Journal of Biological Sciences and Bioconservation
Volume 11, Number 1, 2019
ISSN: 2277-0143

EFFECTS OF TITANIUM DIOXIDE NANOPARTICLES ON POPULATION GROWTH RATE OF *BRACHIONUS CALYCIFLORUS*, Pallas, 1766

Oluwagbemiga Areo
Email: olaitanareo@yahoo.com

ABSTRACT

This study was carried out to determine the toxicity of Titanium dioxide nanoparticles (TiO_2 NPs) to a freshwater planktonic rotifer, *Brachionus calyciflorus*. Reconstituted water was used as the medium for the toxicity tests for both acute and chronic exposures. Acute concentrations of 0.01, 0.05, 0.10, 0.50 and 1.00mg/l of TiO_2 NPs were used, from which the value of LC_{50} was obtained to establish chronic concentrations of 0.0065, 0.008 and 0.016mg/l of TiO_2 NPs. The result of the acute toxicity test showed that TiO_2 NPs was toxic on *Brachionus calyciflorus* with 81.67% mortality recorded as the concentration of the toxicant increased from 0.01mg/l to 1.00mg/l and LC_{50} of 0.065mg/l. For chronic exposure, population growth rate was determined. The exposure lasted 18 days, after which the population growth rate of the test organism significantly declined with increasing concentrations of TiO_2 NPs, with the best population growth rate of $0.304d^{-1}$ at 0.0065mg/l concentration. This proves that TiO_2 NPs are toxic on *Brachionus calyciflorus*, which paves way for a potential inference in offsetting the entire freshwater habitat. However, for more efficiency, further studies should be directed towards measures of preventing and or controlling the accumulation of TiO_2 NPs in freshwater bodies and on aquatic organism

INTRODUCTION

Nanoparticles (NPs) are particles of size less than 100nm. Some occur naturally in rocks and mineral sands or have entered into the environment through anthropogenic activities. Studies have shown that as the surface area of nanoparticles increases, the material can become more toxic (Dillon, 2008). Generally, NPs have negative impact on health on the environment (Handy *et al*., 2008). Titanium dioxide nanoparticles are used in sunscreens and light stabilization for wood coatings (Trouiller *et al*., 2009). Titanium dioxide NPs in recent years have been widely used in industrial and consumer products due to their strong catalytic activity; this is attributed to their smaller sizes, which has allowed for larger surface area per unit mass. Also, this has raised concerns about these properties of TiO_2 NPs posing a unique bioactivity and challenges to life (Maynard and Kuempel, 2005, Tsuji *et al*., 2006). An increase in the number of published studies on the safety of TiO_2 NPs confirms its importance; as studies have shown that TiO_2 NPs are more toxic than fine particles (Oberdorster, 2001, Zhao *et al*., 2009).

TiO_2 NPs are white non-combustible and odourless powder with poorly soluble particulate that has been used as a white pigment. Anatase and rutile are two basic crystal structures of TiO_2, with anatase known to be the most photocatalytically reactive (Warheit *et al*., 2007). The photocatalytic applications of the anatase structure make TiO_2 NPs the most widely used. Also, due to easy charge transfer on the surface of nano-sized particles, which makes the reaction efficient, NPs of anatase TiO_2 are commercially produced and widely used (Fujishima and Zhang, 2006).

The zooplankton, *Brachionus calyciflorus* is a planktonic rotifer species occurring in freshwater. It was used as the test organism; it is commonly used as a model organism in toxicology, ecology and evolutionary biology. Rotifers are outstanding model organisms for ecotoxicological research because they are easily and rapidly cultured in the laboratory, and produce sensitive test results, rotifer population health is a good indicator of water quality, their distinct phylogeny gives them toxicological sensitivity that differs from other groups and their feeding and ability to exploit a wide variety of niches makes them important links in many ecosystems. As such, rotifers have been the subject of a number of studies on aquatic signaling and water quality (Snell *et al.,* 2006). Due to its vast array of usefulness in the aquatic ecosystem, *B. calyciflorus* has been used by several researchers especially in toxicological research. Toxicity tests with rotifers of the genus *Brachionus* are more easily performed than with many other aquatic animals because of their rapid reproduction, short generation times, sensitivity and the commercial availability of rotifer cysts (Halbach *et al.,* 1983). *B. calyciflorus* have been successfully reared on the microalgae *Chlorella spp. Scenedesmus costatogranulatus*, as well as Yeast (Arimoro and Ofojekwu, 2004).

