ZERO-VALENT METAL NANOMATERIALS IN WATERWAYS:

USING MICROPLASTICS AS A CASE STUDY

TO DEVELOP THE OVERDUE POLICY PLATFORM

by

Shanyese Trujillo

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Shanyese Trujillo

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by

Kathleen M. Saul
MA, MES

Date

ABSTRACT

Zero-Valent Metal Nanomaterials in Waterways:
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Shanyese Trujillo

Zero-Valent Metal Nanomaterials (nZVs) are growing contaminant of concern and using Microplastics as a case study demonstrates a need for regulatory measures for their introduction into waterways. The recent amendment to the Federal Food, Drug, and Cosmetic Act to ban the manufacturing of microbeads (spherical microplastics) serve as an example for framing legislations regarding the manufacturing of nZVs. It was important to identify a case study because there is minimal federal regulation for nZVs. In addition to describing how nZVs enter and interact in water systems, this research will offer a synthesis of current nZVs research regarding the extent of ecological risks within aquatic systems, lakes, and rivers, including impacts to aquatic and terrestrial species and present a summation of the gaps in the current research. Finally, in light of the risks that might be presented by nZVs, I will propose a set of recommendation for nZV legislation and describe any research that must be completed prior to the enactment of that legislation. Proposed recommendations for future policy that are put forth in this thesis include creating a tax to generate a financial pool for research, mandatory labeling of nZVs containing products, look at regulating point sources, and developing universal monitoring methodology and universal terminology.

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1. Introduction

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1.1 Introduction

Nanomaterial technologies (NMs), their use, and their development are increasing at an exponential rate since their creation in 1985 (Buckminsterfullerene, C_{60}). Due to the unique chemical properties, nanomaterials have become common components in medicine, electronics, cosmetics, nutrition, suntan lotion, and much more. Since 1994, they have been used to remove a variety of common contaminates (Table 1.1) from lakes, rivers, aquifers, groundwater, and soil (Duvall & Wyatt, 2011).

Zero valent nanomaterials (nZVs) possess a high “surface-to volume” ratio (Richardson & Ternes, 2014) that allows researchers to customize the nZVs surface coating to individual clean-up requirements (Duvall & Wyatt, 2011). The easiest way to define nZVs is metal or metal oxides. The materials possess a natural ability to reduce chemicals of halogenated nature (that is, to transfer electrons to them) or oxidize other types of pollutants (Temsah and Joner, 2012). The chemical processes involving nZVs will be discussed later; the main take away is nZVs can be chemically altered for site-specific clean up. While these materials hold promise as a less invasive method of remediation, a growing literature base reveals negative implications to the environment that were not identified prior to environmental introduction.

Ahamed (2014) explained that NMs enter the environment through several uptake pathways. Soils become contaminated through introduction and application of fertilizers, plant protection products, and liquid suspension from contaminated sites, or through the application of sludge and biosolids. This broad category would then cover groundwater contamination; wastewater effluents, industrial discharges and emissions; and waterways contaminated by combustion processes (i.e. volcanic eruptions) (Ahamed, 2014). Other studies have looked into the release of NMs from consumer products, plastics and textiles into wastewater treatment facilities (Westerhoff et al., 2009; Sinclair et al., 2007) and then into the environment.

Despite the growing use of NMs, policy regulating NMs and their effect on waterways and aquatic ecosystems is lagging. I propose that the recent amendment to the Federal Food, Drug, and Cosmetic Act to ban the manufacturing of microbeads (another tiny man-made material incorporated into consumer products that can enter the water supply, waterways, and the marine food chain) will serve as a warning and an excellent case study for the need for regulatory measures and could serve as an example for framing legislation regarding the manufacture of nZVs. By examining current environmental concerns raised because of nZVs entrance into aquatic ecosystems and waterways, identifying gaps in the research of nZVs, the existing waterway regulation policies of the United States, and identifying the similarities of microplastics concerns, I will demonstrate that microplastics are a viable and important case study for nZVs.

1.2 Methods

The methods that I adapted for my thesis come from previous Evergreen students (Allen, 2014; Bateman, 2011, Tilley, 2007) including those of my advisor, Kathleen Saul (2009). This review and analysis will outline, (1), discuss the nature of microplastics (2) examine currency policies regulating their introduction (3) identify current research of zero-valent metals affecting a variety of aquatic systems, (4) the current research gaps for zero-valent metals, and the (5) compare and contrast microplastics with nanomaterials (6) provide recommendations for future policy. The information outlined above will be used, hopefully, in the future to create a policy specifically for Washington State to monitor the introduction of nanomaterials into our waterways.

The information referenced in this thesis includes investigation into federal laws such as *The Toxics Substance Control Act*, the *Safe Drinking Water Act,* the *Clean Water Act,* the *National Nanotechnology Initiative.* Along with international laws like the *European Union (EU) Policy, Water Framework Directive, Directive on Environmental Quality Standards in the Field of Water Pollution, Directive on the protection of Groundwater Against Pollution and Deterioration, Drinking Water Directive,* and *Classification, Labelling and Packaging of Substances and Mixtures (CLP) Regulation.* This provides the basis for understanding the current policies in place for the regulation of nanomaterials in waterways. By examining technical journals like *Chemical Engineering, Nanotechnology, Nanoparticle Research* and many others, the impact of nanomaterials on a variety of environmental aspects was noted. *Science and Policy Report* and *Research Policy* provided analysis of current gaps in policies to inform readers what needs to be addressed in order to generate a successful monitoring policy in the future.

2.0 Microplastics

Microplastics (MPs) can serve as a major case study for the necessary regulation of nZVs. Microplastics have been located within every major open ocean, many freshwater lakes and rivers (Rochman et al., 2015). Carpenter and Smith identified the first small plastic fragment in the open ocean in 1972; Thompson et al. coined the term in 2004 in *Lost at sea: Where is all the Plastic*. These products are plastic fragments (or beads) made from synthetic polymers (polyethylene, polyactic acid, polypropylene, polystyrene, polyethylene terephthalate) roughly 5μm-1mm in size (Barboza and

Gimenez, 2015). Microplastics are found in everyday household items typically abrasive scrubbers like face washes or cleaning supplies, beads for cleaning boats, and as byproducts of macroplastic degradation (fragment from larger plastic debris through photothermal degradation, oxidation, and/or mechanical abrasion) (Barboza and Gimenez, 2015).

The degradation of MPs can occur through five major processes: biodegradation, photodegradation, thermooxidation, thermal degradation, or hydrolysis. A plastic particle can undergo a single or multiple degradation processes, each reducing the polymer's weight through chemical change. Biodegradation typically achieves this through the utilization of a living microbe, which breaks down a material through natural processes. Photodegradation uses light to break down the plastic material (Andrady, 2011). Thermooxidation and thermal oxidation are similar; the first breaks down material through slow, oxidative moderate temperatures while the latter involves high temperature degradation. The last process is hydrolysis, water's innate ability to solicit a reaction from a material (Andrady, 2011). Each process described above can produce different size particles, strands or fibers can influence the impacts these materials have with the environment.

The problem with MPs is not the sudden influx of particles into the waterways, but rather that they have been accumulating in the oceans for at least the last four decades (Andrady, 2011). The statistics are alarming: 80% of land-based litter contributes this plastic debris and 18% can be directly linked to the fishing industry (Andrady, 2011).Due to their small size, MPs easily flush down the drain where they will eventual be littered throughout the environment through final effluent or biosolids of Waste Water Treatment

Plants (WWTPs) used for landfilling, fertilizer, and surface runoff. Inland sources can include WWTPs, runoff from urban, agricultural, touristic, industrial, and shipping.

The exact amount of contamination is unknown, but Rochman et al. (2015) made a conservative estimate of the total contamination per day. During the WWTPs process, 95-99.9% of the microplastics will settle out into the sludge, leaving 0-7 individual microbeads residing within the final effluent. WWTPs in the United States treat >160 trillion liters of water every day. Assuming all WWTPs operate at half-capacity and 0.1 individual microbeads are found per liter of effluent, 8 trillion microbeads per day could be emitted into aquatic habitats. Emitted 100µm plastic spheres would cover over 300 tennis courts daily.

