

D 2025



BIOMONITORING FIREFIGHTERS' OCCUPATIONAL EXPOSURE

FILIPA COELHO ESTEVES

TESE DE DOUTORAMENTO APRESENTADA

À FACULDADE DE MEDICINA DA UNIVERSIDADE DO PORTO

PROGRAMA DOUTORAL EM SAÚDE PÚBLICA

Programa Doutoral em Saúde Pública

Biomonitoring Firefighters' Occupational Exposure

Filipa Coelho Esteves

Orientação:

Solange Cristina Bastos da Costa, PhD

*Departamento de Saúde Ambiental, Instituto Nacional de Saúde Doutor Ricardo Jorge,
Porto, Portugal*

Co-orientação:

Joana Georgete Vieira Madureira, PhD

*Departamento de Saúde Ambiental, Instituto Nacional de Saúde Doutor Ricardo Jorge,
Porto, Portugal*

PORTO | 2025

Dissertação de candidatura ao grau de Doutor em Saúde Pública apresentada à
Faculdade de Medicina da Universidade do Porto

Declaration of Honour

I declare that this thesis is my own and has not been previously submitted to another course or curricular unit, in FMUP or another institution. All references to the statements, ideas, or thoughts of other authors are properly cited in both the text and bibliography. I am aware that plagiarism and auto-plagiarism are considered academic misconduct.

Porto, 22 January 2025

Filipa Coelho Esteves

Filipa Coelho Esteves

*“Learning is the only thing the mind
never exhausts, never fears and never regrets.”*

- Leonardo da Vinci -

Acknowledgements

I would first like to express my sincere gratitude to my supervisors, Dr Solange Costa and Dr Joana Madureira, for their unwavering support from the very first day of this PhD adventure. Thank you for sharing your knowledge, assisting me in overcoming obstacles, for your guidance and motivation, and for always believing in me. It was a true pleasure and honour to walk this path with you.

A special and heartfelt thanks to all my colleagues at the Department of Environmental Health, of the National Institute of Health Dr Ricardo Jorge, who warmly welcomed me nearly a decade ago and continue to do so with a smile on their faces every day. Thank you for your support, for the encouragement and the friendship. My sincere thanks to those who have mentored me throughout my career, as they contributed to sparking my passion for science and inspired me to pursue this academic journey. Thank you.

To Professor Blanca Laffon and the entire team, a warm thank you. I would also like to extend my acknowledgements to Professor Stefano Bonassi for welcoming me so warmly at the San Raffaele Institute in Rome. I'm grateful for all the support and valuable lessons.

È stato un piacere!

To my colleagues from the BioFirEx Project. It was a pleasure to collaborate with you on this adventure and this special work. A special thanks to all Firefighters, the true Heroes, whose generosity, enthusiasm, and hospitality at the fire stations made this research possible.

To my dear office colleagues and friends, thank you for your companionship, support, laughs, and friendship, and for making my days so much brighter! Of course, I could not forget those in Rome, who received me in the best way possible, always with a smile, *un caffettino e una passeggiata - un grande bacio a tutti! Arrivederci Ragazzi!*

Finally, my deepest thanks to my closest friends and my beloved family. For the patience, support and love. For everything. This work is dedicated to you.

"Wherever you go, go with all your heart."

- Confucius -

This work entitled “Bio4FOX - Biomonitoring Firefighters’ Occupational Exposure”, was supported by the PhD grant Reference UI/BD/150783/2020, by national funds through Fundação para a Ciência e Tecnologia I.P. (FCT), Fundo Social Europeu (FSE) and Projects Ref. UIDB/04750/2020 (DOI: 10.54499/UIDB/04750/2020), Ref. LA/P/0064/2020 (DOI: 10.54499/LA/P/0064/2020) and Ref. PCIF/SSO/0017/2018 (DOI: 10.54499/PCIF/SSO/0017/2018). This thesis was developed within the framework of the Doctoral Programme in Public Health at the University of Porto, at the Epidemiology Research Unit (EPIUnit) and at the Laboratory for Integrative and Translational Research in Population Health (ITR) of the Institute of Public Health of the University of Porto (ISPUP).

The present PhD work describes research conducted at the Department of Environmental Health of the National Institute of Health Dr Ricardo Jorge (INSA, Porto) under the supervision of Dr Solange Costa and Dr Joana Madureira, affiliated with the Department of Environmental Health, of the National Institute of Health Dr. Ricardo Jorge, and the Institute of Public Health of the University of Porto (ISPUP). Under the FCT PhD grant, additional research activities were developed at the University of A Coruña (A Coruña, Spain), DICOMOSA Research Group, supervised by Professor Blanca Laffon (3 months). Furthermore, the statistical analysis was performed under the supervision of Professor Stefano Bonassi, at the Unit of Clinical and Molecular Epidemiology, IRCCS San Raffaele Roma (Rome, Italy) (3 months).

In compliance with what is stated in Decree-Law no. 707/2018 of October 23, it is hereby declared that for this PhD thesis, the candidate participated in the definition of the study’s objectives and hypothesis, in the fieldwork namely in the recruitment of study’s participants and data collection, in the execution of experimental work, and the respective statistical analysis, interpretation of data, writing of the original manuscripts as well as in the dissemination of results. The manuscripts provided in this thesis were not previously included in other theses and correspond to their integral versions.



EDUCAÇÃO, CIÊNCIA E INOVAÇÃO



Table of Contents

List of Publications.....	xv
Communications and Awards.....	xix
List of Figures.....	xxiii
List of Abbreviations.....	xxv
Abstract.....	xxvii
Resumo.....	xxx
Thesis Structure.....	xxxv
CHAPTER I. STATE OF THE ART.....	1
A. GENERAL INTRODUCTION.....	3
A. 1. Firefighters' Burden of Disease.....	3
A. 2. Human Exposure Assessment: A Crucial Tool.....	5
→ <i>Environmental Monitoring</i>	5
→ <i>Biological Monitoring</i>	5
A. 3. Firefighters' Exposure Assessment.....	8
→ <i>Evaluating the Exposure</i>	8
→ <i>From the Exposure to the Biological Effect</i>	10
B. WILDLAND FIREFIGHTERS.....	11
B. 1. Wildland Fires: Dealing With a New Era.....	11
B. 2. Wildland Fires: A Major Source of Pollutant Exposure.....	13
B. 3. Wildland Firefighters: A Brief Contextualization.....	14
B. 4. Wildland Firefighters' Occupational Exposure.....	15
B. 5. Firefighters – Portuguese Reality.....	17
C. REFERENCES.....	19
CHAPTER II. MAIN GOALS.....	35
CHAPTER III. STUDY POPULATION.....	39
CHAPTER IV. ORIGINAL RESEARCH.....	43
A. FIREFIGHTERS' OCCUPATIONAL EXPOSURE IN PREPARATION FOR WILDFIRE SEASON: ADDRESSING BIOLOGICAL IMPACT.....	45
A. 1. <i>Firefighters' Occupational Exposure in Preparation for Wildfire Season: Addressing Biological Impact (Paper I)</i>	47
B. INVESTIGATING THE EXPOSURE-INDUCED BIOLOGICAL MECHANISMS ASSOCIATED WITH WILDLAND FIREFIGHTING OCCUPATIONAL EXPOSURE.....	65
B. 1. <i>Impact of Occupational Exposure to Wildfire Events on Systemic Inflammatory Biomarkers in Portuguese Wildland Firefighters (Paper II)</i>	67

<i>B. 2. Occupational Exposure to Wildland Firefighting and Its Effects on Systemic DNA Damage (Paper III)</i>	81
<i>B. 3. Micronucleus Assay in Buccal and Urothelial Cells in Wildfire-Exposed Firefighters (Paper IV)</i>	101
CHAPTER V. GENERAL DISCUSSION	123
A. SUMMARY OF MAIN FINDINGS	125
B. INTEGRATED DISCUSSION.....	128
C. LIST OF RECOMMENDATIONS	133
D. REFERENCES	135
CHAPTER VI. FINAL REMARKS AND FUTURE PERSPECTIVES	139
APPENDICES.	143

List of Publications

Within the scope of this PhD study, the following manuscripts/chapters were either published or submitted to International Peer-Reviewed Journals:

A. As **first** or **last** author:

A.1. Published manuscripts ($n=3$):

3. **Esteves, F.**, Madureira, J., Barros, B., Alves, S., Pires, J., Martins, S., Oliveira, M., Vaz, J., Slezakova, K., Pereira, M.C., Fernandes, A., Morais, S., Guimarães, J.T., Bonassi, S., Teixeira, J.P. & Costa, S. (2025). Impact of Occupational Exposure to Wildfire Events on Systemic Inflammatory Biomarkers in Portuguese Wildland Firefighters. *Environmental Research*, 277, 121608. <https://doi.org/10.1016/j.envres.2025.121608>
2. **Esteves, F.**, Madureira, J., Costa, C., Pires, J., Barros, B., Alves, S., Vaz, J., Oliveira, M., Slezakova, K., Fernandes, A., Pereira, M.C., Morais, S., Valdiglesias, V., Bonassi, S., Teixeira, J.P. & Costa, S. (2025). Occupational Exposure to Wildland Firefighting and Its Effects on Systemic DNA Damage. *International Journal of Hygiene and Environmental Health*, 266, 114576. <https://doi.org/10.1016/j.ijheh.2025.114576>
1. **Esteves, F.**, Slezakova, K., Madureira, J., Vaz, J., Fernandes, A., Morais, S., Pereira, M.C., Teixeira, J.P. & Costa, S. (2024). Firefighters' Occupational Exposure in Preparation for Wildfire Season: Addressing Biological Impact. *Toxics*, 12(3), 201. <https://doi.org/10.3390/toxics12030201>

A.2. Published book chapters ($n=3$):

3. **Esteves, F.**, Madureira, J., Teixeira, J.P. & Costa, S. (2024) Wildland Firefighters: a crucial weapon for Forest Fire Management. What risks do they face? *Fire Hazards: Socio-economic and Regional Issues*. Springer Nature, pp107-121. https://doi.org/10.1007/978-3-031-50446-4_9
2. Costa, S. & **Esteves, F.** (2024) Biomarkers, human health. *Encyclopedia of Toxicology* (Fourth Edition), Volume 2, 133-140. <https://doi.org/10.1016/B978-0-12-824315-2.00965-9>

1. **Esteves, F.**, Madureira, J., Teixeira, J.P. & Costa, S. (2022). The Role of Biomarkers in the Assessment of Potential Health Risks of Wildland Firefighters' Occupational Exposure: An Overview. In an "Essential Guide to Occupational Exposure". Nova Science Publishers, Inc., pp 47-79. <https://doi.org/10.52305/VGMC6738>

A.3. Manuscripts submitted (n=1):

1. **Esteves, F.**, Madureira, J., Barros, B., Alves, S., Vaz, J., Oliveira, M., Slezakova, K., Fernandes, A., Pereira, M.C., Morais, S., Bonassi, S., Teixeira, J.P. & Costa, S. (2025). Micronucleus Assay in Buccal and Urothelial Epithelial Cells of Wildfire-Exposed Firefighters.

B. As **co-author** (*these publications are not part of the core structure of this thesis*):

B.1. Manuscripts (n=4):

4. Paiva, A., Barros, B., Azevedo, R., Oliveira, M., Alves, S., **Esteves, F.**, Fernandes, F., Vaz, J., Alves, M.J., Slezakova, K., Pereira, M.C., Teixeira, J.P., Costa, S., Almeida, A. & Morais, S. (2024) Biomonitoring of firefighters' exposure to priority pollutant metal(loid)s during wildland fire combat missions: impact on urinary levels and health risks. Science of the Total Environment, 953, 176105. <https://doi.org/10.1016/j.scitotenv.2024.176105>
3. Alves, S., Silva, F., **Esteves, F.**, Costa, S., Slezakova, K., Alves, M., Pereira, M., Teixeira, J.P., Morais, S., Fernandes, A., Queiroga, F. & Vaz, J. (2024) The Impact of Sleep on Haematological Parameters in Firefighters. Clocks & Sleep, 6(3), 291-311. <https://doi.org/10.3390/clockssleep6030021>
2. Paiva, A., Barros, B., Oliveira, M., Alves, S., **Esteves, F.**, Fernandes, A., Vaz, J., Slezakova, K., Teixeira, J.P., Costa, S., & Morais, S. (2024) Biomonitoring of polycyclic aromatic hydrocarbons exposure and short-time health effects in wildland firefighters during real-life fire events. Science of Total Environment, 926, 171801. <https://doi.org/10.1016/j.scitotenv.2024.171801>
1. Barros, B., Paiva, A., Oliveira, M., Alves, S., **Esteves, F.**, Fernandes, A., Vaz, J., Slezakova, K., Costa, S., Teixeira, J.P. & Morais, S. (2024) Baseline data and associations between urinary biomarkers of polycyclic aromatic hydrocarbons, blood pressure, hemogram, and lifestyle among wildland firefighters. Frontiers Public Health, 12, 1338435. <https://doi.org/10.3389/fpubh.2024.1338435>

B.2. Book chapters ($n=2$):

2. Slezakova, K., **Esteves, F.**, Vaz, J., Costa, S., Alves, M., Madureira, J., Barros, B., Fernandes, A., Teixeira, J.P., Morais, S. & Pereira, M.C. (2023) Indoor Air quality at Portuguese Firehouses. In Occupational and Environmental Safety and Health V, Springer Nature Switzerland, vol. pp. 433-442. https://doi.org/10.1007/978-3-031-38277-2_35
1. Slezakova, K., **Esteves, F.**, Vaz, J., Alves, M., Madureira, J., Costa, S., Fernandes, A., Teixeira, J.P., Morais, S. & Pereira, M.C. (2022) Occupational Exposure of Firefighters in Non-fire Settings. In Occupational and Environmental Safety and Health IV, Springer Cham, pp. 79-88. https://doi.org/10.1007/978-3-031-12547-8_7

Communications and Awards

During the PhD, the candidate authored Communications (presenting) at numerous Scientific Meetings, both National and International, including an invited Keynote Communication at an International Conference:

A. **Communications** at scientific meetings as **presenting author**:

A1. Oral Communications ($n=10$):

10. **Esteves, F.**, Madureira, J., Pires, J., Rotondi, C., Alves, S., Barros, B., Vaz, J., Slezakova, K., Fernandes, A., Pereira, M.C., Morais, S., Bonassi, S., Teixeira, J.P. & Costa, S. (2024) The firefighters on the frontline of forest fires: from the exposure to adverse health effects. 3rd International Congress on Fire in the Earth System: Humans and Nature, Cáceres, Spain, 3rd-6th June. (*Online*)
9. **Esteves, F.**, Costa, S., Madureira, J., Pires, J. & Teixeira, J.P. (2023) Heróis, mas não super-humanos: um primeiro olhar sobre a exposição e o efeito nos bombeiros florestais Portugueses. Seminário BioFirEx "Um painel de (Bio)marcadores para a vigilância da saúde e segurança do bombeiro", Bragança, Portugal, 12th December.
8. **Esteves, F.** & Costa, S. (2023) What we have learned from our data thus far- preliminary results on Portuguese firefighters. "Last FIRElinks Workshop: The Global Fire Partnership", Spain, Gandia, 22nd October.
7. **Esteves, F.**, Madureira, J., Pires, J., Teixeira, J.P. & Costa, S. (2023) Tackling the increasing threat of Smoke Health Impacts from Forest fires and Climate Change." Annual ERA-ENVHEALTH Open Conference - Forest Fires – Climate change and Health impacts" Aveiro, Portugal, 18th October.
6. **Esteves, F.**, Madureira, J., Pires, J., Teixeira, J.P., Costa, S. (2023) Protecting Wildland Firefighters' Health: saving the lives of those who fight to save us. "2nd International Congress on Fire in the Earth System: Humans and Nature", Granada, Spain, 4-8th July.
5. **Esteves, F.**, Madureira, J., Pires, J., Teixeira, J.P. & Costa, S. (2023) Wildland Firefighters: Who are they? Why should we care?."2nd International Congress on Fire in the Earth System: Humans and Nature", Granada, Spain. 4-8th July. (**Keynote Speaker**)

4. **Esteves, F.**, Slezakova, K., Madureira, J., Vaz, J., Fernandes, A., Pereira, M.C, Morais, S., Teixeira, J.P. & Costa, S. (2023) Fighting occupational risks among Portuguese wildland firefighters: looking at cytogenetic effects. II TOXRUN International Congress 2023. Hotel Cristal, Porto, Portugal, 27-28th April.
3. **Esteves, F.**, Madureira, J., Teixeira, J.P. & Costa, S. (2022) The firefighters on the frontline of forest fires – preliminary characterisation of Bio4FOX study population. In: "Advances in Forest Fire Research 2022". Hotel Vila Galé, Coimbra, Portugal in 14-17th November.
2. **Esteves, F.**, Madureira, J., Teixeira, J.P. & Costa, S. (2022) Wildland Firefighters: a Crucial Weapon for Forest Fire Management. What risks do they face? WG5 Meeting FIRElinks "Socioeconomic impacts and regional issues of fire management". Centro de Documentación Científico, Granada, Spain in 25-26th February.
1. **Esteves, F.**, Madureira, J., Teixeira, J.P. & Costa, S. (2021) Assessment of Potential Health Risks of Portuguese Wildland Firefighters' Occupational Exposure: Biomonitoring Approach. 4th Doctoral Congress in Engineering, Symposium on Occupational Safety and Health, 28-29th of June (*Online*)

A2. Poster Communications (n=2):

2. **Esteves, F.**, Costa, S., Madureira, J. & Teixeira, J.P. (2022) Biomonitoring Portuguese Wildland Firefighters in Pre-fire season: Preliminary results. 3^o Dia do Jovem Investigador do Instituto Ricardo Jorge. INSA, Lisboa, Portugal, 8th November.
1. **Esteves, F.**, Madureira, J., Teixeira, J.P. & Costa, S. (2021) (Bio)monitoring Wildland Firefighters' Occupational Exposure. 56th Congress of the European Societies of Toxicology. Online. 27th September-5th October.

B. Awards/ Distinctions (n=2):

2. **Manuscript Cover of the Special Issue “Firefighters’ Occupational Exposures and Health Risks”** selected by the Editor. Featured in *Toxics*, Volume 12, Issue 3. **Esteves, F.**, Slezakova, K., Madureira, J., Vaz, J., Fernandes, A., Morais, S., Pereira, M.C., Teixeira, J.P. & Costa, S. (2024). Firefighters’ Occupational Exposure in Preparation for Wildfire Season: Addressing Biological Impact. *Toxics*. 12(3), 201. <https://www.mdpi.com/2305-6304/12/3>
1. **Best Oral Presentation Award** at 4th Doctoral Congress in Engineering (Symposium on Occupational Safety and Health). **Esteves, F.**, Madureira, J., Teixeira, J.P., & Costa, S. (2021) Assessment of Potential Health Risks of Portuguese Wildland Firefighters’ Occupational Exposure: Biomonitoring Approach. 28-29th June, Porto, Portugal.

List of Figures

Figure 1 - Diagram illustrating the cascade of events from exposure to clinical disease, showing the relationships among exposure, effect and susceptibility biomarkers. Adapted from Costa and Esteves (43).	6
Figure 2 - Firefighters' Routes of Exposure. Retrieved from Esteves et al. (145).	16
Figure 3 - Historical and future trends of Forest fire danger in Europe (164).	18
Figure 4 - Number of Portuguese Firefighters over the years. Retrieved from Esteves et al. (145).	19

List of Abbreviations

2-OHFlu	2-hydroxyfluorene
1-OHNaph	1-hydroxynaphthalene
1-OHPhen	1-hydroxyphenanthrene
1-OHPyr	1-hydroxypyrene
γ H2AX	Phosphorylated form of histone H2AX
BMCyt assay	Buccal Mucosa Cytome assay
BMI	Body Mass Index
BN	Binucleated
CC	Condensed Chromatin
COPD	Chronic Obstructive Pulmonary Disease
Cs	Cesium
DNA	Deoxyribonucleic Acid
DSBs	Double Strand Breaks
FPG	Formamidopyrimidine DNA Glycosylase
HBM	Human Biomonitoring
hs-CRP	High-sensitivity C-Reactive Protein
IARC	International Agency for Research on Cancer
IL-6	Interleukin-6
IL-8	Interleukin-8
KHC	Karyorrhexis
KYL	Karyolitic
MN	Micronucleus
NBUDS	Nuclear BUDS
OHPAHs	Hydroxylated Polycyclic Aromatic Hydrocarbons
PAHs	Polycyclic Aromatic Hydrocarbons
PIC	Pyknotic
PM	Particulate Matter
PPE	Personal Protective Equipment
ROS	Reactive Oxygen Species
SBs	Strand Breaks
SCBA	Self-Contained Breathing Apparatus
VOCs	Volatile Organic Compounds
TNF- α	Tumour necrosis factor- α

In recent decades, wildfires have become more frequent and intense, partly due to climate change. Wildland fires release harmful pollutants into the environment, posing significant health risks, particularly for those directly exposed, such as firefighters. Epidemiological studies have increasingly associated this occupation with various health risks. In 2022, after a re-evaluation, the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) classified firefighting occupational exposure as carcinogenic to humans (Group 1). Despite this classification, there is still a substantial need for biomonitoring studies, particularly those involving wildland firefighters. The current PhD study, Bio4FOX, aimed to address this gap by exploring the biological mechanisms associated with wildland firefighters' occupational exposure and identifying a set of biomarkers for monitoring Portuguese wildland firefighters.

A group of 225 wildland firefighters was recruited from 14 fire stations in the northeastern region of Portugal. This longitudinal study was conducted at two assessment time points: Phase I – before the wildfire season (baseline), and Phase II – following exposure to a real wildfire event, during the wildfire season. Biological samples, namely blood, urine, and buccal cells, were collected at both time points. In addition, during Phase I, data on particulate matter (PM) fractions, specifically PM_{2.5} and PM₁₀, were collected both indoors and outdoors in fire stations, during firefighters' regular activities (e.g., rest areas, living rooms, garage). Data on individual characteristics, occupational factors, lifestyle, and health status were assessed via a comprehensive questionnaire. Moreover, in Phase II, a short, self-administered questionnaire was used to collect information on firefighters' participation in the wildfire event after combat, including details such as duration of fire and exposure, as well as reported symptoms.

In Phase I, a preliminary characterisation of firefighters' occupational exposure, at fire stations, was performed. The biological impact of estimated inhaled doses of PM₁₀ and PM_{2.5} was investigated in a subgroup of 80 wildland firefighters using the Buccal Micronucleus Cytome (BMCyt) assay, a cytogenetic technique that evaluates the frequency of micronuclei (MN), a biomarker of chromosomal damage and genome instability. The use of MN frequency in epithelial cells, such as buccal or urothelial cells has become particularly interesting in biomonitoring studies due to its minimally invasive character and correlation with MN frequency in lymphocytes, a well-established early cancer risk biomarker. Results showed that concentrations of both PM₁₀ and PM_{2.5} (indoor and outdoor) were below

regulatory thresholds, and no significant correlation was observed between inhaled doses of PM10 and PM2.5 and respective BMCyt assay endpoints.

During Phase II, the biological mechanisms induced by occupational exposure to real wildfire events were investigated in paired firefighters who participated in both study phases (n=59). Inflammatory biomarkers [i.e. interleukin-6 (IL-6), interleukin-8 (IL-8), tumour necrosis factor α (TNF- α) and high-sensitivity C-reactive protein (hs-CRP)] were analysed in blood. Systemic genotoxicity was evaluated in blood via comet assay, both primary DNA damage and oxidative DNA damage (oxidised purines), as well as phosphorylated histone H2Ax (γ H2Ax) via flow cytometry. Cytogenetic alterations in local contact tissues, namely buccal (inhalation) and urothelial cells (excretion), were evaluated through MN assay. Both levels of pro-inflammatory biomarkers IL-6 and IL-8 were significantly increased after wildfire combat. Moreover, the increase in IL-8 post-exposure levels was significantly associated with wildfire combat exposure duration (>12 hours). Mean plasma hs-CRP concentrations in both study phases were above 3.0 mg/L, suggesting an increased risk of cardiovascular diseases/events. Comet assay endpoints were significantly higher after occupational exposure to a wildfire event compared to baseline levels; no significant effect was observed for γ H2Ax. Exposure duration (hours) and breathing difficulties were significantly associated with increased primary and oxidative DNA damage, correspondingly. Both endpoints were increased, although only the frequency of MN in urothelial cells reached statistical significance, more than doubling in Phase II compared to baseline levels. The relationship between the studied endpoints and dose biomarkers, namely, urinary hydroxylated polycyclic aromatic hydrocarbon metabolites (OHPAHs) and urinary metal levels, was also explored. A significant positive correlation was identified between post-exposure urinary 2-hydroxyfluorene (2-OHFlu) levels - an OHPAH byproduct - and IL-8 and oxidative DNA damage. Although γ H2Ax levels did not significantly differ between study phases, a significant positive correlation was observed between post-exposure γ H2Ax levels and urinary 2-OHFlu concentrations. Furthermore, MN frequency in urothelial cells was significantly associated with total OHPAHs levels, suggesting MN in this tissue as a suitable early-effect biomarker for monitoring firefighters' occupational exposure. Regarding metals in urine, post-exposure cesium (Cs) levels were significantly associated with increased primary and oxidative DNA damage.

Bio4FOX provides novel data on the biological effects of wildland firefighting exposure following real wildfire scenarios, offering insights into biological mechanisms and potential health risks. Furthermore, a set of suitable biomarkers for monitoring wildland firefighters' occupational exposure was identified. These endpoints demonstrated potential in effectively reflecting both acute and chronic effects associated with exposure. Preventive measures,

such as decontamination protocols, among others, are essential to reduce exposure to harmful pollutants. In conclusion, the findings of the present study can inform health surveillance programmes and guide policy development to mitigate occupational health risks in this essential workforce. Additionally, it also offers guidelines that might help firefighting command in making local strategic and operational decisions to implement preventive measures that protect the health of their teams.

Keywords: wildland firefighters; firefighting; occupational exposure; health effects; pollutants; smoke exposure; biomarkers; inflammation; genotoxicity biomarkers; DNA damage; biological effects

Os incêndios florestais têm aumentado nas últimas décadas, em parte devido às alterações climáticas. A exposição aos poluentes libertados por estes eventos constitui um fator de risco para diversos grupos da população, nomeadamente para os bombeiros que se encontram na linha da frente no combate aos incêndios. Recentemente, alguns estudos epidemiológicos têm explorado a associação entre esta ocupação e os respetivos riscos para a saúde. Em 2022, após uma reavaliação, a Agência Internacional de Investigação do Cancro (IARC) da Organização Mundial da Saúde (OMS), classificou a exposição ocupacional dos bombeiros como carcinogénica para humanos (Grupo 1). No entanto, são ainda poucos os estudos de biomonitorização neste grupo ocupacional, particularmente no que concerne aos bombeiros florestais. Assim, este estudo (Bio4FOX), teve como principal objetivo identificar os mecanismos biológicos subjacentes à exposição ocupacional dos bombeiros florestais e identificar biomarcadores adequados para monitorizar a exposição em bombeiros florestais portugueses.

Para o efeito, foi recrutado um grupo de 225 bombeiros florestais pertencentes a 14 corporações de bombeiros da região nordeste de Portugal. Os participantes foram estudados em dois momentos distintos da sua atividade: Fase I – antes da época de incêndios e Fase II – após o combate a um incêndio florestal, durante a época de incêndios. Em ambas as fases do estudo foram recolhidas amostras biológicas, especificamente sangue, urina e células da mucosa bucal. Na Fase I, foi ainda realizada uma avaliação da concentração de material particulado (PM) no ar interior e exterior dos quartéis, incluindo áreas de descanso, salas de estar e garagens. Os dados sobre as características individuais, ocupacionais, estilos de vida e estados de saúde foram obtidos através de um questionário autoadministrado. Foi ainda aplicado um segundo questionário, mais curto, durante a Fase II, para recolha de informações sobre o combate ao incêndio, nomeadamente a duração do fogo, o tempo de exposição no terreno e a sintomatologia reportada.

Na Fase I, foi realizada uma caracterização preliminar da exposição ocupacional dos bombeiros nos respetivos quartéis, com o objetivo de avaliar o impacto biológico das doses inaladas estimadas de PM₁₀ e PM_{2.5} num subgrupo de 80 bombeiros. O indicador biológico utilizado foi a frequência de micronúcleos (MN) na mucosa bucal, avaliado através do ensaio *Buccal Micronucleus Cytome* (BMCyt) assay que permitiu a análise da instabilidade genética e do dano cromossómico nas células deste tecido de primeiro contacto. A utilização deste biomarcador em células epiteliais, como as células bucais ou

uroteliais, é particularmente relevante em estudos de biomonitorização, devido ao seu caráter pouco invasivo e à sua correlação com a frequência de MN em linfócitos, um biomarcador precoce do risco de cancro. Verificou-se que as concentrações de PM10 e PM2.5 (tanto no ar interior como no ar exterior dos quartéis) estavam abaixo dos limites regulamentares, tanto nacionais como internacionais, não se tendo observado associação entre as doses inaladas de PM10 e PM2.5 e a frequência de MN ou outros parâmetros citogenéticos avaliados pelo ensaio BMCyt.

Na Fase II, foram investigados os mecanismos biológicos induzidos pela exposição ocupacional a incêndios florestais nos bombeiros que participaram em ambas as fases do estudo (n=59). No sangue, foram avaliados parâmetros imunológicos [interleucina-6 (IL-6), interleucina-8 (IL-8), fator de necrose tumoral alfa (TNF- α) e proteína C reativa de alta sensibilidade (hs-CRP)], bem como de dano no ADN, incluindo o dano primário e oxidativo, avaliados através do ensaio do cometa, e a fosforilação da histona H2AX (γ H2AX). Foram também analisadas alterações citogenéticas em tecidos-alvo, nomeadamente em células bucais (inalação) e uroteliais (excreção), através do ensaio de MN. A exposição ao combate a incêndio florestal aumentou significativamente os níveis séricos de IL-8 e IL-6. Os níveis séricos de IL-8 foram superiores em bombeiros que reportaram ter estado mais tempo no combate ao incêndio (>12 horas). A média dos níveis de hs-CRP, no plasma, mostraram-se acima de 3.0 mg/L em ambas as fases do estudo, indicando um maior risco de doenças cardiovasculares. Ambos os biomarcadores avaliados pelo ensaio do cometa revelaram um aumento significativo após a exposição ao combate a incêndio, comparativamente com níveis obtidos na Fase I. A duração da exposição e as dificuldades respiratórias reportadas durante o combate a incêndios estiveram associadas a níveis elevados do dano primário no ADN. Verificou-se ainda, um aumento da frequência de MN em células bucais e uroteliais durante a época de incêndios, embora esse aumento só tenha atingido significância estatística nas células uroteliais, onde a frequência de MN mais do que duplicou em relação à Fase I. Neste estudo, avaliou-se também a possível associação entre os biomarcadores de efeito e os biomarcadores de dose, nomeadamente os metabolitos hidroxilados de hidrocarbonetos aromáticos policíclicos (OHPAHs) e metais presentes na urina, na Fase II. Observou-se uma correlação positiva significativa entre os níveis urinários de 2-hidroxifluoreno (2-OHFlu), os níveis séricos de IL-8 e de dano oxidativo no ADN. Foi também observada uma correlação positiva significativa entre os níveis de γ H2Ax e os níveis urinários de 2-OHFlu e entre os OHPAHs totais na urina e a frequência de MN nas células uroteliais. No que concerne aos metais na urina, encontrou-se uma associação significativamente positiva entre os níveis de cério (Cs) e o aumento de dano primário e oxidativo do ADN.

Face ao exposto, o presente estudo fornece dados sobre os efeitos biológicos resultantes da exposição ao combate a incêndios florestais, bem como sobre os mecanismos biológicos subjacentes e os potenciais riscos para a saúde. Além disso, sugere um conjunto de biomarcadores úteis para a vigilância da exposição ocupacional dos bombeiros florestais, os quais demonstraram ter potencial para refletir as alterações biológicas associadas à exposição a incêndios florestais. Em suma, os resultados deste estudo sublinham a necessidade de implementar medidas preventivas, como procedimentos de descontaminação, e reforçam a importância de programas de vigilância da saúde e de políticas públicas, fundamentadas em evidências científicas, que visem mitigar os riscos para a saúde ocupacional dos bombeiros florestais. Este estudo apresenta orientações que poderão ser úteis para a tomada de decisões estratégicas e operacionais, com o objetivo de implementar medidas preventivas capazes de proteger a saúde e segurança dos bombeiros.

Palavras-chave: bombeiros florestais; combate a incêndio; exposição ocupacional; efeitos na saúde; exposição a fumo; biomarcadores; inflamação; biomarcadores genotoxicidade; dano no ADN; efeitos biológicos

This thesis is organised into six different chapters:

Chapter I – State of the Art

This chapter includes the content of two book chapters published during the PhD divided into two different sections:

General Introduction: This first part introduces the burden of disease among firefighters in general, emphasising the importance of biomonitoring studies to assess occupational exposures. It also highlights the lack of studies focused on wildland firefighters.

Wildland Firefighters: This section characterises the wildland firefighters' occupation and the associated risks, with a particular focus on the growing challenges posed by the increasing frequency of wildfires due to climate change. It underscores the urgent need for biomonitoring studies to better understand and mitigate the health risks faced by this workforce.

Chapter II – Thesis Main Goals

This chapter outlines the general and specific objectives of the thesis.

Chapter III – Study Population

This chapter briefly describes the study population included in this study.

Chapter IV – Original Research

This chapter is divided into two main sections:

Characterisation of Firefighters' Occupational Exposure at Fire Stations, Before Wildfire Season: This section includes an original published paper that characterises the occupational exposure of firefighters at fire stations before the wildfire season (baseline).