MATERIALS AND METHODS

Source of Nanoparticles

Dry Titanium dioxide-anatase nano-powder was purchased from Sigma-Aldrich, USA; (CAS number 637254), particle size <25nm (St. Louis, MO, USA).

Test organism

The cysts of the zooplankton, *Brachionus calyciflorus* were obtained from Brineshrimp Direct, Odgen, Utah, U.S.A. *Brachionus calyciflorus* were obtained by hatching cysts.

Food source

The algae, *Chlorella vulgaris* was used as feed to the zooplankton. (Arimoro and Ofojekwu, 2004).

Culture of Algae

Algae, *Chlorella vulgaris* are very easy to culture and this is why researchers use them in investigations. They also contain most of the vitamins except vitamin C. Therefore, due to its photosynthetic efficiency and easiness to culture it becomes an important food source. The green algae, *Chlorella vulgaris* were cultivated in Bold Basal Medium. The growth of the algae was observed taking note of a bloom or a decline in growth for necessary steps to be taken. The optimal temperature of culture will be regulated between 20-31°C with electric fans and at a pH of 8.0.

Dilution water

Reconstituted freshwater was prepared with high quality distilled water to which 96mg $NaHCO_3$, 60mg $CaSO_4.2H_2O$, 60mg $MgSO_4.7H_2O$ and 4mg KCl were added per litre (Peltier and Weber, 1985). It was then stirred for 24 hours and adjusted to pH 7.5 using concentrated Hydrochloric acid and Sodium Hydroxide. This prepared dilution water has a life span of seven (7) days after which it was discarded.

Rotifer Cyst Hatching

In obtaining best results, the cysts were placed in a shallow wide dish (petri-dish) that provided it a high surface area to volume ratio, allowed for sufficient oxygen exchange and a clear lid to reduce evaporation. The use of reconstituted freshwater served as the liquid medium (Arimoro, 2006). Unexpected and inconsistent results can often be traced to problems with the dilution water, so it was prepared and stored very carefully (Peltier and Weber, 1985). 20ml of the reconstituted water into the petri dish was sufficient to hatch 1000-5000 eggs.

Hatching was initiated by placing the rotifer cysts in the dilution water & incubated at 25°C at an illumination level of 7000lux. Hatching began after approximately 24hour and by 48hour approximately 50% of the cysts were hatched. It was however important to commence the collection of the hatched test organisms, after 50% cysts have hatched in carrying out the acute toxicity test, because there was no feeding during the acute toxicity test.

Collection of *B. calyciflorus* into Petri-dish

B. calyciflorus are small animals of approximately 250 micrometer in length, which is one-fourth the size of newborn Daphnia. Due to this size, they require some magnification for transferring the new hatched into the test medium, using a micropipette. The newly hatched are white and are mostly visible against a dark background. A stereomicroscope with 15X magnification, dark field and substantial illumination was ensured. The test organisms were collected with micropipette and transferred to the petri-dishes containing the

appropriate concentration of toxicants using a stereomicroscope.

Toxicity Test

Experimental Design

Acute toxicity tests of Titanium dioxide nanoparticles was conducted based on the modified OECD standard procedure of seven (7) test concentrations of 0.01, 0.05, 0.1, 0.5, 1.0, 5.0, and 10mg/L plus a blank control (OECD, 2004). However, a pilot study was carried out and was discovered that concentrations of 5.0 and 10mg/L were too Lethal on the test organisms. Therefore, for the purpose of this study, five (5) test concentrations of 0.01, 0.05, 0.1, 0.5, 1.0mg/L plus a blank control was used in three (3) replicates and were observed within 96 hours. The test apparatus used were petri dishes of which ten (10) test organisms per petri dish were introduced.

