UNIVERSITI PUTRA MALAYSIA

EMBRYOTOXICITY OF ZINC OXIDE NANOPARTICLES ON JAVANESE MEDAKA (Oryzias javanicus BLEEKER, 1854)

AMIN NAWEEDULLAH

FS 2022 2

EMBRYOTOXICITY OF ZINC OXIDE NANOPARTICLES ON JAVANESE MEDAKA (*Oryzias javanicus* BLEEKER, 1854)

By

AMIN NAWEEDULLAH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

September 2021

DEDICATION

To my beloved father Aminullah Ziai,

My beloved mother Farida Alam Ziai,

&

Strong supporters from my siblings,

Wajiha Amin,

Qudratullah Amin,

Samina Amin,

Samiullah Amin

and

Hasina Amin

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

EMBRYOTOXICITY OF ZINC OXIDE NANOPARITCLES ON JAVANESE MEDAKA (*Oryzias javanicus* BLEEKER, 1854)

By

AMIN NAWEEDULLAH

September 2021

Chair : Syaizwan Zahmir Zulkifli, PhD
Faculty : Science

Zinc oxide nanoparticles (ZnO NPs) are widely applied in various industrial products such as cosmetics, pharmaceutical, and rubber production. However, this nanoparticle is classified as extremely toxic for the aquatic environment, particularly fish. Moreover, to date, there have been no systematic studies of the toxicity of ZnO NPs to the same species of organism in saltwater *versus* freshwater conditions, or during graded salinity changes. The objectives of this study are 1) to determine the median lethal concentration (LC_{50}) of ZnO NPs for the embryos of Javanese medaka (*Oryzias javanicus*) in different types of water (ultra-pure, deionized and dechlorinated tap water), 2) to evaluate developmental toxicity of ZnO NPs by chronic exposure on the embryo of Javanese medaka, and 3) to assess the interactive effects of salinity and ZnO NPs by chronic exposure on the embryo of Javanese medaka. The experiments were conducted in a completely randomized design with three replicates for each concentration. The acute toxicity tests were conducted to determine the 96 h median concentration (LC_{50}) of ZnO NPs for Javanese medaka embryos in different types of water. Results demonstrated the 96 h LC_{50} of ZnO NPs for Javanese medaka embryos were determined 0.6438 mg/L, 1.333 mg/L, and 2.251 mg/L in ultra-pure, deionize, and dechlorinated tap water, respectively. Chronic exposure of ZnO NPs on Javanese medaka's embryos at concentration of 0-25 µg/L was also conducted for 21 days. The heart rate of exposed Javanese medaka embryos increased as the concentration of ZnO NPs increased and showed significantly higher heart rate when it compared with control at 5, 8 and 11 dpe. In general, the mortality of embryos increased and the hatching rates were decreased as the concentration of ZnO NPs were increased. Also, series of abnormalities such as low pigmentation, fin rot, spinal deformities, cranial oedema, yolk sac oedema, precordial oedema, and cranial facial were observed in treatment groups. Javanese medaka embryos were also exposed to 25, 50, and 100 µg/L of ZnO NPs in two different salinity levels (5 and 18 ppt) in this study. The results showed that the toxicity of ZnO NPs on Javanese medaka embryos decreased as salinity of

suspension increased. For instance, in contrast to control, exposed embryos to ZnO NPs at 5 ppt showed significantly higher heart rates to 25, 50 and 100 µg/L of ZnO NPS at 5, 8, and 11 dpe. However, at 18 ppt significantly lower heart rate to 25, 50 and 100 µg/L of ZnO NPs were only observed at 5 dpe. In addition, mortality of exposed Javanese medaka embryos were increased by increasing concentration of ZnO NPs at both salinity level but in contrast to control, significantly higher mortality were observed at 5 ppt at the end of the experiment. Moreover, hatching of exposed embryos decreased as the concentration of ZnO NPs increased at both salinity level. Although, only significantly lower hatching rate were observed at 5 ppt when it compared to control. Furthermore, abnormalities such as low pigmentation, oedema, and tail malformation were observed in treatment groups at both salinity levels throughout the experiment, but abnormalities were more obvious at 5 ppt compared to 18 ppt. This study has revealed that ZnO NPs were extremely toxic to the embryo of Javanese medaka in different types of water, and there was a strong correlation between toxicity of ZnO NPs and salinity of suspension. The finding of this study can strengthen the creation of Javanese medaka as a model organism for tropical areas in aquatic nanoecotoxicological studies. Furthermore, the LC50 value is a valuable criterion for nanoecotoxicity; it is not a representative concentration of contaminants in aquatic environments, but it is essential for indicating the toxicity of certain pollutants. Although the concentrations of ZnO NPs in Malaysia aquatic ecosystem is not yet reported, the result of this showed that this hazardous chemical has the potential to have significant impacts on aquatic ecosystems and its living organisms even at environmental relevant concentration.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

EMBRIOTOKISITI NANOPARTIKEL ZINK OKSIDA TERHADAP MEDAKA JAWA (*Oryzias javanicus* BLEEKER, 1854)