Investigating the Exposure-Induced Biological Mechanisms Associated with Wildland Firefighting Occupational Exposure: This section includes three original research papers (two published, and one submitted) that explore the biological effects of wildland firefighting occupational exposure. The first paper discusses the systemic inflammatory response, while the other two focus on DNA damage and genomic instability, respectively.

Chapter V – General Discussion

This chapter is divided into four sections:

Summary of Main Findings: This section synthesises the main findings obtained in the four original research papers.

Integrated Discussion: This part provides an overall and integrated discussion of the results obtained throughout the thesis. It also addresses the main strengths and limitations of the study.

Recommendations to the Sector: This section offers practical recommendations for the sector.

Chapter VI – Final Remarks and Future Perspectives

This chapter presents the key take-home messages from the research project, discusses the need for future studies, and highlights the relevance of the present study for advancing the knowledge in the field.

CHAPTER I.

State of the art

This chapter was adapted from the following published works:

- **Esteves, F., Madureira, J., Teixeira, J.P. & Costa, S. (2024)** *Wildland Firefighters: a crucial weapon for Forest Fire Management. What risks do they face? Fire Hazards: Socio-economic and Regional Issues.* Springer Nature, pp 107-121. https://doi.org/10.1007/978-3-031-50446-4_9 (Appendix I)

Copyright © 2024 – The Author(s). Open Access article distributed under the terms of the Creative Commons CC BY license (<http://creativecommons.org/licenses/by/4.0/>)



- **Esteves, F., Madureira, J., Teixeira, J.P. & Costa, S. (2022).** *The Role of Biomarkers in the Assessment of Potential Health Risks of Wildland Firefighters' Occupational Exposure: An Overview.* In an "Essential Guide to Occupational Exposure". Nova Science Publishers, Inc., pp 47-79. <https://doi.org/10.52305/VGMC6738> (Appendix II)

Copyright © 2022 – With Kind Permission from Nova Science Publishers, Inc. (www.novapublishers.com)

A. GENERAL INTRODUCTION

A.1. *Firefighters' Burden of Disease*

Firefighters play a crucial role in the community, protecting lives, goods, and the environment (1). The demanding nature of their activity exposes them to a variety of hazards, including chemical, physical, biological, and psychosocial risks (1). Such exposures may result in acute or long-term health effects.

Annually, around 70.000 firefighters worldwide are injured or lose their lives during firefighting activities (2). Cardiac events have been identified as an important cause of death among firefighters (3, 4). In the United States of America (USA), for instance, sudden cardiac events (e.g., heart attack) are the leading cause of fatalities among firefighters on duty (5). These events have been associated with different factors such as high temperatures, physical exertion, dehydration, stress (6), and pre-existing health conditions (e.g., hypertension, diabetes, cholesterol) (7). For example, Fahs et al. (8), found a correlation between short-term firefighting activities and adverse cardiovascular outcomes, noting increased arterial stiffness and vasodilation after three hours of firefighting. Additionally, fatalities in the field may also result from other type of incidents, such as entrapments, vehicle collisions (9), internal trauma, and asphyxia (5). In addition to the immediate risks that firefighters encounter in the field, long-term adverse health outcomes have also been identified, including respiratory disorders (e.g., asthma, chronic obstructive pulmonary disease) (10) and cardiovascular outcomes (8, 11-14). Also, elevated core body temperature, due to exposure to high temperatures and physically challenging activities (15, 16), may trigger cardiovascular events in firefighters who are particularly susceptible (17). The prevalence of health effects has been directly correlated with the duration of firefighting careers, with those serving longer periods having an increased risk of developing adverse cardiovascular outcomes (18). Semmens et al. (18) found that firefighters with more years of service are more likely to develop cardiovascular outcomes (e.g., hypertension and arrhythmia) compared to those with fewer years in the profession. Indeed, exposure to cardiotoxic-inducing agents, such as particulate matter (PM) from fire smoke, has been considered an important risk factor for vascular diseases (19). Both short- and long-term exposure to these pollutants has been associated with cardiovascular and respiratory morbidity (20). PM with smaller diameter is particularly harmful to human health due to its increased ability to penetrate the lungs and enter the bloodstream (20, 21). PM_{2.5} (particles with aerodynamic diameter of 2.5 µm or less), for example, enters the human body through

inhalation reaching the lungs and bloodstream, causing adverse health effects in the cardiovascular, respiratory, immune, and nervous systems (22).

Further long-term health effects related to occupational firefighting exposure have been described (1, 23), including a higher incidence of miscarriage among female firefighters (24), and increased vulnerability to mental health problems due to high levels of stress and exposure to traumatic events (23). These adverse outcomes in firefighters have been associated with a higher risk of substance abuse (25), depression (26), post-traumatic stress disorders (27), sleep disturbance (28), and even suicidal behaviours (29).

Other significant long-term health effects have been associated with firefighters' occupational exposure, including cancer (1). Cancer is one of the leading causes of death globally (30). Carcinogenesis is a multistage process that involves modification and mutation of genes that regulate normal cellular function (31, 32). Although genetic predisposition may play a role in carcinogenesis, a wide range of environmental and occupational factors also contribute to the development of cancer (1). In fact, the World Health Organization (WHO) estimate that 90% of all cancers are caused by environmental and lifestyle factors, which provides major opportunities for prevention (33).

Firefighters are exposed to a wide range of harmful substances, many of them classified by the International Agency for Research on Cancer (IARC) as known (e.g., benzene, benzo[a]pyrene, formaldehyde, and PM_{2.5}) or probable/possible (e.g., acetaldehyde, benzofuran, ethylbenzene, furan, black carbon and styrene) human carcinogens (34). In addition, several epidemiological studies have consistently shown an increased incidence of various types of cancer among firefighters (35-37), such as melanoma, leukaemia (38), colon, rectum, testis, bladder, thyroid, pleura (36), laryngeal and hypopharyngeal (39). Thus, recently, in June 2022, IARC re-evaluated occupational exposure as a firefighter and listed it as carcinogenic to humans (Group 1) based on sufficient evidence for cancer in humans (1, 40).

Despite the IARC's classification and substantial epidemiological evidence linking firefighting to adverse health outcomes, research on monitoring firefighters' occupational exposure remains limited. This highlights the need for comprehensive studies to characterise these exposures and to inform science-based, targeted interventions and protective measures to mitigate associated health risks.

The research gap is even more pronounced for wildland firefighters, as most existing studies focus on structural firefighters. Although studies in this area are particularly challenging, the increasing frequency and severity of wildland fires underscore the urgent need for exposure assessments among this workforce.

A.2. Human Exposure Assessment: A Crucial Tool

Exposure assessment plays a central role in the identification, evaluation, and control of occupational risks. Human monitoring is a crucial approach to identify potential signals of exposure to toxic compounds and to predict certain health risks (41). This approach involves a routine evaluation and interpretation of environmental and/or biological parameters to identify possible health risks before the occurrence of a disease (41). The exposure can be assessed by measuring the concentration of any chemical agent in environmental samples, such as air (environmental monitoring) and/or by measuring biological endpoints (biological monitoring), called biomarkers or biological indicators. Generally, these two methods are used simultaneously, since they provide different but complementary information (42).

→ Environmental Monitoring

Environmental monitoring refers to the quantification of certain pollutant(s) or contaminant(s) in the occupational environment and aims to estimate the hazard level to which individuals are exposed, comparing measured concentrations with established reference values (43). These measurements can be conducted during a single task as well as during an entire work shift. However, environmental monitoring represents only a snapshot of exposure in time, not taking into consideration variations over time and other factors that may have a substantial influence on compound uptake, such as individual characteristics or other contextual factors such as the use of personal protective equipment (PPE).

Monitoring firefighters' exposure during fire combat is particularly challenging due to the dynamics and hazardous nature of the environment, including the variability in the fire location, intensity, and unpredictable weather conditions. In this context, biological monitoring constitutes an important tool that helps to overcome these difficulties.

→ Biological Monitoring

Biological Monitoring, also known as biomonitoring, has been extensively used to assess chemical exposure resulting from different exposure routes (e.g., inhalation, ingestion, and dermal absorption) and through different pathways (absorption, distribution, metabolism, and excretion). Biological monitoring provides measurable data on the individual levels of the internal dose resulting from the exposure to a xenobiotic (biomarkers of exposure) (43), the effects caused by the interaction with cellular/molecular components (biomarkers of effect), and the potential susceptibility related to acquired factors (e.g., diet) or genetic predispositions (biomarkers of susceptibility) (43).

The main purpose of biomonitoring is not only to characterise exposure but also to guide the development of preventive measures to reduce the risk of both short- and long-term adverse health outcomes in exposed populations, particularly, in susceptible groups such as children, older adults, pregnant women and workers.

Biomarkers act as intermediates between exposure and clinical outcomes, representing a sequence of events that begin with the exposure and end with the clinical outcome, the known concept of a “*continuum of biological events*” (**Figure 1**) (43, 44). These biomarkers are essential in occupational studies because they not only signal exposure to a compound but may also provide data to assess the risk of potential adverse health effects associated with such exposures (45). According to the International Program of Chemical Safety, biomarkers can be categorised as: **biomarkers of exposure**, **biomarkers of effect** and **biomarkers of susceptibility** (46).

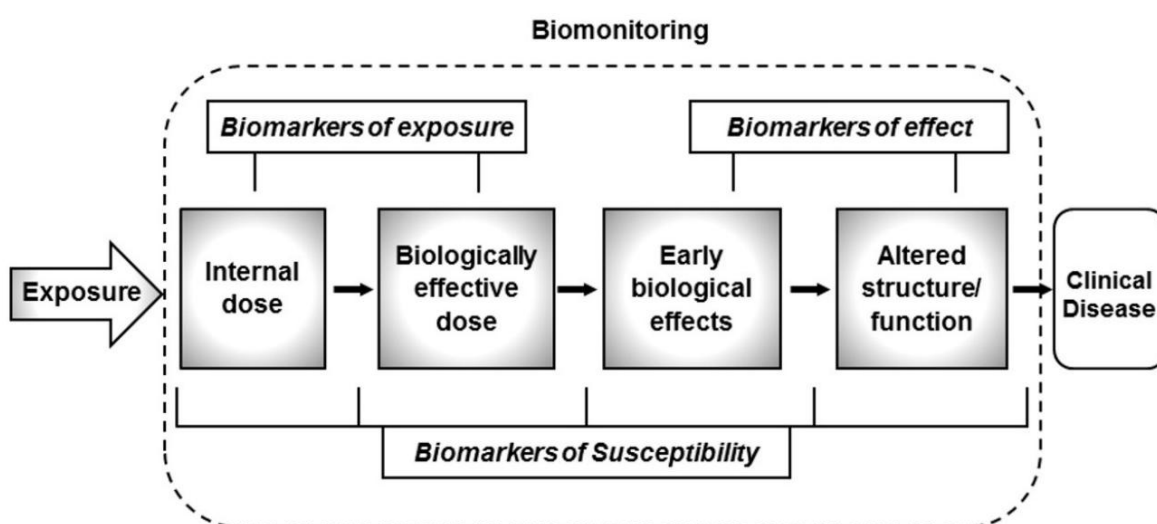


Figure 1 - Diagram illustrating the cascade of events from exposure to clinical disease, showing the relationships among exposure, effect and susceptibility biomarkers. Adapted from Costa and Esteves (43).

Biomarker of exposure is the substance itself, its metabolites or the product of an interaction between a chemical and its target molecule or cell that is measured in a compartment within an organism (43). Their presence usually only indicates that an exposure has occurred. A biomarker of exposure can be defined as a biomarker of internal dose when the amount of a substance or its metabolites is quantified in a biological tissue or fluid (e.g., t, t-muconic acid in the urine, lead in the blood) or as a biomarker of biologically effective dose when it concerns the interactions between the chemical or its metabolites with target cellular macromolecules (e.g. DNA-adducts or protein-adducts) (43).

A **biomarker of effect** is defined as a measurable biochemical, physiological, behavioural, or other alteration in an organism that, depending upon the magnitude, can be associated with a possible health impairment or disease (46). It may represent an event that can be correlated with, and possibly predictive of, a deleterious health effect (47). Effect biomarkers are extensively used in occupational studies to evaluate the genotoxic potential of hazardous chemicals. The measurements of biomarkers of effect generally reflect events that take place at the latter end of the *continuum* between exposure and disease manifestation. They are not chemical specific, and therefore the association with exposure must be established by an independent measure, such as environmental monitoring or simultaneous measurement of a biomarker of exposure, such as the internal dose. An effect biomarker may either indicate early processes that precede disease or predict the development and presence of disease (by altered structure and/or function) (48). In terms of prevention, it is considered ideal if the biomarker can detect reversible biological alterations.

Cytogenetic endpoints such as micronuclei (MN) frequency or chromosomal aberrations have long been applied in the surveillance of human genotoxic exposure and early effects of hazardous compounds (49). The relevance of cytogenetic alterations as potential early cancer risk biomarkers has been supported by various epidemiological studies (50, 51). MN frequency, for instance, is a well-established biomarker used extensively in human biomonitoring studies to assess genomic instability. The relevance of this assay is further supported by epidemiologic data linking the increased frequency of MN in lymphocytes to cancer risk (52). Moreover, evidence of a strong positive correlation between MN frequency in lymphocytes and MN frequency in exfoliated cells, namely buccal cells, suggests that the application of this MN frequency in such local tissues may also serve as a potential indicator of cancer risk (53, 54). The use of this type of biological matrices in human biomonitoring studies is particularly relevant due to its minimally invasive character, and especially because most cancers are of epithelial origin (55).

The single-cell gel electrophoresis assay or comet assay has also been proven to be a sensitive tool in human biomonitoring studies for the detection of different levels of DNA damage at the individual cell level (56). The simultaneous use of comet assay and cytogenetic tests allows a comparison of the presence of DNA strand breaks (due to both acute and chronic exposure), and of chromosome damage (due to clastogenic and aneugenic events), respectively (57, 58).

Biomarkers of susceptibility indicate the inherent or acquired ability of an organism to respond to a specific agent(s) (59). This class of biomarkers help to understand the

individual variations between biomarkers of exposure and biomarkers of effect. These differences may arise from genetic (e.g., polymorphisms that may lead to different capacities to detoxify or repair the DNA) or non-genetic factors (e.g., lifestyle, and age). These factors are independent of exposure but may help identify those individuals in a population who are more susceptible or at risk (45).

Each class of biomarkers provides different information, and it has a set of advantages and disadvantages, concerning its relevance, sensitivity, specificity, reliability, practicality, and analytical complexity (60). The basic principles of biomarker-based exposure assessment are illustrated in the figure above (**Figure 1**).

A.3. Firefighters' Exposure Assessment

As stated herein, the challenging and dynamic conditions inherent to firefighting make real-time monitoring extremely difficult. As a result, most of the existing data on firefighters' exposure originates from biomonitoring studies.

→ Evaluating the Exposure

As with any biomarker of dose, a biomarker of smoke exposure should exhibit adequate sensitivity, specificity, and robustness (61). Some compounds found in human biological samples have been proposed as adequate biomarkers of smoke exposure, namely polycyclic aromatic hydrocarbons (PAHs) metabolites (1, 62).

PAHs are largely emitted during incomplete combustion processes of organic materials (63). Over 100 different PAHs have been identified, from which 16 species have been classified as priority pollutants (34). PAHs are a major concern, being some classified by IARC as known (i.e., benzo[a]pyrene), probable (e.g., dibenz[a,h]anthracene, dibenzo[a,l]pyrene) or possible human carcinogenic (e.g., chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benz[a]anthracene) (34). PAHs and their metabolites can be quantified in different biological matrices, such as blood, urine, and skin (64). Additionally, the metabolism of some PAHs may produce reactive intermediates that can bind to cellular macromolecules (DNA and proteins), generating PAHs adducts (65), such as benzo[a]pyrene diol epoxide (BPDE) (66). These adducts are considered key indicators for assessing exposure to PAHs and are linked to mutagenesis and carcinogenesis (66).

Currently, the most used and validated biomarkers for assessing PAHs exposure are hydroxylated PAHs metabolites (OHPAHs) in urine (67, 68). PAHs are metabolised in the

liver and mostly excreted through urine mainly as their glucuronide conjugates. Among the existent urinary OHPAHs metabolites, some are frequently measured due its prevalence and sensivity, including 1-hydroxypyrene (1-OHPyr), 1-hydroxynaphthalene (1-OHNaph), 1-hydroxyacenaphthene (1-OHAce), 1-hydroxyphenanthrene (1-OHPhen), and 2-hydroxyfluorene (2-OHFlu) (64).

Among wildland firefighters, Oliveira et al. (69), found significantly higher levels of urinary total OHPAHs post-shift, up to 340% higher, in those actively involved in firefighting activities compared to non-exposed firefighters. The metabolites that contributed most to the increase were 1-OHNaph and 1-OHAce, followed by 2-OHFlu, 1-OHPhen, and 1-OHPyr (69).

Despite OHPAHs being the most used biomarkers for monitoring firefighters' occupational exposure, other biomarkers have been identified (1), such as carboxyhaemoglobin (blood), methoxyphenols (urine) and levoglucosan (urine), however, their application has been limited and therefore remains inconclusive (1). Carboxyhaemoglobin, for example, is formed when carbon monoxide (CO) binds to haemoglobin, impairing oxygen transport to vital organs, and causing hypoxia (62). Its potential application has been debated, with some authors suggesting that carboxyhaemoglobin is only suitable for application when exposure levels to CO are high (62, 70). Methoxyphenols, another potential biomarker of wildland smoke exposure, result from the pyrolysis of wood lignin. Associations were found between the exposure to woodsmoke among wildland firefighters and the levels of this biomarker in urine (71). Fernando et al. (72) used a set of methoxyphenols to determine the effect of woodsmoke exposure among firefighters. These compounds were identified as sensitive markers of smoke exposure, as their levels were significantly higher ($p < 0.05$) in urine samples collected 24 hours post-exposure (72). Similarly to carboxyhaemoglobin, this biomarker is considered suitable for high-exposure woodsmoke scenarios (1). Levoglucosan, a byproduct of cellulose pyrolysis, has been proposed as another potential biomarker for woodsmoke exposure (1, 73). However, the feasibility of using levoglucosan as a urinary biomarker is limited by inconsistencies in post-shift increases observed in some studies (72). Naeher et al. (73), for example, analysed urine samples from wildland firefighters before and after their work shifts and verified that post-shift concentrations were not consistently higher than pre-shift levels. The lack of biomonitoring studies using these types of smoke biomarkers (e.g., carboxyhaemoglobin, methoxyphenols, levoglucosan) hampers the ability to establish them as effective tools to monitor smoke exposure among wildland firefighters.

Less-invasive biological matrices, such as exhaled air have been investigated to assess biomarkers of smoke exposure (1). Zhou et al. (74) for example, measured exhaled carbon monoxide and nitric oxide using a hand-held, battery-operated meter and a Nano Coulomb Nitric Oxide Analyser, respectively. They found an association between elevated levels of exhaled carbon monoxide and nitric oxide and increased urinary PAH metabolites (74). Other tools have been used in screening strategies to identify metabolites in exhaled air, such as the electronic nose (e-nose) (75). E-nose allows the analysis of the volatile organic compounds (VOC) profile in exhaled air (75). Breath monitoring has several advantages especially in occupational settings, because is a simple and non-invasive tool to measure the extent of chemical exposures. It allows for real-time assessment, providing rapid feedback on individual levels of exposure, which is crucial for safeguarding workers' health, and ensuring a secure and safe workplace.

→ *From the Exposure to the Biological Effect*

During a wildfire, some of the chemicals released are free radicals. Once absorbed and metabolised, these radicals can generate reactive oxygen species (ROS) (76-78), which, if not neutralised by the body's antioxidant system, may react with proteins, lipids and DNA, leading to oxidative stress (79-81). Oxidative DNA damage is one of the most significant types of genetic damage caused by exogenous substances in humans (82) and is associated with the development of several diseases, including cancer (1). Gaughan et al. (83), for example, observed a positive association between predictors of arterial stiffness and biomarkers of oxidative stress, specifically lipid peroxidation (urinary 8-isoprostane) and oxidative DNA damage (urinary 8-hydroxy-2'-deoxyguanosine). A study conducted in Portugal (84) found higher levels of primary DNA damage and oxidative DNA damage, evaluated by comet assay, in blood samples of wildland firefighters exposed to woodsmoke when compared to non-exposed individuals. Similar results were observed by Andersen et al. (85) following a 3-day firefighting course. In their study, the authors found that primary DNA damage was positively associated with urinary excretion of 1-OHPyr, as well as with dermal exposure to pyrene and the concentration of total PAHs (85), suggesting that parameters measured through the comet assay can be suitable biomarkers for monitoring occupational exposure in firefighters.

Another mechanism of action triggered by smoke inhalation is inflammation (1). The initial response of the innate immune system includes the release of pro-inflammatory cytokines, such as interleukin-6 (IL-6), interleukin-8 (IL-8), and tumour necrosis factor α (TNF- α), which are critical for initiating the acute inflammatory response (86). Previous human biomonitoring studies have shown that individuals exposed to woodsmoke exhibited an

increased pro-inflammatory response (87, 88), consistent with *in vitro* studies (92-94). Elevated levels of pro-inflammatory biomarkers, such as blood serum IL-6 and IL-8, were found after smoke exposure (92-94).

Although human biomonitoring studies are crucial for assessing health risks, up to date, there are still limited studies investigating the biological mechanisms associated with firefighters' occupational exposures (1). This gap is even more pronounced among wildland firefighters, particularly those evaluating biological effects following real-world scenarios of wildfire occupational exposure (1).

B. WILDLAND FIREFIGHTERS

B.1. *Wildland Fires: Dealing With a New Era*

Fire has always played an important role in shaping ecosystems serving as an essential component for ecological balance (95). Human ancestors started to use fire intentionally around 1.500.000 years ago for hunting, protection or cooking, helping their survival (96). The ability to control and use fire marked a transformative step in human evolution, influencing social structures, tool innovation, and even brain development. While fire can be a beneficial tool, when uncontrolled, it poses a significant threat to wildlife habitats, property, and human lives.

A “wildfire”, “forest fire”, “bushfire”, “wildland fire” or “rural fire” (the term varies within countries) can be defined as an unplanned, uncontrolled, and unpredictable fire event that occurs in nature (97, 98). Globally, only 4% of forest fires occur due to natural causes (e.g., storms or lightning) (99), being the most part caused by direct (e.g., land clearing practices, slash-and-burn activities, or arson) or indirect human action (e.g., discarded cigarettes, accidental ignitions or unattended bonfires) (99).

Wildfires' dynamics may be influenced by numerous factors namely the type and amount of vegetation, the climate, the topography, and the weather conditions (102). The regions most affected by wildfires include the coniferous forests of California, the eucalyptus forests of Australia, the African savannahs, the dry forests of South Asia, and the temperate forests, shrublands, and woodlands of the Mediterranean region (100).

By the early 20th century, improvement in firefighting activities together with fire prevention actions, fire suppression policies, and changes in agricultural practices, contributed to a decrease in fire occurrences and burned area in temperate regions (e.g., temperate forests

of Europe) (101, 102). However, by the end of the century, there was a shift, with a significant increase of burned areas in tropical forests driven by deforestation for agricultural and livestock expansion, and in some temperate forests (e.g., North America and Europe) (101). In the USA, for example, the annual area burned by wildfires increased from approximately 1.000.000 hectares (1985-2000) to 2.000.000 hectares (2001-2016) (103). Similarly, Europe experienced an average of 65.000 wildland fires every year, burning approximately 500.000 hectares of wildland and forest areas (106), with over 85% of the areas affected located in the Mediterranean region (104). Portugal, for instance, with a forest area of 3.200.000 hectares is one of the European countries most affected by wildfires (105). In fact, over the last 3 decades, more than 3.800.000 hectares were consumed by wildfires (105), showing the country's vulnerability to this threat.

As previously mentioned, weather and climate play a key role in wildfire dynamics. Climate has been warming in the last decades with prolonged periods of dry weather. Consequently, extended and more frequent drought periods have increased the likelihood of wildfire among dry-climate forests (106-108). Wildfire activity and this type of phenomenon are predicted to increase in several regions of the globe due to the progressive increase in temperature and a pronounced decrease in precipitation patterns caused by climate change (109). The inevitability of global warming, with decreasing precipitation levels and prolonged dry seasons (110), has contributed to the increase in both frequency and severity of wildland fires (111, 112). As pointed out by the United Nations Intergovernmental Panel on Climate Change there is a “*greater likelihood of injury, disease, and death due to more intense heat waves and fires*” (113). Furthermore, the rising number of people working and/or living in areas close to forests increases the risk of serious damage to livelihoods or loss of human lives (100, 114).

The growing threat of wildfires highlights the urgent need for improved prevention strategies and fire management practices. Effective wildfire response relies heavily on well-trained and healthy firefighters, who serve as the backbone of fire suppression efforts (115, 116). Although firefighter is considered one of the most dangerous occupations in the world, little is known about the related health risks. In the coming years, the predicted increase in wildland fires, both in number and intensity, will demand greater physical and psychological endurance from this workforce, making it essential to address the health the issue of risks associated with wildland firefighter exposure (117).

B.2. Wildland Fires: A Major Source of Pollutant Exposure

Wildfires occurrence depends on three elements: heat or an ignition source, oxygen, and fuel (e.g., biomass). Wood, the predominant biomass in forests, is mainly composed of two polymers, cellulose, and lignin, and small amounts of low-molecular-weight compounds such as resins, waxes, sugars, and inorganic salts (118). During combustion, the polymers break apart producing a variety of solid, liquid, and volatile compounds. The incomplete combustion of forest fuel results in a very complex mixture of gaseous and particles, commonly referred to as smoke (119).

Biomass combustion occurs through a sequence of phases: ignition, flaming and smouldering (120). Each phase is characterised by distinct physical and chemical processes resulting in different emissions influenced by factors such as oxygen availability, temperature, and fuel composition (120). At the initial stage (ignition) of a wildfire, a complex mixture of products, including VOCs, are released forming a flammable mixture upon contact with air (121). During the flaming combustion (1127°C), this mixture ignites, and most of the black (or elemental) carbon particles and highly oxidised gases such as carbon dioxide, nitrogen oxide (NO_x) and sulphur dioxide (SO₂) are emitted (121). The following phase, smouldering, occurs between 527°C and 727°C. It is a slow, low-temperature, flameless form of combustion that substantially increases the conversion of fuel into toxic compounds (121). During this phase, CO, amines, nitriles, methyl halides (i.e., CH₃Cl, CH₃Br, and CH₃I), non-methane organic compounds (NMHC), methane (CH₄) and ammonia (NH₃) are released (121-124).

As smoke interacts with the atmosphere some intermediate chemicals are produced, mostly in the form of carbon, hydrogen and oxygen free radicals (77). In addition to these chemicals, smoke emissions also include PM (solid or/and liquid particles) which can be classified as either primary particles, directly released during combustion or secondary particles, formed through atmospheric interactions. These interactions often involve photochemical reactions driven by UV radiation from sunlight, transforming smoke pollutants, such as VOCs into secondary particles that contribute to PM (20, 122, 124). PM₁₀ (aerodynamic diameter of 10 µm or less) and PM_{2.5} are the most described in the literature (20), being PM_{2.5} the one more frequently associated with forest biomass burning (125). The composition and size of emitted particles depend on various factors, including fuel type and combustion phase of release (126). Moreover, PM has adsorbent properties acting as a vehicle for toxic combustion compounds such as PAHs (127). PAHs include compounds such as pyrene, benzo[a]pyrene, fluorene, and phenanthrene, among others

(63). Some of the pollutants referred above have been linked to adverse health effects (128), and some are classified carcinogenic to humans (Group 1) by IARC (34).

Exposure to wildfire smoke has been linked to adverse health effects in the general population, including increased all-cause mortality and various comorbidities, such as respiratory and cardiovascular diseases (129, 130). The risk of these health outcomes is particularly high for vulnerable groups, such as children, older adults, individuals with pre-existing health conditions, and those directly exposed to woodsmoke, including wildland firefighters (131-135).

Although the risks associated with woodsmoke exposure are documented, studies on the health effects of wildfire smoke exposure among wildland firefighters remains limited (1).

B.3. Wildland Firefighters: A Brief Contextualization

The first fire brigade, called “*Vigiles*”, was created in 24 B.C. under the rule of Emperor Augustus and consisted of approximately 600 conscripts that patrolled the streets of Rome checking for crimes and fires (136). By 60 A.D., Emperor Nero expanded this workforce to 7.000 men, assigning them additional responsibilities, including fire prevention and building inspection activities (137). In 1666 (17th century), after the Great Fire of London that destroyed nearly 80% of the city, insurance companies formed the first private fire brigades (138).

Over the centuries, firefighting systems were adjusted according to the reality and respective needs of different countries and populations. Currently, there are around 16.000.000 firefighters worldwide, 91% of whom are males and 9% females. Most are volunteers, although some countries, such as Italy, Greece, Israel, and Singapore, only have professional firefighting forces (2). Others, like the United States, have a considerable number of volunteer firefighters compared to professionals, although mostly are located in small towns and rural areas, while larger urban areas are primarily served by professional firefighters (full-time firefighters) (137). In sum, fire departments can be comprised of professional firefighters, volunteer firefighters, or a combination of both. And generally, each fire department is responsible for a designated geographic area to ensure a rapid response time (137).

Their work involves intense physical activities, high temperatures, and exposure to hazardous conditions, requiring significant endurance and resilience. Compared to urban or structural firefighters, wildland firefighters face other unique challenges. They respond to several wildfire occurrences per year often remaining at the fire scene for extended periods

- ranging from days to weeks - under hostile and unpredictable conditions, while working multiple shifts and with minimal rest time (139). The increasing frequency and intensity of wildfires worldwide demand a higher number of wildland firefighters engaged in fire suppression activities (3). Thus, it is essential to look for appropriate occupational health and safety measures to protect these individuals, given the research on long-term health effects is limited and prevention measures are seldom mentioned.

B.4. Wildland Firefighters' Occupational Exposure

Wildland firefighters are at greater risk due to the nature of their work, being considered one of the most dangerous occupations in the world (140). Common tasks include trekking, chainsaw work, and brush removal (34) while carrying a considerable number of heavy equipment and tools (e.g., rakes, axes, shovels, fire hoses) through remote and difficult terrains (15, 34). In addition, wildland firefighters face high temperatures, high levels of noise (e.g., sirens, diesel engines) (141), and high levels of stress, particularly when they confront life-threatening situations or when they need to deal with injuries or fatalities (15).

The common hazards that wildland firefighters face on the fire line can include burning over/entrapment, heat-related outcomes, injuries, mental stress, fatigue, dehydration, and smoke inhalation (3, 142). Firefighters are highly exposed to harmful pollutants in the form of gases and particles (15). As stated herein, the wildfire smoke is a complex mixture containing hundreds of hazardous gases and particles, such as CO, nitrogen dioxide, PAH, aldehydes, and metals (118). In addition, wildland firefighters may be also exposed to other pollutants such as diesel exhaust since they may be working near vehicles on the ground (143).

Wildland firefighters can be exposed to woodsmoke pollutants through different exposure routes (**Figure 2**), namely inhalation, ingestion, and dermal absorption (144).

Inhalation is the main exposure pathway for several harmful compounds (e.g., CO, NO₂, SO₂, formaldehyde, acrolein, ketones and benzo(a)pyrene) that are present in smoke (146). The fraction of pollutants inhaled is proportional to the volume of air inspired and expired, increasing with the physical effort (147). The typical respiratory protection used by wildland firefighters is a cotton bandana which offers very little protection due to its poor filtration efficiency, which does not prevent the inhalation of hazardous compounds, such as PAHs and PM (118, 146, 148). Furthermore, the use of a self-contained breathing apparatus (SCBA) - equipment supplied with fresh air - usually used in other contexts (e.g.,

urban firefighting), is not feasible with wildland firefighting activities due to its weight and limited capacity for use (15-30 minutes) (34, 148, 149).

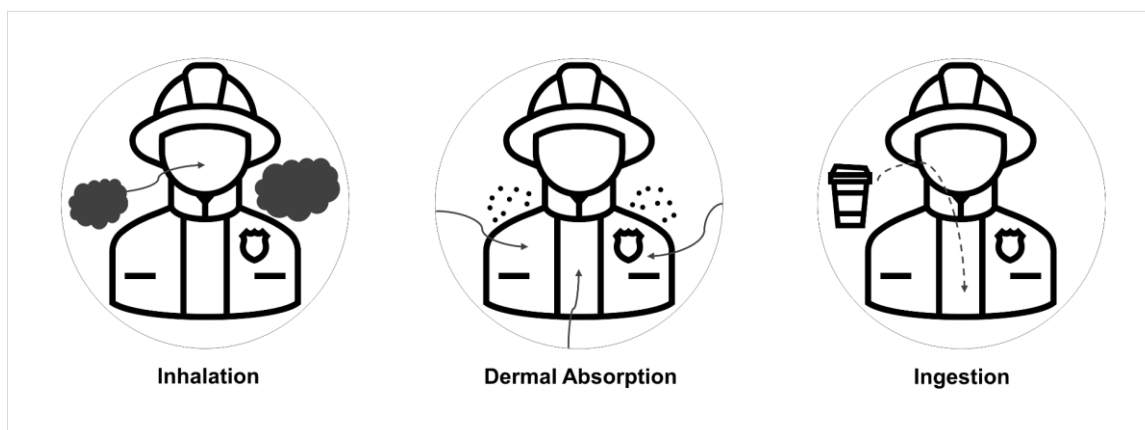


Figure 2 - Firefighters' Routes of Exposure. Retrieved from Esteves et al. (145).

Wildland firefighters might also be exposed to smoke toxicants (e.g., PAHs) via ingestion due to the lack of appropriate PPE (unprotected mouth) or even due to inadequate practices such as eating, drinking, and/or smoking with contaminated hands/gloves during or after firefighting activities (146). Moreover, contaminants that enter the upper respiratory tract may be carried via saliva into the digestive system (147).

