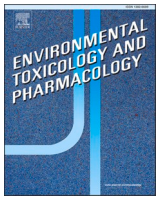
Environmental Toxicology and Pharmacology 109 (2024) 104496

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

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



Enviromental endocrine disruptor risks in the central nervous system: Neurotoxic effects of PFOS and glyphosate

Gianluca Antonio Franco [a], Francesco Molinari [a], Ylenia Marino [b], Nicla Tranchida [b], Francesca Inferrera [b], Roberta Fusco [b], Rosanna Di Paola [b], Rosalia Crupi [a], Salvatore Cuzzocrea [b], Enrico Gugliandolo [a,*], Domenico Britti [c]

[a] Department of Veterinary Sciences, University of Messina, Messina 98168, Italy
[b] Department CHIBIOFARAM, University of Messina, Messina 98166, Italy
[c] Department of Health Sciences, Campus Universitario "Salvatore Venuta" Viale Europa, 4 "Magna Grœcia University" of Catanzaro, Catanzaro 88100, Italy

ARTICLE INFO

Keywords:
Immunotoxicology
Neurotoxicology

ABSTRACT

Endocrine disruptors (EDs) pose significant risks to human and environmental health, with potential implications for neurotoxicity. This study investigates the synergistic neurotoxic effects of perfluorooctane sulfonate (PFOS) and glyphosate (GLY), two ubiquitous EDs, using SHSY5Y neuronal and C6 astrocytic cell lines. While individual exposures to PFOS and glyphosate at non-toxic concentrations did not induce significant changes, their combination resulted in a marked increase in oxidative stress and neuroinflammatory responses. Specifically, the co-exposure led to elevated levels of interleukin-6, tumor necrosis factor alpha, and interferon gamma, along with reduced interleukin-10 expression, indicative of heightened neuroinflammatory processes. These findings underscore the importance of considering the synergistic interactions of EDs in assessing neurotoxic risks and highlight the urgent need for further research to mitigate the adverse effects of these compounds on neurological health.

1. Introduction

To date, the harmful potential of endocrine disruptors on human, animal, and environmental health within the One Health concept is widely recognized (Gao et al., 2024; Sui et al., 2024). These contaminants are causing significant health concerns due to their ability to spread across various systems and compartments and their persistence. Indeed, despite the well-known and demonstrated neurotoxic action of PFOS and glyphosate (GLY), the focus of this study is to test, in an in vitro model, the hypothesis that endocrine disruptors present in the environment at concentrations individually non-toxic may have a synergistic effect in triggering various pathological conditions. Indeed, when considering neurotoxicity, it's crucial to account for toxicity induced by processes such as oxidative stress and inflammation. Even low-grade levels of these processes can have a significant impact on the overall health of the central nervous system, especially over the long term (Lo et al., 2023). GLY is a widely used herbicide, particularly in agriculture. People and animals can be exposed to it through food, water, air, or skin contact. It persists in soil and water. This persistence can lead to contamination of water bodies through runoff, affecting aquatic ecosystems. GLY and its formulations can have neurotoxic effects, including inducing oxidative stress, disrupting mitochondrial function, and causing neural cell death. Additionally, GLY is linked to endocrine disruption, reproductive toxicity, and potential carcinogenic effects. Environmentally, it can harm non-target plant species, aquatic organisms, and beneficial soil microbes, disrupting ecosystems. Due to these health and environmental concerns, GLY is under significant regulatory scrutiny, with some regions restricting or banning its use. Ongoing research aims to better understand its long-term health effects, mechanisms of toxicity (Costas-Ferreira et al., 2022a; Roy et al., 2016). Although some studies suggest neurotoxic effects, the ongoing debate requires comprehensive research to ascertain the full spectrum of effects and safety implications associated GLY (Costas-Ferreira et al., 2022b; Cattani et al., 2014). PFOS is a synthetic chemical used in industrial applications like stain repellents, firefighting foams, and surfactants. Humans and animals can be exposed to PFOS through contaminated food, water, air, or skin contact. Due to its strong carbon-fluorine bonds, PFOS is highly persistent in the environment and resistant to

* Corresponding author.
E-mail address: egugliandolo@unime.it (E. Gugliandolo).

https://doi.org/10.1016/j.etap.2024.104496
Received 24 April 2024; Received in revised form 20 June 2024; Accepted 28 June 2024
Available online 2 July 2024

breakdown, leading to its bioaccumulation in wildlife and humans, particularly in the liver and blood. This persistence allows PFOS to spread globally, far from its original sources. PFOS exposure is associated with neurotoxic effects, including cognitive deficits, behavioral changes, and developmental neurotoxicity. It can induce oxidative stress, disrupt calcium homeostasis, and affect neurotransmitter systems, leading to neuroinflammation and neural damage. Health risks of PFOS include liver damage, immunotoxicity, endocrine disruption, and potential carcinogenicity. Environmentally, PFOS contamination affects wildlife, especially in aquatic ecosystems, causing reproductive and developmental issues in fish and amphibians (Sato et al., 2009). GLY and PFOS, found in both agricultural and industrial areas, can contaminate surface and groundwater, damage soil, and adversely affect aquatic life. People exposed to both contaminants through food, water, air, and direct contact can suffer serious health consequences, including the risk of neurotoxicity, endocrine dysfunction, and development of carcinogenic diseases. Both GLY and PFOS are currently under the scrutiny of environmental authorities, with regulations and monitoring having become focal points of environmental policies in many countries (Iqubal et al., 2020; Starnes et al., 2022). Scientific investigation of the neurotoxicity of these substances is essential for a complete understanding of their risks to human health and the ecosystem. In fact, GLY, after application to soil, can reach initial concentrations between 0.1 and 1 mg/kg. These concentrations decrease over time due to microbial degradation and adsorption to soil particles (Evagelia and Helen, 2020). PFOS, being a persistent pollutant, can be present in soil in concentrations ranging from nanograms per gram (ng/g) to micrograms per gram (μg/g) depending on proximity to sources of contamination (Brusseau et al., 2020). In surface water, GLY can be present in concentrations ranging from a few micrograms per liter (μg/L) to tens of μg/L, especially in agricultural areas after application (Rendon-von Osten and Dzul-Caamal, 2017). PFOS, on the other hand, can range from a few nanograms per liter (ng/L) to hundreds of ng/L in contaminated areas (Yang et al., 2022). In groundwater, GLY concentrations are generally less than 1 μg/L due to adsorption and degradation in soil (Rendon-von Osten and Dzul-Caamal, 2017). PFOS can be found in concentrations ranging from a few ng/L to tens of ng/L, depending on the level of local contamination (Yang et al., 2022).

