

# Broad toxicological effects of per-/poly- fluoroalkyl substances (PFAS) on the unicellular eukaryote, *Tetrahymena pyriformis*

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## ABSTRACT

Per-/Poly- fluoroalkyl substances represent emerging persistent organic pollutants. Their toxic effects can be broad, yet little attention has been given to organisms at the microscale. To address this knowledge shortfall, the unicellular eukaryote *Tetrahymena pyriformis* was exposed to increasing concentrations (0–5000 µM) of PFOA/PFOS and monitored for cellular motility, division and function (i.e., phagocytosis), reactive oxygen species generation and total protein levels. Both PFOA/PFOS exposure had negative impacts on *T. pyriformis*, including reduced motility, delayed cell division and oxidative imbalance, with each chemical having distinct toxicological profiles. *T. pyriformis* represents a promising candidate for assessing the biological effects these emerging anthropogenically-derived contaminants in a freshwater setting.

## 1. Introduction

Whilst drinking water supplies in developed countries are rendered safe through the function of treatment plants, contaminants such as antibiotics, hormones, anti-inflammatory drugs and a number of Persistent Organic Pollutants (POPs) can remain. Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS) are examples of POPs and are a family of synthetic chemicals employed as part of stain- and water-resistant fabric manufacture, cleaning products, paints, fire-fighting foams and in cookware (Gomes et al., 2020). Two of these PFAS, Perfluorooctanoic acid (PFOA) and Perfluorooctanesulfonic acid (PFOS), are of increasing concern as they are now commonly found in water-bodies largely due to industrial waste emissions and they are highly persistent in the environment (Grandjean, 2018). As “bioaccumulants”, they are found in various higher organisms in our ecosystems including earthworms (Karnjanapiboonwong et al., 2018; Navarro et al., 2016), mussels (Liu and Gin, 2018), fish (Teunen et al., 2021), birds (Kannan et al., 2001), plants (Ghisi et al., 2019), marine and land mammals (Giesy and Kannan et al., 2002; Kudo et al., 2003). These “forever chemicals” are linked to the formation of cancer and organ damage in humans and are also associated with the negative impacts on the development of children (Blake and Fenton, 2020). Whilst it is encouraging that there is an increasing awareness of this family of chemicals and their impact on human populations and the environment, there are gaps in our understanding on PFAS effects in microorganisms in aquatic

and non-aquatic environments (Ahrens and Bundschuh et al., 2014).

To study the impact of PFAS on aquatic microorganisms, the unicellular eukaryotic microorganism, *Tetrahymena pyriformis* was adopted. This is a free-living, ciliated model organism, one of the most highly developed protozoans with several specialised organelles that are functionally similar to higher organisms (Sauvant et al., 1999). Many ground-breaking studies into telomerase structure and activity, self-splicing RNA and ribozymes were conducted using the *Tetrahymena* organism as a model organism (e.g., Blackburn and Gall, 1978; Greider and Blackburn, 1985; Latham and Cech, 1989). This organism is also a suitable model to study microbial pathogenesis and host-pathogen interactions (Dayeh et al., 2005; Pang et al., 2012). This is clearly seen in the study of phagocytosis, as *Tetrahymena* can engulf foreign objects through its oral apparatus in an actin-dependent manner, with the involvement of lectins localised on the cell surface, like that of mammalian phagocytes (Cassidy-Hanley, 2012; Csaba, 2016; Gray et al., 2012; Williams et al., 2006). Furthermore, axenic cultures of *Tetrahymena pyriformis* are readily available, cost efficient to culture and thereby allowing larger experimental numbers to be utilised and thus improving statistical discrimination. They are relatively large, easily visible and transparent, allowing us to visualise using basic microscopic techniques and is, in recent years, a popular model to study bacterial virulence (Lainhart et al., 2009; Li et al., 2011; Pang et al., 2012; Woods et al., 2022).

Herein it is shown that PFOA and PFOS decrease growth of

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*Tetrahymena pyriformis* in a dose and time dependent manner. Size and the ability to phagocytose are negatively affected by both chemicals whereas protein concentration and reactive oxygen species production increased in the presence of PFAS. Together, these data provide a basis for further studies investigating how PFAS can impact on aquatic microeukaryote using a simple, controllable experimental system that can be extrapolated to freshwater bodies.