Another route of exposure to pollutants is through the skin, commonly referred to as dermal absorption (146). Previous research has demonstrated that some pollutants, such as PAHs and VOCs, can either accumulate on the skin's surface (150) or even penetrate the firefighting protective ensemble (151), allowing dermal absorption of those contaminants. In addition, exposure to high temperatures compromises the skin barrier facilitating the dermal absorption of toxic compounds (146, 152). In fact, absorption increases by 400% for each 5°C rise in skin temperature (147).

Furthermore, the risk of exposure may be enhanced by the inadequate use of PPE or by inefficient decontamination procedures after fire suppression activities (139, 151). PPE typically comprises protective clothing, respiratory protection, a helmet, a neck shroud, a protective hood, gloves, goggles, and boots (153). However, some individuals may be reluctant to wear PPE as it can induce heat, add physiological stress and hinder communication with other crew members (34). Moreover, the use of more heat-resistant clothing may encourage firefighters to get closer to fire, exposing them to higher levels of combustion products (154).

The risk of exposure to pollutants occurs not only during firefighting and other fire operations (e.g., prescribed burning) but also in their return to the fire station via diesel exhaust and contaminated post-fire gear and/or other equipment. The inefficient decontamination procedures of PPE or other equipment used during firefighting also favour cross-contamination in fire stations (e.g., rooms, bedrooms, offices, and garages) compromising the indoor air quality where firefighters spend long periods of their shift (155, 156). A recent study published by Rogula-Kozłowska et al. (150) verified high levels of VOCs (benzene, toluene, ethylbenzene, xylene, and styrene) both in the changing room and in the garage of the fire station, suggesting that the major sources of indoor air pollution in these settings were contaminated by the PPE or other equipment used during firefighting. This may result from an insufficient awareness of safety procedures, established behavioural patterns, or constraints imposed by logistical conditions (157). It is important to note that, in some cases, fire stations are old buildings with inadequate ventilation systems promoting the dispersion of pollutants within indoor environments (139). Moreover, exposure to hazardous pollutants can also occur in other occupational contexts, such as during fire training activities or from exhaust emissions released by fire service vehicles (e.g., at fire stations during maintenance tasks), further contributing to the overall exposure of firefighters (158).

It is important to emphasise that wildland firefighters often begin their careers at a young age and dedicate many years to the profession, which means they are subject to prolonged and repeated exposure to hazardous pollutants. This exposure can result in cumulative damage over time and potentially lead to long-term health effects (157).

B.5. Firefighters – Portuguese Reality

Portugal has around 27.000 firefighters, with 81% being male and 19% female, distributed across 465 fire stations. Of these 63% are volunteers and 37% are professionals, aged between 26 and 50 years old and holding secondary school degrees (159). In a recent Portuguese study, 80% of a group of wildland firefighters reported being exposed to smoke, gases, and particles during their activities (160). These findings are particularly concerning taking into account their long careers (averaging 16 years) and extended work hours, with the majority claiming to work more than 10 hours/day (160).

As mentioned herein, Portugal is among the European countries most affected by wildfires (115). On average 3% of the Portuguese forests burn annually (161), corresponding to around 100.000 hectares. However, this trend has been increasing through the years (161);

from 2008 to 2017, for example, the annual burned area ranged from 18.000 hectares to 500.000 hectares (161).

The demographic shift of the population moving from rural to urban areas, the highly fragmented and unmanaged private forested lands, the typically evergreen and drought-resistant vegetation, the high number of human-cause ignitions (negligence or intentional) and the climate changes (high temperatures and decreasing precipitation levels), all contribute for the increased risk of forest fires in Portugal (115, 162, 163).

The European Union Joint Research Centre (EU-JRC) forecasts for Southern European countries including Portugal an increase in forest fire danger (**Figure 3**) (164). The country has already experienced severe heat waves, storms, and droughts (161). During the extreme wildfires of 2017, Portugal registered a record total burned area of 500.000 hectares, 120 fatalities including firefighters (165), and numerous injured. The recent events and the numbers revealed the need for enhanced fire prevention and management strategies in Portugal.

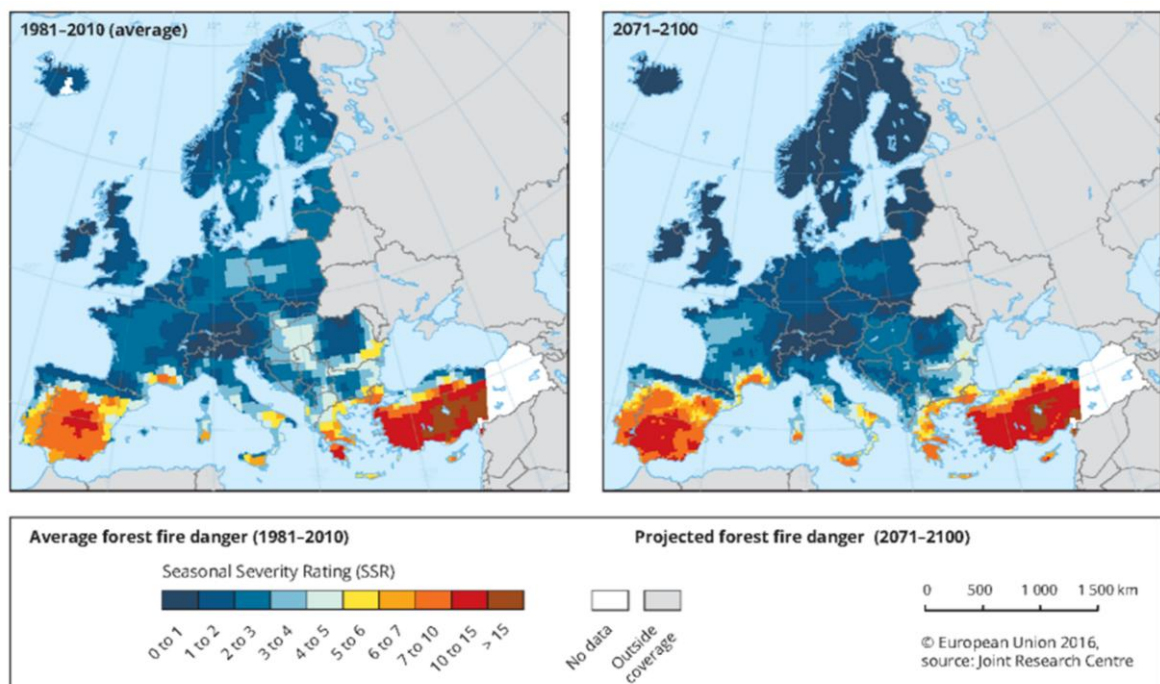


Figure 3 - Historical and future trends of Forest fire danger in Europe (164).

Despite the growing threat of wildfires, the number of Portuguese firefighters, both professional and volunteers, has dropped 33% over the past decade (**Figure 4**) (115, 166).

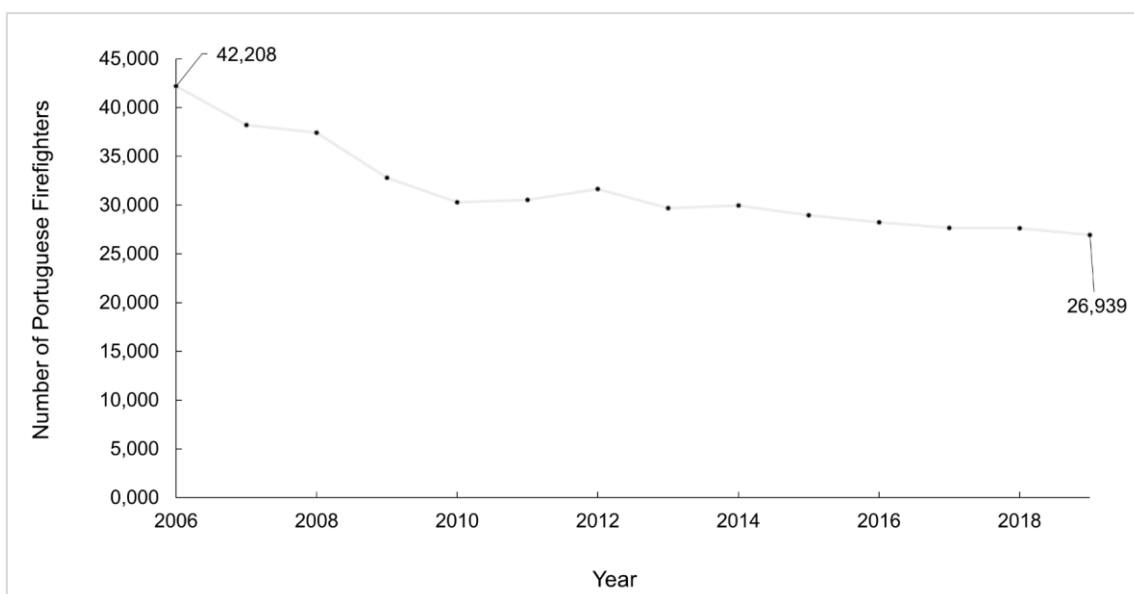


Figure 4 - Number of Portuguese Firefighters over the years. Retrieved from Esteves et al. (145).

This decline highlights the urgency of retaining experienced personnel and attracting younger recruits, creating attractive remuneration conditions (e.g., higher wages) and improving occupational health and safety conditions (115). Surveillance programmes based on biological and environmental monitoring could provide the required insights into potential occupational risks and their impact on firefighters' health and safety.

C. REFERENCES

1. International Agency for Research on Cancer (IARC). Occupational Exposure as a Firefighter. 2023. 1-730 p.
2. CTIF. World Fire Statistics. International Association of Fire and Rescue Services; 2022 2022.
3. Koopmans E, Cornish K, Fyfe TM, Bailey K, Pelletier CA. Health risks and mitigation strategies from occupational exposure to wildland fire: a scoping review. *Journal of Occupational Medicine and Toxicology*. 2022;17(1):1-17.
4. Kales SN, Soteriades ES, Christoudias SG, Christiani DC. Firefighters and on-duty deaths from coronary heart disease: a case control study. *Environmental Health*. 2003;2(1):1-13.

5. Fahy R, Petrillo JT. Firefighter Fatalities in the US in 2021. Quincy, MA: National Fire Protection Association.; 2022.
6. Guidotti T. Health risks and occupation as a firefighter. Adelaide SA: Medical Advisory Services. 2024. Available from: Google Scholar.
7. Kales SN, Soteriades ES, Christophi CA, Christiani DC. Emergency duties and deaths from heart disease among firefighters in the United States. *New England Journal of Medicine*. 2007;356(12):1207-15.
8. Fahs CA, Yan H, Ranadive S, Rossow LM, Agiovlasitis S, Echols G, et al. Acute effects of firefighting on arterial stiffness and blood flow. *Vascular medicine*. 2011;16(2):113-8.
9. Butler C, Marsh S, Domitrovich JW, Helmkamp J. Wildland firefighter deaths in the United States: A comparison of existing surveillance systems. *Journal of occupational and environmental hygiene*. 2017;14(4):258-70.
10. Greven F. Respiratory effects of fire smoke exposure in firefighters and the general population: Utrecht University; 2011.
11. Pedersen JE, Petersen KU, Ebbenhøj NE, Bonde JP, Hansen J. Incidence of cardiovascular disease in a historical cohort of Danish firefighters. *Occupational and environmental medicine*. 2018;75(5):337-43.
12. Soteriades ES, Smith DL, Tsismenakis AJ, Baur DM, Kales SN. Cardiovascular disease in US firefighters: a systematic review. *Cardiology in review*. 2011;19(4):202-15.
13. Navarro KM, Kleinman MT, Mackay CE, Reinhardt TE, Balmes JR, Broyles GA, et al. Wildland firefighter smoke exposure and risk of lung cancer and cardiovascular disease mortality. *Environmental research*. 2019;173:462-8.
14. Andersen MHG, Saber AT, Pedersen PB, Loft S, Hansen ÅM, Koponen IK, et al. Cardiovascular health effects following exposure of human volunteers during fire extinction exercises. *Environmental Health*. 2017;16(1):1-9.
15. Smith DL, Manning T, Petruzzello SJ. Effect of strenuous live-fire drills on cardiovascular and psychological responses of recruit firefighters. *Ergonomics*. 2001;44(3):244-54.
16. Barr D, Gregson W, Reilly T. The thermal ergonomics of firefighting reviewed. *Applied ergonomics*. 2010;41(1):161-72.

17. Drew-Nord DC, Hong O, Froelicher ES, Berryman P, Lukes E. Cardiovascular risk factors among career firefighters. *AAOHN Journal*. 2009;57(10):415-24.
18. Semmens EO, Domitrovich J, Conway K, Noonan CW. A cross-sectional survey of occupational history as a wildland firefighter and health. *American journal of industrial medicine*. 2016;59(4):330-5.
19. Du Y, Xu X, Chu M, Guo Y, Wang J. Air particulate matter and cardiovascular disease: the epidemiological, biomedical and clinical evidence. *Journal of thoracic disease*. 2016;8(1):E8.
20. Costa S, Ferreira J, Silveira C, Costa C, Lopes D, Relvas H, et al. Integrating health on air quality assessment—review report on health risks of two major European outdoor air pollutants: PM and NO₂. *Journal of Toxicology and Environmental Health, Part B*. 2014;17(6):307-40.
21. Scott AF, Reilly CA. Wood and biomass smoke: addressing human health risks and exposures. ACS Publications; 2019.
22. Breyse PN, Delfino RJ, Dominici F, Elder AC, Frampton MW, Froines JR, et al. US EPA particulate matter research centers: summary of research results for 2005–2011. *Air Quality, Atmosphere & Health*. 2013;6(2):333-55.
23. Walker A, McKune A, Ferguson S, Pyne DB, Rattray B. Chronic occupational exposures can influence the rate of PTSD and depressive disorders in first responders and military personnel. *Extreme physiology & medicine*. 2016;5(1):1-12.
24. Jahnke SA, Poston WS, Jitnarin N, Haddock CK. Maternal and child health among female firefighters in the US. *Maternal and child health journal*. 2018;22(6):922-31.
25. Carey MG, Al-Zaiti SS, Dean GE, Sessanna L, Finnell DS. Sleep problems, depression, substance use, social bonding, and quality of life in professional firefighters. *Journal of occupational and environmental medicine/American College of Occupational and Environmental Medicine*. 2011;53(8):928.
26. Kimbrel NA, Steffen LE, Meyer EC, Kruse MI, Knight JA, Zimering RT, et al. A revised measure of occupational stress for firefighters: Psychometric properties and relationship to posttraumatic stress disorder, depression, and substance abuse. *Psychological services*. 2011;8(4):294.
27. Chen Y-S, Chen M-C, Chou FH-C, Sun F-C, Chen P-C, Tsai K-Y, et al. The relationship between quality of life and posttraumatic stress disorder or major depression for firefighters in Kaohsiung, Taiwan. *Quality of life research*. 2007;16(8):1289-97.

28. Vargas de Barros V, Martins LF, Saitz R, Bastos RR, Ronzani TM. Mental health conditions, individual and job characteristics and sleep disturbances among firefighters. *Journal of health psychology*. 2013;18(3):350-8.
29. Boffa JW, Stanley IH, Hom MA, Norr AM, Joiner TE, Schmidt NB. PTSD symptoms and suicidal thoughts and behaviors among firefighters. *Journal of psychiatric research*. 2017;84:277-83.
30. Weiss C. One in four dies of cancer. Questions about the epidemiology of malignant tumours. *Ethical Challenges in Cancer Diagnosis and Therapy*. 2021:15-29.
31. Zacharias F, George D, Michail D, Ioannis P, Marianna T, Arzou B, et al. MicroRNAs determining carcinogenesis by regulating oncogenes and tumor suppressor genes during cell cycle. *MicroRNA*. 2020;9(2):82-92.
32. Weinstein IB. Growth factors, oncogenes, and multistage carcinogenesis. *Journal of cellular biochemistry*. 1987;33(3):213-24.
33. Anand P, Kunnumakara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, et al. Cancer is a preventable disease that requires major lifestyle changes. *Pharmaceutical research*. 2008;25(9):2097-116.
34. IARC. Painting, firefighting, and shiftwork: IARC Press, International Agency for Research on Cancer; 2010.
35. LeMasters GK, Genaidy AM, Succop P, Deddens J, Sobeih T, Barriera-Viruet H, et al. Cancer risk among firefighters: a review and meta-analysis of 32 studies. *Journal of occupational and environmental medicine*. 2006;48(11):1189-202.
36. Jalilian H, Ziaei M, Weiderpass E, Rueegg CS, Khosravi Y, Kjaerheim K. Cancer incidence and mortality among firefighters. *International journal of cancer*. 2019;145(10):2639-46.
37. Pukkala E, Jan Ivar M, Elisabete W, Kristina K, Elsebeth L, Laufey T, et al. Cancer incidence among firefighters: 45 years of follow-up in five Nordic countries. *Occupational and Environmental Medicine*. 2013;oemed-2013-101803.
38. Tsai RJ, Luckhaupt SE, Schumacher P, Cress RD, Deapen DM, Calvert GM. Risk of cancer among firefighters in California, 1988–2007. *American journal of industrial medicine*. 2015;58(7):715-29.
39. Zhao G, Erazo B, Ronda E, Brocal F, Regidor E. Mortality Among Firefighters in Spain: 10 Years of Follow-up. *Annals of work exposures and health*. 2020;64(6):614-21.

40. Demers PA, DeMarini DM, Fent KW, Glass DC, Hansen J, Adetona O, et al. Carcinogenicity of occupational exposure as a firefighter. *The Lancet Oncology*. 2022;23(8):985-6.
41. Teixeira JP, Gaspar J, Silva S, Torres J, Silva SN, Azevedo MC, et al. Occupational exposure to styrene: modulation of cytogenetic damage and levels of urinary metabolites of styrene by polymorphisms in genes CYP2E1, EPHX1, GSTM1, GSTT1 and GSTP1. *Toxicology*. 2004;195(2-3):231-42.
42. WHO. Environmental Health Criteria 155. Biomarkers and risk assessment: concepts and principles. International Programme on Chemical Safety (IPCS). *Ann Occup Hyg*. 1994;38(6).
43. Costa S, Esteves F. Biomarkers, human health. *Encyclopedia of Toxicology* (Fourth Edition). 2023.
44. Henderson R, Hobbie J, Landrigan P, Mattisoti D, Perera F, Pfttaer E, et al. Biological markers in environmental health research. *Environmental Health Perspectives*. 1987;7:3-9.
45. Costa S. Occupational exposure to formaldehyde-genotoxic damage and susceptibility evaluation in anatomical pathology laboratory workers. 2014.
46. Robinson A. Biomarkers and Risk Assessment: Concepts and Principles: World Health Organization; 1993.
47. Council NR, Earth Do, Studies L, Studies BoE, Toxicants CoHBfE. Human biomonitoring for environmental chemicals: National Academies Press; 2006.
48. Kyrtopoulos SA. Biomarkers in environmental carcinogenesis research: striving for a new momentum. *Toxicology letters*. 2006;162(1):3-15.
49. Farmer PB, Emeny J. Biomarkers of carcinogen exposure and early effects. Biomarkers of carcinogen exposure and early effects. 2006.
50. Bonassi S, El-Zein R, Bolognesi C, Fenech M. Micronuclei frequency in peripheral blood lymphocytes and cancer risk: evidence from human studies. *Mutagenesis*. 2011;26(1):93-100.
51. Bonassi S, Hagmar L, Strömberg U, Montagud AH, Tinnerberg H, Forni A, et al. Chromosomal aberrations in lymphocytes predict human cancer independently of exposure to carcinogens. *Cancer Research*. 2000;60(6):1619-25.

52. Bonassi S, Znaor A, Ceppi M, Lando C, Chang WP, Holland N, et al. An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans. *Carcinogenesis*. 2007;28(3):625-31.
53. Ceppi M, Biasotti B, Fenech M, Bonassi S. Human population studies with the exfoliated buccal micronucleus assay: statistical and epidemiological issues. *Mutation Research/Reviews in Mutation Research*. 2010;705(1):11-9.
54. Bonassi S, Coskun E, Ceppi M, Lando C, Bolognesi C, Burgaz S, et al. The HUMAN MicroNucleus project on exfoliated buccal cells (HUMNXL): The role of life-style, host factors, occupational exposures, health status, and assay protocol. *Mutation Research/Reviews in Mutation Research*. 2011;728(3):88-97.
55. Thomas P, Holland N, Bolognesi C, Kirsch-Volders M, Bonassi S, Zeiger E, et al. Buccal micronucleus cytome assay. *Nature protocols*. 2009;4(6):825.
56. Collins AR. The comet assay for DNA damage and repair. *Molecular biotechnology*. 2004;26(3):249.
57. Costa S, Carvalho S, Costa C, Coelho P, Silva S, Santos LS, et al. Increased levels of chromosomal aberrations and DNA damage in a group of workers exposed to formaldehyde. *Mutagenesis*. 2015;30(4):463-73.
58. Albertini RJ, Anderson D, Douglas GR, Hagmar L, Hemminki K, Merlo F, et al. IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. *Mutation Research/Reviews in Mutation Research*. 2000;463(2):111-72.
59. WHO. Biomarkers and Risk Assessment: Concepts and Principles-Environmental Health Criteria 1551993.
60. Bodaghi A, Fattahi N, Ramazani A. Biomarkers: Promising and valuable tools towards diagnosis, prognosis and treatment of Covid-19 and other diseases. *Heliyon*. 2023;9(2).
61. Heo HC, Byun YS, Sohn SH, Jo SM, Park SK, Sakong J. Validity assessment of self-reported smoking status in firefighters using the urine cotinine test. *Annals of occupational and environmental medicine*. 2020;32.
62. Simpson CD, Naeher LP. Biological monitoring of wood-smoke exposure. *Inhalation toxicology*. 2010;22(2):99-103.
63. Simoneit BR. Biomass burning—a review of organic tracers for smoke from incomplete combustion. *Applied Geochemistry*. 2002;17(3):129-62.

64. Gill B, Britz-McKibbin P. Biomonitoring of smoke exposure in firefighters: a review. *Current Opinion in Environmental Science & Health*. 2020.
65. Phillips DH. On the origins and development of the ³²P-postlabelling assay for carcinogen–DNA adducts. *Cancer letters*. 2013;334(1):5-9.
66. Ewa B, Danuta M-Š. Polycyclic aromatic hydrocarbons and PAH-related DNA adducts. *Journal of applied genetics*. 2017;58(3):321-30.
67. Gao P, da Silva E, Hou L, Denslow ND, Xiang P, Ma LQ. Human exposure to polycyclic aromatic hydrocarbons: metabolomics perspective. *Environment international*. 2018;119:466-77.
68. Oliveira M, Capelas S, Delerue-Matos C, Morais S. Grill workers exposure to polycyclic aromatic hydrocarbons: levels and excretion profiles of the urinary biomarkers. *International journal of environmental research and public health*. 2021;18(1):230.
69. Oliveira M, Slezakova K, Alves MJ, Fernandes A, Teixeira JP, Delerue-Matos C, et al. Firefighters' exposure biomonitoring: Impact of firefighting activities on levels of urinary monohydroxyl metabolites. *International journal of hygiene and environmental health*. 2016;219(8):857-66.
70. Dunn K, Devaux I, Stock A, Mott J, Naeher L. Evaluation of exposure to carbon monoxide during prescribed forest burns. *American Industrial Hygiene Conference and Exposition*; May, 2005; Atlanta, GA2005.
71. Neitzel R, Naeher L, Paulsen M, Dunn K, Stock A, Simpson C. Biological monitoring of smoke exposure among wildland firefighters: a pilot study comparing urinary methoxyphenols with personal exposures to carbon monoxide, particulate matter, and levoglucosan. *Journal of exposure science & environmental epidemiology*. 2009;19(4):349-58.
72. Fernando S, Shaw L, Shaw D, Gallea M, VandenEnden L, House R, et al. Evaluation of firefighter exposure to wood smoke during training exercises at burn houses. *Environmental science & technology*. 2016;50(3):1536-43.
73. Naeher LP, Barr DB, Adetona O, Simpson CD. Urinary levoglucosan as a biomarker for woodsmoke exposure in wildland firefighters. *International journal of occupational and environmental health*. 2013;19(4):304-10.
74. Zhou Y, Liu Y, Sun H, Ma J, Xiao L, Cao L, et al. Associations of urinary polycyclic aromatic hydrocarbon metabolites with fractional exhaled nitric oxide and exhaled carbon monoxide: A cross-sectional study. *Science of the Total Environment*. 2018;618:542-50.

75. Scarlata S, Pennazza G, Santonico M, Pedone C, Antonelli Incalzi R. Exhaled breath analysis by electronic nose in respiratory diseases. Expert review of molecular diagnostics. 2015;15(7):933-56.
76. Danielsen PH, Møller P, Jensen KA, Sharma AK, Wallin Hk, Bossi R, et al. Oxidative stress, DNA damage, and inflammation induced by ambient air and wood smoke particulate matter in human A549 and THP-1 cell lines. Chemical research in toxicology. 2011;24(2):168-84.
77. Leonard SS, Castranova V, Chen BT, Schwegler-Berry D, Hoover M, Piacitelli C, et al. Particle size-dependent radical generation from wildland fire smoke. Toxicology. 2007;236(1-2):103-13.
78. Liu P-L, Chen Y-L, Chen Y-H, Lin S-J, Kou YR. Wood smoke extract induces oxidative stress-mediated caspase-independent apoptosis in human lung endothelial cells: role of AIF and EndoG. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2005;289(5):L739-L49.
79. Czerska M, Mikołajewska K, Zieliński M, Gromadzińska J, Wąsowicz W. Today's oxidative stress markers. Medycyna pracy. 2015;66(3).
80. Lamprecht M, Greilberger J, Oetl K. Analytical aspects of oxidatively modified substances in sports and exercises. Nutrition. 2004;20(7-8):728-30.
81. Esteves F, Teixeira E, Amorim T, Costa C, Pereira C, Fraga S, et al. Assessment of DNA damage in a group of professional dancers during a 10-month dancing season. Journal of Toxicology and Environmental Health, Part A. 2017;80(13-15):797-804.
82. Azqueta A, Shaposhnikov S, Collins AR. DNA oxidation: investigating its key role in environmental mutagenesis with the comet assay. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2009;674(1-2):101-8.
83. Gaughan DM, Siegel PD, Hughes MD, Chang CY, Law BF, Campbell CR, et al. Arterial stiffness, oxidative stress, and smoke exposure in wildland firefighters. American journal of industrial medicine. 2014;57(7):748-56.
84. Abreu A, Costa C, Pinho e Silva S, Morais S, do Carmo Pereira M, Fernandes A, et al. Wood smoke exposure of Portuguese wildland firefighters: DNA and oxidative damage evaluation. Journal of Toxicology and Environmental Health, Part A. 2017;80(13-15):596-604.
85. Andersen MHG, Saber AT, Clausen PA, Pedersen JE, Løhr M, Kermanizadeh A, et al. Association between polycyclic aromatic hydrocarbon exposure and peripheral blood

mononuclear cell DNA damage in human volunteers during fire extinction exercises. *Mutagenesis*. 2018;33(1):105-15.

86. Abbas A. Cellular and molecular immunology. HBJ International Edition. 1991:226-42.

87. Ghio AJ, Soukup JM, Case M, Dailey LA, Richards J, Berntsen J, et al. Exposure to wood smoke particles produces inflammation in healthy volunteers. *Occupational and environmental medicine*. 2012;69(3):170-5.

88. Danielsen PH, Bräuner EV, Barregard L, Sällsten G, Wallin M, Olinski R, et al. Oxidatively damaged DNA and its repair after experimental exposure to wood smoke in healthy humans. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*. 2008;642(1-2):37-42.

89. Danielsen PH, Loft S, Kocbach A, Schwarze PE, Møller P. Oxidative damage to DNA and repair induced by Norwegian wood smoke particles in human A549 and THP-1 cell lines. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*. 2009;674(1-2):116-22.

90. Danielsen PH, Møller P, Jensen KA, Sharma AK, Wallin H, Bossi R, et al. Oxidative stress, DNA damage, and inflammation induced by ambient air and wood smoke particulate matter in human A549 and THP-1 cell lines. *Chemical research in toxicology*. 2011;24(2):168-84.

91. Jalava PI, Happonen MS, Kelz J, Brunner T, Hakulinen P, Mäki-Paakkanen J, et al. In vitro toxicological characterisation of particulate emissions from residential biomass heating systems based on old and new technologies. *Atmospheric Environment*. 2012;50:24-35.

92. Swiston JR, Davidson W, Attridge S, Li GT, Brauer M, van Eeden SF. Wood smoke exposure induces a pulmonary and systemic inflammatory response in firefighters. *European respiratory journal*. 2008;32(1):129-38.

93. Main LC, Wolkow AP, Tait JL, Della Gatta P, Raines J, Snow R, et al. Firefighter's acute inflammatory response to wildfire suppression. *Journal of occupational and environmental medicine*. 2020;62(2):145-8.

94. Adetona AM, Adetona O, Gogal JRM, Diaz-Sanchez D, Rathbun SL, Naeher LP. Impact of work task-related acute occupational smoke exposures on select proinflammatory immune parameters in wildland firefighters. *Journal of occupational and environmental medicine*. 2017;59(7):679.

95. Bond WJ, Woodward FI, Midgley GF. The global distribution of ecosystems in a world without fire. *New phytologist*. 2005;165(2):525-38.
96. Gowlett JA. The discovery of fire by humans: a long and convoluted process. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 2016;371(1696):20150164.
97. Šomšák L, Dlapa P, Kollár J, Kubíček F, Šimonovič V, Janitor A, et al. Fire impact on the secondary pine forest and soil in the Borská nížina lowland (SW Slovakia). *Ekologia (Bratislava)*. 2009;28(1):52-65.
98. Team CGT, Group TW. Canadian Wildland Fire Management Glossary. Canadian Interagency Forest Fire Centre: Winnipeg, MB, Canada. 2017:2019-03.
99. Stolton S. Future fires: perpetuating problems of the past: WWF International; 2003.
100. Hirschberger P. Forests ablaze: causes and effects of global forest fires. WWF: Berlin, Germany) Available at <https://www.wwfde/fileadmin/fm-wwf/Publikationen-PDF/WWF-Study-Forests-Ablaze.pdf> [Verified 26 March 2020]. 2016.
101. Mouillot F, Field CB. Fire history and the global carbon budget: a 1× 1 fire history reconstruction for the 20th century. *Global Change Biology*. 2005;11(3):398-420.
102. Flannigan MD, Krawchuk MA, de Groot WJ, Wotton BM, Gowman LM. Implications of changing climate for global wildland fire. *International journal of wildland fire*. 2009;18(5):483-507.
103. Avenbuan ON, Zelikoff JT. Woodsmoke and emerging issues. *Current Opinion in Toxicology*. 2020;22:12-8.
104. San-Miguel-Ayanz J, Schulte E, Schmuck G, Camia A, Strobl P, Liberta G, et al. Comprehensive monitoring of wildfires in Europe: the European forest fire information system (EFFIS). Approaches to managing disaster-Assessing hazards, emergencies and disaster impacts: IntechOpen; 2012.
105. Schmuck G S-M-AJ, Camia A, Durrant T, Boca R, Liberta` G, Petroligkis T, Di Leo M, Rodriguez Aseretto R, Boccacci F, Schulte E. Forest Fires in Europe, Middle East and North Africa 2013. 2014.
106. Littell JS, McKenzie D, Peterson DL, Westerling AL. Climate and wildfire area burned in western US ecoprovinces, 1916–2003. *Ecological Applications*. 2009;19(4):1003-21.

107. Littell JS, Oneil EE, McKenzie D, Hicke JA, Lutz JA, Norheim RA, et al. Forest ecosystems, disturbance, and climatic change in Washington State, USA. *Climatic change*. 2010;102(1):129-58.
108. Littell JS, Peterson DL, Riley KL, Liu Y, Luce CH. A review of the relationships between drought and forest fire in the United States. *Global change biology*. 2016;22(7):2353-69.
109. Flannigan MD, Amiro BD, Logan KA, Stocks BJ, Wotton BM. Forest Fires and Climate Change in the 21ST Century. *Mitigation and Adaptation Strategies for Global Change*. 2006;11(4):847-59.
110. Esteves F, Madureira J, Teixeira JP, Costa S. The Role of Biomarkers in the Assessment of Potential Health Risks of Wildland Firefighters' Occupational Exposure: An Overview. In: Marta Madalena Marques de Oliveira SBM, Francisca Pinto Lisboa Martins Rodrigues, editor. "Essential Guide to Occupational Exposure". *Safety and Risk in Society*. New York, United States: Nova Science Publishers, Inc.; 2022.
111. Westerling AL, Hidalgo HG, Cayan DR, Swetnam TW. Warming and earlier spring increase western US forest wildfire activity. *science*. 2006;313(5789):940-3.
112. San-Miguel-Ayanz J, Durrant T, Boca R, Libertà G, Branco A, De Rigo D, et al. Forest Fires in Europe, Middle East and North Africa 2017. Publications Officer of the European Union <https://doi.org/10.2760/663443>. 2018.
113. Field CB, Barros VR. *Climate change 2014—Impacts, adaptation and vulnerability: Regional aspects*: Cambridge University Press; 2014.
114. Radeloff VC, Hammer RB, Stewart SI, Fried JS, Holcomb SS, McKeefry JF. The wildland–urban interface in the United States. *Ecological applications*. 2005;15(3):799-805.
115. Beighley M, Hyde A. Portugal wildfire management in a new era assessing fire risks, resources and reforms. Centro de Estudos Florestais–Instituto Superior de Agronomia/Universidade de Lisboa: Lisboa, Portugal. 2018.
116. Moreira F, Vaz P, Catry F, Silva JS. Regional variations in wildfire susceptibility of land-cover types in Portugal: implications for landscape management to minimize fire hazard. *International Journal of Wildland Fire*. 2009;18(5):563-74.
117. Withen P. Climate change and wildland firefighter health and safety. *New solutions: a journal of environmental and occupational health policy*. 2015;24(4):577-84.