Chronic toxicity tests of TiO_2 nanoparticles on *B. calyciflorus* was based on the LC_{50} of the acute toxicity test of which 0.065mg/L was obtained and used to determine the test concentrations of (0.0065, 0.008 and 0.016) with a blank control (OECD, 1998). These were also carried out in three (3) replicates and were observed for 18 days of which they were constantly fed, using *Chlorella vulgaris*. The population growth rate of the test organism was carried out within 18 days.

Acute toxicity tests

Acute toxicity tests of Titanium dioxide nanoparticles was conducted based on the modified OECD standard procedure (OECD, 2004). Five (5) test concentrations (0.01, 0.05, 0.10,

0.5 and 1.0 mg/L) plus a blank control was used. The containers used in the test were petri-dishes filled with 20 mL of test solution. The neonates aged less than 24 hour in groups of ten (10) was exposed to each concentration for 96 hour and the test solution will be renewed daily to maintain the same concentration of exposure. *Brachionus calyciflorus* will not be fed during the testing period. Testing was performed in triplicate. The status of immobilization and mortality will be checked after 96 hour. Hydrogen concentration (pH) of 7.5 of the test medium was measured at the very beginning and the end of the test. *Brachionus calyciflorus* that will be unable to swim within 15 seconds of gentle agitation of the test container will be considered immobile. Those whose heartbeats' stops will be considered dead.

Chronic toxicity tests

The effect of Titanium dioxide nanoparticles on the reproductive output was assessed in a semi-static test according to the standard protocol for *Brachionus calyciflorus* Reproduction Test (OECD, 1998). Based on the results of acute toxicity, neonates aged less than 24 hour were exposed to a series of concentrations of Titanium dioxide nanoparticles (0.000, 0.0065, 0.008 and 0.016 mg/L). The containers used in the tests were petri-dishes filled with 20 mL of test solution and ten (10) test organisms, and the testing was performed in three (3) replicates. *Brachionus calyciflorus* was fed daily and the feeding rate was 1.5×10^{6} cells per *B. calyciflorus* a day. Each day, each offspring was carefully separated from the petri dishes and the test solutions renewed.

Survival Experiment

Survival, reproduction (fecundity) and growth of *B. calyciflorus* was observed and recorded for each of the three (3) replicates in 18 days of the study. Days to the first brood and the number of first brood per female and the average offspring in each brood was the criteria used to evaluate the fecundity (population growth rate = *r*).

The *r* value will be calculated with the formula used by El-Bassat *et al.* (2011),

$r = \ln N_{final} - \ln N_{start}/T$ where,

N_{final} is the number of rotifers in each petri-dish at the end of the experiment

N_{start} is the number of rotifers in each petri-dish at the start of the test

T is time of exposure. The mean *r* values were subsequently calculated.

As *r* calculated in *B. calyciflorus* organism after 18 days is indistinguishable from *r* estimated for the entire lifespan, and owing to the great importance of early reproduction (Leeuwen *et al.,* 1985), all the calculations was based on 18-days experiments.

RESULTS

Acute toxicity test

The results of the survival rates are dose-dependent. Survival decreased with increase in concentrations of TiO_2 NPs. At 24 hours, survival rate at 0.01 and 0.05 (mgl^{-1}) was 95% and 91.67% while at 0.10, 0.50 and 1.00 (mgl^{-1}) concentrations; it was 86.67%, 85.00% and 80.00% respectively. There was significant difference (p<0.05) between the control and 1.00mgl^{-1} at 24 hours exposure period. After 48 hours, the highest percentage survival was 91.67% at 0.01mgl^{-1}

concentration and lowest by 73.33% at 1.00mgl^{-1} concentration. There was significant difference (p<0.05) between the control and 1.00mgl^{-1} at 48 hours exposure period.

At 72 hours, the highest percentage survival was 85.00% at 0.01mgl^{-1} concentration and lowest percentage was 55.00% at 1.00mgl^{-1} concentration. There was significant difference (p<0.05) between the control and 1.00mgl^{-1} at 72 hours period of exposure. By 96 hours, the lowest percentage survival was 18.33% at 1.00mgl^{-1} concentration and highest percentage survival of 75.00% at 0.01mgl^{-1}. Similarly, there was significant difference (p<0.05) between the control and 1.00mgl^{-1} and the end of 96 hours exposure.

