Evaluating the use of zinc oxide and titanium dioxide nanoparticles in a metalworking fluid from a toxicological perspective

Citation	Seyedmahmoudi, S. H., Harper, S. L., Weismiller, M. C., & Haapala, K. R. (2015). Evaluating the use of zinc oxide and titanium dioxide nanoparticles in a metalworking fluid from a toxicological perspective. Journal of Nanoparticle Research, 17(2), 104. doi:10.1007/s11051-015-2915-7
DOI	10.1007/s11051-015-2915-7
Publisher	Springer
Version	Accepted Manuscript
Terms of Use	http://cdss.library.oregonstate.edu/sa-termsofuse

Evaluating the use of zinc oxide and titanium dioxide nanoparticles in a metalworking fluid from a toxicological perspective

S.H. Seyedmahmoudi[1], Stacey L. Harper[2], Michael C. Weismiller[3], and Karl R. Haapala[1]

[1] Industrial Sustainability Laboratory, School of Mechanical, Industrial, and Manufacturing Engineering, Oregon State University, Corvallis, Oregon, 97331

[2] Department of Environmental and Molecular Toxicology & School of Chemical, Biological and Environmental Engineering, Oregon State University, Corvallis, Oregon, 97331

[3] Master Chemical Corporation, Perrysburg, Ohio, 43551

Abstract

Adding nanoparticles (NPs) to metalworking fluids (MWFs) has been shown to improve performance in metal cutting. Zinc oxide nanoparticles (ZnO NPs) and titanium dioxide nanoparticles (TiO_2 NPs), for example, have exhibited the ability to improve lubricant performance, decrease the heat created by machining operations, reduce friction and wear, and enhance thermal conductivity. ZnO and TiO_2 NPs are also relatively inexpensive compared to many other NPs. Precautionary concerns of human health risks and environmental impacts, however, are especially important when adding NPs to MWFs. The goal of this research is to investigate the potential environmental and human health effects of these nanoenabled products during early design and development. This research builds on a prior investigation of the stability and toxicity characteristics of NPs used in metalworking nanofluids (MWnF™). The previous study only investigated one type of NP at one level of concentration. This research expands on the previous investigations through the valuation of three different types of NPs that vary in morphology (size and shape) and was conducted over a wide range of concentrations in the base fluid. In the presented work, mixtures of a microemulsion (TRIM® MicroSol® 585XT), two different types of TiO_2 NPs (referred to as TiO_2A and TiO_2B) and one type of ZnO NP were used to evaluate MWnF™ stability and toxicity. Dynamic light scattering (DLS) was used to assess stability over time and an embryonic zebrafish assay was used to assess toxicological impacts. The results reveal that, in general, the addition of these NPs increased toxicity relative to the NP-free formulation. The lowest rate of zebrafish malformations occurred at 5 g/L TiO_2A NP, which was even lower than for the base fluid. This result is particularly promising for future MWnF™ development, given that the mortality rate for 5 g/L TiO_2A was not significantly different than for the base fluid.

Keywords

Nanotoxicology, Nanofluid Stability, Zinc Oxide Nanoparticles, Titanium Dioxide Nanoparticles

Introduction

Metalworking fluids (MWFs) are used to reduce the heat and friction in machining operations (Schey, 1967). Due to the large use of MWFs globally, which is more than 500 million gallons annually, industry is concerned about their sustainability (Clarens et al. 2008). Manufacturing sustainability performance (e.g., environmental, economic, health and safety aspects) can be enhanced by improving the efficiency of MWFs. For instance, improving the heat transfer properties of MWFs can reduce the rate of cutting tool replacement, which is highly expensive (Jen et al. 2002).

Many researchers have undertaken various strategies to improve MWF sustainability performance. To enhance environmental performance, for example, biocide-free MWFs were compared to conventional mineral oil based fluids by Winter et al. (2012), and were found to reduce measured machining forces and workpiece surface roughness. Minimum quantity lubrication (MQL) application of MWFs have been applied to reduce environmental and health impacts and enhance penetration into the cutting zone (Lee et al. 2010; Park et al. 2010; Wang and Clarens, 2013; Weinert et al. 2004). Microfiltration has been shown to reduce health risks and environmental impacts of MWFs (Ham et al. 2010). Studies have shown that nanoparticle (NP) additives decrease friction, improve lubricity and thermal conductivity, reduce cutting forces, and improve the machining performance compared to NP-free MWFs (Kotnarowski, 2008; Mosleh et al. 2009; Rapoport et al. 2005). Thus, advantages of using such metalworking nanofluids (MWnF™) include reduction of energy and fluid consumption, reduction of related economic and environmental impacts, and enhancement of cutting tool life. To identify the potential hazards of using MWnF™, however, they must be evaluated more holistically (Krajnik et al. 2011).

Thus, the goal of this research is to investigate environmental and human health concerns as a part of precautionary MWnF™ development. The research builds on a prior investigation of the stability and toxicity of MWnF™ formulations. Several prior studies that found NPs additives to improve MWF performance are presented. Research exploring the uncertainties surrounding NP life cycle environmental performance is also briefly reviewed. Finally, methods employed to explore the stability and biological responses of several MWnF™ formulations using two different types of TiO_2 NPs and a ZnO NP are presented and the results are discussed.

Advancements in nanolubricants

The tribological (friction and wear) properties of several NP additives have been explored. Tungsten disulfide (WS_2) and molybdenum disulfide (MoS_2) NPs were found to play a key role where fluids were unable to support the loads under severe conditions (Rapoport et al. 2005). Mosleh et al. (2009) showed that using WS_2 and MoS_2 NPs in oil reduced the wear volume in testing of 440C ball-titanium sheet pairs and 440C ball-steel sheet pairs by 25-30% and 55-65%, respectively. While the coefficient of friction (COF) for the 440C ball-titanium sheet pair was reduced by 10%, no improvement was noticed for the 440C ball-steel sheet pair. Kotnarowski (2008) added CuO NPs to SN 100 and Hydrorafinat II basic oils to evaluate the COF reduction. They concluded that adding 0.25% by volume CuO NPs is most effective, resulting in 10-25% reduction in COF. Hu and Dong (1998) found 20 nm titanium oxide NPs added to 500 SN base oil decreased COF by 12%, and the wear resistance and load capacity of the fluid were improved. McCook et al. added different NPs to an epoxy matrix to test the COF and wear resistance by using a linear reciprocating pin-on-disk tribometer (McCook et al. 2006). Optimum wear resistance was observed with a 1% by volume ZnO and 14.5% by volume polytetrafluoroethylene (PTFE) NPs. Similarly, optimal COF reduction was found with 3.5% by volume ZnO and 14.5% by volume PTFE NPs. Turgut et al. showed a 3% volume fraction of TiO_2 in a water-based nanofluid improved thermal conductivity by 7.4% compared to the base fluid (Turgut et al. 2009). The viscosity increased with an increasing volume fraction of NPs. LotfizadehDehkordi et al. (2013) employed a UV–Vis spectrophotometer and observed thermal conductivity enhancement by using a high concentration of TiO_2 in water-based nanofluids. Yang and Du (2012) observed the same characteristic of other water-based nanofluids.

