Environmental Toxicology and Pharmacology 95 (2022) 103954

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap

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

Jenson Lim [1]

Biological and Environmental Sciences, Faculty of Natural Sciences, University of Stirling, Stirling FK9 4LA, Scotland, UK

ARTICLE INFO

Edited by Dr. M.D. Coleman

Keywords:
Tetrahymena
Perfluorooctanoic acid (PFOA)
Perfluorooctanesulfonic acid (PFOS)
Phagocytosis
Cell division
Reactive oxygen species

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 waterbodies 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

E-mail address: jenson.lim@stir.ac.uk.
[1] http://orcid.org/0000-0001-7417-356X.

https://doi.org/10.1016/j.etap.2022.103954
Received 10 May 2022; Received in revised form 3 August 2022; Accepted 6 August 2022
Available online 7 August 2022

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 ^{o}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 μM or 5000 – 39 μM 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 μM 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 μl 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 1×10^6 *T. pyriformis* grown in medium were exposed to 5000 – 39 μM of PFOA or PFOS for 1 h, respectively, in a 25 °C incubator. 100 μl of cell suspension was aliquot into 3 wells of a white 96-well microplate, along with 200 μM luminol and read in plate reader with luminescence capability (GloMax Discover System, Promega) for 10 min at 25 ^{o}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 ^{o}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 μM 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 $CaCl_2H_2O$) for 24 h in a 25 ^{o}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 ^{o}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 16,000*g* 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 (μg)/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. 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). 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 μM to 39 μM in medium. Within 5 min post exposure, motility of *T. pyriformis* was consistently inhibited at concentrations > 2500 μM PFOS and PFOA-intact cells were still visible (Fig. 1A; Supplement video 1). Within 2 h, motility was inhibited by 2500 μM and 156 μM 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 EC_{50} values of PFOA and PFOS estimated at 1724 μM and 103 μM, respectively (Fig. 1C). PFAS are toxic to *T. pyriformis*, with PFOS more potent than PFOA.

Supplementary material related to this article can be found online at doi:10.1016/j.etap.2022.103954.

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 μM 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 μM 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

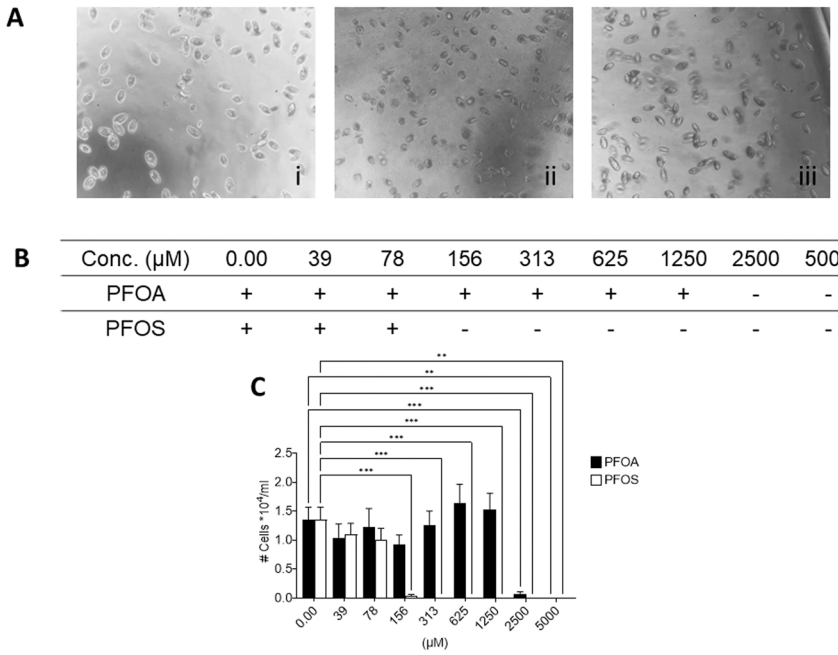

Fig. 1. PFAS affects cell proliferation. *Tetrahymena pyriformis* were exposed to 5000 – 39 μM of PFOA and PFOS, incubated at 25 °C and observed for motility (A, B), cell counts (B) and ROS production (C). Representative images captured at < 5 min, from cells cultured in 2500 μM PFOA (i) and PFOS (ii), 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).