118. Naeher LP, Brauer M, Lipsett M, Zelikoff JT, Simpson CD, Koenig JQ, et al. Woodsmoke health effects: a review. *Inhalation toxicology*. 2007;19(1):67-106.
119. Ward D. Combustion chemistry and smoke. *Forest fires*: Elsevier; 2001. p. 55-77.
120. Koppmann R, Von Czapiewski K, Reid J. A review of biomass burning emissions, part I: gaseous emissions of carbon monoxide, methane, volatile organic compounds, and nitrogen containing compounds. *Atmospheric chemistry and physics discussions*. 2005;5(5):10455-516.
121. Andreae MO, Merlet P. Emission of trace gases and aerosols from biomass burning. *Global biogeochemical cycles*. 2001;15(4):955-66.
122. Akagi S, Yokelson RJ, Wiedinmyer C, Alvarado M, Reid J, Karl T, et al. Emission factors for open and domestic biomass burning for use in atmospheric models. *Atmospheric Chemistry and Physics*. 2011;11(9):4039-72.
123. Yokelson RJ, Griffith DW, Ward DE. Open-path Fourier transform infrared studies of large-scale laboratory biomass fires. *Journal of Geophysical Research: Atmospheres*. 1996;101(D15):21067-80.
124. Durán S, Reisen F, Rideout K. Evidence review: wildfire smoke and public health risk. BC Centre for Disease Control: Vancouver, BC, Canada. 2014.
125. Simões Amaral S, Andrade de Carvalho J, Martins Costa MA, Pinheiro C. Particulate matter emission factors for biomass combustion. *Atmosphere*. 2016;7(11):141.
126. Eck T, Holben B, Reid J, O'Neill N, Schafer J, Dubovik O, et al. High aerosol optical depth biomass burning events: A comparison of optical properties for different source regions. *Geophysical Research Letters*. 2003;30(20).
127. Benefield VL, Perna S, Pham S, Chong NS, Li Z, Zhang M. Correction: Evaluation of Mass Spectrometric Methods for Screening Polycyclic Aromatic Hydrocarbons in the Particulate Phase of Wildfire/Biomass Smoke. *Fire Technology*. 2024;60(3):2255-6.
128. Manisalidis I, Stavropoulou E, Stavropoulos A, Bezirtzoglou E. Environmental and health impacts of air pollution: a review. *Frontiers in public health*. 2020;8.
129. Cascio WE. Wildland fire smoke and human health. *Science of the total environment*. 2018;624:586-95.
130. Reid CE, Brauer M, Johnston FH, Jerrett M, Balmes JR, Elliott CT. Critical review of health impacts of wildfire smoke exposure. *Environmental health perspectives*. 2016;124(9):1334-43.

131. Anderson H, Atkinson R, Bremner S, Marston L. Particulate air pollution and hospital admissions for cardiorespiratory diseases: are the elderly at greater risk? *European Respiratory Journal*. 2003;21(40 suppl):39s-46s.
132. Anderson H, Atkinson R, Bremner S, Carrington J, Peacock J. Quantitative systematic review of short term associations between ambient air pollution (particulate matter, ozone, nitrogen dioxide, sulphur dioxide and carbon monoxide), and mortality and morbidity. Report to Department of Health revised following first review. 2007.
133. Von Klot S, Peters A, Aalto P, Bellander T, Berglind N, D'Ippoliti D, et al. Health Effects of Particles on Susceptible Subpopulations (HEAPSS) Study Group. Ambient air pollution is associated with increased risk of hospital cardiac readmissions of myocardial infarction survivors in five European cities. *Circulation*. 2005;112(20):3073-9.
134. Liu JC, Pereira G, Uhl SA, Bravo MA, Bell ML. A systematic review of the physical health impacts from non-occupational exposure to wildfire smoke. *Environmental research*. 2015;136:120-32.
135. Youssouf H, Liousse C, Roblou L, Assamoi E-M, Salonen RO, Maesano C, et al. Non-accidental health impacts of wildfire smoke. *International journal of environmental research and public health*. 2014;11(11):11772-804.
136. Butgereit L, Moonsamy S, Thomson T, Van Zyl T, McFerren G. Fire hazard notifications via Satellite, Twitter, Citizen Reports, and Android Apps. 2014.
137. Chiefs IAoF. Fundamentals of fire fighter skills: Jones & Bartlett Learning; 2008.
138. Peresun'ko T, Spirkina O. HISTORY OF FIREFIGHTING: KEY POINTS.
139. Demers PA, DeMarini DM, Fent KW, Glass DC, Hansen J, Adetona O, et al. Carcinogenicity of occupational exposure as a firefighter. *The Lancet Oncology*. 2022.
140. Adetona O, Simpson CD, Onstad G, Naeher LP. Exposure of wildland firefighters to carbon monoxide, fine particles, and levoglucosan. *Annals of occupational hygiene*. 2013;57(8):979-91.
141. Broyles G, Butler CR, Kardous CA. Noise exposure among federal wildland fire fighters. *The Journal of the Acoustical Society of America*. 2017;141(2):EL177-EL83.
142. Britton C, Lynch CF, Ramirez M, Torner J, Buresh C, Peek-Asa C. Epidemiology of injuries to wildland firefighters. *The American journal of emergency medicine*. 2013;31(2):339-45.

143. Horn GP, Fent KW, Kerber S, Smith DL. Hierarchy of contamination control in the fire service: Review of exposure control options to reduce cancer risk. *Journal of Occupational and Environmental Hygiene*. 2022;19(9):538-57.
144. Ruby BC, Shriver TC, Zderic TW, Sharkey BJ, Burks C, Tysk S. Total energy expenditure during arduous wildfire suppression. *Medicine and Science in Sports and Exercise*. 2002;34(6):1048-54.
145. Esteves F, Madureira J, Teixeira JP, Costa S. Wildland Firefighters: A Crucial Weapon for Forest Fire Management. Which Health Risks Do They Face? *Fire Hazards: Socio-economic and Regional Issues*: Springer International Publishing Cham; 2024. p. 107-21.
146. Heus R. Exposure to smoke. An overview report of the studies to exposure. 2018.
147. Stec A, Wolffe T, Clinton A. Minimising firefighters' exposure to toxic fire effluents. Interim Best Practice Report University of Central Lancashire. 2020.
148. Austin C. Wildland Firefighter Health Risks and Respiratory Protection (Report R-572). Montreal, Quebec, Canada: IRSST (Institut de recherche Robert-Sauvé en santé et en sécurité du travail). 2008.
149. George B. Wildland firefighters smoke exposure smoke. Logan, Utah: Utah State University; 2013.
150. Rogula-Kozłowska W, Bralewska K, Jureczko I. BTEXS Concentrations and Exposure Assessment in a Fire Station. *Atmosphere*. 2020;11(5):470.
151. Barker RL. A Review of gaps and limitations in test methods for first responder protective clothing and equipment: a final report presented to National Personal Protection Technology Laboratory, National Institute for Occupational Safety and Health (NIOSH). 2005.
152. Wingfors H, Nyholm JR, Magnusson R, Wijkmark CH. Impact of fire suit ensembles on firefighter PAH exposures as assessed by skin deposition and urinary biomarkers. *Annals of work exposures and health*. 2018;62(2):221-31.
153. Carballo-Leyenda B, Villa JG, López-Satué J, Collado PS, Rodríguez-Marroyo JA. Fractional contribution of wildland firefighters' personal protective equipment on physiological strain. *Frontiers in physiology*. 2018;9:1139.

154. Lau C, Anitole K, Hodes C, Lai D, Pfahles-Hutchens A, Seed J. Perfluoroalkyl acids: a review of monitoring and toxicological findings. *Toxicological sciences*. 2007;99(2):366-94.
155. Oliveira M, Slezakova K, Alves MJ, Fernandes A, Teixeira JP, Delerue-Matos C, et al. Polycyclic aromatic hydrocarbons at fire stations: firefighters' exposure monitoring and biomonitoring, and assessment of the contribution to total internal dose. *Journal of hazardous materials*. 2017;323:184-94.
156. Banks AP, Thai P, Engelsman M, Wang X, Osorio AF, Mueller JF. Characterising the exposure of Australian firefighters to polycyclic aromatic hydrocarbons generated in simulated compartment fires. *International Journal of Hygiene and Environmental Health*. 2021;231:113637.
157. Magnusson S, Hultman D. Healthy Firefighters-The Skellefteå Model improves the work environment. Swedish Civil Contingencies Agency. 2015.
158. Fent KW, Toennis C, Sammons D, Robertson S, Bertke S, Calafat AM, et al. Firefighters' and instructors' absorption of PAHs and benzene during training exercises. *International journal of hygiene and environmental health*. 2019;222(7):991-1000.
159. INE. Estatísticas do Ambiente: 2020. Edição 2021 ed. Lisboa2020.
160. Esteves F, Madureira J, Teixeira JP, S C. The firefighters on the frontline of forest fires – preliminary characterisation of Bio4FOX study population. In: Viegas X RL, editor. *Advances in Forest Fire Research Universidade de Coimbra*; 2022. p. 1330-4.
161. Schleussner C-F, Menke I, Theokritoff E, van Maanen N, Lanson A. Climate impacts in portugal. *Climate Analytics*; 2019.
162. Carmo M, Moreira F, Casimiro P, Vaz P. Land use and topography influences on wildfire occurrence in northern Portugal. *Landscape and Urban Planning*. 2011;100(1-2):169-76.
163. Nunes M, Vasconcelos MJ, Pereira J, Dasgupta N, Alldredge RJ, Rego FC. Land cover type and fire in Portugal: do fires burn land cover selectively? *Landscape Ecology*. 2005;20(6):661-73.
164. European Comission. Speech by President von der Leyen in the Plenary of the European Parliament at the debate on the conclusions of the European Council meeting of 12 and 13 December 2019. Available from: https://ec.europa.eu/commission/presscorner/detail/en/speech_19_6802.

165. Turco M, Jerez S, Augusto S, Tarín-Carrasco P, Ratola N, Jiménez-Guerrero P, et al. Climate drivers of the 2017 devastating fires in Portugal. *Scientific reports*. 2019;9(1):1-8.
166. Bombeiros: Quantos bombeiros há profissionais ou voluntários? [Internet]. 2020 [cited 15/06/2021]. Available from: <https://www.pordata.pt/Portugal/Bombeiros-1188>.

CHAPTER II.

Main Goals

A. MAIN GOALS

The current PHD study, “Bio4FOX – *Biomonitoring Firefighters’ Occupational Exposure*”, aims to address the existing knowledge gaps by investigating the potential biological mechanisms associated with wildland firefighting occupational exposure and identifying a comprehensive set of biomarkers suitable for the surveillance of Portuguese wildland firefighters.

To achieve this goal, a longitudinal study, with a pre-post study design, was carried out comprising a group of Portuguese wildland firefighters studied at two distinct time-points of their occupational exposure/activity: **Phase I** - before the wildfire season (baseline levels); and **Phase II** - after exposure to a real wildfire event, during the wildfire season.

Considering our main research aims four specific goals were established, including:

1. **To characterise wildland firefighters’ occupational exposure at fire stations, before the wildfire season.** This characterisation envisaged the estimation of inhaled doses of particulate matter (PM10 and PM2.5, indoor and outdoor) and the associated biological impact, specifically on effect biomarkers. In addition, we examined the association between effect biomarkers and sociodemographic, occupational, and lifestyle factors. (*Paper I*)
2. **To investigate the systemic inflammatory response associated with wildland firefighters’ occupational exposure.** To address this objective, we analysed the response of systemic inflammatory biomarkers, namely serum IL-6, IL-8 and TNF- α and plasma high-sensitivity C-reactive protein (hs-CRP), following wildland firefighting occupational exposure. Additionally, we examined the potential contribution of urinary OHPAHs, a validated smoke exposure biomarker, and other relevant factors (e.g., exposure duration) to the observed inflammatory responses. (*Paper II*)
3. **To assess the effects of occupational exposure to wildland firefighting on systemic DNA damage.** This study comprehended the evaluation of primary and oxidative DNA damage and phosphorylated histone H2Ax (γ H2AX) levels following firefighting occupational exposure to a real wildfire event. Furthermore, we assessed the association between some occupational exposure biomarkers and other occupational-related variables (e.g., exposure duration) on these effect biomarkers. (*Paper III*)

- 4. To evaluate the impact of occupational exposure to wildland firefighting on cytogenetic alterations of locally targeted tissues.** This study specifically investigates the impact of firefighting exposure on wildland firefighters' epithelial tissues, namely buccal cells and urothelial cells, using the micronucleus assay, an early-effect biomarker. Additionally, this research explores the relationship between urinary levels of total OHPAHs and the observed molecular and cellular endpoints. (*Paper IV*)

Bio4FOX study is part of a larger project entitled BioFirEx “*A panel of (bio)markers for the surveillance of firefighter's health and safety*” (FCT Ref PCIF/SSO/0017/2018). Our collective data seeks to provide insights that could inform protective policies, support the development of recommendations and preventive measures to safeguard firefighters' health and serve as the basis for future studies.

CHAPTER III.

Study Population

A. STUDY POPULATION

Bio4FOX comprised a group of wildland firefighters recruited from 14 fire stations located in the northeastern region of Portugal. The region is characterised by mountainous terrain, diverse vegetation, including forests, shrubs, agricultural fields, and a Mediterranean climate with hot and dry summers (1). These conditions have historically made this region particularly vulnerable to wildfires (1). The presence of firefighter units dedicated to wildland firefighting has allowed the study to focus on wildland firefighters.

The criteria for inclusion in the study included, being a firefighter, healthy, and actively participating in wildland firefighting activities. Exclusion criteria included, being a firefighter for less than one year and/or receiving palliative care and/or with oncologic disease history.

The initial group consisted of a convenience sample of approximately 275 firefighters. Of these, around 225 wildland firefighters were recruited (**Appendix III** – Flow Chart of Study Population), resulting in an adherence rate of more than 80%. During Phase II - wildfire season, 63 wildland firefighters were actively involved in wildfire suppression activities. Paired assessments (n=59) i.e., individuals followed in Phase I and Phase II, were critical to understanding changes between pre- and during wildfire season, after participation in a real wildfire event, allowing robust longitudinal analysis of exposure and biological impacts over time.

All subjects were fully informed about the objectives of the study (**Appendix IV** – Information to the participant). An informative leaflet outlining the aims of the study was distributed to all firefighters (**Appendix V** – Informative leaflet). Firefighters were informed about the confidentiality and protection of data, as well as the possibility of leaving the study at any moment. Participation in the study was voluntary, and no financial compensation was offered. Volunteers who agreed to participate in the study signed an informed consent form prior to enrolment in the study (**Appendix VI** – Informed consent). A Comprehensive Questionnaire (**Appendix VII** – Questionnaire A) and a Short- Questionnaire (**Appendix VIII** – Questionnaire B) were designed to be self-administered in Phase I (pre-wildfire season) and Phase II (wildfire season), respectively. The comprehensive questionnaire aimed to collect information on socio-demographic factors, characterisation of the activity as a firefighter, health status, lifestyle (e.g., diet, physical activity) and relevant exposures (occupational and non-occupational). Subjects involved in wildland firefighting activities (Phase II) filled out a short questionnaire to characterise the wildfire occurrence, their exposure duration (hours), if existing, symptoms related to the acute smoke exposure, such as nose and throat irritation, difficulty in breathing, and nausea, among others.

The research was conducted in compliance with ethical guidelines as outlined in the Declaration of Helsinki and the Oviedo Convention. Ethical approval was granted by the Ethics Committee of the University of Porto, Portugal (Reference number 92/CEUP/202).

B. REFERENCES

1. Ermitão T, Páscoa P, Trigo I, Alonso C, Gouveia C. Mapping the most susceptible regions to fire in Portugal. *Fire*. 2023;6(7):254.

CHAPTER IV.

Original Research

This chapter comprises the main outcomes of the PhD study divided into two main sections:

A. Characterisation of Firefighters' Occupational Exposure at Fire Stations, Before Wildfire Season

A.1. In this section, an original research paper, which has been published, presents preliminary data on the characterisation of firefighters' occupational exposure at fire stations before the wildfire season (baseline) (Paper I)



B. Investigating the Exposure-Induced Biological Mechanisms Associated with Wildland Firefighting Occupational Exposure

This section explores the biological impact associated with wildland firefighters' occupational exposure to a real wildfire event. Different biological mechanisms were assessed, including inflammatory response, genotoxicity/oxidative stress, and genetic/chromosomal instability.

The following studies were performed:

B.1. "Impact of Occupational Exposure to Wildfire Events on Systemic Inflammatory Biomarkers in Portuguese Wildland Firefighters" (Paper II)

B.2. "Occupational Exposure to Wildland Firefighting and Its Effects on Systemic DNA Damage" (Paper III)

B.3. "Micronucleus Assay in Buccal and Urothelial Cells in Wildfire-Exposed Firefighters" (Paper IV)

A.

***FIREFIGHTERS' OCCUPATIONAL EXPOSURE IN PREPARATION FOR WILDFIRE
SEASON: ADDRESSING BIOLOGICAL IMPACT***

A. 1. Firefighters' Occupational Exposure in Preparation for Wildfire Season: Addressing Biological Impact (Paper I)

*Esteves F., Slezakova K., Madureira J., Vaz J., Fernandes A., Morais S., Pereira M.C.,
Teixeira J.P. & Costa S.*

Reprinted from Toxics 12(3), 201

Copyright © 2024 by the authors. Licensee MDPI, Basel, Switzerland.

*Open access article distributed under the terms and conditions of the Creative Commons
Attribution (CC BY) license.*

*Esteves, F., Slezakova, K., Madureira, J., Vaz, J., Fernandes, A., Morais, S., Pereira,
M.C., Teixeira, J.P. & Costa, S. (2024). Firefighters' Occupational Exposure in Preparation
for Wildfire Season: Addressing Biological Impact. Toxics, 12(3), 201.*

<https://doi.org/10.3390/toxics12030201>

*The PhD candidate was responsible for the conceptualization and writing of the
manuscript.*

Article

Firefighters' Occupational Exposure in Preparation for Wildfire Season: Addressing Biological Impact

Filipa Esteves ^{1,2,3,4} , Klara Slezakova ⁵, Joana Madureira ^{1,2,3}, Josiana Vaz ^{6,7} , Adília Fernandes ⁸ , Simone Morais ⁹ , Maria do Carmo Pereira ⁵ , João Paulo Teixeira ^{1,2,3,*}  and Solange Costa ^{1,2,3}

- ¹ Environmental Health Department, National Institute of Health, Rua Alexandre Herculano, n° 321, 4000-055 Porto, Portugal; filipa.esteves@insa.min-saude.pt (F.E.); joana.madureira@insa.min-saude.pt (J.M.); solange.costa@insa.min-saude.pt (S.C.)
- ² EPIUnit-Instituto de Saúde Pública, Universidade do Porto, Rua das Taipas, n° 135, 4050-600 Porto, Portugal
- ³ Laboratório para a Investigação Integrativa e Translacional em Saúde Populacional (ITR), Universidade do Porto, Rua das Taipas, n° 135, 4050-600 Porto, Portugal
- ⁴ Department of Public Health and Forensic Sciences, Medical School, Faculty of Medicine, University of Porto, Rua Doutor Plácido da Costa, 4200-450 Porto, Portugal
- ⁵ LEPABE-ALiCE, Faculdade de Engenharia da Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal; slezakok@fe.up.pt (K.S.); mcsp@fe.up.pt (M.d.C.P.)
- ⁶ CIMO, Instituto Politécnico de Bragança, Campus Santa Apolónia, 5300-253 Bragança, Portugal; josiana@ipb.pt
- ⁷ SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
- ⁸ UICISA: E, Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; adilia@ipb.pt
- ⁹ REQUIMTE/LAQV-Instituto Superior de Engenharia, Instituto Politécnico do Porto, Dr. António Bernardino de Almeida 431, 4249-015 Porto, Portugal; sbm@isep.ipp.pt
- * Correspondence: joao.teixeira@insa.min-saude.pt



Citation: Esteves, F.; Slezakova, K.; Madureira, J.; Vaz, J.; Fernandes, A.; Morais, S.; do Carmo Pereira, M.; Teixeira, J.P.; Costa, S. Firefighters' Occupational Exposure in Preparation for Wildfire Season: Addressing Biological Impact. *Toxics* **2024**, *12*, 201. <https://doi.org/10.3390/toxics12030201>

Academic Editor: Vamsi Kodali

Received: 5 February 2024

Revised: 29 February 2024

Accepted: 2 March 2024

Published: 5 March 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: The characterization of wildland firefighters' occupational exposure must consider different exposures, including those at the fire station. The present study aimed to characterize the occupational exposure of 172 Northern Portuguese wildland firefighters in fire stations during the pre-wildfire season of 2021. The biological impact of estimated inhaled doses of PM₁₀ and PM_{2.5} (indoor/outdoor) was accessed through a buccal micronucleus cytome (BMCyt) assay in exfoliated buccal cells of a subgroup of 80 firefighters. No significant association was found between estimated inhaled doses of PM₁₀ and PM_{2.5} (mean $1.73 \pm 0.43 \mu\text{g kg}^{-1}$ and $0.53 \pm 0.21 \mu\text{g kg}^{-1}$, respectively) and biological endpoints. However, increased frequencies of cell death parameters were found among subjects of the Permanent Intervention Teams (full-time firefighters). The intake of nutritional supplements was associated with a significant decrease in micronucleus frequencies (i.e., DNA damage or chromosome breakage). In addition, our findings showed a significantly increased frequency of cell death endpoints (i.e., nuclear fragmentation) with coffee consumption, while daily consumption of vegetables significantly decreased it (i.e., nuclear shrinkage). Our results provide data on the occupational exposure of wildland firefighters while working in fire stations during the pre-wildfire season, providing the essential baseline for further studies throughout the wildfire season.

Keywords: firefighters; non-invasive samples; biomonitoring; buccal micronucleus assay; particulate matter; inhaled dose

1. Introduction

Occupational exposure as a firefighter was recently classified as “carcinogenic to humans” (Group 1) by the International Agency for Research on Cancer (IARC) [1]. However, only a few studies shed light on the exposure-induced biological mechanisms that may lead to such adverse health effects [2]. Wildland firefighters are typically exposed to a wide range of air pollutants during their activities [3]. In addition to fire suppression, firefighters may be exposed to various hazardous agents at fire stations, including particulate matter

(PM). The presence of PM in fire stations can be attributed to various sources, including contaminated gear, equipment, and vehicles and diesel exhaust fumes originating from vehicles, fire engines, and trucks. This exposure may be particularly higher when facilities do not have adequate ventilation systems [4]. It is known that exposure to PM may induce various biological effects, including inflammation, DNA damage, and genomic instability [5]. Several studies have reported an association between human adverse health effects and exposure to PM₁₀ and PM_{2.5} [6,7]. In the general population, PM₁₀ exposure has been associated with respiratory illnesses, particularly in vulnerable groups [8], whereas exposure to PM_{2.5} has been linked with respiratory (e.g., chronic obstructive pulmonary disease and asthma) [9], cardiovascular (e.g., myocardial infarction, stroke, and arrhythmias) outcomes [10] and cancer [2]. Notwithstanding, few studies have focused on the biological impact of occupational exposure to PM on firefighters' health [2]. So far, limited research exists regarding the assessment of firefighters' occupational exposure to PM at fire stations [11–13], and up to date, none have studied its impact on effect biomarkers.

Human biomonitoring studies can provide critical information on the potential risks occupational exposures may pose to human health [14,15]. The use of effect biomarkers is of utmost importance to detect early events associated with disease-related outcomes [14–16]. Classic cytogenetic assays, such as the micronucleus (MN) test, have been extensively used in molecular epidemiological studies [17]. MN frequency is a biomarker of chromosomal damage, genome instability, and cancer risk [17]. MNi are extra-nuclear DNA-containing bodies morphologically similar to the main nucleus but smaller, formed during cell division from chromosome fragments or whole chromosomes lagging at anaphase. Traditionally analyzed in blood, MNi have been successfully assessed in recent years in other non-invasive biological matrices, e.g., urine, nasal, and buccal cells. The use of MN frequency in exfoliated buccal cells has become particularly interesting due to its minimally invasive character [18] and because it has been strongly correlated with MN frequency in lymphocytes, an early cancer risk biomarker [19,20].

Moreover, since humans are nasal–oral breathers, the mouth may be the first tissue in contact with gaseous compounds during inhalation [21], making MNi in buccal cells an attractive biomarker to assess in occupational settings where workers are exposed to these agents, allowing the identification of individuals with a higher risk of developing adverse health outcomes (e.g., cancer of the upper aerodigestive tract) [14]. Several authors have found statistically significant correlations between MNi in peripheral blood lymphocytes and MNi in buccal cells of occupationally exposed populations [19,22]. This strong link can be interpreted as an indication that MNi in exfoliated buccal cells are also a predictive marker of cancer [20]. In addition, the buccal micronucleus cytome (BMCyt) assay, besides chromosomal instability, allows the detection/evaluation of other cytochrome parameters, such as gene amplification (nuclear buds frequency), cytokinetic defects (binucleated cells frequency), proliferative potential (basal cells frequency), and cell death parameters (condensed chromatin, karyorrhexis and karyolytic and pycnotic cells) [23,24].

The concurrent evaluation of the exposure and effect endpoints among wildland firefighters in different settings (i.e., non-fire; fire suppression activities) and different time-points of a wildfire season (e.g., before the season and during wildfire season) are of utmost importance for the health risk characterization of this carcinogenic occupation.

The present study aims to characterize wildland firefighters' occupational exposure at fire stations before the wildfire season. The biological impact of estimated inhaled doses (indoor/outdoor) of PM₁₀ and PM_{2.5} in the buccal cells of a group of Northern Portuguese wildland firefighters was assessed. In addition, the influence of demographic-, occupational-, and lifestyle-related variables on MN frequency and other BMCyt endpoints was evaluated. To our knowledge, this is the first study accessing MN frequency in buccal epithelial cells among wildland firefighters, as well as the first relating this effect biomarker with firefighters' occupational exposure to PM₁₀ and PM_{2.5}, before the wildfire season.

2. Materials and Methods

2.1. Study Population

Wildland firefighters ($n = 176$) with at least one year of service were recruited from fourteen Portuguese fire stations in Portugal's northern region. All individuals who agreed to participate in the study were fully informed about the procedures and objectives of the study. Each participant signed an informed consent prior to the study. The research was conducted in full accordance with the Declaration of Helsinki [25,26]. Ethical approval for this study was obtained by the University of Porto Ethics Committee (reference number 92/CEUP/2020).

After giving written consent, participants were asked to complete a comprehensive questionnaire to obtain data on demographic-, occupational-, and lifestyle-related variables. Afterwards, participants were requested to provide one sample of blood, buccal mucosa cells, and urine, which were coded and analyzed under blind conditions. Confidentiality and privacy were guaranteed for all participants. Participants with cancer history ($n = 1$), with insufficient buccal cells to perform the BMCyt assay ($n = 2$), or with a missing questionnaire ($n = 1$) were excluded from the present analysis, reaching a total of 172 individuals. The biological and environmental samples were performed concurrently over two weeks in late spring of 2021.

2.2. Particulate Matter Monitoring

PM₁₀ and PM_{2.5} were measured in seven fire stations before the wildfire season began (i.e., during the pre-fire season) over the period of two weeks in late spring of 2021. In each fire station, indoor and outdoor monitoring of PM were conducted concurrently over 1–2 consecutive days. Indoor PM levels were assessed by stationary monitoring within various communal indoor spaces, including rest areas and living quarters, which were commonly utilized as primary indoor areas where firefighters await deployment into service; in each indoor space, PM monitoring was conducted for at least 8 consecutive hours. Outdoor PM was continuously monitored in outdoor open-area zones directly adjacent to the main buildings, where workers regularly performed various technical or operational activities (e.g., equipment maintenance and vehicle services.).

Indoor and outdoor real-time PM (PM₁₀ and PM_{2.5}) concentrations were continuously monitored by a laser photometer (DustTrakTM DRX, model 8533, TSI Inc., Minnesota, USA; measuring range of $1\text{--}150 \times 10^3 \mu\text{g m}^{-3}$; accuracy of reading $\pm 0.1\% 1 \mu\text{g m}^{-3}$) and a Lighthouse Handheld particle counter (model 3016 IAQ; Lighthouse Worldwide Solutions, Fremont, CA, USA; aerodynamic diameter of 300 nm–10 μm), respectively; one 1 min data resolution was used.

All equipment was positioned and mounted on supports at approximately 1.5 ± 0.2 m above the floor or ground surface and at least 1.5 m from the walls or any obstacles in order to minimize the influence on particle dispersion [27,28]. All direct emission sources that might interfere with data acquisition indoors and outdoors (i.e., air conditioners, ventilation points, entrance exits, walls, equipment, etc.) were avoided; outdoor equipment was positioned in a manner to be sheltered from direct sunlight and precipitation.

Before the sampling campaign, all equipment was calibrated at the manufacturers. In order to minimize the occurrences of sudden artifact jumps in particle concentrations [29], all particle counters were zeroed daily (using external zeroing modules).

Finally, all relevant information for indoor (e.g., layout, dimensions, construction materials, ventilation, and heating) and outdoor characterizations (e.g., levels of urbanization, presence of possible emission sources, etc.) of fire stations were collected. Other data, including occupancies and workers' activities, were recorded.

2.3. Inhalation Dose Assessment

PM inhalation doses were estimated for 80 firefighters from the seven fire stations where air monitoring evaluation took place, using data from the questionnaires on gender, age, body weight (kg), and time spent at the fire station per day. The calculation of PM

inhalation doses was performed based on methods described elsewhere [30]; the following equation was used:

$$\text{Dose (D)} = (\text{BR}/\text{BW}) \times \text{C} \times \text{t},$$

where D corresponds to the age-specific dose ($\mu\text{g kg}^{-1}$); BR is the age-specific breathing rate ($\text{m}^3 \text{min}^{-1}$); BW is the individual body weight (kg); C is the concentration of the PM in the respective indoor or outdoor spaces ($\mu\text{g m}^{-3}$) (Supplementary Table S1), and t represents the time of exposure (min/day) (i.e., time spent at the fire station/day), considering a permanence of 70% outdoors and 30% indoors (Table S1). Mean BR was obtained from the US Environmental Protection Agency (Supplementary Table S1) [31].

The schedules and associated activities were registered and analyzed daily. In general, the study participants' daily activity patterns were similar.

2.4. BMCyt Assay

2.4.1. Reagents

Sodium chloride (NaCl), sodium hydroxide (NaOH), ethylenediaminetetraacetic acid tetra (EDTA), hydrochloric acid (HCL), Schiff's reagent, Fast green FCF for microscopy, and EntellanTM were acquired from Merck KGaA (Darmstadt, Germany). Tris-hydrochloride (Tris-HCl) was obtained from AppliChem (Darmstadt, Germany). Ethanol absolute and acetic acid glacial were purchased from VWR Chemicals (Radnor, PA, USA).

2.4.2. Collection of Exfoliated Buccal Mucosa

Prior to buccal cell collection, subjects were asked to rinse their mouths with tap water to remove unwanted debris. Buccal sampling was performed separately inside both cheeks, left and right, to eliminate any unknown bias caused by sampling only one cheek. A sample from each cheek (left and right) was collected for every subject using separate cytobrushes (Deltalab) and suspended in 10 mL of cell buffer (0.01 M Tris-HCl, 0.1 M EDTA, 0.02 M NaCl, pH7). After collection, all biological samples were transported in a cooler (4 °C) to the laboratory for further processing. Samples were washed twice at 501 rcf with fresh cell buffer; after the last wash, cells were resuspended in 5 mL of a fixative solution of ethanol: acetic acid (3:1, v/v) at −20 °C and stored until further analysis.

2.4.3. BMCyt Assay

BMCyt assay was performed according to the procedure described by Thomas et al. [23] and Costa et al. [22] with minor modifications. Briefly, fixed cells were centrifuged at 501 rcf for 10 min, and the pellet was dropped onto clear coded glass slides (three slides per cheek). Slides were left to dry horizontally overnight. Air-dried fixed slides were immersed in solutions containing 50% (v/v) and 20% (v/v) ethanol for 1 min each and later submerged in deionized water for 2 min. Next, slides were immersed in a 5M HCl solution for 30 min and washed in running tap water for 3 min. After that, slides were stained with Schiff's reagent (Merck) at room temperature in the dark for 90 to 120 min. Slides were then counterstained for 5 sec in 0.2% (w/v) Fast Green (Merck) solution, dipped 3x in absolute ethanol, allowed to air dry, and mounted with Entellan[®]. The Feulgen-Fast green staining avoids false positive results (commonly found in non-specific DNA staining analysis, such as Giemsa) and consequently contributes to reliable and comparable results. Slides were scored blindly by a single trained operator on a Nikon Eclipse E400 light microscope under 400× magnification. Cells containing micronuclei were confirmed under fluorescence using a G-2A filter to eliminate false positives. Scoring was performed according to criteria proposed by previous studies [23,24]. The frequency of basal cells, binucleated cells (BN), cells undergoing death, such as cells with condensed chromatin, karyorrhectic cells, karyolytic cells, and pycnotic cells was determined in 1000 cells, whereas the genetic instability and gene amplification biomarkers (MNI and NBUDs) were scored in 2000 differentiated cells [23] per individual. In this study, we converted the MN frequency units to MN frequencies per 1000 cells (%). Photographic images obtained during the present analysis, showing typical examples of cells scored in the BMCyt assay, are presented in Figure 1.