## 2. Materials and methods

### 2.1. Reagents

All key reagents, such as proteose peptone (LP0085, Oxoid), tryptone (LP0042, Oxoid), dipotassium phosphate (P3786), potassium chloride (P/4280/53), perfluorooctanoic acid (PFOA, Acros 173960050), perfluorooctanesulfonic acid (PFOS, Aldrich 77283) were purchased from either Fisher Scientific or Sigma-Aldrich, UK (now MERCK) in their purest form.

### 2.2. *Tetrahymena pyriformis* growth and maintenance

*Tetrahymena pyriformis* (Carolina Biological Supply Company, US) was purchased from Blades Biological Ltd (UK) and is maintained in *Tetrahymena* medium, (0.5% (w/v) proteose peptone, 0.5% tryptone, 0.02% dipotassium phosphate, pH 7.2) at 25 °C before use.

### 2.3. Cell viability assay of *Tetrahymena pyriformis* to PFAS

One thousand or  $10^6$  *T. pyriformis* grown in medium were exposed to either 500 – 5  $\mu$ M or 5000 – 39  $\mu$ M of PFOA or PFOS for 2 h or 6 days, respectively, in a 25 °C incubator. Historically PFAS can be found in groundwater from 1 to 15  $\mu$ M in sites where aqueous film-forming foams (containing PFAS) were used (Schultz et al., 2004). Cell viability was assessed by either motility or cell counts. For motility, cells were observed visually using light microscopy (CX31, Olympus). For cell counts, 10  $\mu$ l was taken from each sample and mixed with an equal volume of 2.5% glutaraldehyde. Numbers of *T. pyriformis* were enumerated using a hemacytometer (FastRead-102) under a light microscope (CX31, Olympus).

### 2.4. Generation of reactive oxygen species

Approximately  $1 \times 10^6$  *T. pyriformis* grown in medium were exposed to 5000 – 39  $\mu$ M of PFOA or PFOS for 1 h, respectively, in a 25 °C incubator. 100  $\mu$ l of cell suspension was aliquot into 3 wells of a white 96-well microplate, along with 200  $\mu$ M luminol and read in plate reader with luminescence capability (GloMax Discover System, Promega) for 10 min at 25 °C to obtain the baseline. This is followed by equal volume of a 1:10,000 diluted Black Indian ink (Winsor and Newton, UK) and read for a further 60 min at 25 °C.

### 2.5. Size determination of *Tetrahymena pyriformis*

Twenty microlitres of a mixture containing equal volumes of cell suspension (exposed to 500, 50 or 5  $\mu$ M of PFOA or PFOS) and 2.5% glutaraldehyde was placed on a clean microscope slide without coverslip. Individual *T. pyriformis* were imaged using an upright (light) microscope (CX31, Olympus) with an eyepiece camera (BF960, Swift Optical Instruments Ltd) controlled using the Swift EasyView software (V1.20.08.041615). The area of each *T. pyriformis* was measured using the ImageJ software (National Institutes of Health). At least 48 up to 78 organisms from all samples were measured across three independent experiments.

### 2.6. Phagocytosis assay

After 6 days exposure of *T. pyriformis* to PFAS, cells were diluted 1:10 with nutrient deficient, sterile Chalkey's medium (1.710 mM NaCl, 0.054 mM KCl, 0.060 mM  $\text{CaCl}_2 \cdot \text{H}_2\text{O}$ ) for 24 h in a 25 °C incubator. Cells were mixed with an equal volume of a 1:10,000 diluted Black Indian ink (Winsor and Newton, UK) and incubated for 30 min at 25 °C. Equal volumes 2.5% glutaraldehyde solution were added to fix cells. For counting of phagocytosed ink, *T. pyriformis* were visualised under a microscope using a 40x objective. Between 47 and 61 *T. pyriformis* per sample across three independent experiments were counted for the number of black vesicles located within each cell. Phagocytosis index was defined as the number of black vesicles engulfed per *T. pyriformis* cell.