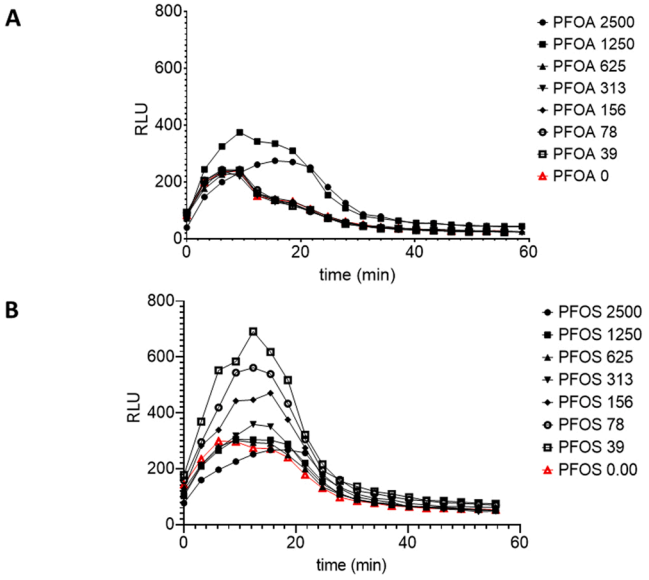

Fig. 2. PFAS causes ROS production. *Tetrahymena pyriformis* were incubated with 25 °C with luminol for a baseline reading in a plate reader before activating with India ink and recorded further for 60 min. Significance in mean cell counts was determined using two-way ANOVA and a Tukey's multiple comparisons 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 ± 0.35 c.f. 2.78 ± 0.30, $P = 0.006$) and PFOS (4.64 ± 0.35 c.f. 3.07 ± 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 ± 342.3 c.f. 7885.7 ± 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 ± 0.002 c.f. 0.022 ± 0.001, $P = 0.99$), although there was a 2-fold increase in protein content with cells cultured in 50 μM PFOA (0.018 ± 0.002 c.f. 0.035 ± 0.003, $P = 0.02$) and 500 μM PFOA (0.018 ± 0.002 c.f. 0.038 ± 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