Oleh

AMIN NAWEEDULLAH

September 2021

Pengerusi : Syaizwan Zahmir Zulkifli, PhD
Fakulti : Sains

Nanopartikel zink oksida (ZnO NP) telah digunakan secara meluas dalam pelbagai produk perindustrian seperti pengeluaran produk kosmetik, farmaseutikal dan getah. Walau bagaimanapun, nanopartikel ini telah diklasifikasikan sebagai sangat toksik pada persekitaran akuatik, terutamanya kepada ikan. Selain itu, sehingga kini, masih belum ada kajian sistematik mengenai ketoksikan ZnO NP terhadap spesies organisma yang sama dalam keadaan air masin berbanding air tawar, atau semasa perubahan perbezaan kemasinan secara berperingkat. Objektif kajian ini adalah 1) untuk menentukan median kepekatan kematian (LC_{50}) bagi ZnO NP terhadap embrio Jawa medaka (*Oryzias javanicus*) dalam jenis air berbeza (ultra-tulen, ternyahion dan air paip ternyahklorin), 2) untuk menilai ketoksikan pengembangan ZnO NP melalui pendedahan kronik terhadap embrio Jawa medaka, dan 3) untuk menilai kesan interaktif kemasinan dan ZnO NP melalui pendedahan kronik terhadap Jawa embrio medaka. Eksperimen telah dijalankan dalam reka bentuk rawak dengan tiga ulangan untuk setiap kepekatan. Ujian ketoksikan akut dilakukan untuk menentukan kepekatan median kematian (LC_{50}) 96 jam ZnO NP pada embrio Jawa medaka dalam pelbagai jenis air berbeza. Keputusan menunjukkan 96 jam LC_{50} ZnO NP pada embrio Jawa medaka ditentukan masing-masing pada 0.6438 mg/L, 1.333 mg/L dan 2.251 mg/L dalam air ultratulen, air ternyahion, dan air paip ternyahklorin. Pendedahan kronik ZnO NP pada embrio Jawa medaka pada kepekatan 0-25 µg/L juga dijalankan selama 21 hari. Kelajuan jantung embrio ikan medaka Jawa telah meningkat apabila kepekatan ZnO NPs meningkat dan keputusan telah menunjukkan peningkatan kelajuan jantung yang signifikan apabila dibandingkan dengan kawalan pada 5, 8 dan 11 hari selepas pendedahan (dpe). Secara amnya, kematian embrio meningkat dan kadar penetasan menurun apabila kepekatan NP ZnO meningkat. Selain itu, siri keabnormalan seperti pigmentasi rendah, reput sirip, kecacatan tulang belakang, edema tengkorak, edema kantung kuning telur, edema prekordial dan kranial diperhatikan dalam kumpulan rawatan. Embrio Jawa medaka juga didedahkan kepada 25, 50, dan 100 µg/L NP ZnO dalam dua tahap kemasinan yang berbeza (5 dan 18 ppt) dalam kajian ini. Keputusan menunjukkan ketoksikan ZnO NPs pada embrio Jawa medaka menurun

apabila kemasinan meningkat. Sebagai contoh, berbanding dengan kawalan, embrio yang terdedah kepada ZnO NPs pada 5 ppt menunjukkan kadar denyutan jantung yang jauh lebih tinggi kepada 25, 50 dan 100 µg/L ZnO NPS pada 5, 8, dan 11 hari selepas pendedahan. Walau bagaimanapun, pada 18 ppt, kadar denyutan jantung yang lebih rendah dengan ketara kepada 25, 50 dan 100 µg/L NP ZnO hanya diperhatikan pada 5 hari selepas pendedahan. Di samping itu, kematian embrio Jawa medaka yang terdedah telah meningkat dengan meningkatkan kepekatan ZnO NP pada kedua-dua tahap kemasinan tetapi berbeza dengan kawalan, kadar kematian yang jauh lebih tinggi diperhatikan pada 5 ppt pada akhir eksperimen. Selain itu, penetasan embrio terdedah menurun apabila kepekatan ZnO NP meningkat pada kedua-dua tahap kemasinan. Namun, hanya kadar penetasan yang jauh lebih rendah diperhatikan pada 5 ppt apabila dibandingkan dengan kawalan. Tambahan pula, keabnormalan seperti pigmentasi rendah, edema dan kecacatan ekor diperhatikan dalam kumpulan rawatan pada kedua-dua tahap kemasinan sepanjang eksperimen, tetapi keabnormalan lebih jelas pada 5 ppt berbanding 18 ppt. Kajian ini telah mendedahkan bahawa ZnO NP adalah sangat toksik kepada embrio Jawa medaka dalam pelbagai jenis air, dan terdapat korelasi yang kuat antara ketoksikan ZnO NP dan kemasinan. Dapatan kajian ini dapat membuktikan Jawa medaka sebagai model organisma untuk kawasan tropika dalam kajian nanoekotoksokologi akuatik. Tambahan pula, nilai LC50 adalah kriteria yang bernilai untuk nanoekotoksikologi; ia bukanlah kepekatan wakil pencemar dalam persekitaran akuatik, tetapi ia adalah penting untuk menunjukkan ketoksikan bahan pencemar tertentu. Walaupun kepekatan NP ZnO di ekosistem akuatik Malaysia masih belum dilaporkan secara terperinci, keputusan ini menunjukkan bahawa bahan kimia berbahaya ini mempunyai potensi untuk memberi kesan yang ketara ke atas ekosistem akuatik dan organisma hidupnya walaupun pada kepekatan yang berkaitan dengan persekitarannya.

ACKNOWLEDGEMENTS

In the name of Allah S.W.T., the Most Gracious and the Most Merciful,

First of all, I would like to thank almighty Allah S.W.T. for giving me the strength, knowledge, ability, and opportunity to undertake this study and to preserve and complete it satisfactorily. This accomplishment would not have been achievable without his blessings.

My deepest appreciation and gratitude to my supervisor, Dr. Syaizwan Zahmir Zulkifli, as well as members of supervisory committee (Prof. Dr. Ahmad Ismail and Assoc. Prof. Dr. Mohammad Noor Amal Azmai) for their invaluable ideas, care, commitment, suggestions, encouragement, and enthusiasm during the whole period of this study program.

My acknowledgment would be incomplete without thanking my senior laboratory-mates (Dr. Musa Adamu Ibrahim, Nur Amiera Kamardin, and Munirah Hanapiah) for the guidance and strong support throughout this study. My unconditional love to my beloved parents Mr. Aminullah Ziai and Mrs. Farida Alam Ziai and my siblings for their everlasting love, care, continuous encouragement, moral support, motivational phrases, and constant prayers, which always cheers me up and drives me to complete this research work successfully. I love you all very much. Finally, for the rest I could not mention, believe me, you are not forgotten.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Syaizwan Zahmir Zulkifli, PhD
Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Mohammad Noor Amal Azmai, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

Ahmad Ismail, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 10 Febuary 2022

Declaration by Members of Supervisory Committee

This is to confirm that:
- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature:	______________________________
Name of Chairman of Supervisory Committee:	Dr. Syaizwan Zahmir Zulkifli

Signature:	______________________________
Name of Member of Supervisory Committee:	Assoc. Prof. Dr. Mohammad Noor Amal Azmai

Signature:	______________________________
Name of Member of Supervisory Committee:	Pro. Dr. Ahmad Ismail

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	Percent
°C	Degree Celsius
µg	Microgram
mg	Milligram
g	Gram
kg	Kilogram
DO	Dissolved oxygen
NPs	Nanoparticles
BPs	Bulk particles
UV	Ultra violet
LC_{50}	Median lethal concentration
nm	Nano meter
µm	Micro meter
MeO-NPs	Metal oxide nanoparticles
eV	Electron volt
mV	Mega volt
WWTPs	Waste Water Treatment Plants
L	Liter
DOM	Dissolved organic material
pH	Potential of hydrogen
mg/L	Milligram per liter
µg/L	Microgram per liter
ppt	Parts per thousand
hr	Hour
hpf	Hour post fertilization
dpe	Day post exposure
ROS	Reactive oxygen species

