RESEARCH ARTICLE

Short-term safety and risk evaluation of engine oil enriched by high concentrations copper nanoparticles on the skin

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ABSTRACT

Objective(s): Copper nano particles are added to ordinary engine oil as an additive to reduce friction and repair damaged surface under friction conditions. However, it is still unclear what environmental effects such a compound might have on conventional engine oils and its toxicity in different animal species has not been determined. The aim of this study is to investigate the effect of short-term exposure of large amounts of nano sized particle-enriched engine oil to transcutaneous animal model on earthworms in order to evaluate its hazards in human contact.

Methods: Screening test (filter paper contact test) involves applying earthworms on the paper to identify potentially toxic chemicals in the soil for earthworms, and artificial soil testing involves holding earthworms in samples of predefined and precise soil. In both tests a range of different concentrations is used. In artificial soil testing the results of loss is obtained 7 and 14 days after the experiment. In the flat paper test the losses are checked 24 and 48 hours or if required up to 72 hours later.

Results: The lethality rate of the engine oil used at a concentration of 1.25 ml or higher was obtained from fresh engine oil containing nano-copper at 24 Hours and 48 was significantly higher ($p < 0.001$).

Conclusions: The toxicity of a new engine oil is higher than that of a new engine oil containing copper nanoparticles, but in the case of used engine oil, the toxicity of nano-oil is higher than that of conventional oil.

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INTRODUCTION

"Nanoscience is the study of phenomena and manipulation of materials at atomic, molecular and macromolecular scales, where properties differ significantly from those at larger" scaleThe scale of dimensions adopted for the applicability of nanotechnology is usually less than 100 nm[1] .One of the hallmarks of nano materials is that they behave differently from the behavior of coarse-grained or micro structured materials. the size of the material will also be one of the factors affecting the properties of the material when the particle size becomes smaller than a specific size [2]. Copper nano particles, one of the metal nano particles produced, is now commercially available.

Copper nano particles have recently been widely used as additives in oils, plastics / polymers, metal coatings and inks [3]. The mechanical properties of nano materials improve with decreasing size [4]. In addition, metal and semiconductor nano particles have lower melting point or transition temperature compared to their mass. A lower melting point is observed when the particle size is less than 100 nm, due to the increase in surface energy with decreasing particle size [5]. Chemically, the range of chemical reactions for nano-materials is much higher than

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for conventional materials. In nano-materials, a high percentage of atoms are at the surface of the material, thus increasing the probability of the atoms colliding, thus increasing the reactivity of the material. In addition, nano materials can be used as catalysts because the nanostructure will increase the amount of voids than usual. Therefore, nanometer-sized catalysts can be used at lower temperatures, which reduces the reactivity of these catalysts, resulting in a longer service life than the usual type [6]. Also, as the magnetic properties increase with increasing surface-to-volume ratio, the magnetic materials of the nano materials exhibit abnormal properties due to the size of the particles and their charge transfer properties [7]. The use of nano particles is increasing day by day, so nano materials may enter the environment intentionally or unintentionally. Recent studies indicate the toxicity of nano materials to aquatic and rodent species, although many of the hidden effects of these substances are not yet known [8]. There is currently limited understanding of the side effects of nano materials in nature and wildlife [9]. As well as much of our information is on the toxicity of nano materials to aquatic organisms, further studies on the toxicity of nano materials to onshore organisms are needed [8]. One way in which nano materials enter the body of living organisms is inhalation, which in itself causes many organ involvement. There is ample evidence that the active surface (size of nano particles) and the number of inhaled nano particles play a decisive role in the deleterious effects they cause. Example: Study of the effect of carbon nano particles and titanium oxide with inter-size measurements 220-12 Nanometers on mice have been shown to lower the defenses in their lungs, as well as working in places where carbon black is used to cause respiratory illnesses such as bronchitis or even lung cancer. [10]. Another way in which nano particles penetrate animals and humans is through the skin. This first frontier is almost impenetrable. Especially ionic substances or water-soluble molecules cannot pass through the skin [11]. But one of the easiest and most important ways for the nano particles to enter the human body is the gastrointestinal tract. This is the most important portal for the transfer of macromolecules into the body. The intestinal boundaries in turn are composed of micropores that have an area of about 200 They produce square meters for food[12]. Combustion-derived nanoparticles can cause adverse health effects in addition to air pollution and its environmental impact [13].