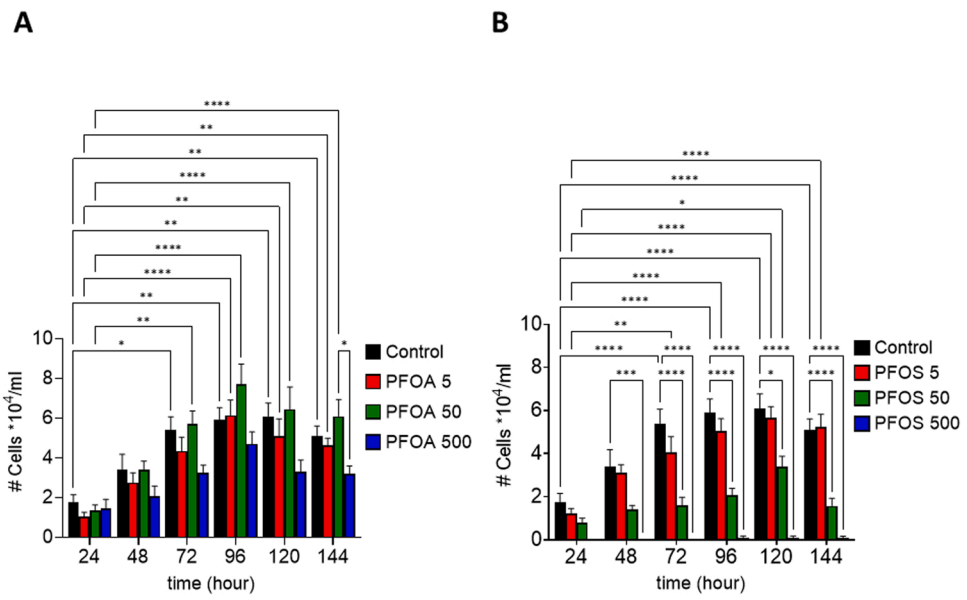

Fig. 3. Long-term impact of PFAS on *Tetrahymena pyriformis* proliferation. Cells were exposed to 500 – 5 μM of PFOA or PFOS 6 days at 25 °C. Cell viability was assessed by cell counts by fixing equal volumes of cell suspension with glutaraldehyde and enumerated using a hemacytometer (FastRead-102) under 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.

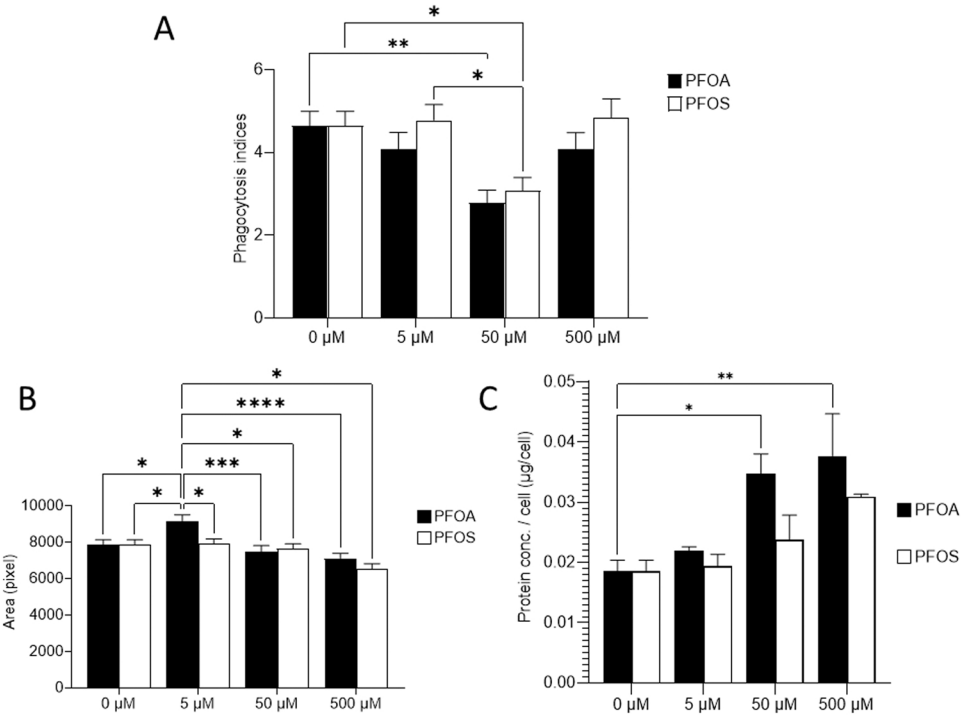

Fig. 4. Size and function changes to *Tetrahymena pyriformis* in the presence of PFAS. Cells were exposed to 500 – 5 μM of PFOA or PFOS 6 days at 25 °C. Samples of cell suspension were taken for size determination by light microscopy (A) and protein quantification by BCA assay (B). Cells were also assessed 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_{50} toxicities between *T. pyriformis* (PFOA: 1724 μM / 714 mg/L and PFOS: 103 μM / 52 mg/L), green neon shrimps (*Neocaridina denticulate*); 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; Wielsoe 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_{50} 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; Barmentlo et al., 2015) although EC_{50} 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^{2+} 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_{50} 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).