XRD	X ray diffraction
TEM	Transmission electron microscopy
DLS	Dynamite light scattering
OECD	Organization for economic co-operation and development
HBpM	Heart beat per minutes

CHAPTER 1

INTRODUCTION

1.1 General introduction

Currently, several dangerous chemicals are considered a global threat to humans, other organisms and also the environment itself. Nevertheless, from time to time, the world is constantly generating and introducing large amounts of chemical substances into the environment. At the same time, the effects on organisms and the environment of these substances are not well known. Among evolving chemicals, nanoparticles (NPs) are one of those which are described as a particle with at least one dimension between 1 and 100 nm with different characteristics from bulk materials (Miao et al., 2010), and nanotechnology is known as the use of these materials (Patibandla et al., 2018). In scientific writing, the word nano, comes from the Greek nanos, which means dwarf, is becoming more prevalent. Nano is also commonly used as an adjective to denote objects, systems, or phenomena that have properties resulting from nano-meter size (one-billionthe of a meter or 10^{-9}) structure (Buzea et al., 2007). Nanotechnology has recently developed as a rapidly growing market with efficient effects on major economic sectors with novel and unique properties that have been used in a diverse group of consumer goods such as agriculture, cosmetics, electronics, textiles, and pharmaceuticals (Kahru et al., 2010; Li et al., 2013; Rajput 2018). Based on the core material, NPs can categorize through inorganic, organic, carbon based and composite based NPs (Rajput et l., 2018).
In recent years, the production and distribution of NPs have gradually increased. According to BCC Research (2019), commercial production of NPs is estimated to increase unitary from approximately 223,060 metric tons in 2014 to approximately 584,984 metric tons in 2019, with a compound annual growth rate of 21.1% from 2014 to 2019. Meanwhile, in 2017 the global market for nanoparticles was \$2.0 billion and is expected to raise \$7.3 billion by 2022. One of the important groups of these new materials are metallic NPs (Shaw et al., 2011), and have been widely implemented and monitored for their toxic effects on the activity, abundance, and diversity of living organisms and due to their antimicrobial activity they have been used as biocides to inhibit or limit the growth of microorganisms (Rajput et al., 2018). Besides metallic NPs, metal oxide NPs (MeO-NPs) are also widely used and implemented in commercial and industrial materials. Although bulk metal oxide products also produce massively in a wide range of commercial and industrial products.

Among several NPs, zinc oxide nanoparticles (ZnO NPs) are known as one of the most efficiently used in the nano-scale range with a wide bandgap and large excitonic binding energy (Sabir et al., 2014), high stability, anticorrosion and photo-catalytic properties (Hao et al., 2013), non-migratory, fluorescent, piezoelectric, absorptive, and scatters ultraviolet light (Li et al., 2018), diverse nanostructures (Bai et al., 2010), and antimicrobial activity (Pereira et al., 2019). Zinc oxide NPs are already extensively implemented in consumer goods such as paints, UV filters, biosensors, paper, plastics, ceramics, building materials, rubber, power electronics, coatings, feed, photocatalytic, degradation of textiles, and printed matter (Brun et al., 2014; Wong et al., 2014). ZnO NPs, which are the third most widely applied metal-based NPs with an approximate world-wide total production of 550 to 33,400 tons (Rajput et al., 2018), can reach the environment, particularly the aquatic environment by (1) wastewater which contains the highest amount of ZnO NPs (0.3–0.4 µg/L), (2) direct use and (3) deposition from the air compartment (Vale et al., 2016; Poynton et al., 2019).

Since ZnO NPs are already introduced directly or indirectly into the environment, particularly the aquatic environment, they may constitute a threat to the aquatic environment (Li et al., 2018). They can be extremely toxic and as an aquatic environmental risk due to their negative impacts on different aquatic living things, including bacteria, algae, crustaceans, ciliates, and fishes (Cong et al., 2017). Meanwhile, studies related to ZnO NPs toxicity on aquatic vertebrate organisms have concentrated largely on fish, in particular zebrafish. Several reports have shown that ZnO NPs can be highly toxic to zebrafish, particularly in the early developmental stages, the key emphasis of those studies has been on acute toxicity studies of aquatic species. At the same time, the chronic toxicity studies of ZnO NPs were surprisingly scarce (Xiong et al., 2011; Zhao et al., 2013). Furthermore, serious threats and higher toxicity compared to other NPs in aquatic environments have been reported in recent studies for ZnO NPs. For instance, Zhu et al. (2008) reported that ZnO NPs showed higher toxicity compared to TiO_2 NPs and Al_2O_3 NPs on the early life stage of zebrafish. Another study that has shown ZnO NPs are more toxic than TiO_2 NPs is the study of Bhuvaneshwari et al. (2017), who reported 27.62 and 71.63 mg/L for ZnO NPs and 117 and 120.9 mg/L for TiO_2 NPs as 48 h LC_{50} on *Artemia salina* under pre-UV-A and visible light conditions.

Oryzias javanicus (Javanese medaka) belongs to the Adrianichthyidae family (Magtoon & Termvidchakorn, 2009). This species is widely distributed in Asian countries and highly adaptable to fresh, brackish, and saltwater (Inoue & Takei, 2002). The sensitivity of the species belonging to this family makes it an ideal test organism for toxicology and ecotoxicology studies. Recent studies have indeed used Javanese medaka as the test organism because of their high adaptability to both freshwater and saltwater, broad geographical range and availability throughout the year (Ismail & Yusof, 2011; Yusof et al., 2012; Woo et al., 2012), short life span and life cycle, fast development (Salleh et al., 2017; Yusuff et al., 2018), hardy, easy to identify and cultivate, short spawning period <1 min, and their transparent eggs (Ibrahim et al., 2020). These properties make it a suitable choice for studies, especially studies that involve early life stages.

They are several significant sources of information on ZnO NPs that can induce mortality at high concentration, while surprisingly data for environment relevant concentration that can caused adverse effects of the whole-body system in a long period are not reported yet. The endpoint of this study is to observe the impairment and abnormalities in the embryo and larva of Javanese medaka. The chosen endpoints provide ecological importance, as the low concentration of ZnO NPs has an environment relevant. Meanwhile, the idea that water chemistry can affect the fate and behavior of chemicals, and their subsequent bioavailability to fishes, is well established but, to date, there have been no systematic studies of the toxicity of NPs to the same species of organism in saltwater versus freshwater, or during graded salinity changes. Moreover, the selected endpoints of ZnO NPs exposure in Javanese medaka have not been studied recently and to date.