### 2.7. Protein quantitation assay

*T. pyriformis* exposed to PFAS for 6 days were counted and washed 3 times in cold PBS. Cells were resuspended in 1 ml lysis buffer (10 mM Tris-Cl, 50 mM NaCl, 1 mM EDTA, 0.5% Triton X-100, protease inhibitors, #88666 from Pierce), sonicated gently, by pulsing for 5 min, before centrifuging for 5 min at 16,000g at 4 °C (3–30KS, Sigma Laborzentrifugen GmbH). Supernatants were recovered and protein content determined using the Micro BCA Protein Assay (#23235, Thermo Fisher) with BSA as a standard. Data across three independent experiments was expressed as amount of protein ( $\mu$ g)/number of cells.

### 2.8. Statistical analyses

ANOVA or non-linear least square fit regression were used, along with Tukey's and sum-of-square F-test multiple comparisons tests, respectively, to assess the effects of PFAS for all experimental endpoints.  $\text{EC}_{50}$  was determined using non-linear fit (variable slope). All analyses were performed in GraphPad Prism 9.4.0., (San Diego, California USA, [www.graphpad.com](http://www.graphpad.com)). Sample sizes can be found within the respective methods sections.

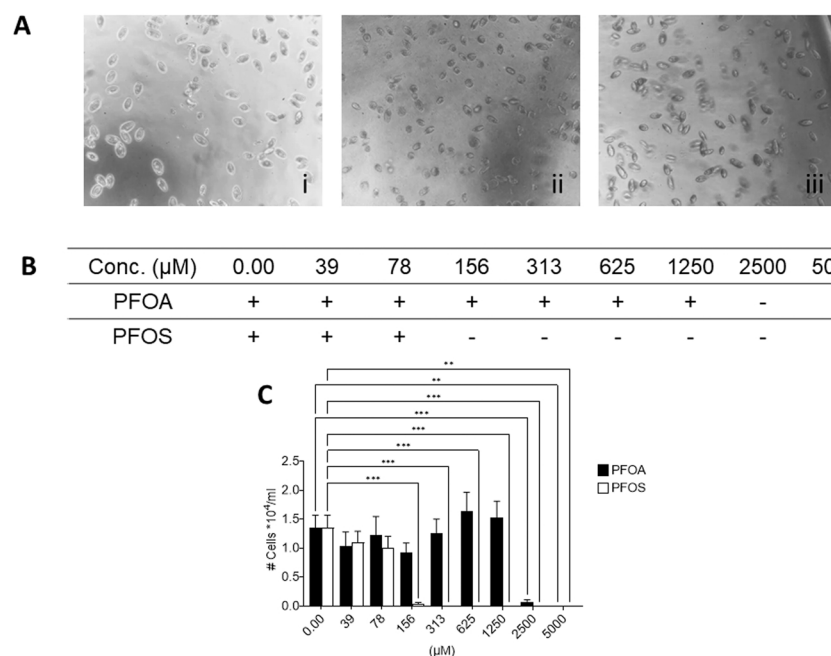
## 3. Results

### 3.1. PFAS toxic effects on *Tetrahymena pyriformis*

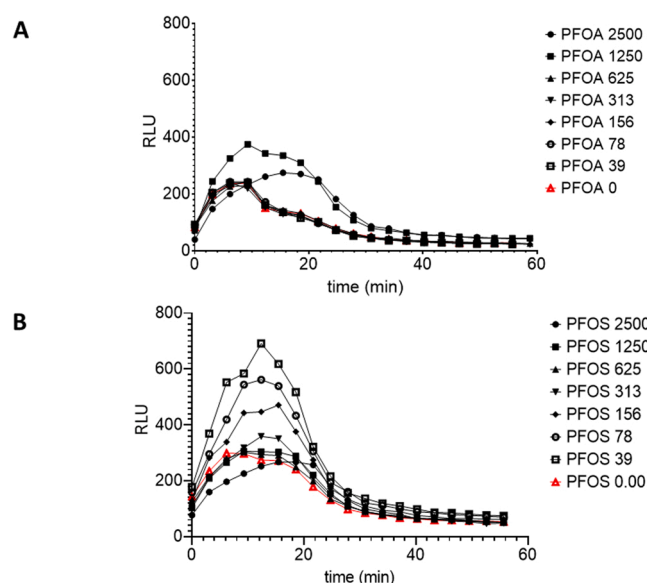
PFAS effects in microorganisms from aquatic and non-aquatic environments are limited (Lau et al., 2007; Ahrens and Bundschuh, 2014). To determine the toxicity of PFAS, PFOA and PFOS were serially diluted from 5000  $\mu$ M to 39  $\mu$ M in medium. Within 5 min post exposure, motility of *T. pyriformis* was consistently inhibited at concentrations > 2500  $\mu$ M PFOS and PFOA-intact cells were still visible (Fig. 1A; Supplement video 1). Within 2 h, motility was inhibited by 2500  $\mu$ M and 156  $\mu$ M of PFOA and PFOS, respectively (Fig. 1B). Concomitantly, there was also a significant difference seen between cell counts from PFOA and PFOS (5.8% of total variation,  $P < 0.0001$ ) with  $\text{EC}_{50}$  values of PFOA and PFOS estimated at 1724  $\mu$ M and 103  $\mu$ M, respectively (Fig. 1C). PFAS are toxic to *T. pyriformis*, with PFOS more potent than PFOA.