These tribological enhancements have led to demonstrated improvements in machining processes. Using 3% by volume alumina and 1% by volume multi-walled carbon nanotube (MWCNT) nanofluids (NFs) in oil significantly reduced the force, energy, and temperature of cutting (Roy and Amitava, 2013). Shen et al. (2008) found that adding 5-20% by volume of 100 nm MoS_2 NPs to three different fluids (paraffin, soybean oil, and CANMIST) decreased the COF in grinding of cast iron by 30-50%. Khandekar et al. (2012) found that adding 1% by volume of Al_2O_3 to a conventional cutting fluid reduced cutting force, tool wear, and surface roughness compared to the conventional cutting fluid.

Precautionary concerns

Although NPs can improve the tribological properties, there are uncertainties about their life cycle sustainability performance. The toxicity of MWnF™ is one of the uncertainties that must be considered in their early development (Niyaghi et al. 2014). Broadly, the potential health risks of NPs are little known since the development of nanomaterials is in its early stages. Hoet et al. (2004) provided a comprehensive review of data analysis on the health risks of nanomaterials. Various nanomaterial attributes, such as size, shape, and morphology, affect toxicity and must be considered in the development of safer nano-based products (Harper et al. 2008; Thomas, 2005).

In previous research by the authors, various concentrations of 20 nm ZnO NPs were added to a commercially available semi-synthetic microemulsion (TRIM® MicroSol® 585XT) to investigate the cutting force and temperature in turning of a titanium alloy (Ti-6Al-4V) (Sahakian, 2011). Machining tests revealed that the lowest temperature and cutting force occurred at 0.5% by weight of ZnO NPs in the MWF. The stability and toxicity of 20 nm ZnO NPs used were also investigated for a range of concentrations (Niyaghi et al. 2014). It was concluded that the surfactants contained in the base fluid were sufficient for dispersion of the NPs. Using a zebrafish embryo assay, it was found that 20 nm ZnO NPs increased the toxicity of the base MWF over a range of concentrations. In related work, Bai et al. (2010) studied the toxicity of 30 nm ZnO NPs in a water suspension using a zebrafish embryo test. The results indicated that concentrations of 0.05 and 0.1% by volume of ZnO NPs cause mortality of zebrafish embryos. In lower concentrations (1–25 mg/L), malformations such as body and tail deformities were observed.

Materials and Methods

The research reported herein focuses on stability and toxicity assessment of different NPs for a variety of concentrations. The objective is to assess NPs that vary in morphology (size and shape) and composition that have been shown to improve machining performance in order to assist precautionary development of MWnF™ that balance both process performance and safety.

Materials

TiO_2 and ZnO NPs (Nanostructured & Amorphous Materials, Inc., Houston, TX) were chosen as MWF additives. The NPs were added to a semi-synthetic microemulsion (TRIM® MicroSol® 585XT by Master Chemical Corp., Perrysburg, OH). The properties of the selected TiO_2 and ZnO NPs are shown in Table 1. The low dissolution rate of TiO_2 and ZnO NPs in water makes them suitable candidates for suspensions at a variety of concentrations, while their availability and low cost makes them amenable for use in a commercially viable metalworking nanofluid.

Suspensions (40 mL) of 5 g/L of NPs (TiO_2A, TiO_2B, and ZnO) were prepared in semi-synthetic microemulsion with nanopure water (1:13 by volume ratio). The nanoparticles were dispersed into the suspensions under 15 minutes of sonication using a Sonics® VCX 750 horn ultrasonicator (Sonics and Materials Inc., Newtown, CT). The suspensions were then allowed to cool for two hours, allowing large particles to settle out of suspension to prevent fluctuations in size measurements. This procedure was repeated for 7.5 and 10 g/L concentrations of NPs in the semi-synthetic microemulsion and nanopure water. In all suspensions, the concentration of the initial microemulsion was 7.5% by volume (1:13 ratio) in nanopure water.

Table 1 Properties of the selected nanoparticles (NanoAmor 2013)

Property	ZnO	TiO_2A	TiO_2B
Purity	99.5%	99%	99%
Average Particle Size	20 nm	20 nm	15 nm
Specific Surface Area	50 m^2/g	210 m^2/g	240 m^2/g
Color	Milky White	White	White
Morphology	Nearly Spherical	Spherical	Irregular-angular
True Density	5.606 g/cm^3	3.9 g/cm^3	3.9 g/cm^3
Solubility in Water	Insoluble	Insoluble	Insoluble

Fluid stability evaluation procedure

A dynamic light scattering (DLS) method was used to measure the size (hydrodynamic diameter) of NPs dispersed in the MWnF™ colloids with a ZetaSizer Nano ZS DLS device (Malvern Instruments, Westborough, MA). A significant decrease in average particle size over time was interpreted as fluid instability due to the settling out of large particles. Thus, measuring the average hydrodynamic diameter (Z_{ave}) indicates lower agglomeration and smaller colloids. From each 40 mL suspension, samples (1.6 mL) were pipetted into cuvettes and inserted into the DLS instrument. Z_{ave} measurements were replicated eight times and each of the replicates was repeated three times. To evaluate the stability of the suspensions, these tests were repeated after five days and one month. After evaluation, the safety performance of stable suspensions was assessed using toxicity tests as described below.

Nanofluid toxicity evaluation procedure

Volume-based serial dilution zebrafish assay experiments were conducted for MWF and MWnF™ formulations to identify the relative toxic potential of the selected NPs. Various concentrations of NPs with a 7.5% volume concentration of semi-synthetic fluid were used in the preparation of suspensions. Suspensions required dilution to obtain meaningful zebrafish assay results. To dilute the MWnF™ suspensions, fishwater, a 0.26 g/L mixture of sea salt (Instant Ocean®, Blacksburg, VA) and deionized water, was used. The suitable range of pH for zebrafish embryos is 6.5 to 7.5. Thus, pH was adjusted to that range using hydrochloric acid.

The toxicity evaluation procedure used in this study has been previously reported (Truong et al. 2011). Briefly, the chorionic membrane surrounding the embryo was enzymatically removed at seven hours post-fertilization (hpf) to yield more accurate results. The chorion is natural layer, which protects the embryo. Next, the prepared solutions were pipetted into two 96-well plates, which allowed 24 replicates for each concentration, enabling statistical analysis. The dechorionated embryos were then placed into the plate suspensions to be tested. A control group was placed in fishwater without any MWnF™ suspensions. Plates were kept at standard laboratory conditions of 28°C on a 14h light:10h dark photoperiod. To perform the toxicology assessment, 21 developmental endpoints were considered, and were examined at 24 hpf and 120 hpf. Developmental endpoints included morphological malformations, behavioral abnormalities, and embryo mortality.

Results

Using the techniques described above, the stability and toxicity of various formulations were evaluated as reported below.

Fluid stability results

The Z_{ave} (hydrodynamic diameter) values for each MWnF™ suspension was measured using DLS at 7 hours, 120 hours, and 1 month after preparation (Figs. 1-3). It can be seen that the Z_{ave} of all suspensions has a decreasing trend over time due to aggregation and precipitation of NP clusters. The effect is statistically insignificant (95% confidence interval), however, indicating good fluid stability in each case.