GLY concentrations in air are generally in the nanogram per cubic meter (ng/m^3) range during application periods, decreasing rapidly thereafter (Evagelia and Helen, 2020). PFOS concentrations in air are very low, often on the order of picograms per cubic meter (pg/m^3) down to a few nanograms per cubic meter (ng/m^3) (Costanza et al., 2019). The aim of this study was to evaluate any potential synergism in neurotoxicity between GLY and PFOS. We focused on PFOS and GLY because their co-exposure is not a rare event but can occur quite frequently, as both are contaminants that easily enter the human and animal food chains as described above (Tarazona et al., 2017; Martins-Gomes et al., 2022; Saikat et al., 2013; Beach et al., 2006; Rawat et al., 2023; Yang et al., 2024). To test our hypothesis, we utilized two cell lines, SHSY5Y neurons to assess direct neural toxicity (Park et al., 2024) and C6 astrocytic cells, as these cells play a key role in neuroinflammation and can respond to various stimuli by triggering an neuro inflammatory response (Dey and Singh, 2022; Kabata and Artis, 2019).

2. Materials and methods

2.1. Cell lines and culture

Rat astroglioma cell line (C6 cells) was obtained from the American Type Culture Collection (ATCC) and maintained according to the procedure previously described (Dos Santos et al., 2006). This cell line is one of the glial cell models of astrocytic lineage. It has demonstrated astrocytic behavior and responds to neurotoxic and inflammatory stimuli to produce cytokines (Park et al., 2017; Goswami et al., 2015).

Cells were seeded in flasks (BioLite 75 cm^2 Flask) and cultured in DMEM F12 pH 7.4(Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham, Sigma-Aldrich S.p.a. Milan, Italy) containing 5 % fetal bovine serum and 2 % Penicillin-Streptomycin (P0781–100 mL, Sigma-Aldrich S.p.a. Milan, Italy). The human SH-SY5Y neuroblastoma cells used in this study were purchased from (ATCC). This cells, derived from human neuroblastomas, offer an unobstructed view of the realm of neurons in vitro (Shipley et al., 2016). These cells prove particularly useful in the evaluation of neurotoxicity, allowing the impact of chemicals or drugs on nerve cells to be assessed and providing valuable insights into potential impacts on the health of the nervous system (Sayre et al., 2008). The cells were cultured in 75 cm^2 flasks (BioLite 75 cm^2 Flask) and maintained in high glucose (4.5 gL^{-1}) Dulbecco's modified Eagle medium (DMEM, Sigma-Aldrich S.p.a. Milan, Italy), supplemented with 10 % (*v/v*) fetal bovine serum (FBS, Sigma-Aldrich S.p.a. Milan, Italy), 4 mM of L-glutamine (Sigma-Aldrich S.p.a. Milan, Italy), and 1 % (*v/v*) antibiotic solution penicillin-streptomycin (Sigma Aldrich). Cells were kept at 37°C in an atmosphere of 5 % CO2/ 95 % air. Exponentially growing cells were detached from the culture flasks using scraper and seeded in 6, 24 or 96-well plates, accordingly with experimental design. After 24 h since we sowed them, culture medium was removed and cells pre-incubated in the presence of a heptadecafluorooctansulforic acid solution (Sigma-Aldrich) and *N*-(Phosphonomethyl) glycine, Glyphosate (Sigma-Aldrich)

2.2. Cell viability

To study the impact of PFOS and GLY on cell viability, the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphe- nyltetrazolic bromide) reduction assay was used (Saxena et al., 2024). A solution of MTT (5 mg/mL) was prepared in medium under light-protected conditions. SH-SY5Y and C6 cells were seeded at a density of 4 $\times 10^4$ cells per well in a 96-well plate in a 150 μL volume of DMEM F12. After a 24-hour incubation at 37°C, 5 % CO2, culture medium containing various concentrations (0.01–1000 μM) of PFOS and GLY alone and in combination in a 1:1 ratio was replaced. Viability was performed after 24 h and 48 h of exposure. After 24 h and 48 h of exposure, respectively, the medium was removed and 150 μL of MTT dissolved in DMEM/F12 was added. The plates were incubated for 3 h, after which the medium was removed and replaced with 150 μL of dimethyl sulfoxide to dissolve the formazan salts produced by cell metabolism during MTT incubation. Subsequently, the absorbance was measured at 540 nm (Gugliandolo et al., 2020). The value that keeps cells viable at 80 % (IC20) were individuated and used for the future assays. In particular, At 24 hours, we obtained 10 μM for PFOS and 37 μM for GLY in C6 cell. For SHSY-5 J we obtained a IC20 value of 10 μM and 39 μM for PFOS and GLY respectively.

2.3. Elisa

For enzyme-linked immunosorbent assay (ELISA) analysis of IL-6, TNF, and IL-10 levels, medium samples were prepared according to the manufacturer's instructions (Rat IL-6 Cat #88–50625–88 ThermoFisher SCIENTIFIC, Human IL-6 Cat #EH2IL6 ThermoFisher SCIENTIFIC, Rat TNF-a Cat #BMS622 ThermoFisher SCIENTIFIC, Human TNF-a Cat #88–7346–88 ThermoFisher SCIENTIFIC, Rat IL-10 Cat #88–50629–88 ThermoFisher SCIENTIFIC, Human IL-10 Cat #88–7106–88 ThermoFisher SCIENTIFIC). Absorbance was measured at 450 nm (Gugliandolo et al., 2020; Paterniti et al., 2017).

