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# Evidence on the genotoxic and ecotoxic effects of PFOA, PFOS and their mixture on human lymphocytes and bacteria

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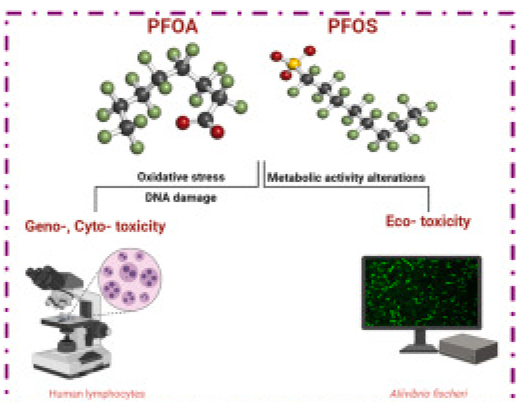
## Highlights

- The genotoxic and ecotoxic effects of PFOA and PFOS and their mixture were studied.
- PFOS was found to be more genotoxic than PFOA.
- The mixture was more cytotoxic and genotoxic than the individual compounds.
- PFOA and PFOS presented very low acute effects to *Aliivibrio fischeri.*
- Geno- and eco-toxicological studies of PFAS mixtures are of high interest.

## Abstract

human and environmental health as well as the increasing interest of the scientific community in the implications that might present especially when they co-exist, this study aims to assess their harmful impacts, both individually and as a mixture on human lymphocytes and aquatic microorganisms. The cytokinesis-block micronucleus (CBMN) assay was used to examine their potential for cytotoxicity and genotoxicity towards human cells, and Microtox assay using *Aliivibrio fischeri* assay was used to estimate the environmental risk. Regarding the human lymphocytes, the tested concentrations ranged between 250 and 1000 $\mu g L^{-1}$, for all cases. PFOA increased slightly the frequency of micronuclei (MN) but without statistical significance. In the case of PFOS, our results showed a dose-dependent increase in the frequency of micronuclei which showed a statistically significant difference ($p<0.001$) at 1000 $\mu g L^{-1}$, which is the highest studied concentration. Regarding the CBPI index, statistically significant ($p<0.05$, $p<0.01$, and $p<0.001$ respectively) differences were observed at all studied concentrations of PFOS, compared to the control. The mixture was found to be more cytotoxic and genotoxic than the individual tested compounds, causing a higher decrease at the CBPI index even in lower concentrations and increase at the MN frequencies. *Aliivibrio fischeri* was exposed to various concentrations in the range of 0.5 $\mu g\ L^{-1}$- 20 $mg L^{-1}$, for 5 and 15 min and significant increase in the inhibition percentage at the highest tested concentration of their mixture after 15 min was observed.

## Graphical abstract

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## Introduction

organofluorine substances that are frequently utilized in various consumer products and industrial applications. Their molecules' C–F bonds provide them with significant physical characteristics like low coefficients of friction, low polarizabilities, and chemical inertness and consequently they have a multitude of applications (Glüge et al., 2020; Papadopoulou et al., 2011; Stahl et al., 2011; Buser and Morf, 2009; Prevedouros et al., 2006). In the PFAS group, Perfluorooctanoic acid (PFOA) and Perfluorooctane sulfonic acid (PFOS) are the most well-known substances in this group (Brunn et al., 2023; Wang et al., 2021). PFOA and PFOS are "long-chain" chemicals, with the first consisting of a hydrophobic carbon-fluorine (C–F) chain and a hydrophilic carboxyl group and the second having sulfonate as a hydrophilic functional group (Leung et al., 2022). Their applications are numerous and include non-stick cookware, paints, foams for fighting fires, textiles that resist stains, food packaging, insecticides, flame retardants, and additives for synthetic industrial goods (Mhadhbi et al., 2012; Centers for Disease Control and Prevention, 2017; Glüge et al., 2020; Jarvis et al., 2021).

PFOA and PFOS have been characterized as "forever" chemicals as they present high persistence in the environment due to the high stability of the C–F bond. Although they have been phased out in many countries, they are often detected in the environment over the years (Harada et al., 2003; Flores et al., 2013; Cao et al., 2019; Bai and Son, 2021), posing a serious risk to the environment and human health (Bonato et al., 2020 and references therein) . Traces of PFOA and PFOS have been detected in numerous environmental samples, such as water (Hepburn et al., 2019; Bai and Son, 2021) and soil (Sammut et al., 2019; Cao et al., 2019). As PFOA is more water soluble than PFOS, it has a reduced potential for bioaccumulation (Martin et al., 2003; Ahrens, 2011).