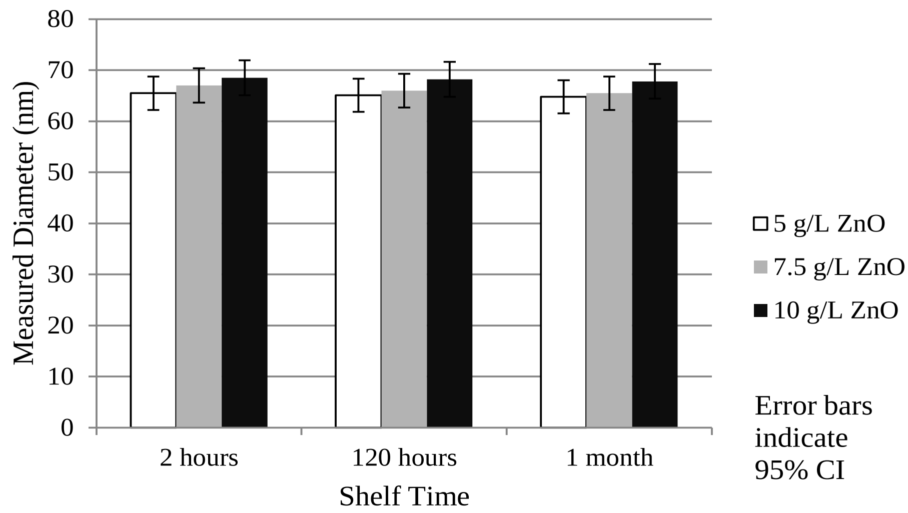

Fig. 1 Effect of time on average ZnO NP size

From the results, it can be seen that average measured size increases with increasing NP concentration. It is speculated that opportunities for aggregation increase as numbers of particles increase. It can be seen that the smaller TiO_2B NPs, which are irregular angular in shape, result in larger average measured size and greater reduction in measured size over time. This indicates a greater tendency for agglomeration. Table 2 presents the change in measured size for each NP type after 120 hours and one month compared to two hours after preparation. It can be seen that the percent reductions were low (0.37-2.99%) and the variance was small. As a result, the mixture of NPs in the base fluid remained consistent over a one month period, which is necessary for this type of product.

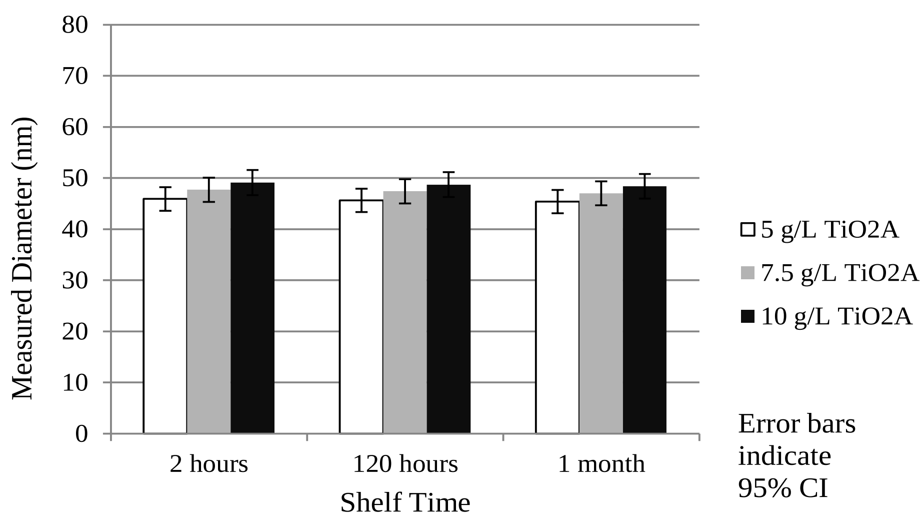

Fig. 2 Effect of time on average TiO_2A NP size

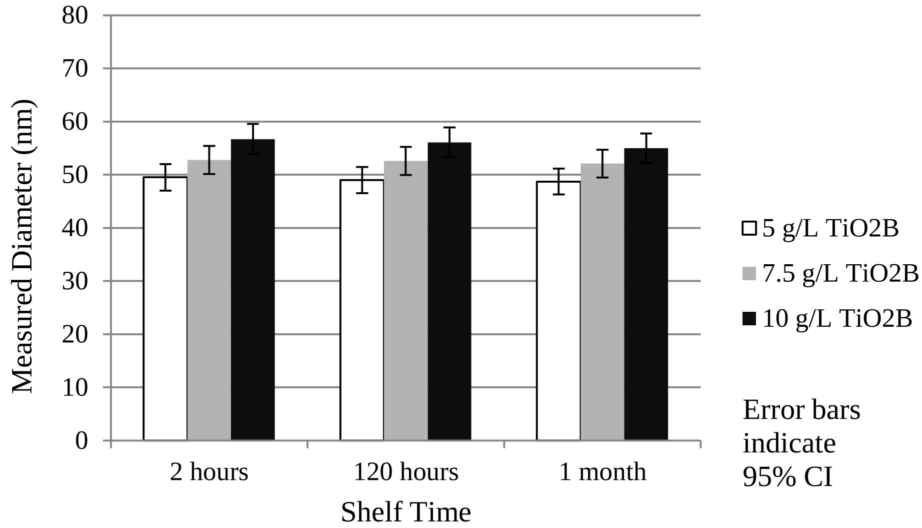

Fig. 3 Effect of time on average TiO_2B NP size

Toxicity results

Toxicity evaluation proceeded with stable formulations identified in the stability evaluation phase. The zebrafish model was used to examine the toxicity effect of all three NPs at different concentrations in the base fluid. First, the critical toxic concentration region of the base fluid was determined by employing a dose range-finding experiment. The critical range is defined as the range of concentrations that bounds the lethal point.

Table 2 Effect of time on average nanoparticle size

Type of Suspension	% Reduction compared to 2 hours after preparation	
	120 hours	1 month
5 g/L ZnO	0.61	1.06
7.5 g/L ZnO	1.49	2.23
10 g/L ZnO	0.43	1.02
5 g/L TiO_2A	0.65	1.08
7.5 g/L TiO_2A	0.62	1.46
10 g/L TiO_2A	0.81	1.42
5 g/L TiO_2B	1.01	1.61
7.5 g/L TiO_2B	0.37	1.32
10 g/L TiO_2B	1.05	2.99

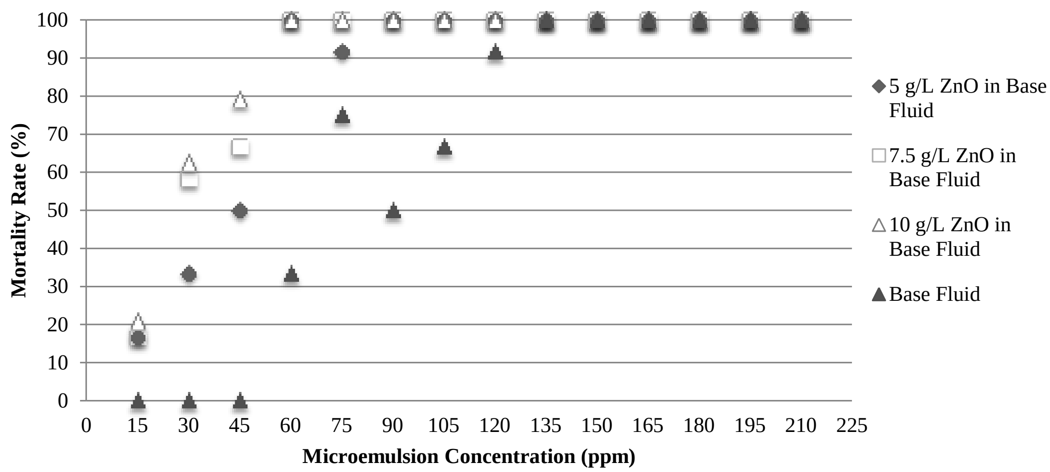

Fig. 4 Mortality rates among samples exposed to suspensions with and without ZnO NPs

The mixture was diluted in a serial manner based on semi-synthetic fluid concentration until the critical region was found. The lethal point for the base fluid was found to be 120 parts per million (ppm). The fluid did not affect zebrafish embryos at microemulsion concentrations below 15 ppm. With the critical region identified, NPs were added to the base fluid in varying concentrations (i.e., 0 g/L, 5 g/L, 7.5 g/L, and 10 g/L) to assess the effect of NP additions on the biological responses of zebrafish embryos. Figures 4-6 show the mortality results for each of the concentrations evaluated. Suspensions with NPs resulted in considerably higher mortality than formulations without the NPs added for each of the three NP types examined except for the case of 5 g/L TiO_2A.