2.4. Determination of intracellular ROS

For the assessment of intracellular reactive oxygen species (ROS) production, using the carboxy-H2DCFDA assay, cells were seeded at a density of 40,000 cells per well in a 96-well plate and incubated with various concentrations of PFOS and GLY for 24 hours. Following treatment, the medium was replaced with a 10 μM fluorescent probe (carboxy-H2DCFDA, Life Technologies Corporation) for ROS detection.

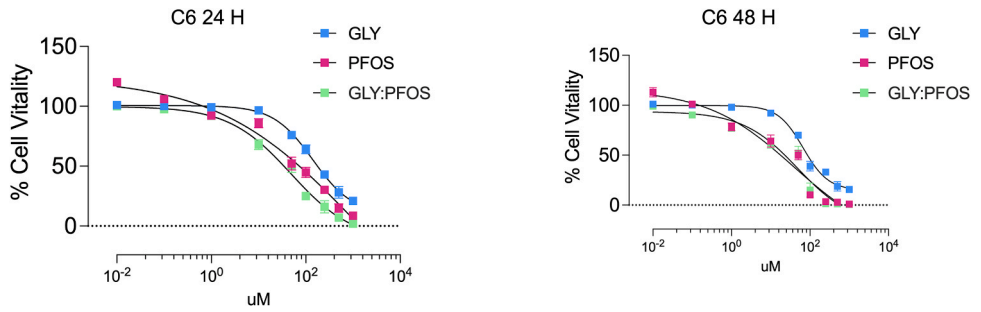


Fig. 1. Dose-response curves of C6 cells exposed to GLY, PFOS, and GLY+PFOS combination at 24 h and 48 h, respectively. IC50 value of 32.4 μM at 24 h and 10.25 μM at 48 h for PFOS. And a IC50 value of 174.5 μM at 24 h and 86.78 μM at 48 h for GLY. The results are represented as mean +\- SEM.

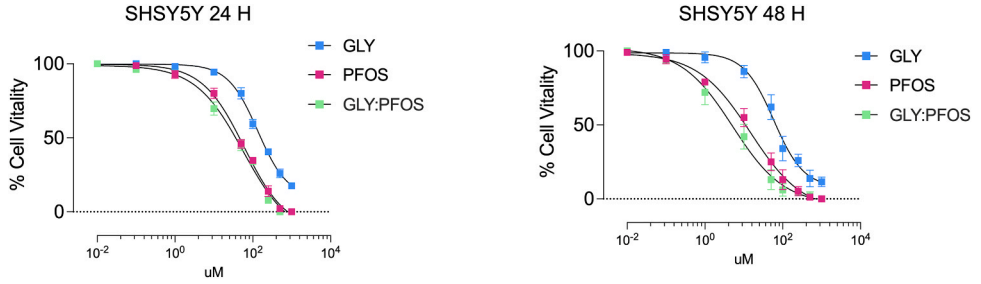


Fig. 2. Dose-response curves of SH-SY5Y cells exposed to GLY, PFOS and GLY+PFOS combination at 24 h and 48 h, respectively. IC50 value of 161,6 μM at 24 h and 68,14 μM at 48 h for GLY. And a IC50 value of 45,73 μM at 24 h and 11,43 μM at 48 h for PFOS. The results are represented as mean +\- SEM (Fig. 2B). The other results are presented as a violin graph as mean with confidence interval.

