](#)]
5. Shipway, A.N.; Lahav, M.; Gabai, R.; Willner, I. Investigations into the Electrostatically Induced Aggregation of Au Nanoparticles. *Langmuir* **2000**, *16*, 8789–8795. [[CrossRef](#)]
6. Kowalska-Góralaska, M.; Zygadlik, K.; Dobrzański, Z.; Patkowska-Sokoła, B.; Kowalski, Z. Metody otrzymywania nanozwiązków i ich praktyczne zastosowania. *Przem. Chem.* **2010**, *89*, 430–433.
7. Roco, M.C. Nanotechnology: Convergence with modern biology and medicine. *Curr. Opin. Biotechnol.* **2003**, *14*, 337–346. [[CrossRef](#)]
8. Baker, T.J.; Tyler, C.R.; Galloway, T.S. Impacts of metal and metal oxide nanoparticles on marine organisms. *Environ. Pollut.* **2014**, *186*, 257–271. [[CrossRef](#)]
9. Keller, A.A.; McFerran, S.; Lazareva, A.; Suh, S. Global life cycle releases of engineered nanomaterials. *J. Nanopart. Res.* **2013**, *15*, 1692. [[CrossRef](#)]
10. Bundschuh, M.; Filser, J.; Lüderwald, S.; McKee, M.S.; Metreveli, G.; Schaumann, G.E.; Schulz, R.; Wagner, S. Nanoparticles in the environment: Where do we come from, where do we go to? *Environ. Sci. Eur.* **2018**, *30*, 6. [[CrossRef](#)]
11. De Oliveira-Filho, E.C.; Lopes, R.M.; Paumgarten, F.J.R. Comparative study on the susceptibility of freshwater species to copper-based pesticides. *Chemosphere* **2004**, *56*, 369–374. [[CrossRef](#)]
12. Baeg, E.; Sooklert, K.; Sereemasapun, A. Copper Oxide Nanoparticles Cause a Dose-Dependent Toxicity via Inducing Reactive Oxygen Species in *Drosophila*. *Nanomaterials* **2018**, *8*, 824. [[CrossRef](#)]
13. Hordyjewska, A.; Popiołek, Ł.; Kocot, J. The many “faces” of copper in medicine and treatment. *BioMetals* **2014**, *27*, 611–621. [[CrossRef](#)]
14. Morsi, R.E.; Alsaabagh, A.M.; Nasr, S.A.; Zaki, M.M. Multifunctional nanocomposites of chitosan, silver nanoparticles, copper nanoparticles and carbon nanotubes for water treatment: Antimicrobial characteristics. *Int. J. Biol. Macromol.* **2017**, *97*, 264–269. [[CrossRef](#)]
15. Yoon, K.Y.; Hoon Byeon, J.; Park, J.H.; Hwang, J. Susceptibility constants of *Escherichia coli* and *Bacillus subtilis* to silver and copper nanoparticles. *Sci. Total Environ.* **2007**, *373*, 572–575. [[CrossRef](#)]
16. Cohen, D.; Soroka, Y.; Ma’or, Z.; Oron, M.; Portugal-Cohen, M.; Brégégère, F.M.; Berhanu, D.; Valsami-Jones, E.; Hai, N.; Milner, Y. Evaluation of topically applied copper(II) oxide nanoparticle cytotoxicity in human skin organ culture. *Toxicol. In Vitro.* **2013**, *27*, 292–298. [[CrossRef](#)]
17. Llorens, A.; Lloret, E.; Picouet, P.A.; Trbojevich, R.; Fernandez, A. Metallic-based micro and nanocomposites in food contact materials and active food packaging. *Trends Food Sci. Technol.* **2012**, *24*, 19–29. [[CrossRef](#)]
18. Gupta, U.C.; Gupta, S.C. Trace element toxicity relationships to crop production and livestock and human health: Implications for management. *Commun. Soil Sci. Plant Anal.* **1998**, *29*, 1491–1522. [[CrossRef](#)]
19. Seth, R.; Yang, S.; Choi, S.; Sabeen, M.; Roberts, E. In vitro assessment of copper-induced toxicity in the human hepatoma line, Hep G2. *Toxicol. In Vitro.* **2004**, *18*, 501–509. [[CrossRef](#)]
20. Gaetke, L.M.; Chow, C.K. Copper toxicity, oxidative stress, and antioxidant nutrients. *Toxicology* **2003**, *189*, 147–163. [[CrossRef](#)]

21. Fraga, C.G. Relevance, essentiality and toxicity of trace elements in human health. *Mol. Asp. Med.* **2005**, *26*, 235–244. [[CrossRef](#)]
22. WHO. *Copper in Drinking-Water. Background Document for Preparation of WHO Guidelines for Drinking-Water Quality*; (WHO/SDE/WSH/03.04/88); World Health Organization: Geneva, Switzerland, 2004.