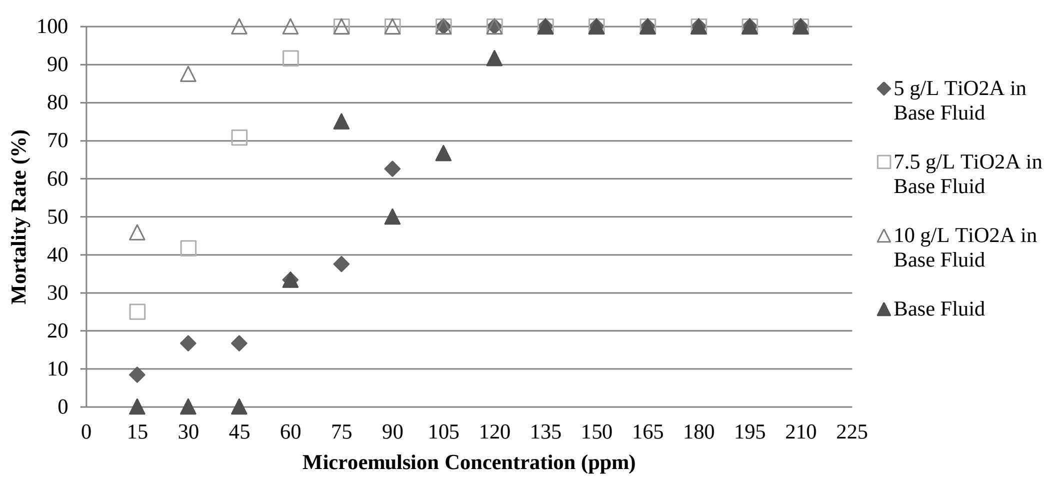

Fig. 5 Mortality rates among samples exposed to suspensions with and without TiO_2A NPs

For the base fluid, 5 and 10 g/L TiO_2A, and 5g/L ZnO NPs, the rate of zebrafish embryo malformations was recorded for dilution levels that did not cause mortality. Due to resource limitations, malformation comparison between suspensions was only possible for these experimental conditions. Figure 7 shows representative images of normal and malformed zebrafish embryos from the study.

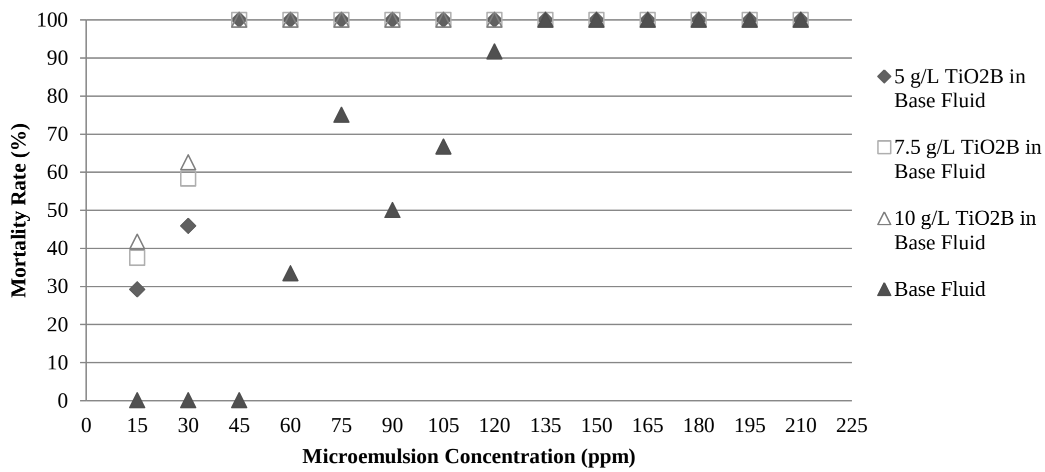

Fig. 6 Mortality rates among samples exposed to suspensions with and without TiO_2B NPs

Figure 8 presents the average number of malformations observed in the surviving zebrafish embryos after five days of exposure for TiO_2A and ZnO suspensions. The results revealed embryos exposed to the suspensions with NPs have higher rates of malformation than those exposed to only the base fluid. The lowest rate of malformation occurred at 5 g/L TiO_2A NP, which was, remarkably, consistently lower than for the base fluid. This result is particularly promising, given that the mortality rate for 5 g/L TiO_2A was similar for the base fluid. Figures 9-11 provide a comparison of mortality results for samples exposed to MWnF™ suspensions with 5, 7.5, and 10 g/L NP concentrations.

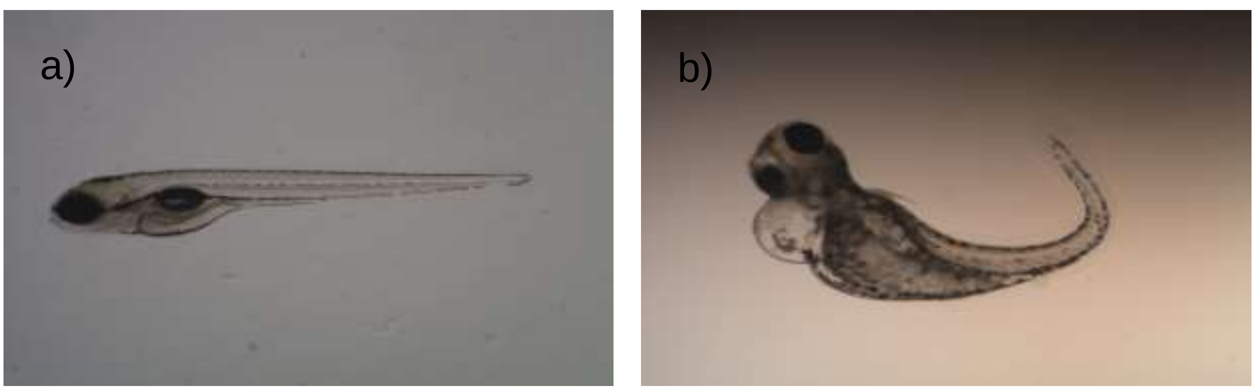

Fig. 7 Representative images of a) normal b) malformed zebrafish embryo development

It can be seen that, for each concentration of 5 g/L NP MWnF™ formulations, TiO_2A NPs have the lowest mortality rate followed by ZnO and TiO_2B, respectively. At a 90 ppm microemulsion concentration the mortality rate is 60% for TiO_2A NPs, for example, while it is 100% for ZnO and TiO_2B NPs. For the 7.5 g/L NP MWnF™ formulations, the mortality rates for each NP vary; it cannot be concluded which is safest. For instance, while the mortality rate for ZnO NPs is lower than other NPs at a 15 ppm microemulsion concentration, at a 30 ppm concentration TiO_2A NPs have the lowest mortality rate.