Upon entry into waterways, MPs are readily available for uptake by plankton, invertebrates (molluscs, polychaetes, crustaceans, echinoderms), fish, birds, mammals, and and then accumulation in the food web (Wagner et al., 2014; Moos et al., 2012; Bhattacharya et al. 2012; Thompson et al., 2004; Graham and Thompson et al., 2009; Teiten et al., 2009; Murray and Cowie, 2011). MPs can travel one of three avenues within waterways: 1) accumulate in gyres at the water's surface; 2) sink to the sea bed as a result of waterlogging or surface fouling that ultimately ends in colonization and accumulation in sediments (may wash ashore); 3) buoyant plastics end can be transmitted through winds or currents for long distances (Moos et al., 2012). Buoyant plastics often sit at the microlayer of the sea-surface "…where hydrophobic compounds can be concentrated 500 times that of the underlying water column" (Teuten et al., 2007). Often these plastics are fouled (by hydrophobic chemical contaminants), allowing them to transport contaminates to other locations and deposit them. Serious issues arise during turbulent weather events that can re-suspend MPs that have settled on the seafloor. A combination of wave action and UV radiation can result in the degradation of large plastic debris, compounding the already large MPs problems (Moos et al., 2012; Wegner et al., 2012).

A study at Plymouth, United Kingdom, collected sediment samples from beaches, estuarine, and subtidal areas to test for the presence of synthetic polymers; 23 of 30 samples revealed the presence of synthetic polymers. Findings were at 17 other beaches (Thompson et al., 2004). This MP problem is not new: researchers inspected plankton samples dating back to 1960 and found significant increase over time in amount of plastic identified within each sample (Thompson et al., 2004). In 1962, Edward et al. examined polystyrene spherules found throughout the water column, sea surface, and they presumed in the sediments, and noticed eight of 14 species of fish and a Chaetognatha had ingested the plastic spherules. They also noted the potential for intestinal blockage for small fish. In addition, in 1972, Carpenter, Smith, and Smith Jr. identified an average of 3500 pieces and 290 grams per sq. kilometer of MPs throughout the Sargasso Sea. These researchers cautioned that the increase in plastic production and the poor waste management would lead to ever-increasing MPs into the ocean. In addition, the MPs throughout this area possessed surfaces for diatom and hydroid attachment and thus the possibility of introduction polychlorinated biphenyls into the aquatic environment. Even plastics deemed biodegradable leave behind copious non-degradable plastic fragments (Thompson et al., 2004).

To date more than 260 species have been documented as having ingested or become entangled by plastic (Teuten et al., 2007); 44% of known marine birds have ingested plastic (Andrady, 2011). MPs’ impacts on several species including zooplankton, lugworm, blue mussel, and sand hopper have been studied. The particles can obstruct feeding appendages, aggregate, block the alimentary canal, limit the food intake of an organism, and translocate into circulatory system (Cole et al., 2013). For example, *Daphnia magna*, simply referred to as a water flea have been observed ingesting MPs, which cross the gut epithelium and accumulate in the lipid storage (Wagner et al., 2014). As a result, the accumulation within the organisms fats opens up the opportunity for a decline in enzyme production, "…permanent cellular and tissue damage, particularly in the brain, peripheral nervous system, spleen, and bone marrow" (NINDS, 2016) and raises potential concern for passage of MPs onto higher trophic level predators. In addition, MPs "can act as a vector for water borne pathogens" which will ultimately influence the water quality (Wagner et al., 2014). Any potential impact on water quality should be investigated, especially since preliminary studies are indicating there is an ability for microorganisms generate MPs films surrounding themselves while several human pathogens (such as *Vibri)* are attaching to MPs for transport (Wagner et al., 2014). MPs possess the ability to accumulate contaminates such as metals and toxic compounds in conjunction with plastics innate ability to leach EDCs increasing their heavy metal load and serve as carriers of terrestrial contaminants into waterways (Wagner et al., 2014). The following review of the literature demonstrates the known implications of MPs within waterways on aquatic species; a later comparison will reveal shockingly similar results for NMs within waterways.

2.1 Sediment Interactions

During a study of vertical sediment, researchers' analyzed cores from the beaches of Hawaii for the presence of plastic debris. The plastic debris was found in nearly all samples, increasing the permeability of the sediment but decreasing the sediment's ability to absorb heat (Carson et al., 2011). By lowering the maximum temperature of the sediment, marine biota could be affected (i.e. sex determination in turtle eggs). Likewise, increased permeability could lead to desiccation of sediment dwellers (Carson et al., 2011).

2.2 Interactions with Aquatic Species

2.2.1 Chlorella and Scendesmus

Bhattacharya et al. (2012) noted that New Zealand beaches were identified to have 100,000 plastic granules per meter of coastal zone (Bhattacharya et al., 2012). Moreover, beaches in the South Pacific Islands were found to be at comparable levels to industrialized areas in regards to MPs quantity. Researchers removed all plastic from the beach to determine the rate of plastic deposition. At the experimentally cleared beaches of Panama, 50% of the original plastic in 3 months was regained (Bhattacharya et al., 2012).

Twenty nanometer polystyrene beads hindered photosynthesis and promoted a reactive oxygen species (ROS) within the Chlorella and Scendesmus (Bhattacharya et al., 2012). ROS is a natural side product of oxygen metabolism, utilized for cell signaling and maintain cellular homeostasis. Often because of the electron transport chain within the chloroplasts, O_2 diverts electrons instead of CO_2 during stress events, resulting in the decline of photosynthetic efficiency (Bhattacharya et al., 2012).

2.2.2 Zooplankton

Another aquatic organism study focused on zooplankton due to their vital role within the food web, their ability to use both chemo- and mechanoreceptors to select their prey, and their preferentially ability to select one prey over another (Cole et al. 2013). To test impact of the collected MPs on zooplankton, five zooplankton taxa (15 species in total) were selected and exposed to polystyrene MPs. Of the 15 exposed species, 13 ingested the microplastics. Often found adhering to the copepod, decapod larvae, and euphausid extremities were the MPs. The copepods that died during MPs exposure were found to possess carapace's (hard upper shell of the copepod) coated in MPs, remaining on the carapace once shed (Cole et al. 2013). Of living zooplankton, the MPs adhered to swimming legs, feeding apparatus, antennae and furca. Overall, MPs adhering to the copepods extremities may pose a threat to key functions such as locomotion, ingestion, mating, mechanopreception, reproduction, foraging behavior, and predator evasion (Cole et al. 2013).

Because of the presence of MPs on the zooplankton, the filament hairs and setae (of antennules, furca, and swimming legs), the organisms had trouble with movement, ingestion, mating, and mechanoreceptor. Decrease of the mechanoreceptor leads to an impact on the ability for zooplankton to find food, feed, reproduce, and survive. There is potential for the faecal pellets (particle excretions by gastropods, annelids, and crustaceans) to change in density and structure altering the vertical carbon flux, increasing or decreasing rate of deep sediment burial and the rate of mineral release and retention. Due to the varied plastic shapes identified during sampling (i.e. fibers, granules, and fragments), the shapes and structure can tangle the intestinal tract, ultimately leading to organism death if the intestine is completely strangled (WEB MD, 2016). Of great concern is the potential for MP to transfer contaminants up the food chain as a result of ingestion and storage of MPs leading to possible "…bioaccumulation and therefore adverse health consequences in higher trophic organisms" (Cole et al., 2013).

2.2.3 Dispastrea--Scleractinian Corals

Coral reefs are unique ecosystems now becoming inundated with MPs: larger plastics fragment into MPs and lodge within the reefs (Hall et al., 2015). Coral reefs provide vital habitat for a variety of species and attract tourism, recreational vessels, and fishing trawlers (Hall et al., 2015). Unfortunately, the MPs are the same size as both sand grains and planktonic organisms as well as the organisms ingested by corals. This 2015 study is the first to examine MPs ingestion by corals.

Corals form clustered colonies of calcium carbonate skeleton; each cluster is a polyp made of genetically identical organisms. The coral possess a symbiotic relationship with S*ymbiodinium spp.,* commonly known as Dinoflagellate, which produces photosynthetic carbon; however, coral are still heterotrophs (Hall et al., 2015). That means the coral will feed on mesozooplankton for approximately 50% of their daily carbon (Hall et al., 2015). *Scleratinian corals* have been documented to prefer a diet of pico-and nanoplankton typically feeding on particles within 10-100µm.

Hall et al. (2015) examined the Great Barrier Reef to assess whether MPs were present in its structure (Hall et al., 2015). They found the MPs localized within the polyps of the coral and wrapped within the mesenterial tissue, increasing considerably the possibility that coral digestion would be interrupted (Hall et al., 2015). The outer surface of the coral receives an aggregate film of MPs—likely adhering to the coral's mucus layer. In sum, the evidence suggests that corals will ingest and house the MPs for 24 hours, but the effects of MPs on the corals' energetics and growth are unknown (Hall et al., 2015).