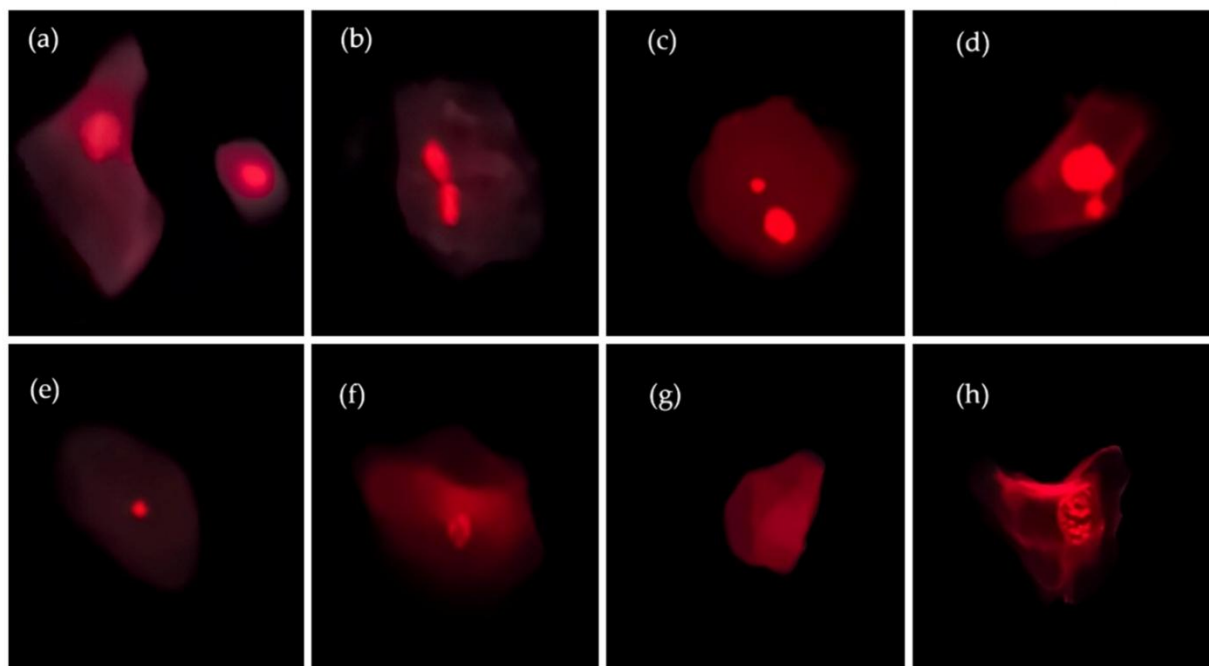


Figure 1. Photographic images of exfoliated buccal cells: (a) differentiated and basal cells; (b) binucleated cell; (c) differentiated cell with micronuclei; (d) NBUDs; (e) pycnotic cell; (f) karyorrhectic cell; (g) karyolytic cell; (h) differentiated cell with condensed chromatin.

2.5. Statistical Analysis

A descriptive analysis was performed both for quantitative and qualitative variables. The normality of continuous variables was determined using the Kolmogorov–Smirnov test, complemented with data dispersion graphs and outlying values analysis. *T*-test (for continuous variables) and chi-square test of independence (for categorical variables) were used for the comparison between groups (i.e., total group and subgroup from the seven fire stations where PM measurements were conducted). Body mass index (BMI) [$\text{weight (kg)} \times \text{height (m)}^{-2}$] and nutritional status (i.e., underweight, normal weight, overweight, and obese) were calculated and classified according to the World Health Organization [32]. Concerning smoking habits, subjects were classified as smokers, non-smokers, or ex-smokers. Ex-smokers were defined as those who had quit smoking at the time of the interview and had smoked at least 100 cigarettes during his/her lifetime [33]. Dependent variables (BM cyt assay outputs) departed from the normal distribution, so non-parametric tests were used. Differences were analyzed by the Man–Whitney U-test (two groups) and the Kruskal–Wallis test (more than two groups) with the Bonferroni adjustment. Spearman's rank correlation test was used to determine any possible correlation between BM cyt assay parameters and other continuous variables. In addition, simple linear regression analyses were performed to investigate the possible influence of independent variables (e.g., demographic-, occupational-, and lifestyle-related) on the BM cyt assay outputs. Categorical variables were transformed into dummy variables. A significance level of 0.05 was considered. All statistical analyses were performed using IBM SPSS Statistics 26.0 for Windows (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Population Characterization

Table 1 presents the general characteristics of the study participants. No significant differences were observed between the study population and the subgroup.

Table 1. General characteristics of the study population ($n = 172$) and subgroup from the seven fire stations where air monitoring evaluation occurred ($n = 80$).

Study Participant Characteristics		Total (<i>n</i> = 172)		Subgroup (<i>n</i> = 80)	
		mv *		mv *	
Sociodemographic					
Gender, <i>n</i> (%)	-		-		
Females		31 (18.0%)		12 (15.0%)	
Males		141 (82.0%)		68 (85.0%)	
Age (years) **	2	37.5 ± 10.9 (19.0–65.0)	-	37.6 ± 11.9 (19.0–65.0)	
BMI (kg m ⁻²) **	11	27.4 ± 4.1 (18.5–41.3)	-	27.7 ± 4.0 (18.5–41.3)	
Occupational exposure:					
Years of service (as a firefighter) **	7	15.9 ± 10.3 (1.0–43.0)	3	16.2 ± 11.5 (1.0–43.0)	
Environmental exposure:					
Secondhand smoke exposure, <i>n</i> (%)	7	144 (87.3%)	1	69 (87.3)	
X-ray exposure last year, <i>n</i> (%)	7	68 (41.2%)	1	32 (40.5%)	
Lifestyle characterization:					
Alcohol intake, <i>n</i> (%)	3	53 (31.4%)	-	26 (32.5%)	
Smoking habits	2		-		
Non-smoker, <i>n</i> (%)		74 (43.5%)		37 (46.3%)	
Ex-smoker, <i>n</i> (%)		34 (20.0%)		25 (22.5%)	
Current smoker, <i>n</i> (%)		62 (36.5%)		25 (31.3%)	
Physical activity, <i>n</i> (%)	3	128 (75.7%)	-	60 (75.0%)	
Diet					
Vegetable intake	23		8		
Never, <i>n</i> (%)		12 (8.1%)		8 (11.1%)	
Daily, <i>n</i> (%)		101 (67.8%)		50 (69.4%)	
Weekly, <i>n</i> (%)		36 (24.2%)		14 (19.4%)	
Coffee intake, <i>n</i> (%)	1	147 (86.0%)	-	71 (88.8%)	
N° times/week **	57	23.6 ± 13.4 (3.0–70.0)	24	23.2 ± 12.3 (3.0–56.0)	
Tea intake, <i>n</i> (%)	1	70 (40.7%)	-	32 (40.0%)	
N° times/week **		5.2 ± 5.7 (1.0–28.0)		5.8 ± 6.6 (1.0–28.0)	
Smoked food consumption, <i>n</i> (%)	1	129 (75.4%)	-	61 (76.3%)	
N° times/week **		3.1 ± 4.5 (1.0–35.0)		2.3 ± 3.1 (1.0–14.0)	
Nutritional supplements intake, <i>n</i> (%)	4	19 (11.3%)	-	11 (13.8%)	

* Missing values. ** Mean $\pm$ SD (standard deviation), (min-max).

BMI values among males and females were $27.6 \pm 3.9 \text{ kg m}^{-2}$ and $26.4 \pm 4.5 \text{ kg m}^{-2}$, respectively. According to World Health Organization criteria [27], only 29% of firefighters ($n = 47$) had normal weight (range, $18.5\text{--}24.9 \text{ kg m}^{-2}$), with the remaining 44% ($n = 71$) and 27% ($n = 43$) being either pre-obese (range, $25.0\text{--}29.9 \text{ kg m}^{-2}$) or obese ($\geq 30.0 \text{ kg m}^{-2}$), respectively. Most firefighters (75%) declared having some physical activity. Nearly 90% of the firefighters reported being secondhand smokers (i.e., those exposed to environmental tobacco smoke, also called passive smokers) mostly at work and during leisure activities. Regarding dietary behaviors and practices, the majority of firefighters consumed vegetables daily, coffee, and smoked food between 2–3 times a week.

Around 15.0% of the firefighters reported starting their training between the ages of 12 and 16 (i.e., as a junior or a cadet, respectively). However, the majority, 55.8%, started between 17 and 22 years old, while the rest (29.4%) started after that age. Besides fire combat, firefighters reported being involved in other activities, namely telephone operator ($n = 17$; 11.1%), driver ($n = 53$; 34.6%), pre-hospital emergencies ($n = 47$; 30.7%), rescue

($n = 12$; 7.8%), and command tasks ($n = 12$; 7.8%). Concerning the average time spent at the fire station during the off-season, a meaningful proportion of participants (49.1%) reported spending more than 10 h daily in their duties at the fire station, 37.1% within 8–9 h, and the remaining, less than 8 h (13.8%). Most firefighters were volunteers, whereas 30.1% ($n = 46$) reported to be full-time contract employees and members of the Permanent Intervention Team.

3.2. PM Inhaled Doses vs. Effect Biomarker

Detailed results of indoor and outdoor PM₁₀ and PM_{2.5} levels measured in the seven fire stations were described previously by Slezakova et al. [34]. Mean concentrations of indoor PM₁₀ ranged between 8 and 15 $\mu\text{g m}^{-3}$ (overall mean: 10 $\mu\text{g m}^{-3}$), while mean indoor PM_{2.5} concentrations at each fire station ranged between 6 and 13 $\mu\text{g m}^{-3}$ (overall mean: 8.5 $\mu\text{g m}^{-3}$). Regarding the outdoor concentrations of PM₁₀ and PM_{2.5} measured in the seven fire stations, means varied between 16 to 27 $\mu\text{g m}^{-3}$ (overall mean: 21 $\mu\text{g m}^{-3}$) and between 2 and 6 $\mu\text{g m}^{-3}$ (overall mean: 5 $\mu\text{g m}^{-3}$) for PM₁₀ and PM_{2.5}, respectively.

Table 2 presents the descriptive statistics regarding the estimated inhaled doses of PM₁₀ and PM_{2.5} with the respective indoor and outdoor contributions (expressed as %).

Table 2. Estimated inhaled doses of PM₁₀ and PM_{2.5} for 80 firefighters from seven fire stations.

	Mean $\pm$ SD	Min.–Max.	Median	P25–P75
Inhaled Dose PM ₁₀ ($\mu\text{g kg}^{-1}$)	1.73 $\pm$ 0.43	0.85–2.70	1.68	1.45–2.02
Contribution of Indoor (%)	18.12 $\pm$ 4.08	10.67–26.79	18.00	17.06–17.95
Contribution of Outdoor (%)	81.87 $\pm$ 4.08	73.21–89.33	82.42	82.05–82.94
Inhaled Dose PM _{2.5} ($\mu\text{g kg}^{-1}$)	0.53 $\pm$ 0.21	0.22–1.09	0.49	0.37–0.72
Contribution of Indoor (%)	49.09 $\pm$ 9.09	26.22–57.69	54.00	44.74–53.94
Contribution of Outdoor (%)	50.90 $\pm$ 9.09	42.31–73.78	46.39	46.05–55.26

SD: standard deviation; Min: minimum; Max: maximum; P25: percentile 25; P75: percentile 75.

A higher mean of inhaled doses of PM₁₀ was observed when compared with PM_{2.5}. The mean of estimated inhaled doses due to exposure to PM₁₀ outdoors was four times higher than indoors. For PM_{2.5}, the contributions from indoor and outdoor exposures were similar.

Simple linear regression analyses were performed to investigate the influence of the estimated PM₁₀ and PM_{2.5} doses on the frequency of the effect biomarker endpoints. No significant statistical association was found. Table 3 shows the data obtained for the MN frequencies.

Table 3. Influence of inhaled PM₁₀ and PM_{2.5} on buccal MN frequency in a sub-group of 80 firefighters.

Variables	β	t	p-Value	95% CI
Inhaled dose ($\mu\text{g kg}^{-1}$)				
PM ₁₀	−0.01	−0.04	0.97	−0.08 to 0.78
PM _{2.5}	0.07	0.29	0.77	−0.42 to 0.57

95% CI= confident interval; β = regression coefficient.

3.3. Influence of Demographic, Occupational, and Lifestyle Variables on Effect Biomarker Endpoints

In the present study, the frequency of MNi in buccal cells was not significantly influenced by age, gender, lifestyle (e.g., smoking habits), or years of service. However, firefighters using nutritional supplements in the last year showed a significantly lower frequency of MN than those who did not (mean $\pm$ S.E., 0.16 $\pm$ 0.07 vs. 0.40 $\pm$ 0.04; $p = 0.04$). Furthermore, firefighters of the Permanent Intervention Team showed a significantly increased frequency of cell death parameters (i.e., nuclear fragmentation) compared to others (mean $\pm$ S.E., 3.54 $\pm$ 2.91 vs. 1.32 $\pm$ 0.10; $p = 0.01$). A significantly higher frequency of this endpoint was also found in firefighters who reported drinking coffee (mean $\pm$ S.E.,

2.97 ± 0.96 vs. 0.25 ± 1.12 ; $p = 0.02$). Moreover, higher frequencies of nuclear shrinkage (cell death parameter) were observed among individuals consuming smoked meat daily ($p = 0.03$); this endpoint was also higher in firefighters who reported not eating vegetables ($p = 0.05$). Female firefighters revealed significantly higher frequencies of binucleated cells than males (mean $\pm$ S.E., 0.52 ± 0.15 vs. 0.11 ± 0.03 ; $p < 0.01$). Pre-obese firefighters presented a slight increase in karyorrhectic cells compared to those with normal weight (mean $\pm$ S.E., 0.14 ± 0.05 vs. 0.00 ± 0.00 ; $p = 0.04$).

Simple regression analyses were applied to assess the extent of the effect of independent variables (e.g., demographic-, occupational-, and lifestyle-related variables) on the frequencies of micronucleus (MN), nuclear buds (NBUDs), binuclear cells, and cell death endpoints; the results of this analysis are displayed in the form of Pareto charts of t-values (Figure 2). The Pareto charts comprise a series of bars whose lengths indicate the impact of each predictable variable on the dependent variable. Due to the high number of tested variables, here, we only present important theoretical confounders that are typically used in these types of biomonitoring studies (i.e., age, gender, smoking habits, BMI, physical activity, and alcohol consumption) and variables that showed to be statistically significantly associated with some of the BMCyt parameters evaluated, namely the intake of nutritional supplements and tea (cups/week).

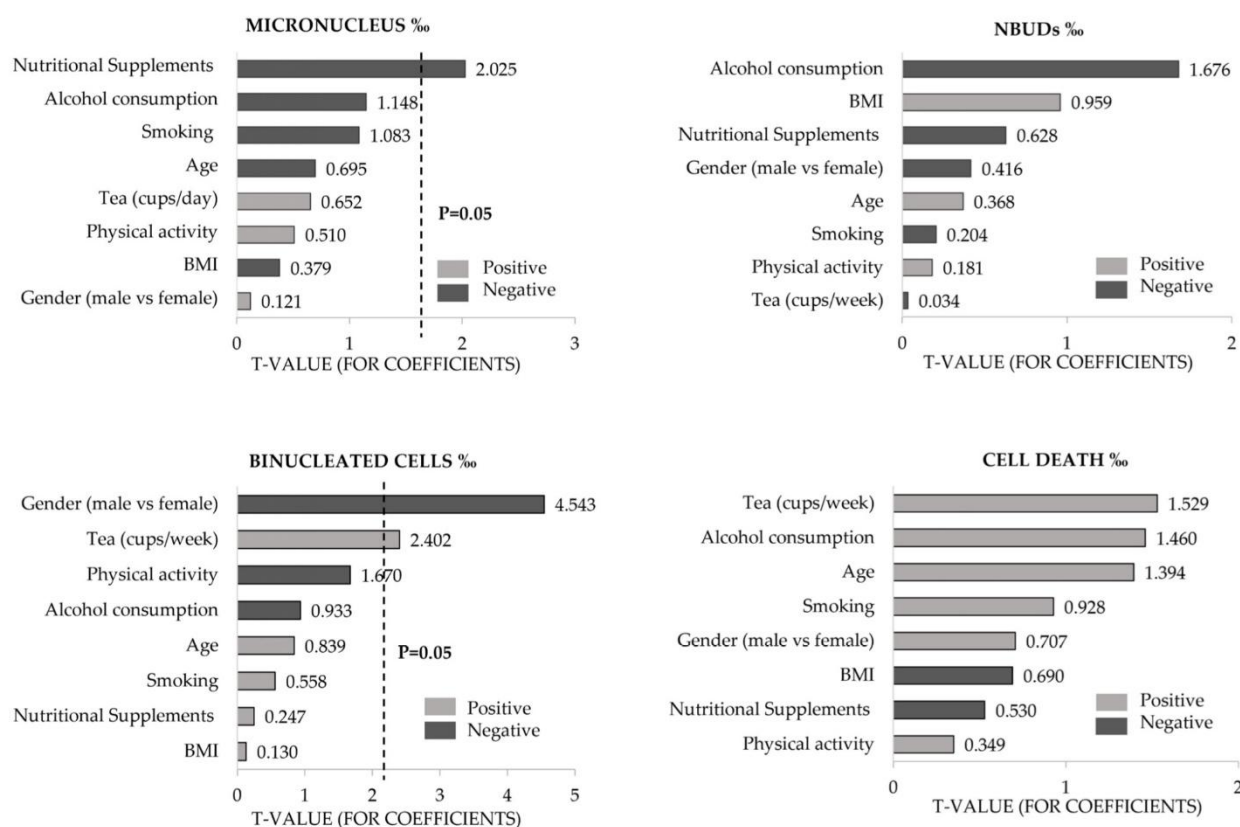


Figure 2. Simple regression analysis results considering the total sample ($n = 172$). Correlation between the frequency of each BMCyt endpoint (micronuclei, nuclear buds, binucleated cells, and sum of cell death endpoints) and selected predictor variables.

Some dietary habits influenced some BMCyt assay markers; the intake of nutritional supplements had a statistically significant negative contribution to micronuclei frequency ($\beta = -0.24$; $p = 0.04$), whereas ingestion of tea (cups/week) was a predictor of binucleated cells ($\beta = 0.03$; $p = 0.02$). Gender, i.e., female, was a predictor of the occurrence of binucleated cells ($\beta = -0.41$; $p < 0.01$).

4. Discussion

Wildland firefighters may be exposed to genotoxic compounds in different settings during their activities. When working at fire stations, firefighters can be exposed to diesel exhaust or other hazardous materials, such as PM and other known carcinogenic agents. Thus, any risk assessment of firefighters' occupational exposure should include these activities. To our knowledge, no studies have yet estimated the inhaled doses of PM₁₀ and PM_{2.5} (indoor/outdoor) at fire stations or even correlated them with a biomarker of effect. The frequency of MNi in buccal cells is particularly important as it has been proven to be a sensitive biomarker for detecting early biological effects of air pollutant exposure [35].

In this study, indoor PM₁₀ and PM_{2.5} concentrations were below the levels set in the Portuguese Law No. 138-G/2021 (PM₁₀ = 50 µg m⁻³ and PM_{2.5} = 25 µg m⁻³, respectively) that establishes the requirements for the assessment of indoor air quality in public buildings [36]. Regarding outdoor PM₁₀ and PM_{2.5} levels, the observed concentrations fulfilled the recommendations proposed by the World Health Organization in 2021 (45 and 15 µg m⁻³, respectively) [37].

No association was found between inhaled doses (indoor/outdoor) of PM₁₀ and PM_{2.5} and MN frequency, which could be related to the low PM levels.

The level of MN frequency found in our population was within the expected range of spontaneous events [0.30–1.70‰], according to Bonassi et al. [38]. Understanding the MN frequency distribution in a population is crucial [39], particularly if MN frequencies are to be used as biomarkers to evaluate the biological impact of workers' exposure to hazardous agents [40].

Regarding the influence of variables on BMCyt endpoints, here, we observed that the number of years in service as a firefighter did not influence the dependent variables. Nevertheless, we found that subjects of the Permanent Intervention Teams had statistically significantly higher levels of karyolytic cells, a cell death parameter, compared to other colleagues. These teams comprise full-time contracted firefighters who may perform different tasks throughout the year, including firefighting, rescue, medical emergencies, vehicle maintenance, support to natural disasters, and other emergency response operations. The level of commitment, the extent of occupational exposure to harmful substances, and the stressful nature of their work may justify the difference found.

Aside from occupational exposure, there are endogenous (e.g., age and gender) and exogenous factors (e.g., smoking, drinking habits, and diet) that must be taken into consideration in any human biomonitoring study [19,41].

Some studies have shown that age may influence MN frequencies in buccal cells [42]; however, no association was found in the present study. Similar to our results, Haveric et al. [43] assessed the cytogenetic levels in exfoliated buccal cells in young individuals (aged 20–37) and found no association. In our study, no statistically significant differences were observed in MN frequency between males and females, which aligns with other studies [44,45]. The HUMNXL project (Human MicroNucleus project; 'XL' referring to eXfoliated cells) evaluated the impact of host factors, occupation, and lifestyle on the occurrence of MNi in exfoliated buccal cells and also found no effect of gender [38]. Notwithstanding, in this study, a significant increase in binucleated cells was found among female firefighters. Few studies have mentioned differences concerning occupational aspects between female and male firefighters. Perroni et al. [46] reported that due to physiological sex differences, female firefighters have a higher level of physical demands to perform expected firefighter tasks. In addition, a working group of IARC recently concluded that a lack of proper personal protective equipment (PPE) fitting is likely among females, which could result in more significant contamination and exposure [2]. However, to date, a lack of evidence exists concerning the effect of gender in BMCyt endpoints, and therefore, caution is warranted when interpreting the results obtained here; further studies using BMCyt endpoints are needed.

Very few studies have explored the influence of BMI on the frequency of MNi in buccal cells [47]. Idolo et al. (2018) assessed the association between the frequency of MN cells and BMI categories among a group of one hundred and twenty-two healthy Italian children

aged 6–8 years and found that obesity was strongly associated with this biomarker (OR = 3.849; CI = 1.140–12.994). Similarly to our results, Ernst et al. [48] found no association between BMI and MN frequency in a group of male individuals (aged 33.6 ± 8.6). Yet, we found that overweighted firefighters had statistically increased frequencies of cell death endpoints (i.e., condensed chromatin) compared with those with normal weight.

Despite the numerous genotoxic chemicals in tobacco smoke [49], scientific findings on smoking and MN frequency are controversial [45,50]. Bloching et al. [50] found a strong positive correlation between smoking and MN frequency in buccal cells of tumor patients and healthy controls. However, most studies found no association between MNi in buccal cells and smoking habits [45,51]. Furthermore, in a pooled re-analysis of 24 databases of the HUMN international collaborative project [52], the authors found no effect of smoking nor the number of daily cigarettes on MN frequency analyzed in lymphocytes [52]. In agreement, our results also demonstrated that smoking, as well as the number of cigarettes smoked and the duration of smoking in years, did not have a significant impact on the frequencies of the studies' endpoints. A possible explanation raised by some authors is related to a possible adaptive response (<30 cigarettes per day) showing that the MN frequency in lymphocytes was not influenced by the number of cigarettes smoked per day among subjects occupationally exposed to genotoxic agents [52].

Alcohol intake is a risk factor often collected in biomonitoring studies and has previously been linked to MN frequency in lymphocytes [53]. However, in line with previous works [54–58], alcohol consumption was not significantly associated with the increase in any BMCyt parameter. It is important to highlight that our study sample did not include any heavy alcohol drinkers; most firefighters reported drinking alcohol moderately (one to two cups of wine/beer).

The influence of tea and coffee consumption on BMCyt endpoints was also evaluated. Neither tea nor coffee intake was a predictor of buccal MN frequencies. Nevertheless, other BMCyt parameters, such as binucleated cell frequency and cell death endpoints (i.e., nuclear fragmentation), were significantly higher with tea (cups/day) and coffee intake (yes vs. no), respectively. A possible explanation for these findings may be related to the consumption of hot beverages; in fact, IARC classified hot beverage consumption as probably carcinogenic to humans [59]. Ernst et al. [48] found a significant association between temperature (>60 °C) and the increase in BMCyt endpoint frequencies in a group of 73 individuals before and after consuming hot beverages, i.e., tea and coffee.

Firefighters who regularly eat vegetables revealed statistically significantly lower cell death levels than those who never eat vegetables. In line with our results, results published by the HUMNXL project [38] revealed that the consumption of vegetables had a protective role on exfoliated buccal cells (lower MN frequencies in daily consumers when compared with those that reported no consumption at all). Conversely, firefighters who reported daily consumption of smoked food showed statistically higher frequencies of this endpoint; smoked food (e.g., ham and sausages) is known to be a dietary source of several hazardous compounds [60].

In this study, the intake of nutritional supplements was associated with lower MN frequencies. This finding is consistent with earlier reports; for example, Fenech et al. [61] demonstrated that low intake of calcium, folate, nicotinic acid, vitamin E, retinol, and beta-carotene and high intake of pantothenic acid, biotin, and riboflavin increased genome instability. In addition, the results of a review carried out by Thomas et al. [62] suggested that supplementation with antioxidant vitamins may cause a decrease in MN frequency both in buccal cells and in lymphocytes. The association of either nutritional supplement and vegetable intake with BMCyt assay outputs may be explained by the higher concentration of antioxidants and other nutrients that play an important role in genome health maintenance [63].

Strengths and Limitations

The combination of biological monitoring with questionnaires and environmental monitoring data allows a comprehensive characterization of wildland firefighters before the wildland season. To the best of our knowledge, no studies have yet analyzed the impact of estimated inhaled doses of PM₁₀ and PM_{2.5} (outdoor/indoor) in MN frequency or explored the influence of potential confounder variables or risk factors. Moreover, this is the first study accessing MN frequency in buccal epithelial cells among wildland firefighters; so far, only a previous work comprising urban firefighters ($n = 47$) has performed it [64].

However, our findings should be interpreted in light of some limitations. Data were collected through self-reported questionnaires; thus, some possible biases may have affected the variables (underestimate or overestimate). It is important to note that this study was conducted in a post-COVID-19 deconfinement period (2021) with an overall lower particulate air pollution. Thus, the association between the respective pollutants and biological markers may have been underestimated. In addition, as this is a cross-sectional study, it is impossible to establish a temporal relationship between exposure and the outcome. Still, this work offers valuable insights for guiding future research and stands as a foundational study for prospective longitudinal comparisons (e.g., pre-fire vs. during a fire season).

5. Conclusions

Our findings contributed to a baseline characterization of firefighters' occupational exposure in preparation for the wildfire season. Given the multitude of PM sources (e.g., exhaust fumes from operational vehicles and contaminated PPE/equipment/vehicles), a comprehensive understanding of respective exposure within fire stations becomes imperative. The inclusion of a sensible biological endpoint, such as the BMCyt assay, may help to estimate the health risks faced by firefighters. We found no significant correlation between estimated inhaled doses of PM₁₀ and PM_{2.5} and biological endpoints, as assessed by the BMCyt assay, probably due to the environmental levels found below established threshold values. Exposure to PM₁₀ and PM_{2.5} has been linked to various adverse health effects, including respiratory and cardiovascular problems, cancer, as well as premature death. However, it is also worth noting that even exposure to levels below these limits may still pose health risks, and cumulative exposure effects over the years cannot be excluded. Improving air quality within fire stations through measures such as enhanced ventilation systems and regular air quality monitoring is essential for fostering healthier work environments.

The incorporation of sensitive biological endpoints, such as the BMCyt assay, can provide valuable insights into the health risks faced by firefighters. Our study revealed that certain occupational groups, such as full-time firefighters (Permanent Intervention Teams), exhibited higher levels of cell death endpoints compared to the other firefighters. Interestingly, we found that those reporting healthy dietary habits, particularly regular vegetable consumption, showed lower levels of cell death endpoints. Maintaining a healthy lifestyle, including proper nutrition and exercise, is crucial for enhancing overall health and resilience to occupational demands among firefighters. Moreover, it is imperative to implement occupational health and safety procedures at both individual and organizational levels.

In summary, (bio)monitoring programs are a crucial tool to identify firefighters at high risk for developing adverse health outcomes (short- and long-term). Further studies analyzing the two main points of firefighters' occupational exposure, either during resting or off-season as during the fire season, are warranted to comprehensively evaluate occupational exposures holistically and refine strategies for safeguarding firefighter health and well-being.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/toxics12030201/s1>, Table S1: Summary of parameters considered for inhalation dose estimation, specifically the concentration of PM both indoor and outdoor in the

seven sampled fire stations (A); the time of exposure both indoor and outdoor (B); and the inhalation rate in males and in females, by age category, unadjusted for body weight (C).

Author Contributions: F.E.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Writing—review and editing, Writing—original draft. K.S., J.M. and S.C.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Writing—review and editing, Writing—original draft, Project Administration, Supervision. J.V.: Methodology, Project Administration, Resources, Writing—review and editing. A.F. and M.d.C.P.: Funding acquisition, Methodology, Project administration, Resources, Writing—review and editing. S.M.: Funding acquisition, Project administration, Writing—review and editing. J.P.T.: Funding acquisition, Project administration, Resources, Writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This work received financial support from the project PCIF/SSO/0017/2018 (<http://doi.org/10.54499/PCIF/SSO/0017/2018>) by the Fundação para a Ciência e a Tecnologia (FCT), Ministério da Ciência, Tecnologia e Ensino Superior (MCTES) through national funds. Filipa Esteves, the PhD grant UI/BD/150783/2020 recipient, is supported by FCT and the European Social Fund (ESF). This work is financed by national funds through the FCT within the scope of projects UIDB/04750/2020 (<http://doi.org/10.54499/UIDB/04750/2020>) and LA/P/0064/2020 (<http://doi.org/10.54499/LA/P/0064/2020>), LA/P/0045/2020 (<http://doi.org/10.54499/LA/P/0045/2020>), UIDP/00511/2020 (<http://doi.org/10.54499/UIDP/00511/2020>) and UIDB/00511/2020 (<http://doi.org/10.54499/UIDB/00511/2020>), LA/P/0008/2020 (<http://doi.org/10.54499/LA/P/0008/2020>), UIDP50006/2020 (<http://doi.org/10.54499/UIDP/50006/2020>) and UIDB/50006/2020 (<http://doi.org/10.54499/UIDB/50006/2020>).

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the University of Porto, Portugal, Report Nr. 92/CEUP/2020, under the BioFirEx project (PCIF/SSO/0017/2018): “A panel of (bio)markers for the surveillance of firefighter’s health and safety”.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The authors do not have permission to share data.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

PM: Particulate matter; BMCyt: Buccal micronucleus cytome assay; DNA: Deoxyribonucleic acid; IARC: International Agency for Research on Cancer; MN: Micronucleus; BN: Binucleated cells; NBUDs: Nuclear buds; BMI: Body mass index; PPE: Personal protective equipment; NaCl: Sodium chloride; NaOH: Sodium hydroxide; EDTA: ethylenediaminetetraacetic acid tetra; HCL: hydrochloric acid; Tris-HCL: Tris-hydrochloride.