1.2 Objectives

The objectives of this study are:

i. To determine the lethal concentration (LC_{50}) value of ZnO NPs by acute exposure on the embryo of Javanese medaka in different types of water (ultra-pure, deionized, and dechlorinated tap water).
ii. To determine the developmental toxicity of ZnO NPs by chronic exposure on the embryo of Javanese medaka.
iii. To evaluate the interactive effects of salinity and ZnO NPs by chronic exposure on the embryo of Javanese medaka.

REFERENCES

Adams, L. K., Lyon, D. Y., & Alvarez, P. J. (2006). Comparative eco-toxicity of nanoscale TiO2, SiO2, and ZnO water suspensions. *Water Research*, *40*(19), 3527-3532.

Amal, M. N. A., Ismail, A., Saad, M. Z., Yasin, I. S. M., Nasruddin, N. S., Mastor, S. S., Rahman, M. H. A., & Mohamad, N. (2019). Study on *Streptococcus agalactiae* infection in Javanese medaka (*Oryzias javanicus* Bleeker, 1854) model. *Microbial Pathogenesis*, *131*, 47-52.

Amal, M. N. A., Zarif, S. T., Suhaiba, M. S., Aidil, M. R. M., Shaqinah, N. N., Zamri-Saad, M., & Ismail, A. (2018). The effects of fish gender on susceptibility to acute Streptococcus agalactiae infection in Javanese medaka Oryzias javanicus. *Microbial Pathogenesis*, *114*, 251-254.

Amde, M., Liu, J. F., Tan, Z. Q., & Bekana, D. (2017). Transformation and bioavailability of metal oxide nanoparticles in aquatic and terrestrial environments. A review. *Environmental Pollution*, *230*, 250-267.

Appierot, G., Lipovsky, A., Dror, R., Perkas, N., Nitzan, Y., Lubart, R., & Gedanken, A. (2009). Enhanced antibacterial activity of nanocrystalline ZnO due to increased ROS-mediated cell injury. *Advanced Functional Materials*, *19*(6), 842–852.

Applerot, G., Lipovsky, A., Dror, R., Perkas, N., Nitzan, Y., Lubart, R., & Gedanken, A. (2009). Enhanced antibacterial activity of nanocrystalline ZnO due to increased ROS- mediated cell injury. *Advanced Functional Materials*, *19*(6), 842-852.

Auffan, M., Rose, J., Wiesner, M. R., & Bottero, J. Y. (2009). Chemical stability of metallic nanoparticles: a parameter controlling their potential cellular toxicity in vitro. *Environmental Pollution*, *157*(4), 1127-1133.

Azam, A., Ahmed, F., Arshi, N., Chaman, M., & Naqvi, A. H. (2010). Formation and characterization of ZnO nanopowder synthesized by sol–gel method. *Journal of Alloys and Compounds*, *496*, 399-402.

Aziz, F. Z. A., Zulkifli, S. Z., Mohamat-Yusuff, F., Azmai, M. N. A., & Ismail, A. (2017). A histological study on mercury-induced gonadal impairment in Javanese medaka (*Oryzias javanicus*). *Turkish Journal of Fisheries and Aquatic Sciences*, *17*(3), 621-627.

Aziz, S., Abdullah, S., Abbas, K., & Zia, M. A. (2020). Effects of engineered zinc oxide nanoparticles on freshwater fish, *Labeo rohita*: characterization of zno nanoparticles, acute toxicity and oxidative stress. *Pakistan Veterinary Journal*, *40*(4), 479-483.

Bai, W., Zhang, Z., Tian, W., He, X., Ma, Y., Zhao, Y., & Chai, Z. (2010). Toxicity of zinc oxide nanoparticles to zebrafish embryo: a physicochemical study of toxicity mechanism. *Journal of Nanoparticle Research*, *12*(5), 1645-1654.

BCC Research. (2019) Nanotechnology and Advanced Materials Research Review. Retrieved from https://www.bccresearch.com/market-research/nanotechnology/nanotechnology-fuel-cell-research-review.html

BCC Research. (2021). Nanobiotechnology: Applications and Global Markets. Retrieved from https://www.bccresearch.com/market-research/nanotechnology/nanobiotechnology-market.html

Blinova, I., Ivask, A., Heinlaan, M., Mortimer, M., & Kahru, A. (2010). Ecotoxicity of nanoparticles of CuO and ZnO in natural water. *Environmental Pollution*, *158*(1), 41-47.

Brun, N. R., Lenz, M., Wehrli, B., & Fent, K. (2014). Comparative effects of zinc oxide nanoparticles and dissolved zinc on zebrafish embryos and eleuthero-embryos: importance of zinc ions. *Science of the Total Environment*, *476*, 657-666.

Buikema, A. L. J., Niederlehner, B. R., & Cairns, J. J. (1982). Biological monitoring part IV—toxicity testing. *Water Research*, *16*(3), 239-262.

Buzea, C., Pacheco, I. I., & Robbie, K. (2007). Nanomaterials and nanoparticles: sources and toxicity." *Biointerphases 2*(4), MR17-MR71.

Caballero-Guzman, A., & Nowack, B. (2016). A critical review of engineered nanomaterial release data: Are current data useful for material flow modeling?. *Environmental Pollution*, *213*, 502-517.

Callaghan, N. I., & MacCormack, T. J. (2017). Ecophysiological perspectives on engineered nanomaterial toxicity in fish and crustaceans. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *193*, 30-41.

Callaghan, N. I., & MacCormack, T. J. (2017). Ecophysiological perspectives on engineered nanomaterial toxicity in fish and crustaceans. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *193*, 30-41.

Chen, T. H., Lin, C. C., & Meng, P. J. (2014). Zinc oxide nanoparticles alter hatching and larval locomotor activity in zebrafish (*Danio rerio*). *Journal of Hazardous Materials*, *277*, 134-140.

Clifford, M., & McGeer, J. C. (2009). Development of a biotic ligand model for the acute toxicity of zinc to *Daphnia pulex* in soft waters. *Aquatic Toxicology*, *91*(1), 26-32.

Cong, Y., Jin, F., Wang, J., & Mu, J. (2017). The embryotoxicity of ZnO nanoparticles to marine medaka, *Oryzias melastigma*. *Aquatic Toxicology*, *185*, 11-18.

Degen, A., & Kosec, M. (2000). Effect of pH and impurities on the surface charge of zinc oxide in aqueous solution. *Journal of the European Ceramic Society*, *20*(6), 667-673.