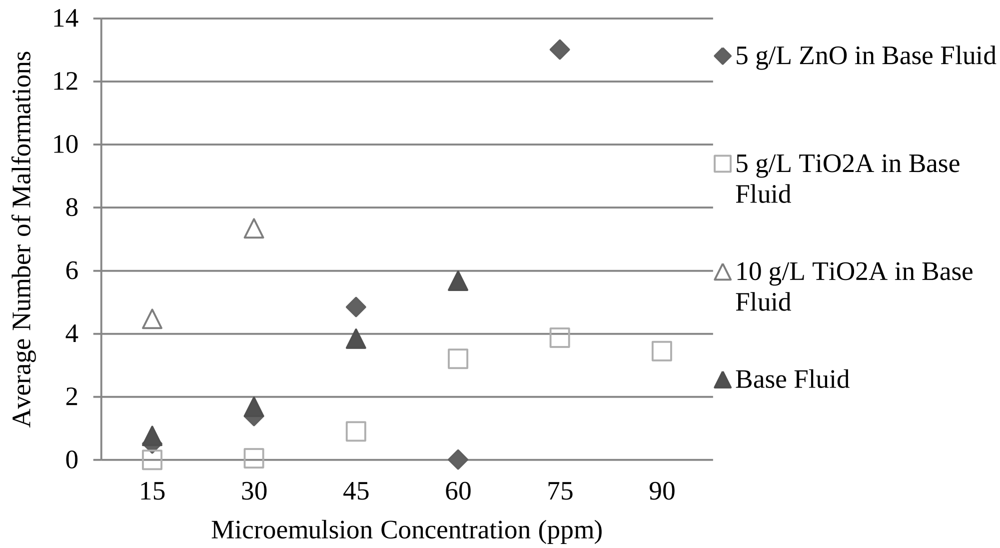

Fig. 8 Average number of malformations among surviving samples exposed to suspensions with and without ZnO and TiO_2A NPs

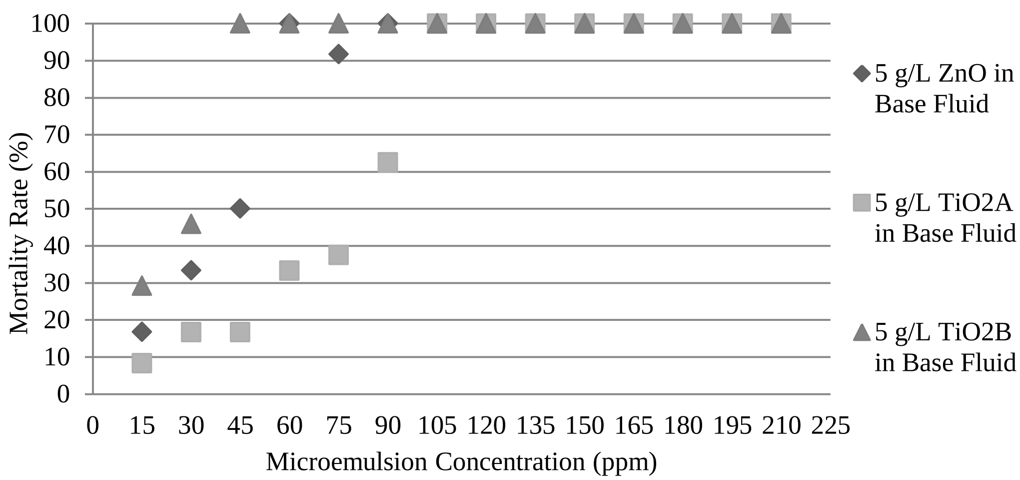

Fig. 9 Mortality rate among samples exposed to suspensions with 5 g/L NPs

Similar to the 7.5 g/L NP MWnF™ formulations, mortality rates for suspensions with 10 g/L NP vary. Regardless of NP type, for 10 g/L NP MWnF™ formulations the mortality rate for each concentration is significantly higher than the base fluid.

Discussion

This research investigates the precautionary environmental and human health concerns of several MWnF™ formulations. The results presented above build upon prior research that demonstrated good stability, but reduced toxicity, with the addition of ZnO NPs to a semi-synthetic microemulsion. The above results show that the two different types of TiO_2 NPs and the ZnO NPs again exhibit good stability in the base fluid and reduced toxicity. Interestingly, however, one of the NPs evaluated (TiO_2A) reduced the toxicity compared to the base fluid at a low concentration. Below, the methods applied and statistical analysis of the results are discussed.

Nanofluid stability

As stated above, two different types of TiO_2 NPs and a ZnO NPs were chosen as potential additives to an MWF. Their low cost compared to the other NPs is one advantage (NanoAmor 2013). In addition, Abdullah et al. (2013) showed that both TiO_2 and ZnO can be dispersed stably in oil-based fluid and can be used as lubricant additives. Further, adding TiO_2 was shown to decrease the COF and increase wear resistance and load carrying capacity of MWFs (Hu and Dong 1998), while adding ZnO to a lubricant enhanced the lubricity and reduced friction (Hernandez Battez et al. 2006). In the work presented, a key aspect to be understood before assessing toxicity was the stability of the selected NPs in the MWF.