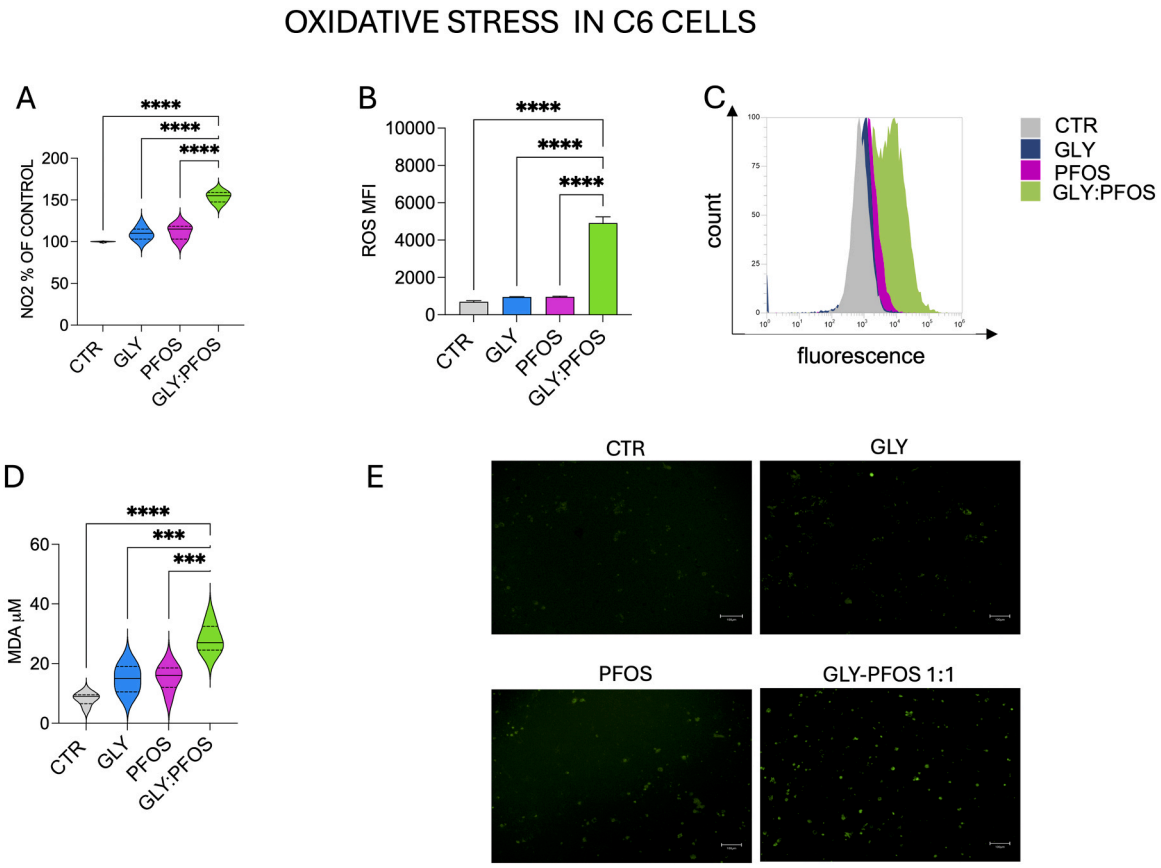


Fig. 3. Graphs representing the results obtained in the assessment of oxidative stress by assay of nitrite (A), ROS (B), and MDA (D) in C6 cells exposed to GLY, PFOS, and association of GLY+PFOS. Graph of fluorescence assessment of ROS by flow cytofluorometer (C) with representative photos of ROS (E). The results are represented as mean +\- SEM (Fig. 3B). The other results are presented as a violin graph as mean with confidence interval. ****p < 0.0001; ***p < 0.001.

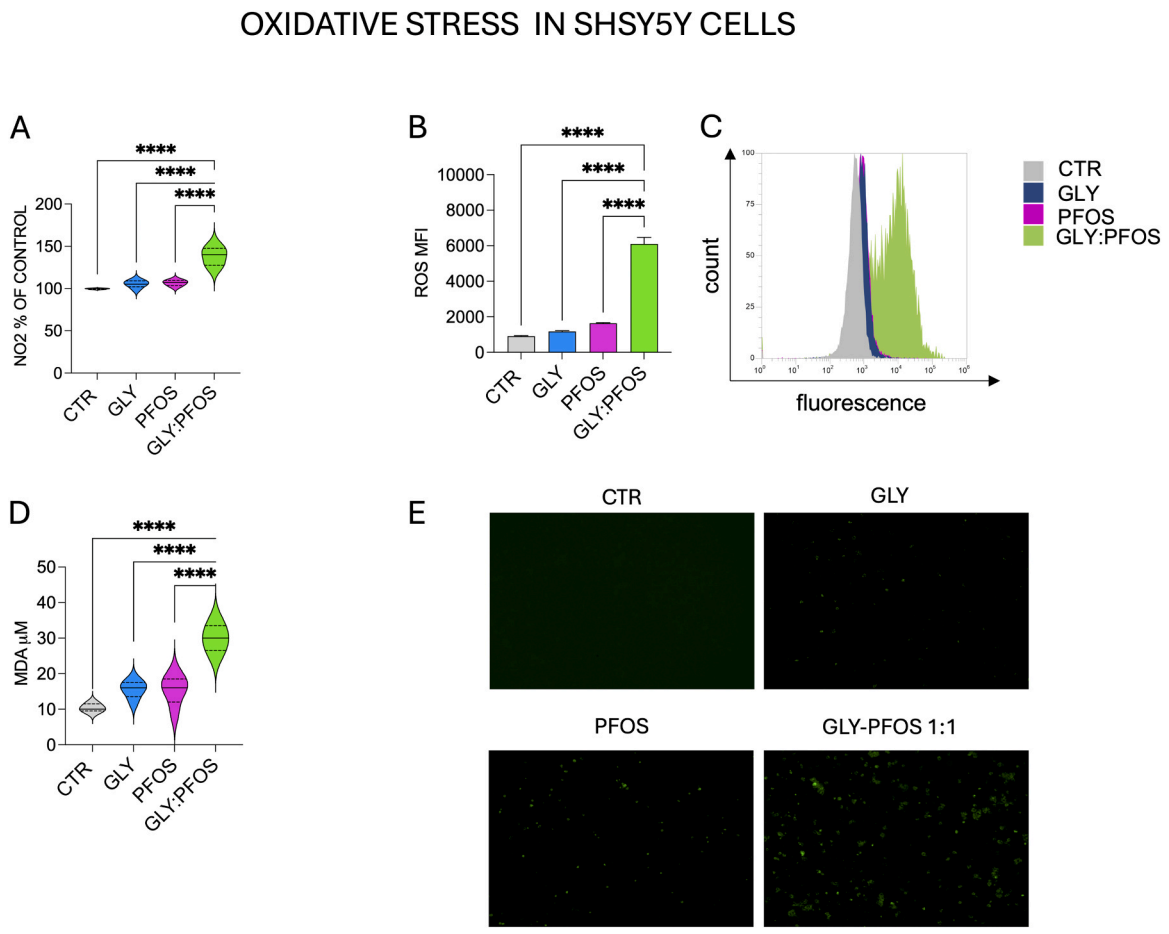


Fig. 4. Graphs representing the results obtained in the assessment of oxidative stress by assay of nitrite (A), ROS (B), and MDA (D) in SH-SY5Y cells exposed to GLY, PFOS, and association of GLY+PFOS. Graph of fluorescence assessment of ROS (C) by flow cytofluorometer with representative photos of ROS (E). The results are represented as mean +\- SEM (Fig. 4B). The other results are presented as a violin graph as mean with confidence interval. ****p < 0.0001; ***p < 0.001.

After an incubation time of 2 h, the fluorescence intensity signals were measured using the Attune NxT cytometer (Thermo Fischer Scientific) and observed under a fluorescence microscope (Evos M5000, Thermo Fisher) (Gugliandolo et al., 2020).

2.5. Nitrite evaluation

Nitrite levels were determined using the Griess reaction. Cells were plated at 4 $\times 10^4$ cells per well in a 96-well plate and treated with PFOS and glyphosate for 24 hours. Supernatant from each sample was collected and subjected to the Griess reaction. Specifically, 50 µL of supernatant was added to another 96-well plate, followed by the addition of 50 µL of 1 % sulfanilamide solution (in 5 % phosphoric acid). After a 10-minute incubation, 50 µL of (0,1 % N-1-napthylethylenediaminedihydrochloride (NED) solution was added to each well, followed by another 10-minute incubation. Absorbance was measured at 540 nm within 30 minutes using a plate reader (Bogdan, 2001).