Acknowledgements

JL would like to thank his family for their help and support and to Christopher Coates for his support with editing and proof-reading of the manuscript. The University of Stirling and the Carnegie Trust for the Universities of Scotland (RIG008296) provided some financial support.

References

Ahrens, L., Bundschuh, M., 2014. Fate and effects of poly- and perfluoroalkyl substances in the aquatic environment: a review. Environ. Toxicol. Chem. 33, 1921–1929.
Aldea, M., Jenkins, K., Csikasz-Nagy, A., 2017. Growth rate as a direct regulator of the start network to set cell size. Front. Cell Dev. Biol. 5, 57.
Augustsson, A., Lennqvist, T., Osbeck, C.M.G., Tibblin, P., Glynn, A., Nguyen, M.A., Westberg, E., Vestergren, R., 2021. Consumption of freshwater fish: a variable but significant risk factor for PFOS exposure. Environ. Res. 192, 110284.
Barmentlo, S.H., Stel, J.M., van Doorn, M., Eschauzier, C., de Voogt, P., Kraak, M.H., 2015. Acute and chronic toxicity of short chained perfluoroalkyl substances to Daphnia magna. Environ. Pollut. 198, 47–53.
Bearden, A.P., Gregory, B.W., Schultz, T.W., 1997. Population growth kinetics of Tetrahymena pyriformis exposed to selected nonpolar narcotics. Arch. Environ. Contam. Toxicol. 33, 401–406.
Blackburn, E.H., Gall, J.G., 1978. A tandemly repeated sequence at the termini of the extrachromosomal ribosomal RNA genes in Tetrahymena. J. Mol. Biol. 120, 33–53.
Blake, B.E., Fenton, S.E., 2020. Early life exposure to per- and polyfluoroalkyl substances (PFAS) and latent health outcomes: a review including the placenta as a target tissue and possible driver of peri- and postnatal effects. Toxicology 443, 152565.