23. Kunachowicz, H.; Nadolna, I.; Przygoda, B.; Iwanow, K. *Tabele Składu i Wartości Odżywczej Żywności*; Wydawnictwo Lekarskie PZWL: Warszawa, Poland, 2005.
24. Dykman, L.A.; Khlebtsov, N.G. Gold nanoparticles in biology and medicine: Recent advances and prospects. *Acta Nat.* **2011**, *3*, 34–55. [[CrossRef](#)]
25. Libralato, G.; Galdiero, E.; Falanga, A.; Carotenuto, R.; de Alteriis, E.; Guida, M. Toxicity Effects of Functionalized Quantum Dots, Gold and Polystyrene Nanoparticles on Target Aquatic Biological Models: A Review. *Molecules* **2017**, *22*, 1439. [[CrossRef](#)]
26. Kong, F.Y.; Zhang, J.W.; Li, R.F.; Wang, Z.X.; Wang, W.J.; Wang, W. Unique Roles of Gold Nanoparticles in Drug Delivery, Targeting and Imaging Applications. *Molecules* **2017**, *22*, 1445. [[CrossRef](#)]
27. Nune, S.K.; Gunda, P.; Thallapally, P.K.; Lin, Y.Y.; Forrest, M.L.; Berkland, C.J. Nanoparticles for biomedical imaging. *Expert Opin. Drug Deliv.* **2009**, *6*, 1175–1194. [[CrossRef](#)]
28. Zhang, X. Gold Nanoparticles: Recent Advances in the Biomedical Applications. *Cell Biochem. Biophys.* **2015**, *72*, 771–775. [[CrossRef](#)]
29. Farooq, M.U.; Novosad, V.; Rozhkova, E.A.; Wali, H.; Ali, A.; Fateh, A.A.; Neogi, P.B.; Neogi, A.; Wang, Z. Gold Nanoparticles-enabled Efficient Dual Delivery of Anticancer Therapeutics to HeLa Cells. *Sci. Rep.* **2018**, *8*, 2907. [[CrossRef](#)]
30. Huff, T.B.; Tong, L.; Zhao, Y.; Hansen, M.N.; Cheng, J.X.; Wei, A. Hyperthermic effects of gold nanorods on tumor cells. *Nanomedicine* **2007**, *2*, 125–132. [[CrossRef](#)]
31. Ajnai, G.; Chiu, A.; Kan, T.; Cheng, C.C.; Tsai, T.H.; Chang, J. Trends of Gold Nanoparticle-based Drug Delivery System in Cancer Therapy. *J. Exp. Clin. Med.* **2014**, *6*, 172–178. [[CrossRef](#)]
32. Huang, X.; El-Sayed, I.H.; Qian, W.; El-Sayed, M.A. Cancer Cell Imaging and Photothermal Therapy in the Near-Infrared Region by Using Gold Nanorods. *J. Am. Chem. Soc.* **2006**, *128*, 2115–2120. [[CrossRef](#)]
33. Hwang, S.; Nam, J.; Jung, S.; Song, J.; Doh, H.; Kim, S. Gold nanoparticle-mediated photothermal therapy: Current status and future perspective. *Nanomedicine* **2014**, *9*, 2003–2022. [[CrossRef](#)] [[PubMed](#)]