In one study, the effect of gold nanoparticles on dermal fibroblast cells has been demonstrated. In this study, morphology of nanoparticles in contact with these cells was evaluated by increasing the concentration of nanoparticles from 0.1 mg / ml to 0.6 mg / ml, resulting in a much thinner cellular attachment. It showed less adhesion to the surface; in addition, the number of mature cells decreased, leading to a decrease in the ability of cells to proliferate. [14]. Copper nano particles are one of the commercially produced nano particles used in various industries, for example due to their high speed, low cost and recyclable properties as one of the polar anode materials used in lithium batteries. Placed [15]. Another characteristic of copper nano particles that has been of interest to researchers is the lubricating properties of copper nano particles mixed with the lubricants used in the lubrication industry [16]. Due to the healing effects of copper nano particles, they are added to the lubricating oils as an additive to reduce friction and repair the damaged surface in the friction position. Domestic researchers at the Petroleum Industry Research Institute have enriched conventional engine oil with nano materials, hoping that this product will be able to replace conventional engine oil used in cars due to better performance. However, it remains unclear what the environmental effects of such a compound may be on conventional engine oil and its toxicity in different animal species. Therefore, the present study was designed to investigate the effect of acute cutaneous toxicity of copper nano particles on earthworm animal model. Some studies have shown that cutaneous inflammation and oxidative stress are one of the most important causes of nanoparticle toxicity that can be caused by several primary pathways: A) Particle surface contact causes oxidative stress as a result of increased intracellular calcium and gene activation; (B) oxidative stress, increased intracellular calcium, and gene activation due to the released metals from the particles; (C) activation of cell surface receptors by the intermediate released from the particles and subsequent activation of the gene; (D) Intracellular Distribution of Nanoparticles (NSPS) to Mitochondria and Induce Oxidative Stress; In other words, the bio-effects of the nanoparticles are largely influenced by their unique physicochemical properties that can be affected individually or in combination with [17,18,19]. Combustion-derived nanoparticles in addition to air pollution and its impact on the environment can cause adverse health effects [18]. In one study, the effect of gold nanoparticles on skin fibroblast cells has been demonstrated. In this study, morphology of nanoparticles in contact with these cells was evaluated by increasing the concentration of nanoparticles from 0.1 mg / ml to 0.6 mg / ml, resulting in a much narrower cellular attachment. It showed less adhesion to the surface; in addition, the number of grown cells decreased, leading to a decrease in the ability of cells to proliferate [14]. Therefore, the present study aimed to investigate the effect of acute cutaneous toxicity of motor oil containing copper nanoparticles on animal model of earthworm.

MATERIALS AND METHODS

Proposed animal species for testing

Eisenia foetida (Michaelsen) earthworm lives in organic rich soil and its sensitivity to chemicals is similar to real earthworm species, has a short life cycle of coconut within 3–4 The week comes out, and it takes about 7-8 weeks to reach maturity (at 20 ° C). [20, 21]

Engine oil

Engine oil containing fresh copper nanoparticles, engine oil without copper nanoparticles, engine oil containing copper nanoparticles and engine oil without copper nanoparticles was obtained from Oil Industry Research Institute.

Screening test (filter paper contact test)

Earthworms were placed on the filter paper to test the desired materials, to identify potentially toxic chemicals in the soil for earthworms. And a range of different concentrations are used in the test. The concentration test that gives no casualties, and the concentration that results in casualties, are used to determine the LC50.

