# Modulation of the uptake and toxicity of PFOA by Polystyrene and Titanium dioxide nanoparticles in Pacific oysters (*Magallana gigas*) and Daphnia (*Daphnia magna*)

by

Arian Farajizadeh

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

Department of Biological Sciences
University of Alberta

## Abstract

Nanomaterial toxicity is a major concern in today's world. The benefits of nanoparticle use have led to the production of various kinds of nanomaterials including a high volume of nanoplastics, $TiO_2$ nanoparticles, and CeO nanoparticles. Different nanomaterials are shown to be a suitable vector for various toxicants. The effects of plastic pollution on marine organisms are of growing concern. The hydrophobic surface of plastics has been shown to adsorb phenanthrene and increase the rate of uptake into fish. To date, the potential for other nanoparticles to associate with POPs in water and affect POP transport has not been investigated. Titanium dioxide ($TiO_2$) nanoparticles are known to form different eco-coronas by adsorption of various constituents in water. Therefore, I hypothesized that the presence of nano-sized plastic particles could also enhance PFOA uptake in animals. I measured the uptake rate of $^{14}$C-PFOA in juvenile Pacific Oysters at different concentrations, and in different periods of exposure time, and investigated whether different concentrations of either 500 nm or 20 nm polystyrene nanoparticles (PS-NPs) altered the uptake rate of PFOA. My results demonstrate that PS-NPs have both a high sorption capacity for PFOA and also can significantly enhance the uptake of PFOA at environmentally realistic exposure concentrations. I found that PFOA uptake at 100 μg/l was increased 2.3-fold in the presence of 1000 μg/L 500 nm PS-NPs and 3.2 -fold increase was seen in the presence of 1000 μg/L 20 nm PS-NPs. Based on the previous data, I also hypothesized that PFOA would adsorb to the hydrophobic surface of nano sized $TiO_2$ particles and affect the uptake of PFOA into Daphnia magna. I measured the accumulation of PFOA and $TiO_2$ compound in Daphnia using a radiotracer-based method involving $^{14}$C-labelled PFOA over multiple concentrations, flux times, and different $TiO_2$ particle sizes. My results showed that $TiO_2$ *NP*s have a high sorption capacity for PFOA and also meaningfully modulate PFOA uptake at environmentally relevant concentrations. Uptake of

10 μg/L PFOA was found to be 45% higher in the presence of 500 μg/L 5 nm $TiO_2$ which is 20% higher than the uptake enhancement caused by adsorption of PFOA to the 25 nm $TiO_2$, respectively. Results from my uptake experiments demonstrated the exacerbated uptake rate of PFOA by adsorption onto the surface of the plastic and $TiO_2$ nanoparticles in two different organisms. Furthermore, I investigated whether the presence of the *NP* potentiated accumulation of PFOA, which would result in an intensified PFOA-induced toxicological impact on aquatic animals. PFOA is shown to induce oxidative stress and alter the metabolism of various organisms. I showed that the presence of PS-NPs increased the oxidative stress induced by 1 mg/L PFOA by 2.5-fold and 3-fold in the presence of either 100 mg/L 500 or 20 nm PS-NPs, respectively. These findings demonstrate that micro and nanoplastics as co-contaminants in marine Pacific Oysters can significantly potentiate organic contaminate uptake and toxicity. Additionally, PFOA sorption to $TiO_2$ *NP*s also potentiated the decrease in metabolic oxygen consumption ($MO_2$) by 0.31-fold, compared to PFOA alone, when co-contaminated with 5nm $TiO_2$ particles. These results also showed for the first time that $TiO_2$ nanoparticles can act as vectors for organic pollutants and significantly modulate their accumulation and toxicity in Daphnia. Overall, my data demonstrated the nanoparticles' capacity to adsorb organic pollutants and showed that adsorption of POPs to *NP*s accelerated the uptake of organic toxicants and intensified the toxicity of the accumulated toxicant in different aquatic organisms.

## Preface

Arian Farajizadeh is the principal researcher for the research done within this thesis and therefore assumes all responsibilities. The components of Radio tracer-based experiment and analyses were modified from previous publications by Zhang et al. (2018) to suit the conditions, resources, and research goals of the experiments. All experimental work in chapter 2 uptake experiments (on Pacific oysters) was conducted at Bamfield Science center situated at the Bamfield, British Colombia, Canada. Additionally, the rest of the study were held on the Department of Biological Sciences located at the University of Alberta, North Campus, Edmonton, Alberta, Canada.

Dr. Marina Giacomin provided help over. analyses of uptake and toxicity experiments of chapter two. Additionally, Lazarus Siu and Jonas Wang provided assistance in toxicity evaluation experiment (including TBARS assay and Oxygen consumption assay) and Daphnia colony maintenance . The entirety of this research was conducted under the supervision of Dr. Greg Goss.

No part of this thesis has been published as of the submission of this work to the University of Alberta.

## Acknowledgments

My very first and special thanks go to my supervisor, Dr. Greg Goss, without whose support and kindness not only couldn't I execute my research but also couldn't make it through my years of graduate studies. Thank you, Greg, for all your support and guidance. My words cannot describe my gratitude towards you!

Thank you, my committee members, Dr. Keith Tierney, and Dr. Jeffrey Farner who generously dedicated your time to helping me finishing my graduate studies and kindly provided guidance and support through this research.

My next most sincere gratitude goes to Dr. Ebrahim (Ebi) Lari, my very first mentor and teacher of biological sciences, who helped and guided me to indescribable extents. Thanks, Ebi!

I would also like to appreciate Dr. Marina Giacomin, for all her support and valuable instructions. Thank you, Marina, for all the tips and help in both Bamfield and Edmonton!

Also, a very especial thank you for Tad Plesowicz from the university animal care facility for all his help and support through my graduate studies!

Subsequently, I would like to thank Dr. Yueyang (Brian) Zhang, Laszarus Sui, Jonas Wang and all past and present Goss lab members who helped me throughout this research in many ways!

Lastly, I would like to thank my family, especially my mom, who supported me all my life in any way possible and beyond!

# Table of Contents

## List of Figures

## List of Abbreviation

| | |
|---|---|
| µL | Microliter |
| µm | Micrometer |
| µg | Microgram |
| µg/L | Micromole per Liter |
| % | Percentage |
| ~ | Approximately |
| [] | Concentration |
| ATP | Adenosine Triphosphate |
| BHT | Butylated Hydroxytoluene |
| °C | Celsius |
| $^{14}C$ | Carbon-14 |
| $^{14}C$ | Carbon-14 |
| $^{14}C$-PFOA | Carbon-14 Labelled Perfluorooctanoic Acid |
| CAT | Catalase activity |
| CeO | Cerium Oxide |
| CD | Compact Disc |
| cpm | Counts per Minute |
| DLS | Dynamic Light Scattering |
| EC50 | Half Maximal Effective Concentration |

| | |
|---|---|
| EDTA | Ethylenediaminetetraacetic Acid |
| EPA | Environmental Protection Agency |
| EROD | Ethoxyresorufin-O-deethylase |
| FDA | (U.S.) Food and Drug Administration |
| Fig | Figure |
| g | Gram |
| g/L | Gram per Liter |
| $H^3$ | Tritiated Hydrogen |
| HA | Humic Acid |
| HCl | Hydrochloric Acid |
| $HNO_3$ | Nitric Acid |
| h | Hour(s) |
| KCl | Potassium Chloride |
| $K_{ow}$ | n-octanol/water partition coefficient |
| L | Liters |
| LC50 | Lethal Concentration in 50% of an exposed population |
| LDPE | Low-density Polyethylene |
| LPO | Lipid Peroxidization |
| min | Minute |
| mg | Milligrams |

| | |
|---|---|
| mg/L | Milligrams per Liter |
| mL | Milliliter |
| mV | Millivolts |
| M | Molar |
| MDA | Malondialdehyde |
| MP | Microplastic |
| MPT | Mitochondrial Permeability Transition Pore |
| $MO_2$ | metabolic oxygen consumption |
| N | Normal |
| NP | Nanoplastic |
| *NP* | Nanoparticles |
| NM | Nanomaterial |
| ng/L | Nanogram per Liter |
| nmol/g/h | Nanomoles per Gram per Hour |
| nm | Nanometer |
| PAH | Polycyclic Aromatic Hydrocarbons |
| PBS | Phosphate-Buffered Saline |
| PDI | Polydispersity Index |
| PET | Polyethylene Terephthalate |
| PFAS | Per- and Polyfluorinated Substances |

| | |
|---|---|
| PFOA | Perfluorooctanoic acid |
| PO | Phenol oxidases |
| POPs | Persistent organic pollutants |
| PP | Polypropylene |
| PS | Polystyrene |
| PS-NPs | Polystyrene Nanoparticles |
| PS | Polystyrene |
| PVC | Polyvinyl Chloride |
| pH | Potential Hydrogen |
| pmol | Picomole |
| pmol/g | Picomole per gram |
| pmol/g/h | Picomole per gram per hour |
| ppm | Parts Per Million |
| REACH | Registration, Evaluation, Authorisation and Restriction of Chemicals |
| ROS | Reactive Oxygen Species |
| SEM | Standard Error of Mean |
| SSA | Specific Surface Area |
| TBA | 2-Thiobarbituric Acid |
| TBARS | Thiobarbituric Acid Reactive Substances |
| $TiO_2$ | Titanium Dioxide |

| | |
|---|---|
| $TiO_2$ *NP*s | Titanium Dioxide nanoparticles |
| TM | Trademark |
| U/mL | Units per milliliter |
| Uci/kg/min | Micro Currie per Kilogram per Minute |
| U | Uranium |
| UV | Ultra Violet |
| X | Times |

# 1 Chapter 1: General introduction

## 1.1 Plastics

For over 80 years, plastics have been produced in large quantities and production has expanded widely in the last 50 years (Brandts et al. 2018; Geyer, Jambeck, and Law 2017; Mattsson et al. 2018). Plastic production is estimated to be more than 320 million tonnes, annually (Brandts et al. 2018; Geyer et al. 2017). Plastics are used in a wide range of products such as agricultural plastic mulch, car tires, microfibers from textiles, and other objects (including plastic bottles, rope, bags, nets, and artificial sculptures) (Chen et al. 2020; E. Hernandez, Nowack, and Mitrano 2017; McDevitt et al. 2017; Surendran et al. 2023; Zhou et al. 2020). Roughly 50% of these plastics are considered one-time-use materials only (Geyer et al. 2017; Surendran et al. 2023). Of the total world production, about 10% of the annual production of plastic is estimated to reach the marine environment (Chidambarampadmavathy, Karthikeyan, and Heimann 2017; Mattsson et al. 2018; Wang et al. 2019)**.** Plastics are estimated to form more than half of marine litter globally, making them one of the most abundant marine pollutants (Chen et al. 2017; Mattsson et al. 2018; Zhang et al. 2020). UV light exposure and mechanical degradation are the primary reasons for the degradation of plastics in the environment. Plastics break down gradually creating smaller particles including microplastics (MPs) (Chae and An 2017; Zhang et al. 2020). MPs are particles that are less than 5 mm as defined by The National Oceanic and Atmospheric Administration. These MPs can undergo further degradation initiated through microbiological activity, mechanical force, or UV radiation creating nanosized plastic particles (nanoplastics) which have an extremely high surface area per gram of plastic compared to MPS (da Costa et al. 2016; Manfra et al. 2017; Mattsson et al. 2018). Nano plastics (NPs) are defined as plastic particles smaller than 100 nm in at least one dimension(Brandts et al. 2018; Chen et al. 2017; da Costa et al. 2016; Mattsson et al.

2018; Zhang et al. 2020). In addition to the breaking down of larger plastic particles, NPs are also introduced to the environment as direct ubiquitous nanoparticle toxicants (Cole et al. 2011) generated from commercial products such as toothpaste, cosmetics, personal care products, etc. which, until recent bans by many countries, contain nanoplastics beads (Cole et al. 2011; Luo et al. 2019; Miao et al. 2019).

### 1.2 Micro and Nano plastic as pollutants

Micro and nano plastics have been detected in various marine ecosystems, including the Pacific Ocean, Southern Caspian, and Mediterranean Sea coasts, and both the Arctic and Antarctic environments (da Costa et al. 2016; Geyer et al. 2017; Mattsson et al. 2018). The uptake of these plastics has been demonstrated to result in adverse effects on a variety of different aquatic organisms (Chae and An 2017; Chen et al. 2017; Cole et al. 2011; da Costa et al. 2016; Deloid et al. 2022; Luo et al. 2019; Manfra et al. 2017; Zhang et al. 2020; Zhou et al. 2020). The dominant route of exposure to MPs and NPs in marine organisms is ingestion (Cole et al. 2011; Pikuda et al. 2019; Zhang et al. 2020). Microplastic toxicity is normally caused by physical blockage of the intestinal tract (Jovanović 2017) although MPs toxicity also includes decreased growth or photosynthesis rates (Besseling et al. 2014; Zhang et al. 2020), reduced reproduction (Besseling et al. 2014), reduced body size(Besseling et al. 2014), altered muscle and liver metabolism, and altered feeding rate (Mattsson et al. 2015) in *Scenedesmus obliquus, Daphnia magna,* and *Carassius carassius,* respectively. Degraded plastic particles with smaller sizes are generally shown to be associated with higher toxicity (Chae and An 2017; Cole et al. 2011; da Costa et al. 2016; Jovanović 2017; Xue et al. 2020). For example, 50 nm NPs have been demonstrated to result in more significant membrane damage compared to 1 μm MPs in exposed *Halomonas alkaliphile* (Gonçalves and Bebianno 2021). Another proposed mechanism

of acute NP toxicity is an exacerbated immune response (Brandts et al. 2020). In addition to direct toxicity resulting from exposure to MPs and NPs, another recently proposed mechanism is that MPs and NP can act as a carrier for other pollutants (Qiao et al. 2019; Zarfl and Matthies 2010; Zhang et al. 2020), and this forms the subject of my thesis.

### 1.3 Polystyrene nanoparticles (PS-NP)

Plastics are usually manufactured using synthetic organic polymers including low-density polyethylene (LDPE), polypropylene (PP), polyethylene terephthalate (PET), polyvinyl chloride (PVC), and polystyrene (PS) (Kik, Bukowska, and Sicińska 2020). Plastics can be shaped into any required form due to their thermoplastic features. Global plastic manufacture has been constantly expanding, with plastic production increased about 178-fold in roughly 63 years (1950-2013) (da Costa et al. 2016; Kik et al. 2020) and plastic production has risen 12% in the past 3 years. Furthermore, this production rate is hypothesized to increase significantly (approximately 100%) in the coming years (Hesler et al. 2019; Kik et al. 2020). Vinylbenzene, known as Styrene, is formed from Ethylene and Benzene. Styrene monomers are polymerized to an aromatic polymer form called PS. Mass PS polymerization is done through catalytic dehydrogenation of ethylbenzene (J.R Wunsch 2000a; Kik et al. 2020). Stability, translucency, and being smoothly dyable are significant features of PS. Toothbrushes, toys, and CDs are examples of PS utilization. Styrofoam, known for limited elasticity, is an important product when PS is exposed to rapid heating, making it a good choice for the manufacture of kitchenware including dishes, trays, and mugs. Food transport, packing materials, clips, and office supplies are also some of the PS's other products (Domininghaus 1992; Johannaber and Michaeli 2004; J.R Wunsch 2000a). Full degradation of PS only results from exposure to temperatures over 330 oC, forming styrene monomers. Styrene concentrations above 300 ppm (1000 µg/L) are known to be toxic to human

health, as determined by Environmental Protection Agency (EPA) (Gurman, Baier, and Levin 1987; Mutti et al. 1992). While average styrene consumption is ~ 9 μg/person/day, this is lower than the FDA-approved admissible daily intake, making them a suitable polymer for food and non-food products (Lickly, Breder, and Rainey 1995; US Food and Drug Administration 2002; World Health Organization (WHO) 2000), and hence a risk for ingestion. Furthermore, PS particles with a broad size diversity can be easily produced making them model particles for the investigation of particle surface feature effects and effects on numerous biological organisms (Brandts et al. 2020; Kik et al. 2020; Luo et al. 2019; Manfra et al. 2017). PS-NPs also have broad technological and biomedical applications due to their ease of modulating the shape and size of the particles. Recently developed functions include bio-sensory products, photonics, and other different nanostructures (C Loss 2014; Gurman et al. 1987; J.R Wunsch 2000b).

Different sizes of PS-NPs have been detected in almost all aquatic ecosystems, with concentrations of up to 9200 particles/m3 detected on the shores of British Columbia, Canada (Zellers 2005). They have also been shown to be present in high concentrations in wastewater plants (Mahon et al. 2017). Exposure of zebrafish (*Danio rerio)* embryos to 25, 50, 250, and 700 nm PS-NPs have been shown to result in significant adverse effects where particles larger than 50 nm in size were only found in the gastrointestinal tract but25 and 50 nm particles were shown to penetrate tissue membranes and were detected in different organs (van Pomeren et al. 2017). In a similar study by Lee et al. (2019), zebrafish embryos were exposed to three different sizes of 50, 200, and 500 nm PS NPs alone or co-contaminated with Au ions. It was found that the smaller the size of the nanoplastic, the easier the penetration of tissues and increased accumulation of plastics and Au in the body, especially in lipid-rich organs (Lee et al. 2019). A similar supporting study by Mattsson et al. (2017) showed PS-NP accumulation in the brain of fish, with a higher NP uptake

demonstrated for 53 nm PS-NPs compared to 180 nm NPs. This was associated with acute behavioral and morphological changes, followed by excess weight loss and reduced brain fluids (Mattsson et al. 2017).

### 1.4 Titanium Dioxide ($TiO_2$)

More than %0.5 of the earth's crust is made of natural titanium existing as oxides. (Völz et al. 2000). Nanosized titanium dioxide is manufactured for many different purposes such as solar cells, biomaterials, memory devices, and photocatalysts (Chen and Mao 2007; Diebold 2003; Gong, Selloni, and Vittadini 2006; Lin et al. 2009; Liu et al. 2004; Martin et al. 1996; Martyanov et al. 2004; Nakamura and Nakato 2004; Tan and Wu 2006; Wang, Groenzin, and Shultz 2005; Zhang et al. 1999) and worldwide production exceeds now 330 million tons [gg1] /year. Nano $TiO_2$ is present in the majority of sunscreens and cosmetic products due to its UV light-absorbing abilities (Chen and Mao 2007). While cosmetic pigments including $TiO_2$ in sunscreen are washed off directly into the environment after use (Tourinho et al. 2012), due to their broad scale uses in consumer products, $TiO_2$ mainly enters the environment through wastewater treatment plants as nanoparticles (Tourinho et al. 2012).

### 1.5 TiO2 nanoparticles

Nano-sized $TiO_2$ particles (especially less than 10 nm) have unique size-dependent characteristics, such as surface reactivity and particle morphology, and have a high band gap (Erik Lucas 2001; Pettibone et al. 2008). Given that nano sized $TiO_2$ is used in many commercial, medicinal, and environmental applications, studying $TiO_2$ surface chemistry is of utmost importance for the safe application of this helpful nanomaterial (Akakuru, Iqbal, and Wu 2020; Hussain et al. 2010). $TiO_2$ nanoparticles are usually poorly dispersed in water due to their significant hydrophobic nature, and therefore, applying surfactants is necessary to use in most applications (Akakuru et al. 2020;

Hussain et al. 2010; Khan et al. 2020) . Additionally, multiple studies have demonstrated that $TiO_2$ NM can form an eco-corona of proteins and other substances following entrance into the environment (Khan et al. 2020; Pettibone et al. 2008). The fact that the hydrophobic surface of $TiO_2$ can bind materials in the environment leads to the possibility that the $TiO_2$ in combination with a co-contaminant will result in the adsorption and partitioning of the co-contaminant to the surface of the $TiO_2$ *NP*s (Pettibone et al. 2008; Zhang et al. 1999).

### 1.6 Hydrophobic persistent organic pollutants (POPs)

The deposition of hydrophobic persistent organic pollutants (POPs), including polycyclic aromatic hydrocarbons (PAHs), is a significant environmental issue in the world (Mattsson et al. 2018; Wang et al. 2019). POPs have been shown to have significant adverse effects on a wide variety of marine and terrestrial organisms, including developmental defects, chronic illnesses, and even death, have been demonstrated by many studies (AL Andarady 2011; Cole et al. 2011; Ma et al. 2016a; Zhang et al. 2020). Exposure to some POPs can result in disruption within the endocrine system, the central nervous system, or the immune system (Cole et al. 2011; Ma et al. 2016a).

### 1.7 Perfluorooctanoic acid (PFOA)

Perfluorooctanoic acid (PFOA) is a widely known hydrophobic persistent organic pollutant (POPs) known to be highly persistent and abundant in the environment (Cai et al. 2020; Domingo and Nadal 2017a; Gebbink and van Leeuwen 2020a; de la Torre et al. 2020). It has been found in soils, sediment, the atmosphere, both groundwater and drinking water, plants, and animal body fluids (Awad et al. 2021; Bai and Son 2021; Franco et al. 2020; Galloway et al. 2020; Hagenaars et al. 2013a; Lindim, van Gils, and Cousins 2016; Xie et al. 2021). The half-life of PFOA is reported to be ~3 years in human blood, 250 years in ocean water, and even longer in soils (Bartell et al. 2010; Brede et al. 2010; Kennedy et al. 2010; Steenland, Fletcher, and Savitz 2010). PFOA's main known

environmental sources consist of landfill leachate, film-forming foams such as fire retardants, and wastewater releases from industrial companies and municipal wastes. As a result of these depositions, organisms are exposed to PFOA through food, drinking water, air, and dust (Agency for Toxic Substances and Disease Registry (ATSDR) 2018; D'eon and Mabury 2011; Langer, Dreyer, and Ebinghaus 2010; Renner 2007; Schecter et al. 2010). High concentrations of PFOA exposure can result in severe toxicological effects (Shanmuganathan et al. 2011a). PFOA is known to bioaccumulate in different organs and has been shown to act as a carcinogen ,a liver toxicant ,an immune system modulator ,or an endocrine disrupter (K. Betts 2007; K. S. Betts 2007; Hood 2008; Lau et al. 2007; Ryu et al. 2021). PFOA, a widely used PFAS, was officially added to the 2017 and 2019 REACH regulatory restrictions and Annex A of the Stockholm Convention on carcinogens (UNEP, 2019). (Fiedler et al. 2019; Fiedler and Sadia 2021)(UNEP, 2019). While having PFOA excluded from daily use would reduce their adverse effects, PFOA will remain persistent and minimally degradable in the environment and would still be accumulated by organisms for the foreseeable future, affecting their physiological well-being(Darnerud et al. 2001a; Domingo and Nadal 2017b). PFOA is known to be present in high concentrations, up to µg/L levels in air, water, soil, food (Awad et al. 2021; Bai and Son 2021; Franco et al. 2020; Galloway et al. 2020; Hagenaars et al. 2013a; Lindim et al. 2016; Xie et al. 2021). PFOA has been shown to demonstrate greater uptake and accumulation in aquatic organisms compared to similar terrestrial animals (Geng et al. 2021a; Guo et al. 2019a; Wang et al. 2014). Marine organisms such as bivalves (e.g., shellfish are a major source of human PFOA intake(Du et al. 2021; Shanmuganathan et al. 2011b), with values up to 40 times more than average contaminant accumulation in Baltic marine organisms (including mussels and oysters) (Darnerud et al. 2001b). The highest human blood PFOA concentration was recorded as 147 ng/L (Gebbink and van

Leeuwen 2020b), while PFOA has been found in human breast milk in North America, Sweden, and China with concentrations of 40.1 ng/g lipid(Zhang et al. 2017), 89 pg/mL, and 976 pg/L(Gebbink and van Leeuwen 2020c). The detected presence in breast milk is particularly concerning for breastfeeding infants. Although no PFOA limits for industrial facilities wastewater discharges have been established, the US EPA plans to create wastewater discharge standards to encourage more effective PFOA removal during wastewater treatment.

### 1.8 Oxidative stress, Lipid peroxidation

Many aquatic pollutants lead to the production of reactive oxygen species (ROS) in various animals (Cavaletto et al. 2002; Cheung et al. 2004; Gebbink and van Leeuwen 2020c; Lushchak 2011; Regoli and Giuliani 2014; Verlecar, Jena, and Chainy 2007). Excess ROS results in damage to biomolecules such as lipids and proteins(Halliwell, medicine, and 2015 2015; Jones 2006; Klein et al. 2019; Verlecar et al. 2007). The increased level of oxidative damage can be analyzed through the measurement of either lipid peroxidation or through the formation of protein carbonyls which are widely used indicators for exposure to increase oxidative stress. When lipids are exposed to increased ROS, it results in the formation of malondialdehyde (MDA) which can be measured by the thiobarbituric acid reactive substance (TBARS) assay. Increased TBARS is used as an indicator of increased ROS exposure in the cells of an organism (Aguilar Diaz De Leon and Borges 2020; Devasagayam, Boloor, and Ramasarma 2003; Janero 1990).

### 1.9 PFOA-induced lipid peroxidation

The most common source of ROS production is the mitochondrion due to its role in aerobic respiration (Liu et al. 2007; Panaretakis et al. 2001). The mechanism of PFOA-induced toxicity is related to acute mitochondrial disruption (Kelly et al. 1998). However, the exact mechanism by which PFOA induces increased LPO remains relatively understudied. PFOA is assumed to trigger

a change in the mitochondrial permeability transition pore (MPT) due to PFOA's structural similarity to long-chain fatty acids (O'Brien and Wallace 2004; Panaretakis et al. 2001), thus leading to mitochondrial membrane damage, followed by excess ROS production and increased LPO (Liu et al. 2007; O'Brien and Wallace 2004), as witnessed by an increase in malondialdehyde production measured by the TBARS assay (Aguilar Diaz De Leon and Borges 2020; Janero 1990).

### 1.10 Metabolic stress altered (reduced) oxygen consumption

Respiration is an essential function in the physiological well-being of aerobic organisms (Armitage and Lei 1979; J. C. Martins et al. 2007). Differences in routine metabolic oxygen consumption rates are seen between different species and even between individuals or groups of the same species (Armitage and Lei 1979; J. C. Martins et al. 2007). Oxygen consumption measurements are an applicable method of detecting metabolic disruption by various toxicants (Hernando et al. 2005; J. C. Martins et al. 2007). This method offers multiple benefits compared to other toxicity detection methods due to its accurate and quick responses, even at very low detection limits for some chemicals. Respiration rate is altered through intrinsic (including size, age, and sex) and/or extrinsic (including pH, light, temperature, environmental stress levels, food availability, and physical activity rate (Armitage and Lei 1979). The presence of toxicants in the environment affects both the physiological and behavioral functions of organisms in a dose-dependent manner. Respiration-related assays have been used in numerous studies to examine the effects of various aquatic toxicants on organisms (Ahern and Morris 1999; Chinni, Khan, and Yallapragada 2002; Hyne and Maher 2001; Racotta and Hernández-Herrera 2000; Reyes, Dalla-Venezia, and Alvarez 2002; Rodrigues da Silva et al. 2004; Wu and Chen 2004). The Multispecies Freshwater Bio Monitor Test (U.S. Army Center for Environmental Health Research, Fort Detrick, MD, USA) is

an example of the usage of respiration rate to measure the impact of a toxicant(Gerhardt et al. 2002).

### 1.11 PFOA induced $MO_2$

PFOA exposure at or above 0.1 mg/L has been previously demonstrated to result in a significant decrease in whole-body metabolic oxygen consumption, suggesting PFO induces metabolic stress. For example, pollutant-induced metabolic stress has been demonstrated by Martins and colleagues (2009) showing that Daphnia magna had a significantly lowered $MO_2$ when exposed to several different toxicants and several concentrations (J.C Martins et al. 2007). A similar increase in metabolite alteration was also seen in PFOA-exposed zebrafish embryos (*Danio rerio*) whereby the metabolic rate dropped to about 70% of the paired control fish (Gebreab et al. 2020). While the exact mechanism by which PFOA induces decreased $MO_2$ stays relatively understudied, PFOA exposure is hypothesized to alter metabolites through neurotransmitters and/or their precursors including Tyrosine, Tryptophan, Glutamate, Glutamine, choline, and GABA (Souders et al. 2021). Oxygen consumption measurement is an applicable method of detecting the impacts of many toxicants (J. C. Martins et al. 2007). This method offers multiple benefits compared to other detection methods due to its accurate and rapid response even at very low exposure concentrations.