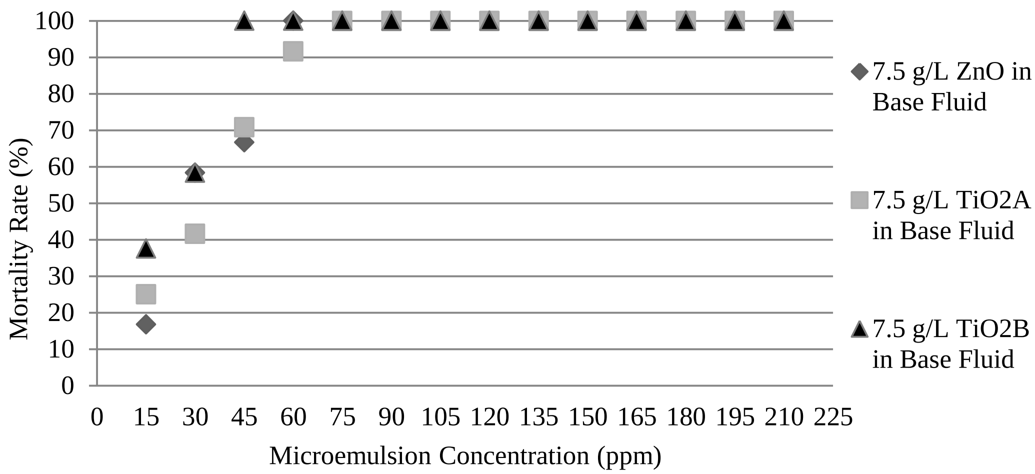

Fig. 10 Mortality rates among samples exposed to suspensions with 7.5 g/L NPs

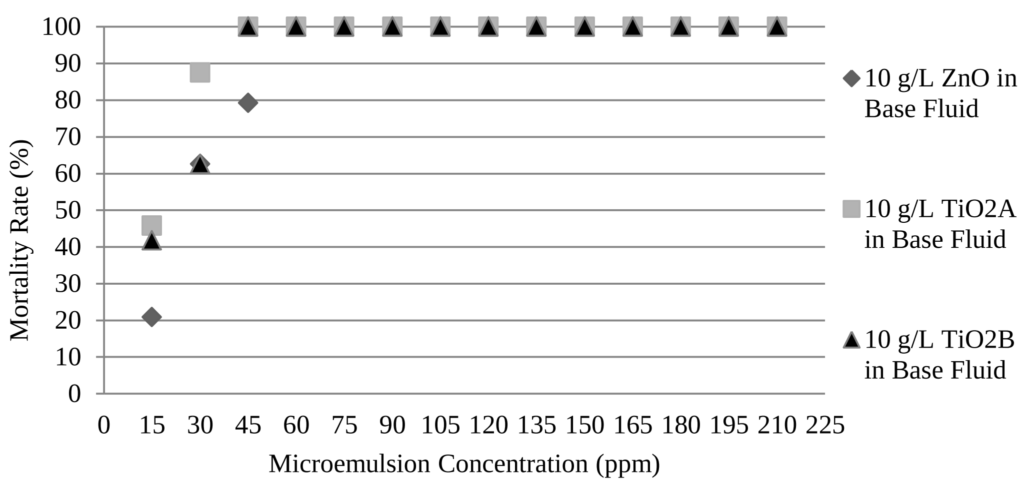

Fig. 11 Mortality rate among samples exposed to suspensions with 10 g/L NPs

Clusters of NPs form due to particle aggregation, and settle out of suspension based on Stokes' Law. This law shows that settling velocity (υ_s) is proportional to the square of particle size (R) (Eq. 1):

$$\upsilon_s = \frac{2(\rho_p - \rho_f)}{9\mu} g \cdot R^2 \qquad [1]$$

where v_s is the particle's velocity, g is the gravitational acceleration, μ is the dynamic viscosity, R is the radius of the spherical object, and ρ_p and ρ_f are the mass density of the particles and fluid, respectively. Unsuspended aggregated particles cause machining efficiency reduction and result in inaccurate toxicity test results (Bai et al. 2010; Eastman et al. 2004).

In this research, it was assumed that measuring particle hydrodynamic diameter over time would indicate any agglomeration and settling of NPs. Hydrodynamic diameter, the diameter of a hypothetical sphere, can be calculated by measuring the intensity variation of scattered laser light through a fluid (Sattelle 1988). Thus, in DLS, the hydrodynamic diameter is measured based on the speed of the variations of the laser travel time. The DLS device used calculated the particle sizes using Stokes-Einstein equation (Eq. 2):

$$D = \frac{k_B T}{6\pi\eta r} \qquad [2]$$

where D is the diffusion constant, k_B is Boltzmann's constant, T is the absolute temperature, η is a viscosity, and r is the radius of the spherical particle. To calculate the hydrodynamic diameter, fluid viscosity for each suspension was provided as an input to the DLS instrument; temperature and radius are measured by the device.

To disperse the NPs and improve the stability of the MWnF™, ultrasonication was applied. Ultrasonic frequency waves help to fully disperse the NPs in the mixture and fragment the agglomerated particles (Kusters et al. 1993). Xia et al. (2010) studied the preparation of stable nitrendipine nanosuspensions by using ultrasonication and demonstrated an improved dissolution rate of the oral bioavailability drug. Nanofluid stability improvement was reported by Sahakian in preparation of ZnO NPs for an MWnF™ by using the ultrasonication (Sahakian 2011). Finally, the effect of shelf time, defined as the time after preparation of the suspension, on the stability of suspensions was investigated to assess stability over time. Longer term stability is necessary for a viable, commercializable product. While the DLS results indicated good stability, future work can further assess variation in size distribution and particle count to validate the initial assumption relating size to stability. Further, longitudinal machining tests will assist in indicating fluid stability and viability.

Nanomaterial toxicity

Since the environmental and human health impacts of NP-based products are often uncertain or unknown, it is especially critical to better understand and mitigate harm during product development of MWnF™ formulations. Investigating the fluid use phase is important since machinists come into contact with MWFs (Calvert et al. 1998; Silverstein et al. 1988; Simpson et al. 2003). Hazards, such as flammability and acute or chronic toxicity, are considered in early MWF development. To ensure the safety of using NPs in MWFs, the authors posit that toxicological assessments of MWnF™ are also critical. Potential health hazards due to inhalation and dermal routes of exposure must be investigated (Thomas 2005). NP characteristics, such as size, shape, dissolution rate, and agglomeration state must be known (Boverhof and David 2010).

Hussain et al. (2009) presented a review of physical and chemical characteristics of NPs necessary to determine the exposure protocols and how nanomaterials affect living organisms. A study by Lademann et al. (1999) revealed that TiO_2 used in sunscreens can penetrate the hair follicle and stratum corneum. Hussain et al. (2005) studied the toxic effect of NPs including TiO_2 for use in industry with the in vitro rat liver derived cell line method. The results revealed that lower doses (10-50 mg/mL) of 40 nm TiO_2 NPs had no effect on mitochondrial function, while at higher doses (100-250 mg/mL), significant effects existed. Chen et al. (2009) investigated the in vivo acute toxicity of TiO_2 NPs on adult mice by injecting various doses. Passive behavior, loss of appetite, tremors, and lethargy were the signs that were exhibited in the treated mice when the deposited NPs were found in liver, kidney, and lung. Wu et al. (2009) studied the penetration of P25 (21 nm) TiO_2 NPs in mice skin and, after 60 days, the results revealed that NPs can penetrate to the skin and reach several organs. Beckett et al. (2005) tested a 500 $\mu g/m^3$ concentration of ZnO NPs for two hours on healthy human subjects, and found no significant effects on the respiratory or cardiovascular systems. A study by Wang et al., however, found that oral exposure to varying doses of 20 nm and 120 nm ZnO NPs given to mice can cause damage to the liver, spleen, and pancreas (Wang et al. 2008). Bai et al. (2010) exposed zebrafish to 30 nm ZnO NPs in water and assessed mortality and malformations of the fish elicited at varying concentrations of NPs. The study revealed that the mortality range for ZnO NPs was between 50 to 100 mg/L and the malformation range is between 1 to 25 mg/L.

As seen above, mammals (and even humans) have been used to assess health risks and toxicological effects, which is cost and time intensive. In this research, the zebrafish model is used a suitable alternative. It is a rapid toxicity evaluation model and the organisms are morphologically and physiologically similar to mammals (Zon and Peterson 2005). Zebrafish organs are genetically similar to human organs, thus the model allows researchers to investigate a variety of human diseases (Lieschke and Currie 2007). Each female zebrafish can spawn hundreds of eggs per day and the fish organs develop within 48 hours (Kimmel et al. 1995). Thus, to have statistically powerful assays, toxicological tests using zebrafish embryos can be repeated multiple times. It must be noted that the zebrafish model is not a comprehensive solution to investigate all variety of human diseases, however, and other standard human toxicity testing should be completed before commercializing nano-based products.