Derjaguin, B., & Landau, L. (1993). Theory of the stability of strongly charged lyophobic sols and of the adhesion of strongly charged particles in solutions of electrolytes. *Progress in Surface Science*, *43*(1-4), 30-59.

Du, J., Cai, J., Wang, S., & You, H. (2017). Oxidative stress and apotosis to zebrafish (*Danio rerio*) embryos exposed to perfluorooctane sulfonate (PFOS) and ZnO nanoparticles. *International Journal of Occupational Medicine and Environmental Health*, *30*(2), 213-229.

Ealia, S. A. M., & Saravanakumar, M. P. (2017, November). A review on the classification, characterisation, synthesis of nanoparticles and their application. In *IOP Conference Series: Materials Science and Engineering* (Vol. 263, No. 3, p. 032019). IOP Publishing.

Fabrega, J., Tantra, R., Amer, A., Stolpe, B., Tomkins, J., Fry, T., Lead, J. R., Tyler, C. R., & Galloway, T. S. (2012). Sequestration of zinc from zinc oxide nanoparticles and life cycle effects in the sediment dweller amphipod *Corophium volutator*. *Environmental Science & Technology*, *46*(2), 1128-1135.

Fabrega, J., Tantra, R., Amer, A., Stolpe, B., Tomkins, J., Fry, T., Lead, J, R., Tyler, C, R., & Galloway, T. S. (2012). Sequestration of zinc from zinc oxide nanoparticles and life cycle effects in the sediment dweller amphipod *Corophium volutator*. *Environmental Science and Technology*, 46(2), 1128-1135.

Fairbairn, E. A., Keller, A. A., Mädler, L., Zhou, D., Pokhrel, S., & Cherr, G. N. (2011). Metal oxide nanomaterials in seawater: linking physicochemical characteristics with biological response in sea urchin development. *Journal of Hazardous Materials*, *192*(3), 1565-1571.

Franklin, N. M., Rogers, N. J., Apte, S. C., Batley, G. E., Gadd, G. E., & Casey, P. S. (2007). Comparative toxicity of nanoparticulate ZnO, bulk ZnO, and ZnCl2 to a freshwater microalga (*Pseudokirchneriella subcapitata*): the importance of particle solubility. *Environmental Science & Technology*, *41*(24), 8484-8490.

García-Gómez, C., García-Gutiérrez, S., Obrador, A., & Fernández, M. D. (2020). Study of Zn availability, uptake, and effects on earthworms of zinc oxide nanoparticle versus bulk applied to two agricultural soils: Acidic and calcareous. *Chemosphere*, *239*, 124814.

Griffitt, R. J., Hyndman, K., Denslow, N. D., & Barber, D. S. (2009). Comparison of molecular and histological changes in zebrafish gills exposed to metallic nanoparticles. *Toxicological Sciences*, *107*(2), 404-415.

Guo, S., Dong, S., & Wang, E. (2009). Polyaniline/Pt hybrid nanofibers: High- efficiency nanoelectrocatalysts for electrochemical devices. *Small*, *5*(16), 1869-1876.

Gupta, G. S., Senapati, V. A., Dhawan, A., & Shanker, R. (2017). Heteroagglomeration of zinc oxide nanoparticles with clay mineral modulates the bioavailability and toxicity of nanoparticle in *Tetrahymena pyriformis*. *Journal of Colloid and Interface Science*, *495*, 9-18.

Haaker, J., Gaburro, S., Sah, A., Gartmann, N., Lonsdorf, T. B., Meier, K., Singewald, N., Pape, H., Morellini, F., & Kalisch, R. (2013). Single dose of L-dopa makes extinction memories context-independent and prevents the return of fear. *Proceedings of the National Academy of Sciences*, *110*(26), E2428-E2436.

Handy, R. D., Kammer, F. V. D., Lead, J. R., Hassellöv, M., Owen, R., & Crane, M. (2008). The ecotoxicology and chemistry of manufactured nanoparticles. *Ecotoxicology*, *17*(4), 287-314.

Hao, L., Chen, L., Hao, J., & Zhong, N. (2013). Bioaccumulation and sub-acute toxicity of zinc oxide nanoparticles in juvenile carp (*Cyprinus carpio*): a comparative study with its bulk counterparts. *Ecotoxicology and Environmental Safety*, *91*, 52-60.

Heijerick, D. G., Schamphelaere, K. A. C. D., & Janssen, C. R. (2002). Biotic ligand model development predicting Zn toxicity to the alga *Pseudokirchneriella subcapitata*: possibilities and limitations. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *133*(1-2), 207-218.

Heinlaan, M., Ivask, A., Blinova, I., Dubourguier, H. C., & Kahru, A. (2008). Toxicity of nanosized and bulk ZnO, CuO and TiO2 to bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*. *Chemosphere, 71*(7), 1308-1316.

Hogstrand, C., Wilson, R. W., Polgar, D., & Wood, C. M. (1994). Effects of zinc on the kinetics of branchial calcium uptake in freshwater rainbow trout during adaptation to waterborne zinc. *Journal of Experimental Biology*, *186*(1), 55-73.

Hou, J., Liu, H., Zhang, S., Liu, X., Hayat, T., Alsaedi, A., & Wang, X. (2019). Mechanism of toxic effects of Nano-ZnO on cell cycle of zebrafish (*Danio rerio*). *Chemosphere*, *229*, 206-213.

Ibrahim, M. A., Zulkifli, S. Z., Azmai, M. N. A., Mohamat-Yusuff, F., & Ismail, A. (2020). Embryonic toxicity of 3, 4-dichloroaniline (3, 4-DCA) on Javanese medaka (*Oryzias javanicus* Bleeker, 1854). *Toxicology Reports*, *7*, 1039-1045.

Imai, S., Koyama, J., & Fujii, K. (2007). Effects of estrone on full life cycle of Java medaka (Oryzias javanicus), a new marine test fish. *Environmental Toxicology and Chemistry*, *26*(4), 726-731.

Inoue, K., & Takei, Y. (2002). Diverse adaptability in *Oryzias* species to high environmental salinity. *Zoological Science*, *19*(7), 727-734.

Ismail, A., & Safahieh, A. (2004). Copper and zinc in intertidal surface sediment and Telescopium telescopium from Lukut River, Malaysia. *Coastal Marine Science*, *29*(2), 111-115.

Ismail, A., & Yusof, S. (2011). Effect of mercury and cadmium on early life stages of Java medaka (*Oryzias javanicus*): A potential tropical test fish. *Marine Pollution Bulletin*, *63*(5-12), 347-349.

Iwamatsu, T., & Kobayashi, H. (2002). Electron microscopic observations of karyogamy in the fish egg. *Development, Growth & Differentiation*, *44*(5), 357-363.