### 1.12 Trojan Horse effect

Some hydrophobic POPs tend to adsorb to the hydrophobic surface of micro and nanoparticles (AL Andarady 2011; Cole et al. 2011; Ma et al. 2016b). This sorption is considered a potential toxicity mechanism by potentiating the transport of the POPs into the animal (AL Andarady 2011; Cole et al. 2011; Garbovskiy 2017; Ma et al. 2016b; Mattsson et al. 2018; Wang et al. 2019; Zhang and Xu 2020). This potentiation is termed the “Trojan horse effect” (Fig 1.1) whereby uptake of certain POPs has been demonstrated to be enhanced by the presence of micro and nanoparticles.

Nanoparticles are shown to have considerably higher adsorption capacity by delivering POPs to the exposed organisms due to their much smaller size in comparison to MPs and their larger specific surface area (SSA) (Brandts et al. 2018; Ma et al. 2016b; Mattsson et al. 2018; Wang et al. 2019; Zhang and Xu 2020). Furthermore, nano-sized particles can pass through bio-membranes, resulting in a higher uptake rate of POPs (Binelli et al. 2017; Cole et al. 2011; da Costa et al. 2016). Several studies have demonstrated a higher accumulation of PAHs in tissues while co-contaminated with *NP*s (Brandts et al. 2018; Ma et al. 2016b; Zhang and Xu 2020). $TiO_2$ nanoparticles are known to have an increased dispersion in the presence of higher concentrations of dissolved organic matter (Preočanin and Kallay 2006; Völz et al. 2000). They are shown to be coated with different (organic (Pettibone et al. 2008)) substances, forming different versions of eco-coronas (Khan et al. 2020). A direct relation between nanoparticle size and surface adsorption properties is predicted for $TiO_2$ nanoparticles. Solution phase adsorption studies using numerous organic acids support this hypothesis (Wang et al. 2005; Wu and Chen 2004; Zhang et al. 1999). The growing rate of various nanomaterial releases results in increased interaction with chemicals in surface waters, the atmosphere, and soils (Astefanei, Núñez, and Galceran 2014; Bäuerlein et al. 2017; Gondikas et al. 2014; Kaegi et al. 2010; Sanchís et al. 2012). This interaction leads to the adsorption of toxicants onto the surface of the nanomaterial which is hypothesized to facilitate the uptake and transportation of pollutants. Co-contamination is predicted to increase the adverse toxicological effects of the pollutant (Hartmann and Baun 2010; Naasz, Altenburger, and Kühnel 2018). The results of co-contamination caused by the Trojan Horse Effect are as yet relatively understudied, especially in marine organisms.

### 1.13 Pacific Oysters (Maggallana gigas)

The use of bivalves including mussels and oysters as a toxicological indicator and model of marine environmental pollution is a growing trend (Bouallegui et al. 2017; DP 2000; Geng et al. 2021b; Luna-Acosta et al. 2010; Vidal-Liñán et al. 2015). The filter-feeding mechanism of bivalves exposes them to most of the elements present in their environment since bivalves constantly filter the surrounding water. This property makes them an ideal test organism for measuring the accumulation of the toxicants and examining their implications on toxicity (Geng et al. 2021b; Jeon et al. 2010a; Lemos et al. 2022; Zhou et al. 2008) Bivalves are known to take up micropollutants from the environment (Garnier et al. 2007; Jeon et al. 2010b), including trace metals (Boyden and Philips 1981), antibiotics (Watkinson et al. 2007) and plastic particles (Ward et al. 2019). Mollusks including mussels and oysters are great test organisms for eco-toxicological studies, specifically for examination of POPs uptake and accumulation experiments (Geng et al. 2021b; Jeon et al. 2010b; de Souza, Windmöller, and Hatje 2011). PFOA has been shown to have the greatest rate of detection in mollusks compared to other marine biomarkers/POPs (Guo et al. 2019b; Lee and Kim 2015; Shanmuganathan et al. 2011c). The Pacific oyster (*Maggallana gigas*) is a marine bivalve mollusk in the family Osteiddae, originally native to the Pacific coast of Asia (Salvi, Macali, and Mariottini 2014), although it has become a widely introduced cosmopolitan species. Pacific oysters have special characteristics including being easy to breed and grow, having high environmental tolerance, and being easily adaptable to different eco-systems, making them one of the most widely commercially farmed oysters (Pacific Oyster Factsheet, Food and Agriculture Organization of the United Nations (FAO)). Coastal waters and the nearby estuaries have been known to be primary habitats for filter-feeding sessile animals including Pacific oysters (Boyden and Philips 1981; Salvi et al. 2014). The filter-feeding lifestyle means oysters have constant interaction with various pollutants existing in their inhabited

ecosystems. This has made Pacific oysters a frequently used test organism by the France National Monitoring Network (RNO) to evaluate the presence of pollutants in the aquatic environment. Nevertheless, numerous mortality reports of this ecologically and economically significant species, especially juveniles, have become a well-known concern for the last few decades (DP 2000; Garnier et al. 2007; J.A., J.H., and K.K. 1981; Luna-Acosta et al. 2010). Different estuarine commercial production sites for this species, including Marennes Oleron Bay, meaning they are exposed to numerous toxicants including pesticides, metals, and polycyclic aromatic hydrocarbons (PAHs) through inputs into rivers that are receiving waste waters from municipal and commercial activities (Miramand, Guyot, and Pigeot 2003; Munaron 2004; Munaron et al. 2006). Environmental stress caused by pollutants is known to be a significant cause of incidence and/or increase in severity of different abnormalities (Lacoste et al. 2001). Bivalve molluscs, including Pacific oysters, have been known to have different types of coping mechanisms to POP exposure including cellular mechanisms such as encapsulation and/or phagocytosis, and humoral mechanisms such as synthesis of nitric oxide, plus heat shock proteins and antimicrobial peptides. Reactive oxygen species (ROS) are demonstrated to be helpful in phagocytosis as these are normal antibacterial cell-killing responses in the immune system. Other immune system factors such as phenol-oxidases (PO) are activated and can also be altered in organisms exposed to organic toxicants (Stabili and Pagliara 2009). The utilization rate of these immune response parameters can serve as biomarkers to help in detecting signals of environmental pollutants. Their use has grown in recent decades (Bernier et al. 1995; Luster et al. 1989) and is now included in the National Oceanic and Atmospheric Administration's National Status and Trends Program (Cajaraville et al. 2000). Thus, oysters are excellent model organisms for determining the potential risk of the co-contaminant uptake of POPS in the presence of NPs in the environment.

### 1.14 Water flea (*Daphnia magna*)

*Daphnia magna* of the genus *Daphnia* is a water flea. *Daphnia* is found in ecosystems rich in organic matter and making it a key organism in northern hemisphere lentic habitats. This animal is acknowledged as a sensitive organism to a wide range of aquatic toxicants (Dodson and Hanazato 1995; J. C. Martins et al. 2007; J. Martins et al. 2007), including organic pollutants and nanoparticles (Lin et al. 2020). The use of this animal resulted in the modification of numerous water-quality tests including the Dynamic Daphnia Test by Michels et al. (Michels et al. 2000) and Martins et al. (J. Martins et al. 2007) phototactic analysis of Daphnia behavior. Daphnia has an ecologically important role in various aquatic environments (J. C. Martins et al. 2007; J. Martins et al. 2007) including their ease of breeding and maintenance in the laboratory, and high fertility making them a beneficial test organism in the field of toxicology (Enserink, Maas-Diepeveen, and Van Leeuwen 1991; J. C. Martins et al. 2007). Daphnia are relatively transparent animals, thereby allowing observation of their inner structures using a microscope. Daphnia asexual reproduction (Parthenogenesis) allows them to generate identical colonies in all aspects including sex and size (Bronmark and Hansson 2012; J. C. Martins et al. 2007; J. Martins et al. 2007; Michels et al. 2000). The production of clones enables us to examine replicate responses on different individuals' morphological or physiological differences. OECD guidelines for the testing of chemicals utilize *Daphnia* as a specified test organism (OECD Guidelines for Testing of Chemicals 2004a), in various 48-hour acute toxicity experiments(OECD Guidelines for Testing of Chemicals 2004b). *D. magna* filters the suspended particles present in its environment through an evolved filtering apparatus. A water current is generated within the carapace's thoracic opening facilitating foraging and digestion of different nutrients(Fryer 1991). *D. magna* is one of the dominant filter feeders due to its mobile feature and specialized filtering system, making them a suitable test

organism for uptake experiments (Lin et al. 2020). The Martins et al (2007) data demonstrated the effects of various environmental toxicants on daphnia metabolic oxygen consumption. This data showed a decreased rate in Daphnia oxygen consumption (J.C Martins et al. 2007).

### 1.15 Radiotracer based techniques

An element isotope is only different from the same atom in its mass number. As an example, $^{14}C$ is formed by the absorption of neutrons by the atomic nucleus of 13C, in which the element of concern would have a greater mass number by one unit (Schlyer et al. 1990). A radioactive tracer substance is a chemical compound in which one (or more) atom(s) is replaced with a radioactive isotope of the same chemical element; the process is also known as radioactive labeling (Pathak and Sen 2017). Low concentrations of radioactive isotopes can be present and sensitive radiation detectors such as Geiger or Scintillation counters can detect the presence of the low concentrations of them due to their highly energetic radioactive decay. The 1943 Nobel Prize in Chemistry was awarded to George de Havesy for his research on the "Use of isotopes as tracers in the study of chemical processes" (The Nobel Prize 1943). The use of radioisotope tracers is a common technique in biological research(Rennie 1999a; Zhang et al. 2020). Its purpose and mechanism of use can differ depending on the study details and methods (Rennie 1999b; Zhang et al. 2020). A radiotracer compound that enters an organism through channels such as digestion or respiration and its distribution can be easily tracked to specific cells or tissues by simply measuring radioactive decay (Magkos and Sidossis 2004; Zhang et al. 2020). Radioactive compounds are frequently used in metabolism research including lipoprotein metabolism research in human and experimental animals (Collins et al. 1992).$^{14}C$-labelled urea is commonly used for breath tests for *helicobacter pylori* to investigate h. pylori infection (Rennie 1999a).

### 1.16 Thiobarbituric Acid Reactive Substances (TBARS)

The TBARS assay has been broadly used for the detection and measurement of lipid peroxidation. This method is an acceptable metric of the oxidative stress levels in different organisms (Antus et al. 2015; Kaur, Politis, and Jacobs 2016; Khoubnasabjafari, Ansarin, and Jouyban 2015; Khoubnasabjafari, Soleymani, and Jouyban 2018; Kilic et al. 2014; Morales and Munné-Bosch 2019; Wade and van Rij 1989). Lipid peroxidation results in the production of multiple compounds including lipid peroxyl radicals, hydroperoxides, and malondialdehyde (MDA) (Tsikas 2017). MDA goes through a reaction in the presence of thiobarbituric acid (TBA) to form the MDA-TBA compound (Ohkawa, Ohishi, and Yagi 1978). The mixture then results in a reddish-pink color change which when measured at 532 nm, is an indicator of LPO/oxidative stress (Devasagayam et al. 2003; Kaur et al. 2016; Kilic et al. 2014).

### 1.17 Dynamic light scattering (DLS)

Understanding the properties of nanomaterials in solution helps determine much of their impact and one of the most common means to examine these properties is the use of dynamic light scattering (L. M. Hernandez, Yousefi, and Tufenkji 2017; Zhang, Wang, and Chen 2022). Measurement of light-scattering is typically conducted by sample exposure to a monochromatic light wave, detection of the signal using a suitable detector, and mathematical interpretation of sample size, polydispersity, and zeta potential (net charge in solution) (Berne and Pecora 1976; Stetefeld, McKenna, and Patel 2016). DLS is a common method for the determination of the size and aggregation of *NP*s based on the peptides, nucleic acids, and viruses' hydrodynamic behavior (Bloomfield 1981; Fujime 1972; Harding and Jumel 1998; Harvey 1973; Jamieson et al. 1972; Lorber et al. 2012; Nieuwenhuysen and Clauwaert 1981; Nobbmann et al. 2007; Rimai et al. 1970; Schurr 1977; Serdyuk et al. 2007; Van Holde 1970; Zakharov and Scheffold 2009). DLS holds various advantages over other techniques due to its workability with a variety of sample buffers,

temperatures, and concentrations and its relative ease of use (Stetefeld et al. 2016). DLS is not an invasive procedure and only needs low sample concentrations and small volumes but produces a reliable, consistent estimation of the physical-chemical qualities of various particles in solution (Stetefeld et al. 2016).

### 1.18 Hypotheses and Aims

In this study, we aimed to develop a method to track the accumulation of PFOA and this was accomplished using radiolabelled $^{14}$C-PFOA as a model hydrophobic organic pollutant. Our first aim was to determine if the presence of different nanoparticles in the environment results in an altered accumulation rate of PFOA. My study aimed to: 1. Design a modified assay to measure PFOA uptake in various aquatic organisms using $^{14}$C-labelled PFOA; and 2. Examine the effects of PFOA co-contamination with and without $TiO_2$ nanoparticles of varying sizes and concentrations on the rate of PFOS accumulation and resultant toxicological responses to PFOA exposure in relevant marine and freshwater aquatic test organisms.

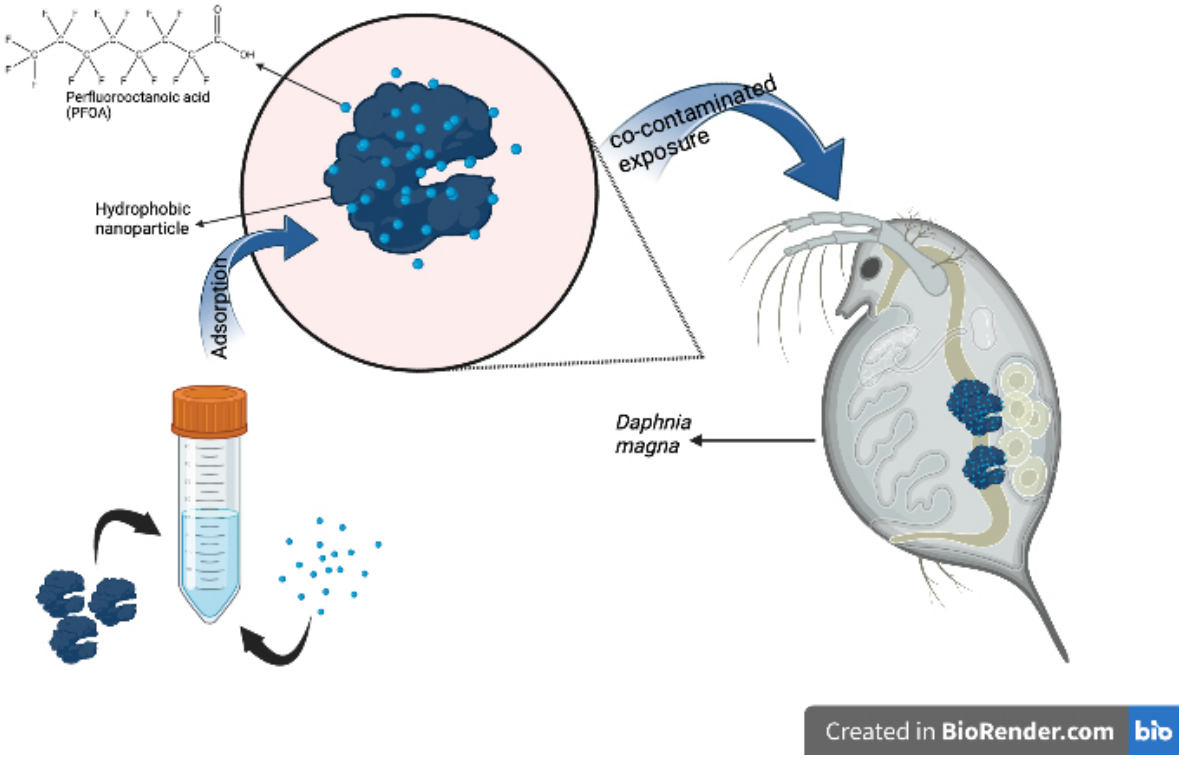



**Figure 1-1**

**Demonstration of PFOA adsorption on different nanoparticles and exacerbated uptake of the co-contaminated compound in exposed organism.**

# 2 Chapter 2: Modulation of the uptake of PFOA by polystyrene nanoparticles in Pacific Oyster (*Magallana gigas*)

## 2.1 Introduction

Plastics have been produced in large quantities since the 1940s and its production has expanded widely since the 1970s (Brandts et al. 2018; Mattsson et al. 2018) with plastic production estimated to be more than 320 million tonnes in 2015 annually (Brandts et al. 2018). Approximately 50% of these plastics are made for a one-time use only. Furthermore, ~10% of the annual plastic production is estimated to reach the marine environment (Chidambarampadmavathy et al. 2017; Mattsson et al. 2018; Wang et al. 2019). Plastics are estimated to form 60% of marine litter globally, turning them to one of the most dominant pollutants in the marine environment (Chen et al. 2017; Mattsson et al. 2018; Zhang et al. 2020).

These plastics break down gradually due to both UV light exposure and mechanical degradation to produce microplastics (MPs; less than 5 mm, defined by The National Oceanic and Atmospheric Administration) (Chae and An 2017; Zhang et al. 2020). MPs can go under further degradation caused by microbiological activity, mechanical force, and UV radiation to generate even a lower sized group of plastics which have a higher surface area per gram of plastic (da Costa et al. 2016; Manfra et al. 2017; Mattsson et al. 2018). These nanoplastics (NPs) are defined as plastic particles smaller than 100 nm in at least one aspect (da Costa et al. 2016; Mattsson et al. 2018; Zhang et al. 2020). The dominant route of exposure to MPs and NPs in marine organisms is by ingestion (Cole et al. 2011; Pikuda et al. 2019). Microplastics can exert their toxicological effects by physical blockage of the intestinal tract (Jovanović 2017). while some other noted adverse biological effects of MPs include reduced growth, reproduction, metabolism, and feeding rate (Besseling et al. 2014; Mattsson et al. 2015; Zhang et al. 2020).

Hydrophobic persistent organic pollutants (POPs), including polycyclic aromatic hydrocarbons (PAHs), are also a significant issue in the environment (Mattsson et al. 2018; Wang et al. 2019). POPs have been extensively studied and have been demonstrated to have significant adverse effects on a wide variety of marine and terrestrial organisms including developmental defects, chronic illnesses, and even death (Andrady 2011; Cole et al. 2011; Ma et al. 2016b; Zhang et al. 2020). Exposure to some POPs can result in disruption within the endocrine system(K. S. Betts 2007), the central nervous system (K. Betts 2007) or the immune system (Cole et al. 2011; Ma et al. 2016b).

Some hydrophobic POPs adhere to the surface of NPs (8, 15, 16) and sorption of these organic pollutants on the surface of plastics is considered as a potential toxicity mechanism by exacerbating transport of the POPs into the animal (Andrady 2011; Cole et al. 2011; Ma et al. 2016b; Mattsson et al. 2018; Wang et al. 2019). This has been variously termed the "Trojan horse effect" (AL Andarady 2011; Cole et al. 2011; Ma et al. 2016b), whereby uptake of certain POPs has been demonstrated to be enhanced by the presence of MPs and NPs (AL Andarady 2011; Cole et al. 2011; Garbovskiy 2017; Ma et al. 2016b; Mattsson et al. 2018; Wang et al. 2019; Zhang and Xu 2020). NPs are hypothesized to have considerably higher capacity to adsorb and deliver POPs to the exposed organisms due to their much smaller size in comparison to MPs and their larger specific surface area (SSA) (Brandts et al. 2018; Ma et al. 2016b; Mattsson et al. 2018; Wang et al. 2019). Furthermore, nano-sized particles have the ability to pass through bio-membranes and this could result in a higher uptake rate of POPs (Binelli et al. 2017; Cole et al. 2011; da Costa et al. 2016; Zarfl and Matthies 2010; Zhang et al. 2020). Several studies have demonstrated a higher uptake rate of PAHs into tissues while exposed to POPs in the presence of NPs (Lin et al. 2020; Ma et al. 2016b; Zhang et al. 2020).

Bivalves are filter feeding animals which exposes them to the elements present in their environment. They are ideal animals for measuring the uptake of the pollutants and examining their effects due to their constant filtration of the water (Bayne et al. 1976; Geng et al. 2021b; Jeon et al. 2010a; Lemos et al. 2022; Zhou et al. 2008) and thus are considered model organisms for determining the potential risk of the co-contaminant uptake of POPS in the presence of NPs in the environment (Brandts et al. 2018; Vidal-Liñán et al. 2015). Pacific Oyster (*Maggallana gigas*) is a marine bivalve mollusk in the family Osteiddae, originally native to the Pacific coast of Asia but now a widely introduced cosmopolitan species (Salvi et al. 2014).

In this study, we developed a method to track the uptake of PFOA using radiolabelled $^{14}$C-PFOA as a model hydrophobic POP contaminant. Our goal was to determine if the presence of NPs results in altered uptake of PFOA due to its adsorption to NPs. The aims of our study were to: 1. develop an optimized assay to measure PFOA uptake in Pacific oysters using $^{14}$C-PFOA; and 2.investigate the effects of NP size and concentration on the uptake rate and toxicological response to PFOA exposure.

## 2.2 Material and Methods

### 2.2.1 Animal collection and maintenance

Juvenile Pacific oysters (*Magallana Gigas < ~2 mm in size*) were provided from NOVA Harvest Ltd, Bamfield BC and were housed in Bamfield Marine Science Centre standard wet tables with flow through seawater from their natural habitat (pH 8, temperature 12 °C). Oysters were fed every 2 days with a mixture or local algae given an acclimation time of at least 48 hours to minimize stress from transportation. We selected oysters as our model organism due to that fact they are filter feeders with high fluid filtering rates (Bayne et al. 1976; Geng et al. 2021b; Jeon et al. 2010a; Lemos et al. 2022; Zhou et al. 2008) (ref), they live in the near shore environment where micro

and nanoplastics are being formed from mechanical and solar degradation, and they occur at the shore nearest where most organic pollutants are discharged (Brandts et al. 2018; Vidal-Liñán et al. 2015).

### 2.2.2 Chemicals

Two sizes of carboxylated polystyrene nano plastics (PSNP) (500 nm and 20 nm) were purchased for this study (ThermoFisher). The stock solution of $^{14}$C-PFOA (55 mCi/mmol) was purchased from American Radiolabelled Chemicals Inc. and prepared to a final working concentration of 0.1mCi/mL which corresponds to 0.784 g/L of PFOA. All other chemicals used in the study were purchased from Sigma chemical.

### 2.2.3 Experimental Protocol

#### 2.2.3.1 <u>Development of a method to measure uptake rate of PFOA:</u>

To determine the optimal uptake time and concentration to measure PFOA uptake in the oysters, 3 different concentrations (25, 50 and 100 µg/L) of PFOA and 4 different time intervals of 1, 2, 4 and 6 h exposure periods were tested. Three beakers were filled with 25 mL filtered seawater and brought to 25, 50 and 100 µg/L concentrations of PFOA by addition from the $^{14}$C-PFOA stock (above). Two 1 mL samples were removed from each beaker for measurement of initial $^{14}$C-PFOA specific activity. For each exposure, 60 oysters (< 10 mg) were randomly selected and placed into an 18 mL glass scintillation vials, each containing 2mL of filtered sea water. Oysters were then given 0.5 h acclimation which re-established the oysters' normal behaviour of filtration (18). After the acclimation period, the water around the oysters was replaced with 2 mLs of either the 25, 50 of 100 µg/L $^{14}$C PFOA diluted solutions and the animals were exposed for one of 4 different time periods (1, 2, 4, 6 h) in 12 $^{o}$C incubator. At the prescribed time, a single water sample (0.5 mL) was taken to determine final $^{14}$C-PFOA specific activity. To measure $^{14}$C-PFOA

activity in water samples, Optifase scintillation cocktail (Perkinelmer) was added at a ratio of 5 parts Optiphase:1part $^{14}C$- containing water) to water samples, incubated for at least 2 h in the dark to remove chemiluminescence before measuring counts per minute (CPM) in a B-counter (Beckman-Wallace LS-6500).

At the end of each flux period, oysters were removed and washed twice with non-radioactive PFOA (2 mg/L) and then once with filtered sea water to remove any surface bound $^{14}C$-PFOA. Extra fluid around the oysters was wicked away using a Kimwipe, they were then weighed and placed in 1 mL of HNO3 (2 N) at 65 °C for 24 h. After digestion, samples were briefly vortexed, and 0.25 mL of the digest added to 1.5 mL Ultima Gold scintillation cocktail (Perkinelmer). Samples were incubated for 2 h before measuring CPM in the beta-counter as above. All measurements were corrected for background CPM using a blank sample.

Unidirectional influx of PFOS was calculated by the following equation:

Equation 1: $J_{in}$ = (($CPM_{tissue}$)/Weight/Time) x (nmol PFOA/CPM $^{14}C$-PFOA)

#### 2.2.3.2 Characterization of PS-NPs and effects of co-contaminant exposure on particle behaviour

##### 2.2.3.2.1 Transmission electron microscopy

PS-NPs were diluted in ddH2O at 2g/L and placed on a polymer-coated copper grid (TED PELLA, INC, Lot # 151122) air-dried and counterstained with 4% uranyl acetate to allow imaging. They were placed in a transmission electron microscope (Phillips Morgagni 268) and imaged at 10,000x and 180,000 x using DigitalMicrograph ™ 1.81.78 for GMS 1.8.0 software.

##### 2.2.3.2.2 Dynamic light scattering

To validate the size of the PS-NPs and if there is an effect of co-contaminant exposure, 5 mL solutions of 1000 μg/L of PS-NP in the presence and absence of co-contamination with 100 μg/L PFOA was prepared and analyzed using Dynamic Light Scattering (Malvern Nano Series Zetasizer). Six 1 mL aliquots replicate of each solution were then tested in the DLS for average particle size, zeta potential and polydispersity index.

#### 2.2.3.3 Sorption of PFOA onto the surface of NPs

Stock Polystyrene nanoparticles (PS-NP) solutions were first dialyzed overnight in distilled water using dialysis membrane (3kDa MWCO) with at least 8 water changes to remove sodium azide preservative. The day of the experiment, 25 mL of filtered seawater was diluted to 100 μg/L $^{14}$C-PFOA into each of six glass beakers. PS-NPs (either 500 nm or 20 nm size) were then added to three beakers to reach final concentrations of 100, 500 and 1000 μg/L. The mixtures were then incubated at 12 °C for 30 minutes to allow sorption equilibration between the PFOA and the PS-NP.

#### 2.2.3.4 Measurement of uptake rate of PFOA from seawater co-contaminated with PS-NPs

To examine the effect of each of the 3 concentrations of PS-NP on the uptake rate of PFOA, 60 oysters (< 10 mg each) were randomly selected and placed into an 18 mL glass scintillation vials, each containing 2mL of filtered sea water. They were then exposed to a co-contaminant mixture of $^{14}$C PFOA-spiked PS-NPs for either 4 or 6 h. The uptake rate of oysters was calculated as per Section a and equation 1 above.

#### 2.2.3.5 48 h exposure to PFOA and effects of co-contaminating PS-NPs on oxidative stress

To determine if PFOA alone resulted in oxidative stress, 5 different concentrations of PFOA alone (0, 0.5, 1, 2, and 4 mg/L) were prepared in filtered sea water. To measure the effect of co-contamination with PS-NPs, three different concentrations of either 20 nm or 500 nm PS-NPs (1, 10, and 100 mg/L) were co-contaminated with 1 mg/L PFOA. In addition, to determine if PS-NPs alone caused increased oxidative stress, two PS-NP-only control groups of 10 mg/L/20 nm PS-NP or 10 mg/L 500 nm PS-NP were also tested. For each exposure, 300 oysters (< 10 mg each) were randomly selected and placed into 250 mL glass beaker filled with 200 mL filtered seawater. Oysters were then given 0.5 h acclimation which re-established the oyster's normal behaviour of filtration. After the acclimation period, the water around the oysters was replaced with 200 mLs of the experimental groups (above) and the animals exposed for 48h in 12 $^{o}$C incubator, with aeration inside each beaker. At the end of each flux period, oysters were removed and washed twice with filtered sea water, extra fluid around the oysters was wicked away using a Kimwipe. Oysters were then weighed, placed in 2 mL microfuge tubes, frozen using Liquid Nitrogen, and put in -80 freezer.