Statistical analysis of toxicity evaluation results

As mentioned above, an initial dose range-finding experiment was undertaken for the base fluid without NPs to indicate the concentration beyond which significant impacts on zebrafish occur, known as the critical toxic concentration. The base fluid was diluted iteratively by order of magnitude, based on semi-synthetic fluid concentration, until discovering the range of critical toxic concentration. Meticulous inspections were then carried out to indicate the critical toxic concentration with more accuracy. It was found that the fluid significantly affected zebrafish at concentrations above 15 ppm, and concentrations over 120 ppm were found to be lethal for zebrafish. Thus, dilutions of 5, 7.5, and 10 g/L NPs suspension were prepared at concentrations of 15-210 ppm to evaluate the effect of NP additions on biological responses of zebrafish.

To verify the significance of the measured biological responses for each formulation, statistical analysis was conducted using mortality results (Seyedmahmoudighomi 2014). Fitted models of the recorded mortality rates for each combination of NP and initial concentration were compared using the Tukey test method. From the above figures, it appears increasing NP concentration does increase mortality rate, however, due to the scarcity of data, differences were only found to be statistically significant when comparing 10 g/L TiO_2A, 7.5 g/L TiO_2B, and 10 g/L TiO_2B to the base fluid. Prior work compared the mortality rate for a ZnO MWnF™ to the base fluid, and found that toxicity significantly increased for each dilution level (Niyaghi 2013). Thus, to compare the relative toxicity of each combination of NP type and initial concentration, future work would need to examine more levels within the critical concentration range to establish accurate models for the effect of concentration level on mortality rate.

Conclusions

This research investigated the precautionary development of a nano-based products (i.e., MWnF™ formulations) due to the attendant environmental and human health concerns. One purpose was to identify potential NP additives to improve MWF machining performance, while maintaining or improving the shelf life and safety performance to ensure consumer acceptability. Stability and toxicity were used as indicators of shelf life and safety. Formulation stability was evaluated using dynamic light scattering (DLS), and biological responses were evaluated using zebrafish assays, for two types of TiO_2 NPs and one type of ZnO NP at several concentrations (5 g/L, 7.5 g/L, and 10 g/L). The measured size reduction of NPs in the mixtures after 120 hours and after one month was less than a 2% and 3%, respectively, for all formulations compared to the measured size at 2 hours post-preparation. Thus, it was concluded that mixtures of each of the NPs in the base fluid were sufficiently stable to proceed to toxicity evaluation.

Addition of NPs was found to cause higher mortality than the NP-free formulations. Increased mortality rates were also observed with increasing concentrations for all three NP types evaluated. Higher initial NP concentrations led to an increase in mortality at lower dilutions, indicating that the relative amount of NPs in the mixture impacts the level of exposure, as would be expected. A bit more surprisingly, however, the relationship between concentration and mortality rate was found to be nonlinear, and reveals existence of phenomena to be explored. A higher average number of malformations was also observed with addition of NPs. Among all 5 g/L NP concentration formulations, TiO_2A NP exhibited the lowest mortality rate. The lowest number malformations also occurred for 5 g/L TiO_2A NP, which was even lower than for the base fluid. This result is particularly promising for future MWnF™ development, since it indicates the potential for maintaining, and possibly reducing, the toxicity of the base fluid.

This is the first study to explore the stability and toxicity of different MWnF™ formulations using a prepared MWF microemulsion (TRIM® MicroSol 585XT in water) as the base fluid, and varying NP chemistry and morphology (size and shape), and concentration. Previously, only one type of NP and one concentration level was reported. Regardless of the NP type evaluated, higher concentrations of NPs were found to significantly increase toxicity. Although all three NP types enhance tribological characteristics, the safety of the MWnF™ formulations from a toxicological point of view remains a concern. The findings of this research reiterate, due to the uncertainties in environmental and human health impacts, that it is especially critical to understand these implications and to mitigate harm during the early development of nano-based products.

The approach reported in this research can be used to assist sustainable design decision makers in reducing use phase product environmental impacts and human health risks from the early stages of product development. For future research, different NPs with different morphologies and a variety of concentrations should be explored to elucidate the effect on fluid stability, toxicity, and machining performance. Life cycle assessment approaches also should be applied to understand comprehensive costs and environmental impacts of the production, use, and end of life of MWnF™ formulations. This information can eventually lead to data-driven design of lower cost, safer, and environmentally friendly nano-based MWFs. In closing, it must be iterated that the safety of MWnF™ and other nano-based products can be enhanced by a training programs and appropriate personal protective equipment use within industry.

References

Abdullah SF, Nomanbhay SM, Ishak IS, Radiman S (2013) Structural and tribology properties of WO_3, TiO_2 and ZnO composite nanoparticles as lubricant additives. J Energy Environ *4:26-30*

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. J Nanoparticle Res 12:1645–1654

Beckett WS, Chalupa DF, Pauly-Brown A, Speers DM, Stewart JC, Frampton MW, Utell MJ, Huang LS, Cox C, Zareba W, et al (2005) Comparing inhaled ultrafine versus fine Zinc Oxide particles in healthy adults. Am J Respir Crit Care Med *171*:1129–1135

Boverhof DR, David RM (2010) Nanomaterial characterization: Considerations and needs for hazard assessment and safety evaluation. Anal Bioanal Chem *396*:953–961

Calvert GM, Ward E, Schnorr TM, Fine LJ (1998) Cancer risks among workers exposed to metalworking fluids: a systematic review Am J Ind Med *33*:282–292

Chen J, Dong X, Zhao J, Tang G (2009) In vivo acute toxicity of titanium dioxide nanoparticles to mice after intraperitioneal injection. J Appl Toxicol *29*:330–337

Clarens AF, Zimmerman JB, Keoleian GA, Hayes KF, Skerlos SJ (2008) Comparison of Life Cycle Emissions and Energy Consumption for Environmentally Adapted Metalworking Fluid Systems. Environ Sci Technol *42*:8534–8540

Eastman JA, Phillpot SR, Choi SUS, Keblinski P (2004) Thermal transport in nanofluids. Annu Rev Mater Res *34*:219–246

Ham S, Kapoor SG, DeVor RE, Wentz J (2010) The impact of surface forces on particle flow and membrane fouling in the microfiltration of metalworking fluids. J Manuf Sci Eng *132*:011006

Harper S, Usenko C, Hutchison JE, Maddux BLS, Tanguay RL (2008) In vivo biodistribution and toxicity depends on nanomaterial composition, size, surface functionalisation and route of exposure. J Exp Nanosci *3*:195–206.

Hernandez Battez A, Fernandez Rico J, Navas Arias A, Viesca Rodriguez J, Chou Rodriguez R, Diaz Fernandez J (2006) The tribological behaviour of ZnO nanoparticles as an additive to PAO6. Wear *261*:256–263.