Janusz, W., & Sędłak, A. (2011). Specific adsorption of carbonate ions at the hematite/aqueous electrolyte solution interface. *Physicochemical Problems of Mineral Processing*, *46*, 65-72.

Jeevanandam, J., Barhoum, A., Chan, Y. S., Dufresne, A., & Danquah, M. K. (2018). Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. *Beilstein Journal of Nanotechnology*, *9*(1), 1050-1074.

Jiang, J., Oberdörster, G., & Biswas, P. (2009). Characterization of size, surface charge, and agglomeration state of nanoparticle dispersions for toxicological studies. *Journal of Nanoparticle Research*, *11*(1), 77-89.

Joo, H. S., Kalbassi, M. R., Yu, I. J., Lee, J. H., & Johari, S. A. (2013). Bioaccumulation of silver nanoparticles in rainbow trout (*Oncorhynchus mykiss*): Influence of concentration and salinity. *Aquatic Toxicology*, *140*, 398-406.

Kahru, A., & Dubourguier, H. C. (2010). From ecotoxicology to nanoecotoxicology. *Toxicology*, *269*(2-3), 105-119.

Kamarudin, N. A., Zulkifli, S. Z., Azmai, M. N. A., Aziz, F. Z. A, & Ismail, A. (2020). Herbicide diuron as endocrine disrupting chemicals (EDCs) through histopathalogical analysis in gonads of Javanese medaka (*Oryzias javanicus*, Bleeker 1854). *Animals*, *10*(3), 525.

Keller, A. A., & Lazareva, A. (2014). Predicted releases of engineered nanomaterials: from global to regional to local. *Environmental Science & Technology Letters*, *1*(1), 65-70.

Keller, A. A., Wang, H., Zhou, D., Lenihan, H. S., Cherr, G., Cardinale, B. J., Miller, R., & Ji, Z. (2010). Stability and aggregation of metal oxide nanoparticles in natural aqueous matrices. *Environmental Science & Technology*, *44*(6), 1962-1967.

Khalfalla, M. M., Hammouda, Y. A., Tahoun, A. M., & State, A. H. A. M. (2008, October). Effect of broodstock sex ratio on growth and reproductive performance of blue tilapia *Oreochromis aureus* (Steindachner) rered in hapas. In *from the pharaohs to the future. Eighth International Symposium on Tilapia in Aquaculture. Proceedings. Cairo, Egypt* (pp. 12-14).

Kinoshita, M., Murata, K., Naruse, K., & Tanaka, M. (Eds.). (2009). *Medaka: biology, management, and experimental protocols*. John Wiley & Sons.

Kool, P. L., Ortiz, M. D., & Gestel, C. A. M. V. (2011). Chronic toxicity of ZnO nanoparticles, non-nano ZnO and ZnCl2 to *Folsomia candida* (Collembola) in relation to bioavailability in soil. *Environmental Pollution*, *159*(10), 2713-2719.

Koyama, J., Imai, S., Fujii, K., Kawai, S. I., Yap, C. K., & Ismail, A. (2006). Pollution by estrogens in river and estuarine waters around Kuala Lumpur, Malaysia, and their effects on the estuarine Java-medaka, *Oryzias javanicus*. *Japanese Journal of Environmental Toxicology*, *9*(2), 141-147.

Li, J., Chen, Z., Huang, R., Miao, Z., Cai, L., & Du, Q. (2018). Toxicity assessment and histopathological analysis of nano-ZnO against marine fish (*Mugilogobius chulae*) embryos. *Journal of Environmental Sciences*, *73*, 78-88.

Li, M., Lin, D., & Zhu, L. (2013). Effects of water chemistry on the dissolution of ZnO nanoparticles and their toxicity to *Escherichia coli*. *Environmental pollution*, *173*, 97-102.

Ma, H., Williams, P. L., & Diamond, S. A. (2013). Ecotoxicity of manufactured ZnO nanoparticles–a review. *Environmental Pollution*, *172*, 76-85.

Magtoon, W., & Termvidchakorn, A. (2009). A revised taxonomic account of Ricefish *Oryzias* (Beloniformes; Adrianichthyidae), in Thailand, Indonesia and Japan. *Tropical Natural History*, *9*(1), 35-68.

Miao, A. J., Zhang, X. Y., Luo, Z., Chen, C. S., Chin, W. C., Santschi, P. H., & Quigg, A. (2010). Zinc oxide–engineered nanoparticles: dissolution and toxicity to marine phytoplankton. *Environmental Toxicology and Chemistry*, *29*(12), 2814-2822.

Mohamat-Yusuff, F., Sarah-Nabila, A. G., Zulkifli, S. Z., Azmai, M. N. A., Ibrahim, W. N. W., Yusof, S., & Ismail, A. (2018). Acute toxicity test of copper pyrithione on Javanese medaka and the behavioral stress symptoms. *Marine Pollution Bulletin*, *127*, 150-153.

Morgalev, Y. N., Gosteva, I. A., Morgaleva, T. G., Morgalev, S. Y., Kostenko, E. V., & Kudryavtsev, B. A. (2018). Parameters of embryogenesis in zebrafish *Danio rerio* as indicators of the ecological toxicity of zinc oxide nanoparticles. *Nanotechnologies in Russia*, *13*(5), 311-316.

Nair, S., Sasidharan, A., Rani, V. V. D., Menon, D., Nair, S., Manzoor, K., & Raina, S. (2009). Role of size scale of ZnO nanoparticles and microparticles on toxicity toward bacteria and osteoblast cancer cells. *Journal of Materials Science: Materials in Medicine*, *20*(1), 235.

Nakayama, K., Oshima, Y., Yamaguchi, T., Tsuruda, Y., Kang, I. J., Kobayashi, M., Imada, I., & Honjo, T. (2004). Fertilization success and sexual behavior in male medaka, Oryzias latipes, exposed to tributyltin. *Chemosphere*, *55*(10), 1331-1337.

Nugegoda, D., & Rainbow, P. S. (1989). Salinity, osmolality, and zinc uptake in *Palaemon elegans* (Crustacea: Decapoda). *Marine Ecology Progress Series. 55*(2), 149-157.

OECD Test (1992). 210: Fish, early-life stage toxicity test. *OECD Guidelines for the Testing of Chemicals, Section*, *2*.

Omar, F. M., Aziz, H. A., & Stoll, S. (2014). Stability of ZnO nanoparticles in solution. Influence of pH, dissolution, aggregation and disaggregation effects. *Journal of Colloid Science and Biotechnology*, *3*(1), 75-84.