#### 2.2.3.6 TBARS assay

The standard test for increased oxidative stress used the measurement of Thio-barbituric acid-reactive substances (TBARS) where malondialdehyde is generated by increased lipid peroxidization (LPO). TBARS were performed as per Janero DR, 1990 (Ref.) with some modifications. To make sure final absorbance was lower than the limits of the fluorometer, 125 µl of potassium phosphate buffer (0.1 M, pH 7.5) was added to 200 mg of whole oysters. The oysters were then homogenised 4x, 30 second each time, using a bead beater homogenizer (name, manufacturer). The homogenates were briefly centrifuged at low speed (1000 g for 120 seconds) and the supernatant used for the assay. Aliquots (70 µl) of either samples or accompanying

standards were transferred to 1.5 mL tubes kept on ice (~0 C) and 17 µl of freshly made 1mM Butylated hydroxytoluene (BHT), 230µl PBS and 83 µl of 50% Trichloroacetic acid (TCA) was added in order and the mixture cooled for 15 minutes on ice. The mixture was centrifuged at 13000 g for 2 minutes. After that 370 µl of the supernatant was added to screw cap centrifuge tubes and 240 µl of freshly made Thiobarbituitic acid (TBA) was added. The final mixture was heated for at least 60 minutes in a heated (> 90 °C) water bath. Triplicate 150 ul aliquots of the supernatant were plated into 96 well plates and fluorescence (Victor V, Molecular Dynamics) was measured 4x at 532 nm excitation and 545 nm emission. TBARS level was expressed as nmol TEP equivalents/ mg protein using a molar extinction coefficient of 1.56 × 105 mol−1 cm−1.

### 2.2.4 Statistical analysis

Statistical analysis was done using Prism 9 for macOS (version 9.5.1). All data were checked for normality using Shapiro Wilk/Kolmogorov-Smirnov test for normality (all data were normal). Afterwards, Data were analyzed for different factors using a T-test, one-way ANOVA, or two-way ANOVA based on the data set number and variability (specified in figure captions), followed by Turkey's multiple comparison post-hoc test for all data.

## 2.3 Results

### 2.3.1 Particle characterization

We verified the nanoplastic size using TEM and representative images are shown in Fig 2.1. The 500 nm and 20 PS-NPs approximated the size as reported by the manufacturer with consistent sizes and relatively little variation in size. Solution specific characterization was conducted by dynamic light scattering (Fig 2.2) where the hydrated size of the nominal 500 nm particles was 706.1 nm +/- 17.2 and the nominal 20 nm particles had an average hydrated size of 56.5 nm +/- 2.0 The polydispersity index (PDI) for the 500 and 20 nm particles in solution was found to be

similar (0.253 +/- 0.03 and 0.213 +/- 0.01), respectively while the zeta potential was also similar (-25.3 +/- 1.04 mV and -23.9 +/0- 0.52 mV) for the 500 and 20 nm particles without PFOA present. Addition 100 µg/L PFOA resulted in increases in the average hydrated diameter of the 500 nm particles to 721 +/- 20.1 nm and 79.7 +/- 4.9 nm for the 20 nm particles. The presence of PFOA resulted in an increased PDI to 0.386 +/- 0.04 and 0.334 +/- 0.02 for the 500 and 20 nm, respectively. This was also associated with a reduced zeta potential in both the 500 (-34.4 +/ 2.02 mV) and 20 nm (-33.1 +/- 0.03 mV) particles (Fig 2.2).

### 2.3.2 Uptake rate of PFOA - effect of time and PFOA concentration:

PFOA uptake (in the absence of PS-NPs) consistently occurred at all time points with uptake increasing with both increasing [PFOA] and time of exposure demonstrating an ability to accurately measure PFOA uptake rate. At each of 1, 2, 4 and 6 h of exposure (Fig 2.3A-D), there was a significantly greater uptake of PFOA at both 50 and 100 µg/L compared to 25 µg/L. However, the 100 µg/L PFOA uptake was only significantly greater than the 50 µg/L exposure at the 2, 4, and 6 h time periods. At 6 h, the uptake of PFOA in 100 µg/L exposed oysters was 37% higher than the 50 µg/L PFOA and 177% higher than the 25 µg/L PFOA exposed oysters. This suggests that the 100 µg/L was the optimal concentration to examine uptake, and this is the concentration used in subsequent experiments. To determine the optimal time of exposure, PFOA accumulation was compared at the 100 µg/L concentration and the results are shown in Fig 4. Direct comparison of PFOA uptake at each time in 100 µg/L exposed oysters demonstrated significantly greater uptake at 6 h (85.4 +/- 3.9 pmol/mg/h) when compared to 1 h (50.3 +/- 2.5 pmol/mg/h), 2 h (65.9 +/- 2.6 pmol/mg/h) and 4 (73.2 +/- 7.2 pmol/mg/h) h of exposure (Fig 2.4).

### 2.3.3 PFOA uptake rate in presence of 20 nm and 500 nm PS-NP:

PFOA uptake with co-contamination of PS-NP demonstrated consistently higher uptake with increasing [PS-NP] and lower particle size. At both 4 and 6 h of exposure (Fig 2.5A-B), there was a significant increase in PFOA accumulation in the presence of both 500 and 1000 μg/L 500 nm PS-NP compared to 0 and 100 μg/L PS-NP. However, in the presence of 20 nm PS-NP, PFOA accumulated at a greater rate with significant increases noted at each PS-NP concentration (1000, 500, 100 and 0 μg/L), at both 4 and 6 h time intervals, when compared with each other. Furthermore, PFOA uptake was meaningfully higher when exposed to 20 nm PS-NP in comparison to when exposed to 500 nm at all time intervals and PS-NP concentrations tested. At 4 h, the uptake of PFOA in presence of 1000 μg/L 500 nm PS-NP exposed oysters was 2.3X higher than the uptake of 100 μg/L PFOA in the presence of 0 μg/L 500 nm PS-NP and 2X higher than the PFOA and 100 μg/L PS-NP co-contaminated exposed oysters. Direct evaluation of PFOA uptake at 6 h in the presence of different concentrations of 20 nm PS-NPs showed significantly higher accumulation at 1000 μg/L (307.6 +/- 9.3 pmol/mg/h) when compared to 500 (246.9 +/- 17.9 pmol/mg/h), 100 (162.7 +- 9.2 pmol/mg/h) and 0 (96.3 +/- 5.0 pmol/mg/h) μg/L 20 nm PS-NP (Fig 2.5 A-B). This shows that PS-NP can modulate PFOA uptake, and this increases with the higher nanoparticles' concentration and a lower nano plastics size.

### 2.3.4 Effect of PFOA on lipid peroxidation

Lipid peroxidation (LPO) as measured by the TBARS assay, was consistently triggered at all PFOA concentrations with an increasing rate as [PFOA] increased. At 48h of exposure (Fig 2.6), there was a significantly greater TBARS concentration at 1, 2 and 4 mg/L PFOA compared to 0 mg/L PFOA exposed oysters. The TBARS concentration in 4 mg/L PFOA exposed oysters was significantly higher while being 52%, 107%, 230% and 279% higher than the 2, 1, 0.5 and 0 mg/L

PFOA exposed oysters, respectively. This data demonstrates that sub chronic exposure to higher [PFOA] results in greater LPO.

### 2.3.5 Effect of 20 nm and 500 nm PS-NP on PFOA-induced LPO:

Lipid peroxidation consistently increased as an indicator of oxidative stress with both incremental [PS-NP] and smaller particle size when co-contaminated with 1 mg/L PFOA. At 48h of exposure (Fig 2.7), there was a significantly greater TBARS concentration at 100 mg/L PS-NP compared to oysters co-contaminated with 1 mg/L PFOA, but at lower concentrations of PS-NP . TBARS in 1 mg/L PFOA oysters co-exposed with either 100 mg/L 500 nm or 20 nm PS-NP was 2.5X and 3X higher than 1 mg/L PFOA exposed oysters, respectively. Co-contamination with 100 mg/L 20 nm PS-NP significantly exacerbated lipid peroxidation ([TBARS] = 449.17 +- 7.73 nmol/mg protein$^{-1}$) compared to 100 mg/L 500 nm PS-NP ([TBARS] = 365.58 +- 8.45 nmol/mg protein$^{-1}$). This data demonstrates that sub chronic exposure to PFOA co-contaminated with higher [PS-NP] results in intensified oxidative stress (higher [TBARS]).

## 2.4 Discussion

### 2.4.1 PFOA uptake by Pacific oysters and effects on lipid peroxidation

As PFOA concentration in the water increased, there was a general increase in the rate of accumulation of PFOA. This is the expected result, and it is reasonable that a higher concentration would result in a higher accumulation rate due to a higher bioavailability (Zhang et al. 2020). It was also shown that the optimal time for our radiotracer assay was between 4 and 6 hours with shorter periods showing lower rates and greater variability. The likely reason for the increased rate at 4 and 6 h is due to both the high ventilation rate for oysters (Geng et al. 2021b; Zhang et al. 2020) and the fact we are operating in a fixed volume static system, which then allows for resampling of the water and increases in the apparent rate of uptake of the organic pollutant. Due

to the small size of these oysters (< 20 mg), we are unable to distinguish the specific organs that take up the PFOA and their effects on specific biochemical pathways. However, PFOA exposure at or above 1 mg/L did cause a significant increase in whole body TBARS suggesting an increase in oxidative stress as result of PFOA exposure. PFOA induced oxidative stress been previously demonstrated by Amraoui and colleagues (2018) showed that the gill of freshwater mussels (*Unio ravoisieri*) had significantly increased LPO and CAT when exposed to PFOA concentrations above 2 mg/L PFOS (Amraoui, Khalloufi, and Touaylia 2018). A similar increased in LPO was found in 10 mg/L PFOA exposed adult male Murray River rainbow fish (*Melanotaenia fluviatilis*) whereby TBARS concentration was 2 and 4 times higher in gill and liver, respectively, compared to 0 mg/L PFOA exposed fish (Miranda et al. 2020). The mechanism by which PFOA is thought to induce LPO is not completely defined but it is thought to stimulate reactive oxygen species (ROS) production, such as superoxide and hydrogen peroxide, ultimately leading to increased markers of oxidative damage such as LPO (Liu et al. 2007; Panaretakis et al. 2001).

The most common source of ROS production is the mitochondrion due to its role in aerobic respiration (Kelly et al. 1998). The mechanism of PFOA-induced toxicity has been suggested to be associated with mitochondrial disruption (Kelly et al. 1998). However, the exact mechanism by which PFOA induces increased LPO remains relatively understudied. PFOA is thought to act by triggering a change in the mitochondrial permeability transition (MPT) because of its long-chain fatty acid similar structure (O'Brien and Wallace 2004; Panaretakis et al. 2001), thus leading to membrane damage followed by LPO (Liu et al. 2007; O'Brien and Wallace 2004) as witnessed by a significant) increase in malondialdehyde production measured by the TBARS assay (Aguilar Diaz De Leon and Borges 2020; Janero 1990).

### 2.4.2 Association between PFOA and polystyrene nanoplastics

One of the primary methods for toxicity in aquatic invertebrates is through occlusion/blockage of the intestinal tract (Jovanović 2017) although numerous reports exist of translocation of nanoplastics across integumental barriers with unknown direct effects on cellular physiology (Besseling et al. 2014; Mattsson et al. 2015; Zhang et al. 2020). This has also raised concerns regarding direct toxicity of some of the plastic which constituent themselves such as metals, hardeners, and polymerization agents (Gurman et al. 1987). Recently, numerous studies have also highlighted the potential for association between hydrophobic organic contaminants and the hydrophobic surfaces of plastics, thereby creating a vector for enhanced organic exposure/uptake (Lin et al. 2020; Ma et al. 2016b; Wang et al. 2019; Zhang et al. 2020). Normally, PS-NPs will remain as relatively mono-dispersed particles in water and have low rates of aggregation. This was confirmed by both the relatively similar sizes between the nominal (500 and 20 nm particles) and their respective hydrodynamic radius (721 and 56 nm) and the similar PDI (0.25 and 0.21) in the absence of PFOA. Given that PFOA has a terminal carboxylic acid functional group, it was predicted that adsorption of PFOA onto hydrophobic polystyrene nanoparticles would result in an outward facing negative surface charge, a subsequent decrease in zeta potential and an increase in dispersion as measured by PDI. The surface coating of the particle with PFOA explains the nominal increase in the average hydrodynamic size as seen for both sized particles. As predicted, the addition of a PFOA coating resulted in greater charge:charge repulsion between particle and this explains both the decrease in aggregation as reflected in increase in the polydispersity index and the decrease in zeta potential resulting from increased surface negative charges as a result of PFOA binding to the surface (Pettibone et al. 2008).

### 2.4.3 Effects of PS-NP co-contamination on uptake rates of PFOA and TBARS expression

Our study demonstrates that sorption of the PFOA onto the surface of PS-NP alters both the particle behaviour in solution and confirms previous research showing potentiation of organic toxicant uptake into the animal by the presence of nanoplastics (Ma et al. 2016b; Zhang et al. 2020). In general, the smaller the NP size, the potential for greater organic toxicant binding capacity due to their increased surface area/mg NP (Ma et al. 2016b; Pettibone et al. 2008; Wang et al. 2019; Zhang et al. 2020). However, other factors likely also determine the binding of each particular organic with each different type of plastics. There are many types of plastics (e.g., polystyrene, polypropylene, polyethylene etc), each with specific formulations of hardeners, metals, colourants, plasticizers etc, complicating our ability to predict association with various organics. It is thought that plastics with more hydrophobic surfaces will interact strongly with organics with high $K_{ow}$. A recent study by Lin et al., (2020) demonstrated that a combination of humic acid (HA) and PS nanoparticles facilitated transfer to the lipids of daphnia exposed to 6 different PAHs with differing hydrophobicity (Lin et al. 2020). Those PAHs with lower hydrophobicity (lower Log $K_{ow}$) had higher accumulation in the presence of either HA or HA plus PS plastics. Since PFOA is negatively charged, it is likely that the surface negative charge afforded by either HA or PFOA association to the PS facilitates transfer of the organics into the animal (Lin et al. 2020). Our results clearly support and extend these findings that NPs can potentiate the uptake of organic contaminants into animals.

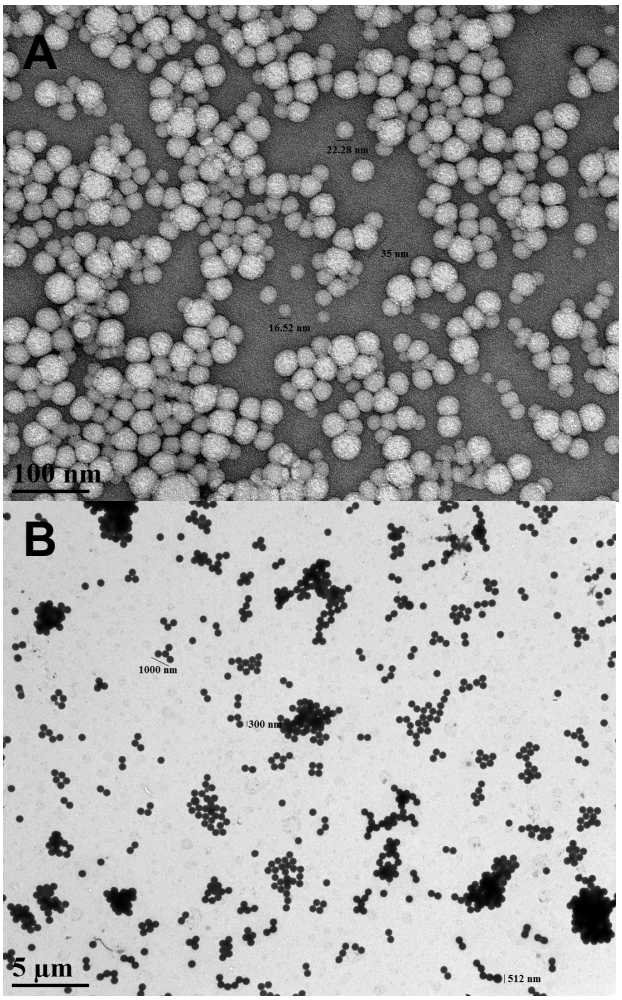
Unfortunately, we did not differentiate if the plastics remain associated with the PFOA once they enter into the animal's digestive tract or pass by the gill/mantle epithelia, or if the PFOA dissociates from the plastics once inside the animal. It is known that small nanoplastics (< 500nm) are able to cross the intestinal membrane in some studies (Besseling et al. 2014; Mattsson et al. 2015; Zhang

et al. 2020), but this study did not examine if co-contaminant exposure alters the rate of nanoplastic translocation, this remains to be investigated.

## 2.5 Conclusion

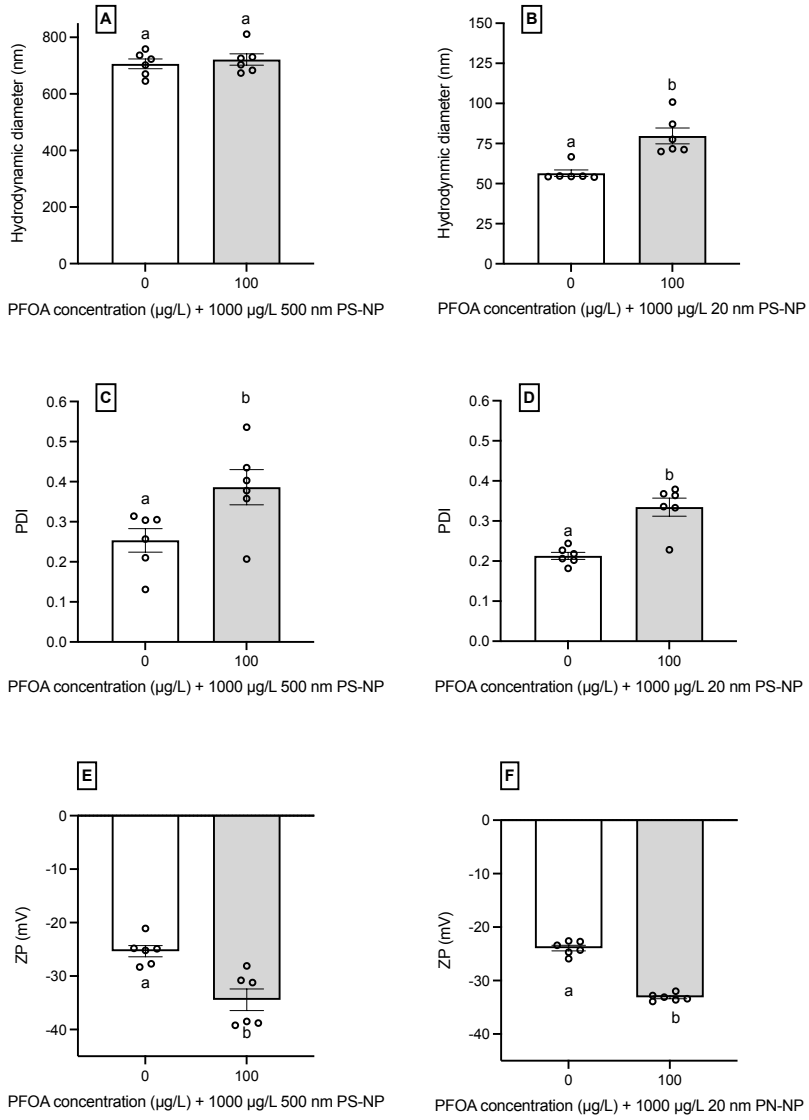
We have developed a radiotracer-based method to measure unidirectional PFOA uptake in oysters at very low and environmentally realistic concentrations (Geng et al. 2021b). This study clearly demonstrates that Pacific oysters rapidly take up PFOA from the water in a time and concentration dependent manner. Furthermore, the presence of nanoplastics significantly potentiates this uptake, with the smaller 20 nm sized PS-NPs resulting in even greater potentiation of uptake when compared to 500 nm sized PS-NPs. PFOA is known to increase lipid peroxidation as evidenced by increased malondialdehyde production, which can be detected by the TBARs assay. As shown by the TBARS assay, exposure to the co-contaminant mixture also potentiated the TBARS response suggesting increased toxicological impact. We consider NPs to be better vectors for the organic transport and uptake due their much smaller size and their larger specific surface area (SSA), and their increased mobility in solution when compared to MPs. Many other nanoparticles are also present in the environment and have been demonstrated to have their own toxicological impacts (Gao et al. 2004; Kaegi et al. 2010; Khan et al. 2020; Liu et al. 2004; Naasz et al. 2018; Zhang et al. 1999) (refs). Some nanoparticles, such as $TiO_2$ which is heavily used in a broad variety of industrial applications such as paint, sunscreen, and food colourings (Akakuru et al. 2020; Pettibone et al. 2008), may also act as a vector for the organic pollutants due to the relatively hydrophobic surface(Khan et al. 2020; Pettibone et al. 2008). Future directions should investigate the potential for the sorption of PFOA and other POPs onto hydrophobic nanoparticles (e.g., $TiO_2$, CeO) and whether the formation of co-associated materials leads to a higher accumulation and toxicity of the POP. This study evaluated the possibility of PFOA sorption to the surface of PS-

NPs and demonstrates that PFOA uptake and transport are assisted in presence of PS-NPs. The potential complexity and impacts of co-contamination in organic pollutant toxicity is a major concern and requires further investigation to integrate into models of both hazard and risk assessment.



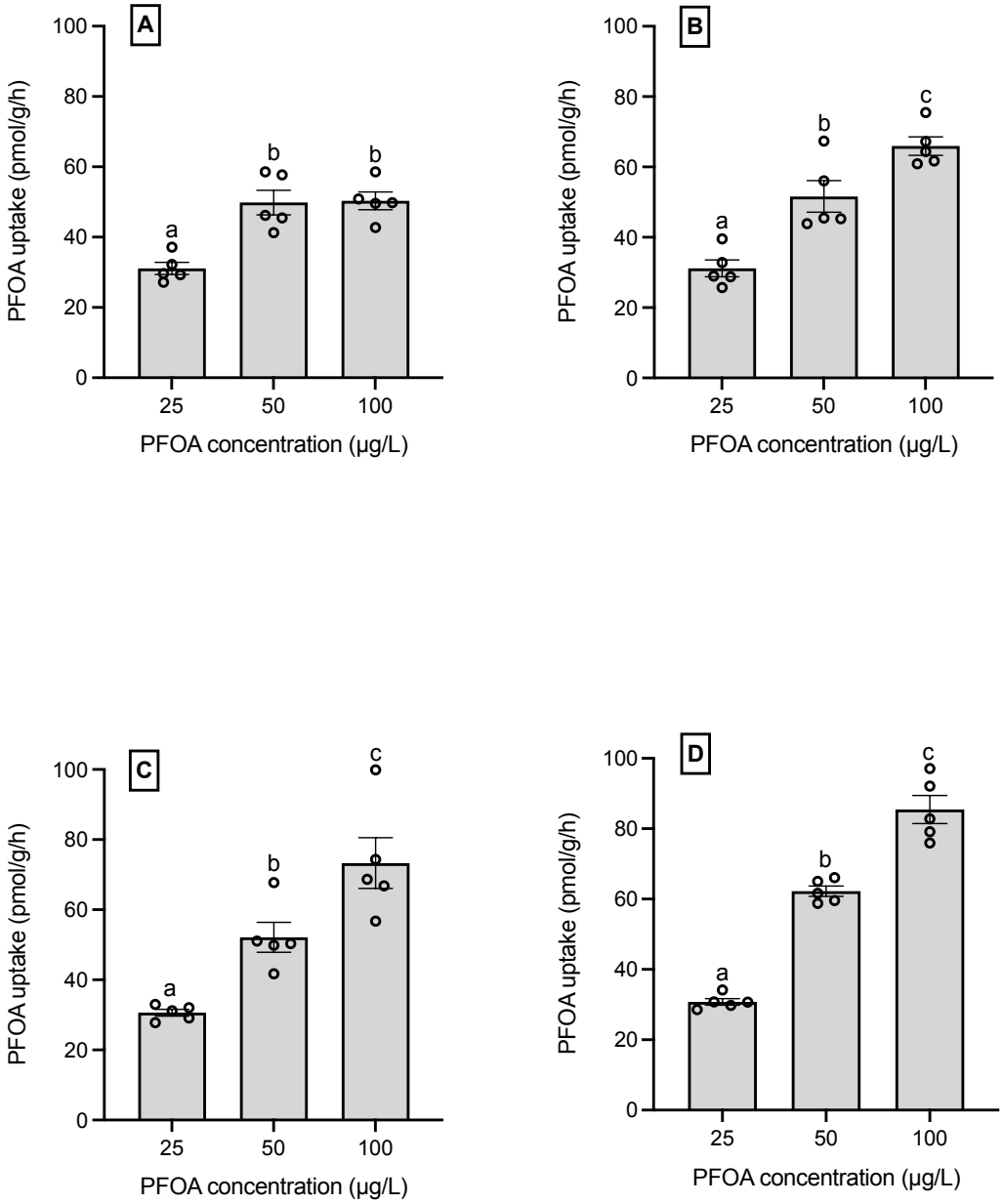

## Figure 2-1

**Transmission Electronic Microscope (TEM) pictures of 2 g/L (A) 20 nm, and (B) 500 nm PS-NPs using 18000X, and 10000X magnification, respectively.**




## Figure 2-2

**Evaluation of 1000 µg/L PS-NPs only and 1000 µg/L PS-NPs co-contaminated with 100 µg/L PFOA difference in size (of (A) 500nm, and (B) 20 nm), Polydispersity index (PDI) (of (C) 500nm, and (D) 20 nm), and Zeta-potential (ZP (of (E) 500nm, and (F) 20 nm), PS-NPs using Dynamic light scattering (DLS) method.** (T-test : P-value_A=0.5760; P-value_B=0.0015; P-value_C=0.0.0309; P-value_D=0.0005; P-value_E=0.0025; P-value_F=<0.0001). Mean values sharing the same lower-case letter are not significantly different among PFOA concentrations. Data are means ± SEM (n = 6). Symbols are individual data points.