2.2.4 Talitrus saltator—Sand hopper

The sand hopper is an amphipod crustacean commonly found on sandy beaches, dwelling under rocks and among seaweed. Its typical movement gave the species its name: hopping by extending the abdomen. Ugolini et al. (2013) tested the sand hopper's ability to ingest microspheres mixed with their typical diet. The researchers found the sand hoppers were not selective about microsphere size and consumed all spheres in addition to the natural food. The plastic was expelled within 24 hours of ingestion. The microspheres did not kill the sand hoppers but long-term effects still need to be studied (Ugolini et al., 2013).

2.2.5 *Tigriopus japonicas—Copepod*

Copepod is a general term used to describe a group of crustaceans found commonly in all freshwater habitats and the sea. Copepods are filter-feeding omnivores that transport energy and pollutants across the food chain (Lee et al., 2013). Due to the vast numbers and dual-feeding capabilities, they are commonly used as biodiversity indicators and are considered suitable "for assessing environmental risk in W. Pacific coastal regions" (Lee et al., 2013). Lee et al. studied copepod survival, development, and fecundity noting both adult and nauplius (first larval stage of crustaceans) ingested MPs even when MPs were presented alongside phytoplankton. The two largest sizes of MP's significantly decreased fecundity at all concentrations tested. The findings of most interest were those closest to the nanoplastic range, an area about which very little is known:

> *…0.05-µm PS beads at a concentration greater than 12.5 µg/mL caused the mortality of nauplii and copepodites in the F0 generation and even triggered mortality at a concentration of 1.25 µg/mL in the next generation. In the 0.5-µm PS bead treatment, despite there being no significant effect on the F0 generation, the highest concentration (25 µg/mL) induced a significant decrease in survival compared with the control population in the F1 generation (Lee et al., 11278).*

These findings reveal that nanoplastics of a size similar to that of NMs may have serious impacts on marine organisms.

2.2.6 Arenicola Marina—Lugworm

The buoyancy of plastic allows for easy transportation via wind and storm events in which, it is frequently distributing along coastlines around the globe. These particles wash ashore, and due to their bright colors and resemblance to natural prey for a variety of species cause negative effects on marine organisms (i.e. birds, mammals, and turtles). This transfer of microplastics occurs along all rungs of the food chain, but it is unclear at which particle size the transfer will occur and the effects it will have. A number of studies have documented the uptake of MPs by lugworms, mussels, amphipods, barnacles, sea cucumbers, and fish. Besseling et al. (2012) focused on identifying how MPs were taken up by marine (epi)benthic organisms and the effects of MPs on their survival, growth, activity, and Polychlorinated biphenyls (PCB) concentrations (Besseling et al., 2012).

The species selected for the Besseling study was *Arenicola Marina*, a large marine worm that is a significant North Sea deposit feeder at the base of the food web (Besseling et al., 2012). The study revealed a positive relationship between the concentration of MPs within the sediment and, both, the lugworm's ability to uptake plastic particles and weight loss—revealing a decrease in organic matter content

(Besseling et al., 2012). Organic matter is the soils nutrient supply, the soil's structure, ability for water-holding capacity, and erosion prevention, it is the health of the soil (Funderburg, 2001). A decrease in organic matter content can result from the lugworms need to process more sediment to meet nutritional demand, process the larger MPs, or may signal a reduction in energy assimilation (demonstrating loss of energy assimilation) (Besseling et al., 2012). As a direct result of having to process these plastic particles, the lugworm's system is placed under stress causing weight loss; excess processing decreases the overall soil health. All three may decrease the longevity of the lugworm population, since growing numbers of MPs impacts food availability. An additional concern of MPs consumption is the aspect of bioaccumulation which was noted that worms retained more MPs lower concentrations of MPs does while at higher concentrations bioaccumulation decreased-this ingestion of MPs presents exposure pathways for predators (Besseling et al., 2012).

2.2.7 Echinodermata—Sea Cucumbers

Known facts about plastic fragments include their ability to accumulate biofilms (bacterial layer on the surface), increase their density, and sink, and then mix with sediments. These properties have important implications for deposit and suspension feeders, as demonstrated in a study conducted by Graham and Thompson (2009) to understand the effects of MPs (specifically nylon and polyvinyl chlorides shavings/pellets) on four species of sea cucumbers . Unfortunately, all species exhibited ingestion of plastic fragments for two of the three types (nylon and shavings) of plastics (Graham and Thompson, 2009). The cucumbers also preferred ingesting plastic to sand grains; the particle size did not influence the desire for ingestion. Through their research, Graham and Thompson concluded that MPs residing in sediment around the globe are an exposure pathway for benthic marine invertebrates and MPs will readily adsorb PCBs and other organic pollutants for additional exposure (Graham and Thompson, 2009).

2.2.8 Mytilus edulis L. –Blue Mussel

MPs have been identified to be ingested by marine invertebrates such as polycheates, crustaceans' echinoderms, bryozoans, bivalves, as such; Moos et al. focused on *Mytilus edulis L.,* commonly known as the blue mussel. During blue mussel exposure to MPs, the authors identified two uptake pathways. First, microvilli particles transfer MPs to the gills via endocytosis. The small particles were found in the blood lacunae of the gills and the larger particles on the gills surface. The second uptake pathway is ciliae movement— in this instance particles travel to the stomach intestine through primary and secondary tubules. Particles identified in the intestines were numerous aggregate particles specifically located in the lumica of the primary and secondary ducts and the tubules. The findings of this study suggested that mussels formed granulocytes in the connective surrounding the ducts and tubules after only 96 hours of exposure to MPs. This consequently led to a decrease in lysosomal membrane stability. A destabilization in the lysosomal membrane can increase autophagy; release of hydrolases into the mussels surrounding tissue leading to necrotic processes (Moos et al., 2012). Simply put, MPs lead to unregulated cell death within the organism.

Wegner et al. (2012) completed a second study of the blue mussel, recognizing the significance of this mussel as a filter feeder and prey to numerous intertidal species and humans across a wide geographic range. This Wegner et al. study aimed to identify the effects of nano-MPs uptake on blue mussel feeding behaviors. The findings revealed that for all treatments containing nano-MPs, the mussels produced pseudofeces (suspended particles considered unnecessary by the organism); filtering activity was reduced in the presence of nano-MPs-- MPs were present in the mussels' foot. Future studies must focus on the chronic effects of MPs to understand the longer-term effects and consequences.

2.2.9 Mytilus edulis vs Symphodus melops

One single tank on board a bulk chemical tanker can hold up to 3,000 metric tons of chemicals while the ship itself can house over 40,000 metric tons of chemicals (Mamaca et al., 2005). Converting these figures to pounds, a single tank will hold over 6.5 million pounds while the ship will house over 88 million pounds. Nanoparticle weight determined through viscosity and light scattering of ($8.1\ x10^{-19}$g) and nanoparticle weight through reference methods ($8.6x10^{-19}$g) the average weight for an example nanoparticle of amino-CLIO which contains an iron core is $8.35x10^{-19}$g. If we go by the reference, weight of $8.1x10^{-19}$ grams converting to metric tons $8.6x10^{-6}$ and finally to pounds 0.019pounds for a single amino-CLIO (Reynolds et al., 2005). Therefore, the ship would contain 4,631,578,947.37, or four billion, six hundred thirty one million, five hundred seventy eight thousand, nine hundred forty seven single amino-CLIO.

Because of the constant shipping of chemicals via boats and barges along numerous coastal routes, spills are inevitable. Mamaca et al. (2005) sought to understand the environmental effects of a spill within a small amount of time and in turn evaluate the marine fauna. Styrene was the chemical selected for the spill simulation because it is considered a chemical of no concern in regards to chronic toxicity in aquatic environments (low bioaccumulation, readily volatized, and biodegradable) (Mamaca et al., 2005).

The findings revealed no mortality; however, there was DNA damage in haemocytes, blood cells, and the stability of the lysosomal membrane was altered in both species (Mamaca et al., 2005). These observations serve as indicators of impaired immunocompetence and cell injury. The greatest DNA breaks occurred in mussels. This revealed that mussel's bioaccumulate a greater quantity of MPs than the fish. Likely due to the fish/crustacean's ability to biotransform organic xenobiotic elements and excretes more polar metabolites (Mamaca et al., 2005). Simply put the MPs are expelled quicker from fish and are less likely to damage. This information also indicates that bioaccumulation does not accurately reflect the uptake of fish or crustaceans due to their biotransformative nature. The metabolized pollutant likely produced a byproduct that deleted some of the cells or structural macromolecules leading to strands breaking or covalent bindings of radicals to the mussels DNA (Mamaca et al., 2005).