References

1. Demers, P.A.; DeMarini, D.M.; Fent, K.W.; Glass, D.C.; Hansen, J.; Adetona, O.; Andersen, M.H.; Freeman, L.E.; Caban-Martinez, A.J.; Robert, D.; et al. Carcinogenicity of occupational exposure as a firefighter. *Lancet Oncol.* **2022**, *23*, 985–986. [[CrossRef](#)]
2. IARC. Occupational Exposure as a Firefighter. In *IARC Monographs on the Identification of Carcinogenic Hazards to Humans*; International Agency for Research on Cancer: Lyon, France, 2023; Volume 132, pp. 1–730.
3. Esteves, F.; Madureira, J.; Teixeira, J.P.; Costa, S. The Role of Biomarkers in the Assessment of Potential Health Risks of Wildland Firefighters’ Occupational Exposure: An Overview. In *Essential Guide to Occupational Exposure*; Moraes, S., Oliveira, M., Rodrigues, F., Eds.; Nova Science Publishers, Inc.: New York, NY, USA, 2022.
4. Bott, R.C.; Kirk, K.M.; Logan, M.B.; Reid, D.A. Diesel particulate matter and polycyclic aromatic hydrocarbons in fire stations. *Environ. Sci. Process Impacts* **2017**, *19*, 1320–1326. [[CrossRef](#)] [[PubMed](#)]
5. Santibáñez-Andrade, M.; Quezada-Maldonado, E.M.; Osornio-Vargas, Á.; Sánchez-Pérez, Y.; García-Cuellar, C.M. Air pollution and genomic instability: The role of particulate matter in lung carcinogenesis. *Environ. Pollut.* **2017**, *229*, 412–422. [[CrossRef](#)] [[PubMed](#)]
6. Kim, K.-H.; Kabir, E.; Kabir, S. A review on the human health impact of airborne particulate matter. *Environ. Int.* **2015**, *74*, 136–143. [[CrossRef](#)] [[PubMed](#)]

7. Von Klot, S.; Peters, A.; Aalto, P.; Bellander, T.; Berglind, N.; D'Ippoliti, D.; Elosua, R.; Hörmann, A.; Kulmala, M.; Lanki, T.; et al. Ambient air pollution is associated with increased risk of hospital cardiac readmissions of myocardial infarction survivors in five European cities. *Circulation* **2005**, *112*, 3073–3079. [CrossRef]
8. Mott, J.A.; Meyer, P.; Mannino, D.; Redd, S.C.; Smith, E.M.; Gotway-Crawford, C.; Chase, E. Wildland forest fire smoke: Health effects and intervention evaluation, Hoopa, California, 1999. *West J. Med.* **2002**, *176*, 157. [CrossRef]
9. Cascio, W.E. Wildland fire smoke and human health. *Sci. Total Environ.* **2018**, *624*, 586–595. [CrossRef]
10. Du, Y.; Xu, X.; Chu, M.; Guo, Y.; Wang, J. Air particulate matter and cardiovascular disease: The epidemiological, biomedical and clinical evidence. *J. Thorac. Dis.* **2016**, *8*, E8.
11. Oliveira, M.; Slezakova, K.; Alves, M.J.; Fernandes, A.; Teixeira, J.P.; Delerue-Matos, C.; Pereira, M.C.; Morais, S. Polycyclic aromatic hydrocarbons at fire stations: Firefighters' exposure monitoring and biomonitoring, and assessment of the contribution to total internal dose. *J. Hazard. Mater.* **2017**, *323*, 184–194. [CrossRef]
12. Rogula-Kozłowska, W.; Bralewska, K.; Rogula-Kopiec, P.; Makowski, R.; Majder-Lopatka, M.; Łukawski, A.; Brandyk, A.; Majewski, G. Respirable particles and polycyclic aromatic hydrocarbons at two Polish fire stations. *Build. Environ.* **2020**, *184*, 107255. [CrossRef]
13. Sparer, E.H.; Prendergast, D.; Apell, J.N.; Bartzak, M.R.; Wagner, G.R.; Adamkiewicz, G.; Hart, J.E.; Sorensen, G. Assessment of ambient exposures firefighters encounter while at the fire station: An exploratory study. *J. Occup. Environ. Med.* **2017**, *59*, 1017. [CrossRef]
14. Bolognesi, C. Genotoxicity of pesticides: A review of human biomonitoring studies. *Mutat. Res./Rev. Mutat. Res.* **2003**, *543*, 251–272. [CrossRef]
15. Gajski, G.; Gerić, M.; Oreščanin, V.; Garaj-Vrhovac, V. Cytokinesis-block micronucleus cytome assay parameters in peripheral blood lymphocytes of the general population: Contribution of age, sex, seasonal variations and lifestyle factors. *Ecotoxicol. Environ. Saf.* **2018**, *148*, 561–570. [CrossRef] [PubMed]
16. Hopf, N.B.; Bolognesi, C.; Danuser, B.; Wild, P. Biological monitoring of workers exposed to carcinogens using the buccal micronucleus approach: A systematic review and meta-analysis. *Mutat. Res./Rev. Mutat. Res.* **2019**, *781*, 11–29. [CrossRef] [PubMed]
17. Albertini, R.J.; Anderson, D.; Douglas, G.R.; Hagmar, L.; Hemminki, K.; Merlo, F.; Natarajan, A.T.; Norppa, H.; Shuker, D.E.; Tice, R.; et al. IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. *Mutat. Res./Rev. Mutat. Res.* **2000**, *463*, 111–172. [CrossRef]
18. Torres-Bugarín, O.; Ramos-Ibarra, M.L. Utilidad de la prueba de micronúcleos y anomalías nucleares en células exfoliadas de mucosa oral en la evaluación de daño genotóxico y citotóxico. *Int. J. Morphol.* **2013**, *31*, 650–657. [CrossRef]
19. Ceppi, M.; Biasotti, B.; Fenech, M.; Bonassi, S. Human population studies with the exfoliated buccal micronucleus assay: Statistical and epidemiological issues. *Mutat. Res./Rev. Mutat. Res.* **2010**, *705*, 11–19. [CrossRef]
20. Nersesyan, A.; Kundi, M.; Fenech, M.; Stopper, H.; da Silva, J.; Bolognesi, C.; Mišák, M.; Knasmueller, S. Recommendations and quality criteria for micronucleus studies with humans. *Mutat. Res./Rev. Mutat. Res.* **2022**, *789*, 108410. [CrossRef] [PubMed]
21. Esteban, M.; Castaño, A. Non-invasive matrices in human biomonitoring: A review. *Environ. Int.* **2009**, *35*, 438–449. [CrossRef]
22. Costa, S.; Brandão, F.; Coelho, M.; Costa, C.; Coelho, P.; Silva, S.; Porto, B.; Teixeira, J.P. Micronucleus frequencies in lymphocytes and buccal cells in formaldehyde exposed workers. *WIT Trans. Biomed. Health* **2013**, *16*, 83–94.
23. Thomas, P.; Holland, N.; Bolognesi, C.; Kirsch-Volders, M.; Bonassi, S.; Zeiger, E.; Knasmueller, S.; Fenech, M. Buccal micronucleus cytome assay. *Nat. Protoc.* **2009**, *4*, 825. [CrossRef] [PubMed]
24. Bolognesi, C.; Knasmueller, S.; Nersesyan, A.; Thomas, P.; Fenech, M. The HUMNxl scoring criteria for different cell types and nuclear anomalies in the buccal micronucleus cytome assay—An update and expanded photogallery. *Mutat. Res./Rev. Mutat. Res.* **2013**, *753*, 100–113. [CrossRef] [PubMed]
25. WMA. World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. *JAMA* **2013**, *310*, 2191–2194. [CrossRef] [PubMed]
26. Williams, J.R. The Declaration of Helsinki and public health. *Bull. World Health Organ.* **2008**, *86*, 650–652. [CrossRef] [PubMed]
27. Holmberg, S.; Li, Y. Modelling of the indoor environment—particle dispersion and deposition. *Indoor Air.* **1998**, *8*, 113–122. [CrossRef]
28. Jin, H.; He, C.; Lu, L.; Fan, J. Numerical investigation of the wall effect on airborne particle dispersion in a test chamber. *Aerosol Air Qual. Res.* **2013**, *13*, 786–794. [CrossRef]
29. Rivas, I.; Mazaheri, M.; Viana, M.; Moreno, T.; Clifford, S.; He, C.; Bischof, O.F.; Martins, V.; Reche, C.; Alastuey, A. Identification of technical problems affecting performance of DustTrak DRX aerosol monitors. *Sci. Total Environ.* **2017**, *584*, 849–855. [CrossRef]
30. Slezakova, K.; Peixoto, C.; Oliveira, M.; Delerue-Matos, C.; Pereira, M.C.; Morais, S. Indoor particulate pollution in fitness centres with emphasis on ultrafine particles. *Environ. Pollut.* **2018**, *233*, 180–193. [CrossRef]
31. USEPA. *Exposure Factors Handbook 2011 Edition (Final)*; U.S. Environmental Protection Agency: Washington, DC, USA, 2011.
32. World Health Organization (WHO). Body Mass Index—BMI. 2022. Available online: <https://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi> (accessed on 20 October 2022).
33. CDC. Adult Tobacco Use Information—Glossary. Center for Disease Prevention and Control. 2017. Available online: https://www.cdc.gov/nchs/nhis/tobacco/tobacco_glossary.htm (accessed on 25 October 2023).

34. Slezakova, K.; Esteves, F.; Vaz, J.; Alves, M.J.; Madureira, J.; Costa, S.; Fernandes, A.; Teixeira, J.P.; Morais, S.; Pereira, M.C. Occupational Exposure of Firefighters in Non-fire Settings. In *Occupational and Environmental Safety and Health IV*; Springer: Berlin/Heidelberg, Germany, 2022; pp. 79–88.
35. O'Callaghan-Gordo, C.; Fthenou, F.; Pedersen, M.; Espinosa, A.; Chatzi, L.; Beelen, R.; Chalkiadaki, G.; Decordier, I.; Hoek, G.; Merlo, D.F.; et al. Outdoor air pollution exposures and micronuclei frequencies in lymphocytes from pregnant women and newborns in Crete, Greece (Rhea cohort). *Environ. Res.* **2015**, *143*, 170–176. [\[CrossRef\]](#)
36. Secretaria de Estado da Saúde e Ambiente e Ação Climática. *Portaria No. 138-G/2021*; Diário da República: Lisbon, Portugal, 2021.
37. World Health Organization. *WHO Global Air Quality Guidelines: Particulate Matter (PM_{2.5} and PM₁₀), Ozone, Nitrogen Dioxide, Sulfur Dioxide and Carbon Monoxide: Executive Summary*; World Health Organization: Geneva, Switzerland, 2021; ISBN 9789240034228.
38. Bonassi, S.; Coskun, E.; Ceppi, M.; Lando, C.; Bolognesi, C.; Burgaz, S.; Holland, N.; Kirsh-Volders, M.; Knasmueller, S.; Cavallo, D.; et al. The HUMAN MicroNucleus project on exfoliated buccal cells (HUMNXL): The role of life-style, host factors, occupational exposures, health status, and assay protocol. *Mutat. Res./Rev. Mutat. Res.* **2011**, *728*, 88–97. [\[CrossRef\]](#)
39. Bolognesi, C.; Bonelli, L.; Compalati, A.; Ferla, V.; Stagnaro, L.; Ubezio, G.; Bruzzi, P. “Normal values” for the lymphocyte cytokinesis-block micronucleus cytome parameters: Repeatability and reproducibility in a healthy reference population. *Sci. Total Environ.* **2019**, *652*, 513–522. [\[CrossRef\]](#)
40. Bolognesi, C.; Bruzzzone, M.; Ceppi, M.; Kirsch-Volders, M. The lymphocyte cytokinesis block micronucleus test in human populations occupationally exposed to vinyl chloride: A systematic review and meta-analysis. *Mutat. Res./Rev. Mutat. Res.* **2017**, *774*, 1–11. [\[CrossRef\]](#)
41. Ceppi, M.; Gallo, F.; Bonassi, S. Study design and statistical analysis of data in human population studies with the micronucleus assay. *Mutagenesis* **2011**, *26*, 247–252. [\[CrossRef\]](#)
42. Hopf, N.B.; Danuser, B.; Bolognesi, C.; Wild, P. Age related micronuclei frequency ranges in buccal and nasal cells in a healthy population. *Environ. Res.* **2020**, *180*, 108824. [\[CrossRef\]](#)
43. Haveric, A.; Haveric, S.; Ibrulj, S. Micronuclei frequencies in peripheral blood and buccal exfoliated cells of young smokers and non-smokers. *Toxicol. Mech. Methods* **2010**, *20*, 260–266. [\[CrossRef\]](#)
44. Ferraz, G.A.; Neto, A.O.C.; Cerqueira, E.M.M.; Meireles, J.R.C. Effects of age on the frequency of micronuclei and degenerative nuclear abnormalities. *Rev. Bras. Geriatr. E Gerontol.* **2016**, *19*, 627–634. [\[CrossRef\]](#)
45. Burgaz, S.; Karahalil, B.; Bayrak, P.; Taşkın, L.; Yavuzaslan, F.; Bökesoy, I.; Anzion, R.B.; Bos, R.P.; Platin, N. Urinary cyclophosphamide excretion and micronuclei frequencies in peripheral lymphocytes and in exfoliated buccal epithelial cells of nurses handling antineoplastics. *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* **1999**, *439*, 97–104. [\[CrossRef\]](#)
46. Perroni, F.; Cardinali, L.; Cignitti, L.; Gobbi, E.; Grugni, F.; Amatori, S.; Rocchi, M.B.L.; Grandinetti, L.; Lunetta, F.; Stocchi, V.; et al. Are there sex differences in physiological parameters and reaction time responses to overload in firefighters? *PLoS ONE* **2021**, *16*, e0249559. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Idolo, A.; Grassi, T.; Bagordo, F.; Panico, A.; De Giorgi, M.; Serio, F.; Guido, M.; Piscitelli, P.; De Filippis, G.; Raho, A. Micronuclei in exfoliated buccal cells of children living in a cluster area of Salento (Southern Italy) with a high incidence of lung cancer: The IMP. AIR Study. *Int. J. Environ. Res. Public Health* **2018**, *15*, 1659. [\[CrossRef\]](#) [\[PubMed\]](#)
48. Ernst, B.; Setayesh, T.; Nersesyan, A.; Kundi, M.; Fenech, M.; Bolognesi, C.; Mišík, M.; Ghane, M.; Mousavi, S.F.; Knasmüller, S. Investigations concerning the impact of consumption of hot beverages on acute cytotoxic and genotoxic effects in oral mucosa cells. *Sci. Rep.* **2021**, *11*, 23014. [\[CrossRef\]](#) [\[PubMed\]](#)
49. Pappas, R.; Polzin, G.M.; Zhang, L.; Watson, C.H.; Paschal, D.C.; Ashley, D.L. Cadmium, lead, and thallium in mainstream tobacco smoke particulate. *Food Chem. Toxicol.* **2006**, *44*, 714–723. [\[CrossRef\]](#) [\[PubMed\]](#)
50. Bloching, M.; Hofmann, A.; Lautenschläger, C.; Berghaus, A.; Grummt, T. Exfoliative cytology of normal buccal mucosa to predict the relative risk of cancer in the upper aerodigestive tract using the MN-assay. *Oral. Oncol.* **2000**, *36*, 550–555. [\[CrossRef\]](#) [\[PubMed\]](#)
51. Lucero, L.; Pastor, S.; Suárez, S.; Gómez, C.; Parrón, T.; Creus, A.; Marcos, R. Cytogenetic biomonitoring of Spanish greenhouse workers exposed to pesticides: Micronuclei analysis in peripheral blood lymphocytes and buccal epithelial cells. *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* **2000**, *464*, 255–262. [\[CrossRef\]](#) [\[PubMed\]](#)
52. Bonassi, S.; Neri, M.; Lando, C.; Ceppi, M.; Lin, Y.; Chang, W.; Holland, N.; Kirsch-Volders, M.; Zeiger, E.; Fenech, M. Effect of smoking habit on the frequency of micronuclei in human lymphocytes: Results from the Human MicroNucleus project. *Mutat. Res./Rev. Mutat. Res.* **2003**, *543*, 155–166. [\[CrossRef\]](#)
53. Fenech, M.; Bonassi, S. The effect of age, gender, diet and lifestyle on DNA damage measured using micronucleus frequency in human peripheral blood lymphocytes. *Mutagenesis* **2011**, *26*, 43–49. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Garcia, P.; Linhares, D.; Amaral, A.F.S.; Rodrigues, A.S. Exposure of thermoelectric power-plant workers to volatile organic compounds from fuel oil: Genotoxic and cytotoxic effects in buccal epithelial cells. *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* **2012**, *747*, 197–201. [\[CrossRef\]](#)
55. Hallare, A.; Gervasio, M.K.R.; Gervasio, P.L.G.; Acacio-Claro, P.J.B. Monitoring genotoxicity among gasoline station attendants and traffic enforcers in the City of Manila using the micronucleus assay with exfoliated epithelial cells. *Environ. Monit. Assess.* **2009**, *156*, 331–341. [\[CrossRef\]](#)

56. Martínez-Valenzuela, C.; Gómez-Arroyo, S.; Villalobos-Pietrini, R.; Waliszewski, S.; Calderón-Segura, M.E.; Félix-Gastélum, R.; Alvarez-Torres, A. Genotoxic biomonitoring of agricultural workers exposed to pesticides in the north of Sinaloa State, Mexico. *Environ. Int.* **2009**, *35*, 1155–1159. [[CrossRef](#)]
57. Çelik, A.; Çavaş, T.; Ergene-Gözükara, S. Cytogenetic biomonitoring in petrol station attendants: Micronucleus test in exfoliated buccal cells. *Mutagenesis* **2003**, *18*, 417–421. [[CrossRef](#)]
58. Corrêa, N.S.; Bassan, J.S.; Cunha, C.J.; Fernández, R.R.; Bachettini, P.S.; Garcias, G.L.; Martino-Roch, M.G. Monitoring the genotoxic action in shoe workers by micronucleus test, Pelotas, Rio Grande do Sul State/Monitoramento da acao genotoxica em trabalhadores de sapatarias atraves do teste de micronucleos, Pelotas, Rio Grande do Sul. *Ciência Saúde Coletiva* **2009**, *14*, 2251–2261. [[CrossRef](#)]
59. IARC. Drinking Coffee, Mate, and Very Hot Beverages. In *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*; International Agency for Research on Cancer: Lyon, France, 2018; Volume 116.
60. Iko Afé, O.H.; Kpoclou, Y.E.; Douny, C.; Anihouvi, V.B.; Igout, A.; Mahillon, J.; Hounhouigan, D.J.; Scippo, M.-L. Chemical hazards in smoked meat and fish. *Food Sci. Nutr.* **2021**, *9*, 6903–6922. [[CrossRef](#)] [[PubMed](#)]
61. Fenech, M.; Baghurst, P.; Luderer, W.; Turner, J.; Record, S.; Ceppi, M.; Bonassi, S. Low intake of calcium, folate, nicotinic acid, vitamin E, retinol, β -carotene and high intake of pantothenic acid, biotin and riboflavin are significantly associated with increased genome instability—Results from a dietary intake and micronucleus index survey in South Australia. *Carcinogenesis* **2005**, *26*, 991–999. [[PubMed](#)]
62. Thomas, P.; Wu, J.; Dhillon, V.; Fenech, M. Effect of dietary intervention on human micronucleus frequency in lymphocytes and buccal cells. *Mutagenesis* **2011**, *26*, 69–76. [[CrossRef](#)] [[PubMed](#)]
63. Fenech, M. Genome health nutrigenomics and nutrigenetics—diagnosis and nutritional treatment of genome damage on an individual basis. *Food Chem. Toxicol.* **2008**, *46*, 1365–1370. [[CrossRef](#)]
64. Ray, M.R.; Basu, S.; Mukherjee, S.; Roychowdhury, S.; Lahiri, T. Micronucleus frequencies and nuclear anomalies in exfoliated buccal epithelial cells of firefighters. *Int. J. Hum. Genet.* **2005**, *5*, 45–48. [[CrossRef](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

Supplementary Material

Table S1. Summary of parameters considered for inhalation dose estimation, specifically the concentration of PM both indoor and outdoor in the seven sampled fire station (A); the time of exposure both indoor and outdoor (B) and the Inhalation Rate in males and in females, by age category, unadjusted for body weight (C).

Parameters considered for inhalation dose estimation:								
A. Mean concentration of PM (µg/m³):								
		FS1	FS2	FS3	FS4	FS5	FS6	FS7
Indoors	PM ₁₀	16.10	7.90	7.50	11.90	9.30	11.50	9.20
	PM _{2.5}	14.10	6.30	6.40	10.50	7.70	9.20	6.70
Outdoors	PM ₁₀	19.00	15.80	27.00	25.00	17.00	23.00	21.60
	PM _{2.5}	4.00	2.30	7.70	6.00	6.00	3.00	2.70
B. Time of exposure:								
Daily time spent in FC (hours/day) ^a	Daily time spent indoors (hours/day) ^b	Daily time spent indoors (min/day)	Daily time spent outdoors (hours/day) ^c	Daily time spent outdoors (min/day)				
12.00	3.60	216	8.40	504				
8.00	2.40	144	5.60	336				
C. Inhalation Rate by Age Group:								
Age (years)	Males		Females					
	Daily Inhalation Rate (m³/day) ^d	Inhalation Rate Min (m³/min)	Daily Inhalation Rate (m³/day) ^e	Inhalation Rate Min (m³/min)				
16 to <21	17.21	1.19 × 10 ⁻²	13.59	9.44 × 10 ⁻³				
21 to <31	18.82	1.31 × 10 ⁻²	14.57	1.01 × 10 ⁻²				
31 to <41	20.29	1.41 × 10 ⁻²	14.98	1.04 × 10 ⁻²				
41 to <51	20.94	1.45 × 10 ⁻²	16.20	1.13 × 10 ⁻²				
51 to <61	20.91	1.45 × 10 ⁻²	16.19	1.12 × 10 ⁻²				
61 to <71	17.94	1.25 × 10 ⁻²	12.99	9.02 × 10 ⁻³				

^a Information retrieved from the questionnaire; ^b Considering a reported permanence of 30%; ^c Considering a reported permanence of 70%; ^d Adapted from Table 6.14 of Exposure Factors Handbook (USEPA, 2011); ^e Adapted from Table 6.15 from Exposure Factor Handbook (USEPA, 2011).

B.

**INVESTIGATING THE EXPOSURE-INDUCED BIOLOGICAL MECHANISMS
ASSOCIATED WITH WILDLAND FIREFIGHTING OCCUPATIONAL EXPOSURE**

B. 1. Impact of Occupational Exposure to Wildfire Events on Systemic Inflammatory Biomarkers in Portuguese Wildland Firefighters (Paper II)

Esteves F., Madureira J., Barros B., Alves S., Pires J., Martins S., Oliveira M., Vaz J., Slezakova K., Pereira M. C., Fernandes A., Morais S., Guimarães J. T., Bonassi S., Teixeira J. P. & Costa S.

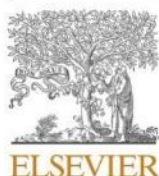
Reprinted from Environmental Research 277, 121608

Copyright © 2025 The Authors. Published by Elsevier Inc.

Open access article under the CC BY license.

Esteves, F., Madureira, J., Barros, B., Alves, S., Pires, J., Martins, S., Oliveira, M., Vaz, J., Slezakova, K., Pereira, M.C., Fernandes, A., Morais, S., Guimarães, J.T., Bonassi, S., Teixeira, J.P. & Costa, S. (2025). Impact of Occupational Exposure to Wildfire Events on Systemic Inflammatory Biomarkers in Portuguese Wildland Firefighters. Environmental Research, 277, 121608. <https://doi.org/10.1016/j.envres.2025.121608>

The PhD candidate was responsible for the conceptualization and writing of the manuscript.



Contents lists available at ScienceDirect

Environmental Research

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

Impact of occupational exposure to wildfire events on systemic inflammatory biomarkers in Portuguese wildland firefighters

Filipa Esteves^{a,b,c,d}, Joana Madureira^{a,b,c}, Bela Barros^e, Sara Alves^f,
Joana Pires^{a,b,c}, Sandra Martins^{b,g}, Marta Oliveira^e, Josiana Vaz^{h,i}, Klara Slezakova^j,
Maria do Carmo Pereira^j, Adília Fernandes^h, Simone Morais^e, João Tiago Guimarães^{b,g,k},
Stefano Bonassi^{l,m}, João Paulo Teixeira^{a,b,c,*}, Solange Costa^{a,b,c}

^a Environmental Health Department, National Institute of Health, Rua Alexandre Herculano, n° 321, 4000-055, Porto, Portugal

^b EPIUnit - Instituto de Saúde Pública, Universidade do Porto, Rua das Taipas, n° 135, 4050-600, Porto, Portugal

^c Laboratório para a Investigação Integrativa e Translacional em Saúde Populacional (ITR), Universidade do Porto, Rua das Taipas, n° 135, 4050-600, Porto, Portugal

^d Department of Public Health and Forensic Sciences, and Medical School, Faculty of Medicine, University of Porto, Rua Doutor Plácido da Costa, 4200-450, Porto, Portugal

^e REQUIMTE/LAQV, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Dr. António Bernardino de Almeida 431, 4249-015, Porto, Portugal

^f Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253, Bragança, Portugal

^g Department of Clinical Pathology, São João University Hospital Centre, 4200-319, Porto, Portugal

^h Research Centre for Active Living and Wellbeing (LiveWell), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253, Bragança, Portugal

ⁱ CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253, Bragança, Portugal

^j LEPABE-ALICE, Faculdade de Engenharia da Universidade do Porto, Rua Dr. Roberto Frias, 4200-465, Porto, Portugal

^k Unit of Biochemistry, Department Biomedicine, Faculty of Medicine, University of Porto, 4200-319, Porto, Portugal

^l Unit of Clinical and Molecular Epidemiology, IRCCS San Raffaele Roma, 00163, Rome, Italy

^m Department of Human Sciences and Quality of Life Promotion, San Raffaele University, 00166, Rome, Italy

ARTICLE INFO

Keywords:

Wildland firefighters
Inflammation
Cytokines
Biomarkers
Woodsmoke exposure
Firefighting

ABSTRACT

While occupational exposure as a firefighter is considered a dangerous occupation, research on the underlying mechanisms remains limited, particularly in wildland firefighters. Inflammation, a key effect of wildfire exposure, plays a significant role in the development of various diseases. The current study aims to investigate the impact of wildland firefighting exposure on the levels of pro-inflammatory systemic biomarkers. A pre-post study design investigated 59 wildland firefighters comparing data collected after participation in a wildfire event (Phase II) with data obtained before wildfire season (Phase I). Data on demographics, lifestyle, health and occupational-related factors were assessed. Exposure factors, such as fire combat (e.g., exposure duration), were also registered. Inflammatory biomarkers (i.e. interleukin-6 [IL-6], interleukin-8 [IL-8], tumor necrosis factor α [TNF- α] and high-sensitivity C-reactive protein [hs-CRP]) and hydroxylated polycyclic aromatic hydrocarbons metabolites (1-OHNaph+1-OHAce, 2-OHFlu, 1-OHPhen, 1-OHPyr) were analysed in blood and urine samples, respectively. Serum IL-8 and IL-6 levels were significantly increased after wildland fire combat. IL-8 levels were 2.62 times higher (95 % CI: 1.96–3.50; $p < 0.01$), whereas IL-6 levels were 1.25 times higher (95 % CI: 1.00–1.57; $p = 0.04$). Furthermore, IL-8 levels were significantly correlated with urinary 2-hydroxyfluorene levels and fire combat duration (>12 h). In addition, the mean hs-CRP level, in both phases, was above 3.0 mg/L, indicating a potential risk for cardiovascular events. Given the long-term health implications of firefighting occupational exposure, biomonitoring and early detection of occupational risks are essential for protecting firefighters' health. Protective measures must be urgently implemented to enhance occupational health and strengthen preventive strategies in this sector.

* Corresponding author. Environmental Health Department, National Institute of Health, Rua Alexandre Herculano, n° 321, 4000-055, Porto, Portugal.
E-mail address: joao.teixeira@insa.min-saude.pt (J.P. Teixeira).

<https://doi.org/10.1016/j.envres.2025.121608>

Received 10 November 2024; Received in revised form 25 March 2025; Accepted 12 April 2025

Available online 14 April 2025

0013-9351/© 2025 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Over recent decades, the intensity and severity of wildland fires have been increasing (Knorr et al., 2017). This trend is expected to persist in a progressively warmer world facing the challenges of global climate change (Ellis et al., 2022). Firefighting is a physically and psychologically demanding occupation, requiring firefighters to face dangerous and complex exposures (Reinhardt and Ottmar, 2004; Adetona et al., 2016; Navarro et al., 2019). Wildfire smoke contains a wide range of toxic compounds (e.g., carbon monoxide, methane, nitrogen oxides) (International Agency for Research on Cancer (IARC), 2023; Naeher et al., 2007) some of which are known carcinogens such as particulate matter (PM_{2.5}), heavy metals and polycyclic aromatic hydrocarbons (PAHs) (International Agency for Research on Cancer (IARC), 2010; Esteves et al., 2022). The exposure to hazardous contaminants, along with long work shifts and high physiological demands in challenging environments, contribute to the occupational health risk observed among wildland firefighters (Adetona et al., 2016; Esteves et al., 2022; Reid et al., 2016). Apart from respiratory and cardiovascular outcomes, firefighters, in general, are at a higher risk than the general population for developing certain types of cancer (International Agency for Research on Cancer (IARC), 2023). In 2022, the International Agency for Research on Cancer (IARC) classified firefighting occupational exposure as "carcinogenic to humans" (Group 1) (International Agency for Research on Cancer (IARC), 2023). However, studies on exposure-induced biological mechanisms among wildland firefighters remain limited (International Agency for Research on Cancer (IARC), 2023). This gap may be attributed to the unpredictability of wildfire events and the difficulty in collecting data in such challenging environments (Taeger et al., 2023). Thus, most available studies have focused on structural fire exposures, simulated/training conditions, or exposure to controlled fire (e.g., prescribed burns) (International Agency for Research on Cancer (IARC), 2023).

Wildland firefighters face various occupational stressors such as heat stress, intensive physical exercise (Aisbett et al., 2012), mental stress (Orysiak et al., 2022), and exposure to woodsmoke-air contaminants (Naeher et al., 2007). Previous studies have found an association between the inhalation of woodsmoke pollutants and local inflammatory responses in both upper and lower airways (Gianniou et al., 2018), linking it to respiratory problems such as asthma, chronic obstructive pulmonary disease, and sinusitis (Naeher et al., 2007). One of the initial responses of the innate immune system is the secretion of pro-inflammatory cytokines, such as IL-6, IL-8, and TNF- α , which are essential for initiating the acute inflammatory response (Abbas, 1991). Some of these pro-inflammatory cytokines (IL-6, TNF- α) stimulate the liver production of acute phase proteins, such as CRP (Akira and Kishimoto, 1992; Shephard and Shek, 1995), which play a fundamental role in the inflammatory response and in the modulation of immune system activity (Nehring et al., 2017). However, when acute inflammation is not effectively resolved by anti-inflammatory mediators (e.g., anti-inflammatory cytokines, glucocorticoids) it can progress to chronic inflammation (Orysiak et al., 2022), particularly in the case of recurrent acute stimuli. This continued over-stimulation of the immune system may lead to chronic low-grade systemic inflammation which has been associated with numerous health implications (Brenner et al., 1994; Bauer, 2005), such as cardiovascular (Guarner and Rubio-Ruiz, 2015; Kim et al., 2018), respiratory (Orysiak et al., 2022), metabolic disorders (Hotamisligil, 2006), mental health (Renna et al., 2018; Passos et al., 2015; Slavich and Irwin, 2014) and cancer (International Agency for Research on Cancer (IARC), 2023; Orysiak et al., 2022; Chen et al., 2018; Singh et al., 2018; Greten and Grivennikov, 2019).

Human biomonitoring studies are crucial for evaluating the association between occupational exposure and adverse health outcomes (Vorkamp et al., 2021). The evaluation of biomarkers of exposure (i.e., the xenobiotic and/or its metabolites, or the products of the interaction between the xenobiotic and target molecules) is an important tool

widely used in occupational health risk assessment and preventive medicine (Esteves et al., 2022), since it quantifies the real dose to which the organism is exposed to, taking in account the individual characteristics. Most of the biomonitoring studies studying firefighting occupational exposure were focused on the assessment of hydroxylated metabolites of PAHs in urine (OHPAHs) (Caux et al., 2002; Fent et al., 2014, 2019; Fernando et al., 2016; Keir et al., 2017), reflecting all the PAH exposure routes (i.e., inhalation, dermal absorption and ingestion) (Wingfors et al., 2018; Hwang et al., 2022).

However, biomarkers of exposure alone do not provide information about the effects that may be induced by the exposure (Esteves et al., 2022), but only estimate the organism's exposure to the xenobiotic. The integration of biomarkers of effect is crucial for evaluating the biological events that occur later in the continuum between exposure and disease (Esteves et al., 2022). A biomarker of effect is defined as a measurable biochemical, physiological, behavioural, functional, or other alteration within an organism that might be elicited by exposure (Costa and Esteves, 2023). It may represent an event that can be correlated with, and possibly predictive of, a deleterious health effect (Costa and Esteves, 2023).

High-sensitivity C-reactive protein (hs-CRP) is a sensitive biomarker of systemic inflammation and a valuable biomarker for predicting cardiovascular risk (Silva and de Lacerda, 2012; Ridker et al., 2007), identifying adults with CRP levels greater than 3.0 mg/L as at higher risk for developing cardiovascular disease (e.g., heart attack, stroke) (Li et al., 2017; Ridker, 2003; Cushman et al., 2009). The application of this effect biomarker in firefighters is particularly interesting because cardiac events have been considered an important cause of death among these professionals (Esteves et al., 2024a). According to the National Fire Protection Association, cardiovascular events such as heart attacks, are among the most common causes of death among on-duty firefighters (Fahy, 2005). Circulating cytokines (e.g., interleukin-6 [IL-6], interleukin-8 [IL-8], and tumour necrosis factor- α [TNF- α]) and hs-CRP have been used as biomarkers of inflammation after woodsmoke exposure and ambient particulates (van Eeden et al., 2005; Swiston et al., 2008). Despite some studies have measured inflammatory biomarkers among wildland firefighters (Gianniou et al., 2018; Swiston et al., 2008; Wolkow et al., 2015; Cherry et al., 2021), most have been confined to changes over a single shift/event (Swiston et al., 2008; Adetona et al., 2017a), not considering their assessment in distinct time points of the wildfire season.

The use of biomarkers is of utmost value to understand the possible mechanisms involved in the cellular events resulting from firefighting occupational exposure (Bocato et al., 2019), especially due to the lack of occupational studies among firefighters integrating both biomarkers of exposure and effect (Andersen et al., 2018a; Oliveira et al., 2020; Esteves et al., 2024b). To the best of the authors' knowledge, no studies have explored the association between systemic levels of inflammatory biomarkers and urinary PAH metabolites in the context of wildland firefighters' occupational exposure to real wildfire events.

Thus, this study aims to investigate the impact of wildfire exposure on systemic pro-inflammatory biomarkers in paired samples of wildland firefighters from the northern region of Portugal. Additionally, we aim to examine the potential relationship between levels of systemic inflammatory biomarkers and urinary PAH metabolites following wildfire occupational exposure, as well as with other self-reported exposure-related variables.

2. Material and methods

2.1. Study population

Our study enrolled 59 healthy wildland firefighters from five fire stations located in a predominantly rural district in the Northern region of Portugal. All participants had at least one year of service and were actively involved in wildfire combat activities. This pre-post study

design involved paired assessments, with each firefighter serving as their own control. Participation involved biomonitoring the firefighters at two distinct points: Phase I - before the wildfire season, during late spring (low exposure); and Phase II - after wildfire combat, during the wildfire season, in summer (higher exposure). The summer period, specifically from July to September, is recognised as the critical fire season in Portugal (Bergonse et al., 2021). Data collection was carried out between 2021 and 2023 with sampling intervals varying according to wildland firefighters' involvement in firefighting activities. During this period, firefighters were deployed to wildfires occurring in their respective regions assigned to each fire station; besides wildfires considered for the present study, no other major wildfires, in this period, occurred in this region of Portugal.

In Phase I, during the off-season, firefighters were fully informed about the study objectives, procedures, potential benefits, and associated risks. They were also instructed on data confidentiality and protection, as well as the possibility to withdraw from the study at any time without any consequences. Each participant who agreed voluntarily to participate signed an informed consent form before the start of the study. A self-administered questionnaire was used in Phase I to collect data on sociodemographic characteristics (e.g., sex, age), lifestyle habits (e.g., smoking), anthropometric measurements (i.e., weight and height) and occupational-related information (e.g., shift duration, years of service). In this Phase (off-season), firefighters did report no recent occupational exposure to fire occurrences.