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PFAS-induced toxicity was further explored for the presence of reactive oxygen species using a luminol assay to detect hydrogen peroxide, one of the products of this process (Redza-Dutordoir and Averill-Bates, 2016). 1250  $\mu$ M of PFOA generated the highest and fastest peak in luminescence production at 9.3 min compared to all the other doses including its PFOA-free control whereas 2500  $\mu$ M of PFOA showed delayed (18.6 min) response (Fig. 2A). Results for *T. pyriformis* exposed to PFOS were interesting as while there was a minor delay in peak luminescence production compared to its PFOS-free control (12.4 min c. f. 9.3 min), the intensity of luminescence produced was inversely proportional to concentration of PFOS with the highest peak coming from



**Fig. 1.** PFAS affects cell proliferation. *Tetrahymena pyriformis* were exposed to 5000 – 39 µM of PFOA and PFOS, incubated at 25 °C and observed for motility (A, B), cell counts (B) and ROS production (C). Representative images captured at < 5 min, from cells cultured in 2500 µM PFOA (i) and PFOS (ii), or in no-PFAS control conditions (iii) (A). Representative table where motile cells (+) and non-motile cells (-) from 5 independent experiments after 2 h is shown (B), after which cells were fixed and counted using a hemocytometer, with a total of 30 of the “16-squares” were enumerated, as described in the Section 2 (C).



**Fig. 2.** PFAS causes ROS production. *Tetrahymena pyriformis* were incubated with 25 °C with luminol for a baseline reading in a plate reader before activating with India ink and recorded further for 60 min. Significance in mean cell counts was determined using two-way ANOVA and a Tukey's multiple comparisons test. \*\*\*  $p \leq 0.001$ ; \*\*  $p \leq 0.01$ . Results were based on the average of 5 independent experiments.

39 µM PFOS (Fig. 2B). The decline in luminescence could be due to increasing cell death, which suggests a higher level of oxidative stress generated by PFOS compared to PFOA.

### 3.2. Long-term effects of PFAS on *Tetrahymena pyriformis*

As the doubling time for *T. pyriformis* was reported to be 3–4 h at 27 °C (Bearden et al., 1997), to understand the long-term effects of PFAS on *T. pyriformis*, they were cultured for up to 6 days in the presence of subtoxic concentrations of PFOA and PFOS (5, 50, 500 µM). During the first 48 h, there were minor, non-significant increases in cell counts in