## Figure 2-3

**Uptake rate (pmol/h $g^{-1}$) 25, 50, 100 µg/L of PFOA at (A) 1, (B) 2, (C) 4 and (D) 6h in the Pacific oyster (*Magallana gigas*).** (One-way ANOVA: P-value_A=0.0003; P-value_B=<0.0001; P-value_C=0.0002; P-value_D=<0.0001). Mean values sharing the same lower-case letter are not significantly different among PFOA concentrations. Data are means ± SEM (n = 5). Symbols are individual data points

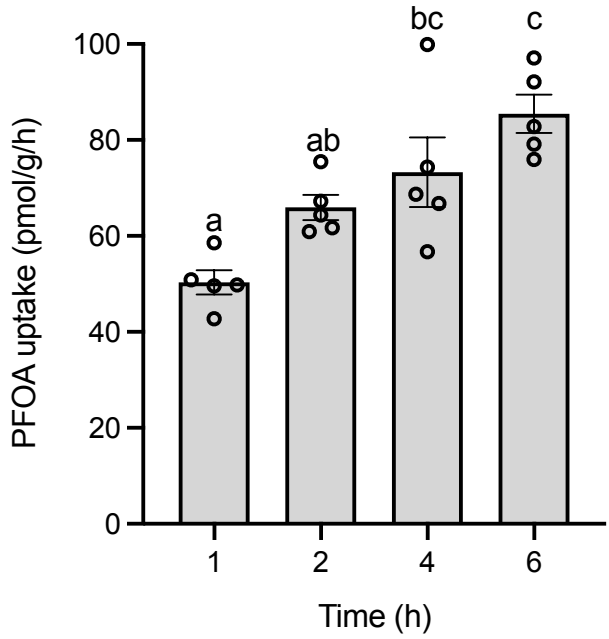
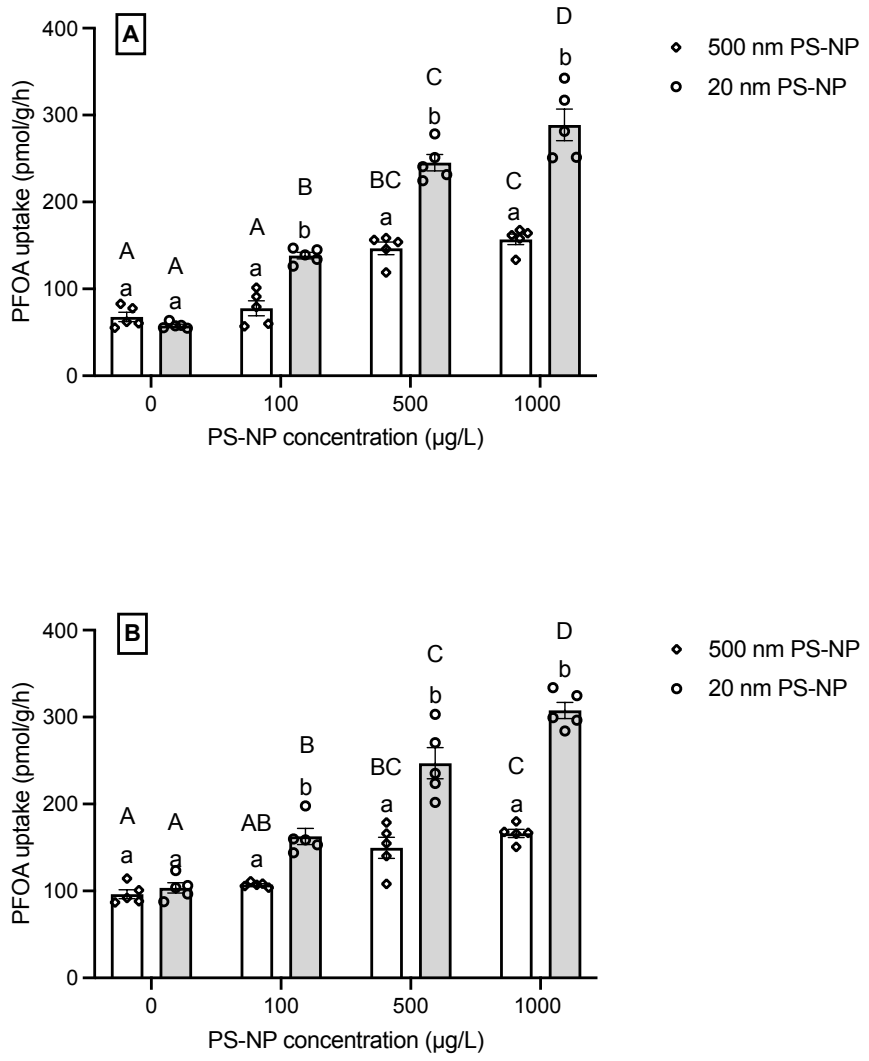



**Figure 2-4**

**Uptake rate (pmol/h g-1) of, 100 µg/L PFOA at 1, 2, 4 and 6 in the Pacific oyster (Magallana gigas).** (One-way ANOVA: P-value =0.0004). Mean values sharing the same lower- case letter are not significantly different among PFOA concentrations. Data are means ± SEM (n = 5). Symbols are individual data points.




## Figure 2-5

**Uptake rate (pmol/h g-1) of 100 µg/L PFOA co-contaminated with 0, 100, 500, 1000 µ/L of 20 nm and 500 nm PS-NPs in (A) 4 and (B) 6 hours of exposure in the Pacific oyster (Magallana gigas).** Mean values sharing the same upper-case letter are not significantly different among different PS-NP concentrations of the same size. Mean values sharing the same lower-case letters are not significantly different among different PS-NP sizes with the same concentrations. (Two-way ANOVA P-values _ A: Pinteraction=<0.0001, Psize=<0.0001, Pconcentration=<0.0001; P-values_Y: Pinteraction=<0.0001, Psize=<0.0001, Pconcentration=<0.0001). Data are means ± SEM (n = 5). Symbols are individual data points.

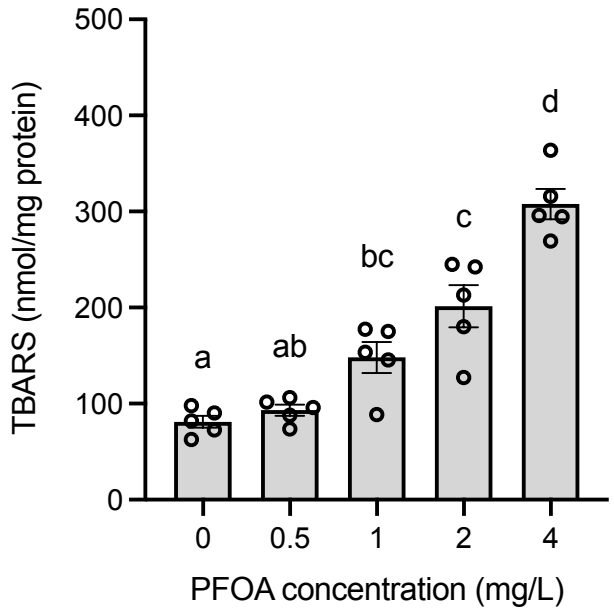



**Figure 2-6**

**TBARS concentration (NM/mg protein$^{-1}$) in the Pacific oyster (*Magallana gigas*) after 48 h of exposure to 0, 0.5, 1, 2, and 4 mg/L PFOA.** (One-way ANOVA: P-value = =<0.0001). Mean values sharing the same lower- case letter are not significantly different among PFOA concentrations. Data are means ± SEM (n = 5). Symbols are individual data points.

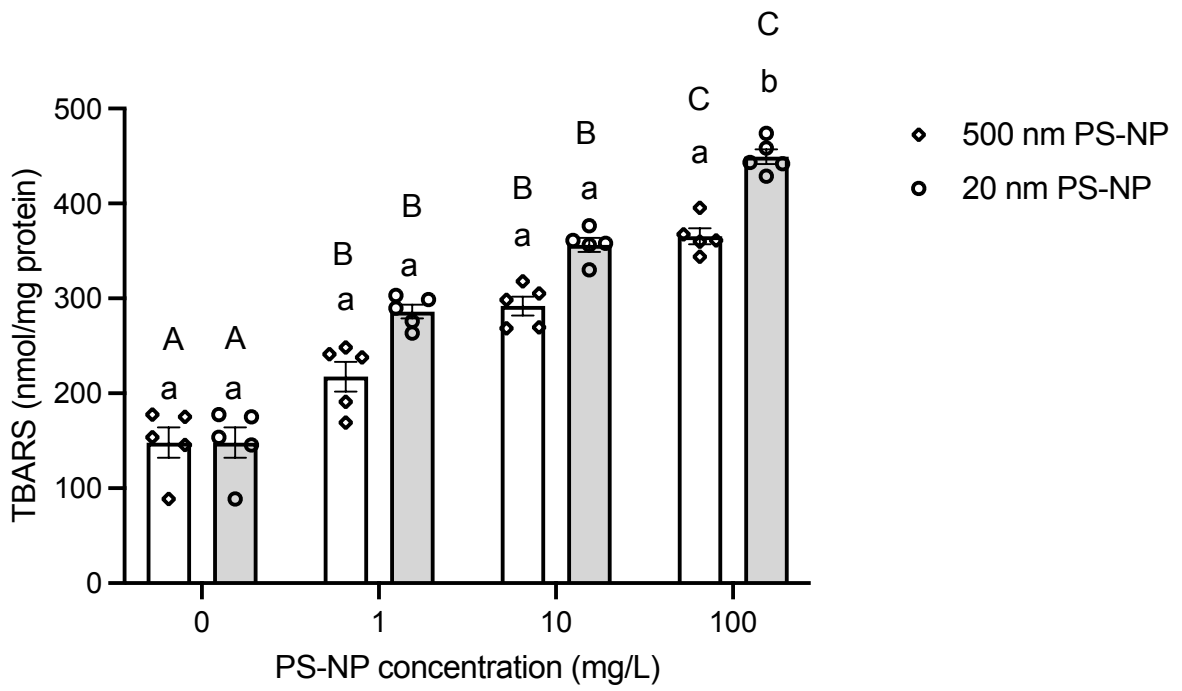



**Figure 2-7**

**TBARS concentration (NM/mg protein−1) in the Pacific oyster (*Magallana* gigas) exposed to 1 mg/L PFOA co-contaminated with 0, 1, 10, 100 mg/L of PS-NP (20 and 500 nm) after 48 h of exposure**. Mean values sharing the same upper-case letter are not significantly different among different PS-NP concentrations of the same size. Mean values sharing the same lower-case letters are not significantly different among different PS-NP sizes with the same concentrations. (Two-way ANOVA P-values _ A: $P_{interaction}$=0.0060, $P_{size}$=<0.0001, $P_{concentration}$=<0.0001). Data are means ± SEM (n = 5). Symbols are individual data points.

# 3 Chapter 3: Modulation of the uptake of PFOA by $TiO_2$ nanoparticles in Daphnia (*Daphnia magna*)

## 3.1 Introduction

More than %0.5 of the earth's crust is made of natural titanium existing only as oxides (34). Manufactured nanosized titanium dioxide is used for many different purposes including solar cells, biomaterials, memory devices, and photocatalysts (Chen and Mao 2007; Diebold 2003; Gong et al. 2006; Lin et al. 2009; Liu et al. 2004; Martin et al. 1996; Martyanov et al. 2004; Nakamura and Nakato 2004; Tan and Wu 2006; Wang et al. 2005; Zhang et al. 1999). $TiO_2$ is also present in majority of sunscreens and cosmetics products due to their UV light absorbing abilities (Jaroenworaluck et al. 2006). These cosmetic pigments including $TiO_2$ are washed off into the environment after use (Tourinho et al. 2012), while nanosized $TiO_2$ also enters the environment through wastewater treatment plants (Tourinho et al. 2012). In general, $TiO_2$ particles have not been associated with significant health concerns in humans (Völz et al. 2006; Warheit and Donner 2015). Nano-sized $TiO_2$ particles, especially those less than 25 nm, display novel size-dependent characteristics including photocatalytic ability, increased mobility in solutions, and altered surface reactivity (Erik Lucas 2001). The surface of anatase $TiO_2$ is generally hydrophobic and aggregates rapidly in aqueous solutions (Grassian et al. 2009; Kiadó and Vol 2006; Rachel, Subrahmanyam, and Boule 2002). Perfluorooctanoic acid (PFOA) is considered a hydrophobic persistent organic pollutant (POP) of concern given its highly resistance to degradation and nearly ubiquitous presence in the environment, being found in soil, air, and ground water (Cai et al. 2020; Domingo and Nadal 2017a; Gebbink and van Leeuwen 2020a; de la Torre et al. 2020). Organisms are exposed to PFOA through food, drinking water, air, and dust (Awad et al. 2021; Bai and Son 2021; Franco et al. 2020; Galloway et al. 2020; Hagenaars et al. 2013a; Lindim et al. 2016; Xie et al.

2021). Exposure to PFOA leads to bioaccumulation in different organs where it can act as a possible carcinogen, a liver and immune system toxicant, and as an endocrine disrupter (K. Betts 2007; K. S. Betts 2007; Hood 2008; Lau et al. 2007; Ryu et al. 2021). *Daphnia magna* of the genus Daphnia is a filter feeding water flea (Armitage and Lei 1979; J. C. Martins et al. 2007). Daphnia are found in aquatic eco-systems rich in organic matter, making it a key model organism for toxicology studies(Dodson and Hanazato 1995; Lin et al. 2020; J. C. Martins et al. 2007). As a filter feeding organism, *D. magna* will also filter any suspended particles from its environment utilizing their evolved filtering apparatus (Fryer 1991; Lin et al. 2020).

$TiO_2$ particles are known to be coated with different substances when placed in environmental solutions to form an Eco corona (Khan et al. 2020; Pettibone et al. 2008). This ecocorona has been show to both alter $TiO_2$ behaviour in solution (e.g., dispersion/aggregation) (Gong et al. 2006; Pettibone et al. 2008; Zhang et al. 1999)and aquatic toxicity (Khan et al. 2020). For example, $TiO_2$ nanoparticles are known to have a better dispersion/increase in poly-dispersity index in the presence of higher concentrations of dissolved organic matter (Preočanin and Kallay 2006; Tourinho et al. 2012). The effect of co-contaminants other than dissolved organic matter is relatively understudied. PFOA is an 8-carbon chain molecule with saturating fluorine molecules at the hydrophobic end with a negatively charged terminal carboxylic acid at the other end (Bai and Son 2021; Gebreab et al. 2020). Given the relative hydrophobic nature of both $TiO_2$ and PFOA, we hypothesized that PFOA would form a corona with $TiO_2$ when both were placed in aqueous solutions. In this study, we used radioactively labelled $^{14}$C-PFOA to develop a method to induce PFOA adsorption onto $TiO_2$ nanoparticles. We then tracked both the effects of PFOA on the properties of $TiO_2$ in solution and quantified the rate of uptake of either PFOA alone or PFOA in association with $TiO_2$. Our goal was to determine if the coating of $TiO_2$ results in altered uptake

of PFOA due to its adsorption to the nanoparticles. The aims of our study were to: 1. develop an optimized assay to measure PFOA adsorption on $TiO_2$ nanoparticles, 2) to measure PFOA uptake and toxicological response in D. magna; and 3) to investigate the effects of nanoparticle size and concentration on the PFOA uptake rate and toxicological response during combined $TiO_2$/PFOA exposure.

## 3.2 Material and Methods

### 3.2.1 Chemicals

The stock solution of $^{14}$C-PFOA (55mCi/mmol) was provided from American Radiolabelled Chemicals Inc. and diluted to a suitable concentration of 0.784 g/L PFOA (0.1mCi/mL). Three different sizes of Titanium dioxide ($TiO_2$), 5, 25, and 100 nm were acquired from JRC European Commission, Evonik Industries, and mk Nano, respectively. All other chemicals used were from Sigma-Aldrich unless otherwise specified.

### 3.2.2 Daphnia maintenance

Adult Water flea (*Daphnia magna*) were obtained from Aquatic Research Organisms (ARO; Hampton, NH, USA) and were then housed and reared at room temperature in moderately hard OECD water in the Goss Lab at the University of Alberta. New-born neonates were removed from the adult colonies daily and transferred to a 1 L beaker filled freshly made OECD water. Daphnia were fed using a mixed food of Algae (ARO) and house made yeast, Cerophyl, trout chow (YCT). The water was changed every 2 days with a freshly made OECD water and there was a maximum occupancy of 100 and 50 daphnia per beaker at 3-4 and 7-8 days of age, respectively. Daphnia of use in this study were 10-15 days old, chosen based their optimal metabolism and moving behavior.

### 3.2.3 Experimental Protocol

#### 3.2.3.1 Development of a method to match PFOA adsorption onto $TiO_2$ nanoparticles to control exposures

To allow a direct comparison of PFOA uptake with and without $TiO_2$, we needed to adjust the amount of $^{14}C$-PFOA added to each $TiO_2$ solution so that the amount of absorbed PFOA in the $TiO_2$ exposure solutions was the same as the non-$TiO_2$ exposure solution. This was validated for each test by counting a 100 µL subset of each stock solution in a B-counter and ensuring that the amount of PFOA in each test vessel was the same, allowing direct comparison. To coat $TiO_2$ with PFOA, $TiO_2$ was first dispersed DMSO and vortexed, then 10 uL suspension was added to 10 mLs of an OCED solution containing 10 µg/L $^{14}C$ -PFOA, vortexed for 2 min, allowed to sit for 2 h, vortexed again and then then centrifuged for 2 h at 14,000 g. A 100 uL subset of the $TiO_2$ pellet coated with PFOA was counted and this allowed us to pair the PFOA adsorbed to $TiO_2$ with each control PFOA alone exposure. $TiO_2$ pellet was diluted using dd$H_2O$ to make needed concentration of the co-contaminated $TiO_2$-PFOA.

#### 3.2.3.2 Characterization of $TiO_2$ nanoparticles and effects of co-contaminant exposure on particle behaviour

##### 3.2.3.2.1 Transmission electron microscopy

A 2g/L $TiO_2$ stock was made using ddH2O and mixed using a Vortex and then sonicated 3X using a bath sonication right before addition to minimize aggregation. The sample was then placed on a polymer-coated copper grid (TED PELLA, INC, Lot # 151122) and air-dried. They were imaged at 10,000 X and 180,000 X using a transmission electron microscope (Phillips Morgagni 268) and imaged at 10,000 X and 180,000 X using DigitalMicrograph ™ 1.81.78 for GMS 1.8.0 software.

##### 3.2.3.2.2 Dynamic light scattering (DLS)

To certify the size of the $TiO_2$ nanoparticles and if there is an effect of co-contaminant exposure on particle behaviour, 5 mL solutions of 500 µg/L $TiO_2$ either coated with PFOA or not were prepared. A test PFOA solution (10 µg/L) was prepared and added where appropriate. Five replicate 1 mL aliquots were then tested using DLS Malvern Nano Series Zetasizer and average particle size, zeta potential and polydispersity index measured.

#### 3.2.3.3 Development of a method to measure uptake rate of PFOA

The rate of uptake of PFOA was determined using radioactively labelled $^{14}$C-PFOA as a tracer and this method was adapted from previous research on oysters (Farajizadeh et al, submitted). To determine the optimal time interval for measurement of PFOA uptake in daphnia, one PFOA concentration (10 µg/L) was tested over 5 different exposure time intervals (0.25, 0.5, 1, 2, 4 h). At the beginning of each exposure period, 20 daphnia (10-15 days old) were randomly selected and placed into a 10 mL glass vial containing 5 mL of freshly made OECD water. Daphnia were given 0.5 h acclimation to allow the animal to re-establish its normal behavior and metabolism. PFOA exposure was initiated by removal of OECD water and replacement with 5 mL of $^{14}$C-PFOA containing (10 µg/L) working solution. Concentration of the working solution was verified by removal of two 1 mL samples, counting $^{14}$C-PFOA total activity and PFOA concentration calculated based on specific activity of the ARC stock solution. Daphnia were then exposed for one of 5 different time intervals (1, 2, 4, 6 h) at room temperature. At the end of each flux period, a water sample (0.5 mL) was removed to determine final $^{14}$C-PFOA activity. To measure $^{14}$C-PFOA activity, Optifase (company) was added water samples at a ratio of 5 parts Optiphase:1part $^{14}$C- containing water) and incubated at least 2 h in the dark to remove chemiluminescence before determining activity (counts per minute (CPM)) in a B-counter (Hitachi Beckman-Wallace LS-6500).

At the end of each exposure interval, Daphnia were taken out and washed twice with solution of 2mg/L non-radioactive PFOA and then once with OECD water to remove any surface attached $^{14}$C-PFOA. Extra fluid around the daphnia was wicked away using a Kimwipe for at least 90 seconds. They were then weighed and placed in 1 mL of 2 N HNO3 solution at 65 °C for 24 h. After digestion, samples were vortexed for at 0.5 minutes, and 1.5 mL Ultima Gold scintillation cocktail (Perkinelmer) was added to 0.25 mL of the digest. Samples were incubated for 2 h before measuring CPM in the beta-counter as above. All measurements were corrected for background CPM using a blank sample.

Unidirectional influx of PFOA was analysed by the following formula:

E1uation 1: $J_{in}$ = (($CPM_{tissue}$)/Weight/Time) x (nmol PFOA/CPM $^{14}$C-PFOA)

#### 3.2.3.4 Measurement of PFOA uptake rate with or without TiO2 nanoparticles present.

For each test, 20 daphnia (10-15 days old) were randomly selected and placed into a 10 mL glass scintillation vials, each containing 5mL of freshly made OECD water. After 30 min acclimation time, Daphnia were then exposed to either PFOA alone or a matching combination of PFOA-spiked $TiO_2$ for the time interval (h). The uptake rate of PFOA was then calculated as per equation 1 above. First, we tested the possible modulation of 10 µg/L PFOA uptake by the presence of 5 nm $TiO_2$ at 5 different $TiO_2$ concentrations (0, 125, 250, 500 or 1000 µg/L). Next, we tested for the effect of particle size on PFOA (10 µg/L) uptake using 3 nominal particle sizes (5 nm, 25 nm, or 100 nm) at 500 µg/L $TiO_2$. Additionally, we tested for the effect of 500 µg $TiO_2$ as a co-contaminant on the uptake rate of 10µg/L PFOA over 0.25, 0.5, 1, 2, and 4 h.

#### 3.2.3.5 48 h exposure to PFOA and effects of co-contaminating $TiO_2$ nanoparticle on metabolic status

To measure metabolic oxygen consumption ($MO_2$), fifty randomly selected 10–15-day old daphnia were transferred into two 300 mL glass beaker filled with 250 mL filtered freshly made OECD water. Animals were then given 0.5 h acclimation to re-establish the daphnia normal filtration and foraging behaviour. The water around them was then replaced with 250 mL of the appropriate treatment groups (above). The daphnia were exposed to the treatments for 48h in a 21 $^{o}$C temperature-controlled room. After the treatment period, animals were removed, washed twice with OECD water, and extra fluid around the daphnia was wicked away using a Kimwipe. Daphnia were then randomly sorted in groups of two. Each group was then placed into sensor cells of an oxygen consumption SDR- meter, filled with 500 µl freshly made clean OECD water and sealed. Oxygen concentration in each cell was then recorded using SDR$^{TM}$-Sensor Dish Reader software every 15 seconds, for 1 h and $MO_2$ calculated on the rates of O2 decline in the metabolic chamber. To determine if PFOA exposure affected the metabolic status, daphnia were exposed to 5 different concentrations of PFOA alone (0.1, 0.25, 0.5, and 1 mg/L) and $MO_2$ measured. Based on these results, $MO_2$ was measured in daphnia comparing the effects of exposure to either 0.5 mg/L PFOA or 0.5 mg/L PFOA sorbed to 5 nm $TiO_2$ nanoparticles at three different concentrations (25, 50, and 100 mg/L). We also measured $MO_2$ in daphnia exposed to either 0.5 mg/L PFOA or 0.5 mg/L PFOA sorbed to 50 mg/L $TiO_2$ in three sizes (5, 25,100 nm). As a control, we also tested if $TiO_2$ (50 mg/L) alone of either 5 nm, 25 nm, or 100 nm $TiO_2$ nanoparticles affected $MO_2$ metabolic and no significant effect of $TiO_2$ on $MO_2$ was noted (data not shown).

### 3.2.4 Statistical analysis

Statistical analysis was done using Prism 9 for macOS (version 9.5.1). All data were normal and were checked for normality using Shapiro Wilk/Kolmogorov-Smirnov tests for normality. Data were then analyzed for different factors using a T-test, one-way ANOVA, or two-way ANOVA

based on requirements of the data set (specified in figure captions), followed by Turkey's multiple comparison post-hoc test for all data.

## 3.3 Results

### 3.3.1 Particle characterization

$TiO_2$ nanoparticles size were verified using TEM and Fig 3.1 demonstrates the supportive images. The 5, 25, and 100 $TiO_2$ nominal manufacturer provided dimensions were closely matched with our images with little variation in sizes. DLS was used to determine if the presence PFOA altered behaviour of $TiO_2$ in solution (Fig 3.2). The measured hydrodynamic radius of the nominal 5, 25, and 100 nm particles alone was 1330.5 nm +/- 81.8, 1553.6 nm +/- 78.3, and 1127.17 +/- 32.2, respectively, suggesting significant aggregation. The recorded polydispersity index (PDI) (0.249 +/- 0.01, 0.196 +/- 0.03, and 0.34 +/- 0.01) and Zeta potential (ZP) (-11.2 +/- 0.64, -9.9 +/- 0.34, and -8.3 +/- 0.23 mV) for present non-PFOA-coated 5, 25, and 100 nm $TiO_2$ *NP*s in solution was found to be similar, respectively. However, the presence of a 10 μg/L PFOA coating was associated with a 34%, 20%, and 27% decrease in the average hydrated diameter of the 5, 25, and 100 nm particles, respectively. Adsorption of PFOA resulted in an increased PDI and decreased ZP, whereas PDI for 5, 25, and 100 nm PFOA-spiked $TiO_2$ *NP*s was increased by 1.31, 1.67, and 1.26 times. Similarly, ZP of the $TiO_2$ was reduced by 13.8 mV, 7.32 mV, and 4.07 mV, respectively (Fig 3.2) in the presence of PFOA.

### 3.3.2 Development of a PFOA uptake assay

10 μg/L PFOA was chosen as an optimal concentration for measuring uptake based on both preliminary experiments and the fact that this slightly above (in order to ease the uptake measurement) measured environmental levels of organic pollutants (Dodson and Hanazato 1995) including PFOA (Geng et al. 2021b). The effect of flux time on PFOA uptake (without $TiO_2$ co-

contamination) is shown in Fig 3.3. A higher PFOA uptake rate was associated with an increasing time of exposure (up to 2 h) demonstrating the efficacy of our method to measure PFOA uptake. a greater rate of uptake of PFOA was observed at each of 0.25, 0.5, 1, and 2 h of exposure (Fig 3.3) while PFOA uptake at 4 h of exposure was slightly decreased compared to the 2 h period. This suggests that the 10 µg/L PFOA exposure for 2h was the optimal time and concentration to study PFOA uptake.

### 3.3.3 Effect of [$TiO_2$] on PFOA uptake.

We next examined if the amount of PFOA-sorbed $TiO_2$ altered the rate of PFOA uptake (at same PFOA concentration). Daphnia exposed for 2 h to increasing concentrations of $TiO_2$ showed a significant increase in their uptake rate compared to control. When the PFOA exposure was co-contaminated with 0.5 or 1 mg/L $TiO_2$, uptake rate increased by 65%, and 123% respectively (Fig 3.4).

### 3.3.4 Effect of $TiO_2$ size on PFOA uptake

Accumulation of PFOA in Daphnia showed a significantly greater rate as the $TiO_2$ co-contaminant with reduced particle size (Fig 3.5). 5 nm $TiO_2$ nanoparticles as a co-contaminant resulted in 45% greater PFOA uptake rate over two hours when compared controls without $TiO_2$ while 25 nm $TiO_2$ *NP*s showed a modest 20% increase and 100 nm $TiO_2$ *NP*s did not significantly potentiate uptake.

### 3.3.5 $TiO_2$ potentiates PFOA uptake in Daphnia magna

Based on the method development described above, we examined effect of an exposure to 5 nm $TiO_2$ (0.5 mg/L) coated with PFOA (10 mg/L) over increasing time intervals (Fig 3.6). At each time interval (0.5 h to 4 h), 10 µg/L PFOA sorbed to 500 µg/L $TiO_2$ nanoparticles showed significant and consistently higher rates of transport compared to the time-matched control without $TiO_2$. Similar to above, a 2 h period of exposure showed the highest PFOA uptake rate when

compared to other time intervals (0.25, 0.5, 1, and 4 h). $TiO_2$ co-contaminated with 10 µg/L PFOA, showed 1.81-, 1.64-, 1.65-, and 1.61-fold greater uptake when compared to the non-adsorbed PFOA when exposed for 0.5, 1, 2, and 4 h, respectively.

### 3.3.6 Effect of PFOA on $MO_2$

After 48h of exposure (Fig 3.7A), there was a significant depression of respiration at each of 0.1, 0.25, 0.5, and 1 mg/L PFOA compared to 0 mg/L PFOA. Daphnia had a significantly lower oxygen consumption when exposed to 0.5 mg/L (0.13 +/- 0.00 µg/daphnia/h) and 1 mg/L PFOA (0.10 +/- 0.01) compared to daphnia treated with 0.25 (0.21+- 0.0), 0.1 (0.22 +- 0.0), and 0 (0.28 +/- 0.01) mg/L PFOA, respectively. From this information, a [PFOA] of 0.5 mg/L was chosen to investigate the possible effects of $TiO_2$ as a co-contaminant.

**Effects of co-contamination with 5 nm $TiO_2$ on PFOA-induced $MO_2$**

Addition of 0.5 mg/L PFOA-coated 5 nm sized $TiO_2$ particles consistently decreased $MO_2$, an indicator of daphnia metabolic stress. Moreover, as [$TiO_2$] increased, there was a stepwise decreased in daphnia $MO_2$ with 50 mg/L $TiO_2$ resulting a 32% decrease and 100 mg/L $TiO_2$ resulting in a 55% decrease in $MO_2$ compared to the daphnia treated to 0.5 mg/L PFOA only (no $TiO_2$) (Fig 3.7B).