Screening Test Method

Glass bottom vials with around 8 cm long and 3 cm in diameter were used, and the paper was cut to the appropriate size. The paper must be embedded in the vials in such a way that they do not come out of the vial. In this study we used chloroform solvent to dilute the oils to the following ratios. Ratios of oil to chloroform (solvent): It was done at 2 to 0, 1.75 to 0.25, 1.5 to .5, 1.25 to 0.75, 1 to 1, 0.75 To 1.25, 0.5. To 1.5, 0.25 to 1.75 and 0 to 2. Two ml of solution in each sample vial was subjected to a gentle flow of air for drying. Sample glass should begin to rotate horizontally until dry. The drying step was repeated three times, and each concentration was repeated 10 times in each operation to obtain a precipitate. That is, each concentration was repeated 30 times, and each worm was used in one vial (more than one worm per vial could not be used because the death of one worm may affect the other worm). After drying, one ml of deionized water was added to each vial to moisten the filter paper. The sample vial was coated with a lid or plastic membrane with a small hole for ventilation. The test temperature was set at 20 ± 2 °C, and the test was performed in the dark for 48 h The worms were diagnosed for being alive or dead on the basis of a gentle stimulus mechanism on the worm's head, known as the needle test.

Artificial soil testing

7 and 14 days after mortality test. In the flat paper test the losses are checked 24 and 48 hours or if required up to 72 hours later. The concentration test that gives no casualties, and the concentration that results in casualties, are used to determine the LC50.

Synthetic soil materials

Includes: 10% moss fertilizer, 20% kaolin clay soil, 70% industrial sand (the amount of fine sand should be over 50% and their size is between 50-50 microns), calcium bicarbonate (to adjust soil pH about 6±0.5).

Method of Artificial Soil Testing

Proposed concentrations by protocol based on artificial soil dry weight (1000, 100, 10, 1, 0.1, 0.01 mg / kg), which according to the paper test results of higher concentrations in the soil test we used (5 concentrations were used in a geometric progression), the concentrations used in this study were based on ppm (1, 10, 100, 1000, 10000 and 100000) and the different amounts of oil were finally mixed with 750 g of soil. Twenty-four hours prior to the experiment, the worms were placed in artificial oil-free soil, and after 24 hours the worms were washed, and 10 earthworms were placed in each glass container containing soils mixed with different concentrations of oil. The containers were covered with a perforated plastic membrane to prevent drying of the test environment, and were kept for 14 days in the test condition. Four replicates were used for each treatment, and this was the case for four control dishes. No test was added to the containers in this test. The mortality assessment was adjusted on days 7 and 14 and the experimental temperature was 20± 2 ° C. The experiment was conducted in continuous light. Mortality was diagnosed by emptying the test medium on a tray or glass plate with a needle test. After estimating mortality at day 7, the worms were returned to the test medium. Any significant pathological or behavioral and morphological signs were recorded.

Conditions for test validity

The number of casualties in controls should not exceed 10% at the end of both tests, otherwise the test is not performed correctly.