The lethal concentration (LC_{50}) of 0.065mg/l for the rotifer was determined graphically from the acute toxicity with percentage mortality against log of concentration. This was used to determine the chronic concentrations of (0.0065, 0.008 and 0.016) mg/l.

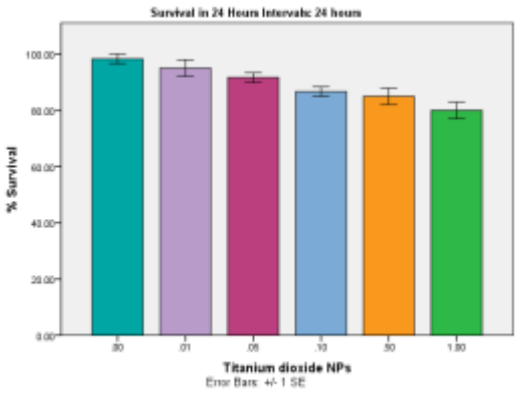

Fig.1

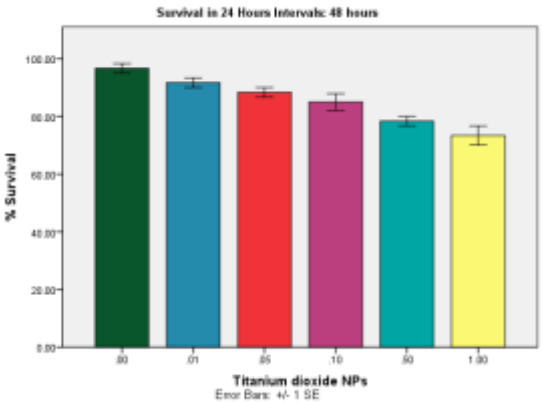

Fig.2

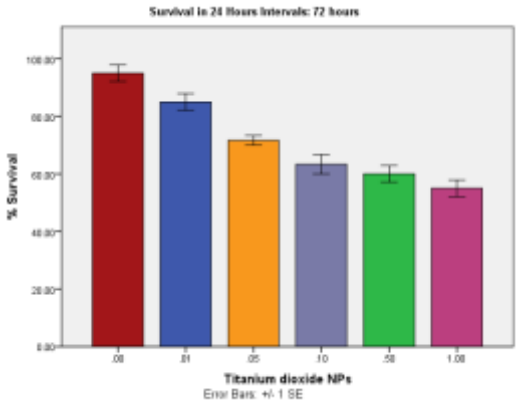

Fig.3

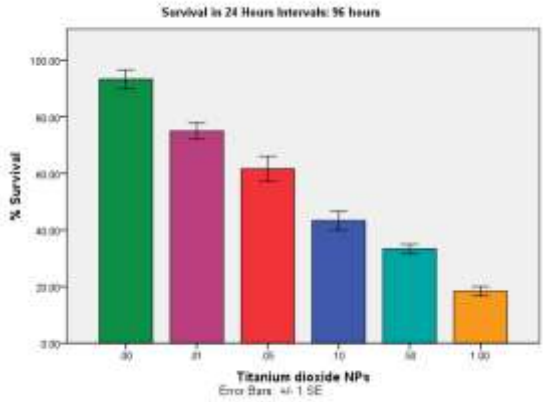

Fig.4

Fig. 1 - The survival of *Brachionus calyciflorus* exposed to TiO_2 NPs at acute dose-dependent rates after 24 hours.

Fig. 2 - The survival of *Brachionus calyciflorus* exposed to TiO_2 NPs at acute dose-dependent rates after 48 hours.

Fig. 3 - The survival of *Brachionus calyciflorus* exposed to TiO_2 NPs at acute dose-dependent rates after 72 hours.

Fig. 4 - The survival of *Brachionus calyciflorus* exposed to TiO_2 NPs at acute dose-dependent rates after 96 hours.