Bonato, M., Corra, F., Bellio, M., Guidolin, L., Tallandini, L., Irato, P., Santovito, G., 2020. PFAS environmental pollution and antioxidant responses: an overview of the impact on human field. Int. J. Environ. Res. Public Health 17.
Bots, J., De Bruyn, L., Snijkers, T., Van den Branden, H., Van Gossum, B., 2010. Exposure to perfluorooctane sulfonic acid (PFOS) adversely affects the life-cycle of the damselfly Enallagma cyathigerum. Environ. Pollut. 158, 901–905.
Bozzone, D.M., 2000. Investigating phagocytosis in tetrahymena: an experimental system suitable for introductory & advanced instruction. Am. Biol. Teach. 62, 136–139.
Brase, R.A., Schwab, H.E., Li, L., Spink, D.C., 2022. Elevated levels of per- and polyfluoroalkyl substances (PFAS) in freshwater benthic macroinvertebrates from the Hudson River Watershed. Chemosphere 291, 132830.
Calabrese, E.J., Baldwin, L.A., 2001. U-shaped dose-responses in biology, toxicology, and public health. Annu. Rev. Public Health 22, 15–33.
Cassidy-Hanley, D.M., 2012. Tetrahymena in the laboratory: strain resources, methods for culture, maintenance, and storage. Methods Cell Biol. 109, 237–276.
Csaba, G., 2016. Lectins and tetrahymena - a review. Acta Microbiol. Immunol. Hung. 63, 279–291.
Dayeh, V.R., Grominsky, S., DeWitte-Orr, S.J., Sotornik, D., Yeung, C.R., Lee, L.E., Lynn, D.H., Bols, N.C., 2005. Comparing a ciliate and a fish cell line for their sensitivity to several classes of toxicants by the novel application of multiwell filter plates to Tetrahymena. Res. Microbiol. 156, 93–103.
Ding, G.H., Fromel, T., van den Brandhof, E.J., Baerselman, R., Peijnenburg, W.J., 2012. Acute toxicity of poly- and perfluorinated compounds to two cladocerans, Daphnia magna and Chydorus sphaericus. Environ. Toxicol. Chem. 31, 605–610.
Fenton, S.E., Ducatman, A., Boobis, A., DeWitt, J.C., Lau, C., Ng, C., Smith, J.S., Roberts, S.M., 2021. Per- and polyfluoroalkyl substance toxicity and human health review: current state of knowledge and strategies for informing future research. Environ. Toxicol. Chem. 40, 606–630.
Florentin, A., Deblonde, T., Diguio, N., Hautemaniere, A., Hartemann, P., 2011. Impacts of two perfluorinated compounds (PFOS and PFOA) on human hepatoma cells: cytotoxicity but no genotoxicity? Int. J. Hyg. Environ. Health 214, 493–499.
Ghisi, R., Vamerali, T., Manzetti, S., 2019. Accumulation of perfluorinated alkyl substances (PFAS) in agricultural plants: a review. Environ. Res. 169, 326–341.
Giesy, J.P., Kannan, K., 2002. Perfluorochemical surfactants in the environment. Environ. Sci. Technol. 36, 146A–152A.
Gomes, I.B., Maillard, J.-Y., Simões, L.C., Simões, M., 2020. Emerging contaminants affect the microbiome of water systems—strategies for their mitigation. npj Clean Water 3, 39.
Grandjean, P., 2018. Delayed discovery, dissemination, and decisions on intervention in environmental health: a case study on immunotoxicity of perfluorinated alkylate substances. Environ. Health 17, 62.
Gray, R., Gray, A., Fite, J.L., Jordan, R., Stark, S., Naylor, K., 2012. A simple microscopy assay to teach the processes of phagocytosis and exocytosis. CBE Life Sci. Educ. 11, 180–186.
Greider, C.W., Blackburn, E.H., 1985. Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. Cell 43, 405–413.
Hellung-Larsen, P., Andersen, A.P., 1989. Cell volume and dry weight of cultured Tetrahymena. J. Cell Sci. 92 (Pt. 2), 319-24.
Iwamoto, M., Sugino, K., Allen, R.D., Naitoh, Y., 2005. Cell volume control in Paramecium: factors that activate the control mechanisms. J. Exp. Biol. 208, 523–537.
Ji, K., Kim, Y., Oh, S., Ahn, B., Jo, H., Choi, K., 2008. Toxicity of perfluorooctane sulfonic acid and perfluorooctanoic acid on freshwater macroinvertebrates (Daphnia magna and Moina macrocopa) and fish (Oryzias latipes). Environ. Toxicol. Chem. 27, 2159–2168.
Kane, A.J., Brennan, C.M., Xu, A.E., Solis, E.J., Terhorst, A., Denic, V., Amon, A., 2021. Cell adaptation to aneuploidy by the environmental stress response dampens induction of the cytosolic unfolded-protein response. Mol. Biol. Cell 32, 1557–1564.