2.2.10 Trophic Level Transfer Mytilus edulis (L.) to Carcinus maenas (L.)

Farrell and Nelson (2013) recognized that MPs have been documented to be taken up through "…the gastrointestinal epithelium of rodents into the lymphatics systems, showing cellular damage and thrombosis …" (clotting of the blood) but failed to investigate the ability of MPs to transfer between trophic levels. In the study, the authors selected the blue mussel because it serves as the shore crab's food source. Overall, the findings suggest further studies are needed about implications to the food web at large. The MPs were found in crab tissue samples (including the stomach, hepatopancreas, ovary, and gills), with the greatest concentration in the gut (Farrell and Nelson 2013).

There were MPs present in the haemolymph tissue samples revealing there is a small transference, which translocated to the haemolymph and tissue of the Shore crab (Farrell and Nelson 2013). However, by day 21 no MPs were found in either samples.

2.2.11 Daphnia magna—Planktonic Crustacean

Lithner, Nordensvan, and Dave (2011) sought to assess the chemical hazards and risks associated with the presence of plastics in waterways. Through the examination of five plastic products, they identified the toxicity compound classes, while simultaneously determining the toxicity and comparing the toxicity of the leachates. They found 42% of the leachates, all Polyvinyl chloride (PVC) leachates, and 20% of High-density polyethylene (HDPE) leachates were toxic while none of the polypropylene fell into that category, Both Acrylonitrile butadiene styrene (ABS) and rigid PVC showed toxicity. The chemicals leached from plastics even during the short-term (24-72 hour) testing of plasticized PVC and epoxy products were mainly as result of the hydrophobic organics and metals (Lithner, Nordensvan, and Dave, 2011).

Global plastic production has doubled in 15 years; plastic production was at 245 million tons in 2008 (Lithner, Nordensvan, and Dave, 2011). Plastic products are of concern not solely for their degradation potential but also for the additives used to give plastic polymers certain properties for their designated application (Lithner, Nordensvan, and Dave, 2011). The research of Lithner, Nordensvan, and Dave had indicated that those additives could leach into the marine environment and have the potential for causing further harm.

2.3 Future Concerns

2.3.1 Seafood Industry

Oceans and sea are sources of raw materials, biomaterials, and potential energy; however, pollution has increasingly invaded these areas in the form of chemical substances. Chemicals enter water systems through rivers, direct discharge and/or atmospheric deposition with each material providing potential health hazards (Vandermeersch et al., 2015). Seafood is an important food commodity, consumed worldwide. Chemicals in the waterways may change their chemical form or fail to degrade over time (persist or bioaccumulate) (Vandermeersch et al., 2015).Once MPs enter waterways they are readily available to adhere to organism or be ingested by them.

Numerous toxic chemicals have been shown to accumulate within tissues (i.e. heavy metals) of some shellfish. The fishing industry regulates[1] for toxins in an organism's edible tissues, but does not consider any additional tissues that may house the majority of chemicals of concern (Vandermeersch et al., 2015). This leads to the growing concern that MPs will become the next toxin, as more studies reveal that MPs enter the marine food chain in a manner similar to that of chemicals (Vandermeersch et al., 2015). Additionally, studies are showing MPs accumulate in higher trophic levels, as in the littoral crab after feeding on MPs exposed mussels. Unfortunately, there are no studies reporting on in vivo or in vitro toxicological studies. However, early predications are being made that MPs enter the body and are stored. These stored MPs exist within the gut, leaving potential for leachable additives to harm the organism. Alternatively, MPs

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[1] These authors looked at European Industry regulations not United States

may adhere to persistent contaminants within the waterway during transport and serve as another introduction mechanism into an organism (Vandermeersch et al., 2015).

There is a long way to go before the issue of MPs is rectified. To begin to achieve this end all goal, we should be monitoring microplastics presence, investigating source and environmental fate (assessment) of MPs exposure to species, evaluate biological effects of MPs exposure, understand interaction between MPs, and other contaminates, risk assessment of MPs (Wagner et al, 2014). Before moving on to the next selection the following page contains a table that summarizes all previously discussed studies with key findings for a quick summation of this section.

Table 1. *This table provides a summary of the previously discussed Microplastics studies containing the title, organism studied, and the key findings from each.*

Study	**Organism**	**Key Findings**
Bhattacharya et al. (2012)	*Chlorella and Scendesmus*	• Remote Beaches containing as much MPs as industry land • After removal, 50% of MPs returned in 3 months • Decline in photosynthetic activity
Cole et al. (2013)	*Zooplankton*	• Adhered to filament hairs and setae (of antennules, furca, and swimming legs) • Impacted movement, ingestion, mating, and mechanoreceptor • Alter vertical carbon flux
Hall et al. (2015)	Dispastrea--Scleractinian Corals	• MPs localized within the polyps of the coral • Wrapped within the mesenterial tissue • Thought to impede ingestion • Adhere to coral surface • Long term impacts unknown
Ugolini et al. (2013)	*Talitrus saltator—Sand hopper*	• Ingested MPs with regular food • Expelled • Unknown long term effects
Lee et al. (2013)	*Tigriopus japonicas—Copepod*	• Significantly decreased fecundity • Smallest MPs caused mortality • Similar in size to NMs
Besseling et al. (2012)	*Arenicola Marina—Lugworm*	• Weight loss • Decreased organic matter content • Bioaccumulation potential • Decreased soil health
Graham and Thompson (2009)	*Echinodermata—Sea Cucumbers*	• Preferred ingestion of MPs to traditional food source • MPs readily adsorb PCBs and other organic pollutants • New exposure pathway
Moos et al. (2012) Wegner et al. (2012)	*Mytilus edulis L. – Blue Mussel*	• MPs attached to gills and stomach • Altered lysosomal membrane • Cell Death • Reduced Filtering Activity
Mamaca et al. (2005)	*Mytilus edulis vs Symphodus melops*	• DNA damage in haemocytes, blood cells • Lysosomal membrane altered
Farrell and Nelson (2013)	*Mytilus edulis (L.) to Carcinus maenas (L*	• Trophic level transfer occurs • Found in the stomach, hepatopancreas, ovary, and gills • MPs exit system
Lithner, Nordensvan, and Dave (2011)	*Daphnia magna—* Planktonic Crustacean	• Chemical additives in MPs have potential to leach into marine environments • Several known leachates are toxic

2.5 Microbead Ban: A Case Study for Policy Implementation

Halden (2012) produced a paper that examined emerging contaminates of concern, two of which were MPs and NMs. Specifically, Halden noted that a topics publishing activity could serve as a method of tracking and quantifying a chemicals "concern" (meaning "…interest, importance, or concern) (Halden, 2012). The author selected twelve case studies to analyze. These were selected based on three components: 1) possess emergence history over four decades 2) public health importance 3) possess spectrum of chemical compositions/properties (Halden, 2012). Halden revealed that published activity (scientific literature) on Nanomaterials (NMs) will peak no later than 2016 while that regarding MPs is expected to peak around 2022. Current laws regulating MPs only exist at the state level (i.e. California, Washington, and New York) while in 2013 NMs in 2013 were placed under Significant New Use Rule (SNUR) to gather more information and Engineered Nanomaterial (ENMs) manufactures were asked to notify the federal government of materials being used. No Chemical Abstracts Service (CAS) registry numbers were available for NMs or MPs (Halden, 2012). This projection serves for potential projection of scientific developments based on predicted chemicals of emerging concern.

One such development needing to occur resides in the realm of politics, more specifically the aspect of legislation. Current MPs law leaves plenty of room for error--it does not cover all sources of microbeads, microbeads that can be sent down the drain through products not defined as "personal care" or "rinse off" products (Rochman et. al, 2015). Nor are the terms of "plastic" and "biodegradeable" clearly defined. In the Illinois statute for example, plastic has been defined as "something that retains its shape during its life cycle and after disposal" allowing for companies to create a bead that only slightly degrades and still be compliant with this law (Rochman et al., 2015). Even the law's definition of "biodegradable" does not explicitly state full degradation as a requirement; it only requires slight degradation within a year (Rochman et al., 2015).

Microplastics, serve as an excellent case study for Zero-Valent Metal Nanomaterials as their method of introduction into waterways is the same, their impacts on aquatic systems is the same, leading to concern for the longevity of the food chain, and in fact, these nanomaterials, specifically nZVs are in far more products than microplastics. By analyzing the current MPs law, we are establishing a starting point for nZVs regulation as none currently exists. The similarities between these technologies allows for their comparison and demonstrates a need for policy to be implemented.

3.0 Nanomaterials

3.1 Nanomaterial Definition

Currently, there is no comprehensive set of criteria accepted by the scientific criteria accepted by the scientific community as to what constitutes nanomaterials (NMs). Some definitions only reference size while others look to make a more specific targeted definition. For example, Lu et al. (2012), discuss the novel properties (such as change in melting point or explosivity) possessed by NMs, while Hendren et al. (2015) attribute their novel properties to their small size.