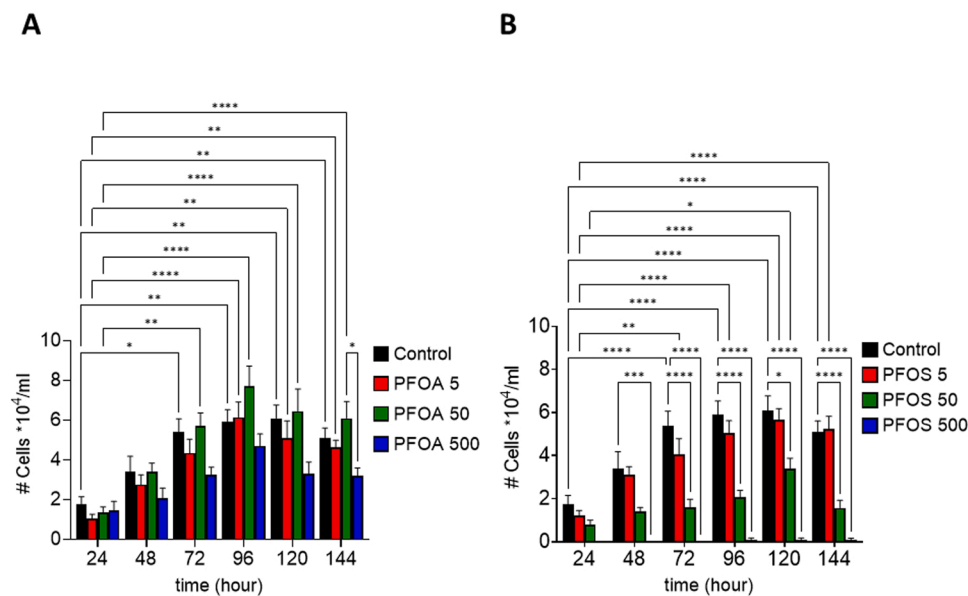
samples cultured in both PFOA ( $P = 0.98$ ) and PFOS ( $P = 0.72$ ) as well as the control. From 72 h onwards, cell counts were significantly higher with *T. pyriformis* cultured in 5 and 50, but not 500 µM PFOA compared to 24 h (Fig. 3A). Interestingly, with PFOS, from 72 h onwards, cell counts were significantly higher with *T. pyriformis* cultured in 5, but not 50 µM and furthermore, fewer viable cells were counted in medium containing 500 µM PFOS (Fig. 3B). Furthermore, from 72 h onwards, fewer cells were observed in medium containing 50 and 500 µM PFOS but not PFOA. From the data obtained after 96-day exposure period,  $EC_{50}$  values of 157.2 µM (65.1 mg/L) and 26.4 µM (13.2 mg/L) for PFOA and PFOS were obtained. This suggests there are different long-term effects of PFOA and PFOS on cell growth.