### 3.3.7 Effect of co-contamination with different sizes of $TiO_2$ on PFOA-induced depression of $MO_2$

Using 50 mg/L $TiO_2$ and 0.5 mg/L PFOA as common elements in our assay, we investigated the effect of different sized $TiO_2$ as a co-contaminant on $MO_2$ (Fig 3.7C). $MO_2$ was significantly reduced by 22% and 11%, in the 25 nm and 100 nm $TiO_2$ treatment groups, respectively. However, 5 nm $TiO_2$ resulted in a much greater (32%) significant reduction in $MO_2$ compared to the absence of $TiO_2$.

## 3.4 DISCUSSION

In this study, we have demonstrated that PFOA is rapidly taken up by Daphnia magna. Importantly, this uptake can be significantly potentiated by the presence of $TiO_2$, with the smaller $TiO_2$ particles resulting in a greater potentiation of PFOA uptake. Moreover, the presence of $TiO_2$ in PFOA containing solutions resulted in a greater reduction of the PFOA-induced $MO_2$, again, with the smaller sized 5 nm $TiO_2$ showing greater reductions in $MO_2$ compared to 25 and 100 nm $TiO_2$.

### 3.4.1 Measurement of PFOA uptake and effects on whole body metabolism

Measurement of PFOA uptake by Daphnia magna was accomplished by modification of our previously published method on Pacific oysters (Farajizadeh et al, submitted). This method allows for acute measurement of unidirectional PFOA uptake rates at environmentally relevant concentrations. Aquatic PFOA concentrations have been often measured at low µg/L (Geng et al. 2021b) although measurements are usually higher near the sources of production. We chose 10 µg/L as an optimal exposure concentration for uptake experiments to increase sensitivity but remain close to the environmentally relevant range. Unidirectional uptake of PFOA increased with exposure time up until 2 h and then levelled off or decreased after this time, likely due to increase in excretion rate by the Daphnia to match the uptake rate. Lower uptake rates in the shorter exposure time periods are likely the result of the experiment is being done in a fixed volume static system where longer exposure period would result in resampling of the water and hence the accumulation rate increases with exposure time. In our experiments, PFOA exposure at or above 0.1 mg/L caused a significant decrease in whole body metabolic oxygen consumption suggesting a metabolic stress as result of PFOA exposure. Pollutant-induced reductions in metabolic rate have been previously demonstrated by numerous researchers (Armitage and Lei 1979; J. C. Martins et

al. 2007; Reyes et al. 2002; Wu and Chen 2004). Martins and colleagues (2007) showed that Daphnia magna had significantly lowered $MO_2$ when exposed to several different toxicants (J. C. Martins et al. 2007). With respect to PFOA, Hagenarrs et al (2013) found an ~ 60% reduction in mitochondrial oxygen consumption in zebrafish exposed to 1 mg/L PFOA with proteomic analysis suggesting the principal target o PFOA disruption is metabolic processes (Hagenaars et al. 2013b). Similarly, Gebreab et al (2020) that 25-200 ppm PFOA exposed zebrafish embryos (*Danio rerio*) displayed a 70% reduction in mitochondrial metabolic oxygen demand(Gebreab et al. 2020). While the exact mechanism by which PFOA induces decreased $MO_2$ is relatively understudied, the findings above correlates with our significant reductions in whole animal metabolic oxygen consumption and suggests impairment of mitochondrial function (Souders et al. 2021). PFOA exposure has also been demonstrated to significantly alter metabolite concentrations in the muscles of fish with Tyrosine, Tryptophan, Glutamate, Glutamine, choline, and GABA all being affected by PFOA exposure (Souders et al. 2021).

### 3.4.2 Association between PFOA and $TiO_2$ nanoparticles and effects on particle behaviour

It is well known that nanoparticles in environmental solutions can associated with various pollutants (POPs, proteins, dissolved organic carbon etc) and inorganics (metals) to form an eco-corona (Cedervall et al. 2007; Chetwynd et al. 2020; Khan et al. 2020; Pettibone et al. 2008). Similarly, recent studies have also demonstrated association of POPs with the hydrophobic surfaces of nanoplastics (Lin et al. 2020; Ma et al. 2016b; Zhang et al. 2020). Generally, $TiO_2$ *NP*s tend to form a larger cluster in water and have high aggregation rates. This was demonstrated by the relatively large hydrodynamic size of the particles in solution compared to the actual physical size (~5 nm, ~25 nm, and ~100 nm) as seen in the TEM. The presence of PFOA, which has a terminal carboxylic acid functional group which results in an outward facing negative surface

charge. As we saw in the results, PFOA surface coating of the $TiO_2$ particles led to a decreased zeta potential (increased negative charge), a diminished average hydrodynamic size of each of the three particle sizes, and an increase in the poly dispersion index, using DLS technique. DLS is not the most accurate technique for particle size analysis but since we are comparing the hydrodynamic characteristics of PFOA -spiked TiO2 with non-co-contaminated TiO2, DLS is an acceptable method. These changes would be predicted to increase the bioavailability of both the $TiO_2$ and PFAO. The mechanisms by which this happens is likely due to increase repulsion between PFOA-spiked $TiO_2$ particles by a greater charge:charge interaction. This increase repulsion would reduce aggregation as revealed in the improved polydispersity and smaller average clusters formed shown as average particle size (Pettibone et al. 2008; Wang et al. 2005).

### 3.4.3 Effects of $TiO_2$ co-contamination on PFOA uptake and toxicity

It has been previously demonstrated that many POPs sorb onto the surface of a variety of NPs (Cole et al. 2011; Ma et al. 2016b; Wang et al. 2019; Zhang et al. 2020). We have also previously demonstrated that 20 and 500 nm nanoplastics can modulate the uptake of PFOA into marine oysters, with 20 nm nanoplastics having a greater effect compared to 500 nm nanoplastics. Together, these findings confirm that the presence of particulate matter, specifically very small nano-sized materials (< 100 nm), have the potential to significantly exacerbate organic toxicity. Given that regulatory limits are often based on acute exposures (e.g., $LC_{50}$, $EC_{50}$) of contaminants in the relative absence of nano sized particles, that means that regulatory limits for contaminant releases of high particulate effluents (e.g., municipal waste, mining wastewater) may need to be revisited. A higher particulate co-contamination rate can lead to a higher accumulation of organics (Cole et al. 2011; Ma et al. 2016b; Wang et al. 2019; Zhang et al. 2020). It is likely that the relatively high hydrophobicity of the surface of some nanoparticles (e.g., $TiO_2$, CeO) will interact

more strongly with organics with high $K_{ow}$ (Lin et al. 2020). Therefore, the interaction between organics and particulate matter, specifically nano-sized materials, should be further investigated to determine the relative importance of physico-chemical characteristics on uptake of various POPs and their associated toxicity. While PFOA alone has been shown by many others to negatively affect $MO_2$ in a variety of organisms (Gebreab et al. 2020; Hagenaars et al. 2013b), our results demonstrate that the presence of $TiO_2$ particles co-associated with PFOA resulted in an even more substantial inhibition of $MO_2$.

At this stage, we are unsure of the mechanism responsible for increased uptake of PFOA in the presence of $TiO_2$ and the effect of particle size on PFOA uptake. Possible explanations are that smaller size nano particles (< 500nm) are known to cross cell membranes (Besseling et al. 2014; Mattsson et al. 2015; Zhang et al. 2020) and these could serve as a "trojan horse" ferrying the PFOA into the animal (Ma et al. 2016a; Zhang et al. 2020). Other explanations include increase polydispersity and mobility in solutions for smaller particles resulting in increased uptake or alternatively, simply having more smaller particles close to the cell membranes, resulting in dissociation of the PFOA from the $TiO_2$ near the membrane and an increased rate of transfer of the hydrophobic PFOA (and not the TiO2 particle per se). The mechanism by which $TiO_2$ potentiate organic uptake is understudied and needs to be further investigated.

### 3.5 Conclusion

In this study, a radiotracer-based method was developed to measure unidirectional accumulation of PFOA at environmentally relevant concentrations (Geng et al. 2021b). It is clearly shown that PFOA in the water is transferred rapidly into daphnia with time- and concentration-dependent rates. We also demonstrated that PFOA sorption to $TiO_2$ nanoparticles accelerates its accumulation rate in the animal. A greater increase of uptake rate is seen in co-contamination with

a smaller particle size (5 nm $TiO_2$) when compared to bigger sizes (25 nm and 100 nm $TiO_2$). PFOA is known to alter the metabolic status of many organisms as demonstrated by a reduced MO2. Interestingly, exposure to PFOA-spiked $TiO_2$ nanoparticles also exacerbated the decrease in oxygen consumption suggesting that organic contaminants in the presence of nanoparticles can significantly increase toxicological impact. Smaller sized particles have a greater specific surface area and also a high mobility in solution and can serve as vectors for transport and uptake of different toxicants (including POPs) (Pettibone et al. 2008; Zhang et al. 1999). Future directions include investigating the underlying properties affecting co-contaminant uptake in a variety of nano-sized materials and POPs. The possible potentiation of various pollutants (specifically POPs) uptake and toxicity by different hydrophobic particles is a growing concern and requires further research to modify and/or create relevant hazard and risk assessment models.

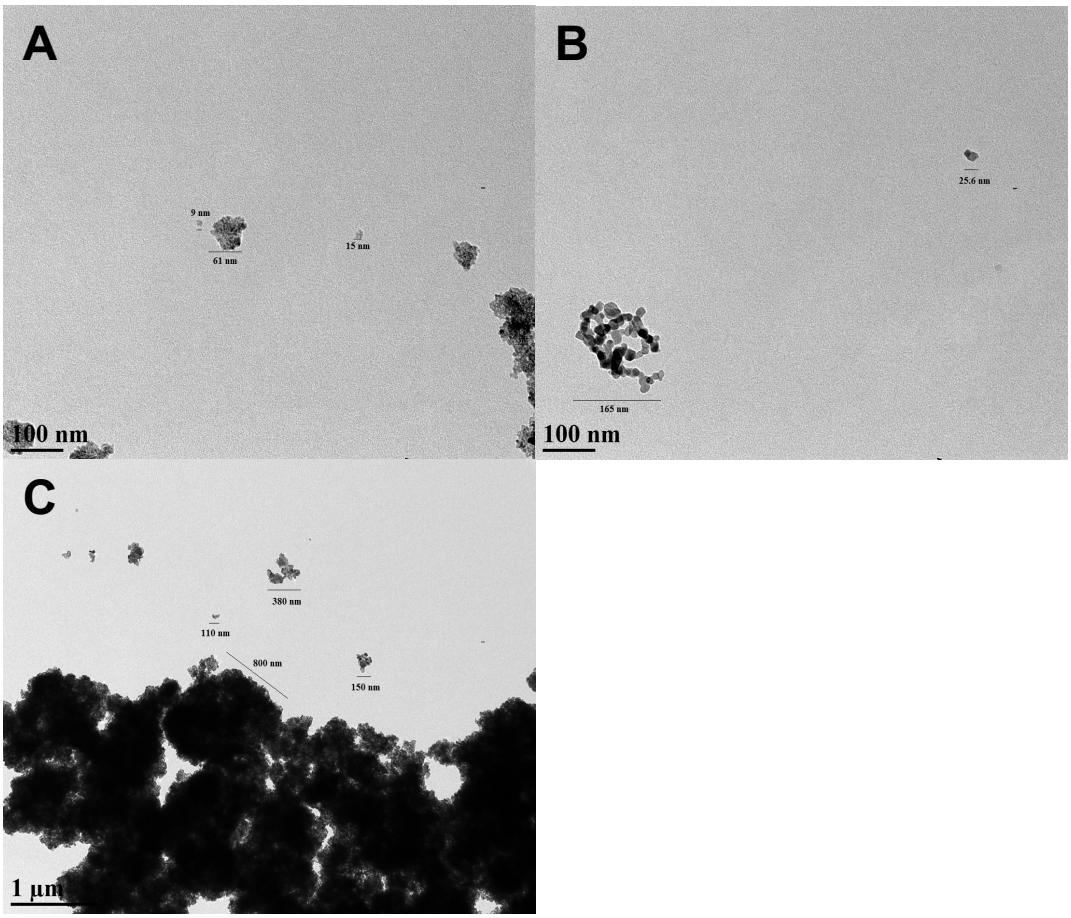
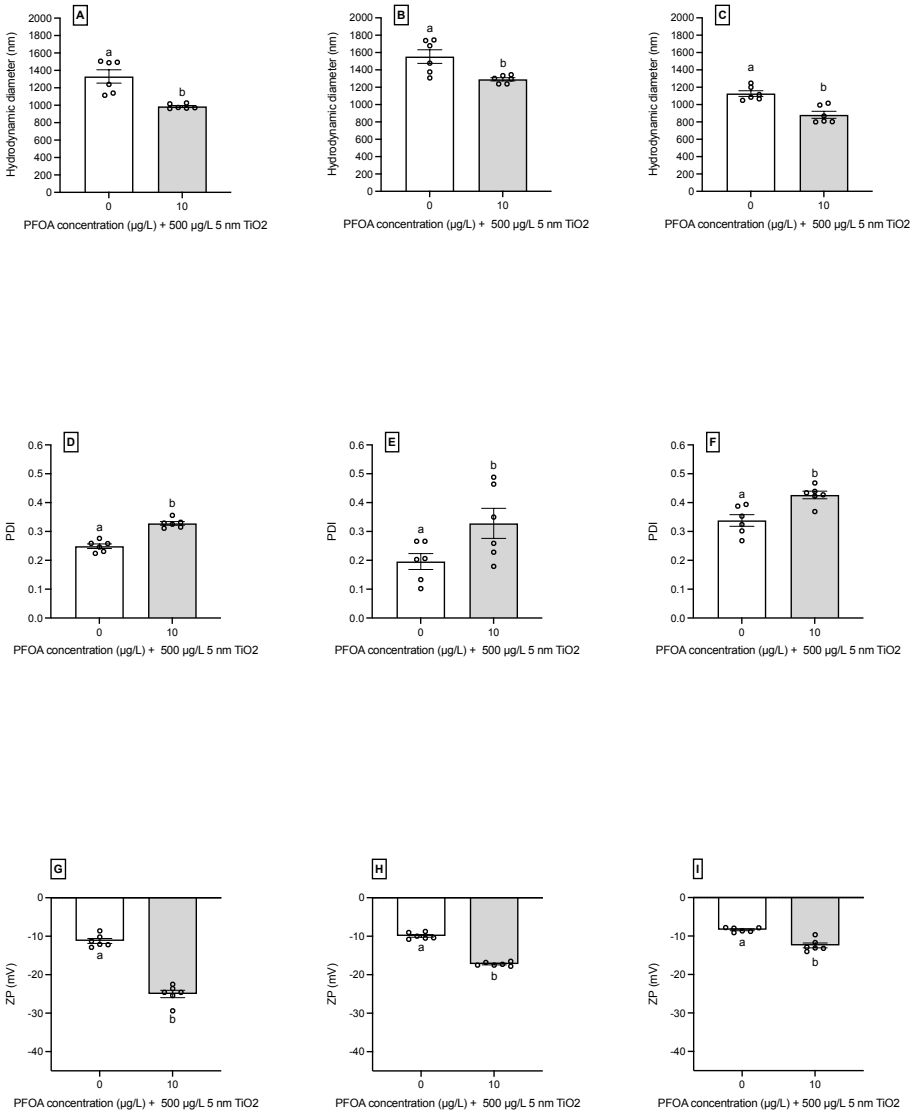



**Figure 3-1**

**Transmission Electronic Microscope (TEM) pictures of 2 g/L (A) 5 nm, and (B) 25 and (C) 100 nm $TiO_2$ nanoparticles using 18000X, 18000X, and 10000X magnification, respectively.**




## Figure 3-2

**Evaluation of 0.5 mg/L $TiO_2$ nanoparticle only and 0.5 mg/L $TiO_2$ nanoparticle co-contaminated with 10 µg/L PFOA difference in size (of (A) 5 nm, (B) 25 nm and (C) 100 nm), Polydispersity index (PDI) (of (D) 5 nm, (E) 25 nm and (F) 100 nm), and Zeta-potential (ZP) (of (G) 5 nm, (H) 25 nm and (I) 100 nm) $TiO_2$ nanoparticles using Dynamic light scattering (DLS) method.** (T-test : P-value_A=0.0.0012; P-value_B=0.0087; P-value_C=0.0008; P-value_D=<0.0001; P-value_E=0.0491; P-value_F=0.0044; P-value_G=<0.0001; P-value_H =<0.0001; P-value_I=0.0001). Mean values sharing the same lower-case letter are not significantly different among PFOA concentrations. Data are means ± SEM (n = 6). Symbols are individual data points.

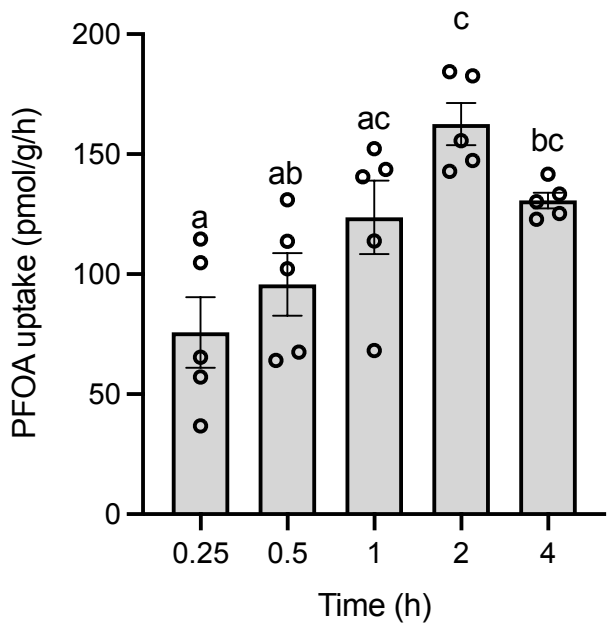



**Figure 3-3**

**Uptake rate (pmol/h $g^{-1}$) of, 10 µg/L PFOA at 0.25, 0.5, 1, 2, and 4 h in the daphnia (*Daphna magna*).** (One-way ANOVA: P-value =0.0006). Mean values sharing the same lower- case letter are not significantly different among PFOA concentrations. Data are means ± SEM (n = 5). Symbols are individual data points.

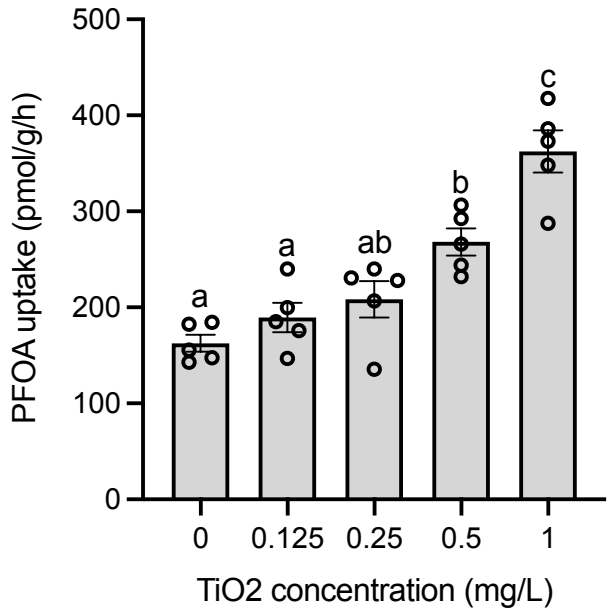



**Figure 3-4**

**Uptake rate (pmol/h $g^{-1}$) of 10 µg/L PFOA (10 µg/L) co-contaminated with 0, 0.125, 0.25, 0.5, and 1 mg/L of 5 nm $TiO_2$ nanoparticles in 2 hours of exposure in daphnia (*Daphnia magna*)**. Mean values sharing the same lower-case letters are not significantly different among different $TiO_2$ nanoparticles concentrations. (One-way ANOVA: P-value =<0.0001). Data are means ± SEM (n = 5). Symbols are individual data points.

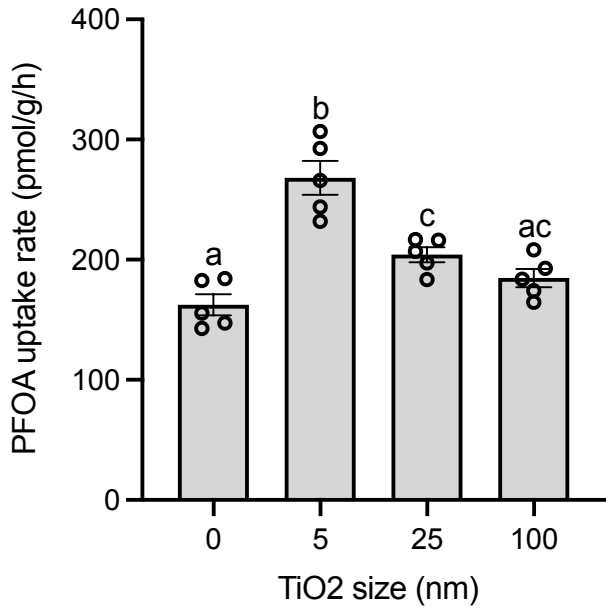
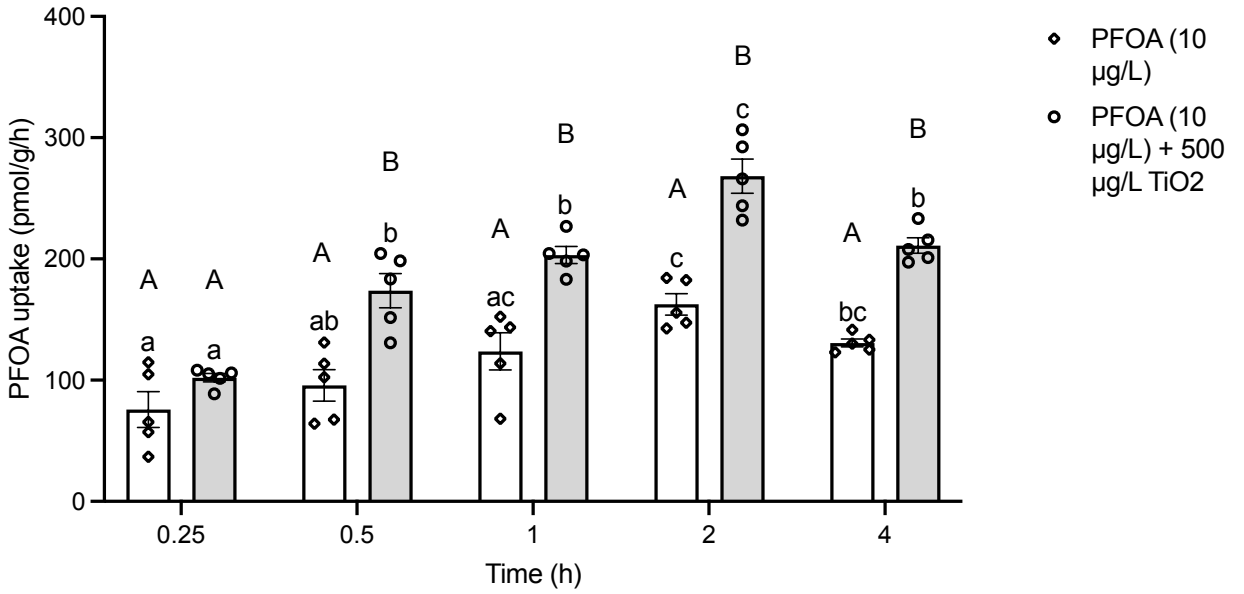


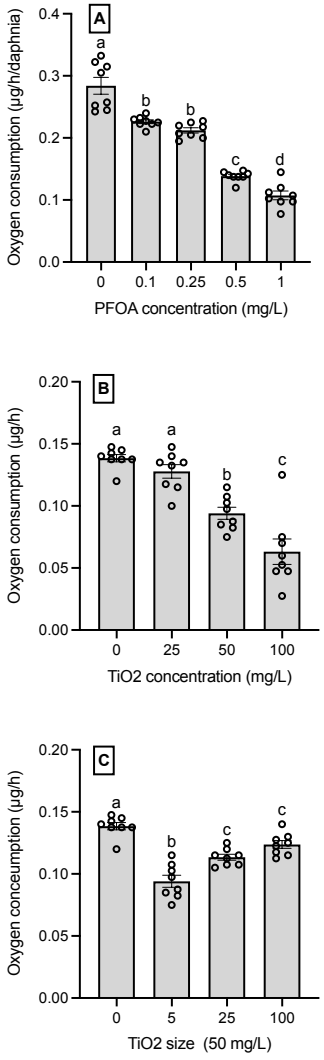

**Figure 3-5**

**Uptake rate (pmol/h $g^{-1}$) of 10 µg/L PFOA (10 µg/L) co-contaminated with 0.5 mg/L of 5, 25, and 100 nm $TiO_2$ nanoparticles in 2 hours of exposure in daphnia (*Daphnia magna*)**. Mean values sharing the same lower-case letters are not significantly different among different $TiO_2$ nanoparticles sizes. (One-way ANOVA: P-value =<0.0001). Data are means ± SEM (n = 5). Symbols are individual data points.




## Figure 3-6

**Uptake rate (pmol/h $g^{-1}$) of 10 µg/L $^{14}$C-PFOA (100 µg/L) co-contaminated with 0, and 500 mg/L 5 nm $TiO_2$ nanoparticles in 0.25, 0.5, 1, 2, and 4 hours of exposure in the daphnia (*Daphnia magna*)**. Mean values sharing the same upper-case letter are not significantly different among different exposure periods for same $TiO_2$ concentrations. Mean values sharing the same lower-case letters are not significantly different among different 5 nm $TiO_2$ concentrations for same exposure periods. (Two-way ANOVA P-values : $P_{interaction}$=<0.0156, $P_{time}$=<0.0001, $P_{concentration}$=<0.0001). Data are means ± SEM (n = 5). Symbols are individual data points.




## Figure 3-7

**Metabolic oxygen consumption ($MO_2$) (µg/h daphnia$^{-1}$) concentration in the daphnia (*daphnia magna*) after 48 h of exposure to (A) 0, 0.1, 0.25, 0.5, and 1 mg/L PFOA, (B) 0.5 mg/L PFOA co-contaminated with 0, 25, 50, and 100 mg/L 5 nm $TiO_2$ nanoparticles, and (C) 0.5 mg/L PFOA co-contaminated with 50 mg/L of 5, 25, and 100 nm $TiO_2$ nanoparticles.** (One-way ANOVA: P-value_A =<0.0001; P-value_B=<0.0001; P-value_C=<0.0001). Mean values sharing the same lower- case letter are not significantly different among different (A) PFOA concentrations, (B) 5 nm $TiO_2$ nanoparticles concentrations, and (C) $TiO_2$ nanoparticles sizes. Data are means ± SEM (n = 8). Symbols are individual data points.

# 4 Chapter 4: General conclusion

## 4.1 Conclusion

Nanoparticles (NM), alongside other pollutants including persistent organic pollutants, are frequently co-released into the environment (Ma et al. 2016b; Pettibone et al. 2008; Zhang et al. 2020). These persistent organic pollutants can interact with the nanoparticles in the aquatic environment, forming an ecocorona on the surface of the *NP* (Khan et al. 2020; Pettibone et al. 2008; Zhang et al. 2020). The goal of my series of experiments was to determine if there is a Trojan horse effect of the nanomaterial through the adsorption of different pollutants, and a subsequent increase in the exposure and their exposure effects on different aquatic organisms. The adsorption of PFOA onto the hydrophobic surface of both polystyrene nanoparticles (PS-NPs) and Titanium dioxide ($TiO_2$) was demonstrated by the changes in the characteristics of the dispersed particles as demonstrated through changes in properties found by dynamic light scattering (DLS). My results demonstrate a significantly decreased zeta potential, increased polydispersity index, and an increased or decreased particle size for PS-NPs or $TiO_2$ nanoparticles, respectively in the presence of PFOA. Given that PFOA possesses a negative carboxylic group, I also hypothesized that exposure to this mixture of *NP*s and the PFOA toxicant would modulate/potentiate the effect of the chemical in exposed organisms. My data for both types of hydrophobic *NP*s demonstrated that the co-occurrence of the PFOA contaminant with these *NP*s results in an accelerated transport and bioconcentration of the adsorbed toxicant in the exposed animals. During my research program, I was able to develop a novel radiotracer-based method to unidirectionally measure organic toxicant (PFOA) uptake in different aquatic organisms at various environmentally and scientifically realistic concentrations (Geng et al. 2021b). My data then clearly demonstrates that the presence of PFOA in the environment results in a time and concentration-dependent accumulation in both

the Pacific oyster (*Magallana gigas*) and the water flea Daphnia (*Daphnia magna*). Furthermore, my data clearly shows that the Trojan horse effect of NM can significantly enhance the uptake of PFOA in exposed animals and exacerbate the effects of PFOA compared to PFOA exposure alone. For example, I have demonstrated that PFOA sorption to 20 nm and 500 nm sized PS-NPs potentiate PFOA uptake in Pacific oysters by 196% and 72%, respectively. Additionally, I showed that PFOA adsorption to 5 nm, 25 nm, and 100 nm $TiO_2$ nanoparticles increased the uptake rate of the PFOA in daphnia by 65%, 25%, and 13%, respectively.