Kannan, K., Franson, J.C., Bowerman, W.W., Hansen, K.J., Jones, P.D., Giesy, J.P., 2001. Perfluorooctane sulfonate in fish-eating water birds including bald eagles and albatrosses. Environ. Sci. Technol. 35, 3065–3070.
Karnjanapiboonwong, A., Deb, S.K., Subbiah, S., Wang, D., Anderson, T.A., 2018. Perfluoroalkylsulfonic and carboxylic acids in earthworms (Eisenia fetida): accumulation and effects results from spiked soils at PFAS concentrations bracketing environmental relevance. Chemosphere 199, 168–173.
Kawamoto, K., Nishikawa, Y., Oami, K., Jin, Y., Sato, I., Saito, N., Tsuda, S., 2008. Effects of perfluorooctane sulfonate (PFOS) on swimming behavior and membrane potential of Paramecium caudatum. J. Toxicol. Sci. 33, 155–161.
Kleszczynski, K., Skladanowski, A.C., 2009. Mechanism of cytotoxic action of perfluorinated acids. I. alteration in plasma membrane potential and intracellular pH level. Toxicol. Appl. Pharmacol. 234, 300–305.
Kleszczynski, K., Skladanowski, A.C., 2011. Mechanism of cytotoxic action of perfluorinated acids. III. Disturbance in Ca(2)+ homeostasis. Toxicol. Appl. Pharmacol. 251, 163–168.
Kleszczynski, K., Stepnowski, P., Skladanowski, A.C., 2009. Mechanism of cytotoxic action of perfluorinated acids II. Disruption of mitochondrial bioenergetics. Toxicol. Appl. Pharmacol. 235, 182–190.
Koch, A., Jonsson, M., Yeung, L.W.Y., Karrman, A., Ahrens, L., Ekblad, A., Wang, T., 2020. Per- and polyfluoroalkyl-contaminated freshwater impacts adjacent riparian food webs. Environ. Sci. Technol. 54, 11951–11960.
Kudo, N., Kawashima, Y., 2003. Toxicity and toxicokinetics of perfluorooctanoic acid in humans and animals. J. Toxicol. Sci. 28, 49–57.
Kumar, E., Koponen, J., Rantakokko, P., Airaksinen, R., Ruokojarvi, P., Kiviranta, H., Vuorinen, P.J., Myllyla, T., Keinanen, M., Raitaniemi, J., Mannio, J., Junttila, V., Nieminen, J., Venalainen, E.R., Jestoi, M., 2022. Distribution of perfluoroalkyl acids in fish species from the Baltic Sea and freshwaters in Finland. Chemosphere 291, 132688.
Lainhart, W., Stolfa, G., Koudelka, G.B., 2009. Shiga toxin as a bacterial defense against a eukaryotic predator, Tetrahymena thermophila. J. Bacteriol. 191, 5116–5122.
Latham, J.A., Cech, T.R., 1989. Defining the inside and outside of a catalytic RNA molecule. Science 245, 276-82.
Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., Seed, J., 2007. Perfluoroalkyl acids: a review of monitoring and toxicological findings. Toxicol. Sci. 99, 366–394.
Li, J., Zhang, X.L., Liu, Y.J., Lu, C.P., 2011. Development of an Aeromonas hydrophila infection model using the protozoan Tetrahymena thermophila. FEMS Microbiol. Lett. 316, 160–168.
Li, M.H., 2009. Toxicity of perfluorooctane sulfonate and perfluorooctanoic acid to plants and aquatic invertebrates. Environ. Toxicol. 24, 95–101.
Liu, C., Gin, K.Y., 2018. Immunotoxicity in green mussels under perfluoroalkyl substance (PFAS) exposure: Reversible response and response model development. Environ. Toxicol. Chem. 37, 1138–1145.
Lopez-Arellano, P., Lopez-Arellano, K., Luna, J., Flores, D., Jimenez-Salazar, J., Gavia, G., Teteltitla, M., Rodriguez, J.J., Dominguez, A., Casas, E., Bahena, I., Betancourt, M., Gonzalez, C., Ducolomb, Y., Bonilla, E., 2019. Perfluorooctanoic acid disrupts gap junction intercellular communication and induces reactive oxygen species formation and apoptosis in mouse ovaries. Environ. Toxicol. 34, 92–98.
MacDonald, M.M., Warne, A.L., Stock, N.L., Mabury, S.A., Solomon, K.R., Sibley, P.K., 2004. Toxicity of perfluorooctane sulfonic acid and perfluorooctanoic acid to Chironomus tentans. Environ. Toxicol. Chem. 23, 2116–2123.
Marziali, L., Rosignoli, F., Valsecchi, S., Polesello, S., Stefani, F., 2019. Effects of perfluoralkyl substances on a multigenerational scale: a case study with Chironomus riparius (Diptera, Chironomidae). Environ. Toxicol. Chem. 38, 988–999.