2.6. MDA

Lipid peroxidation was determined by assessing MDA production by thiobarbituric acid assay. The cells were detached from the plate with KCl 1.15 % and homogenised. The thiobarbituric acid assay (TBARS), as previously described (Amoroso et al., 1999), was performed on the supernatant for the assessment of MDA levels for lipid peroxidation.

2.7. Statistical analysis

Data presented are representative of a minimum of three independent experiments. Data were assessed for normality of the data distribution by using the Shapiro– Wilk test and reported as the mean ± SEM (standard error of the mean) or as mean with confidence interval (violin plot). The results are representative of at least five independent experiments of n=5. Results were analyzed by one-way ANOVA and Bonferroni for multiple comparison. GraphPad Prism statistical software was employed for data analysis. A p value less than 0.05 was considered significant. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0,0001.

3. Results

3.1. Effect on cell vitality

The effects on cell viability after exposure to PFOS and GLY against C6 (Fig. 1) and SH-SY5Y (Fig. 2) cells during 24 and 48 hours of exposure were studied using the MTT assay, by which we evaluated increasing concentrations of GLY and PFOS (0.01–1000uM) in both cases. The MTT assay for C6 cell showed a IC50 value of 32.4 µM at 24 h and 10.25 µM at 48 h for PFOS. And a IC50 value of 174.5 µM at 24 h and 86.78 µM at 48 h for GLY. While combination of PFOS and GLY showed a IC50 value of 34,54 µM at 24 and 25,78 µM at 48 h. The MTT assay for SH-SY5Y cell showed a IC50 value of 161,6 µM at 24 h and 68,14 µM at 48 h for GLY. And a IC50 value of 45,73 µM at 24 h and 11,43 µM at 48 h for PFOS. While combination of PFOS and GLY showed a IC50 value of 37,15 µM at 24 and 4,59 µM at 48 h. To test our hypotheses that the low

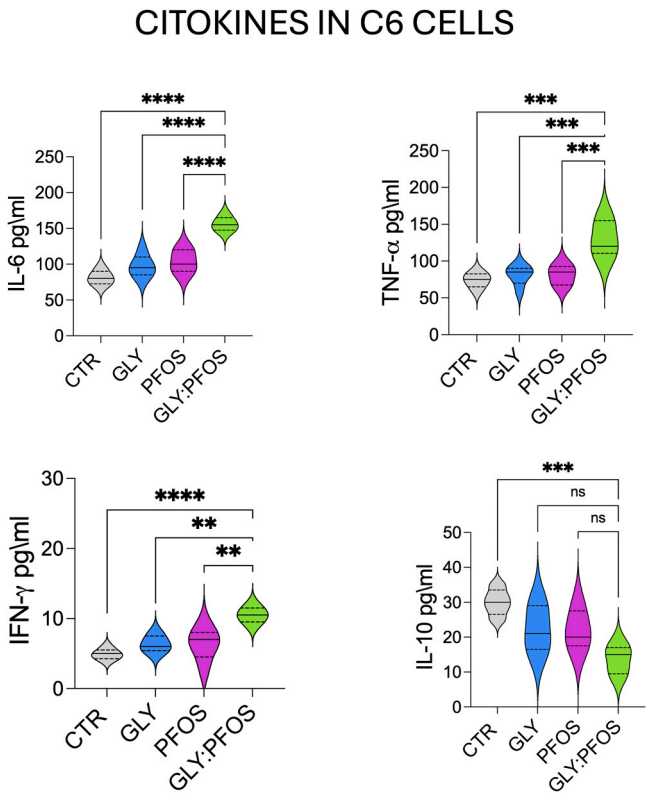


Fig. 5. Graphs representing the results obtained in the evaluation of pro-inflammatory cytokines such as IL-6, TNF-a, IFN-y and the anti-inflammatory one IL-10 in C6 cells exposed to GLY, PFOS and combination of GLY+PFOS. NS: Non-Significative; ****p < 0.0001; ***p < 0.001; **p < 0.01; The results are presented as violin plot as mean with confidence interval.

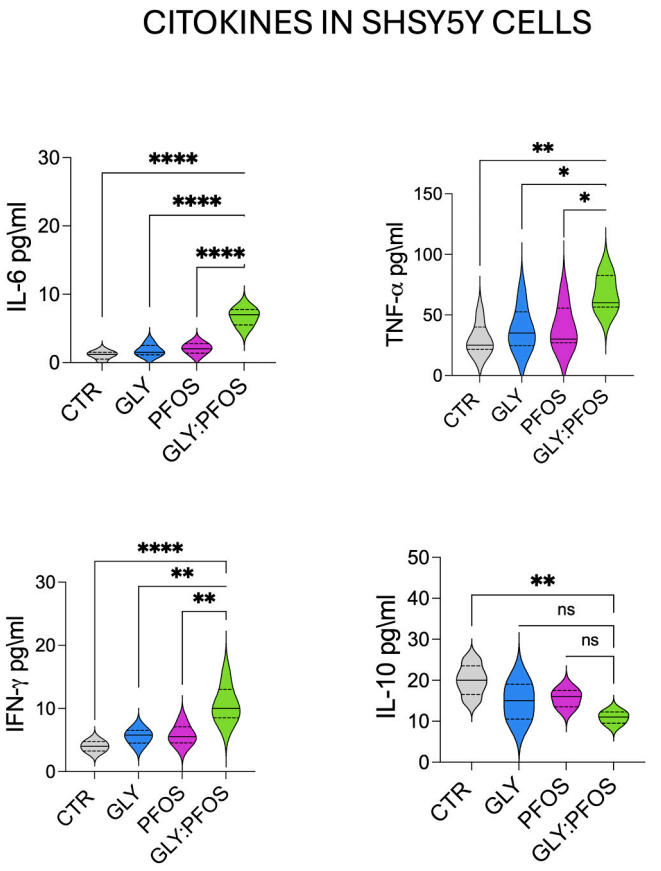


Fig. 6. Graphs representing the results obtained in the evaluation of pro-inflammatory cytokines such as IL-6, TNF-a, IFN-y and the anti-inflammatory one IL-10 in SH-SY5Y cells exposed to GLY, PFOS and combination of GLY+PFOS. NS: Non significative; ****p < 0.0001; **p < 0.01; *p < 0.1; The results are presented as violin plot as mean with confidence interval.

doses of the substances used are not toxic by themselves, but that in 1:1 combination they may be toxic, we identified a value that keeps cells viable at 80 % (IC20). At 24 hours, we obtained 10 μM for PFOS and 37 μM for GLY in C6 cell. For SHSY-5 J we obtained a IC20 value of 10 μM and 39 μM for PFOS and GLY respectively.