Numerous investigations have also been carried out concerning the occurrence of PFOA and PFOS in human samples. Their presence is confirmed, amongst others, in blood serum and plasma (Bartell et al., 2010; Pan et al., 2010; Wu et al., 2017; Poothong et al., 2017) and breast milk (Kärrman et al., 2007; Lee et al., 2018; Antignac et al., 2013; Fujii et al., 2012), at ng $mL^{-1}$. Exposure of humans to PFAS can take place through numerous sources, including water, dust and air, and consumer products such as personal care products, cosmetics, stain resistant fabrics and cleaning products (ATSDR, 2022; Sunderland et al., 2019; Ojo et al., 2022b; US EPA, 2023a). Primary ways of exposure to PFOA and PFOS, amongst others, are the contaminated food and food packed in materials containing PFAS (Emmett et al., 2006; Houde et al., 2006; Rahman et al., 2014; ATSDR, 2022; Sunderland et al., 2019; Ojo et al., 2022b; US EPA, 2023a). PFAS are absorbed in the gastrointestinal tract, and since they cannot be metabolized, they accumulate in the human bodies (Knutsen et al., 2018; EEA, (Knutsen et al., 2018). It was observed that the category 'Fish and other products' was mainly responsible for the chronic exposure, for both PFAS. Other food categories that had an important contribution were 'Milk and dairy products' and 'Drinking water' for PFOA, and 'Meat and meat products' and 'Eggs and egg products' for PFOS. The concentrations, across all categories, were ranging from 0.005 to 789 μg/kg, and from 0.002 to 3480 μg/kg, for PFOA and PFOS respectively. PFAS can also be transferred from the mother to the child in the womb, as well as from the consumption of breast milk that contains PFAS (EEA, 2023; Koponen et al., 2018; Knutsen et al., 2018).

Nowadays, PFOA and PFOS are being studied because of the concerns about their possible harm to people and the environment. They have been connected to a number of detrimental health outcomes, including liver damage, developmental and reproductive harm, and hormone disruption (US EPA, 2016a; US EPA, 2016b). Other adverse effects on human health that have been documented are thyroid disease, increased cholesterol levels, kidney, testicular cancer, as well as pancreatic cancer (Barry et al., 2013, Kamendulis et al., 2022, C8 Science Panel Website, 2012, Eea should be presented as EEA).

Limitations and restrictions have been set in place for several PFAS, considering the implications that they have for humans and the environment. In 2016, the United States Environmental Protection Agency (US EPA), calculated the lifetime drinking water health advisory for PFOA and PFOS to be 0.07 $\mu g L^{-1}$ (US EPA, 2016a; US EPA, 2016b). Additionally, the European Food Safety Authority (EFSA) in 2018, set the standards for the tolerable weekly intake for PFOA and PFOS. That intake was 6 and 13 ng/kg of body weight per week, respectively (Knutsen et al., 2018). In 2020, that threshold became, by EFSA, 4.4 ng/kg of body weight per week, for both PFAS, due to the growing knowledge and considering the simultaneous exposure to them (EFSA, 2020). European Union (EU), considering the recommendations of EFSA, set the maximum levels of PFOA and PFOS in different food groups with the Regulations a) (EU) 2022/2388, and b) (EU) 2022/1431. EU through the Persistent Organic Pollutants (POPs) Regulation restricted the use of PFOS (Regulation (EU) 2020/1203) and banned PFOA (Regulation (EU) 2020/784). More recently, in 2023, a proposal from the European Chemicals Agency (ECHA, 2023) was made for the further restriction of the manufacture and use of PFAS. In United States of America (USA), regulations vary from State to State. In example, Alaska with the Senate Bill No. 67 prohibited the use of firefighting materials containing PFAS. California with the Assembly Bill No. 246 , as well as Georgia with the House Bill No. 257, banned the usage of PFAS in menstrual products. Hawaii with the House Bill No. 748 and the Senate Bill No. 504 bans the production of personal care products and food packaging containing PFAS. US EPA (2023b), better understanding about them.