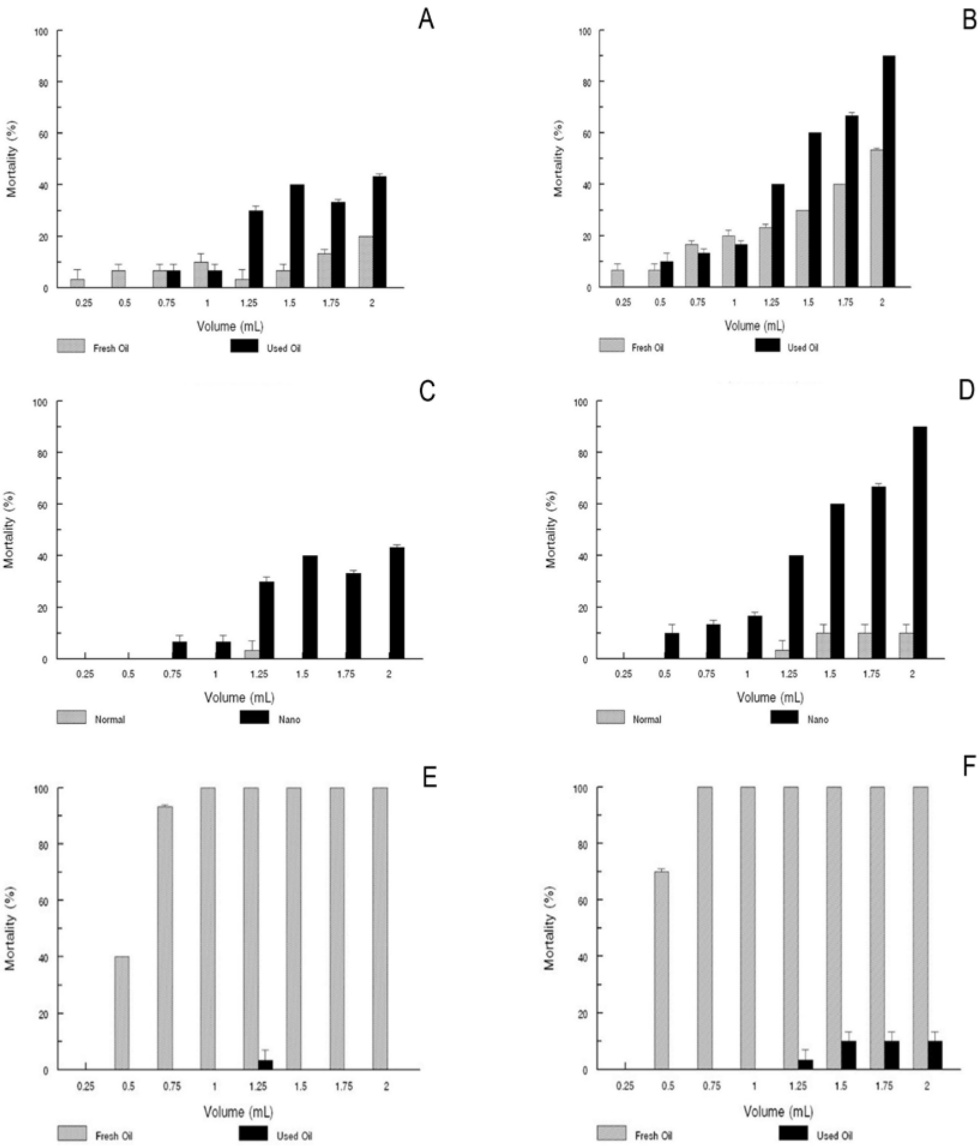

Fig. 1: A) Comparative effects of engine oil containing fresh and working copper nano particles in 24 hours
B) Comparative effects of engine oil containing fresh and operated copper nano particles at 48 hours
C) Comparative effects of engine oil containing copper nano particles with and without copper nano particles in 24 hours
D) Comparative effects of engine oil with and without copper nano particles in 48 h
E) Comparative effects of running engine oil and fresh copper nano particles in 24 hours
F) Comparative effects of running and fresh engine oil without copper nano particles in 48 hours

RESULTS AND DISCUSSION

The results of the study with different concentrations of nano particle-free and non-nano particulate motor oils were described in both paper and artificial soil tests. And to oil to solvent ratios: 2 to 0, 1.75 to 0.25 , 1.5 to .5, 1.25 to 0.75, 1 to 1, 0.75 To 1.25, 0.5. To 1.5, 0.25 to 1.75, shown as a diagram (Figs. 2, 1 and 3).