Chronic toxicity test

Population growth rate

The population growth rate shows that there is a dramatic significant inhibition of population growth rate as the concentration increases with the highest population growth rate observed at 0.0065mg/l ($0.304\pm3.3\times10^{-5}$ d^{-1}) and the lowest at 0.0160mg/l ($0.222\pm3.3\times10^{-5}$ d^{-1}).

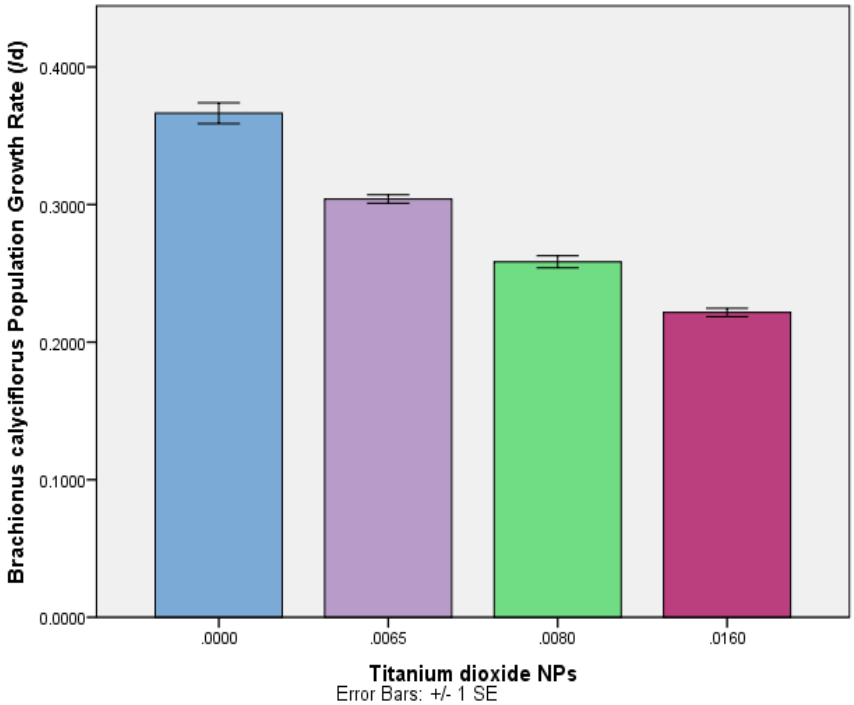

Fig. 5 - The population growth rate of *Brachionus calyciflorus* exposed to chronic toxicity of TiO_2 NPs.

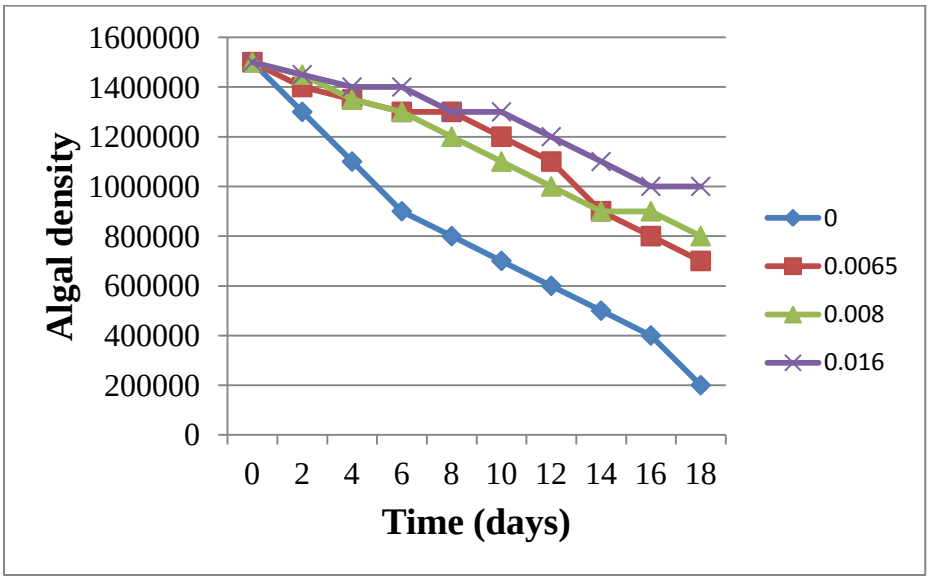

Fig. 6 Effect of TiO_2 NPs on Algal density during the chronic toxicity exposure at 0.00mgl^{-1} (control), 0.0065 mgl^{-1}, 0.008mgl^{-1} and 0.016mgl^{-1}

DISCUSSIONS

Acute toxicity of Titanium dioxide nanoparticles on *Brachionus calyciflorus*.