34. Riley, R.S.; Day, E.S. Gold Nanoparticle-Mediated Photothermal Therapy: Applications and Opportunities for Multimodal Cancer Treatment. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* **2017**, *9*. [[CrossRef](#)] [[PubMed](#)]
35. Kim, H.; Lee, D.; Kim, H.S.; Lee, D.Y. Near-Infrared-Responsive Cancer Photothermal and Photodynamic Therapy Using Gold Nanoparticles. *Polymers* **2018**, *10*, 961. [[CrossRef](#)] [[PubMed](#)]
36. Singh, P.; Pandit, S.; Mokkapati, V.R.S.S.; Garg, A.; Ravikumar, V.; Mijakovic, I. Gold Nanoparticles in Diagnostics and Therapeutics for Human Cancer. *Int. J. Mol. Sci.* **2018**, *19*, 1979. [[CrossRef](#)] [[PubMed](#)]
37. Han, G.; Ghosh, P.; Rotello, V.M. Functionalized gold nanoparticles for drug delivery. *Nanomedicine* **2007**, *2*, 113–123. [[CrossRef](#)] [[PubMed](#)]
38. Hussain, K.; Hussain, T. Gold Nanoparticles: A Boon to Drug Delivery System. *South Indian J. Biol. Sci.* **2015**, *1*, 128. [[CrossRef](#)]
39. Kumar, A.; Zhang, X.; Liang, X.J. Gold nanoparticles: Emerging paradigm for targeted drug delivery system. *Biotechnol. Adv.* **2013**, *31*, 593–606. [[CrossRef](#)]
40. Brown, S.D.; Nativo, P.; Smith, J.A.; Stirling, D.; Edwards, P.R.; Venugopal, B.; Flint, D.J.; Plumb, J.A.; Graham, D.; Wheate, N.J. Gold Nanoparticles for the Improved Anticancer Drug Delivery of the Active Component of Oxaliplatin. *J. Am. Chem. Soc.* **2010**, *132*, 4678–4684. [[CrossRef](#)]
41. Dreaden, E.C.; Austin, L.A.; Mackey, M.A.; El-Sayed, M.A. Size matters: Gold nanoparticles in targeted cancer drug delivery. *Ther. Deliv.* **2012**, *3*, 457–478. [[CrossRef](#)]
42. Comber, J.D.; Bamezai, A. Gold Nanoparticles (AuNPs): A New Frontier in Vaccine Delivery. *J. Nanomed. Biother. Discov.* **2015**, *5*, 4. [[CrossRef](#)]
43. Nowack, B.; Bucheli, T.D. Occurrence, behavior and effects of nanoparticles in the environment. *Environ. Pollut.* **2007**, *150*, 5–22. [[CrossRef](#)] [[PubMed](#)]
44. Dayal, N.; Singh, D.; Patil, P. Effect of bioaccumulation of gold nanoparticles on ovarian morphology of female zebrafish (*Danio rerio*). *World J. Pathol.* **2017**, *6*, 1–12.