Tread rate of engine oil used at a concentration of 1.25 ml or more Nano-containing motor oil was significantly ($p < 0.001$) higher (Fig. 1A). Tread rate of engine oil used at a concentration of 1.25 ml or more From fresh engine oil to nano in 48 hours It was also significantly ($p < 0.001$) higher (Fig. 1B). Lethality rate in 24 hours in engine oil containing copper nano particles at concentrations of 1.25

to the top There was a significant difference ($p < 0.001$) for normal and higher engine oil (Fig. 1C). Lethality in 48 hours in engine oil containing copper nano particles at concentrations of 0.75 to the top There was a significant difference ($p < 0.001$) from normal engine oil and more (Fig. 1D). Lethality rate in fresh engine oil without copper nano particles at concentrations of 0.5 for 24 hours to the top There was a significant difference ($p < 0.001$) with normal engine oil And more (Fig.

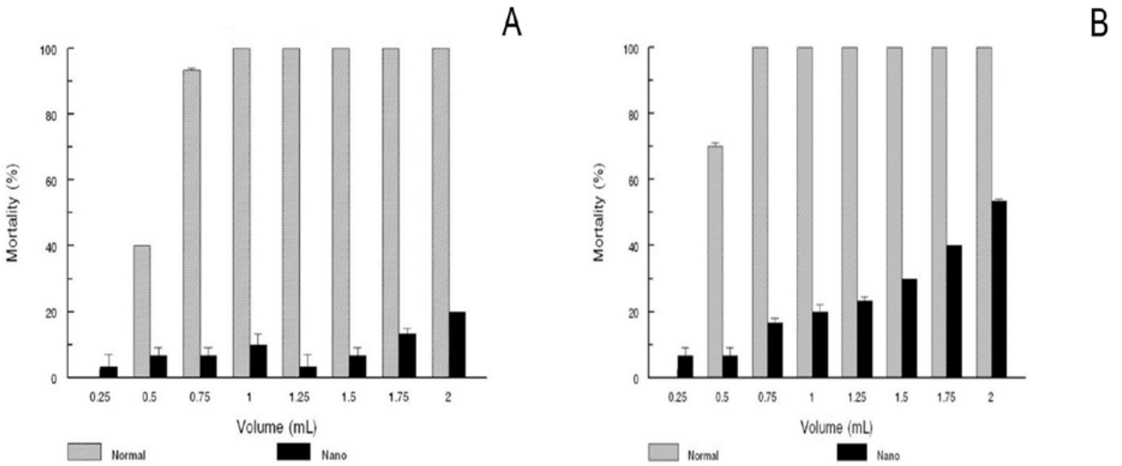

Fig. 2: A) Comparative Effects of New Engine Oil Containing Copper Nanoparticles with and without Copper Nano particles In 24 hours
B) Comparative effects of new engine oil containing copper nanoparticles with and without copper nano particles In 48 hours