In an attempt to create a cohesive definition of these particles, the Joint Research Centre of the European Commission developed the following working definition,

> *'Nanomaterial' means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size*

> *range 1 nm- 100 nm. In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50 % may be replaced by a threshold between 1 and 50 %. The Recommendation further specifies: By derogation [...], fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials. [...] 'particle', 'agglomerate' and 'aggregate' are defined as follows: (a) 'particle' means a minute piece of matter with defined physical boundaries; (b) 'agglomerate' means a collection of weakly bound particles or aggregates where the resulting external surface area is similar to the sum of the surface areas of the individual components; (c) 'aggregate' means a particle comprising of strongly bound or fused particles. Where technically feasible and requested in specific legislation, compliance with the definition [...] may be determined on the basis of the specific surface area by volume. A material should be considered as falling under the definition [...] where the specific surface area by volume of the material is greater than 60 m2/cm3. However, a material which, based on its number size distribution, is a nanomaterial should be considered as complying with the definition [...] even if the material has a specific surface area lower than 60 m2 /cm 3. (Rauscher,p.15)*

The Commission developed this definition in the first of a series of three papers that concluded with several recommendations for improving the future development of nanotechnology. They generated prioritization processes and are continuing to improve the definition as information is gathered. However, adoption of the NNI definition can be achieved through the passing of legislation making the definition legally binding (Bleeker et al., 2013). The European Union (EU) definition of NMs incorporates natural nanoparticles, incidental generation of nanomaterial (human activity), and their intentional introduction into the environment. Other regulations in place for the EU that do define nanomaterials include:

- Cosmetics (EC No 1223/3009;EU, 2009c): intentionally manufactured insoluble or bio-persistent materials with one dimension 1 to 100nm (Bleeker et al., 2013).

- Food (EU, 2011b; EU, 2011c; 2011d): intentionally manufactured material made of discrete functional parts with one dimension 1 to 100 nm in size, this definition does include aggregates and agglomerates that are above 100 nm but still possess novel nano-properties(Bleeker et al., 2013).

- Biocidal: Member State can ask the Commission in accordance with Article 81 (3) with Recommendation 2011/696 whether a substance is a nanomaterial (Bleeker et al., 2013).

Several common themes appear in the above definitions of nanomaterials that would be appropriate for a universal definition:

- Can be intentionally manufactured or naturally occurring,
- Possess novel properties (vary from bulk form), and
- At least one dimension is 1-100 nm.

This will be the definition of NMs used throughout this paper unless otherwise stated. Scientists traditionally categorize NMs into four groups, *Carbon Nanotubes (CNTs), Quantum Dots, Zero-Valent Metals (nZVs),* and *Dendrimers.* Preliminary research for this thesis indicates that nZVs would provide the largest academic set of studies, allowing for a more thorough analysis. This categorization is a further refinement of the definition but this refinement will be discussed in the next section.

4.0 Zero-Valent Metals

4.1 Zero Valent Metal Definition

Zero-valent metals (nZVs) have been studied and utilized the most in research, and thus provide a fair representation of available information, data, and policies on nanomaterials. nZVs can be characterized by their natural ability to reduce chemicals of halogenated nature or oxidize other types of pollutants. nZVs have been successfully used in remediating polycyclic aromatic hydrocarbons, halogenated organic compounds, pesticides, metalloids, and heavy metals (El-Temsah and Joner, 2012). nZVs have the ability to immobilize, destroy, or transform the particulate nature of pollutants in water. For example, zero-valent metal iron (nZVI) can be used to transform chlorinated methane from wastewater treatment facilities to reduce the tetrachlormethane and trichloromethane.

nZVs can be customized during manufacturing to meet specific requirements, this could be to increase the melting point or improve durability (Salieri et al, 2015) In the laboratory setting, when nZVs are altered for a specific intent they become what are known as engineered nanoparticles (or nanomaterials) (ENPs/ENMs). ENPs used in water remediation include zinc oxide, titanium dioxide, cerium dioxide, chromium dioxide, and many more (Salieri et al., 2015). These materials can either adhere to the contaminates and neutralize the chemical or interact and transformed into another chemical. The nZVs that are filled with contaminants are assumed by scientists to become immobilized, as it is considered an inorganic material. Unfortunately, this line of reasoning does not take into account weathering, exchange between additionally compounds etc. ENPs can be enhanced further with a metallic coating that allows for contaminant interaction (Keane, 2009); this characteristic makes them vital for environmental remediation efforts.

4.2 Current Zero-Valent Metal Research

4.2.1 Issues in Studying Nanomaterials

Klaine et al. (2008) examined the available literature to identify studies completed on the concentration of NMs in sediment or natural waterways; there was no available literature. The problems with being able to fine NMs in the waterways were identified by Klaine et al., such as "…characterizing environmental behavior, fate, and bioavailability," sharing of data from nZV manufacturers, and generating standardized test media, are still relevant today as studies continue to identify monitoring nZVs as an issue. Researchers continue to seek answers, in differentiating naturally occurring nZVs with those that were manufactured, generating technology that can monitor these particles outside of the lab, and tracking the life of these particles. To understand the difficulties these researchers face look at the following. As an example, consider the naturally occurring element nano-Fe. Iron can be identified in either dissolved or a solid phase under natural conditions. If one was to introduce FeO^{2}_{nZV} (nZVI) into a waterway, researchers could not distinguish the naturally dissolved iron phase and the engineered iron oxide (Klaine et al., 2008). There are several studies (Aruoja et al., 2009; (Zhu, Tian, and Cai, 2012) discussed later that compare the impacts of bulk and nZV on aquatic organisms.

Part of what causes researchers difficulty in their ability to differentiate the natural and engineered nZV is its colloidal nature. Briefly, colloids consist of insoluble molecules that will not mix with a surrounding liquid. This trait (small size, great surface area, and similarly conformational behavior) makes tracing nZVs difficult (Klaine et al., 2008). Researchers cannot get an accurate representation within waterways of the concentration (current amount) of nZV's in the waterway because nZVs will readily interact with natural colloids in which two typical outcomes are expected. The colloids with nZVs will aggregate and settle out typically in the sediment or will interact with one another changing the particles behavior into something else altogether. Toxicologists who generate models and risk assessments of NMs need to be able to predict the contaminant's soluble portion. This is impossible if they cannot estimate the concentration of NMs in sediment or natural waterways.

4.2.2 Marine System Studies

Marine environments generally have increased ionic strength, alkalinity, and contain a variety of organic matter and colloids (Klaine et al., 2008). These environmental traits are potentially susceptible to detrimental change because of introduce nZVs, which can infiltrate into marine systems through coastal runoff, chemical spills, or through intentional introduction during remediation efforts.

These nZVs are subject to geochemical processes presenting the potential for global dispersion of particles (Klaine et al., 2008). For example, nano-Fe present in fresh water run-off that coincides with coastal erosion can move freely throughout the oceanic water column. This allows free interaction between the nano-Fe and marine organisms through ingestion, uptake or contamination (Klaine et al., 2008). The rate at which aggregated NMs settle to the seabed (known due to aggregation and colloid chemistry) or how they react with varying temperature currents has yet to be determined (Klaine et al., 2008). The concern arises to organisms that reside at the pelagic zone because these areas of accumulation are their feeding areas and the deposition may affect benthic species.

Beyond ocean currents, the properties of oceanic microlayer[2] result in potential detrimental reactions with nZVs upon their introductions. nZVs can become trapped on the ocean's surface where they can transform through aerosol exposure, these aerosolized compounds are than readily available to marine birds, mammals, and organisms residing in the microlayer. The surface layer can be exposed to photochemical interactions that are not thoroughly understood and believed to vary widely among nZVs (Klaine et al., 2008). Typically, nZVs are primarily made of metals; their behavioral tendencies are not well understood. Researchers believe nZVs react similarly to metal colloids, which adsorb or absorb into smaller colloids, resulting in a transfer from colloids in the water column to sediments (Klaine et al., 2008). nZVs have the potential to adhere to sediment particulates allowing transport to exit waterways.

4.2.3 Freshwater System Studies

Only two studies Eckleman et al. (2012) and Walser et al., 2011 demonstrated the capacity to calculate the Characterization Factor of Engineered Nanoparticles (ENPs) (Salieri et al., 2015). The characterization factor is an important ecotoxicological freshwater formula that expresses the toxicity of a substance in terms of potentially affected fraction of species, resident time in a particular environment, and exposure to humans and other species. Because nZVs do not possess these traits, the generation of life cycle models and assessments for the introduction of nZVs into waterways cannot be determined. These models are typically used, but for nZVs they do not apply, thus new models need to be generated. nZVs do not have a model assessment.