45. Dedeh, A.; Ciutat, A.; Treguer-Delapierre, M.; Bourdineaud, J.P. Impact of gold nanoparticles on zebrafish exposed to a spiked sediment. *Nanotoxicology* **2015**, *9*, 71–80. [[CrossRef](#)] [[PubMed](#)]
46. Pan, Y.; Leifert, A.; Ruau, D.; Neuss, S.; Bornemann, J.; Schmid, G.; Brandau, W.; Simon, U.; Jahnen-Dechent, W. Gold nanoparticles of diameter 1.4 nm trigger necrosis by oxidative stress and mitochondrial damage. *Small* **2009**, *5*, 2067–2076. [[CrossRef](#)] [[PubMed](#)]
47. Bakri, S.J.; Pulido, J.S.; Mukherjee, P.; Marler, R.J.; Mukhopadhyay, D. Absence of histologic retinal toxicity of intravitreal nanogold in a rabbit model. *Retina* **2008**, *28*, 147–149. [[CrossRef](#)] [[PubMed](#)]
48. Cho, W.S.; Cho, M.; Jeong, J.; Choi, M.; Cho, H.Y.; Han, B.S.; Kim, S.H.; Kim, H.O.; Lim, Y.T.; Chung, B.H.; et al. Acute toxicity and pharmacokinetics of 13 nm-sized PEG-coated gold nanoparticles. *Toxicol. Appl. Pharmacol.* **2009**, *236*, 16–24. [[CrossRef](#)] [[PubMed](#)]
49. Huang, H.; Quan, Y.; Wang, X.; Chen, T. Gold Nanoparticles of Diameter 13 nm Induce Apoptosis in Rabbit Articular Chondrocytes. *Nanoscale Res. Lett.* **2016**, *11*, 249. [[CrossRef](#)] [[PubMed](#)]
50. Moore, M.N. Do nanoparticles present ecotoxicological risks for the health of the aquatic environment? *Environ. Int.* **2006**, *32*, 967–976. [[CrossRef](#)]
51. Handy, R.D.; von der Kammer, F.; Lead, J.R.; Hassellöv, M.; Owen, R.; Crane, M. The ecotoxicology and chemistry of manufactured nanoparticles. *Ecotoxicology* **2008**, *17*, 287–314. [[CrossRef](#)]
52. Van Pomeran, M.; Peijnenburg, W.J.G.M.; Vlieg, R.C.; van Noort, S.J.T.; Vijver, M.G. The biodistribution and immuno-responses of differently shaped non-modified gold particles in zebrafish embryos. *Nanotoxicology* **2019**, 558–571. [[CrossRef](#)]
53. Heinlaan, M.; Muna, M.; Knöbel, M.; Kistler, D.; Odzak, N.; Kühnel, D.; Müller, J.; Gupta, G.S.; Kumar, A.; Shanker, R.; et al. Natural water as the test medium for Ag and CuO nanoparticle hazard evaluation: An interlaboratory case study. *Environ. Pollut.* **2016**, *216*, 689–699. [[CrossRef](#)] [[PubMed](#)]
54. Xia, T.; Li, N.; Nel, A.E. Potential Health Impact of Nanoparticles. *Annu. Rev. Public. Health* **2009**, *30*, 137–150. [[CrossRef](#)] [[PubMed](#)]
55. Jo, H.J.; Choi, J.W.; Lee, S.H.; Hong, S.W. Acute toxicity of Ag and CuO nanoparticle suspensions against *Daphnia magna*: The importance of their dissolved fraction varying with preparation methods. *J. Hazard. Mater.* **2012**, *227*, 301–308. [[CrossRef](#)] [[PubMed](#)]
56. Khoshnood, R.; Jaafarzadeh, N.; Jamili, S.; Farshchi, P.; Taghavi, L. Nanoparticles Ecotoxicity on *Daphnia magna*. *Transylv. Rev. Syst. Ecol. Res.* **2016**, *18*, 29–38. [[CrossRef](#)]
57. Heinlaan, M.; Ivask, A.; Blinova, I.; Dubourguier, H.C.; Kahru, A. Toxicity of nanosized and bulk ZnO, CuO and TiO₂ to bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*. *Chemosphere* **2008**, *71*, 1308–1316. [[CrossRef](#)] [[PubMed](#)]
58. Blinova, I.; Ivask, A.; Heinlaan, M.; Mortimer, M.; Kahru, A. Ecotoxicity of nanoparticles of CuO and ZnO in natural water. *Environ. Pollut.* **2010**, *158*, 41–47. [[CrossRef](#)]
59. Zhao, H.; Lu, G.; Xia, J.; Jin, S. Toxicity of Nanoscale CuO and ZnO to *Daphnia magna*. *Chem. Res. Chin. Univ.* **2012**, *28*, 209–213.