3.2. Evaluation on oxidative stress parameters

Oxidative stress was assessed through nitrite, ROS, and MDA levels. The combination of PFOS and GLY resulted in a significant increase in nitrites compared to the control and individual exposure groups. Similarly, the ROS assay demonstrated a notable rise in ROS levels in the combined exposure group compared to individual exposures. MDA levels, indicative of lipid peroxidation, were also significantly elevated in the group exposed to both PFOS and GLY, reinforcing the presence of heightened oxidative stress in this combined exposure scenario.(Fig. 3 and Fig. 4)

3.3. Effect on IL-6, TNF-a, INF-y and IL-10 release

We investigated on C6 and SH-SY5Y cells the levels of cytokines involved in inflammatory processes. Both the pro-inflammatory ones, such as IL-6, IFN-gamma, TNF-a, and the main one involved in the anti-inflammatory process, namely IL-10. The first three, as can be seen from the figure below (Figs. 5 and 6, in C6 and SH-SY5Y, respectively), increase in the group in which the cells were exposed to the association of the two substances, PFOS and GLY in a 1:1 ratio, presenting a significant increase compared with the PFOS and GLY groups.

In a mirror manner, we performed the same process with SH-SY5Y cells. The results were superimposable, again having a significant increase in pro-inflammatory parameters in the group exposed to the association of the two substances compared to the others, in which we exposed C6 cells and SH-SY5Y cells to only one of two substances, PFOS and GLY, respectively.

As for the anti-inflammatory cytokine IL-10, we have a significant decrease in the group exposed to PFOS and GLY compared with those in which exposure occurred with only one of the two substances.

4. Discussion

Neuroinflammation is a complex condition caused by multiple mechanisms (Marino et al., 2022). The relationship between environmental contaminants and these neuroinflammatory mechanisms is a topic of growing interest and concern in the scientific and medical fields (Mahapatra et al., 2023; Izumi et al., 2024). The objective of this work was to test for any potential synergism in the neurotoxicity of these contaminants, taking into consideration the synergism between GLY and PFOS. To test our hypothesis we used two cell lines, SHSY5Y neurons to evaluate direct neural toxicity and C6 astrocytic cells, since these cells play a key role in neuroinflammation (Sun et al., 2019; Martínez et al., 2020). Our cell viability results demonstrate that the combination of PFOS and GLY evokes a toxic effect, but at a lower dosage than individual exposures both at 24 and 48 hours of exposure. Having identified the toxic dose, we then decided to identify the doses that would not have caused a decrease in vitality greater than 80 %, to evaluate how this influences the induction of oxidative stress in both SHSY5Y and C6 cells. These condition corresponded to a concentratrion of 10 μM for PFOS and 37 μM for GLY in C6 cells, and 10 μM and 39 μM for PFOS and glyphosate respectively in SHSY-5Y cells. Oxidative stress is

characterized by the increase of intracellular oxygen reactive species (ROS) (Cobb and Cole, 2015) and an overproduction of nitric oxide, leading to increased nitrosative stress (Morris et al., 2018). In addition to damaging DNA and key components of these cells (Sayre et al., 2008), oxidative stress can cause lipid peroxidation, which is an important mechanism in central nervous system neurotoxicity (Butterfield, 2020). In fact, many endocrine disruptors manifest their toxic effects through this mechanism, inducing oxidative and nitrosative stress and the subsequently cellular events (Masuo and Ishido, 2011). In fact, the hypothesis of this study encompasses a fundamental aspect: substances or mixtures of substances, such as endocrine disruptors, at concentrations that do not manifest toxic actions, may be capable of inducing cellular responses such as oxidative stress and inflammation. This can then lead to a cascade of secondary events that may contribute to the onset of pathological conditions or impact overall well-being (Raja et al., 2022). Consider the link between neuroinflammation and neurodegenerative diseases, where non-pathological conditions of neuroinflammation, when prolonged over time, lead to events that compromise normal homeostasis (de Oliveira et al., 2015; Marogianni et al., 2020).

In accordance with previously studies (Costas-Ferreira et al., 2022b; Li et al., 2017) on oxidative stress, induced by these compaunds, our results demonstrate a similar effect on both SHSY-5Y cells and C6 cells, where the combination of PFOS and GLY has a significantly greater effect in inducing oxidative stress, compared to individual exposures.

Indeed, for both cell lines SHSY-5Y and C6, we have a significant increase in all parameters associated with nitrosative and oxidative stress, such as nitrites, ROS and MDA. The Griess assay showed that the association leads to a significant increase in nitrites. This could be due to both a direct action of the compounds on oxidative stress induction and the endogenous production of NO in response to exposure. The increase in ROS and nitrites observed is consistent with the increased levels of lipid peroxidation that we found following exposure. Interesting, individual exposures do not cause significant variations compared to the control group. This suggests a potential synergistic effect of PFOS and GLY in inducing oxidative stress, highlighting the importance of considering interactions between endocrine disruptors when evaluating their neurotoxic effects. The inflammatory process is tightly and finely regulated in the central nervous system, where it is mediated by the secretion of cytokines that act as important messengers in the communication between immune cells resident in the central nervous system and neuronal cells. As shown in previously studies (Wang et al., 2015; Peillex and Pelletier, 2020; Chen et al., 2018a), an important involvement of inflammation in the toxicity given by interferents such as GLY and PFOF has been seen and therefore in our study, to evaluate how inflammation is influenced by the synergism between these two contaminants, we considered the main inflammatory cytokines, including interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), interferon gamma IFN-γ, and interleukin 10 (IL-10). In agreement with previous studies (Chen et al., 2018b; Costas-Ferreira et al., 2022b), we found that individual concentrations of PFOS and GLY do not produce significant changes in cytokine levels. However, co-exposure to PFOS and GLY at the same concentration causes a significant imbalance in the levels of cytokines secreted in both SHSY5J and C6 cells. IL-6, TNF-α, IFN-γ and IL-10 are all involved in neuroinflammatory processes and an alteration of their levels can lead to neurodegeneration or an alteration of central nervous system homeostasis. Our results thus demonstrate how endocrine disruptors such as PFOS and GLY, at individually non-toxic concentrations, can pose a serious risk when combined, leading to the induction of neuroinflammatory processes and oxidative stress in the central nervous system.