McCarthy, C.J., Roark, S.A., Wright, D., O'Neal, K., Muckey, B., Stanaway, M., Rewerts, J.N., Field, J.A., Anderson, T.A., Salice, C.J., 2021. Toxicological response of chironomus dilutus in single-chemical and binary mixture exposure experiments with 6 perfluoralkyl substances. Environ. Toxicol. Chem. 40, 2319–2333.
Miettinen, T.P., Caldez, M.J., Kaldis, P., Bjorklund, M., 2017. Cell size control - a mechanism for maintaining fitness and function. Bioessays 39.
Navarro, I., de la Torre, A., Sanz, P., Pro, J., Carbonell, G., Martinez, M.L.A., 2016. Bioaccumulation of emerging organic compounds (perfluoroalkyl substances and halogenated flame retardants) by earthworm in biosolid amended soils. Environ. Res. 149, 32–39.
Pang, M.D., Lin, X.Q., Hu, M., Li, J., Lu, C.P., Liu, Y.J., 2012. Tetrahymena: an alternative model host for evaluating virulence of Aeromonas strains. PLoS One 7, e48922.
Peters, A., Nawrot, T.S., Baccarelli, A.A., 2021. Hallmarks of environmental insults. Cell 184, 1455–1468.
Qian, Y., Ducatman, A., Ward, R., Leonard, S., Bukowski, V., Lan Guo, N., Shi, X., Vallyathan, V., Castranova, V., 2010. Perfluorooctane sulfonate (PFOS) induces reactive oxygen species (ROS) production in human microvascular endothelial cells: role in endothelial permeability. J. Toxicol. Environ. Health A 73, 819–836.
Redza-Dutordoir, M., Averill-Bates, D.A., 2016. Activation of apoptosis signalling pathways by reactive oxygen species. Biochim. Biophys. Acta 1863, 2977–2992.
Reistad, T., Fonnum, F., Mariussen, E., 2013. Perfluoroalkylated compounds induce cell death and formation of reactive oxygen species in cultured cerebellar granule cells. Toxicol. Lett. 218, 56–60.
Ruehle, M.D., Orias, E., Pearson, C.G., 2016. Tetrahymena as a unicellular model eukaryote: genetic and genomic tools. Genetics 203, 649–665.
Sauvant, M.P., Pepin, D., Piccinni, E., 1999. Tetrahymena pyriformis: a tool for toxicological studies. A review. Chemosphere 38, 1631–1669.
Schultz, M.M., Barofsky, D.F., Field, J.A., 2004. Quantitative determination of fluorotelomer sulfonates in groundwater by LC MS/MS. Environ. Sci. Technol. 38, 1828–1835.
Seyfert, H.M., Hipke, H., Schmidt, W., 1984. Isolation and phenotypic characterization of Tetrahymena thermophila size mutants: the relationship between cell size and regulation of DNA content. J. Cell Sci. 67, 203–215.
Shi, X., Zhou, B., 2010. The role of Nrf2 and MAPK pathways in PFOS-induced oxidative stress in zebrafish embryos. Toxicol. Sci. 115, 391–400.
Shibata, Y., Morimoto, R.I., 2014. How the nucleus copes with proteotoxic stress. Curr. Biol. 24, R463–R474.
Shor, E., Fox, C.A., Broach, J.R., 2013. The yeast environmental stress response regulates mutagenesis induced by proteotoxic stress. PLoS Genet. 9, e1003680.
Solan, M.E., Lavado, R., 2022. The use of in vitro methods in assessing human health risks associated with short-chain perfluoroalkyl and polyfluoroalkyl substances (PFAS). J. Appl. Toxicol. 42, 1298–1309.
Suh, K.S., Choi, E.M., Kim, Y.J., Hong, S.M., Park, S.Y., Rhee, S.Y., Oh, S., Kim, S.W., Pak, Y.K., Choe, W., Chon, S., 2017. Perfluorooctanoic acid induces oxidative damage and mitochondrial dysfunction in pancreatic beta-cells. Mol. Med. Rep. 15, 3871–3878.
Tang, J., Lu, X., Chen, F., Ye, X., Zhou, D., Yuan, J., He, J., Chen, B., Shan, X., Jiang, J., Liu, W., Zhang, H., 2018. Effects of perfluorooctanoic acid on the associated genes expression of autophagy signaling pathway of Carassius auratus lymphocytes in vitro. Front. Physiol. 9, 1748.
Terhorst, A., Sandikci, A., Keller, A., Whittaker, C.A., Dunham, M.J., Amon, A., 2020. The environmental stress response causes ribosome loss in aneuploid yeast cells. Proc. Natl. Acad. Sci. USA 117, 17031–17040.
Teunen, L., De Jonge, M., Malarvannan, G., Covaci, A., Belpaire, C., Focant, J.F., Blust, L., Bervoets, R., 2021. Effect of abiotic factors and environmental concentrations on the bioaccumulation of persistent organic and inorganic compounds to freshwater fish and mussels. Sci. Total Environ. 799, 149448.