60. Liu, J.; Fan, D.; Wang, L.; Shi, L.; Ding, J.; Chen, Y.; Shen, S. Effects of ZnO, CuO, Au, and TiO₂ nanoparticles on *Daphnia magna* and early life stages of zebrafish *Danio rerio*. *Environ. Prot. Eng.* **2014**, *40*, 139–149.
61. Sovová, T.; Kocí, V.; Kochánková, L. Ecotoxicity of nano and bulk forms of metal oxides. In Proceedings of the 2009 NANOCON Conference, Roznov pod Radhostem, Czech Republic, 20–22 October 2009; pp. 62–71.
62. Griffitt, R.J.; Hyndman, K.; Denslow, N.D.; Barber, D.S. Comparison of Molecular and Histological Changes in Zebrafish Gills Exposed to Metallic Nanoparticles. *Toxicol. Sci.* **2009**, *107*, 404–415. [[CrossRef](#)]
63. Kowalska-Górska, M.; Dziewulska, K.; Kulasza, M. Effect of copper nanoparticles and ions on spermatozoa motility of sea trout (*Salmo trutta m. Trutta L.*). *Aquat. Toxicol.* **2019**, *211*, 11–17. [[CrossRef](#)]
64. Mortimer, M.; Kasemets, K.; Vodovnik, M.; Marinšek-Logar, R.; Kahru, A. Exposure to CuO Nanoparticles Changes the Fatty Acid Composition of Protozoa *Tetrahymena thermophila*. *Environ. Sci. Technol.* **2011**, *45*, 6617–6624. [[CrossRef](#)] [[PubMed](#)]
65. Mortimer, M.; Kasemets, K.; Kahru, A. Toxicity of ZnO and CuO nanoparticles to ciliated protozoa *Tetrahymena thermophila*. *Toxicology* **2010**, *269*, 182–189. [[CrossRef](#)]
66. Shaw, B.J.; Handy, R.D. Physiological effects of nanoparticles on fish: A comparison of nanometals versus metal ions. *Environ. Int.* **2011**, *37*, 1083–1097. [[CrossRef](#)] [[PubMed](#)]
67. Notter, D.A.; Mitrano, D.M.; Nowack, B. Are nanosized or dissolved metals more toxic in the environment? A meta-analysis. *Environ. Toxicol. Chem.* **2014**, *33*, 2733–2739. [[CrossRef](#)] [[PubMed](#)]

68. Mastin, B.J.; Rodgers, J.H. Toxicity and bioavailability of copper herbicides (Clearigate, Cutrine-Plus, and copper sulfate) to freshwater animals. *Arch. Environ. Contam. Toxicol.* **2000**, *39*, 445–451. [[CrossRef](#)] [[PubMed](#)]
69. Harmon, S.M.; Specht, W.L.; Chandler, G.T. A comparison of the daphnids *Ceriodaphnia dubia* and *Daphnia ambigua* for their utilization in routine toxicity testing in the Southeastern United States. *Arch. Environ. Contam. Toxicol.* **2003**, *45*, 79–85. [[CrossRef](#)] [[PubMed](#)]
70. Taylor, N.S.; Kirwan, J.A.; Johnson, C.; Yan, N.D.; Viant, M.R.; Gunn, J.M.; McGeer, J.C. Predicting chronic copper and nickel reproductive toxicity to *Daphnia pulex-pulicaria* from whole-animal metabolic profiles. *Environ. Pollut.* **2016**, *212*, 325–329. [[CrossRef](#)]
71. Skjolding, L.M.; Kern, K.; Hjorth, R.; Hartmann, N.; Overgaard, S.; Ma, G.; Veinot, J.G.C.; Baun, A. Uptake and depuration of gold nanoparticles in *Daphnia magna*. *Ecotoxicology* **2014**, *23*, 1172–1183. [[CrossRef](#)]