5. Conclusion

In conclusion, we believe that understanding these aspects is crucial for better comprehending the role of endocrine disruptors and their hazardous nature, especially in long-term processes and pathologies such as neurodegenerative diseases. This is particularly significant considering the widespread presence of these endocrine disruptors in the environment, which enables them to interact with biological systems even at very low concentrations, often in synergy or in the presence of numerous other endocrine disruptors. Certainly, future studies are necessary to better understand the toxic action of these two dangerous endocrine disruptors and to better evaluate their neurotoxic aspects, thus improving health from a One Health perspective.

CRediT authorship contribution statement

Rosalia Crupi: Data curation. **Rosanna Di Paola:** Supervision. **Enrico Gugliandolo:** Writing – original draft, Conceptualization. **Salvatore Cuzzocrea:** Resources. **Francesca Inferrera:** Methodology. **Nicla Trankida:** Methodology. **Roberta Fusco:** Software. **Gianluca Antonio Franco:** Investigation. **Domenico Britti:** Conceptualization. **Ylenia Marino:** Formal analysis. **Francesco Molinari:** Formal analysis.

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.

Data availability

No data was used for the research described in the article.

References

Amoroso, S., et al., 1999. In the neuronal cell line SH-SY5Y, oxidative stress-induced free radical overproduction causes cell death without any participation of intracellular Ca2+ increase. Biochim. Et. Biophys. Acta (BBA) Mol. Cell Res. 1452 (2), 151–160.
Beach, S.A., et al., Ecotoxicological evaluation of perfluorooctanesulfonate (PFOS). Reviews of Environmental Contamination and Toxicology: Continuation of Residue Reviews, 2006: p. 133-174.
Bogdan, C., 2001. Nitric oxide and the immune response. Nat. Immunol. 2 (10), 907–916.
Brusseau, M.L., Anderson, R.H., Guo, B., 2020. PFAS concentrations in soils: Background levels versus contaminated sites. Sci. Total Environ. 740, 140017.
Butterfield, D.A., 2020. Brain lipid peroxidation and alzheimer disease: synergy between the butterfield and mattson laboratories. Ageing Res. Rev. 64, 101049.
Cattani, D., et al., 2014. Mechanisms underlying the neurotoxicity induced by glyphosate-based herbicide in immature rat hippocampus: involvement of glutamate excitotoxicity. Toxicology 320, 34–45.
Chen, X., et al., 2018a. Perfluorooctanesulfonate induces neuroinflammation through the secretion of TNF-α mediated by the JAK2/STAT3 pathway. Neurotoxicology 66, 32–42.
Chen, X., et al., 2018b. Per fl uorooctanesulfonate induces neuroinflammation through the secretion of TNF-alpha mediated by the JAK2/STAT3 pathway. Neurotoxicology 66, 32–42.
Cobb, C.A., Cole, M.P., 2015. Oxidative and nitrative stress in neurodegeneration. Neurobiol. Dis. 84, 4–21.
Costanza, J., et al., 2019. Accumul. PFOA PFOS Air Water Interface Environ. Sci. Technol. Lett. 6 (8), 487–491.
Costas-Ferreira, C., Duran, R., Faro, L.R.F., 2022b. Toxic effects of glyphosate on the nervous system: a systematic review. Int. J. Mol. Sci. 23 (9).
Costas-Ferreira, C., Durán, R., Faro, L.R., 2022a. Toxic effects of glyphosate on the nervous system: a systematic review. Int. J. Mol. Sci. 23 (9), 4605.
Dey, M., Singh, R.K., 2022. Exposure of aluminium to C6 glioma cells modulates molecular and functional neurotoxic markers. J. Biochem. Mol. Toxicol. 36 (12), e23210.
Evagelia, T., Helen, K., 2020. In: Dimitrios, K., Anna, K., Kassio Ferreira, M. (Eds.), Glyphosate Residues in Soil and Air: An Integrated Review, in Pests, Weeds and Diseases in Agricultural Crop and Animal Husbandry Production. IntechOpen, Rijeka p. Ch. 8.
Gao, M., et al., 2024. Perfluorooctane sulfonate (PFOS) induces apoptosis and autophagy by inhibition of PI3K/AKT/mTOR pathway in human granulosa cell line KGN. Environ. Pollut. 344, 123333.
Goswami, P., et al., 2015. Astrocyte activation and neurotoxicity: a study in different rat brain regions and in rat C6 astroglial cells. Environ. Toxicol. Pharmacol. 40 (1), 122–139.
Gugliandolo, E., et al., 2020. Protective effect of hydroxytyrosol on LPS-induced inflammation and oxidative stress in bovine endometrial epithelial cell line. Vet. Sci. 7 (4), 161.
Iqubal, A., et al., 2020. Environmental neurotoxic pollutants. Environ. Sci. Pollut. Res. 27, 41175–41198.