The potential for *NP*s to intensify accumulation and exposure translates to increased measures of organisms' toxicity of the adsorbed pollutant as measured by potentiates TBARs and $MO_2$ measured in exposed animals. My data is the first to study PFOA toxicity using radiotracer-based means and is one of only a handful of studies that examine the effects of PFOA on the physiology of organisms at environmentally relevant levels. I have demonstrated that higher concentrations of PFOA result in intensified lipid peroxidation (LPO) and reduced metabolic oxygen consumption ($MO_2$) in both marine (oysters) and freshwater (daphnia) organisms. Moreover, my data also demonstrates that co-contaminant mixtures of PFOA and PS-NPs will increase LPO in oysters by at least 3-fold. Similarly, the reduced $MO_2$ response in my data also suggests that the presence of $TiO_2$ as a co-contaminant will increase the toxicological impact of PFOA by at least 55%. Overall, my research clearly demonstrates that nanomaterial can be vectors for enhancing the transport of organic substances into organisms, and this accumulation is enhanced when smaller particles with large specific surface areas (SSA), high dispersions rates, and increased mobility are present.

There is a very large variety of possible adsorbable pollutants in the environment that are generated from sources like industrial or domestic wastewater (Gao et al. 2004; Jaroenworaluck et al. 2006; Lin et al. 2020; Ma et al. 2016b; Pettibone et al. 2008; Wang et al. 2019; Zhang et al. 2020), with

each toxicant demonstrated to have a specific toxicological effect (Geng et al. 2021b; Hagenaars et al. 2013b; Liu et al. 2007; J. C. Martins et al. 2007; Wu and Chen 2004). Similarly, different types of nanoparticles are abundantly present in the environment as a result of release from multiple applications including medicine, pharmacy, paint, sunscreen, food colourings, etc. (Gurman et al. 1987; Hussain et al. 2010; Mattsson et al. 2018; Preočanin and Kallay 2006), with each having demonstrated to have their own toxicological impacts (Brandts et al. 2018; Chae and An 2017; Ma et al. 2016b). While the diversity of outcomes from these interactions between the NM and various toxicants is daunting, I believe that the potential effects of co-contamination in nanoparticles with various toxic chemicals are a major concern and further investigation is needed to examine the underlying properties (e.g. . surface reactivity of NPA, $K_{ow}$, charge) of toxicants to create modified models for the prediction of both hazard and risk assessment.

### 4.2 Future directions

As mentioned, there is an incredibly high variety of nanomaterials with a hydrophobic surface making them possible candidates for being a suitable vector for different hydrophobic pollutants (Gurman et al. 1987; Hussain et al. 2010; Mattsson et al. 2018; Preočanin and Kallay 2006). Additionally, there are many toxicants with adsorption capacity onto the surface on nanomaterials (Gao et al. 2004; Jaroenworaluck et al. 2006; Lin et al. 2020; Ma et al. 2016b; Pettibone et al. 2008; Wang et al. 2019; Zhang et al. 2020). Future directions should explore the potential for the adsorption of the mentioned pollutants (e.g., heavy metals, other POPs) onto the hydrophobic surface of different nanomaterials (e.g., CeO, nAg) and whether the formation of co-contaminated compounds resulting in a potentiated accumulation and toxicity of the mixture on different exposed aquatic (e.g., mussels, shrimps, and fish) and/or terrestrial (e.g., rodents and/or primates) organism.

Given that co-contamination between nanomaterials and various toxicants is constantly happening in the environment, especially near the source of the pollutant to surface waters, samples from wastewater should be analyzed for the presence of these co-contaminated compounds c to demonstrate the percentage and actual level of the pollutant sorption onto the surface of NM in the presence of high natural organic matter which likely also affects the sorption capacity and enhanced toxicity of co-contamination.

My thesis demonstrated that adsorption to NMs does happen and results in higher uptake and toxicity of the pollutant. However, the potential for *NP*s to affect the excretion of the PFOA in the exposed animals remains unstudied. Additionally, I did not investigate if the POP and the *NP* remain associated inside the animal or if there is dissociation, and if so, to what extent.

Finally, although co-contamination results in various Trojan horse effects and potentiates the toxicity of various organisms in laboratory conditions(Ma et al. 2016b; Pettibone et al. 2008; Zhang et al. 2020), we are unsure of the impact in real-world scenarios. Given that applications of nanomaterial and their uses is highly beneficial for multiple fields including medicine and industry, it is unwise to ask for production to be slowed as the benefits likely outweigh the harm as this point and current regulations have considerable safety factors worked into regulations. Nontoxic chemicals (including proteins, DOC, etc.) are known to form Eco-coronas by sorption to the surface of the nanomaterial including Plastic and $TiO_2$ nanoparticles (Cai et al. 2022; Khan et al. 2020). This adsorption likely results in the blockage of the surface and inhibition of the adsorption of other toxicants. Future research conducting experiments to evaluate the possibility of formation and effects of other possible protein or non-protein coronas when *NP*s are released into the environment is recommended.

# 5 References


Agency for Toxic Substances and Disease Registry (ATSDR). 2018. 'Federal Register :: Availability of Draft Toxicological Profile: Perfluoroalkyls'. *Department of Health and Human Services (HHS)*. Retrieved 12 May 2023 (https://www.federalregister.gov/documents/2018/06/21/2018-13385/availability-of-draft-toxicological-profile-perfluoroalkyls).

Aguilar Diaz De Leon, Jesús, and Chad R. Borges. 2020. 'Evaluation of Oxidative Stress in Biological Samples Using the Thiobarbituric Acid Reactive Substances Assay'. *JoVE (Journal of Visualized Experiments)* 2020(159):e61122. doi: 10.3791/61122.

Ahern, Mark D., and Steve Morris. 1999. 'Respiratory, Acid–Base and Metabolic Responses of the Freshwater Crayfish Cherax Destructor to Lead Contamination'. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 124(2):105–11. doi: 10.1016/S1095-6433(99)00101-4.

Akakuru, Ozioma U., Zubair M. Iqbal, and Aiguo Wu. 2020. 'TiO2 Nanoparticles'. *Wiley TiO* 1–66. doi: 10.1002/9783527825431.CH1.

Amraoui, Imen, Noureddine Khalloufi, and Samir Touaylia. 2018. 'Effects to Perfluorooctane Sulfonate (PFOS) on the Mollusk Unio Ravoisieri under Laboratory Exposure'. *Https://Doi.Org/10.1080/02757540.2018.1433168* 34(4):324–39. doi: 10.1080/02757540.2018.1433168.

AL Andarady. 2011. 'A. L. Andrady, Microplastics in the Marine Environment,... - Google Scholar'. *Mar Pollute Bull*. Retrieved 12 May 2023 (https://scholar.google.ca/scholar?hl=en&as_sdt=0%2C5&q=A.+L.+Andrady%2C+Micropl

astics+in+the+marine+environment%2C+Mar.+Pollut.+Bull.%2C+2011%2C+62%3A+1596-1605.+&btnG=).

Andrady, Anthony L. 2011. 'Microplastics in the Marine Environment'. *Marine Pollution Bulletin* 62(8):1596–1605. doi: 10.1016/J.MARPOLBUL.2011.05.030.

Antus, Balazs, Orsolya Drozdovszky, Imre Barta, and Krisztina Kelemen. 2015. 'Comparison of Airway and Systemic Malondialdehyde Levels for Assessment of Oxidative Stress in Cystic Fibrosis'. *Lung* 193(4):597–604. doi: 10.1007/S00408-015-9739-1/TABLES/3.

Armitage, Kenneth B., and Chi Hsiang Lei. 1979. 'Temperature Acclimatization in the Filtering Rates and Oxygen Consumption of Daphnia Ambigua Scourfield'. *Comparative Biochemistry and Physiology Part A: Physiology* 62(4):807–12. doi: 10.1016/0300-9629(79)90007-0.

Astefanei, Alina, Oscar Núñez, and Maria Teresa Galceran. 2014. 'Analysis of C60-Fullerene Derivatives and Pristine Fullerenes in Environmental Samples by Ultrahigh Performance Liquid Chromatography–Atmospheric Pressure Photoionization-Mass Spectrometry'. *Journal of Chromatography A* 1365:61–71. doi: 10.1016/J.CHROMA.2014.08.089.

Awad, Raed, Yihui Zhou, Elisabeth Nyberg, Shahla Namazkar, Wu Yongning, Qianfen Xiao, Yaije Sun, Zhiliang Zhu, Åke Bergman, and Jonathan P. Benskin. 2021. 'Correction: Emerging per- and Polyfluoroalkyl Substances (PFAS) in Human Milk from Sweden and China'. *Environmental Science: Processes & Impacts* 23(1):188–188. doi: 10.1039/D0EM90043E.

Bai, Xuelian, and Yeongkwon Son. 2021. 'Perfluoroalkyl Substances (PFAS) in Surface Water and Sediments from Two Urban Watersheds in Nevada, USA'. *Science of The Total Environment* 751:141622. doi: 10.1016/J.SCITOTENV.2020.141622.

Bartell, Scott M., Antonia M. Calafat, Christopher Lyu, Kayoko Kato, P. Barry Ryan, and Kyle Steenland. 2010. 'Rate of Decline in Serum PFOA Concentrations after Granular Activated Carbon Filtration at Two Public Water Systems in Ohio and West Virginia'. *Environmental Health Perspectives* 118(2):222–28. doi: 10.1289/EHP.0901252.

Bäuerlein, Patrick S., Erik Emke, Peter Tromp, Jan A. M. H. Hofman, Andrea Carboni, Ferry Schooneman, Pim de Voogt, and Annemarie P. van Wezel. 2017. 'Is There Evidence for Man-Made Nanoparticles in the Dutch Environment?' *Science of The Total Environment* 576:273–83. doi: 10.1016/J.SCITOTENV.2016.09.206.

Bayne, B. L., C. J. Bayne, C. T. Carefoot, and R. T. Thompson. 1976. 'The Physiological Ecology of Mytilus Californianus Conrad. 2. Adaptations to Low Oxygen Tension and Air Exposure on JSTOR'. *Oecologia*. Retrieved 20 May 2023 (https://www.jstor.org/stable/4215211?casa_token=-ii9eFZIP2kAAAAA%3AtxtjfVDWO0MxUX6j561EXWdp_jcnd1Yv0VGKbzfyjXBtqC99xvI3ar7bsMWKYmvnOzDq6cy15vx3DacyPp3htyskCKZ-ZDyfFoyPUhcbwDwVYEV-KW4).

Berne, B. J., and R. Pecora. 1976. 'Dynamic Light Scattering: With Applications to Chemistry, Biology, and Physics - Bruce J. Berne, Robert Pecora - Google Books'. *Dover Publications*. Retrieved 19 May 2023 (https://books.google.ca/books?hl=en&lr=&id=vBB54ABhmuEC&oi=fnd&pg=PA1&dq=Berne+BJ,+Pecora+R+(1976)+Dynamic+light+scattering:+with+applications+to+chemistry,+biology,+and+physics.+John+Wiley+%26+Sons,+Inc,+New+York,+USA&ots=L7qFI4qmtg&sig=3-fl7e2CYBNf3QlUVPskdr6Efys#v=onepage&q&f=false).

Bernier, J., P. Brousseau, K. Krzystyniak, H. Tryphonas, and M. Fournier. 1995. 'Immunotoxicity of Heavy Metals in Relation to Great Lakes.' *Environmental Health Perspectives* 103(SUPPL. 9):23–34. doi: 10.1289/EHP.95103S923.

Besseling, Ellen, Bo Wang, Miquel Lürling, and Albert A. Koelmans. 2014. 'Nanoplastic Affects Growth of S. Obliquus and Reproduction of D. Magna'. *Environmental Science and Technology* 48(20):12336–43. doi: 10.1021/ES503001D/SUPPL_FILE/ES503001D_SI_001.PDF.

Betts, Kellyn. 2007. 'PFOS and PFOA in Humans: New Study Links Prenatal Exposure to Lower Birth Weight'. *Environmental Health Perspectives* 115(11). doi: 10.1289/EHP.115-A550A.

Betts, Kellyn S. 2007. 'PERFLUOROALKYL ACIDS: What Is the Evidence Telling Us?' *Environmental Health Perspectives* 115(5). doi: 10.1289/EHP.115-A250.

Binelli, A., L. Del Giacco, N. Santo, L. Bini, S. Magni, M. Parolini, L. Madaschi, A. Ghilardi, D. Maggioni, M. Ascagni, A. Armini, L. Prosperi, C. Landi, C. La Porta, and C. Della Torre. 2017. 'Carbon Nanopowder Acts as a Trojan-Horse for Benzo(α)Pyrene in Danio Rerio Embryos'. *Https://Doi.Org/10.1080/17435390.2017.1306130* 11(3):371–81. doi: 10.1080/17435390.2017.1306130.

Bouallegui, Younes, Ridha Ben Younes, Houda Bellamine, and Ridha Oueslati. 2017. 'Histopathological Indices and Inflammatory Response in the Digestive Gland of the Mussel Mytilus Galloprovincialis as Biomarker of Immunotoxicity to Silver Nanoparticles'. *Https://Doi.Org/10.1080/1354750X.2017.1409803* 23(3):277–87. doi: 10.1080/1354750X.2017.1409803.

Boyden, C. R., and D. J. H. Philips. 1981. 'Seasonal Variation and Inherent Variability of Trace Elements in Oysters and Their Implications for Indicator Studies on JSTOR'. *Marine Ecology Progress Series*. Retrieved 18 May 2023 (https://www.jstor.org/stable/24812913?casa_token=g733PiMh7IUAAAAA%3AsuFTFFWWcnVK-mu-JVoH5BmG7oCJyn_ymCxAVwgQ_9kEWDrPN3FFfTAJnK1ZkF7XAE9s1BqD85B9J9RXm6lGPA7b618rMCJGsOSuaLaWoQNcDccf7Nw).

Brandts, I., M. Teles, A. P. Gonçalves, A. Barreto, L. Franco-Martinez, A. Tvarijonaviciute, M. A. Martins, A. M. V. M. Soares, L. Tort, and M. Oliveira. 2018. 'Effects of Nanoplastics on Mytilus Galloprovincialis after Individual and Combined Exposure with Carbamazepine'. *Science of The Total Environment* 643:775–84. doi: 10.1016/J.SCITOTENV.2018.06.257.

Brandts, Irene, Marlid Garcia-Ordoñez, Lluis Tort, Mariana Teles, and Nerea Roher. 2020. 'Polystyrene Nanoplastics Accumulate in ZFL Cell Lysosomes and in Zebrafish Larvae after Acute Exposure, Inducing a Synergistic Immune Response in Vitro without Affecting Larval Survival in Vivo'. *Environmental Science: Nano* 7(8):2410–22. doi: 10.1039/D0EN00553C.

Brede, Edna, Michael Wilhelm, Thomas Göen, Johannes Müller, Knut Rauchfuss, Martin Kraft, and Jürgen Hölzer. 2010. 'Two-Year Follow-up Biomonitoring Pilot Study of Residents' and Controls' PFC Plasma Levels after PFOA Reduction in Public Water System in Arnsberg, Germany'. *International Journal of Hygiene and Environmental Health* 213(3):217–23. doi: 10.1016/J.IJHEH.2010.03.007.

Bronmark, Christer., and Lars-Anders. Hansson. 2012. 'Chemical Ecology in Aquatic Systems - Google Books'. *Oxford*. Retrieved 19 May 2023

(https://books.google.ca/books?hl=en&lr=&id=3bCRvgOh2v4C&oi=fnd&pg=PA250&dq=L%C3%BCrling,+M.+Infodisruption:+pollutants+interfering+with+the+natural+chemical+information+conveyance+in+aquatic+systems.+In+Br%C3%B6nmark,+C.,+Hansson,+L.-A.,+Eds.+Chemical+Ecology+in+Aquatic+Systems%3B+Oxford+University+Press:+Oxford,+UK%3B+2012%3B+pp+250%E2%88%92271.&ots=nbFAPOSIxS&sig=DnzMpNJ1Z4eQFkEr4JkFEL7UIhQ#v=onepage&q&f=false).

C Loss, T. Syrovets, A. Musyanovich, K. landfester. 2014. 'Loss: Functionalized Polystyrene Nanoparticles As... - Google Scholar'. *Nanotechnology*. Retrieved 11 May 2023 (https://scholar.google.com/scholar_lookup?title=Functionalized%20polystyrene%20nanoparticles%20as%20a%20platform%20for%20studying%20bio-nano%20interactions&author=C.%20Loss&publication_year=2014&pages=2403-2412).

Cai, Kaihan, Qingbin Song, Wenyi Yuan, Jujun Ruan, Huabo Duan, Ying Li, and Jinhui Li. 2020. 'Human Exposure to PBDEs in E-Waste Areas: A Review'. *Environmental Pollution* 267:115634. doi: 10.1016/J.ENVPOL.2020.115634.

Cai, Rong, Jiayu Ren, Mengyu Guo, Taotao Wei, Ying Liu, Chunyu Xie, Peng Zhang, Zhiling Guo, Andrew J. Chetwynd, Pu Chun Ke, Iseult Lynch, and Chunying Chen. 2022. 'Dynamic Intracellular Exchange of Nanomaterials' Protein Corona Perturbs Proteostasis and Remodels Cell Metabolism'. *Proceedings of the National Academy of Sciences of the United States of America* 119(23):e2200363119. doi: 10.1073/PNAS.2200363119/SUPPL_FILE/PNAS.2200363119.SD01.XLSX.

Cajaraville, Miren P., Maria J. Bebianno, Julián Blasco, Cinta Porte, Carmen Sarasquete, and Aldo Viarengo. 2000. 'The Use of Biomarkers to Assess the Impact of Pollution in Coastal

Environments of the Iberian Peninsula: A Practical Approach'. *Science of The Total Environment* 247(2–3):295–311. doi: 10.1016/S0048-9697(99)00499-4.

Cavaletto, M., A. Ghezzi, B. Burlando, V. Evangelisti, N. Ceratto, and A. Viarengo. 2002. 'Effect of Hydrogen Peroxide on Antioxidant Enzymes and Metallothionein Level in the Digestive Gland of Mytilus Galloprovincialis'. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 131(4):447–55. doi: 10.1016/S1532-0456(02)00030-3.

Cedervall, Tommy, Iseult Lynch, Stina Lindman, Tord Berggård, Eva Thulin, Hanna Nilsson, Kenneth A. Dawson, and Sara Linse. 2007. 'Understanding the Nanoparticle-Protein Corona Using Methods to Quntify Exchange Rates and Affinities of Proteins for Nanoparticles'. *Proceedings of the National Academy of Sciences of the United States of America* 104(7):2050–55. doi: 10.1073/PNAS.0608582104/SUPPL_FILE/IMAGE97.GIF.

Chae, Yooeun, and Youn Joo An. 2017. 'Effects of Micro- and Nanoplastics on Aquatic Ecosystems: Current Research Trends and Perspectives'. *Marine Pollution Bulletin* 124(2):624–32. doi: 10.1016/J.MARPOLBUL.2017.01.070.

Chen, Qiqing, Michael Gundlach, Shouye Yang, Jing Jiang, Mirna Velki, Daqiang Yin, and Henner Hollert. 2017. 'Quantitative Investigation of the Mechanisms of Microplastics and Nanoplastics toward Zebrafish Larvae Locomotor Activity'. *Science of The Total Environment* 584–585:1022–31. doi: 10.1016/J.SCITOTENV.2017.01.156.

Chen, Xiaobo, and Samuel S. Mao. 2007. 'Titanium Dioxide Nanomaterials: Synthesis, Properties, Modifications and Applications'. *Chemical Reviews* 107(7):2891–2959. doi: 10.1021/CR0500535/ASSET/IMAGES/MEDIUM/CR0500535F00041.GIF.

Chen, Yuling, Yifei Leng, Xiaoning Liu, and Jun Wang. 2020. 'Microplastic Pollution in Vegetable Farmlands of Suburb Wuhan, Central China'. *Environmental Pollution* 257:113449. doi: 10.1016/J.ENVPOL.2019.113449.

Chetwynd, Andrew J, Wei Zhang, James A Thorn, Iseult Lynch, Rawi Ramautar, A J Chetwynd, J A Thorn, I Lynch, W Zhang, and R Ramautar. 2020. 'The Nanomaterial Metabolite Corona Determined Using a Quantitative Metabolomics Approach: A Pilot Study'. *Small* 16(21):2000295. doi: 10.1002/SMLL.202000295.

Cheung, C. C. C., W. H. L. Siu, B. J. Richardson, S. B. De Luca-Abbott, and P. K. S. Lam. 2004. 'Antioxidant Responses to Benzo[a]Pyrene and Aroclor 1254 Exposure in the Green-Lipped Mussel, Perna Viridis'. *Environmental Pollution* 128(3):393–403. doi: 10.1016/J.ENVPOL.2003.09.010.

Chidambarampadmavathy, Karthigeyan, Obulisamy Parthiba Karthikeyan, and Kirsten Heimann. 2017. 'Sustainable Bio-Plastic Production through Landfill Methane Recycling'. *Renewable and Sustainable Energy Reviews* 71:555–62. doi: 10.1016/J.RSER.2016.12.083.

Chinni, Satyavathi, Ritindra N. Khan, and Prabhakara Rao Yallapragada. 2002. 'Acute Toxicity of Lead on Tolerance, Oxygen Consumption, Ammonia-N Excretion, and Metal Accumulation in Penaeus Indicus Postlarvae'. *Ecotoxicology and Environmental Safety* 51(2):79–84. doi: 10.1006/EESA.2000.2019.

Cole, Matthew, Pennie Lindeque, Claudia Halsband, and Tamara S. Galloway. 2011. 'Microplastics as Contaminants in the Marine Environment: A Review'. *Marine Pollution Bulletin* 62(12):2588–97. doi: 10.1016/J.MARPOLBUL.2011.09.025.

Collins, A., S. Venkatesan, P. Garcia, P. J. Pacy, and D. Halliday. 1992. 'Very Low Density Lipoprotein Apolipoproteins B-100 and B Turnover in Control Subjects Using l-[1-13C]-Leucine'. *Biochemical Society Transactions* 20(2):102S-102S. doi: 10.1042/BST020102S.

da Costa, João Pinto, Patrícia S. M. Santos, Armando C. Duarte, and Teresa Rocha-Santos. 2016. '(Nano)Plastics in the Environment – Sources, Fates and Effects'. *Science of The Total Environment* 566–567:15–26. doi: 10.1016/J.SCITOTENV.2016.05.041.

Darnerud, Per Ola, Gunnar S. Eriksen, Torkell Johannesson, Poul B. Larsen, and Matti Viluksela. 2001a. 'Polybrominated Diphenyl Ethers: Occurrence, Dietary Exposure, and Toxicology'. *Environmental Health Perspectives* 109:49. doi: 10.2307/3434846.

Darnerud, Per Ola, Gunnar S. Eriksen, Torkell Johannesson, Poul B. Larsen, and Matti Viluksela. 2001b. 'Polybrominated Diphenyl Ethers: Occurrence, Dietary Exposure, and Toxicology'. *Environmental Health Perspectives* 109:49. doi: 10.2307/3434846.

Deloid, Glen M., Xiaoqiong Cao, Roxana Coreas, Dimitrios Bitounis, Dilpreet Singh, Wenwan Zhong, and Philip Demokritou. 2022. 'Incineration-Generated Polyethylene Micro-Nanoplastics Increase Triglyceride Lipolysis and Absorption in an in Vitro Small Intestinal Epithelium Model'. *Environmental Science and Technology* 56(17):12288–97. doi: 10.1021/acs.est.2c03195.

D'eon, Jessica C., and Scott A. Mabury. 2011. 'Exploring Indirect Sources of Human Exposure to Perfluoroalkyl Carboxylates (PFCAs): Evaluating Uptake, Elimination, and Biotransformation of Polyfluoroalkyl Phosphate Esters (PAPs) in the Rat'. *Environmental Health Perspectives* 119(3):344–50. doi: 10.1289/EHP.1002409.

Devasagayam, T. P. A., K. K. Boloor, and T. Ramasarma. 2003. 'Methods for Estimating Lipid Peroxidation: An Analysis of Merits and Demerits'. *IJBB Vol.40(5) [October 2003]* 40:300–308.

Diebold, Ulrike. 2003. 'The Surface Science of Titanium Dioxide'. *Surface Science Reports* 48(5–8):53–229. doi: 10.1016/S0167-5729(02)00100-0.

Dodson, S. I., and T. Hanazato. 1995. 'Commentary on Effects of Anthropogenic and Natural Organic Chemicals on Development, Swimming Behavior, and Reproduction of Daphnia, a Key Member of Aquatic Ecosystems.' *Environmental Health Perspectives* 103(SUPPL. 4):7–11. doi: 10.1289/EHP.95103S47.

Domingo, José L., and Martí Nadal. 2017a. 'Per- and Polyfluoroalkyl Substances (PFASs) in Food and Human Dietary Intake: A Review of the Recent Scientific Literature'. *Journal of Agricultural and Food Chemistry* 65(3):533–43. doi: 10.1021/ACS.JAFC.6B04683/ASSET/IMAGES/MEDIUM/JF-2016-04683W_0001.GIF.

Domingo, José L., and Martí Nadal. 2017b. 'Per- and Polyfluoroalkyl Substances (PFASs) in Food and Human Dietary Intake: A Review of the Recent Scientific Literature'. *Journal of Agricultural and Food Chemistry* 65(3):533–43. doi: 10.1021/ACS.JAFC.6B04683/ASSET/IMAGES/MEDIUM/JF-2016-04683W_0001.GIF.

Domininghaus, Hans. 1992. 'Plastics and Their Properties. 4. Rev. Ed. Die Kunststoffe Und Ihre Eigenschaften'. *ETDE* 873.

DP, CHENEY. 2000. 'Summer Mortality of Pacific Oysters, Crassostrea Gigas (Thunberg) : Initial Finding on Multiple Environmental Stressors in Puget Sound, Washington, 1998'. *J Shellfish Res* 19:353–59.

Du, Di, Yonglong Lu, Yunqiao Zhou, Qifeng Li, Meng Zhang, Guoxiang Han, Haotian Cui, and Erik Jeppesen. 2021. 'Bioaccumulation, Trophic Transfer and Biomagnification of Perfluoroalkyl Acids (PFAAs) in the Marine Food Web of the South China Sea'. *Journal of Hazardous Materials* 405:124681. doi: 10.1016/J.JHAZMAT.2020.124681.

Enserink, E. L., J. L. Maas-Diepeveen, and C. J. Van Leeuwen. 1991. 'Combined Effects of Metals; an Ecotoxicological Evaluation'. *Water Research* 25(6):679–87. doi: 10.1016/0043-1354(91)90043-P.

Erik Lucas, Shawn Decker, Abbas Khaleel. 2001. 'Nanocrystalline Metal Oxides as Unique Chemical Reagents/Sorbents - Lucas - 2001 - Chemistry – A European Journal - Wiley Online Library'. *Chemistry Europe*. Retrieved 12 May 2023 (https://chemistry-europe.onlinelibrary.wiley.com/doi/abs/10.1002/1521-3765(20010618)7:12%3C2505::AID-CHEM25050%3E3.0.CO;2-R?casa_token=byq5vGcNcyUAAAAA:798vZgrl6mX8ZNnDejIz3LbFzbzKCAT6RHu_BrdU71JFJXFxtoc1htHXdKvY0BrLeJVTjgrS7k257w).

Fiedler, Heidelore, Roland Kallenborn, Jacob de Boer, and Leiv K. Sydnes. 2019. 'The Stockholm Convention: A Tool for the Global Regulation of Persistent Organic Pollutants'. *Chemistry International* 41(2):4–11. doi: 10.1515/CI-2019-0202.