Osborne, O. J., Johnston, B. D., Moger, J., Balousha, M., Lead, J. R., Kudoh, T., & Tyler, C. R. (2013). Effects of particle size and coating on nanoscale Ag and TiO2 exposure in zebrafish (*Danio rerio*) embryos. *Nanotoxicology*, *7*(8), 1315-1324.

Othman, F., Chowdhury, M. S. U, Jaafar, W. Z. W, Faresh, E. M. M, & Shirazi, S. M. (2018). Assessing risk and sources of heavy metals in a tropical river basin: A case study of the Selangor river, Malaysia. *Polish Journal of Environmental Studies, 27*(4), 1659-1671.

Parenti, L. R. (2008). A phylogenetic analysis and taxonomic revision of ricefishes, *Oryzias* and relatives (Beloniformes, Adrianichthyidae). *Zoological Journal of the Linnean Society*, *154*(3), 494-610.

Park, J., Kim, S., Yoo, J., Lee, J. S., Park, J. W., & Jung, J. (2014). Effect of salinity on acute copper and zinc toxicity to *Tigriopus japonicus*: the difference between metal ions and nanoparticles. *Marine Pollution Bulletin*, *85*(2), 526-531.

Patibandla, S., Zhang, Y., Tohari, A. M., Gu, P., Reilly, J., Chen, Y., & Shu, X. (2018). Comparative analysis of the toxicity of gold nanoparticles in zebrafish. *Journal of Applied Toxicology*, *38*(8), 1153-1161.

Pereira, A. C., Gomes, T., Machado, M. R. F., & Rocha, T. L. (2019). The zebrafish embryotoxicity test (ZET) for nanotoxicity assessment: From morphological to molecular approach. *Environmental Pollution*, *252*, 1841-1853.

Peters, R., Dam, G. T., Bouwmeester, H., Helsper, H., Allmaier, G., Kammer, F. V., Ramsch, R., Solans, C., Tomaniova, M., Hajslova, J., & Weigel, S. (2011). Identification and characterization of organic nanoparticles in food. *TrAC Trends in Analytical Chemistry*, *30*(1), 100-112.

Poynton, H. C., Chen, C., Alexander, S. L., Major, K. M., Blalock, B. J., & Unrine, J. M. (2019). Enhanced toxicity of environmentally transformed ZnO nanoparticles relative to Zn ions in the epibenthic amphipod *Hyalella azteca*. *Environmental Science: Nano*, *6*(1), 325-340.

Rajput, V. D., Minkina, T. M., Behal, A., Sushkova, S. N., Mandzhieva, S., Singh, R., Gorovtsov, A., Tsitsuashvili, V. S., Purvis, W. O., Ghazaryan, K. A., & Movsesyan, H. S. (2018). Effects of zinc-oxide nanoparticles on soil, plants, animals and soil organisms: a review. *Environmental Nanotechnology, Monitoring & Management*, *9*, 76-84.

Ren, Z. M., Fu, X. E., Zeng, Y., Liu, Y. D., Kim, H. S., & Chon, T. S. (2012). The stepwise behavioral responses of Medaka (*Oryzias latipes*) to organophosphorus pesticides in an online monitoring system. *Procedia Environmental Sciences*, *13*, 1122-1133.

Sabir, S., Arshad, M., & Chaudhari, S. K. (2014). Zinc oxide nanoparticles for revolutionizing agriculture: synthesis and applications. *The Scientific World Journal*, *2014*, 925494.

Saddick, S., Afifi, M., & Zinada, O. A. A. (2017). Effect of Zinc nanoparticles on oxidative stress-related genes and antioxidant enzymes activity in the brain of *Oreochromis niloticus* and *Tilapia zillii*. *Saudi Journal of Biological Sciences*, *24*(7), 1672-1678.

Salleh, A. F. M., Amal, M. N. A., Nasruddin, N. S., Zulkifli, S. Z., Yusuff, F. M., Ibrahim, W. N. W., & Ismail, A. (2017). Water pH effects on survival, reproductive performances, and ultrastructure of gonads, gills, and skins of the Javanese medaka (*Oryzias javanicus*). *Turkish Journal of Veterinary and Animal Sciences*, *41*(4), 471-481.

Schartl, M. (2014). Beyond the zebrafish: diverse fish species for modeling human disease. *Disease Models & Mechanisms*, *7*(2), 181-192.

Shaw, B. J., & Handy, R. D. (2011). Physiological effects of nanoparticles on fish: a comparison of nanometals versus metal ions. *Environment International*, *37*(6), 1083-1097.

Shazili, N. Z. M., Yunus, K., Ahmad, A. S., Abdullah, N., & Rashid, M. K. A. (2006). Heavy metal pollution status in the Malaysian aquatic environment. *Aquatic Ecosystem Health & Management*, *9*(2), 137-145.

Subashkumar, S., & Selvanayagam, M. (2014). First report on: Acute toxicity and gill histopathology of fresh water fish *Cyprinus carpio* exposed to Zinc oxide (ZnO) nanoparticles. *International Journal of Scientific and Research Publications*, *4*(3), 1-4.

Taherian, S. M. R., Hosseini, S. A., Jafari, A., Etminan, A., & Birjandi, M. (2019). Acute toxicity of Zinc Oxide nanoparticles from *Satureja hortensis* on Rainbow Trout (*Oncorhynchus mykiss*). *Turkish Journal of Fisheries and Aquatic Sciences*, *20*(6), 481-489.

Takehana, Y., Naruse, K., & Sakaizumi, M. (2005). Molecular phylogeny of the medaka fishes genus *Oryzias* (Beloniformes: Adrianichthyidae) based on nuclear and mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, *36*(2), 417-428.

Tang, F., Uchikoshi, T., & Sakka, Y. (2002). Electrophoretic deposition behavior of aqueous nanosized zinc oxide suspensions. *Journal of the American Ceramic Society*, *85*(9), 2161-2165.

Vale, G., Mehennaoui, K., Cambier, S., Libralato, G., Jomini, S., & Domingos, R. F. (2016). Manufactured nanoparticles in the aquatic environment-biochemical responses on freshwater organisms: a critical overview. *Aquatic Toxicology*, *170*, 162-174.

Waalewijn- Kool, P. L., Ortiz, M. D., Lofts, S., & Gestel, C. A. V. (2013). The effect of pH on the toxicity of zinc oxide nanoparticles to *Folsomia candida* in amended field soil. *Environmental Toxicology and Chemistry*, *32*(10), 2349-2355.