Many people in USA have been found to have blood levels of PFAS, which they were exposed to (ATSDR, 2023). Biomonitoring studies have identified the levels of PFAS in different population groups in the USA. The average geometric values of PFOA and PFOS concentrations detected in the blood of manufacturing workers at PFAS production facilities in 1998 were 899 and 941 $\mu g L^{-1}$, respectively (Olsen et al., 2003). In groups of people who exposed to PFAS through drinking water between 2005 and 2018, the geometric and/or numeric mean of PFOA and PFOS concentrations that were detected in blood ranged from 227.6 to 1.5 $\mu g L^{-1}$ and from 23.5 to 6.6 $\mu g L^{-1}$, respectively (Frisbee et al., 2009; ATSDR, 2013; Pennsylvania Department of Health, 2019; New Hampshire Department of Health and Human Services, 2015; New York Department of Health, 2016/2017; New York Department of Health, 2018). The geometric and/or numerical value of the concentrations of PFOA and PFOS detected in the blood of the general U.S. population ranged from 5.2 to 1.4 and from 30.4 to 4.3 $\mu g L^{-1}$, respectively (Centers for Disease Control, NHANES, 1999–2018).

There hasn't been much research done on the genotoxic effects of PFAS in international literature. Most studies of the genotoxic effects of PFAS have focused on PFOA and PFOS with the distinctive disadvantage that they demonstrate contradictory results. In addition, there are limited studies of genotoxic effects on primary human cells as well as in humans exposed to PFAS (ATSDR, 2021). Ecotoxicity assessments for both PFOA and PFOS have been performed in different organisms, including zebrafish (Ulhaq et al., 2013), mice (Kamendulis et al., 2014, 2022), algae (Latała et al., 2009), and bacteria (Rosal et al., 2010; Mulkiewicz et al., 2007). Although data for the toxicity of the individual compounds can be found easily, the available literature for their mixtures potential adverse effects are limited.

Taking into consideration (a) the data from the international literature which link the exposure to PFAS, and more specifically PFOA and PFOS, with harmful toxic effects on mammalian cells, organs and tissues including the human ones (ATSDR, 2021) (b) the conflicting results regarding its genotoxicity, particularly for PFOA and PFOS, when tested separately in different mammalian cells under either *in vitro* or *in vivo* conditions (c) the classification of PFOA by the International Agency for Research on Cancer (IARC) as a Group 2 agent “possibly carcinogenic to humans” (IARC, 2017) (d) the data from the United States Environmental Protection Agency (US EPA) indicating that PFOS and PFOA could be carcinogenic (US EPA, 2016a; US EPA, 2016b) and (e) the presence of PFAS, including PFOA and PFOS, in different environmental media despite restrictions on their production in recent years (Panieri et al., 2022) and (f) the limited data on the impact of PFAS mixtures on effect of PFOA, PFOS and their mixture in primary human peripheral blood lymphocytes and marine bacteria.

## Section snippets

### Chemicals and reagents

Perfluorooctane sulfonic acid potassium (96.4 %) was obtained from LGC/DR EHRENSTORFER and Pentadecafluorooctanoic acid (96 %) by Acros Organics. For the preparation of the solutions ultrapure water (18.2 MΩ/cm 25 °C) was used. Other solvents i.e., methanol (99.8 %) and ethanol (98 %) was purchased by Fisher Scientific and Honeywell, respectively. The freeze-dried culture of *Aliivibrio fischeri* marine bacteria which is reconstituted before use was purchased from Modern Water. NaCl, phenol, $ZnSO_4$…

### Genotoxic and cytotoxic effects of PFAS on human lymphocytes

The genotoxic and cytotoxic properties of PFOA and PFOS and their mixtures, in cultured human lymphocytes from healthy donors, were evaluated using the Cytokinesis Block Micronucleus (CBMN) assay. The CBMN assay is a validated method which is widely used to determine the genotoxicological effects of a variety of chemical compounds and factors (OECD, 2016; Spyrou et al., 2022; Antonopoulou et al., 2022a; Antonopoulou et al., 2022b; Dailianis et al., 2023).

The concentrations chosen to be studied…

### Conclusions

The potential ecotoxic, cytotoxic and genotoxic effects of PFOA, PFOS and their mixture were assessed in marine bacteria and human lymphocytes. A clear genotoxic effect of PFOS and the mixture of PFOA and PFOS at the highest tested concentration ($1000\,\mu g L^{-1}$ or $1\,\mu g mL^{-1}$) and cytotoxic activity at all tested concentrations both for individual compounds and their mixture were observed. PFOA induced a slight increase in the frequency of MN which is not statistically significant. Regarding the…

### CRediT authorship contribution statement

**Dimitris Vlastos:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Maria Antonopoulou:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project…

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.…

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