Fiedler, Heidelore, and Mohammad Sadia. 2021. 'Regional Occurrence of Perfluoroalkane Substances in Human Milk for the Global Monitoring Plan under the Stockholm Convention on Persistent Organic Pollutants during 2016–2019'. *Chemosphere* 277:130287. doi: 10.1016/J.CHEMOSPHERE.2021.130287.

Franco, Marco E., Grace E. Sutherland, Maria T. Fernandez-Luna, and Ramon Lavado. 2020. 'Altered Expression and Activity of Phase I and II Biotransformation Enzymes in Human

Liver Cells by Perfluorooctanoate (PFOA) and Perfluorooctane Sulfonate (PFOS)'. *Toxicology* 430:152339. doi: 10.1016/J.TOX.2019.152339.

Fryer, G. 1991. 'Functional Morphology and the Adaptive Radiation of the Daphniidae (Branchiopoda: Anomopoda)'. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 331(1259):1–99. doi: 10.1098/RSTB.1991.0001.

Galloway, Jason E., Anjelica V. P. Moreno, Andrew B. Lindstrom, Mark J. Strynar, Seth Newton, Andrew A. May, Andrew A. May, Linda K. Weavers, and Linda K. Weavers. 2020. 'Evidence of Air Dispersion: HFPO-DA and PFOA in Ohio and West Virginia Surface Water and Soil near a Fluoropolymer Production Facility'. *Environmental Science and Technology* 54(12):7175–84. doi: 10.1021/ACS.EST.9B07384/SUPPL_FILE/ES9B07384_SI_001.PDF.

Gao, Y., R. Wahi, A. T. Kan, J. C. Falkner, V. L. Colvin, and M. B. Tomson. 2004. 'Adsorption of Cadmium on Anatase Nanoparticles-Effect of Crystal Size and PH'. *Langmuir* 20(22):9585–93. doi: 10.1021/LA049334I/ASSET/IMAGES/MEDIUM/LA049334IN00001.GIF.

Garbovskiy, Yuriy. 2017. 'Biological Contamination of Nanoparticles and Its Manifestation in Optical Absorbance Measurements'. *Analytical Chemistry* 89(14):7282–85. doi: 10.1021/ACS.ANALCHEM.7B01766/ASSET/IMAGES/AC-2017-01766S_M007.GIF.

Garnier, M., Y. Labreuche, C. Garcia, M. Robert, and J. L. Nicolas. 2007. 'Evidence for the Involvement of Pathogenic Bacteria in Summer Mortalities of the Pacific Oyster Crassostrea Gigas'. *Microbial Ecology* 53(2):187–96. doi: 10.1007/S00248-006-9061-9/FIGURES/6.

Gebbink, Wouter A., and Stefan P. J. van Leeuwen. 2020a. 'Environmental Contamination and Human Exposure to PFASs near a Fluorochemical Production Plant: Review of Historic and Current PFOA and GenX Contamination in the Netherlands'. *Environment International* 137:105583. doi: 10.1016/J.ENVINT.2020.105583.

Gebbink, Wouter A., and Stefan P. J. van Leeuwen. 2020b. 'Environmental Contamination and Human Exposure to PFASs near a Fluorochemical Production Plant: Review of Historic and Current PFOA and GenX Contamination in the Netherlands'. *Environment International* 137:105583. doi: 10.1016/J.ENVINT.2020.105583.

Gebbink, Wouter A., and Stefan P. J. van Leeuwen. 2020c. 'Environmental Contamination and Human Exposure to PFASs near a Fluorochemical Production Plant: Review of Historic and Current PFOA and GenX Contamination in the Netherlands'. *Environment International* 137:105583. doi: 10.1016/J.ENVINT.2020.105583.

Gebreab, Kiflom Y., Muhamed N. H. Eeza, Tianyu Bai, Zain Zuberi, Jörg Matysik, Kevin E. O'Shea, A. Alia, and John P. Berry. 2020. 'Comparative Toxicometabolomics of Perfluorooctanoic Acid (PFOA) and next-Generation Perfluoroalkyl Substances'. *Environmental Pollution* 265:114928. doi: 10.1016/J.ENVPOL.2020.114928.

Geng, Qianqian, Mengmeng Guo, Haiyan Wu, Jixing Peng, Guanchao Zheng, Xiaoyu Liu, Yuxiu Zhai, and Zhijun Tan. 2021a. 'Effects of Single and Combined Exposure to BDE-47 and PFOA on Distribution, Bioaccumulation, and Toxicity in Blue Mussel (Mytilus Galloprovincialis)'. *Ecotoxicology and Environmental Safety* 228:113014. doi: 10.1016/J.ECOENV.2021.113014.

Geng, Qianqian, Mengmeng Guo, Haiyan Wu, Jixing Peng, Guanchao Zheng, Xiaoyu Liu, Yuxiu Zhai, and Zhijun Tan. 2021b. 'Effects of Single and Combined Exposure to BDE-47

and PFOA on Distribution, Bioaccumulation, and Toxicity in Blue Mussel (Mytilus Galloprovincialis)'. *Ecotoxicology and Environmental Safety* 228:113014. doi: 10.1016/J.ECOENV.2021.113014.

Gerhardt, A., L. Janssens de Bisthoven, Z. Mo, C. Wang, M. Yang, and Z. Wang. 2002. 'Short-Term Responses of Oryzias Latipes (Pisces: Adrianichthyidae) and Macrobrachium Nipponense (Crustacea: Palaemonidae) to Municipal and Pharmaceutical Waste Water in Beijing, China: Survival, Behaviour, Biochemical Biomarkers'. *Chemosphere* 47(1):35–47. doi: 10.1016/S0045-6535(01)00223-5.

Geyer, Roland, Jenna R. Jambeck, and Kara Lavender Law. 2017. 'Production, Use, and Fate of All Plastics Ever Made'. *Science Advances* 3(7). doi: 10.1126/SCIADV.1700782/SUPPL_FILE/1700782_SM.PDF.

Gonçalves, Joanna M., and Maria João Bebianno. 2021. 'Nanoplastics Impact on Marine Biota: A Review'. *Environmental Pollution* 273:116426. doi: 10.1016/J.ENVPOL.2021.116426.

Gondikas, Andreas P., Frank Von Der Kammer, Robert B. Reed, Stephan Wagner, James F. Ranville, and Thilo Hofmann. 2014. 'Release of TiO2 Nanoparticles from Sunscreens into Surface Waters: A One-Year Survey at the Old Danube Recreational Lake'. *Environmental Science and Technology* 48(10):5415–22. doi: 10.1021/ES405596Y/SUPPL_FILE/ES405596Y_SI_001.PDF.

Gong, Xue Qing, Annabella Selloni, and Andrea Vittadini. 2006. 'Density Functional Theory Study of Formic Acid Adsorption on Anatase TiO2(001): Geometries, Energetics, and Effects of Coverage, Hydration, and Reconstruction'. *Journal of Physical Chemistry B* 110(6):2804–11. doi: 10.1021/JP056572T/SUPPL_FILE/JP056572TSI20051202_092909.PDF.

Grassian, Vicki H., Andrea Adamcakova-Dodd, John M. Pettibone, Patrick I. O'Shaughnessy, and Peter S. Thorne. 2009. 'Inflammatory Response of Mice to Manufactured Titanium Dioxide Nanoparticles: Comparison of Size Effects through Different Exposure Routes'. *Http://Dx.Doi.Org/10.1080/17435390701694295* 1(3):211–26. doi: 10.1080/17435390701694295.

Guo, Mengmeng, Guanchao Zheng, Jixing Peng, Di Meng, Haiyan Wu, Zhijun Tan, Fengling Li, and Yuxiu Zhai. 2019a. 'Distribution of Perfluorinated Alkyl Substances in Marine Shellfish along the Chinese Bohai Sea Coast'. *Https://Doi.Org/10.1080/03601234.2018.1559570* 54(4):271–80. doi: 10.1080/03601234.2018.1559570.

Guo, Mengmeng, Guanchao Zheng, Jixing Peng, Di Meng, Haiyan Wu, Zhijun Tan, Fengling Li, and Yuxiu Zhai. 2019b. 'Distribution of Perfluorinated Alkyl Substances in Marine Shellfish along the Chinese Bohai Sea Coast'. *Https://Doi.Org/10.1080/03601234.2018.1559570* 54(4):271–80. doi: 10.1080/03601234.2018.1559570.

Gurman, Joshua L., Laura Baier, and Barbara C. Levin. 1987. 'Polystyrenes: A Review of the Literature on the Products of Thermal Decomposition and Toxicity'. *Fire and Materials* 11(3):109–30. doi: 10.1002/FAM.810110302.

Hagenaars, A., L. Vergauwen, D. Benoot, K. Laukens, and D. Knapen. 2013a. 'Mechanistic Toxicity Study of Perfluorooctanoic Acid in Zebrafish Suggests Mitochondrial Dysfunction to Play a Key Role in PFOA Toxicity'. *Chemosphere* 91(6):844–56. doi: 10.1016/J.CHEMOSPHERE.2013.01.056.

Hagenaars, A., L. Vergauwen, D. Benoot, K. Laukens, and D. Knapen. 2013b. 'Mechanistic Toxicity Study of Perfluorooctanoic Acid in Zebrafish Suggests Mitochondrial Dysfunction to Play a Key Role in PFOA Toxicity'. *Chemosphere* 91(6):844–56. doi: 10.1016/J.CHEMOSPHERE.2013.01.056.

Halliwell, B., JMC Gutteridge-Free radicals in biology and medicine, and undefined 2015. 2015. 'Redox Chemistry: The Essentials'. *Free Radicals in Biology and Medicine* .

Hartmann, Nanna B., and Anders Baun. 2010. 'The Nano Cocktail: Ecotoxicological Effects of Engineered Nanoparticles in Chemical Mixtures'. *Integrated Environmental Assessment and Management* 6(2):311–13. doi: 10.1002/IEAM.39.

Hernandez, Edgar, Bernd Nowack, and Denise M. Mitrano. 2017. 'Polyester Textiles as a Source of Microplastics from Households: A Mechanistic Study to Understand Microfiber Release during Washing'. *Environmental Science and Technology* 51(12):7036–46. doi: 10.1021/ACS.EST.7B01750/ASSET/IMAGES/LARGE/ES-2017-01750C_0006.JPEG.

Hernandez, Laura M., Nariman Yousefi, and Nathalie Tufenkji. 2017. 'Are There Nanoplastics in Your Personal Care Products?' *Environmental Science and Technology Letters* 4(7):280–85. doi: 10.1021/ACS.ESTLETT.7B00187/ASSET/IMAGES/LARGE/EZ-2017-00187H_0003.JPEG.

Hernando, M. D., A. R. Fernández-Alba, R. Tauler, and D. Barceló. 2005. 'Toxicity Assays Applied to Wastewater Treatment'. *Talanta* 65(2):358–66. doi: 10.1016/J.TALANTA.2004.07.012.

Hesler, Michelle, Leonie Aengenheister, Bernhard Ellinger, Roland Drexel, Susanne Straskraba, Carsten Jost, Sylvia Wagner, Florian Meier, H. von Briesen, Claudia Büchel, P. Wick, Tina Buerki-Thurnherr, and Yvonne Kohl. 2019. 'Multi-Endpoint Toxicological Assessment of

Polystyrene Nano- and Microparticles in Different Biological Models in Vitro'. *Toxicology in Vitro* 61:104610. doi: 10.1016/J.TIV.2019.104610.

Hood, Ernie. 2008. 'Alternative Mechanism for PFOA?: Trout Studies Shed Light on Liver Effects'. *Environmental Health Perspectives* 116(8):A351.

Hussain, M., R. Ceccarelli, D. L. Marchisio, D. Fino, N. Russo, and F. Geobaldo. 2010. 'Synthesis, Characterization, and Photocatalytic Application of Novel TiO2 Nanoparticles'. *Chemical Engineering Journal* 157(1):45–51. doi: 10.1016/J.CEJ.2009.10.043.

Hyne, R. V, and W. A. Maher. 2001. 'Macroinvertebrate Biomarkers: Links to Toxicosis and Changes in Populations or Communities'. *Citeseer*.

J.A., Perdue, Beattie J.H., and Chew K.K. 1981. 'Some Relationships between Gametogenic Cycle and Summer Mortality Phenomenon in the Pacific Oyster (Crassostrea Gigas) in Washington State.' *Journal of Shellfish Research*. doi: 10.3/JQUERY-UI.JS.

Janero, David R. 1990. 'Malondialdehyde and Thiobarbituric Acid-Reactivity as Diagnostic Indices of Lipid Peroxidation and Peroxidative Tissue Injury'. *Free Radical Biology and Medicine* 9(6):515–40. doi: 10.1016/0891-5849(90)90131-2.

Jaroenworaluck, A., W. Sunsaneeyametha, N. Kosachan, and R. Stevens. 2006. 'Characteristics of Silica-Coated TiO2 and Its UV Absorption for Sunscreen Cosmetic Applications'. *Surface and Interface Analysis* 38(4):473–77. doi: 10.1002/SIA.2313.

Jeon, Junho, Kurunthachalam Kannan, Han Kyu Lim, Hyo Bang Moon, Jin Sung Ra, and Sang Don Kim. 2010a. 'Bioaccumulation of Perfluorochemicals in Pacific Oyster under Different Salinity Gradients'. *Environmental Science and Technology* 44(7):2695–2701. doi: 10.1021/ES100151R/SUPPL_FILE/ES100151R_SI_001.PDF.

Jeon, Junho, Kurunthachalam Kannan, Han Kyu Lim, Hyo Bang Moon, Jin Sung Ra, and Sang Don Kim. 2010b. 'Bioaccumulation of Perfluorochemicals in Pacific Oyster under Different Salinity Gradients'. *Environmental Science and Technology* 44(7):2695–2701. doi: 10.1021/ES100151R/SUPPL_FILE/ES100151R_SI_001.PDF.

Johannaber, Friedrich, and Walter Michaeli. 2004. 'Anhang'. *Handbuch Spritzgießen* 1269–73. doi: 10.3139/9783446440982.017.

Jones, Dean P. 2006. 'Redefining Oxidative Stress'. *Https://Home.Liebertpub.Com/Ars* 8(9–10):1865–79. doi: 10.1089/ARS.2006.8.1865.

Jovanović, Boris. 2017. 'Ingestion of Microplastics by Fish and Its Potential Consequences from a Physical Perspective'. *Integrated Environmental Assessment and Management* 13(3):510–15. doi: 10.1002/IEAM.1913.

J.R Wunsch. 2000a. 'Polystyrene: Synthesis, Production and Applications - J. R. Wünsch - Google Books'. *RAPRA Technology LTD.* Retrieved 11 May 2023 (https://books.google.ca/books?hl=en&lr=&id=9Oal8DG_7GAC&oi=fnd&pg=PA5&ots=8nOJVOWxnp&sig=6ajFYDtunJQZEoCk3cOhS2gL6SI&redir_esc=y#v=onepage&q&f=false).

J.R Wunsch. 2000b. 'Polystyrene: Synthesis, Production and Applications - J. R. Wünsch - Google Books'. *RAPRA Technology LTD.* Retrieved 11 May 2023 (https://books.google.ca/books?hl=en&lr=&id=9Oal8DG_7GAC&oi=fnd&pg=PA5&ots=8nOJVOWxnp&sig=6ajFYDtunJQZEoCk3cOhS2gL6SI&redir_esc=y#v=onepage&q&f=false).

Kaegi, Ralf, Brian Sinnet, Steffen Zuleeg, Harald Hagendorfer, Elisabeth Mueller, Roger Vonbank, Markus Boller, and Michael Burkhardt. 2010. 'Release of Silver Nanoparticles

from Outdoor Facades'. *Environmental Pollution* 158(9):2900–2905. doi: 10.1016/J.ENVPOL.2010.06.009.

Kaur, Jasdeep, Constantinus Politis, and Reinhilde Jacobs. 2016. 'Salivary 8-Hydroxy-2-Deoxyguanosine, Malondialdehyde, Vitamin C, and Vitamin E in Oral Pre-Cancer and Cancer: Diagnostic Value and Free Radical Mechanism of Action'. *Clinical Oral Investigations* 20(2):315–19. doi: 10.1007/S00784-015-1506-4/TABLES/5.

Kelly, Sue A., Christine M. Havrilla, Todd C. Brady, Kimberly Harris Abramo, and Edward D. Levin. 1998. 'Oxidative Stress in Toxicology: Established Mammalian and Emerging Piscine Model Systems.' *Environmental Health Perspectives* 106(7):375–84. doi: 10.1289/EHP.98106375.

Kennedy, Gerald L., John L. Butenhoff, Geary W. Olsen, John C. O'Connor, Andrew M. Seacat, Roger G. Perkins, Lisa B. Biegel, Sandra R. Murphy, and David G. Farrar. 2010. 'The Toxicology of Perfluorooctanoate'. *Http://Dx.Doi.Org/10.1080/10408440490464705* 34(4):351–84. doi: 10.1080/10408440490464705.

Khan, Abdullah O., Alessandro Di Maio, Emily J. Guggenheim, Andrew J. Chetwynd, Dan Pencross, Selina Tang, Marie France A. Belinga-Desaunay, Steven G. Thomas, Joshua Z. Rappoport, and Iseult Lynch. 2020. 'Surface Chemistry-Dependent Evolution of the Nanomaterial Corona on TiO2 Nanomaterials Following Uptake and Sub-Cellular Localization'. *Nanomaterials 2020, Vol. 10, Page 401* 10(3):401. doi: 10.3390/NANO10030401.

Khoubnasabjafari, Maryam, Khalil Ansarin, and Abolghasem Jouyban. 2015. 'Reliability of Malondialdehyde as a Biomarker of Oxidative Stress in Psychological Disorders'. *BioImpacts : BI* 5(3):123. doi: 10.15171/BI.2015.20.

Khoubnasabjafari, Maryam, Jafar Soleymani, and Abolghasem Jouyban. 2018. 'Avoid Using Spectrophotometric Determination of Malondialdehyde as a Biomarker of Oxidative Stress'. *Https://Doi.Org/10.2217/Bmm-2017-0437* 12(6):551–54. doi: 10.2217/BMM-2017-0437.

Kiadó, Akadémiai, and Budapest Vol. 2006. 'THERMOSTABILITY OF NANO-TiO 2 AND ITS PHOTOCATALYTIC ACTIVITY'. *Springer* 89(1).

Kik, Kinga, Bożena Bukowska, and Paulina Sicińska. 2020. 'Polystyrene Nanoparticles: Sources, Occurrence in the Environment, Distribution in Tissues, Accumulation and Toxicity to Various Organisms'. *Environmental Pollution* 262:114297. doi: 10.1016/J.ENVPOL.2020.114297.

Kilic, Nedret, Mine Yavuz Taslipinar, Yildiz Guney, Ercument Tekin, and Erhan Onuk. 2014. 'An Investigation into the Serum Thioredoxin, Superoxide Dismutase, Malondialdehyde, and Advanced Oxidation Protein Products in Patients with Breast Cancer'. *Annals of Surgical Oncology* 21(13):4139–43. doi: 10.1245/S10434-014-3859-3/TABLES/4.

Klein, Roberta Daniele, Lygia S. Nogueira, Fabíola Xochilt Valdez Domingos-Moreira, Patrícia Gomes Costa, Adalto Bianchini, and Chris M. Wood. 2019. 'Effects of Sublethal Cd, Zn, and Mixture Exposures on Antioxidant Defense and Oxidative Stress Parameters in Early Life Stages of the Purple Sea Urchin Strongylocentrotus Purpuratus'. *Aquatic Toxicology* 217:105338. doi: 10.1016/J.AQUATOX.2019.105338.

de la Torre, Adrián, Paloma Sanz, Irene Navarro, and María de los Ángeles Martínez. 2020. 'Investigating the Presence of Emerging and Legacy POPs in European Domestic Air'. *Science of The Total Environment* 746:141348. doi: 10.1016/J.SCITOTENV.2020.141348.

Lacoste, Arnaud, Fabienne Jalabert, Shelagh K. Malham, Anne Cueff, and Serge A. Poulet. 2001. 'Stress and Stress-Induced Neuroendocrine Changes Increase the Susceptibility of Juvenile Oysters (Crassostrea Gigas) to Vibrio Splendidus'. *Applied and Environmental Microbiology* 67(5):2304–9. doi: 10.1128/AEM.67.5.2304-2309.2001/ASSET/7F17D4C7-8C11-41E1-82BC-2E1232070204/ASSETS/GRAPHIC/AM0511818003.JPEG.

Langer, Vera, Annekatrin Dreyer, and Ralf Ebinghaus. 2010. 'Polyfluorinated Compounds in Residential and Nonresidential Indoor Air'. *Environmental Science and Technology* 44(21):8075–81. doi: 10.1021/ES102384Z/SUPPL_FILE/ES102384Z_SI_001.PDF.

Lau, Christopher, Katherine Anitole, Colette Hodes, David Lai, Andrea Pfahles-Hutchens, and Jennifer Seed. 2007. 'Perfluoroalkyl Acids: A Review of Monitoring and Toxicological Findings'. *Toxicological Sciences* 99(2):366–94. doi: 10.1093/TOXSCI/KFM128.

Lee, Hyo Jin, and Gi Beum Kim. 2015. 'An Overview of Polybrominated Diphenyl Ethers (PBDEs) in the Marine Environment'. *Ocean Science Journal* 50(2):119–42. doi: 10.1007/S12601-015-0010-8/METRICS.

Lee, Seyong, John Gounley, Amanda Randles, and Jeffrey S. Vetter. 2019. 'Performance Portability Study for Massively Parallel Computational Fluid Dynamics Application on Scalable Heterogeneous Architectures'. *Journal of Parallel and Distributed Computing* 129:1–13. doi: 10.1016/J.JPDC.2019.02.005.

Lemos, Leila, Laura Gantiva, Catherine Kaylor, Alessandra Sanchez, and Natalia Quinete. 2022. 'American Oysters as Bioindicators of Emerging Organic Contaminants in Florida, United States'. *Science of The Total Environment* 835:155316. doi: 10.1016/J.SCITOTENV.2022.155316.

Lickly, T. D., C. V. Breder, and M. L. Rainey. 1995. 'A Model for Estimating the Daily Dietary Intake of a Substance from Food-Contact Articles: Styrene from Polystyrene Food Contact Polymers'. *Regulatory Toxicology and Pharmacology* 21(3):406–17. doi: 10.1006/RTPH.1995.1055.

Lin, Wei, Ruifen Jiang, Xiaoying Xiao, Jiayi Wu, Songbo Wei, Yan Liu, Derek C. G. Muir, and Gangfeng Ouyang. 2020. 'Joint Effect of Nanoplastics and Humic Acid on the Uptake of PAHs for Daphnia Magna: A Model Study'. *Journal of Hazardous Materials* 391:122195. doi: 10.1016/J.JHAZMAT.2020.122195.

Lin, Yongjing, Sa Zhou, Xiaohua Liu, Stafford Sheehan, and Dunwei Wang. 2009. 'TiO 2/TiSi 2 Heterostructures for High- Efficiencyphotoelectrochemical H 2O Splitting'. *Journal of the American Chemical Society* 131(8):2772–73. doi: 10.1021/JA808426H/SUPPL_FILE/JA808426H_SI_001.PDF.

Lindim, C., J. van Gils, and I. T. Cousins. 2016. 'Europe-Wide Estuarine Export and Surface Water Concentrations of PFOS and PFOA'. *Water Research* 103:124–32. doi: 10.1016/J.WATRES.2016.07.024.

Liu, Chunsheng, Ke Yu, Xiongjie Shi, Jingxian Wang, Paul K. S. Lam, Rudolf S. S. Wu, and Bingsheng Zhou. 2007. 'Induction of Oxidative Stress and Apoptosis by PFOS and PFOA in Primary Cultured Hepatocytes of Freshwater Tilapia (Oreochromis Niloticus)'. *Aquatic Toxicology* 82(2):135–43. doi: 10.1016/J.AQUATOX.2007.02.006.

Liu, Guohong, Jinglin Wang, Yongfa Zhu, and Xinrong Zhang. 2004. 'Destructive Adsorption of Carbon Tetrachloride on Nanometer Titanium Dioxide'. *Physical Chemistry Chemical Physics* 6(5):985–91. doi: 10.1039/B312082A.

Luna-Acosta, Andrea, Paco Bustamante, Joachim Godefroy, Ingrid Fruitier-Arnaudin, and Hélène Thomas-Guyon. 2010. 'Seasonal Variation of Pollution Biomarkers to Assess the Impact on the Health Status of Juvenile Pacific Oysters Crassostrea Gigas Exposed in Situ'. *Environmental Science and Pollution Research* 17(4):999–1008. doi: 10.1007/S11356-009-0287-1/TABLES/1.

Luo, Ting, Caiyun Wang, Zihong Pan, Cuiyuan Jin, Zhengwei Fu, and Yuanxiang Jin. 2019. 'Maternal Polystyrene Microplastic Exposure during Gestation and Lactation Altered Metabolic Homeostasis in the Dams and Their F1 and F2 Offspring'. *Environmental Science and Technology* 53(18):10978–92. doi: 10.1021/ACS.EST.9B03191/ASSET/IMAGES/LARGE/ES9B03191_0006.JPEG.

Lushchak, Volodymyr I. 2011. 'Environmentally Induced Oxidative Stress in Aquatic Animals'. *Aquatic Toxicology* 101(1):13–30. doi: 10.1016/J.AQUATOX.2010.10.006.

Luster, M. I., M. F. Ackermann, D. R. Germolec, and G. J. Rosenthal. 1989. 'Perturbations of the Immune System by Xenobiotics.' *Environmental Health Perspectives* 81:157–62. doi: 10.1289/EHP.8981157.

Ma, Yini, Anna Huang, Siqi Cao, Feifei Sun, Lianhong Wang, Hongyan Guo, and Rong Ji. 2016a. 'Effects of Nanoplastics and Microplastics on Toxicity, Bioaccumulation, and Environmental Fate of Phenanthrene in Fresh Water'. *Environmental Pollution* 219:166–73. doi: 10.1016/J.ENVPOL.2016.10.061.

Ma, Yini, Anna Huang, Siqi Cao, Feifei Sun, Lianhong Wang, Hongyan Guo, and Rong Ji. 2016b. 'Effects of Nanoplastics and Microplastics on Toxicity, Bioaccumulation, and Environmental Fate of Phenanthrene in Fresh Water'. *Environmental Pollution* 219:166–73. doi: 10.1016/J.ENVPOL.2016.10.061.

Magkos, Faidon., and Labros. Sidossis. 2004. 'Measuring Very Low Density Lipoprotein-Triglyceride Kinetics... : Current Opinion in Clinical Nutrition & Metabolic Care'. *Curent Opinion in Clinical Nutrition and Metabolic Care*. Retrieved 19 May 2023 (https://journals.lww.com/co-clinicalnutrition/Abstract/2004/09000/Measuring_very_low_density.7.aspx).

Mahon, A. M., B. O'Connell, M. G. Healy, I. O'Connor, R. Officer, R. Nash, and L. Morrison. 2017. 'Microplastics in Sewage Sludge: Effects of Treatment'. *Environmental Science and Technology* 51(2):810–18. doi: 10.1021/ACS.EST.6B04048/SUPPL_FILE/ES6B04048_SI_001.PDF.

Manfra, L., A. Rotini, E. Bergami, G. Grassi, C. Faleri, and I. Corsi. 2017. 'Comparative Ecotoxicity of Polystyrene Nanoparticles in Natural Seawater and Reconstituted Seawater Using the Rotifer Brachionus Plicatilis'. *Ecotoxicology and Environmental Safety* 145:557–63. doi: 10.1016/J.ECOENV.2017.07.068.

Martin, Scot T., Janet M. Kesselman, David S. Park, Nathan S. Lewis, and Michael R. Hoffmann. 1996. 'Surface Structures of 4-Chlorocatechol Adsorbed on Titanium Dioxide'. *Environmental Science and Technology* 30(8):2535–42. doi: 10.1021/ES950872E/ASSET/IMAGES/MEDIUM/ES950872EE00006.GIF.

Martins, J., M. L. Soares, M. L. Saker, L. OlivaTeles, and V. M. Vasconcelos. 2007. 'Phototactic Behavior in Daphnia Magna Straus as an Indicator of Toxicants in the Aquatic Environment'. *Ecotoxicology and Environmental Safety* 67(3):417–22. doi: 10.1016/J.ECOENV.2006.11.003.