### 3.3. Size and phagocytosis regulated by PFAS

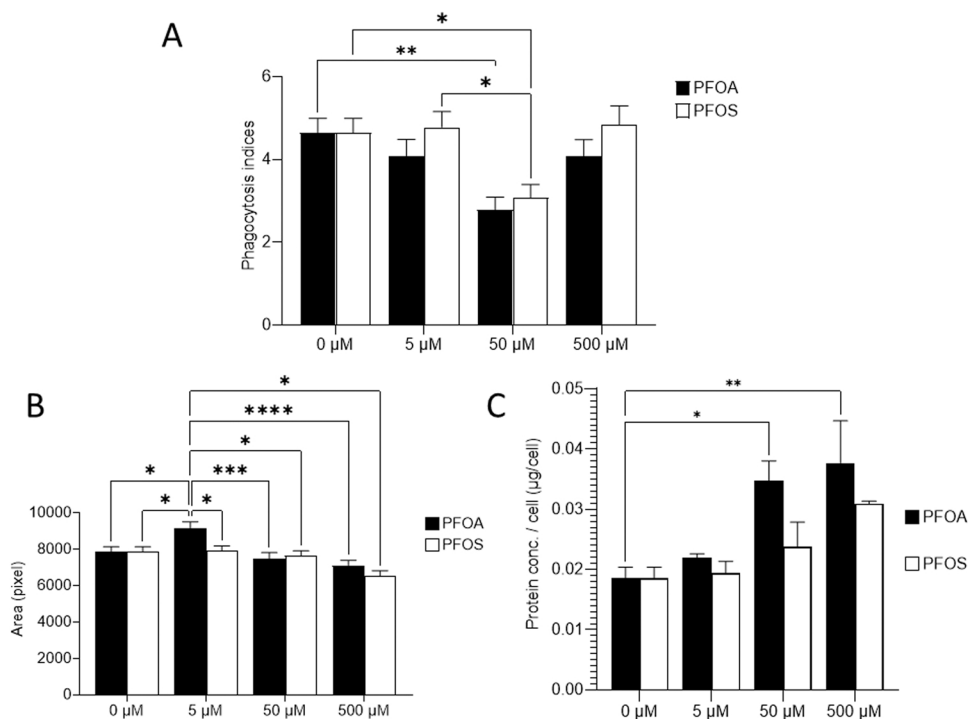
To determine if PFAS regulates phagocytosis, cells were cultured in medium containing 50 – 500 µM PFOA or PFOS for 6 days, starved overnight before being challenged with ink. There were significant decreases in phagocytosis with *T. pyriformis* cultured in both 50 µM PFOA ( $4.64 \pm 0.35$  c.f.  $2.78 \pm 0.30$ ,  $P = 0.006$ ) and PFOS ( $4.64 \pm 0.35$  c.f.  $3.07 \pm 0.33$ ,  $P = 0.03$ ) (Fig. 4A). It was established that the size and dry weight of *Tetrahymena* and *Paramecium* is dependent on several growth conditions including temperature and medium composition (Hellung-Larsen and Andersen, 1989; Iwamoto et al., 2005; Seyfert et al., 1984). *T. pyriformis* cultured in medium containing 5 µM PFOA for 6 days showed small but significant increase in size ( $9164.8 \pm 342.3$  c.f.  $7885.7 \pm 248.8$ ,  $P = 0.03$ ). This was not observed in higher concentrations of PFOA or in PFOS (Fig. 4B). Interestingly, there was no concomitant increase in protein content per cell from *T. pyriformis* cultured in 5 µM PFOA ( $0.018 \pm 0.002$  c.f.  $0.022 \pm 0.001$ ,  $P = 0.99$ ), although there was a 2-fold increase in protein content with cells cultured in 50 µM PFOA ( $0.018 \pm 0.002$  c.f.  $0.035 \pm 0.003$ ,  $P = 0.02$ ) and 500 µM PFOA ( $0.018 \pm 0.002$  c.f.  $0.038 \pm 0.007$ ,  $P = 0.004$ ) when compared to the non-treated control (Fig. 4C). Both PFOA and PFOS perturbs feeding function, only PFOA delayed cell division.

## 4. Discussion

The toxic effects of PFAS on the function of the unicellular protist, *Tetrahymena pyriformis*, is reported in this study. PFAS are persistent



**Fig. 3.** Long-term impact of PFAS on *Tetrahymena pyriformis* proliferation. Cells were exposed to 500 – 5  $\mu$ M of PFOA or PFOS 6 days at 25 °C. Cell viability was assessed by cell counts by fixing equal volumes of cell suspension with glutaraldehyde and enumerated using a hemacytometer (FastRead-102) under a light microscope. Graphs were plotted along with non-linear least square fit regression (second order polynomial) with sum-of-square F-test comparison method. Results were based on the average of 3 independent experiments, with a total of 30 of the “16-squares” enumerated.



**Fig. 4.** Size and function changes to *Tetrahymena pyriformis* in the presence of PFAS. Cells were exposed to 500 – 5  $\mu$ M of PFOA or PFOS 6 days at 25 °C. Samples of cell suspension were taken for size determination by light microscopy (A) and protein quantification by BCA assay (B). Cells were also assessed for phagocytic function by mixing equal volumes of cell suspension with ink for 30 min at 25 °C, before fixing with glutaraldehyde and uptake of ink was determined by light microscopy (C). Significance was determined using two-way ANOVA and a Tukey’s multiple comparisons test. \*\*\*\*  $p \leq 0.0001$ , \*\*\*  $p \leq 0.001$ , \*\*  $p \leq 0.01$ , \*  $p \leq 0.05$ . 48–78 (A) or 47–61 (C) organisms from all samples were measured across 3 independent experiments