Tsuda, S., 2016. Differential toxicity between perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). J. Toxicol. Sci. 41, SP27–SP36.
Van Gossum, H., Bots, J., Snijkers, T., Meyer, J., Van Wassenbergh, S., De Coen, W., De Bruyn, L., 2009. Behaviour of damselfly larvae (Enallagma cyathigerum) (Insecta, Odonata) after long-term exposure to PFOS. Environ. Pollut. 157, 1332–1336.
Wang, M., Law, M.E., Law, B.K., 2022. Proteotoxicity and endoplasmic reticulum stress-mediated cell death. In: Liao, D. (Ed.), Mechanisms of Cell Death and Opportunities for Therapeutic Development. Academic Press, pp. 119–174.
Wen, L.L., Chen, Y.T., Lee, Y.G., Ko, T.L., Chou, H.C., Juan, S.H., 2021. Perfluorooctane sulfonate induces autophagy-associated apoptosis through oxidative stress and the activation of extracellular signal-regulated kinases in renal tubular cells. PLoS One 16, e0245442.
Wielsoe, M., Long, M., Ghisari, M., Bonefeld-Jorgensen, E.C., 2015. Perfluoroalkylated substances (PFAS) affect oxidative stress biomarkers in vitro. Chemosphere 129, 239–245.
Williams, N.E., Tsao, C.C., Bowen, J., Hehman, G.L., Williams, R.J., Frankel, J., 2006. The actin gene ACT1 is required for phagocytosis, motility, and cell separation of Tetrahymena thermophila. Eukaryot. Cell 5, 555–567.
Woods, A.L., Parker, D., Glick, M.M., Peng, Y., Lenoir, F., Mulligan, E., Yu, V., Piizzi, G., Lister, T., Lilly, M.D., Dzink-Fox, J., Jansen, J.M., Ryder, N.S., Dean, C.R., Smith, T. M., 2022. High-throughput screen for inhibitors of Klebsiella pneumoniae virulence using a tetrahymena pyriformis co-culture surrogate host model. ACS Omega 7, 5401–5414.
Ye, L., Wu, L.L., Jiang, Y.X., Zhang, C.J., Chen, L., 2009. Toxicological study of PFOS/PFOA to zebrafish (Danio rerio) embryos. Huan Jing Ke Xue 30, 1727–1732.
Zeng, H.C., Zhu, B.Q., Wang, Y.Q., He, Q.Z., 2021. ROS-triggered autophagy is involved in PFOS-induced apoptosis of human embryo liver L-02 cells. BioMed Res. Int. 2021, 6625952.
Zhao, G., Wang, J., Wang, X., Chen, S., Zhao, Y., Gu, F., Xu, A., Wu, L., 2011. Mutagenicity of PFOA in mammalian cells: role of mitochondria-dependent reactive oxygen species. Environ. Sci. Technol. 45, 1638–1644.