Martins, José C., Martin L. Saker, Luís F. Oliva Teles, and Vítor M. Vasconcelos. 2007. 'Oxygen Consumption by Daphnia Magna Straus as a Marker of Chemical Stress in the

Aquatic Environment'. *Environmental Toxicology and Chemistry* 26(9):1987–91. doi: 10.1897/07-051R.1.

Martyanov, Igor N., Sitharaman Uma, Shalini Rodrigues, and Kenneth J. Klabunde. 2004. 'Structural Defects Cause TiO 2 -Based Photocatalysts to Be Active in Visible Light'. *Chemical Communications* 0(21):2476–77. doi: 10.1039/B409730K.

Mattsson, Karin, Mikael T. Ekvall, Lars Anders Hansson, Sara Linse, Anders Malmendal, and Tommy Cedervall. 2015. 'Altered Behavior, Physiology, and Metabolism in Fish Exposed to Polystyrene Nanoparticles'. *Environmental Science and Technology* 49(1):553–61. doi: 10.1021/ES5053655/SUPPL_FILE/ES5053655_SI_001.PDF.

Mattsson, Karin, Simonne Jocic, Isa Doverbratt, and Lars Anders Hansson. 2018. 'Nanoplastics in the Aquatic Environment'. *Microplastic Contamination in Aquatic Environments: An Emerging Matter of Environmental Urgency* 379–99. doi: 10.1016/B978-0-12-813747-5.00013-8.

Mattsson, Karin, Elyse V. Johnson, Anders Malmendal, Sara Linse, Lars Anders Hansson, and Tommy Cedervall. 2017. 'Brain Damage and Behavioural Disorders in Fish Induced by Plastic Nanoparticles Delivered through the Food Chain'. *Scientific Reports 2017 7:1* 7(1):1–7. doi: 10.1038/s41598-017-10813-0.

McDevitt, Jason P., Craig S. Criddle, Molly Morse, Robert C. Hale, Charles B. Bott, and Chelsea M. Rochman. 2017. 'Addressing the Issue of Microplastics in the Wake of the Microbead-Free Waters Act - A New Standard Can Facilitate Improved Policy'. *Environmental Science and Technology* 51(12):6611–17. doi: 10.1021/ACS.EST.6B05812/SUPPL_FILE/ES6B05812_SI_001.PDF.

Miao, Yang Bao, Wen Yu Pan, Kuan Hung Chen, Hao Ji Wei, Fwu Long Mi, Ming Yen Lu, Yen Chang, and Hsing Wen Sung. 2019. 'Engineering a Nanoscale Al-MOF-Armored Antigen Carried by a "Trojan Horse"-Like Platform for Oral Vaccination to Induce Potent and Long-Lasting Immunity'. *Advanced Functional Materials* 29(43):1904828. doi: 10.1002/ADFM.201904828.

Michels, E., S. Semsari, C. Bin, and L. De Meester. 2000. 'Effect of Sublethal Doses of Cadmium on the Phototactic Behavior of Daphnia Magna'. *Ecotoxicology and Environmental Safety* 47(3):261–65. doi: 10.1006/EESA.2000.1962.

Miramand, P., T. Guyot, and J. Pigeot. 2003. 'Sources et Impacts Potentiels Des Micropolluants Chimiques Sur Un Écosystème Littoral Exploité : L'exemple Des Côtes Des Charente-Maritime'. *Journal de Physique IV (Proceedings)* 108:3–6. doi: 10.1051/JP4:20030584.

Miranda, Ana F., Charlene Trestrail, Sam Lekamge, and Dayanthi Nugegoda. 2020. 'Effects of Perfluorooctanoic Acid (PFOA) on the Thyroid Status, Vitellogenin, and Oxidant–Antioxidant Balance in the Murray River Rainbowfish'. *Ecotoxicology* 29(2):163–74. doi: 10.1007/S10646-020-02161-Z/FIGURES/3.

Morales, Melanie, and Sergi Munné-Bosch. 2019. 'Malondialdehyde: Facts and Artifacts'. *Plant Physiology* 180(3):1246–50. doi: 10.1104/PP.19.00405.

Munaron, Dominique. 2004. 'Etude Des Apports En Herbicides et En Nutriments Par La Charente : Modélisation de La Dispersion de l'atrazine Dans Le Bassin de Marennes-Oléron'. *Oceanologie Chimique et Environement*.

Munaron, Dominique, Jean-François Dubernet, François Delmas, Jean-Yves Stanisière, and Pierre Scribe. 2006. 'Assessment of the Quantities of Herbicides and Nutrients Brought

down by the River Charente to the Coast and Modelling of the Dispersion of Atrazine in the Marennes-Oleron Bay'. *Cahiers de Biologie Marine* 47(1):85–92.

Mutti, A., C. Buzio, F. Perazzoli, E. Bergamaschi, M. C. Bocchi, L. Selis, F. Mineo, and I. Franchini. 1992. 'SOTTOPOPOLAZIONI LINFOCITARIE IN LAVORATORI PROFESSIONALMENTE ESPOSTI A STIRENE'. *Medicina Del Lavoro* 83(2):167–77.

Naasz, Steffi, Rolf Altenburger, and Dana Kühnel. 2018. 'Environmental Mixtures of Nanomaterials and Chemicals: The Trojan-Horse Phenomenon and Its Relevance for Ecotoxicity'. *Science of The Total Environment* 635:1170–81. doi: 10.1016/J.SCITOTENV.2018.04.180.

Nakamura, Ryuhei, and Yoshihiro Nakato. 2004. 'Primary Intermediates of Oxygen Photoevolution Reaction on TiO2 (Rutile) Particles, Revealed by in Situ FTIR Absorption and Photoluminescence Measurements'. *Journal of the American Chemical Society* 126(4):1290–98. doi: 10.1021/JA0388764/ASSET/IMAGES/MEDIUM/JA0388764N00001.GIF.

O'Brien, Timothy M., and Kendall B. Wallace. 2004. 'Mitochondrial Permeability Transition as the Critical Target of N-Acetyl Perfluorooctane Sulfonamide Toxicity in Vitro'. *Toxicological Sciences* 82(1):333–40. doi: 10.1093/TOXSCI/KFH244.

OECD Guidelines for Testing of Chemicals. 2004a. 'OECD Guideline for the Testing of Chemicals: Daphnia... - Google Scholar'. *OECD*. Retrieved 19 May 2023 (https://scholar.google.com/scholar?q=OECD%20Guideline%20for%20the%20Testing%20of%20Chemicals%3A%20Daphnia%20sp.%2C%20Acute%20Immobilisation%20Test).

OECD Guidelines for Testing of Chemicals. 2004b. 'Test No. 202: Daphnia Sp. Acute Immobilisation Test'. *Section 2*. doi: 10.1787/9789264069947-EN.

Ohkawa, H., N. Ohishi, and K. Yagi. 1978. 'Reaction of Linoleic Acid Hydroperoxide with Thiobarbituric Acid'. *Journal of Lipid Research* 19(8):1053–57. doi: 10.1016/s0022-2275(20)40690-x.

Panaretakis, Theoharis, Irina G. Shabalina, Dan Grandér, Maria C. Shoshan, and Joseph W. Depierre. 2001. 'Reactive Oxygen Species and Mitochondria Mediate the Induction of Apoptosis in Human Hepatoma HepG2 Cells by the Rodent Peroxisome Proliferator and Hepatocarcinogen, Perfluorooctanoic Acid'. *Toxicology and Applied Pharmacology* 173(1):56–64. doi: 10.1006/TAAP.2001.9159.

Pathak, Nitu, and R. Sen. 2017. 'Analytical Application of Radioactive Analysis'. *International Journal of Analytical and Applied Chemistry* 3(2):32–35.

Pettibone, John M., David M. Cwiertny, Michelle Scherer, and Vicki H. Grassian. 2008. 'Adsorption of Organic Acids on TiO2 Nanoparticles: Effects of PH, Nanoparticle Size, and Nanoparticle Aggregation'. *Langmuir* 24(13):6659–67. doi: 10.1021/LA7039916/SUPPL_FILE/LA7039916-FILE003.PDF.

Pikuda, Oluwadamilola, Elvis Genbo Xu, Dimitrios Berk, and Nathalie Tufenkji. 2019. 'Toxicity Assessments of Micro- and Nanoplastics Can Be Confounded by Preservatives in Commercial Formulations'. *Environmental Science and Technology Letters* 6(1):21–25. doi: 10.1021/ACS.ESTLETT.8B00614/ASSET/IMAGES/LARGE/EZ-2018-00614M_0003.JPEG.

van Pomeren, M., N. R. Brun, W. J. G. M. Peijnenburg, and M. G. Vijver. 2017. 'Exploring Uptake and Biodistribution of Polystyrene (Nano)Particles in Zebrafish Embryos at Different Developmental Stages'. *Aquatic Toxicology* 190:40–45. doi: 10.1016/J.AQUATOX.2017.06.017.

Preočanin, Tajana, and Nikola Kallay. 2006. 'Point of Zero Charge and Surface Charge Density of TiO2 in Aqueous Electrolyte Solution as Obtained by Potentiometric Mass Titration'. *Croatica Chemica Acta* 79(1):95–106.

Qiao, Ruxia, Kai Lu, Yongfeng Deng, Hongqiang Ren, and Yan Zhang. 2019. 'Combined Effects of Polystyrene Microplastics and Natural Organic Matter on the Accumulation and Toxicity of Copper in Zebrafish'. *Science of The Total Environment* 682:128–37. doi: 10.1016/J.SCITOTENV.2019.05.163.

Rachel, Anita, Machiraju Subrahmanyam, and Pierre Boule. 2002. 'Comparison of Photocatalytic Efficiencies of TiO2 in Suspended and Immobilised Form for the Photocatalytic Degradation of Nitrobenzenesulfonic Acids'. *Applied Catalysis B: Environmental* 37(4):301–8. doi: 10.1016/S0926-3373(02)00007-3.

Racotta, Ilie S., and Roberto Hernández-Herrera. 2000. 'Metabolic Responses of the White Shrimp, Penaeus Vannamei, to Ambient Ammonia'. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 125(4):437–43. doi: 10.1016/S1095-6433(00)00171-9.

Regoli, Francesco, and Maria Elisa Giuliani. 2014. 'Oxidative Pathways of Chemical Toxicity and Oxidative Stress Biomarkers in Marine Organisms'. *Marine Environmental Research* 93:106–17. doi: 10.1016/J.MARENVRES.2013.07.006.

Renner, Rebecca. 2007. 'PFOA in People'. *Environmental Science and Technology* 41(13):4497–4500. doi: 10.1021/ES0725697/ASSET/ES0725697.FP.PNG_V03.

Rennie, Michael J. 1999a. 'An Introduction to the Use of Tracers in Nutrition and Metabolism'. *Proceedings of the Nutrition Society* 58(4):935–44. doi: 10.1017/S002966519900124X.

Rennie, Michael J. 1999b. ‘An Introduction to the Use of Tracers in Nutrition and Metabolism’. *Proceedings of the Nutrition Society* 58(4):935–44. doi: 10.1017/S002966519900124X.

Reyes, José Guillermo Galindo, Luisella Dalla-Venezia, and Ma Guadalupe Lazcano Alvarez. 2002. ‘Effect of Some Organophosphorus Pesticides on Oxygen Consumption of Shrimp, Litopenaeus Vannamei’. *Ecotoxicology and Environmental Safety* 52(2):134–36. doi: 10.1006/EESA.2001.2129.

Rodrigues da Silva, S. L., D. Oliveira Rodrigues, P. Sánchez Castillo, José M. Conde-Porcuna, and Luis Cruz-Pizarro. 2004. ‘Alteraciones En La Tasa de Respiración de Daphnia Magna Bajo Concentraciones Subletales de Anatoxina-a’. *Limnética* 23(1–2):159–66.

Ryu, Heejeong, Baikun Li, Sylvain De Guise, Jeffrey McCutcheon, and Yu Lei. 2021. ‘Recent Progress in the Detection of Emerging Contaminants PFASs’. *Journal of Hazardous Materials* 408:124437. doi: 10.1016/J.JHAZMAT.2020.124437.

Salvi, Daniele, Armando Macali, and Paolo Mariottini. 2014. ‘Molecular Phylogenetics and Systematics of the Bivalve Family Ostreidae Based on RRNA Sequence-Structure Models and Multilocus Species Tree’. *PLOS ONE* 9(9):e108696. doi: 10.1371/JOURNAL.PONE.0108696.

Sanchís, Josep, Naiara Berrojalbiz, Gemma Caballero, Jordi Dachs, Marinella Farré, and Damià Barceló. 2012. ‘Occurrence of Aerosol-Bound Fullerenes in the Mediterranean Sea Atmosphere’. *Environmental Science and Technology* 46(3):1335–43. doi: 10.1021/ES200758M/SUPPL_FILE/ES200758M_SI_001.PDF.

Schecter, Arnold, Justin Colacino, Darrah Haffner, Keyur Patel, Matthias Opel, Olaf Päpke, and Linda Birnbaum. 2010. ‘Perfluorinated Compounds, Polychlorinated Biphenyls, and

Organochlorine Pesticide Contamination in Composite Food Samples from Dallas, Texas, USA'. *Environmental Health Perspectives* 118(6):796–802. doi: 10.1289/EHP.0901347.

Schlyer, David J., Miguel A. V. Bastos, David Alexoff, and Alfred P. Wolf. 1990. 'Separation of [18F]Fluoride from [18O]Water Using Anion Exchange Resin'. *International Journal of Radiation Applications and Instrumentation. Part A. Applied Radiation and Isotopes* 41(6):531–33. doi: 10.1016/0883-2889(90)90034-E.

Shanmuganathan, Devarajan, Mallavarapu Megharaj, Zuliang Chen, and Ravi Naidu. 2011a. 'Polybrominated Diphenyl Ethers (PBDEs) in Marine Foodstuffs in Australia: Residue Levels and Contamination Status of PBDEs'. *Marine Pollution Bulletin* 63(5–12):154–59. doi: 10.1016/J.MARPOLBUL.2011.06.002.

Shanmuganathan, Devarajan, Mallavarapu Megharaj, Zuliang Chen, and Ravi Naidu. 2011b. 'Polybrominated Diphenyl Ethers (PBDEs) in Marine Foodstuffs in Australia: Residue Levels and Contamination Status of PBDEs'. *Marine Pollution Bulletin* 63(5–12):154–59. doi: 10.1016/J.MARPOLBUL.2011.06.002.

Shanmuganathan, Devarajan, Mallavarapu Megharaj, Zuliang Chen, and Ravi Naidu. 2011c. 'Polybrominated Diphenyl Ethers (PBDEs) in Marine Foodstuffs in Australia: Residue Levels and Contamination Status of PBDEs'. *Marine Pollution Bulletin* 63(5–12):154–59. doi: 10.1016/J.MARPOLBUL.2011.06.002.

Souders, Christopher L., Christina L. Sanchez, Wendi Malphurs, Juan J. Aristizabal-Henao, John A. Bowden, and Christopher J. Martyniuk. 2021. 'Metabolic Profiling in Human SH-SY5Y Neuronal Cells Exposed to Perfluorooctanoic Acid (PFOA)'. *NeuroToxicology* 85:160–72. doi: 10.1016/J.NEURO.2021.05.009.

de Souza, Manuel M., Cláudia C. Windmöller, and Vanessa Hatje. 2011. 'Shellfish from Todos Os Santos Bay, Bahia, Brazil: Treat or Threat?' *Marine Pollution Bulletin* 62(10):2254–63. doi: 10.1016/J.MARPOLBUL.2011.07.010.

Stabili, L., and P. Pagliara. 2009. 'Effect of Zinc on Lysozyme-like Activity of the Seastar Marthasterias Glacialis (Echinodermata, Asteroidea) Mucus'. *Journal of Invertebrate Pathology* 100(3):189–92. doi: 10.1016/J.JIP.2009.01.005.

Steenland, Kyle, Tony Fletcher, and David A. Savitz. 2010. 'Epidemiologic Evidence on the Health Effects of Perfluorooctanoic Acid (PFOA)'. *Environmental Health Perspectives* 118(8):1100–1108. doi: 10.1289/EHP.0901827.

Stetefeld, Jörg, Sean A. McKenna, and Trushar R. Patel. 2016. 'Dynamic Light Scattering: A Practical Guide and Applications in Biomedical Sciences'. *Biophysical Reviews* 8(4):409–27. doi: 10.1007/S12551-016-0218-6/FIGURES/1.

Surendran, U., M. Jayakumar, P. Raja, Girish Gopinath, and Padmanaban Velayudhaperumal Chellam. 2023. 'Microplastics in Terrestrial Ecosystem: Sources and Migration in Soil Environment'. *Chemosphere* 318:137946. doi: 10.1016/J.CHEMOSPHERE.2023.137946.

Tan, Bing, and Yiying Wu. 2006. 'Dye-Sensitized Solar Cells Based on Anatase TiO2 Nanoparticle/Nanowire Composites'. *Journal of Physical Chemistry B* 110(32):15932–38. doi: 10.1021/JP063972N/SUPPL_FILE/JP063972NSI20060625_064702.PDF.

The Nobel Prize. 1943. 'NobelPrize.Org'. *The Nobel Prize*. Retrieved 19 May 2023 (https://www.nobelprize.org/prizes/lists/all-nobel-prizes/).

Tourinho, Paula S., Cornelis A. M. van Gestel, Stephen Lofts, Claus Svendsen, Amadeu M. V. M. Soares, and Susana Loureiro. 2012. 'Metal-Based Nanoparticles in Soil: Fate, Behavior,

and Effects on Soil Invertebrates'. *Environmental Toxicology and Chemistry* 31(8):1679–92. doi: 10.1002/ETC.1880.

Tsikas, Dimitrios. 2017. 'Assessment of Lipid Peroxidation by Measuring Malondialdehyde (MDA) and Relatives in Biological Samples: Analytical and Biological Challenges'. *Analytical Biochemistry* 524:13–30. doi: 10.1016/J.AB.2016.10.021.

US Food and Drug Administration. 2002. 'US Food and Drug Administration: The Safety of Styrene-Ba... - Google Scholar'. *US Food and Drug Administration* . Retrieved 11 May 2023 (https://scholar.google.com/scholar_lookup?title=The%20Safety%20of%20Styrene-Based%20Polymers%20for%20Food-Contact%20Use&author=FDA%20(Food%20and%20Drug%20Administration)&publication_year=2002).

Verlecar, X. N., K. B. Jena, and G. B. N. Chainy. 2007. 'Biochemical Markers of Oxidative Stress in Perna Viridis Exposed to Mercury and Temperature'. *Chemico-Biological Interactions* 167(3):219–26. doi: 10.1016/J.CBI.2007.01.018.

Vidal-Liñán, Leticia, Juan Bellas, José Fumega, and Ricardo Beiras. 2015. 'Bioaccumulation of BDE-47 and Effects on Molecular Biomarkers Acetylcholinesterase, Glutathione-S-Transferase and Glutathione Peroxidase in Mytilus Galloprovincialis Mussels'. *Ecotoxicology* 24(2):292–300. doi: 10.1007/S10646-014-1377-5/FIGURES/3.

Völz, Hans G., Jürgen Kischkewitz, Peter Woditsch, Axel Westerhaus, Wolf-Dieter Griebler, Marcel De Liedekerke, Gunter Buxbaum, Helmut Printzen, Manfred Mansmann, Dieter Räde, Gerhard Trenczek, Volker Wilhelm, Stefanie Schwarz, Henning Wienand, Jörg Adel, Gerhard Adrian, Karl Brandt, William B. Cork, Heinrich Winkeler, Wielfried Mayer, Klaus Schneider, Lutz Leitner, Hendrik Kathrein, Ekkehard Schwab, Helmut Jakusch, Manfred

Ohlinger, Ronald Veitch, Günter Etzrodt, Gerhard Pfaff, Klaus-Dieter Franz, Ralf Emmert, Katsuhisa Nitta, Robert Besold, and Harald Gaedcke. 2000. 'Pigments, Inorganic'. *Ullmann's Encyclopedia of Industrial Chemistry*. doi: 10.1002/14356007.A20_243.

Völz, Hans G., Jürgen Kischkewitz, Peter Woditsch, Axel Westerhaus, Wolf-Dieter Griebler, Marcel De Liedekerke, Gunter Buxbaum, Helmut Printzen, Manfred Mansmann, Dieter Räde, Gerhard Trenczek, Volker Wilhelm, Stefanie Schwarz, Henning Wienand, Jörg Adel, Gerhard Adrian, Karl Brandt, William B. Cork, Heinrich Winkeler, Wielfried Mayer, Klaus Schneider, Lutz Leitner, Hendrik Kathrein, Ekkehard Schwab, Helmut Jakusch, Manfred Ohlinger, Ronald Veitch, Günter Etzrodt, Gerhard Pfaff, Klaus-Dieter Franz, Ralf Emmert, Katsuhisa Nitta, Robert Besold, and Harald Gaedcke. 2006. 'Pigments, Inorganic'. *Ullmann's Encyclopedia of Industrial Chemistry*. doi: 10.1002/14356007.A20_243.PUB2.

Wade, C. R., and A. M. van Rij. 1989. 'Plasma Malondialdehyde, Lipid Peroxides, and the Thiobarbituric Acid Reaction.' *Clinical Chemistry* 35(2):336–336. doi: 10.1093/clinchem/35.2.336.

Wang, Chuan Yi, Henning Groenzin, and Mary Jane Shultz. 2005. 'Comparative Study of Acetic Acid, Methanol, and Water Adsorbed on Anatase TiO2 Probed by Sum Frequency Generation Spectroscopy'. *Journal of the American Chemical Society* 127(27):9736–44. doi: 10.1021/JA051996M/SUPPL_FILE/JA051996MSI20050603_101542.PDF.

Wang, Juan, Xinhui Liu, Guannan Liu, Zixuan Zhang, Hao Wu, Baoshan Cui, Junhong Bai, and Wei Zhang. 2019. 'Size Effect of Polystyrene Microplastics on Sorption of Phenanthrene and Nitrobenzene'. *Ecotoxicology and Environmental Safety* 173:331–38. doi: 10.1016/J.ECOENV.2019.02.037.

Wang, Pei, Yonglong Lu, Tieyu Wang, Yaning Fu, Zhaoyun Zhu, Shijie Liu, Shuangwei Xie, Yang Xiao, and John P. Giesy. 2014. 'Occurrence and Transport of 17 Perfluoroalkyl Acids in 12 Coastal Rivers in South Bohai Coastal Region of China with Concentrated Fluoropolymer Facilities'. *Environmental Pollution* 190:115–22. doi: 10.1016/J.ENVPOL.2014.03.030.

Ward, J. Evan, Shiye Zhao, Bridget A. Holohan, Kayla M. Mladinich, Tyler W. Griffin, Jennifer Wozniak, and Sandra E. Shumway. 2019. 'Selective Ingestion and Egestion of Plastic Particles by the Blue Mussel (Mytilus Edulis) and Eastern Oyster (Crassostrea Virginica): Implications for Using Bivalves as Bioindicators of Microplastic Pollution'. *Environmental Science and Technology* 53(15):8776–84. doi: 10.1021/ACS.EST.9B02073/SUPPL_FILE/ES9B02073_SI_008.AVI.

Warheit, David B., and E. Maria Donner. 2015. 'Risk Assessment Strategies for Nanoscale and Fine-Sized Titanium Dioxide Particles: Recognizing Hazard and Exposure Issues'. *Food and Chemical Toxicology* 85:138–47. doi: 10.1016/J.FCT.2015.07.001.

Watkinson, A. J., G. B. Micalizzi, G. M. Graham, J. B. Bates, and S. D. Costanzo. 2007. 'Antibiotic-Resistant Escherichia Coli in Wastewaters, Surface Waters, and Oysters from an Urban Riverine System'. *Applied and Environmental Microbiology* 73(17):5667–70. doi: 10.1128/AEM.00763-07/ASSET/2981B789-8B16-4C66-8EE7-F0D9B2B011EB/ASSETS/GRAPHIC/ZAM0170780820001.JPEG.

World Health Organization (WHO). 2000. 'World Health Organization (WHO): WHO—Toluene: Air... - Google Scholar'. *World Health Organization (WHO)*. Retrieved 11 May 2023

(https://scholar.google.com/scholar_lookup?title=Styrene.%20Air%20Quality%20Guidelines%20for%20Europe&author=WHO&publication_year=2000&pages=1-288).

Wu, Jui Pin, and Hon Cheng Chen. 2004. 'Effects of Cadmium and Zinc on Oxygen Consumption, Ammonium Excretion, and Osmoregulation of White Shrimp (Litopenaeus Vannamei)'. *Chemosphere* 57(11):1591–98. doi: 10.1016/J.CHEMOSPHERE.2004.07.033.

Xie, Lin Na, Xiao Chen Wang, Xiao Jie Dong, Li Qin Su, Hui Juan Zhu, Cong Wang, Dian Ping Zhang, Fang Ying Liu, Sha Sha Hou, Bing Dong, Guo Qiang Shan, Xu Zhang, and Ying Zhu. 2021. 'Concentration, Spatial Distribution, and Health Risk Assessment of PFASs in Serum of Teenagers, Tap Water and Soil near a Chinese Fluorochemical Industrial Plant'. *Environment International* 146:106166. doi: 10.1016/J.ENVINT.2020.106166.

Xue, Baoming, Linlin Zhang, Ruilong Li, Yinghui Wang, Jing Guo, Kefu Yu, and Shaopeng Wang. 2020. 'Underestimated Microplastic Pollution Derived from Fishery Activities And'. *Environmental Science & Technology* 54(4):2210–17. doi: 10.1021/ACS.EST.9B04850.

Zarfl, Christiane, and Michael Matthies. 2010. 'Are Marine Plastic Particles Transport Vectors for Organic Pollutants to the Arctic?' *Marine Pollution Bulletin* 60(10):1810–14. doi: 10.1016/J.MARPOLBUL.2010.05.026.

Zellers, Ann. 2005. 'Density of Plastic Particles Found in Zooplankton Trawls from Coastal Waters of California to the North Pacific Central Gyre'. *Algalita Marine Research Foundation* .

Zhang, Hengzhong, R. Lee Penn, Robert J. Hamers, and Jillian F. Banfield. 1999. 'Enhanced Adsorption of Molecules on Surfaces of Nanocrystalline Particles'. *Journal of Physical Chemistry B* 103(22):4656–62. doi: 10.1021/JP984574Q/ASSET/IMAGES/LARGE/JP984574QF00003.JPEG.

Zhang, Jiyan, Lu Chen, Lin Xiao, Fengxiu Ouyang, Qing Ying Zhang, and Zhong Cheng Luo. 2017. 'Polybrominated Diphenyl Ether Concentrations in Human Breast Milk Specimens Worldwide'. *Epidemiology* 28:S89–97. doi: 10.1097/EDE.0000000000000714.

Zhang, Ming, and Liheng Xu. 2020. 'Transport of Micro- and Nanoplastics in the Environment: Trojan-Horse Effect for Organic Contaminants'. *Https://Doi.Org/10.1080/10643389.2020.1845531* 52(5):810–46. doi: 10.1080/10643389.2020.1845531.

Zhang, Wen, Qi Wang, and Hao Chen. 2022. 'Challenges in Characterization of Nanoplastics in the Environment'. *Frontiers of Environmental Science and Engineering* 16(1):1–3. doi: 10.1007/S11783-021-1445-Z/METRICS.

Zhang, Yueyang, Greg G. Goss, Greg G. Goss, and Greg G. Goss. 2020. 'Potentiation of Polycyclic Aromatic Hydrocarbon Uptake in Zebrafish Embryos by Nanoplastics'. *Environmental Science: Nano* 7(6):1730–41. doi: 10.1039/D0EN00163E.

Zhou, Qunfang, Jianbin Zhang, Jianjie Fu, Jianbo Shi, and Guibin Jiang. 2008. 'Biomonitoring: An Appealing Tool for Assessment of Metal Pollution in the Aquatic Ecosystem'. *Analytica Chimica Acta* 606(2):135–50. doi: 10.1016/J.ACA.2007.11.018.

Zhou, Yujie, Junxiao Wang, Mengmeng Zou, Zhenyi Jia, Shenglu Zhou, and Yan Li. 2020. 'Microplastics in Soils: A Review of Methods, Occurrence, Fate, Transport, Ecological and Environmental Risks'. *Science of The Total Environment* 748:141368. doi: 10.1016/J.SCITOTENV.2020.141368.