Izumi, Y., O'Dell, K.A., Zorumski, C.F., 2024. Glyphosate as a direct or indirect activator of pro-inflammatory signaling and cognitive impairment. Neural Regen. Res. 19 (10), 2212–2218.
Kabata, H., Artis, D., 2019. Neuro-immune crosstalk and allergic inflammation. J. Clin. Investig. 129 (4), 1475–1482.
Li, C., et al., 2017. Protection of taurine against PFOS-induced neurotoxicity in PC12 cells. Adv. Exp. Med. Biol. 975 (Pt 2), 907–916.
Lo, J., et al., 2023. Nordalbergin exerts anti-neuroinflammatory effects by attenuating mapk signaling pathway, NLRP3 inflammasome activation and ROS production in LPS-stimulated BV2 microglia. Int. J. Mol. Sci. 24 (8).
Mahapatra, A., et al., 2023. Unraveling the mechanisms of perfluorooctanesulfonic acid-induced dopaminergic neurotoxicity and microglial activation in developing zebrafish. Sci. Total Environ. 887, 164030.
Marino, M., et al., 2022. Neuroinflammation: molecular mechanisms and therapeutic perspectives. Cent. Nerv. Syst. Agents Med. Chem. 22 (3), 160–174.
Marogianni, C., et al., 2020. Neurodegeneration and inflammation-an interesting interplay in Parkinson's disease. Int. J. Mol. Sci. 21 (22).
Martínez, M.A., et al., 2020. Use of human neuroblastoma SH-SY5Y cells to evaluate glyphosate-induced effects on oxidative stress, neuronal development and cell death signaling pathways. Environ. Int. 135, 105414.
Martins-Gomes, C., et al., 2022. Glyphosate vs. glyphosate-based herbicides exposure: a review on their toxicity. J. Xenobiotics 12 (1), 21–40.
Masuo, Y., Ishido, M., 2011. Neurotoxicity of endocrine disruptors: possible involvement in brain development and neurodegeneration. J. Toxicol. Environ. Health B Crit. Rev. 14 (5-7), 346–369.
Morris, G., et al., 2018. Multiple immune-inflammatory and oxidative and nitrosative stress pathways explain the frequent presence of depression in multiple sclerosis. Mol. Neurobiol. 55 (8), 6282–6306.
de Oliveira, A.C., et al., 2015. Neuroinflammation and neurodegeneration: pinpointing pathological and pharmacological targets. Biomed. Res. Int. 2015, 487241.
Park, S., Oh, H.N., Kim, W.K., 2024. Human coculture model of astrocytes and SH-SY5Y cells to test the neurotoxicity of chemicals. Ecotoxicol. Environ. Saf. 269, 115912.
Park, S., de Perre, C., Lee, L.S., 2017. Alternate reductants with VB12 to transform C8 and C6 perfluoroalkyl sulfonates: Limitations and insights into isomer-specific transformation rates, products and pathways. Environ. Sci. Technol. 51 (23), 13869–13877.
Paterniti, I., et al., 2017. The anti-inflammatory and antioxidant potential of pistachios (Pistacia vera L.) in vitro and in vivo. Nutrients 9 (8), 915.
Peillex, C., Pelletier, M., 2020. The impact and toxicity of glyphosate and glyphosate-based herbicides on health and immunity. J. Immunotoxicol. 17 (1), 163–174.
Raja, G.L., Subhashree, K.D., Kantayya, K.E., 2022. In utero exposure to endocrine disruptors and developmental neurotoxicity: implications for behavioural and neurological disorders in adult life. Environ. Res. 203, 111829.
Rawat, D., et al., 2023. Hazardous impacts of glyphosate on human and environment health: occurrence and detection in food. Chemosphere 329, 138676.
Rendon-von Osten, J., Dzul-Caamal, R., 2017. Glyphosate residues in groundwater, drinking water and urine of subsistence farmers from intensive agriculture localities: a survey in hopelchén, Campeche, Mexico. Int. J. Environ. Res. Public Health 14 (6).
Roy, N.M., Carneiro, B., Ochs, J., 2016. Glyphosate induces neurotoxicity in zebrafish. Environ. Toxicol. Pharmacol. 42, 45–54.
Saikat, S., et al., 2013. The impact of PFOS on health in the general population: a review. Environ. Sci. Process. Impacts 15 (2), 329–335.
Sato, I., et al., 2009. Neurotoxicity of perfluorooctane sulfonate (PFOS) in rats and mice after single oral exposure. J. Toxicol. Sci. 34 (5), 569–574.
Saxena, B., et al., 2024. Neuroprotective effect of taxifolin against aluminum chloride-induced dementia and pathological alterations in the brain of rats: possible involvement of toll-like receptor 4. Toxicol. Mech. Methods 1–14.
Sayre, L.M., Perry, G., Smith, M.A., 2008. Oxidative stress and neurotoxicity. Chem. Res. Toxicol. 21 (1), 172–188.
Shipley, M.M., Mangold, C.A., Szpara, M.L., 2016. Differentiation of the SH-SY5Y human neuroblastoma cell line. JoVE 108, e53193.
Starnes, H.M., et al., 2022. A critical review and meta-analysis of impacts of per-and polyfluorinated substances on the brain and behavior. Front. Toxicol. 37.
Sui, X., et al., 2024. Phenolic compounds induce ferroptosis-like death by promoting hydroxyl radical generation in the Fenton reaction. Commun. Biol. 7 (1), 199.
Sun, P., et al., 2019. ROS-mediated JNK pathway critically contributes to PFOS-triggered apoptosis in SH-SY5Y cells. Neurotoxicol. Teratol. 75, 106821.
Tarazona, J.V., et al., 2017. Glyphosate toxicity and carcinogenicity: a review of the scientific basis of the European Union assessment and its differences with IARC. Arch. Toxicol. 91, 2723–2743.
Wang, C., et al., 2015. Reactive oxygen species mediate nitric oxide production through ERK/JNK MAPK signaling in HAPI microglia after PFOS exposure. Toxicol. Appl. Pharm. 288 (2), 143–151.
Yang, W., et al., 2022. Facile synthesis of nanofiltration membrane with asymmetric selectivity towards enhanced water recovery for groundwater remediation. J. Membr. Sci. 663, 121038.
Yang, H., et al., 2024. Bio-accumulation and health risk assessments of per- and polyfluoroalkyl substances in wheat grains. Environ. Pollut., 124351