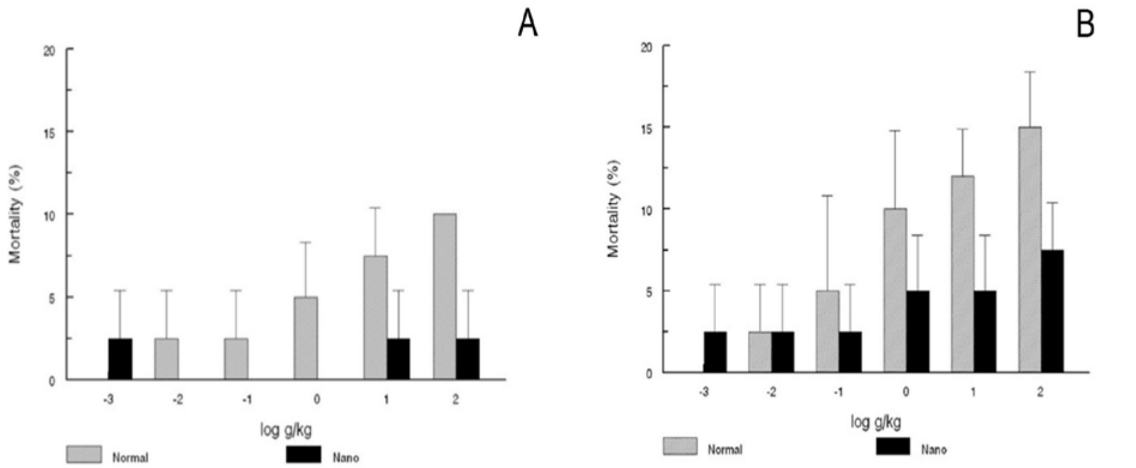

Fig. 3: A) Comparative effects of fresh engine oil containing copper nano particles with and without nano-copper for 7 days on synthetic soil test
B) Comparative effects of fresh engine oil containing copper nano particles with and without copper nano-particle for 14 days on synthetic soil test

1 E). Lethality at 48 h in fresh engine oil without copper nano particles at concentrations of 0.5 to the top There was a significant difference (p <0.001) with normal engine oil and more (Fig. 1F).

The lethality rate at 24 h in fresh engine oil without copper nanoparticles at concentrations above 0.5 was significantly different (p <0.001) from the engine oil containing copper nanoparticles and was higher (Fig. 2A). The lethality rate at 48 h in fresh engine oil without copper nanoparticles at concentrations above 0.5 was significantly different (p <0.001) from the engine oil containing copper nanoparticles and was higher (Fig. 2B).

In artificial soil test the results were evaluated in 7 days and 14 days, the results were analyzed in the engine oil containing copper nano particles with and without copper nano particles at different concentrations. Comparison of the lethal effects between the motor oil containing copper nano particles and the natural motor oil in the first

Table 1: Correlation between lethality and increasing concentration in soil test on days 7 and 14

Factor	14 Day		7 Day	
Type of engine oil	P	R	P	R
Engine oil containing copper nano particles	0/038	0/836	0/317	0/496
Engine oil without copper nanoparticles	0/140	0/676	0/071	0/774

Table 2: Correlation between lethality and increasing concentration in paper test at 24 and 48 h

Factor	48 h		24 h	
Type of engine oil	P	R	P	R
New engine oil without copper nano particles	0/025	0/924	0/014	0/949
Used engine oil without copper nano particles	0/121	0/982	0/268	-0/414
New engine oil containing copper nano particles	<0/001	0/968	0/016	0/767
Used engine oil containing copper nano particles	<0/001	0/954	<0/001	0/916

Table 3: Significance at concentration 0.5 at P<0.001

	Significant within 48 hours					Significant within 24 hours				
	Group 5	Group 4	Group 3	Group 2	Group 1	Group 5	Group 4	Group 3	Group 2	**Group 1**
Group 1				*						
Group 2		*	*				*	*		
Group 3										
Group 4										
Group 5										

Table 4: Significance at concentration 0.75 at P<0.001

	Significant within 48 hours					Significant within 24 hours				
	Group 5	Group 4	Group 3	Group 2	Group 1	Group 5	Group 4	Group 3	Group 2	**Group 1**
Group 1	*	*		*					*	
Group 2		*	*					*		
Group 3	*									
Group 4										
Group 5										

Table 5: Significance at concentration 1 at P<0.001

	Significant within 48 hours					Significant within 24 hours				
	Group 5	Group 4	Group 3	Group 2	Group 1	Group 5	Group 4	Group 3	Group 2	**Group 1**
Group 1	*	*		*					*	
Group 2	*	*	*					*		
Group 3	*	*								
Group 4										
Group 5										

week showed no significant differences at different concentrations in the soil test, but at 48 hours the lethality of motor oil containing copper nano particles increased with increasing concentration compared to the oil. Engine without nano copper grows. In soil test on day 7 in motor oil containing copper nano particles the correlation between lethality and concentration was weak and was not significant with p <0.05. But in 48 hours there was a good and strong correlation which was significant with p <0.05. In motor oil without copper nano particles on days 7 and 14, there was a high correlation between lethality with increasing concentration but not significant at p <0.05 (Fig. 3 A and B).