At the end of the 96 hour test, a receding gradation of survival rates of *Brachionus calyciflorus* across the concentrations was observed with the highest survival rate at 0.01mgl^{-1} of <25nm TiO_2 nanoparticles at 75.00% and the lowest at 1.00mgl^{-1} of TiO_2 nanoparticles at 18.33%. This shows that there was an acute toxicity effect of TiO_2 nanoparticles on the test organism, *B. calyciflorus*, with a significant difference (p<0.05) between the control and 1.00mgl^{-1}. From the aforementioned results, it shows that the duration of exposure plays a major role in the toxicological effect on *B. calyciflorus*. Hence, in a real life situation, a water body suddenly exposed to TiO_2 nanoparticles of these concentrations and extended for more than 96h could cause

high mortality rates on the aquatic system with a vast array of phytoplanktons, zooplanktons, fingerlings and small fishes. Similar observation was reported by Hund-Rinke and Simon (2006) that TiO_2 nanoparticles (25 nm or <100 nm) concentrations of less than 3 mgl^{-1} exhibited little effect on the immobilization of *Daphnids*, and the toxicity of TiO_2 nanoparticles under pre-illumination by simulated sunlight seemed to be higher than that of non-illuminated TiO_2 nanoparticles.

Chronic toxicity of Titanium dioxide nanoparticles on *Brachionus calyciflorus*

Population Growth Rate

There was a dramatic significant inhibition of population growth of the test organism as the concentration increased. This is the most important toxicity test, as a long term exposure/chronic toxicity are given attention to, especially in case of a real life situation. This shows there was a significant difference (p<0.05) between the control and 0.0160mgl^{-1} during the 18 days period. This effect could be related to the size of the nanoparticles (<25nm) which could easily find its way through to the gut of the rotifer. This is related to the work carried out by Snell and Hicks (2009), where their results revealed that the population growth rate of *B. manjavacas* rotifers was substantially reduced when they were exposed to 1µg/ml of 37±4nm particles and the effect disappeared when exposed to particles 83±11nm or larger.

CONCLUSION

Titanium oxide NPs was found to be toxic on *Brachionus calyciflorus* with 90% mortality recorded as the concentration of the toxicant increased from 0.01mgl^{-1} to 1.00mgl^{-1} after 96 hours exposure. This shows that, there are significant toxic

effects of TiO_2 NPs on the test organism, and therefore, nullifies the stated hypothesis that "there are no significant toxic effects of TiO_2 NPs on *Brachionus calyciflorus*. The fecundity (population growth rate) of the test organism was found to be affected with a significant decrease as the concentration of TiO_2 NPs increased. This also negates the hypothesis that stated that, "the reproductive ability of *Brachionus calyciflorus* is not affected upon chronic exposure to TiO_2 NPs."

REFERENCES

Arimoro F.O. and Ofojekwu P.C. (2004). Incidence of feeding, growth, and survival of the toothed carp, *Aphyosemion gairdneri* larvae reared on the freshwater rotifer, *B. calyciflorus*. *Tropical Freshwater Biology*, (12,13): 35-43.

Arimoro Francis O. (2006). Culture of the freshwater rotifer, *Brachionus calyciflorus*, and its application in fish larvi culture technology. *African Journal of Biotechnology*, Vol. 5 (7), 536-541.

Dillon, Lisa (2008). Nanoparticles and the Microenvironment of Marine Ecosystems: The Effect of Titanium Dioxide on *Karlodiniumveneficum*and *Storeatula major*. Plain edge High School 241 Wyngate Drive N. Massapequa, N.Y. 11758.