The globally accepted USEtox framework, which was developed as a result of the UNEP/SETAC Life Cycle Initiative to "…provide a scientific and technical rationale to the comparative assessment of chemicals based on their impacts on human health and on ecosystems…" (USEtox, 2016) was applied to released nano-titanium oxide in areas of freshwater ecotoxicity to determine whether that framework is sufficient for modeling nZVs (Salieri et al., 2015). Due to the lack of solubility of nZVs, the assumption was made that nZVs entering into waterways would be completely bio-unavailable. This research revealed high levels of variability in the toxin data, requiring a model of greater reliability. This study demonstrated the proposed USEtox framework could applied to nZVs.

Oliver et al. completed a key study in 2014 that examined the dietary uptake and toxicity of silver nZV (nZVS) by *Lymaea stagnalis* (a variety of freshwater snail). The principal purpose of the study was to examine the potential for dissolution of nZVS in the presence of oxygen. The researchers categorized the as very soft, moderately hard, and very hard. They found that uptake through bioaccumulation and the toxicity of these nZVSs when ingested with foods were unaffected by water hardness or by humic acids (the dead degraded organic matter in soil). However, both of these variables could affect interactions between the biological membranes and [insert], ultimately triggering a nanoparticle transformation. Oliver et al. noted that nZVSs have the potential to form complexes with ligands, agglomerate, aggregate, and sediment. The implications here are some nZVs ability to adsorb additional chemicals for transmission into waterways, such as DDT or to steal nutrients from marine species. n-TiO_2 showed an ability to adsorb nutrients from a media plate will engulfing algal cells and prevent photosynthetic activity. This particular algae example is from Aruja et al. (2009) which will be discussed in further The knowledge for understanding why nZVs transform, how they transform, and the potential hazards is continuously growing. Not all of the transformations are bad, but the ones that are can lead to organismal injury or death.

4.2.4 River System Studies

One interesting study conducted in Switzerland by Gottschalk et al. (2011) looked at the estimated effects of introducing nano-Ag, TiO_2, and ZnO into 543 native river sections of Switzerland over a span of 20 years. Rather than introducing ENMs into river systems, the authors attempted to illustrate this interaction using a combination of mass balance partitioning models and river box modeling. Mass balance partitioning models determine the input of a NMs within the observed concentration of a water body, in this case a section of river. River box modeling uses boxes divided into an area of moving water, stagnant water, and sediment, allowing researchers to monitor the lifecycle of the target NM.

The Swiss model revealed that upon release into the river, ENMs would likely associate with suspended solids or sediments, accumulate in organisms (none specified), and enter the food chain/water supply while the direct method of introduction was not elaborated upon (Gottschalk et al., 2011). The nZV particles have the potential to migrate based on the flow rates in the rivers and be transported throughout the river system. The authors recommended more research to see how these ENMs are released into rivers and how they are being incorporated into manufactured goods; strongly encouraging industry data to be readily available to the public, which currently it is not.

Gottschalk et al. (2011) compared two variations of TiO_2; the first was a commercial grade TiO_2 that was examined in a 2009 study and the second was a sol-gel synthesized porous anatase[2] TiO_2 (Gottschalk et al., 2011). The latter was modeled to predict environmental concentrations and the risk quotient for its release. The findings revealed that both forms of TiO_2 demonstrate different properties of deposition and agglomeration. The sol-gel TiO_2 did not settle out during the duration of the study. The P25 TiO_2 settled out of suspension within 24 hours. More species should be investigated

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[2] This a hydrolysis formed precipitate that forms a 100% anatase gel, a fancy way for saying a solid was formed from many molecules. The porous nature allows for interaction between materials and increases overall, surface area.

in order to form more accurate fate and behavior models: the researchers acknowledged that nZVs fate and behavior within rivers is not well understood.

4.2.5 Lake System Studies

Sedimentation is also an issue in lake systems. During their examination of engineered nanoparticles (ENP) in lake retention, Koelmans et al. (2015) noted the tendency for nZVs to accumulate within sediment (Koelmans, Quik, & Velzeboer, 2015). In order to better understand nZV accumulation in sediment, the authors examined the processes for several common chemicals (P, N, trace metals, pesticides or hydrophobic chemicals) and examined the ENP's: CeO_2, SiO_2-Ag, and PVP-Ag. Three different scenarios were calculated for these ENPs based upon previous research (Quik et. al, 2014 and Velzeboer et al., 2014). The first consisted of ENPs settling with natural colloids/solid sedimentation. The second was ENPs settling with the suspended entirety of particles from the water column. The last examined seasonal variability (Koelmans, Quik, & Velzeboer, 2015). The findings revealed that there is the potential for ENPs to exit a water system through outflow leading to downstream pollution and accumulation. This study was the first time a combination of sedimentation and hydrodynamic concepts were examined for analyzing how ENPs in the importance of lake retention.

4.2.6 Bacterial System Studies

Holden et al. (2014) touched on terrestrial systems by focusing on bacteria to assess the environmental hazard and fate of nZVs. This is relevant to determining a viable monitoring policy; early monitoring must be cost effective and use an accepted modeling practice. Their research points out five reasons to consider bacteria for species testing when researching nZVs introduction:

1) Bacterial communities to decline in response to nZV presence, suggesting a sensitivity to nZVs (i.e. toxicity—impeding cellular processes)

2) Bacteria's physiology and their nutrient cycle can be altered by nZVs particle interaction;

3) Physical traits and partitioning can be impacted; (i.e. agglomerates—readily sorb)

4) Degradation of nZV's (could remove from environment or solubilize them);

5) Bacteria possess the potential to degrade introduced nZVs and tentatively can transfer nZVs/NMs across trophic levels and into the higher organism.

Bacteria, due to their size and structure, have large surface areas that allow for the strong attachment of nZVs/NMs to a cells surface. Because of this, agglomerates form and are carried through waterways. Bacteria react to the adherence of the nZVs/NMs by releasing "…surfactant-like macromolecules that sorb to and change the manufactured nanomaterial surface hydrophobixity or hydrophilicity" (Holden et al.,2014).

Depending on the route into the waterway and zZVs or additional chemical materials the bacteria agglomerate with determines the concluding reaction of the cell. Accumulation of these nZVs particles can occur within a bacterial cell by bypassing the cellular membrane (Holden et al., 2014). This means that the bacteria can sequester the nZVs or facilitate travel for the nZVs through a system. If the bacteria fail to sequester and stop travel, the agglomerate bacteria can move through the system to a new environment. Bacteria may precipitate new nZVs/NMs by processing the originally introduced nZVs/NMs with a process or element contained within the bacteria. Or the the nZVs/NMs could interact with "…bacterial biofilms on sand surfaces sorb manufacture nanomaterials and thereby retard manufactured nanomaterials transport through pores (Holden et al., 2014).

4.3. Interactions with Aquatic Species

4.3.1 Pseudokirchneriella subcapitata—Microalgae

Aruha et al. (2009) looked at three types of nZVs, ZnO, TiO_2 and CuO and compared them to their bulk form in order to test their toxicities on the microalgae *Pseudokirchneriella subcapitata.* The findings revealed that at low concentrations (<0.1 mg/L) for both nZV-Zinc and the bulk form they were considered toxic while there was total inhibition of algal growth observed at 0.16 mg Zn/L. The TiO_2 was found to be more toxic in the nanoform, however, both forms resulted in aggregates that entrapped almost all algal cells and inhibited their growth. The TiO2 nZVs in combination with UV light resulted in algae inactivation and destroyed the cells surface architecture. Researchers found that TiO_2 adsorbed the Zinc and Phosphorous from the algal growth medium limiting nutrient availability to algae. Finally, the CuO was found to be more toxic in the nanoform and completed inhibition of algal growth was seen at 6.4 mg Cu/L. Solubility plays a key role; the organisms did not internalize the particles (Aruja et al., 2009).

4.3.2 Haliotis diversicolor supertexta—Abalone Embryos

This examined the effect on development of marine benthic embryos of the abalone, which are a common benthic gastropod inhabitant to the coastal environment and considered sensitive to toxins (Zhu, Zhou, and Cai, 2011). At concentrations $\geq$ 10 mg/L hatching inhibition and malformations were seen (Zhu, Zhou, and Cai, 2011). The authors noted nZVs ability to adsorb contaminates from the aquatic environment leading to toxin complexes that transferred through the water column to sediments by aggregation and settling. Zhu, Zhou, and Cai (2011) exposed TiO_2 to Tributyltin (TBT), a common antifouling compound in industrial process which is commonly introduced into waterways. Researchers noted that the toxicity of TBT increased 20-fold when compared to TBT introduction alone. This is a likely result of internalization of the TiO2 aggregates containing TBT by the embryos revealing the indirect effects of on coexisting pollutants.