In the paper test at 24 h in engine oil without copper nano particles, there was a high positive correlation between lethality with increasing oil concentration and significant at p <0.05. Which is 48 hours similar to 24 hours. There was a negative correlation between lethality and increase in oil concentration in the engine oil at 24 hours which was not significant at p <0.05. There was a positive and high correlation at 48 hours but not significant at p <0.05. In the fresh oil containing copper nano particles at 24 hours, there was a high positive correlation between lethality and concentration increase and was significant at p <0.05. At 48 hours, there was a positive and high correlation with p<0.001. In the engine oil containing copper nano particles at 24 and 48 hours, there was a high positive correlation between lethality with increasing concentration and significant with p<0.001.

Table 6: Significance at concentration 1.25 at P<0.001

	Significant within 48 hours					Significant within 24 hours				
	Group 5	Group 4	Group 3	Group 2	Group 1	Group 5	Group 4	Group 3	Group 2	**Group 1**
Group 1	*	*		*		*			*	
Group 2	*	*	*			*	*	*		
Group 3	*	*				*				
Group 4	*					*				
Group 5										

Table 7: Significance at concentration 1.5 at P<0.001

	Significant within 48 hours					Significant within 24 hours				
	Group 5	Group 4	Group 3	Group 2	Group 1	Group 5	Group 4	Group 3	Group 2	**Group 1**
Group 1	*	*		*		*			*	
Group 2	*	*	*				*	*		
Group 3	*	*				*				
Group 4	*					*				
Group 5										

Table 8: Significance at concentration 1.75 at P<0.001

	Significant within 48 hours					Significant within 24 hours				
	Group 5	Group 4	Group 3	Group 2	Group 1	Group 5	Group 4	Group 3	Group 2	**Group 1**
Group 1	*	*		*		*	*		*	
Group 2	*	*	*				*	*		
Group 3	*	*				*				
Group 4	*					*				
Group 5										

Table 9: Significance at concentration 2 at P<0.001

	Significant within 48 hours					Significant within 24 hours				
	Group 5	Group 4	Group 3	Group 2	Group 1	Group 5	Group 4	Group 3	Group 2	**Group 1**
Group 1	*	*		*		*	*		*	
Group 2	*	*	*			*	*	*		
Group 3	*	*				*	*			
Group 4	*					*				
Group 5										

Significance was compared at 24 and 48 h at the same spraying concentrations in different groups, and the results are presented in the following tables, and the names of each group were briefly group 1: control, group 2: fresh engine oil. No Copper Nano particles, Group 3: Used Engine Oil without Copper Nano particles, Group 4: New Engine Oil Containing Copper Nano particles, Group 5: Used Engine Oil Containing Copper Nano particles.

Earthworm is recognized as an important laboratory species for monitoring the potential effects of chemicals on soil environmental systems due to its sensitivity, limited mobility, easy collection and rearing in the soil due to its ability to live in a variety of soils. Therefore, many studies have been done on the effects of chemicals, heavy metals and pesticides on earthworms. Including: aromatic hydrocarbons [22], explosives [23], pesticides [24], cadmium and nickel [25], perchlorate [26], tetraethyl lead [20] and lead [27], hence The amount of work done is also needed to study the environmental impacts of nano materials due to the increasing potential of the nano materials and their potential impacts on the environment, and little information is available. Among the work done on earthworms effects of nano materials contamination on earthworms can be studied the study of acute toxicity of nano zinc oxide and titanium on earthworms. It is titanium nano-oxide but does not show any acute toxicity in artificial soil [20]. In this study, contrary to previous studies of the product, it has been investigated, while earlier studies have been carried out on the nano particles themselves, which makes the results of this research closer to the reality of the environment. The results of this study showed that the toxicity of the new engine oil in the paper test as a comparative test was higher than the toxicity of the new engine oil containing copper nano particles, but in the case of used engine oil, the toxicity of nano-oil was higher than that of conventional oil. Indicates that after consumption of toxic substances due to changes that occur and changes in the study of pollutants should be studied after pollutant product in terms of contamination.