El-Bassat, R.A., Touliabah, H.E., Harisa, G.I. and Sayegh, F.A.Q. (2011). Aquatic toxicity of various pharmaceuticals on some isolated plankton species. *International Journal of Medicine and Medical Sciences,* 3(6), pp. 170-180.

Fujishima A., and Zhang X. (2006). Titanium dioxide photocatalysis: present situation and future approaches. *Congressional Research*, 9: 750-760.

Halbach, U., M. Siebert, M. Westermayer, and C. Wissel (1983). Population ecology of rotifers as a bioassay tool for ecotoxicological tests in aquatic environments. *Ecotoxicology* and *Environmental Safety,* 7:484-513.

Handy, R.D., Owen, R. and Valsami-Jones, E. (2008). The ecotoxicology of nanoparticles and nanomaterials: current status, knowledge gaps, challenges, and future. *Ecotoxicology,* 17:315–325.

Hund-Rinke, K., and Simon M. (2006). Ecotoxicological effects of photocatalytic active nanoparticles TiO_2 on algae and daphnids. *Environmental Science and Pollution Research*, 13(4): 225 – 322.

Leeuwen C. J. V., Luttmer W. J. and Griffioen P. S. (1985). The use of cohorts and popylations in chronic toxicity studies with *Daphnia magna*: a cadmium example. *Ecotoxicology and Environmental Safety*, 9(1): 26 – 39.

Maynard A.D. and Kuempel E.D. (2005). Airborne nano structured particles and occupational health. *Journal of Nanoparticles Research,*(JNR) 2005, 6:587–614.

Oberdorster G. (2001). Pulmonary effects of inhaled ultrafine particles. *International Archives of Occupational and Environmental Health*, 74:1–8.

Organization for Economic Cooperation and Development (OECD), (1998). Guideline for Testing of Chemicals No.211, *OECD Publishing, Paris*.

Organization for Economic Cooperation and Development (OECD), (2004). Guideline for Testing of Chemicals No.202, *OECD Publishing, Paris*.

Peltier.W.H. and Weber C.I. (1985). "Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms."Eds. EPA-60014-85.013, *United States Environmental Protection Agency Washington. DC*, 1985.

Snell, T.W. and Hicks, D.G., (2009). Assessing Toxicity of Nanoparticles using *Brachionus manjavacas* (Rotifera). School of Biology, Georgia Institute of Technology, Atlanta, Georgia 30332 – 0230, USA.

Snell, T.W., Kubanek, J., Carter W., Payne A.B., Kim J., Hicks M. and Stelzer C-P. (2006). "A protein signal triggers sexual reproduction in *Brachionus plicatilis* (Rotifera)." *Marine Biology,* 149: 763-773.

Trouiller B., Reliene R., Westbrook A., Solaimani P. andSchiestl R.H. (2009). Titanium dioxide nanoparticles induce DNA damage and genetic instability in vivo in mice. *Cancer Research,* 69:8784–8789.

Tsuji J.S., Maynard A.D., Howard P.C., James J.T., Lam C.W., Warheit D.B. and Santamaria A.B. (2006). Research strategies for safety evaluation of nanomaterials, part IV: risk assessment of nanoparticles. *Toxicological Sciences,* 89:42–50.

Warheit D.B., Webb T.R., Reed K.L., Frerichs S. and Sayes C.M. (2007). Pulmonary toxicity study in rats with three forms of ultrafine-TiO2 particles: differential

responses related to surface properties. *Toxicology,* 230:90–104.

Zhao J., Bowman L., Zhang X., Vallyathan V., Young S.H., Castranova V. and Ding M. (2009). Titanium dioxide (TiO2) nanoparticles induce JB6 cell apoptosis through activation of the caspase-8/Bid and mitochondrial pathways. *Journal of Toxicology and Environmental Health*, 72:1141–1149.

Reference to this paper should be made as follows: Oluwagbemiga Areo (2019), Effects of Titanium Dioxide Nanoparticles on Population Growth Rate of *Brachionus calyciflorus*, Pallas, 1766. *J. of Biological Science and Bioconservation,* Vol. 11, No. 1, Pp. 26 - 42