4.3.3 *Danio rerio—Zebrafish*

One of the most studied species with respect to nZVs are zebrafish, due to their sensitive nature, their transparent larval stage for easy observation, rapid development, and the fact that they possess a completely sequenced genome and share common genetics with humans.

Zhu, Tian, and Cai (2012) focused on iron oxide NMs in the forms of magnetite and hematite, which due to their super magnetic properties and high catalytic abilities are used for tumor therapy and magnetic storage devices (Zhu, Tian, and Cai, 2012). Unfortunately, the iron oxide aggregates caused significant delay in embryo hatching, some malformation of the embryos and larvae, and can lead to their eventual mortality. Only $\geq$ 10mg/L of iron oxide NMs lead to developmental toxicity in zebrafish. The second study by Zhu et al. (2008) demonstrated some differing results. The researchers examined nano-ZnO, nano-TiO_2 , and nano-Al_2O_3. The nano-TiO_2 and nano-Al_2O_3 did not show toxicity, while the nano-ZnO, the ZnO/Bulk[3] resulted in developmental delay in the embryo and larva, decreased survival, decreased hatching rates, and tissue damage in the zebrafish (Zhu et al., 2008).

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[3] Bulk-the typical manufacturing size

4.3.4 Medaka—Japanese Rice Fish

As discussed earlier, nZVI in the transformation of halogenated solvents and the reducible inorganic contaminants is being increasingly used for groundwater remediation. Researchers assume that the nZVI will stabilize once in the oxygenic environment leading to precipitation within an aquifer; however, it is unclear how these particles will interact with natural water chemistry (Chen, Wu, and Wu, 2013). By examining the impacts of nZVI on Medaka (rice fish), researchers noticed acute mortality, developmental toxicity, sub-lethal oxidative stress in both, embryos and hatchlings (Chen, Wu, and Wu, 2013). The introduction of these materials into waterways must be regulated. The known impacts by nZVs on aquatic species are similar if not worse than the already regulated microplastics and they are detrimentally affecting terrestrial species.

4.4 Future Concerns—Impacts of nZVs on Terrestrial Species

During the course of research examining impacts on aquatic species, several studies were identified on terrestrial species that gave pause for future concern. The negative impacts of nZVs on aquatic species, clearly demonstrated the need for regulation and their similarity to microplastics, which have already been regulated but these terrestrial studies truly solidified it. The following three studies demonstrated that nZVs could affect reproduction and were transferable from parent to offspring indicating there is cause for concern with future generations.

4.4.1 E. fetida and L rubellus—Earthworms

EL-Temsah and Joner (2012) examined how two types of earthworms (*E.fetida and L. rubellus)* would be affected by fresh nZV iron and aged nZV iron. Iron was chosen because it is traditionally used in groundwater and soil remediation. The earthworms were allowed to select soil amended with nZVs and control soil to test for avoidance. The data showed close to a 50:50 split. It is significant to note that the worms did not show clear avoidance to the nZVs: the earthworms seemed incapable of sensing that nZV contamination has occurred. Avoidance did not occur even at the highest levels of nZV Iron (El-Temsah and Joner, 2012).

During the mortality testing of the earthworms at varying nZVs concentration, the results were staggering. All concentrations of freshly added nZVI caused complete reproduction failure for both earthworm species, in both soils with fresh nZVI (no cocoons or juveniles formed). In soil with aged nZVI, researchers observed no juveniles of either species at any concentrations in sandy loam, but did see some cocoons at 100-250 mg kg^{-1} amended sandy loam soil and artificial soil (El-Temsah and Joner, 2012). El-Temsah and Joner explained the cocoons formed before the worm's exposure to nZVI--suggesting that these worms were reproducing before nZV introduction. More importantly, these cocoons could not fully mature because of nZV, demonstrating reproductive failure because no juveniles ultimately formed.

4.4.2. ICR Mice

This study looks at the injection of titanium dioxide (TiO_2) into pregnant mice (Takeda et al., 2009). Two age groups were tested: the first at four days the other at six weeks. The nZVs were identified within the Leydig cells[4], Sertoli cells, and spermatids in the testis at both ages (Takeda et al., 2009). The testicular morphology of the exposed mice was quite abnormal with disorganized, damaged and/or disrupted seminiferous

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[4] Testosterone production occurring in seminiferous tubules

tubules and fewer mature sperm within the lumen (Takeda et al., 2009). These tubes are responsible for housing both, the entirety of germ cells and Sertoli cells. These tubules are the beginning of spermatogenesis and needed to produce immature sperm. Unfortunately, the Sertoli cells are a fixed number of support cells (structure and metabolic) with no ability for regeneration. The mice at six weeks also possessed greater caspase-3 numbers, testing positive for apoptosis reiterating the potential implications for unborn children (Takeda et al., 2009). This may lead to the potential of reduced procreative ability within children and damage to testosterone production within the Leydig cells—low testosterone has been linked to heart disease, high blood pressure, diabetes, infertility, weight gain, low libido, sexual dysfunction, and fatigue (Web M.D., 2016).

4.4.3 Sprague Dawley—Rats

Lee et al. (2012) conducted a studying show the transference of nZVs between mothers to offspring. The researchers examined the transference of AgNPs, treating both the male and female rats orally pre-coitus. The offspring were examined for the presence of AgNPs. Researchers identified AgNPs in the liver, kidney, lung, and brain. The transfer likely occurred through the mother's placenta or milk. Chronic exposure to silver has been known to cause argyria or ardyrosis [permanenet bluish-gray discoloration of the skin or eyes], liver and kidney damage, irritation of the eyes, skin, respiratory and intestinal tract, and changes in the blood cells (Drake and Hazelwood, 2005). Before delving into the next section on gaps within nZV research, the following page contains a summary table of all previously discussed nZV studies and their impacts on the test organism.

Table 2. *This table provides a summary of the previously discussed Zero-Valent Metal Nanomaterial studies containing the title, organism studied, and the key findings from each.*

Study	**Organism**	**Key Findings**
Aruja et al. (2009)	*Pseudokirchneriella subcapitata—Microalgae*	• ZnO toxic • TiO_2 toxic • Inhibited growth • Destroyed cell surface • Adsorb nutrients
Zhu, Zhou, and Cai (2011)	*Haliotis diversicolor supertexta—Abalone Embryos*	• Hatchling Inhibition • Malformations • Increased TBT toxicity
Zhu et al. (2008) Zhu et al. (2012)	*Danio rerio—Zebrafish*	• Significant delay in embryo hatching • Malformation of the embryos and larvae • Tissue Damage • Eventual Mortality
Chen, Wu, and Wu (2013)	*Medaka—Japanese Rice Fish*	• Acute mortality • Developmental toxicity • Sub-lethal Oxidative stress in both, embryos and hatchlings
El-Temsah and Joner (2012)	*E. fetida and L. Rubelus* Earth Worms	• No Avoidance • No juveniles produced • Reproduction Failure
Takeda et al. (2009)	*ICR Mice*	• Offspring Transference Occurred • Present in Leydig, Setoli, and Spermatids • Abnormal testicular morphology • Apoptosis • Damage testosterone production • Potential for reduced procreation
Lee et al. (2012)	*Sprague Dawley—Rats*	• Offspring Transference Occurred • Present in liver, kidney, lung, and brain • Potential chronic silver exposure

4.5 Distinct Gaps/Issues within the Zero-Valent Metal Research

Up until this point, microplastics have been viewed has having negative impacts on aquatic species and as such received regulation. nZVs have been shown to behave similar in introduction into waterways, their behavior once there, and the impacts they are have on aquatic species is the same, if not worse than MPs. The impacts that could result on the food chain and future development of some species are now of concern. This now brings us to looking at gaps and issue within the research.

Currently, research has failed to examine "…complex matrices and real weather conditions, multi-species exposures, competition, predation, and trophic relations" (Bour et al., 2015). When attempting to replicate or test nZV's impacts on water quality, researchers must ensure that the test environment is as authentic as possible to maintain pH, ionic strength, cover variable conditions, sufficient time scale, and contain the appropriate background NMs (Klaine et al., 2008). These are key characteristics necessary to developing safe chemical handling procedures, disposal techniques, and overall, understanding of reactions that could form.