Hoet PH, Brüske-Hohlfeld I, Salata OV (2004) Nanoparticles – known and unknown health risks J Nanobiotechnology *2*:12

Hu ZS, Dong JX (1998) Study on antiwear and reducing friction additive of nanometer Titanium Oxide. Wear *216*:92–96

Hussain SM, Hess KL, Gearhart JM, Geiss KT, Schlager J (2005) In vitro toxicity of nanoparticles in BRL 3A rat liver cells. Toxicol In Vitro *19*:975–983

Hussain SM, Braydich-Stolle LK, Schrand AM, Murdock RC, Yu KO, Mattie DM, Schlager JJ, Terrones M (2009) Toxicity evaluation for safe use of nanomaterials: Recent achievements and technical challenges. Adv Mater *21*:1549–1559

Jen TC, Gutierrez G, Eapen S, Barber G, Zhao H, Szuba PS, Labataille J, Manjunathaiah J (2002) Investigation of heat pipe cooling in drilling applications: Part I: preliminary numerical analysis and verification. Int J Mach Tools Manuf *42*:643–652

Khandekar S, Sankar MR, Agnihotri V, Ramkumar J (2012) Nano-cutting fluid for enhancement of metal cutting perfomance. Mater Manuf Process *27*:963–967

Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF (1995) Stages of embryonic development of the zebrafish. Dev Dyn *203*:253–310

Kotnarowski A (2008) Influence of nanoadditives on lubricants tribological properties. Mater Sci *14*:366–370

Krajnik, P, Pusavec F, Rashid A (2011) Nanofluids: Properties, applications and sustainability aspects in materials processing technologies. Adv Sustain Manuf 107–113

Kusters KA, Pratsinis SE, Thoma SG, Smith DM (1993) Ultrasonic fragmentation of agglomerate powders. Chem Eng Sci *48*:4119–4127

Lademann J, Weigmann H, Rickmeyer C, Barthelmes H, Schaefer H, Mueller G, Sterry W (1999) Penetration of titanium dioxide microparticles in a sunscreen formulation into the horny layer and the follicular orifice. Skin Pharmacol Physiol *12*:247–256

Lee PH, Nam TS, Li C, Lee SW (2010) Environmentally-friendly nano-fluid minimum quantity lubrication (MQL) meso-scale grinding process using nano-diamond particles. In 2010 International Conference on Manufacturing Automation (ICMA) 44–49

Lieschke, GJ, Currie PD (2007) Animal models of human disease: Zebrafish swim into view. Nat Rev Genet *8*:353–367

LotfizadehDehkordi B, Ghadimi A, Metselaar HSC (2013) Box–Behnken experimental design for investigation of stability and thermal conductivity of TiO2 nanofluids. J Nanoparticle Res *15:1-9*

McCook NL, Boesl B, Burris DL, Sawyer WG (2006) Epoxy, ZnO, and PTFE nanocomposite:

Friction and wear optimization. Tribol Lett *22*:253–257

Mosleh M, Atnafu ND, Belk JH, Nobles OM (2009) Modification of sheet metal forming fluids with dispersed nanoparticles for improved lubrication. Wear *267*:1220–1225

NanoAmor (2013) *NanoAmor: Zinc Oxide, Titanium Oxide*, http://www.nanoamor.com/inc/sdetail/19983. Accessed 19 May 2014

Niyaghi F (2013) Stability and Biological Responses of Zinc Oxide Metalworking Nanofluids (ZnO MWnF™). Dissertation, Oregon State University

Niyaghi F, Haapala KR, Harper SL, Weismiller MC (2014) Stability and Biological Responses of Zinc Oxide Metalworking Nanofluids (ZnO MWnF™) using Dynamic Light Scattering and Zebrafish Assays. Tribol Trans *57*:730–739

Park KH, Shantanu J, Kwon P, Drazl LT, Do I (2010) Minimum quantity lubrication (MQL) with nanographene-enhanced lubricants: Ball-milling experiment. Trans NAMRISME *38*:81–88

Rakić R, Rakić Z (2002) The influence of the metal working fluids on machine tool failures. Wear *252*:438–444

Rapoport L, Fleischer N, Tenne R (2005) Applications of WS_2 (MoS_2) inorganic nanotubes and fullerene-like nanoparticles for solid lubrication and for structural nanocomposites. J Mater Chem *15*:1782–1788

Roy S, Amitava G (2013) High speed turning of AISI 4140 steel using nanofluid through twin jet SQL system. In Proceedings of ASME 2013 International Manufacturing Science and Engineering Conference, (Madison, Wisconsin), pp. MSEC2013–1067

Sahakian M (2011) Machining and toxicological performance of a Zinc Oxide metalworking nanofluid. Dissertation, Oregon State University

Sattelle DB (1988) Quasielastic laser light scattering and laser doppler electrophoresis as probes of synaptic and secretory terminal function. J Exp Biol *139*:233–252

Schey JA (1967) Purposes and attributes of metalworking lubricants. Lubr Eng *23*:193–198

Seyedmahmoudighomi S (2014) Sustainability Assessment during Early Product Development: The Manufacturing Case and the Use Case. Dissertation,Oregon State University

Shen B, Malshe AP, Kalita P, Shih A (2008) Performance of novel MoS_2 nanoparticles based grinding fluids in minimum quantity lubrication grinding. Trans NAMRISME *36*:357–365

Silverstein M, Park R, Marmor M, Maizlish N, Mirer F (1988) Mortality among bearing plant workers exposed to metalworking fluids and abrasives. J Occup Environ Med *30*:706–714

Simpson AT, Stear M, Groves AJ, Piney M, Bradley SD, Stagg S, Crook B (2003) Occupational exposure to metalworking fluid mist and sump fluid contaminants. Ann Occup Hyg *17*:17–30

Thomas K (2005) Research strategies for safety evaluation of nanomaterials, Part I: Evaluating the human health implications of exposure to nanoscale materials. Toxicol Sci *87*:316–321

Truong L, Harper SL, Tanguay RL (2011) Evaluation of embryotoxicity using the zebrafish model. Drug Saf Eval *691*:271–279

Turgut A, Tavman I, Chirtoc M, Schuchmann HP, Sauter C, Tavman S (2009) Thermal conductivity and viscosity measurements of water-based TiO_2 nanofluids. Int J Thermophys

30:1213–1226

Wang S. Clarens AF (2013) Analytical model of metalworking fluid penetration into the flank contact zone in orthogonal cutting. J Manuf Process *15*:41–50

Wang B, Feng W, Wang M, Wang T, Gu Y, Zhu M, Ouyang H, Shi J, Zhang F, Zhao Y, et al (2008) Acute toxicological impact of nano- and submicro-scaled zinc oxide powder on healthy adult mice. J Nanoparticle Res *10*:263–276

Weinert K, Inasaki I, Sutherland JW, Wakabayashi T (2004) Dry machining and minimum quantity lubrication. CIRP Ann - Manuf Technol *53:*511–537

Winter M, Bock R, Herrmann C, Stache H, Wichmann H, Bahadir M (2012) Technological evaluation of a novel glycerol based biocide-free metalworking fluid. J Clean Prod *35*:176–182

Wu J, Liu W, Xue C, Zhou S, Lan F, Bi L, Xu H, Yang X, Zeng FD (2009) Toxicity and penetration of TiO2 nanoparticles in hairless mice and porcine skin after subchronic dermal exposure Toxicol Lett *191*:1–8

Xia D, Quan P, Piao H, Sun S, Yin Y, Cui F (2010) Preparation of stable nitrendipine nanosuspensions using the precipitation–ultrasonication method for enhancement of dissolution and oral bioavailability. Eur J Pharm Sci *40*:325–334

Yang L, Du K (2012) Dispersion and thermal conductivity of Al_2O_3 and TiO_2 binary nanofluids. Key Eng Mater *531-532*:442–445

Zhao F, Urbance M, Skerlos SJ (2004) Mechanistic model of coaxial microfiltration for semi-synthetic metalworking fluid microemulsions. J Manuf Sci Eng *126*:435–444

Zon LI, Peterson RT (2005) In vivo drug discovery in the zebrafish. Nat Rev Drug Discov *4*:35–44