72. Li, T.; Albee, B.; Alemayehu, M.; Diaz, R.; Ingham, L.; Kamal, S.; Rodriguez, M.; Bishnoi, S.W. Comparative toxicity study of Ag, Au, and Ag–Au bimetallic nanoparticles on *Daphnia magna*. *Anal. Bioanal. Chem.* **2010**, *398*, 689–700. [[CrossRef](#)]
73. Kim, K.T.; Zaikova, T.; Hutchison, J.E.; Tanguay, R.L. Gold nanoparticles disrupt zebrafish eye development and pigmentation. *Toxicol. Sci.* **2013**, *133*, 275–288. [[CrossRef](#)]
74. AshaRani, P.V.; Lianwu, Y.; Gong, Z.; Valiyaveetil, S. Comparison of the toxicity of silver, gold and platinum nanoparticles in developing zebrafish embryos. *Nanotoxicology* **2011**, *5*, 43–54. [[CrossRef](#)] [[PubMed](#)]
75. Nam, S.H.; Lee, W.M.; Shin, Y.J.; Yoon, S.J.; Kim, S.W.; Kwak, J.I.; An, Y.J. Derivation of guideline values for gold (III) ion toxicity limits to protect aquatic ecosystems. *Water Res.* **2014**, *48*, 126–136. [[CrossRef](#)] [[PubMed](#)]
76. Botha, T.L.; James, T.E.; Wepener, V. Comparative Aquatic Toxicity of Gold Nanoparticles and Ionic Gold Using a Species Sensitivity Distribution Approach. *J. Nanomater.* **2015**, *2015*, 1–16. [[CrossRef](#)]
77. Böhme, S.; Stärk, H.J.; Kühnel, D.; Reemtsma, T. Exploring LA-ICP-MS as a quantitative imaging technique to study nanoparticle uptake in *Daphnia magna* and zebrafish (*Danio rerio*) embryos. *Anal. Bioanal. Chem.* **2015**, *407*, 5477–5485. [[CrossRef](#)] [[PubMed](#)]
78. Fraga, S.; Faria, H.; Soares, M.E.; Duarte, J.A.; Soares, L.; Pereira, E.; Costa-Pereira, C.; Teixeira, J.P.; de Lourdes Bastos, M.; Carmo, H. Influence of the surface coating on the cytotoxicity, genotoxicity and uptake of gold nanoparticles in human HepG2 cells. *J. Appl. Toxicol.* **2013**, *33*, 1111–1119. [[CrossRef](#)] [[PubMed](#)]
79. Harper, S.L.; Carriere, J.L.; Miller, J.M.; Hutchison, J.E.; Maddux, B.L.S.; Tanguay, R.L. Systematic evaluation of nanomaterial toxicity: Utility of standardized materials and rapid assays. *ACS Nano* **2011**, *5*, 4688–4697. [[CrossRef](#)] [[PubMed](#)]
80. Haque, E.; Ward, A.C. Zebrafish as a Model to Evaluate Nanoparticle Toxicity. *Nanomaterials* **2018**, *8*, 561. [[CrossRef](#)] [[PubMed](#)]
81. Bar-Ilan, O.; Albrecht, R.M.; Fako, V.E.; Furgeson, D.Y. Toxicity Assessments of Multisized Gold and Silver Nanoparticles in Zebrafish Embryos. *Small* **2009**, *5*, 1897–1910. [[CrossRef](#)] [[PubMed](#)]
82. Geffroy, B.; Ladhar, C.; Cambier, S.; Treguer-Delapierre, M.; Brèthes, D.; Bourdineaud, J.P. Impact of dietary gold nanoparticles in zebrafish at very low contamination pressure: The role of size, concentration and exposure time. *Nanotoxicology* **2012**, *6*, 144–160. [[CrossRef](#)]