Wang, F., Jin, C., Liang, H., Tang, Y., Zhang, H., & Yang, Y. (2014). Effects of fullerene C60 nanoparticles on A549 cells. *Environmental Toxicology and Pharmacology*, *37*(2), 656-661.

Wehmas, L. C., Anders, C., Chess, J., Punnoose, A., Pereira, C. B., Greenwood, J. A., & Tanguay, R. L. (2015). Comparative metal oxide nanoparticle toxicity using embryonic zebrafish. *Toxicology Reports*, *2*, 702-715.

Wiench, K., Wohlleben, W., Hisgen, V., Radke, K., Salinas, E., Zok, S., & Landsiedel, R. (2009). Acute and chronic effects of nano-and non-nano-scale TiO2 and ZnO particles on mobility and reproduction of the freshwater invertebrate *Daphnia magna*. *Chemosphere*, *76*(10), 1356-1365.

Wittbrodt, J., Shima, A., & Schartl, M. (2002). Medaka a model organism from the Far East. *Nature Reviews Genetics*, *3*(1), 53-64.

Wong, S. W. Y., Leung, P. T. Y, Djurišić, A. B., & Leung, K. M. Y. (2010). Toxicities of nano zinc oxide to five marine organisms: influences of aggregate size and ion solubility. *Analytical and Bioanalytical Chemistry*, *396*(2), 609-618.

Wong, S. W. Y., Leung, P. T. Y., Djurisic, A. B., & Leung, K. M. Y. (2010). Toxicities of nano zinc oxide to five marine organisms: influences of aggregate size and ion solubility. *Analytical and Biological Chemistry*, 396(2010), 609–618.

Woo, S., & Yum, S. (2011). Transcriptional response of marine medaka (*Oryzias javanicus*) on exposure to toxaphene. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *153*(3), 355-361.

Wood, A. K. H., Ahmad, Z., Shazili, N. A. M., Yaakob, R., & Carpenter, R. (1997). Geochemistry of sediments in Johor Strait between Malaysia and Singapore. *Continental Shelf Research*, *17*(10), 1207-1228.

Wu, Y., Zhou, Q., Li, H., Liu, W., Wang, T., & Jiang, G. (2010). Effects of silver nanoparticles on the development and histopathology biomarkers of Japanese medaka (*Oryzias latipes*) using the partial-life test. *Aquatic Toxicology*, *100*(2), 160-167.

Xiong, D., Fang, T., Yu, L., Sima, X., & Zhu, W. (2011). Effects of nano-scale TiO_2, ZnO and their bulk counterparts on zebrafish: acute toxicity, oxidative stress and oxidative damage. *Science of the Total environment*, *409*(8), 1444-1452.

Yoshikawa, T., & Naito, Y. (2002). What is oxidative stress?. *Japan Medical Association Journal*, *45*(7), 271-276.

Yu, L. P., Fang, T., Xiong, D. W., Zhu, W. T., & Sima, X. F. (2011). Comparative toxicity of nano-ZnO and bulk ZnO suspensions to zebrafish and the effects of sedimentation, OH production and particle dissolution in distilled water. *Journal of Environmental Monitoring*, *13*(7), 1975-1982.

Yung, M. M. N, Fougères, P. A., Leung, Y. H., Liu, F., Djurišić, A. B., Giesy, J. P., & Leung, K. M. Y. (2017a). Physicochemical characteristics and toxicity of surface-modified zinc oxide nanoparticles to freshwater and marine microalgae. *Scientific Reports*, *7*(1), 1-14.

Yung, M. M. N., Kwok, K. W. H., Djurišić, A. B., Giesy, J. P., & Leung, K. M. Y. (2017b). Influences of temperature and salinity on physicochemical properties and toxicity of zinc oxide nanoparticles to the marine diatom *Thalassiosira pseudonana*. *Scientific Reports*, *7*(1), 1-9.

Yung, M. M. N., Mouneyrac, C., & Leung, K. M. Y. (2014). Ecotoxicity of zinc oxide nanoparticles in the marine environment. *Encyclopedia of Nanotechnology* (Boca Raton, FL: CRC Press), 1-17.

Yung, M. M. N., Wong, S. W. Y, Kwok, K. W. H., Liu, F. Z., Leung, Y. H., Chan, W. T., Li, X. Y., Djurišić, A. B., & Leung, K. M. (2015). Salinity-dependent toxicities of zinc oxide nanoparticles to the marine diatom *Thalassiosira pseudonana*. *Aquatic Toxicology*, *165*, 31-40.

Yusof, S. B. (2014). Establishment of Java medaka (*Oryzias javanicus* Bleeker) as test organism for ecotoxicological studies in tropical region. Doctor of philosophy. University Putra Malaysia, Department of Biology.

Yusof, S., Ismail, A., & Alias, M. S. (2014). Effect of glyphosate-based herbicide on early life stages of Java medaka (*Oryzias javanicus*): a potential tropical test fish. *Marine Pollution Bulletin*, *85*(2), 494-498.

Yusof, S., Ismail, A., & Rahman, F. (2013). Distribution and localities of Java medaka fish (*Oryzias javanicus*) in Peninsular Malaysia. *Malayan Nature Journal*, 65(2&3), 38-46.

Yusof, S., Ismail, A., Koito, T., Kinoshita, M., & Inoue, K. (2012). Occurrence of two closely related ricefishes, Javanese medaka (*Oryzias javanicus*) and Indian medaka (*O. dancena*) at sites with different salinity in Peninsular Malaysia. *Environmental Biology of Fishes*, *93*(1), 43-49.

Zhao, X., Wang, S., Wu, Y., You, H., & Lv, L. (2013). Acute ZnO nanoparticles exposure induces developmental toxicity, oxidative stress and DNA damage in embryo-larval zebrafish. *Aquatic toxicology*, *136*, 49-59.

Zhu, X., Wang, J., Zhang, X., Chang, Y., & Chen, Y. (2009). The impact of ZnO nanoparticle aggregates on the embryonic development of zebrafish (*Danio rerio*). *Nanotechnology*, *20*(19), 195103.

Zhu, X., Zhu, L., Chen, Y., & Tian, S. (2009). Acute toxicities of six manufactured nanomaterial suspensions to *Daphnia magna*. *Journal of Nanoparticle Research*, *11*(1), 67-75.

Zhu, X., Zhu, L., Duan, Z., Qi, R., Li, Y., & Lang, Y. (2008). Comparative toxicity of several metal oxide nanoparticle aqueous suspensions to Zebrafish (*Danio rerio*) early developmental stage. *Journal of Environmental Science and Health, Part A*, *43*(3), 278-284.