Nanomaterials may enter the body through the respiratory tract, skin (skin contact), digestive tract (intestine) or unwantedly[28] and cause respiratory, skin toxicity, eye and oral toxicity [29]. In target organs, different mechanisms are likely responsible for the biological effects of nanoparticles, such as production of reactive oxygen species (ROS), oxidative stress; mitochondrial dysfunction; inflammation; absorption by the Reticulo-endothelial system; Protein denaturation؛ Defect in alienation; Defect in endothelial cells; Production of neoantigens; Change the order of the cell cycle and DNA damage. For example, fuel-derived nanoparticles include metallic and inorganic nanoparticles with a high surface area and the ability to transmit to the brain and other organs and may cause pulmonary reactions such as Fibrosis, Chronic pulmonary inflammation, Metal fever and cancer. On the other hand, the toxicity of the particles is influenced by many parameters such as the type of particle, Density, Particle size distribution, Solubility in water, Chemical reactivity, Repeat and duration of the call, Interfere with other airborne chemical compounds, Pulmonary ventilation and specific conditions are immunological. Research has shown that the biological effects of nanoparticles are largely influenced by their unique physicochemical properties, which can be either individually or in combination [17, 18, 19]. Dr. Koohi et al (2009) in a study evaluating the excitatory-corrosive property of silver nanoparticles in an animal model investigated the skin effects taking into account the duration and size of nanoparticles, which results: Short-term (3 min) skin contact with

Table 10: LC50 value after 48 hours on paper test in ml/cm^2

Type of engine oil	LC50
Ordinary fresh engine oil	6×10^{-3}
New engine oil containing copper nano particles	23×10^{-3}
Normal working engine oil (8000 km)	Above 24×10^{-3}
Working oil containing nano-copper (8000 km)	16×10^{-3}

Table 11: LC50 levels in artificial soil after 14 in mg/kg artificial soil test

Type of engine oil	LC50
Ordinary fresh engine oil	Above 10000
New engine oil containing copper nano particles	Above 10000

silver nanoparticles lacks cutaneous complications (edema and erythema), Short (3 min) or long (1 or 4 h) exposure to large nanoparticles (30 nm) has no skin effects, Short-term exposure (3min) to fine nanoparticles (10 and 20 nm) lacks cutaneous complications (edema and erythema), Long-term exposure (1 and 4 hours) to fine nanoparticles (10 and 20 nm) has cutaneous complications (edema and erythema), And long-term exposure (1 and 4 hours) to fine nanoparticles (10 and 20 nm) has toxic effects on the internal organs [30]. Further research into the toxicity and penetration of titanium oxide nanoparticles into the skin of mice and pigs after prolonged exposure to titanium oxide nanoparticles, conducted by Wu et al., 2009. The results showed that nanomaterials in the size of 10 nm caused skin damage due to free radicals release, oxidative stress and collagen depletion [31].

CONCLUSIONS

Nanomaterials risk assessment in the field of safety and health including: production of reactive oxygen species (ROS) and oxidative stress, mitochondrial dysfunction, alien deficiency, altered cell cycle order, and DNA damage have led to detailed investigations. In the field of nanomaterial-induced skin toxicity, researchers have proposed in-vitro and in-vitro experiments. Examination of the shape and size of skin cells and their adhesion, lactate dehydrogenase release rate, interleukin 8 release rate, mitochondrial activity, number of living and dead cells, and permeability of skin membranes are methods of in vitro toxicity evaluation of nanoparticles. In vitro methods have also suggested inflammation and skin sensitivity due to the presence of nanoparticles on animal skin. Overall, it can be concluded that conventional engine oil is more toxic than unused nano product, While the usual product has declined sharply since its toxicity, But the nano-product is more toxic than it was before use.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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