contaminants of global concern due to diverse reported negative health effects (reviewed by Fenton et al., 2021). PFAS are a family of chemicals that consist of 4–14 carbon backbones with hydrogen atoms replaced with fluorine and charged functional groups. In the case of PFOA and PFOS, both have an 8-carbon backbone with either a carboxylate or sulphonate charged functional group, respectively. PFAS possess amphipathic structures resembling fatty acids and may affect cell function by activating nuclear receptors or other proteins, altering cell membrane potential, cytosolic pH and/or mitochondrial calcium distribution. This destabilises the antioxidant defence system which leads to oxidative DNA damage and apoptosis (Tsuda, 2016; Bonato et al., 2020; Kleszczyński and Składanowski, 2009, 2011; Kleszczyński et al., 2009). Their non-metabolisable properties means their reaction(s) are

irreversible and persistent (Solan and Lavado, 2022).

While PFAS are monitored in the freshwater environment, organisms like fish, eels, mussels and aquatic insects (e.g., dragonflies, damselflies) that live as larvae in water before emerging after the last metamorphosis have received much attention (e.g. Amphipoda, Araneae, and Coleoptera) (Augustsson et al., 2021; Koch et al., 2020; Kumar et al., 2022; Teunen et al., 2021). An understudied area of focus is freshwater benthic macroinvertebrates (BMIs), bottom-dwelling organisms that consume high levels of pollutants (Brase et al., 2022). The pelagic zone is relatively underexplored due to its heterogeneity, and this was addressed in this report by characterising the impact of PFAS on *T. pyriformis*.

Broadly, PFOS was more toxic towards *T. pyriformis* when compared to PFOA across the same concentration ranges. This differential toxicity



was also reported in other freshwater organisms, e.g., PFAS shared comparable EC<sub>50</sub> toxicities between *T. pyriformis* (PFOA: 1724 µM / 714 mg/L and PFOS: 103 µM / 52 mg/L), green neon shrimps (*Neocaridina denticulata*); PFOA: 2400 µM / 1000 mg/L and PFOS: 400 µM / 200 mg/L) water fleas (*Daphnia magna*; PFOA: 720 µM / 298 mg/L and PFOS: 386 µM / 193 mg/L; *Moina macrocopa*; PFOA: 481 µM / 199.51 mg/L and PFOS: 36 µM / 18 mg/L) and zebrafish (*Danio rerio*; PFOA: 2427 µM / 1005 mg/L and PFOS: 214 µM / 107 mg/L) (Ji et al., 2008; Li, 2009; Ye et al., 2009).

Interestingly, in mammals, PFOA, not PFOS, decreases total antioxidant capacity, though PFOS is only slightly cytotoxic and more haemolytic than PFOA (Florentin et al., 2011; Kawamoto et al., 2008; Wiersoe et al., 2015). In other non-mammalian organisms, both PFOA and PFOS induce reactive oxygen species formation in various vertebrate and invertebrate species, e.g., mice, rat, human, hamster, fish and mussel. This leads to oxidative damage, mitochondrial dysfunction, apoptosis and autophagy (Liu and Gin, 2018; Lopez-Arellano et al., 2019; Qian et al., 2010; Reistad et al., 2013; Shi and Zhou, 2010; Suh et al., 2017; Tang et al., 2018; Wen et al., 2021; Zeng et al., 2021; Zhao et al., 2011). While PFOS and PFOA were both shown to increase ROS generation in *T. pyriformis*, ROS formation differs between the 2 agonists. Lower dose of PFOS or higher dose of PFOA generated higher ROS levels. While the doses of PFOA and PFOS that illicit a ROS response were generally higher (> 156 µM) than some published elsewhere with non-mammalian organisms – 0.8 µM for zebrafish (*Danio rerio*) embryos or 0.02 µM for goldfish (*Carassius auratus*) lymphocytes, those studies used the more oxidant-sensitive probe dichlorodihydrofluorescein diacetate (DCF-DA) unlike the use of luminol in this current study (Shi and Zhou, 2010; Tang et al., 2018).