Kahru and Dubourguier (2010) argue that a more thorough understanding of the nZVs chemical properties needs to be investigated both in the lab and in the field. Coatings and their long-term impacts need to be critically studied. They noted that issues include how the particles agglomerate with one another, how long the particles age and how to handle nZVs during application to a waterway or for another use. Several technical components also should be examined, such as the density of nZV's and how these particles interact within the soil matrix. Researchers must identify the hydraulic properties of the aquifer being investigated, the depth to the water table, presence of organic matter, and other geochemical properties of the aquifer. These additional geochemical properties include pH, dissolved oxygen to determine how interactions with dissolved oxygen will effect organisms and the oxidation-reduction potential (ORP) to see how readily nZVs will lose or gain electrons within an environment (Keane, 2009). For example, in marine environments alterations to modeling of estuarine effluent behavior will be needed, as they are currently applicable to large particles not the nanoscopic (Meesters et al., 2013). The ability to measure, detects, and overall quantifies nZVs in the environment needs substantial research as many assumptions are simply accepted.

In addition to the long list mentioned above, there is a notable lack of models for the aquatic fate[12] of nZVs, necessary for effective risk assessment. Once models have been identified, to provide standard for testing that currently do not exist (Klaine et al., 2008). While the USETox model mentioned earlier looks promising, more research of the model is warranted, such as testing it with a variety of nZVs and working to achieve reliability. Reliability can be achieved through the repetition of nZV studies, which begins building behavior expectations and understanding of occurring reactions for each type of nZVs. This will include nZVs of all types, as was discussed earlier even the same type of metallic components can have an unexpected reaction due to nZV novel properties. These research recommendations should take into account the impact of NMs on marine species, terrestrial species, and impacts on sediment, etc.

Because nZVs are comprised of metals and metal oxides, researchers do not anticipate biodegradation --they are inorganic chemicals. However, more research must be completed to determine the actual fate and biodegradation potential of these materials. nZVs can sorb to soil and sediment particulates. Some nanoparticles (NPs) are subjected to natural biotic and abiotic (hydrolysis and photocatalyzed reaction) degradation (USEPA, 2007). This degradation can result in changes to NM properties under anaerobic conditions, facilitating transformation reactions[26] for other particulates. Additionally, there is potential for a "Trojan Horse effect"-- nZVs may pick up a pollutant and carry it to another area for deposition or interaction (Keane, 2009). United States policies on monitoring nZV impacts on water quality are discussed in the section.

5.0 United States Guidelines and Policy

This section is an examination of several United States policy that regulates safe water practices, substance introduction and nanotechnology. The purpose of this section is to note that these existing policies do not address nZVs and often do not reference the general term of nanomaterials. Even though these laws do not have anything written about nZVs there is still potential for them be the framework for a future amendment the detail of these findings are examined below.

5.1 National Nanotechnology Initiative

The National Nanotechnology Initiative (NNI) was established in 2001 to help develop and coordinate nanotechnology for the federal government (Appendix 3). The NNI works to share strategies, resources, and further NM research and development (Kaiser et al., 2014). The 2014 NNI strategic plan presented four key goals for the organization: 1) To further nanotechnology research, 2) To support new technologies for public/commercial application, 3) To increase education for the public and nanotech workers, and 4) To develop nanotechnology responsibly. This document is used strictly as "…internal prioritization and planning processes" (Kaiser et al., 2014) for member agencies. The plan does not reference regulations to water quality or release of nZV into waterways, the takeaway here is that the prioritization and planning does not include regulate their introduction into waterways. There is no plan in place to monitor these levels of contaminants entering our waterways freely.

5.2 *Clean Water Act (CWA)*

The Clean Water Act (CWA) received its most recent large-scale amendment in 1987 to regulate the nation's water by mitigating pollution, maintain wastewater treatment facilities, and ensure wildlife health. A thorough search of CWA revealed no references to the prefix-nano (Act.C.W., 2008) meaning no reference for NMs nor MPs was found.

5.3 *Safe Drinking Water Act (SDWA)*

The Safe Drinking Water Act (SWDA) was passed in 1974 and most recently amended in 2015[5]. Its purpose is to protect the nation's drinking water and allows the EPA to set national protection standards for natural and engineered contaminants. However, after reviewing SDWA and its amendments the prefix-nano, nZV, Zero-Valent Metals, and Nanomaterials is not referenced at all. The EPA could regulate NMs under this act, but can only do some if a maximum contaminant level (the threshold set for a contaminant within a water system) is set.

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[5] 2015 amendments included the *Drinking Water Protection Act*—specific to addressing algal toxin concerns and *Grassroots Rural and Small Community Water Systems Assistance Act*—provide aid to small public water works

5.4 *Toxic Substances Control Act* (TSCA)

The Toxic Substances Control Act (TSCA) was most recently amended in 2012 to designate the EPA as the regulators of reporting, testing standards restrictions, and recording anything relating to chemical substances (Duvall & Wyatt, 2011). The TSCA excludes pesticides, food, drugs, and cosmetics that contain NMs. In an attempt to regulate some NMs, the EPA passed the Significant New Use Rules (SNUR) under TSCA. The SNUR requires a manufacturer that is using one of the 35 specified chemicals in a new way to notify the EPA. Additional portions of the TSCA regulate a manufacture's ability to create a new chemical; they must first notify and receive approval by the EPA. The EPA is now permitted to gather information from manufactures about their products for EPA's online NM inventory ("including chemical identity, production volume, methods of manufacture, processing, use, exposure and release information, and available health and safety data" (EPA, 2015). No information could be found on penalties for non-compliance.

5.5 Berkeley, California Nano-Regulation

Only one city council in the United States regulates the introduction of NMs into waterways. Berkeley, California amended their hazardous materials law in order to force researchers and manufacturers to disclose the type of nanomaterials with which they were working. In addition, they required these individuals and companies to inform the council how they would handle, monitor, and dispose of their waste or unused materials. This rule is mostly concerned with answering the "what is being released or disposed of".

5.6 Gaps in United States Policy

This brief review of United States policy on nZVs shows that regulation and standards fail to capture the unique properties inherent to NMs, by failing to capture them at all. In 2007, the EPA launched a Nanoscale Materials Stewardship Program and released their Nanotech White Paper. The program attempted to report information on NM use throughout the United States, but concluded in 2009 with little success. Recognizing the growing use and need for nanotechnology, the 2012 Congressional budget allotted $2.1 billion to the National Nanotechnology Initiative[4]. The Clean Water Act (CWA) and Safe Drinking Water Act (SDWA) do not mention NMs at all. TSCA provides limited monitoring of new NMs and none to already introduced nZV. The 2014 strategic plan by the National Nanotechnology Initiative addresses nanotechnology but does not reference legislative change, monitoring NMs in waterways, or removing NMs to maintain water quality.

6.0 Recommendations and Findings

Based on the research of nZVs introduction into waterways, their impacts once there, and the current identified gaps within the research the major recommendation is regulation policy. Regulations must be developed to establish and implement a definition of NMs so all parties involved can understand what is being regulated. If regulations were determined by each state or city, there would be no consistency within testing methodologies, regulatory actions, etc. surrounding the monitoring and classifying of various types of nZVs. This would also allow clear universal terminology to be developed and improve communication between organizations such as researchers, the public, and manufactures.

Berkeley, California's Nano-Regulation should be adopted as a preliminary regulation in order to generate mandatory planning by manufactures and force disclosure of product use and disposal. Manufactures should be required to conduct research, this creates honest business practices, product integrity, and would increase the knowledge base for environmental fate and monitoring techniques. Under TSCA the EPA is now compiling data, unfortunately it is not clear what information they are gaining, this information should be made readily available to the public for study and dialogue on improving and implementing new policy, developing universal monitoring methodologies and more readily see research gaps.

Consumer products should be labeled as containing nZVs this educates the consumer on the product they are buying, allowing choices to be made. To assist in this the Woodrow Wilson database out of Washington D.C., which lists products known to contain nZVs, could be made into an app for easy access on shoppers' mobile device. A product tax could be in place to help generate study revenue as well. The specific of this product tax is beyond the scope of this paper it is merely a recommendation to pursue in the future. One thing that should not be done is cease production altogether, or implement an ban on nZVs in their entirety because it is not realistic, is in far too many things, and would only serve to create an adversarial relationship with manufactures. Instead of continuing to look at nZVs after they enter the waterways, regulating point sources should be considered. This includes increasing data collection a wastewater treatment facilities and conducting environmental surveys at stormwater runoff sites. Another avenue to consider is to generate a cap on the manufacturing of nZVs. The only figure that could be identified was out of Europe where they have a 100-1,000 tonne cap but they caution that any new cap being generated should be far lower.

In conclusion, this thesis served to demonstrate that microplastics make an excellent case study and beginning platform for nZVs legislation into waterways. The commonalities of their small size, introduction methods, impacts on aquatic organisms, and future impacts on the food chain longevity make this comparison ideal. The growing concern over nZVs in waterways is warranted based off the findings identified and the harmful effects extend beyond the aquatic to the terrestrial species. Policy implementation is the most logical step for nZVs in waterways and using the established microplastics ban was useful in generating further recommendations for United States nZVs policy.

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