Concerning potential long-term effects of PFAS on *T. pyriformis* to PFAS, subacute concentrations of PFOA and PFOS showed growth over a 6-day (144 h) period, with no significant difference between the concentrations. However, there was a significant decrease at 500 µM PFOA, 50 and 500 µM PFOS compared to their respective controls. Growth decreased after 96 h, likely due to space constraints of the flask. Interestingly, after 96 h exposure period, the EC<sub>50</sub> value for PFOA 157.2 µM (65.1 mg/L) was lower than that observed in *Daphnia magna* (220 – 239 mg/L; (Ding et al., 2012; Barmiento et al., 2015) although EC<sub>50</sub> of those studies were related to sexual reproduction to form neonates and were not asexual reproduction. Interestingly, the data on the toxicological impact of PFOA/PFOS at environmental levels (up to 15 µM) on organisms are limited and conflicted and is determined by organisms and duration of exposure. The midges (*Chironomus tentans* and *Chironomus dilutus*) and damselfly (*Enallagma cyathigerum*) are sensitive to chronic PFOA/PFOS exposure, with reduced weight, survivability, biomass and total emergence at < 150 µg/L (300 nM) PFOS (MacDonald et al., 2004; McCarthy et al., 2021; Bots et al., 2010). Those that survive and emerge exhibit behavioural changes e.g., reduced rate of swimming, response to predator attack and foraging (Van Gossum et al., 2009). However, in another midge species, *Chironomus riparius*, reduced growth was apparent at most/several generations in a multigeneration study, though survival, development, and reproduction were unaffected (Marziali et al., 2019).

*T. pyriformis* use their cilia to sweep particles (including ink) into their oral groove and into a food vacuole in an actin-dependent process similar to that seen in mammalian phagocytes (Bozzone, 2000; Williams et al., 2006). After 6 days of culture in PFOA or PFOS, there were significant decreases in phagocytosis with *T. pyriformis* cultured in both 50 µM PFOA and PFOS. This U-shaped dose response suggest that any concentration lower (5 µM) or higher (500 µM) than the optimum would either be suboptimal or exhibit compensatory effects, respectively (Calabrese and Baldwin, 2001). Interestingly, in another unicellular protist, *Paramecium caudatum*, PFOS, not PFOA, caused the effect of making the organisms swim backward, largely due to increased intracellular Ca<sup>2+</sup> concentration around the ciliary system (Kawamoto et al., 2008).

Cell size is determined by a finely tuned process between cell growth (mass or volume) and division. Therefore, increased growth rates with a constant rate of division leads to larger cell sizes. In yeast, environmental stressors such as nutrient composition and elevated temperatures can perturb this process with the mechanisms and functional relevance of this phenomenon still controversial (Aldea et al., 2017; Miettinen et al., 2017; Terhorst et al., 2020). Long term exposure of *T. pyriformis* to PFAS coincided with a minor increase in size, when measured microscopically. Interestingly, this increase in size (only at 5 µM PFOA) was also complemented with an increase in protein levels (50 and 500 µM for both PFOA/S). It is possible that this increase in protein levels in *T. pyriformis* causes irreparable cell damage due to an accumulation of impaired and misfolded proteins, a process known as proteotoxicity. Proteotoxicity is known to be triggered by several factors including oxidative stress due to environmental insults (Peters et al., 2021; Wang et al., 2022). Therefore, proteotoxicity would be reflected with cell death via the apparent lack of an increase in cell size at the higher PFOA/S (50 and 500 µM) doses (Kane et al., 2021; Shibata and Morimoto, 2014; Shor et al., 2013).

To conclude, PFAS demonstrated broad toxicity toward the microeukaryote *T. pyriformis*, with differential toxicities being chemical specific (i.e., PFOA versus PFOS). This present study has its limitations with short time scales and relatively high concentrations of PFOA/PFOS in order to obtain EC<sub>50</sub> estimates. However, in real aquatic ecosystems, *Tetrahymena* are chronically exposed to, and bioaccumulate low levels of PFOA/PFOS over its relatively short life cycle (2–3 h under optimal conditions; (Ruehle et al., 2016) and over many generations. *T. pyriformis* represent a promising candidate for assessing the biological effects of anthropogenically-derived contaminants in an aquatic setting.

#### CRediT authorship contribution statement

**Jenson Lim:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization, Project administration, Funding acquisition.

#### Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jenson Lim reports financial support was provided by Carnegie Trust for the Universities of Scotland (RIG008296).

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