In Phase II, after the wildfire combat, firefighters were asked to complete a brief questionnaire with questions about the fire event and activities (e.g., exposure duration). Our study was conducted under the Declaration of Helsinki (World Medical Association, 2013; Williams, 2008). Ethical approval was granted by the Ethics Committee of the University of Porto, Portugal (reference number 92/CEUP/2020).

2.2. Biological sample collection

Blood and urine samples were collected simultaneously for each individual during Phase I and Phase II. In Phase I, blood samples were collected by venipuncture, between 10 and 11 a.m., and transported to the laboratory under refrigerated conditions (4 °C, transport within 2 h) to be immediately processed and consecutively stored at -80 °C for further analysis; a spot urine sample was also collected in sterile polyethylene containers (100 mL) and frozen at -20 °C until analysis.

During Phase II, post-exposure biological samples (blood and urine) were collected from firefighters involved in wildland fire combat/post-combat activities, once they returned to the fire station. After collection, all samples were transported in a cooler to the laboratory to be processed.

2.3. Urinary OHPAHs analysis

Quantification of urinary OHPAHs was performed as in Barros et al. (2024) solid-phase extraction and high-performance liquid chromatography with a fluorescence detector. The following urinary PAH biomarkers were determined: 1-hydroxynaphthalene (1-OHNaph), 1-hydroxyacenaphthene (1-OHAce), 2-hydroxyfluorene (2-OHFlu), 1-hydroxyphenanthrene (1-OHPhen) and 1-hydroxypyrene (1-OHPyr). Calibration curves, along with the limits of detection (LOD) and limits of quantification (LOQ), were defined for each biomarker. Quality control was ensured by assessing intra-day and inter-day precision using relative standard deviation (RSD). Samples were analysed in triplicate for reliability. The methodology's accuracy was verified through recovery assays, with recovery rates ranging from 70 % to 117 %. Concentrations of OHPAHs were normalised to creatinine levels ($\mu\text{mol/mol creatinine}$).

2.4. Serum IL-6, IL-8, TNF- α evaluation

Whole blood collected in serum tubes (Vacuette® Ref#456092) was

allowed to coagulate at room temperature for 1–2 h. After clotting, samples were centrifuged at 720 rcf for 10 min and serum was then aliquoted and frozen at -80 °C until analysis. Pro-inflammatory biomarkers (TNF- α , IL-6, IL-8) were evaluated using multiplex immunoassays on a Luminex 200™ xMAP™ analyser (Luminex Corporation, Austin, TX, USA), following the protocol of the Human High Sensitivity T Cell Magnetic Bead Panel (Milliplex® MAP kit, Millipore Corporation, Billerica, MA, USA). Raw data analysis (mean fluorescence intensity, MFI) was performed using a standard five-parameter logistic (5-PL) curve fit generated by the Luminex xPONENT® Software (version 3.1).

2.5. Plasma hs-CRP quantification

Plasma was obtained from whole blood collected in K2EDTA tubes (Vacuette® Ref#367864) after centrifugation at 720 rcf during 10 min. Afterward, plasma was aliquoted and kept at -80 °C. Plasma levels of hs-CRP were measured by a particle-enhanced immunoturbidimetry method (CRP LX HS, Roche Diagnostics, Rotkreuz, Switzerland) in a Cobas Integra® 400 plus analyser (Roche Diagnostics, Switzerland) according to the manufacturer's instructions with a detection limit of 0.01–2.00 mg/dL.

2.6. Statistical analysis

Descriptive statistics were performed for quantitative and qualitative variables. Individual characteristics, stratified by sex, are presented as mean $\pm$ standard deviation (SD) for continuous variables and as absolute numbers and frequencies for categorical variables. For comparisons between sex groups, the independent *t*-test was used for continuous variables, and the chi-square test of independence was applied for categorical variables. For smoking habits participants were categorised as current smokers and non-current smokers. Non-current smokers included individuals who had never smoked or those who had ceased smoking for more than one year. The normality of data distribution was assessed using the one-sample Kolmogorov-Smirnov test and data dispersion graphs. Data that were not normally distributed are presented as medians with interquartile ranges (IQR). Univariate analysis was conducted to investigate potential associations between inflammatory biomarkers (i.e. IL-6, IL-8, TNF- α , and hs-CRP) and questionnaire retrieved variables (i.e., individual characteristics, lifestyle, and occupational-related factors). Whenever the data did not follow a normal distribution, statistical differences were assessed using the Mann-Whitney *U* test (for two groups) or the Kruskal-Wallis test (for more than two groups). The difference in biomarkers within paired samples (i.e., before and after fire exposure) was evaluated using the Wilcoxon test for non-normally distributed endpoints. Possible correlations among continuous variables were explored using Spearman's rank correlation test. The frequency ratio (FR) (i.e., post-/pre-exposure) and its asymptotic 95 % confidence intervals (95 % CI) were calculated for the studied endpoints i.e. IL-6, IL-8, TNF- α , and hs-CRP.

Inflammatory biomarkers that showed statistically significant differences after fire exposure were further analysed. Linear regression analysis was conducted to identify possible statistically significant associations with exposure variables (i.e. duration of exposure and urinary OHPAHs) and the difference (post-exposure - pre-exposure) of log-transformed inflammatory biomarkers i.e., variation (Δ) inflammatory biomarker = $\log(\text{post-exposure}) - \log(\text{pre-exposure})$. Logarithmic transformation was applied to normalise data distribution and facilitate the calculation of FR using exponentiated coefficients (e^{β}) from linear regression analysis. A statistical significance level of 0.05 was considered. The statistical software used for all analyses was IBM SPSS Statistics 29.0 for Windows (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Population characterisation

Table 1 summarises the general characteristics of the study population. Despite the traditionally male-dominated nature of the firefighting occupation with women often under-represented (International Agency for Research on Cancer (IARC), 2023), nearly a quarter of our participants were women (22.0 %). The overall mean age was 35.5 ± 9.0 years.

Regarding Body Mass Index (BMI) [weight (kg) $\times$ height (m)⁻²] the average was 26.5 ± 3.3 kg/m². Half of our participants were overweight, 11.9 % were obese, and 37.3 % had a normal weight, according to the World Health Organization classification (World Health Organization, 2022). In terms of smoking habits, 54.2 % reported being current smokers, with males accounting for the larger proportion.

Average years of service as a firefighter was 15.9 ± 8.9 years, with no significant differences between males (16.1 ± 9.4 years) and females (14.8 ± 6.7 years). Of the participants, 44.8 % (n = 26) were members of the Permanent Intervention Team (full-time contracted firefighters), while the remaining firefighters were volunteers, with no contract. Moreover, as shown in Table I, a significant association between sex and permanent intervention teams was observed, with a higher proportion of males in these teams. The Permanent Intervention Team members had an average of 16.35 ± 8.59 years of service, while volunteers had an average of 15.37 ± 9.49 years of service (data not shown). On average, firefighters engaged in wildfire suppression activities for 11 h, with 28.8 % (n = 17) of them spending more than 12 h in the field. Wildland firefighters under the age of 36 years spent an average of 10 h at the fire scene, whereas those aged 36 and older spent an average of 12 h in wildfire suppression activities (data not shown).

3.2. Inflammatory biomarkers (IL-6, IL-8, TNF- α and hs-CRP)

Descriptive statistics for the concentrations of IL-6, IL-8, TNF- α , and

Table 1
General characteristics of the study population (n = 59).

Study participant characteristics	Wildland Firefighters (n = 59)	Male (n = 46)	Female (n = 13)
Individual characteristics			
Age (years) ^a	35.5 ± 9.00 (20.0–55.0)	35.6 ± 9.3 (20.0–55.0)	35.1 ± 8.0 (21.0–45.0)
Body Mass Index (BMI) (kg m ⁻²) ^a	26.5 ± 3.3 (19.4–35.9)	26.7 ± 3.3 (19.4–35.9)	26.0 ± 8.0 (21.2–33.0)
Normal weight, n (%)	22 (37.3 %)	17 (37.0 %)	5 (38.5 %)
Overweight, n (%)	30 (50.8 %)	23 (50.0 %)	7 (53.8 %)
Obesity, n (%)	7 (11.9 %)	6 (13.0 %)	1 (7.7 %)
Lifestyle-related			
Smoking habits			
Non-current smoker	27 (45.8 %)	19 (41.3 %)	8 (61.5 %)
Current smoker	32 (54.2 %)	27 (58.7 %)	5 (38.5 %)
Cigarettes/day ^a	16.91 (1.0–35.0)	18.22 (1.0–35.0)	9.80 (4.0–20.0)
Occupational-related			
Years of service ^a	15.9 ± 8.9 (3.0–34.0)	16.1 ± 9.4 (3.0–34.0)	14.8 ± 6.7 (7.0–28.0)
Permanent Intervention Teams			
No, n (%)	32 (55.2 %)	21 (45.7 %)	11 (91.7 %)
Yes, n (%)	26 (44.8 %)	25 (54.3 %)	1 (8.3 %)
Active wildfire exposure			
Exposure duration (hours) ^a	11.0 ± 11.7 (1.0–55.0)	11.7 ± 12.3 (1.0–55.0)	8.7 ± 9.2 (1.0–24.0)
≤ 12 h, n (%)	42 (71.2 %)	32 (69.6 %)	10 (76.9 %)
> 12 h, n (%)	17 (28.8 %)	14 (30.4 %)	3 (23.1 %)

^aStatistical significance was determined using the Chi-square test ($p < 0.05$).

^a Values are expressed as mean $\pm$ SD (standard deviation), with the range presented as min – max.

hs-CRP in both Phase I and Phase II are presented in Table S1 of the supplementary material. A statistically significant increase in IL-8 and IL-6 serum levels was observed after the fire combat compared with the levels accessed before the wildfire season ($p < 0.01$ and $p = 0.04$, respectively). The analysis was performed using paired samples t-tests. Data are presented as mean $\pm$ standard error (SE), and p-values reflect these comparisons between pre- and post-fire combat measurements. Despite no statistically significant difference in hs-CRP levels being observed (Phase I: 3.65 ± 1.05 mg/L; Phase II: 3.24 ± 0.70 mg/L) the mean level of hs-CRP in both phases was greater than 3.0 mg/L indicating a higher risk for cardiovascular events, according to cut-offs values suggested for adults (Silva and de Lacerda, 2012; Cushman et al., 2009; Pearson et al., 2003).

When studying the influence of sex, age, smoking habits, or occupational variables (e.g., years of service, permanent intervention teams) on our endpoints, no significant effect was found (Table S2, Supplementary material). However, TNF- α levels were found to be significantly different between BMI groups ($p < 0.05$). TNF- α levels were significantly increased in overweight firefighters compared to colleagues with normal BMI.

The Phase I/Phase II-observed variations in the individual levels of inflammatory biomarkers, represented as FR, are illustrated in Fig. 1. IL-8 serum levels were significantly increased following the wildfire-combat exposure (FR: 2.62; 95 % CI: 1.96–3.50, $p < 0.01$), showing levels almost three times higher than those found during the pre-wildfire season. IL-6 levels were also significantly increased after the wildfire combat (FR: 1.25; 95 % CI: 1.00–1.57) reflecting a 25 % higher risk of increased IL-6 levels post-exposure ($p = 0.04$). No significant variation was found for TNF- α and hs-CRP between both study phases, with FRs of 0.99 (95 % CI: 0.94–1.04) and 0.89 (95 % CI: 0.60–1.30), respectively.

Correlation between the different variables and endpoints was also tested. A significant positive association was found between Phase II levels of serum TNF- α and IL-6 levels ($r = 0.31$; $p = 0.02$) (data not shown).

3.3. Urinary OHPAHs biomarkers

Creatinine-corrected urinary OHPAHs concentrations within both

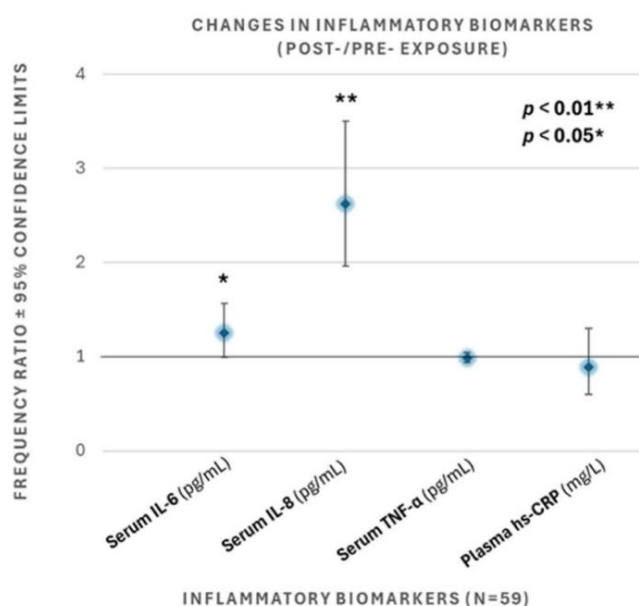


Fig. 1. Frequency ratios showing changes in inflammatory biomarkers [ratio within post (Phase I) and pre-exposure levels (Phase II)]. * and ** indicate the statistical significance of post/pre-exposure ratios, with p-values < 0.05 and < 0.01 , respectively.

phases are presented in Table S3 (Supplementary material). An increase of each studied urinary OHPAHs was observed after firefighting intervention, namely 1-OHNaph+1-OHAce ($\mu\text{mol/mol creatinine}$) (pre: 16.15 ± 2.32 vs. post: 40.34 ± 6.00 ; $p < 0.01$), 2-OHFlu ($\mu\text{mol/mol creatinine}$) (pre: 0.17 ± 0.03 vs. post: 0.21 ± 0.04 ; $p = 0.07$), 1-OHPhen ($\mu\text{mol/mol creatinine}$) (pre: 0.07 ± 0.01 vs. post: 0.13 ± 0.03 ; $p = 0.05$) and 1-OHPyr ($\mu\text{mol/mol creatinine}$) (pre: 0.04 ± 0.01 vs. post: 0.06 ± 0.01 ; $p < 0.01$).

3.4. Impact of firefighting in inflammatory response

The contribution of each exposure-related variable to the variation (Δ) observed on the IL-6 and IL-8 levels was investigated. Δ IL-8 levels were significantly influenced by the exposure duration of the fire-event combat (Phase II). Firefighters who were more than 12 h in the field had approximately two times higher IL-8 levels [FR (95 % CI) = 2.07 (1.12–3.84); $p = 0.02$] compared to those who were 12 h or less (Fig. 2). No significant influence was found for Δ IL-6, regarding exposure-related factors (data not shown).

The association between each urinary OHPAHs on Δ IL-8 was also studied. A significant positive correlation was observed between Δ IL-8 (post- vs. pre-exposure) and post-exposure urinary 2-OHFlu levels ($r = 0.37$; $p < 0.01$) (Fig. 3).

Data from regression analysis confirmed this association, indicating that for every unit increase of 2-OHFlu ($\mu\text{mol/mol creatinine}$), Δ IL-8 increased by 16 % (95 % CI: 1.00, 1.34), after adjusting for age, sex, and smoking habits [FR (95 % CI) = 1.16 (1.00 1.35); $p \leq 0.05$] (data not shown).

In addition, despite no significant differences, a proportional increase in urinary OHPAHs levels was observed across different categories of hs-CRP for cardiovascular risk (low < intermediate < high) as shown in Table 2.

4. Discussion

Our study shows a significant increase in firefighters' serum levels of IL-6 and IL-8 following the wildfire combat. This positive inflammatory response aligns with the scarce previous literature comprising wildland firefighters (Swiston et al., 2008; Main et al., 2020; Adetona et al., 2017b). Swiston et al. (2008) observed a significant increase in both serum IL-6 and IL-8 levels in forest Canadian firefighters ($n = 52$) after an 8-h shift of firefighting activities in a real wildfire scenario. Another study, conducted during prescribed burns in the southeastern United States, also found a significant increase in IL-8 levels in a group of

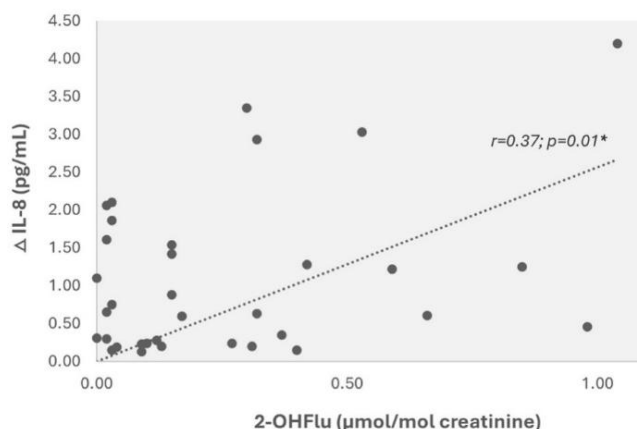


Fig. 3. Correlation within Δ IL-8 levels (pg/mL) with exposure urinary 2-OHFlu levels ($\mu\text{mol/mol creatinine}$). *Statistically significant < 0.05 .

wildland firefighters after their work shift (Hejl et al., 2013). More recently, Main et al. (2020) conducted a study with 38 Australian firefighters enrolled in a real wildfire event, both plasma IL-8 and IL-6 levels were significantly higher after a 12-h shift. Furthermore, Greven et al. (2012) observed among 51 Dutch municipal firefighters (not wildland) an increase in serum IL-8 levels ($p < 0.05$), persisting for up to 3 months.

We also found that, among our group of wildland firefighters, those part of the Permanent Intervention Teams (full-time firefighters) exhibited higher serum IL-8 levels compared to colleagues in Phase I, although this difference was not statistically significant. This finding aligns with previous research, conducted in Greece, reporting greater inflammatory biomarkers (including IL-8) among career firefighters (Gianniou et al., 2016). Our study also showed that firefighters who spent more than 12 h at the wildfire scene had significantly higher IL-8 levels than those with lower exposure duration, showing a potential dependent effect of exposure duration on IL-8 levels. These findings agree with those of Gianniou et al. (2018), who also observed an association between longer participation in forest firefighting and intense systemic inflammation levels among Greek firefighters. Indeed, wildland firefighters' work shifts during a real scenario of wildland fires can last several consecutive days (Betchley et al., 1997). The long-lasting work shifts can place firefighters at greater risk for health effects associated with occupational exposures (Ebert, 2013).

No significant differences were found among our group for plasma hs-CRP levels. The same finding was obtained by two previous studies enrolling wildland firefighters exposed to prescribed burn shifts (controlled fire) (Hejl et al., 2013; Wu et al., 2020). Plasma hs-CRP is an acute-phase protein produced downstream of cytokines release and, thus, likely to undergo a delayed systemic release (van Eeden et al., 2005). IL-6 levels rapidly increase in response to inflammatory stimuli, serving as an early indicator of the systemic inflammatory response (Orysiak et al., 2022). CRP is synthesised by the liver in response to IL-6 and other cytokines, resulting in a delayed peak in its levels (Kramer et al., 2008). However, in both phases, our wildland firefighters had a mean level of hs-CRP above 3.0 mg/L - classified as a high risk for developing cardiovascular disease (Ridker et al., 2007; Li et al., 2017). As described in the literature, wildland firefighters are at high risk of cardiovascular disease due to long-term exposures to cardio-toxic smoke-released pollutants, such as PM_{2.5} and carbon monoxide (Esteves et al., 2024a). Additionally, a sedentary lifestyle, unhealthy diet, high BMI and smoking habits are known risk factors that contribute to high cardiovascular risk (Esteves et al., 2024a). To the best of our knowledge, this is the first study assessing hs-CRP levels - a clinical biomarker of inflammation and cardiovascular risk - among wildland firefighters involved in fire combat of real wildfire scenario.

In accordance with previous studies, urinary PAH levels were

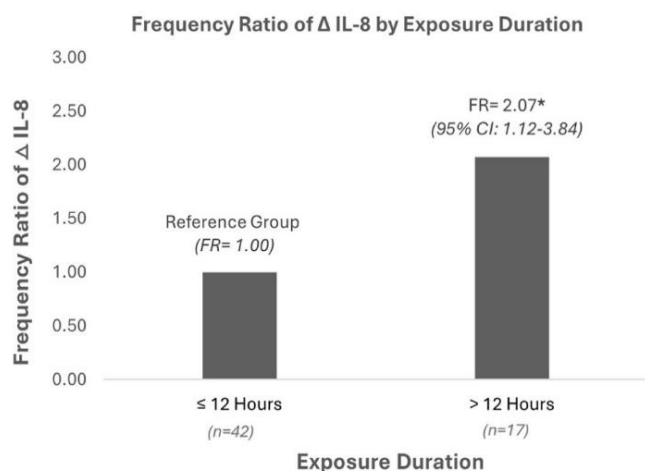


Fig. 2. Frequency ratio of Δ IL8 (post-vs. pre-exposure) by exposure duration: ≤ 12 h vs. > 12 h *Statistically significant < 0.05 .

Table 2

Concentration of urinary OHPAHs metabolites after wildfire exposure by hs-CRP categories for cardiovascular risk (Low < Intermediate < High).

	1-OHNaph+1-OHAce (μmol/mol creatinine)		2-OHFlu (μmol/mol creatinine)		1-OHPhen (μmol/mol creatinine)		1-OHPyr (μmol/mol creatinine)	
	n	Median (IQR ^a)	n	Median (IQR ^a)	n	Median (IQR ^a)	n	Median (IQR ^a)
Low Risk (<1.0 mg/L hs-CRP)	17	22.23 (9.96–40.96)	17	0.06 (0.02–0.31)	17	0.04 (0.02–0.09)	17	0.03 (0.02–0.06)
Intermediate Risk (1.0–3.0 mg/L hs-CRP)	23	36.79 (12.58–43.45)	23	0.10 (0.03–0.17)	23	0.09 (0.04–0.12)	23	0.04 (0.04–0.06)
High Risk (>3.0 mg/L hs-CRP)	15	47.63 (4.34–82.35)	15	0.13 (0.03–0.42)	15	0.10 (0.03–0.07)	15	0.05 (0.03–0.07)

^a Interquartile range (IQR). The Kruskal-Wallis test was used to determine statistical differences between groups.

increased after exposure to woodsmoke (Adetona et al., 2017c). We found a statistically significant association between urinary 2-OHFlu post-fire-levels and IL-8 suggesting that IL-8 may serve as a viable biomarker for monitoring occupational exposure among wildland firefighters. So far, no occupational biomonitoring studies have explored this association, thus, further research is needed to confirm our result.

The use of adequate personal protective equipment (PPE) is essential to mitigate exposure to woodsmoke air contaminants (Esteves et al., 2024a). According to two previous studies conducted by Andersen et al., 2018a,b, no significant changes in IL-8 and IL-6 levels were found in structural firefighters after fire-combat activities. However, in both these studies, firefighters used self-contained breathing apparatus (SCBA). The use of air-supplied SCBA in a wildfire context is not feasible (Esteves et al., 2022), and therefore the lack of complete respiratory protection among wildland firefighters combating a real wildfire may result in a higher pollutant exposure gradient through inhalation.

It is important to note that our study group of wildland firefighters was predominantly male, reflecting the traditionally male-dominated nature of firefighting (International Agency for Research on Cancer (IARC), 2023). Most studies in this field have focused on male firefighters (International Agency for Research on Cancer (IARC), 2023), resulting in limited data on female firefighters. This underscores the need for further research including both sexes. In the present study, we observed no significant differences in the biological endpoints between females and males, though caution is warranted due to the small sample size of female firefighters. Regarding other findings of the present study, no significant association was found between our endpoints and years of service. However, it cannot be dismissed that over the years, firefighters may become less active in combat, potentially leading to a balance in exposure levels. In addition, we observed a significant correlation between IL-6 and TNF-α, confirming their relationship as key pro-inflammatory mediators in response to wildfire exposure. This aligns with earlier research indicating a linked activation of the inflammatory cascade (Abbas, 1991).

Our study has some limitations. Information bias may have been introduced using self-reported questionnaires for data collection. Moreover, the nature of firefighting makes it challenging to isolate other factors that may trigger inflammation, as firefighters are consistently exposed to various co-stressors such as physical exertion, smoke inhalation, psychological stress, heat stress, and sleep restriction, either individually or in combination, which may have contributed to the observed changes. Wildfires are unpredictable events, characterised by variability in timing, intensity, and duration. Wildland firefighting environments are complex and present numerous challenges. Factors such as duration of post-wildfire activities (e.g., mop-up operations), uncertainty regarding return times to the fire station, the need for rest after extended periods of activity, and the fluctuating nature of volunteer firefighter work shifts all contribute to the variability in sample collection timing. Biomarkers such as OHPAHs and inflammatory markers exhibit dynamic temporal patterns following exposure, making the timing of sample collection critical for accurate detection. In our study, although blood and urine samples were collected within a similar time window for all participants, variations in timing may have influenced the biomarker levels observed, potentially leading to an underestimation of some results. In addition, some of our results may potentially be

underestimated since we did not have a “true baseline”, for that only including firefighters before the beginning of their careers. Furthermore, we acknowledge the potential influence of seasonal variations.

However, our study has also significant strengths: This is the first study i) evaluating differences in systemic inflammatory biomarkers among wildland firefighters longitudinally at two distinct time points of wildfire season: during the pre-wildfire season and after fire combat in a real wildfire scenario; ii) evaluating hs-CRP among wildland firefighters – a known clinical marker for cardiovascular risk – considering exposure to real wildfires fire-combat; iii) accessing the association between systemic levels of inflammatory biomarkers and urinary PAH metabolites exposure on wildland firefighters in real scenarios of wildfires. Furthermore, the pre- and post-study design with paired active wildland firefighters enhances the study’s robustness by establishing a temporal relationship between exposures and outcomes. The integration of effect biomarkers with exposure data provides a comprehensive assessment of the inflammatory response.

Evaluation of firefighting occupational exposure is complex and may depend upon multiple factors, such as tasks performed (e.g., front-line firefighter, fire engine driver), duration of exposure, and decontamination procedures after wildfire suppression activities equipment/materials (International Agency for Research on Cancer (IARC), 2023; Fabian et al., 2014). Moreover, other individual factors (e.g., sleep, smoking habits, psychological stress) and external factors (e.g., environmental pollution) may affect inflammatory response (Abbas, 1991). Nonetheless, promoting healthy modifiable behaviours can be an effective strategy to reduce such impact. This involves periodic training sessions, decontamination measures, proper use of PPE, a healthy diet, physical activity, strategies to reduce psychological stress, frequent medical examinations, and healthy sleep hygiene (Esteves et al., 2024a).

5. Conclusions

Although exposure during firefighting is recognised as carcinogenic to humans, the risks associated with this activity are poorly investigated. It is paramount to establish a panel of biomarkers to monitor wildland firefighters’ occupational exposure worldwide, particularly in countries significantly affected by wildfires. The integration of biomarkers of effect with those monitoring exposure in human studies is of utmost value for assessing long-term potential health impacts. Understanding the dynamics of inflammatory biomarkers through time is crucial for their accurate interpretation in acute settings such as wildfire exposure.

Our study found an increased firefighters’ systemic inflammatory response after wildland firefighting activities, a key factor in the development of cardiovascular and other chronic diseases. The data found within exposure-related variables and IL-8 levels suggest it is a suitable biomarker for monitoring occupational exposure of wildland firefighters.

The implementation of preventive strategies and occupational safety measures is crucial to mitigate health risks among firefighters. These strategies should consider the proper use of PPE and adequate postfire decontamination practices. Many occupational diseases, including work-related cancers, have long latency periods, making it difficult to recognise these conditions before clinical symptoms appear. Early detection of occupational risks can significantly reduce the harmful

effects on workers' health, reduce the corresponding health and cost burdens, and improve their quality of life. Further research addressing the risks associated with firefighters' exposure during wildfire season, including the effect of different roles/tasks, is essential to better understand and mitigate occupational hazards, ultimately safeguarding firefighters' health.

CRedit authorship contribution statement

Filipa Esteves: Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Joana Madureira:** Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Bela Barros:** Methodology, Data curation. **Sara Alves:** Methodology, Data curation. **Joana Pires:** Methodology, Data curation. **Sandra Martins:** Software, Resources, Methodology, Data curation. **Marta Oliveira:** Methodology, Data curation. **Josiana Vaz:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Data curation. **Klara Slezakova:** Writing – review & editing, Project administration, Methodology, Investigation, Data curation. **Maria do Carmo Pereira:** Project administration, Funding acquisition. **Adília Fernandes:** Resources, Project administration, Methodology, Funding acquisition, Data curation. **Simone Morais:** Writing – review & editing, Software, Resources, Methodology, Investigation, Funding acquisition, Data curation. **João Tiago Guimarães:** Software, Resources, Methodology, Data curation. **Stefano Bonassi:** Writing – review & editing, Supervision, Software, Resources, Methodology, Formal analysis, Data curation, Conceptualization. **João Paulo Teixeira:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Solange Costa:** Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Ethics approval

This work received approval for research ethics by the Accredited Ethics Committee of the University of Porto, Portugal, Report Nr. 92/CEUP/2020, under the project BioFirEx project (PCIF/SSO/0017/2018): “A panel of (bio)markers for the surveillance of firefighter's health and safety”.

Funding

This work received financial support from the project PCIF/SSO/0017/2018

(<https://doi.org/10.54499/PCIF/SSO/0017/2018>) by the Fundação para a Ciência e a Tecnologia (FCT), Ministério da Ciência, Tecnologia e Ensino Superior (MCTES), through national funds. This work is financed by national funds through the FCT within the scope of projects UIDB/04750/2020 (<https://doi.org/10.54499/UIDB/04750/2020>) and LA/P/0064/2020 (<https://doi.org/10.54499/LA/P/0064/2020>). Filipa Esteves was supported by National Funds through FCT, under the Ph.D. fellowship UI/BD/150783/2020. The work of Stefano Bonassi was supported by the Italian Ministry of Health (Ricerca Corrente), Rome, Italy, and by the Competitive Funding for University Research Projects [Finanziamento competitivo di progetti di ricerca di ateneo (FIN/RIC)] San Raffaele University, Rome, Italy.

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.

Acknowledgments

The authors gratefully acknowledge Marta Alvim, José Vicente Constantino and Armandina Miranda from the Departamento de Promoção da Saúde e Prevenção de Doenças Não Transmissíveis of the National Institute of Health Dr Ricardo Jorge, Lisbon, Portugal, for performing the analysis of hs-CRP.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2025.121608>.

Data availability

The authors do not have permission to share data.

References

- Abbas, A., 1991. *Cellular and Molecular Immunology*, HBJ International Edition, pp. 226–242.
- Adetona, O., Reinhardt, T.E., Domitrovich, J., Broyles, G., Adetona, A.M., Kleinman, M. T., et al., 2016. Review of the health effects of wildland fire smoke on wildland firefighters and the public. *Inhal. Toxicol.* 28 (3), 95–139. <https://doi.org/10.3109/08958378.2016.1145771>.
- Adetona, A.M., Adetona, O., Gogal, Jr RM., Diaz-Sanchez, D., Rathbun, S.L., Naeher, L.P., 2017a. Impact of work task-related acute occupational smoke exposures on select proinflammatory immune parameters in wildland firefighters. *J. Occup. Environ. Med.* 59 (7), 679–690. <https://doi.org/10.1097/JOM.0000000000001053>.
- Adetona, A.M., Adetona, O., Gogal, J.R.M., Diaz-Sanchez, D., Rathbun, S.L., Naeher, L.P., 2017b. Impact of work task-related acute occupational smoke exposures on select proinflammatory immune parameters in wildland firefighters. *J. Occup. Environ. Med.* 59 (7), 679. <https://doi.org/10.1097/JOM.0000000000001053>.
- Adetona, O., Simpson, C.D., Li, Z., Sjödin, A., Calafat, A.M., Naeher, L.P., 2017c. Hydroxylated polycyclic aromatic hydrocarbons as biomarkers of exposure to wood smoke in wildland firefighters. *J. Expo. Sci. Environ. Epidemiol.* 27 (1), 78–83. <https://doi.org/10.1038/jes.2015.75>.
- Aisbett, B., Wolkow, A., Sprajcer, M., Ferguson, S.A., 2012. “awake, smoky, and hot”: providing an evidence-base for managing the risks associated with occupational stressors encountered by wildland firefighters. *Appl. Ergon.* 43 (5), 916–925. <https://doi.org/10.1016/j.apergo.2011.12.013>.
- Akira, S., Kishimoto, T., 1992. IL-6 and NF-IL6 in acute-phase response and viral infection. *Immunol. Rev.* 127, 25–50. <https://doi.org/10.1111/j.1600-065x.1992.tb01407.x>.
- Andersen, M.H.G., Saber, A.T., Pedersen, J.E., Pedersen, P.B., Clausen, P.A., Løhr, M., et al., 2018a. Assessment of polycyclic aromatic hydrocarbon exposure, lung function, systemic inflammation, and genotoxicity in peripheral blood mononuclear cells from firefighters before and after a work shift. *Environ. Mol. Mutagen.* 59 (6), 539–548. <https://doi.org/10.1002/em.22193>.
- Andersen, M.H.G., Saber, A.T., Clausen, P.A., Pedersen, J.E., Løhr, M., Keramanizadeh, A., et al., 2018b. Association between polycyclic aromatic hydrocarbon exposure and peripheral blood mononuclear cell DNA damage in human volunteers during fire extinction exercises. *Mutagenesis* 33 (1), 105–115. <https://doi.org/10.1093/mutage/gev021>.
- Barros, B., Paiva, A.M., Oliveira, M., Alves, S., Esteves, F., Fernandes, A., et al., 2024. Baseline data and associations between urinary biomarkers of polycyclic aromatic hydrocarbons, blood pressure, hemogram, and lifestyle among wildland firefighters. *Front. Public Health* 12, 1338435. <https://doi.org/10.3389/fpubh.2024.1338435>.
- Bauer, M.E., 2005. Stress, glucocorticoids and ageing of the immune system. *Stress* 8 (1), 69–83. <https://doi.org/10.1080/10253890500100240>.
- Bergonse, R., Oliveira, S., Gonçalves, A., Nunes, S., DaCamara, C., Zêzere, J.L., 2021. Predicting burnt areas during the summer season in Portugal by combining wildfire susceptibility and spring meteorological conditions. *Geomat. Nat. Hazards Risk* 12 (1), 1039–1057. <https://doi.org/10.1080/19475705.2021.1909664>.
- Betchley, C., Koenig, J.Q., van Belle, G., Checkoway, H., Reinhardt, T., 1997. Pulmonary function and respiratory symptoms in forest firefighters. *Am. J. Ind. Med.* 31 (5), 503–509. [https://doi.org/10.1002/\(sici\)1097-0274\(199705\)31:5<503::aid-ajim3>3.0.co;2-u](https://doi.org/10.1002/(sici)1097-0274(199705)31:5<503::aid-ajim3>3.0.co;2-u).
- Bocato, M.Z., Bianchi Ximenez, J.P., Hoffmann, C., Barbosa, F., 2019. An overview of the current progress, challenges, and prospects of human biomonitoring and exposome studies. *J. Toxicol. Environ. Health, Part A B* 22 (5–6), 131–156. <https://doi.org/10.1080/10937404.2019.1661588>.
- Brenner, I.K., Shek, P.N., Shephard, R.J., 1994. Infection in athletes. *Sports Med.* 17, 86–107. <https://doi.org/10.2165/00007256-199417020-00002>.
- Caux, C., O'Brien, C., Viau, C., 2002. Determination of firefighter exposure to polycyclic aromatic hydrocarbons and benzene during fire fighting using measurement of biological indicators. *Appl. Occup. Environ. Hyg* 17 (5), 379–386. <https://doi.org/10.1080/10473220252864987>.

- Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., et al., 2018. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget* 9 (6), 7204. <https://doi.org/10.18632/oncotarget.23208>.
- Cherry, N., Beach, J., Galarneau, J.-M., 2021. Are inflammatory markers an indicator of exposure or effect in firefighters fighting a devastating wildfire? Follow-up of a cohort in Alberta, Canada. *Annals of Work Exposures and Health* 65 (6), 635–648. <https://doi.org/10.1093/annweh/wxaa142>.
- Costa, S., Esteves, F., 2023. Biomarkers, human health. *Encyclopedia of Toxicology*, fourth ed.
- Cushman, M., Ballantyne, C.M., Cooper, G.R., Pfeiffer, C., Grundy, S.M., Labarthe, D.R., et al., 2009. National academy of clinical biochemistry laboratory medicine practice guidelines: emerging biomarkers for primary prevention of cardiovascular disease. *Clin. Chem.* 55, 378. <https://doi.org/10.1373/clinchem.2008.115899>.
- Ebert, J.R., 2013. Biomonitoring of Wildland Firefighters: Analysis of Methoxyphenols as Viable Urinary Biomarkers of Wood Smoke Exposure. <https://doi.org/10.1038/jes.2008.21>.
- Ellis, T.M., Bowman, D.M., Jain, P., Flannigan, M.D., Williamson, G.J., 2022. Global increase in wildfire risk due to climate-driven declines in fuel moisture. *Glob. Change Biol.* 28 (4), 1544–1559. <https://doi.org/10.1111/gcb.16006>.
- Esteves, F., Madureira, J., Teixeira, J.P., Costa, S., 2022. The role of biomarkers in the assessment of potential health risks of wildland firefighters' occupational exposure: an overview. In: "Essential Guide to Occupational Exposure". Safety and Risk in Society. Nova Science Publishers, Inc., New York, United States.
- Esteves, F., Madureira, J., Teixeira, J.P., Costa, S., 2024a. Wildland firefighters: a crucial weapon for forest fire management. Which Health Risks do they Face? Fire Hazards: Socio-Economic and Regional Issues. Springer International Publishing, Cham, pp. 107–121. https://doi.org/10.1007/978-3-031-50446-4_9.
- Esteves, F., Slezakova, K., Madureira, J., Vaz, J., Fernandes, A., Morais, S., et al., 2024b. Firefighters' occupational exposure in preparation for wildfire season: addressing biological impact. *Toxics* 12 (3), 201. <https://doi.org/10.3390/toxics12030201>.
- Fabian, T.Z., Borgerson, J.L., Gandhi, P.D., Baxter, C.S., Ross, C.S., Lockey, J.E., et al., 2014. Characterization of firefighter smoke exposure. *Fire Technol.* 50 (4), 993–1019. <https://doi.org/10.1007/s10694-011-0212-2>.
- Fahy, R.F., 2005. US Firefighter Fatalities due to Sudden Cardiac Death, 1995–2004. National Fire Protection Association, Quincy, MA.
- Fent, K.W., Eisenberg, J., Snawder, J., Sammons, D., Pleil, J.D., Stiegel, M.A., et al., 2014. Systemic exposure to PAHs and benzene in firefighters suppressing controlled structure fires. *Ann. Occup. Hyg.* 58 (7), 830–845. <https://doi.org/10.1093/annhyg/meu036>.
- Fent, K.W., Toennis, C., Sammons, D., Robertson, S., Bertke, S., Calafat, A.M., et al., 2019. Firefighters' and instructors' absorption of PAHs and benzene during training exercises. *Int. J. Hyg Environ. Health* 222 (7), 991–1000. <https://doi.org/10.1016/j.ijheh.2019.06.006>.
- Fernando, S., Shaw, L., Shaw, D., Gallea, M., VandenEnden, L., House, R., et al., 2016. Evaluation of firefighter exposure to wood smoke during training exercises at burn houses. *Environ. Sci. Technol.* 50 (3), 1536–1543. <https://doi.org/10.1021/acs.est.5b04752>.
- Gianniu, N., Katsaounou, P., Dima, E., Giannakopoulou, C.-E., Kardara, M., Saltagianni, V., et al., 2016. Prolonged occupational exposure leads to allergic airway sensitization and chronic airway and systemic inflammation in professional firefighters. *Respir. Med.* 118, 7–14. <https://doi.org/10.1016/j.rmed.2016.07.006>.
- Gianniu, N., Giannakopoulou, C., Dima, E., Kardara, M., Katsaounou, P., Tsakitakis, A., et al., 2018. Acute effects of smoke exposure on airway and systemic inflammation in forest firefighters. *J. Asthma Allergy* 11, 81. <https://doi.org/10.2147/JAA.S136417>.
- Greten, F.R., Grivennikov, S.I., 2019. Inflammation and cancer: triggers, mechanisms, and consequences. *Immunity* 51 (1), 27–41. <https://doi.org/10.1016/j.immuni.2019.06.025>.
- Greven, F.E., Krop, E.J., Spithoven, J.J., Burger, N., Rooyackers, J.M., Kerstjens, H.A., et al., 2012. Acute respiratory effects in firefighters. *Am. J. Ind. Med.* 55 (1), 54–62. <https://doi.org/10.1002/ajim.21012>.
- Guarner, V., Rubio-Ruiz, M.E., 2015. Low-grade systemic inflammation connects aging, metabolic syndrome and cardiovascular disease. *Aging and health-a systems biology perspective* 40, 99–106. <https://doi.org/10.1159/000364934>.
- Hejl, A.M., Adetona, O., Diaz-Sanchez, D., Carter, J.D., Commodore, A.A., Rathbun, S.L., et al., 2013. Inflammatory effects of woodsmoke exposure among wildland firefighters working at prescribed burns at the Savannah river site, SC. *J. Occup. Environ. Hyg.* 10 (4), 173–180. <https://doi.org/10.1080/15459624.2012.760064>.
- Hotamisligil, G.S., 2006. Inflammation and metabolic disorders. *Nature* 444 (7121), 860–867. <https://doi.org/10.1038/nature05485>.
- Hwang, J., Xu, C., Grunsted, P., Agnew, R.J., Malone, T.R., Clifton, S., et al., 2022. Urinary metabolites of polycyclic aromatic hydrocarbons in firefighters: a systematic review and meta-analysis. *Int. J. Environ. Res. Publ. Health* 19 (14), 8475. <https://doi.org/10.3390/ijerph19148475>.
- International Agency for Research on Cancer (IARC), 2010. Painting, Firefighting, and Shiftwork. IARC Press, International Agency for Research on Cancer.
- International Agency for Research on Cancer (IARC), 2023. Occupational Exposure as a Firefighter, pp. 1–730.
- Keir, J.L., Akhtar, U.S., Matschke, D.M., Kirkham, T.L., Chan, H.M., Ayotte, P., et al., 2017. Elevated exposures to polycyclic aromatic hydrocarbons and other organic mutagens in Ottawa firefighters participating in emergency, on-shift fire suppression. *Environ. Sci. Technol.* 51 (21), 12745–12755. <https://doi.org/10.1021/acs.est.7b02850>.
- Kim, S., Lee, H., Shin, D., Ku, B., Oh, J., Cho, B., et al., 2018. Cardiovascular risk in fire academy instructors during live-fire simulation activity. *Annals of Burns and Fire Disasters* 31 (4), 313.
- Knorr, W., Dentener, F., Lamarque, J.-F., Jiang, L., Arneth, A., 2017. Wildfire air pollution hazard during the 21st century. *Atmos. Chem. Phys.* 17 (14), 9223–9236. <https://doi.org/10.5194/acp-17-9223-2017>.
- Kramer, F., Torzewski, J., Kamenz, J., Veit, K., Hombach, V., Dedio, J., et al., 2008. Interleukin-1 β stimulates acute phase response and C-reactive protein synthesis by inducing an NF κ B and C/EBP β -dependent autocrine interleukin-6 loop. *Mol. Immunol.* 45 (9), 2678–2689. <https://doi.org/10.1016/j.molimm.2007.12.017>.
- Li, Y., Zhong, X., Cheng, G., Zhao, C., Zhang, L., Hong, Y., et al., 2017. Hs-CRP and all-cause, cardiovascular, and cancer mortality risk: a meta-analysis. *Atherosclerosis* 259, 75–82. <https://doi.org/10.1016/j.atherosclerosis.2017.02.003>.
- Main, L.C., Wolkow, A.P., Tait, J.L., Della Gatta, P., Raines, J., Snow, R., et al., 2020. Firefighter's acute inflammatory response to wildfire suppression. *J. Occup. Environ. Med.* 62 (2), 145–148. <https://doi.org/10.1097/JOM.0000000000001775>.
- Naeher, L.P., Brauer, M., Lipsett, M., Zelikoff, J.T., Simpson, C.D., Koenig, J.Q., et al., 2007. Woodsmoke health effects: a review. *Inhal. Toxicol.* 19 (1), 67–106. <https://doi.org/10.1080/08958370600985875>.
- Navarro, K.M., Cisneros, R., Schweizer, D., Chowdhary, P., Noth, E.M., Balmes, J.R., et al., 2019. Incident command post exposure to polycyclic aromatic hydrocarbons and particulate matter during a wildfire. *J. Occup. Environ. Hyg.* 16 (11), 735–744. <https://doi.org/10.1080/15459624.2019.1657579>.
- Nehring, S.M., Goyal, A., Patel, B.C., 2017. C Reactive Protein.
- Oliveira, M., Costa, S., Vaz, J., Fernandes, A., Slezakova, K., Delerue-Matos, C., et al., 2020. Firefighters exposure to fire emissions: impact on levels of biomarkers of exposure to polycyclic aromatic hydrocarbons and genotoxic/oxidative-effects. *J. Hazard Mater.* 383, 121179. <https://doi.org/10.1016/j.jhazmat.2019.121179>.
- Orysiak, J., Mlynarczyk, M., Piec, R., Jakubiak, A., 2022. Lifestyle and environmental factors may induce airway and systemic inflammation in firefighters. *Environ. Sci. Pollut. Control Ser.* 29 (49), 73741–73768. <https://doi.org/10.1007/s11356-022-22479-x>.
- Passos, I.C., Vasconcelos-Moreno, M.P., Costa, L.G., Kunz, M., Brietzke, E., Quevedo, J., et al., 2015. Inflammatory markers in post-traumatic stress disorder: a systematic review, meta-analysis, and meta-regression. *Lancet Psychiatry* 2 (11), 1002–1012. [https://doi.org/10.1016/S2215-0366\(15\)00309-0](https://doi.org/10.1016/S2215-0366(15)00309-0).
- Pearson, T.A., Mensah, G.A., Alexander, R.W., Anderson, J.L., Cannon, L.I., R.O., Criqui, M., et al., 2003. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the centers for disease control and prevention and the American heart association. *Circulation* 107 (3), 499–511. <https://doi.org/10.1161/01.cir.0000052939.59093.45>.
- Reid, C.E., Brauer, M., Johnston, F.H., Jerrett, M., Balmes, J.R., Elliott, C.T., 2016. Critical review of health impacts of wildfire smoke exposure. *Environ. Health Perspect.* 124 (9), 1334–1343. <https://doi.org/10.1289/ehp.1409277>.
- Reinhardt, T.E., Ottmar, R.D., 2004. Baseline measurements of smoke exposure among wildland firefighters. *J. Occup. Environ. Hyg.* 1 (9), 593–606. <https://doi.org/10.1080/15459620490490101>.
- Renna, M.E., O'Toole, M.S., Spaeth, P.E., Lekander, M., Mennin, D.S., 2018. The association between anxiety, traumatic stress, and obsessive-compulsive disorders and chronic inflammation: a systematic review and meta-analysis. *Depress. Anxiety* 35 (11), 1081–1094. <https://doi.org/10.1002/da.22790>.
- Ridker, P.M., 2003. C-reactive protein: a simple test to help predict risk of heart attack and stroke. *Circulation* 108 (12), e81–e85. <https://doi.org/10.1161/01.CIR.0000093381.57779.67>.
- Ridker, P.M., Buring, J.E., Rifai, N., Cook, N.R., 2007. Development and validation of improved algorithms for the assessment of global cardiovascular risk in women: the reynolds risk score. *JAMA* 297 (6), 611–619. <https://doi.org/10.1001/jama.297.6.611>.
- Shepherd, R., Shek, P., 1995. Exercise, aging and immune function. *Int. J. Sports Med.* 16 (1), 1–6. <https://doi.org/10.1055/s-2007-972954>.
- Silva, D., de Lacerda, A.P., 2012. High-sensitivity C-reactive protein as a biomarker of risk in coronary artery disease. *Rev. Port. Cardiol.* 31 (11), 733–745. <https://doi.org/10.1016/j.repc.2012.02.018>.
- Singh, A., Liu, C., Putman, B., Zeig-Owens, R., Hall, C.B., Schwartz, T., et al., 2018. Predictors of asthma/COPD overlap in FDNY firefighters with world trade center dust exposure: a longitudinal study. *Chest* 154 (6), 1301–1310. <https://doi.org/10.1016/j.chest.2018.07.002>.
- Slavich, G.M., Irwin, M.R., 2014. From stress to inflammation and major depressive disorder: a social signal transduction theory of depression. *Psychol. Bull.* 140 (3), 774. <https://doi.org/10.1037/a0035302>.
- Swiston, J.R., Davidson, W., Attridge, S., Li, G.T., Brauer, M., van Eeden, S.F., 2008. Wood smoke exposure induces a pulmonary and systemic inflammatory response in firefighters. *Eur. Respir. J.* 32 (1), 129–138. <https://doi.org/10.1183/09031936.00097707>.
- Taeger, D., Koslitz, S., K  ferlein, H.U., Pelzl, T., Heinrich, B., Breuer, D., et al., 2023. Exposure to polycyclic aromatic hydrocarbons assessed by biomonitoring of firefighters during fire operations in Germany. *Int. J. Hyg Environ. Health* 248, 114110. <https://doi.org/10.1016/j.ijheh.2023.114110>.
- van Eeden, S.F., Yeung, A., Quinlan, K., Hogg, J.C., 2005. Systemic response to ambient particulate matter: relevance to chronic obstructive pulmonary disease. *Proc. Am. Thorac. Soc.* 2 (1), 61–67. <https://doi.org/10.1513/pats.200406-035MS>.
- Vorkamp, K., Casta  , A., Antignac, J.-P., Boada, L.D., Cequier, E., Covaci, A., et al., 2021. Biomarkers, matrices and analytical methods targeting human exposure to chemicals selected for a European human biomonitoring initiative. *Environ. Int.* 146, 106082. <https://doi.org/10.1016/j.envint.2020.106082>.
- Williams, J.R., 2008. The declaration of Helsinki and public health. *Bull. World Health Organ.* 86, 650–652. <https://doi.org/10.2471/BLT.08.050955>.

- Wingfors, H., Nyholm, J.R., Magnusson, R., Wijkmark, C.H., 2018. Impact of fire suit ensembles on firefighter PAH exposures as assessed by skin deposition and urinary biomarkers. *Annals of work exposures and health* 62 (2), 221–231. <https://doi.org/10.1093/annweh/wxx097>.
- Wolkow, A., Ferguson, S.A., Vincent, G.E., Larsen, B., Aisbett, B., Main, L.C., 2015. The impact of sleep restriction and simulated physical firefighting work on acute inflammatory stress responses. *PLoS One* 10 (9), e0138128. <https://doi.org/10.1371/journal.pone.0138128>.
- World Health Organization, W., 2022. Body Mass Index - BMI. World Health Organization. Available from: <https://www.euro.who.int/en/health-topics/diseases-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi>.
- World Medical Association, 2013. World medical association declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA* 310 (20), 2191–2194.
- Wu, C.-M., Adetona, A., Song, C., Naeher, L., Adetona, O., 2020. Measuring acute pulmonary responses to occupational wildland fire smoke exposure using exhaled breath condensate. *Arch. Environ. Occup. Health* 75 (2), 65–69. <https://doi.org/10.1080/19338244.2018.1562413>.

Supplementary Material

Impact of occupational exposure to wildfire events on systemic inflammatory biomarkers in Portuguese wildland firefighters

Filipa Esteves^{a,b,c,d}, Joana Madureira^{a,b,c}, Bela Barros^c, Sara Alves^f, Joana Pires^{a,b,c}, Sandra Martins^{b,g}, Marta Oliveira^c, Josiana Vaz^{h,i}, Klara Slezakova^j, Maria do Carmo Pereira^j, Adília Fernandes^h, Simone Morais^e, João Tiago Guimarães^{b,g,k}, Stefano Bonassi^l, João Paulo Teixeira^{a,b,c*}, Solange Costa^{a,b,c}

^a Environmental Health Department, National Institute of Health, Rua Alexandre Herculano, n° 321, 4000-055 Porto, Portugal

^b EPIUnit - Instituto de Saúde Pública, Universidade do Porto, Rua das Taipas, n° 135, 4050-600 Porto, Portugal

^c Laboratório para a Investigação Integrativa e Translacional em Saúde Populacional (ITR), Universidade do Porto, Rua das Taipas, n° 135, 4050-600 Porto, Portugal

^d Department of Public Health and Forensic Sciences, and Medical School, Faculty of Medicine, University of Porto, Rua Doutor Plácido da Costa, 4200-450 Porto, Portugal

^e REQUIMTE/LAQV, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Dr. António Bernardino de Almeida 431, 4249-015 Porto, Portugal

^f Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

^g Department of Clinical Pathology, São João University Hospital Centre, 4200-319 Porto, Portugal

^h Research Centre for Active Living and Wellbeing (LiveWell), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

ⁱ CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

^j LEPABE-ALiCE, Faculdade de Engenharia da Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

^k Unit of Biochemistry, Department Biomedicine, Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal

^l Unit of Clinical and Molecular Epidemiology, IRCCS San Raffaele Roma, Rome, Italy, and Department of Human Sciences and Quality of Life Promotion, San Raffaele University, Rome, Italy

*Corresponding author: João Paulo Teixeira; E-mail: joao.teixeira@insa.min-saude.pt

Table S3 - Descriptive statistics of urinary OHPAHs levels in both study phases (n=59).

Outcome measure	Phase I (n=59)		Phase II (n=59)		P-value
	Before wildfire season		After wildfire event		
	<i>n</i>	<i>Median (IQR ^a)</i>	<i>n</i>	<i>Median (IQR ^a)</i>	
Urinary OHPAH levels					
1-OHNaph+1-OHAce (μmol/mol creatinine)	59	9.33 (3.66-23.08)	56	28.45 (8.01-49.51)	<0.01**
2-OHFlu (μmol/mol creatinine)	59	0.05 (0.02-0.23)	56	0.09 (0.03-0.29)	0.07
1-OHPhen (μmol/mol creatinine)	59	0.05 (0.02-0.09)	56	0.07 (0.03-0.12)	0.05*
1-OHPyr (μmol/mol creatinine)	59	0.03 (0.01-0.04)	56	0.05 (0.03-0.07)	<0.01**

^a Interquartile range (IQR). Statistical significance was determined using the Wilcoxon signed-rank test for paired samples, with * $p < 0.05$ and ** $p < 0.01$ indicating statistical significance.

B. 2. Occupational Exposure to Wildland Firefighting and Its Effects on Systemic DNA Damage (Paper III)

Esteves F., Madureira J., Costa C., Pires J., Barros B., Alves S., Vaz J., Oliveira M., Slezakova K., Fernandes A., Pereira M. C., Morais S., Valdiglesias V., Bonassi S., Teixeira J. P. & Costa S.

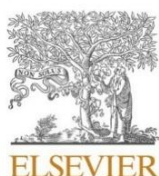
Reprinted from International Journal of Hygiene and Environmental Health 266, 114576

Copyright© 2025 The Authors. Published by Elsevier GmbH.

Open access article under the CC BY license.

Esteves, F., Madureira, J., Costa, C., Pires, J., Barros, B., Alves, S., Vaz, J., Oliveira, M., Slezakova, K., Fernandes, A., Pereira, M.C., Morais, S., Valdiglesias, V., Bonassi, S., Teixeira, J.P. & Costa, S. (2025). Occupational Exposure to Wildland Firefighting and Its Effects on Systemic DNA Damage. International Journal of Hygiene and Environmental Health, 266, 114576. <https://doi.org/10.1016/j.ijheh.2025.114576>

The PhD candidate was responsible for the conceptualization and writing of the manuscript.



Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

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

Occupational exposure to wildland firefighting and its effects on systemic DNA damage

Filipa Esteves^{a,b,c,d}, Joana Madureira^{a,b,c}, Carla Costa^{a,b,c},
Joana Pires^{a,b,c}, Bela Barros^e, Sara Alves^f, Josiana Vaz^{g,h}, Marta Oliveira^e,
Klara Slezakovaⁱ, Adília Fernandes^{g,h}, Maria do Carmo Pereiraⁱ, Simone Morais^e,
Vanessa Valdiglesias^{j,k}, Stefano Bonassi^{l,m}, João Paulo Teixeira^{a,b,c,*}, Solange Costa^{a,b,c}

^a Environmental Health Department, National Institute of Health, Rua Alexandre Herculano, n° 321, 4000-055, Porto, Portugal

^b EPIUnit - Instituto de Saúde Pública, Universidade do Porto, Rua das Taipas, n° 135, 4050-600, Porto, Portugal

^c Laboratório para a Investigação Integrativa e Translacional em Saúde Populacional (ITR), Universidade do Porto, Rua das Taipas, n° 135, 4050-600, Porto, Portugal

^d Department of Public Health and Forensic Sciences, and Medical School, Faculty of Medicine, University of Porto, Rua Doutor Plácido da Costa, 4200-450, Porto, Portugal

^e REQUIMTE/LAQV, Instituto Superior de Engenharia do Instituto Politécnico do Porto, Dr. António Bernardino de Almeida 431, 4249-015, Porto, Portugal

^f Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253, Bragança, Portugal

^g Research Centre for Active Living and Wellbeing (LiveWell), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253, Bragança, Portugal

^h CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253, Bragança, Portugal

ⁱ LEPABE-ALiCE, Faculdade de Engenharia da Universidade do Porto, Rua Dr. Roberto Frias, 4200-465, Porto, Portugal

^j Universidade da Coruña, Grupo NanoToxGen, Centro Interdisciplinar de Química e Biología - CICA, Departamento de Biología, Facultad de Ciencias, Campus A Zapateira s/n, A Coruña, Spain

^k Instituto de Investigación Biomédica de A Coruña (INIBIC), Complejo Hospitalario Universitario de A Coruña (CHUAC), Sergas, As Xubias, A Coruña, 15006, Spain

^l Unit of Clinical and Molecular Epidemiology, IRCCS San Raffaele Roma, 00163, Rome, Italy

^m Department of Human Sciences and Quality of Life Promotion, San Raffaele University, 00166, Rome, Italy

ARTICLE INFO

Keywords:

Wildland firefighters
Firefighting
Occupational exposure
Biomonitoring
Genotoxicity biomarkers
DNA damage

ABSTRACT

Background: Portugal is among the European Union countries more devastated by forest fires. Wildland firefighters are at the forefront of this battle, facing exposure to a wide range of harmful pollutants. Epidemiological studies have highlighted a potential link between occupational firefighting exposure and several diseases, including cancer. To date, very few studies have explored the biological mechanisms associated with such exposure. The present longitudinal study aims to assess changes in early effect biomarkers following wildland firefighters' occupational exposure to a real wildfire event.

Methods: Paired blood samples from 59 healthy Portuguese wildland firefighters were collected at two different time points: before wildfire season and after a fire event during wildfire season. Sociodemographic variables (e.g., age, sex) and work-related factors (e.g., years of service) were assessed via a self-reported questionnaire. Levels of early effect biomarkers, such as primary DNA damage and oxidative DNA damage (oxidised purines) were assessed via comet assay. DNA double-strand breaks (DSBs) were evaluated by phosphorylated H2AX (γH2AX). Moreover, hydroxylated polycyclic aromatic hydrocarbon metabolites (OHPAHs) and metal(loid)s were quantified in urine samples. The influence of urinary OHPAHs, urinary metal(loid)s, and other exposure-related factors (e.g., firefighting duration) on changes (Δ) in early effect biomarkers (post-vs. baseline levels) was investigated.

Results: Firefighting activities led to a significant increase in both primary DNA damage and oxidative DNA damage by 22 % (95 % CI: 1.11–1.35; $p < 0.05$) and 23 % (95 % CI: 1.04–1.45; $p < 0.05$), respectively. Results from linear regression revealed that per each unit increase of urinary 2-hydroxyfluorene (2-OHFlu) (μmol/mol creatinine), the risk of Δ oxidative DNA damage increased by 20 % [FR: 1.20 (1.09–1.32); $p < 0.01$]. Additionally, each unit increase in urinary cesium (Cs) (μg/L) resulted in a significant 4 % increase in Δ primary DNA damage [FR: 1.04 (1.01–1.06); $p < 0.05$] and a 3 % increase in Δ oxidative DNA damage [FR: 1.03 (1.01–1.05); $p < 0.05$]. Post-exposure levels of γH2AX were significantly correlated with urinary 2-OHFlu levels assessed after

* Corresponding author. Environmental Health Department, National Institute of Health, Rua Alexandre Herculano, n° 321, 4000-055, Porto, Portugal.
E-mail address: joao.teixeira@insa.min-saude.pt (J.P. Teixeira).

<https://doi.org/10.1016/j.ijheh.2025.114576>

Received 8 December 2024; Received in revised form 28 March 2025; Accepted 3 April 2025

Available online 9 April 2025

1438-4639/© 2025 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

firefighting ($r = 0.30$; $p < 0.05$). Furthermore, exposure duration and reported breathing difficulties during firefighting were significantly associated with increased levels of primary DNA damage.

Conclusion: Results obtained provide insights into the potential human health effects of wildland firefighting occupational exposure at the genetic and molecular levels, offering new and important mechanistic data. These findings are crucial for implementing health and safety measures, recommendations, and best practices to mitigate occupational risks and protect the health of wildland firefighters.

1. Introduction

In recent years, wildfires have occurred with increasing frequency and unprecedented intensity around the globe (Abatzoglou and Williams, 2016; Bernstein et al., 2019; Radeloff et al., 2018; Zhang et al., 2017). Firefighters are on the frontline of these events, playing a crucial role in wildfire response, and protecting population and goods. Nevertheless, the hazardous nature of their work exposes them to a broad spectrum of occupational risks, including the development of acute and long-term adverse health effects (Esteves et al., 2024). Over the years, epidemiological evidence has linked firefighting occupational exposure to an increased risk of various diseases, including cancer (Casjens et al., 2020; Glass et al., 2017; LeMasters et al., 2006; Navarro et al., 2019).

Recently, in 2022, the International Agency for Research on Cancer (IARC) classified occupational exposure as a firefighter carcinogenic to humans highlighting that such exposure has characteristics of carcinogens, including genotoxicity, induction of epigenetic alterations, oxidative stress, chronic inflammation, and receptor-mediated effects (IARC, 2023). Despite this IARC classification, research on the biological mechanisms induced by occupational exposure in firefighters remains scarce and inconclusive, especially among wildland firefighters (IARC, 2023). Most available studies are focused on firefighters' exposure to structural fires (e.g., municipal), under training/simulated conditions, or during controlled fires such as prescribed burns (IARC, 2023).

During their activities, wildland firefighters are exposed to a wide range of harmful pollutants released by the combustion of natural organic fuels (e.g., vegetation) (Esteves et al., 2022). After being absorbed and metabolised by cells, these contaminants can generate reactive oxygen species (ROS) (Danielsen et al., 2011; Leonard et al., 2007; Liu et al., 2005), which, if not neutralised by the body's antioxidant defense system, may react with proteins, lipids and nucleotides, leading to oxidative stress and genomic instability (Czerska et al., 2015; Esteves et al., 2017; Lamprecht et al., 2004). Oxidative stress is extensively described in the literature as a critical factor in the development of various pathological processes, including cancer (IARC, 2023; Poetsch, 2020; Reid et al., 2016; Senoner and Dichtl, 2019). Among the pollutants released by smoke, some are well known for their carcinogenic potential, such as, polycyclic aromatic hydrocarbons (PAHs), metal (loid)s and particulate matter (PM) (IARC, 2023). PAHs are a large class of ubiquitous pollutants formed during the incomplete combustion of organic matter and released during wildfires (Fabian et al., 2014; Hwang et al., 2022; Kim et al., 2013). It is believed that PAHs (or its metabolites) genotoxicity is primarily due to its ability to interact with DNA (Lewtas, 2007) resulting in genomic instability and alterations that can lead to the development of diseases, including cancer (Kamal et al., 2015; Lewtas, 2007). Metal(loid)s, such as lead (Pb), arsenic (As) or mercury (Hg), are also pervasive in the environment and are associated with various adverse health effects, including cardiovascular diseases, skin and respiratory problems, neurological disorders, kidney dysfunction, adverse pregnancy outcomes and cancer (Ali et al., 2023; Bharti and Sharma, 2022; Chen et al., 2023; Mitra et al., 2022). Furthermore, some metals tend to bioaccumulate, posing an additional health risk, particularly for long-term repetitive exposures (Al-Malki, 2009; Ali et al., 2023).

Human biomonitoring (HBM) is an essential tool in exposure assessment for identifying vulnerable populations, assessing combined exposures, and tracking the effectiveness of interventions to mitigate

health risks posed by the exposure to hazardous substances either in the environment, work settings or other sources (Costa and Esteves, 2023; Esteves et al., 2022). This approach involves the characterisation of exposure to chemicals by measuring their levels or metabolites in biological samples such as blood, urine, hair, saliva, or exhaled air (Costa and Esteves, 2023). HBM provides direct evidence of exposure by quantifying chemicals present in the body - biomarkers of internal dose - rather than relying solely on estimates based on environmental measurements (external dose); it also accounts for exposure through multiple routes while considering individual characteristics (e.g., age, sex) (Costa and Esteves, 2023). Exposure biomarkers provide information on the internal dose, but not on the effects resulting in its interactions with biological molecules biomarkers. In contrast, biomarkers of effect are biochemical alterations associated with pathological processes (Nordberg, 2010) and therefore, may serve as early warning signs or markers of disease (Decker et al., 2013). Genotoxicity evaluation constitutes an essential approach for assessing important occupational hazards (Costa et al., 2011). The comet assay is a valuable method for quantifying DNA strand breaks (SBs), such as single strand-breaks (SSBs) as well as other types of DNA damage, including DNA adducts, alkali-labile sites, and oxidative DNA lesions, in populations exposed to genotoxic agents (A. Collins et al., 2014). This assay is commonly used for the assessment of DNA damage mostly due to its simplicity, sensitivity and low cost (Neri et al., 2014). When coupled with a lesion-specific DNA repair enzyme (Collins, 2004), such as formamidopyrimidine DNA glycosylase (FPG), comet assay is also used to measure oxidised purines allowing for the detection of oxidative DNA damage (Møller et al., 2013). FPG specifically recognises and excises oxidised purines, including 8-oxoguanine - a well-known marker of oxidative stress linked to the development of various diseases (Cadet et al., 2012; Møller et al., 2013).

Other early indicator of genomic instability, specifically double-strand breaks (DSBs), is the phosphorylated histone H2AX (γ H2AX) assay. DSBs are one of the most lethal forms of DNA damage, as they can lead to chromosomal fragmentation, rearrangements, mutations, or aneuploidy (loss of information) if not properly repaired (Cannan and Pederson, 2016; Li et al., 2023). Thus, assessing DSBs levels is crucial for understanding the biological relevance of genetic instability in diseases, particularly cancer (Li et al., 2023). In recent years, γ H2AX has emerged as a key biomarker used in HBM for the evaluation of DSBs, as well as its repair of the activation of DNA damage response pathways (Nikolova et al., 2017; Valdiglesias et al., 2013). γ H2AX is recognised as a robust biomarker for assessing occupational exposure to genotoxic agents and for the early detection of disease risk, including cancer (Valdiglesias et al., 2013). The analysis of γ H2AX by flow cytometry is particularly advantageous for HBM studies because of its high sensitivity, specificity (Sánchez-Flores et al., 2015), and because it allows the collection of data of a large number of samples relatively fast (Laffon et al., 2021) compared to other methods (e.g., ELISA).

Very few studies have assessed DNA damage in firefighters (Abreu et al., 2017; Andersen et al., 2018a, 2018b; Oliveira et al., 2020) and, to the best of our knowledge, none have longitudinally characterised DNA damage levels (SBs and DSBs) and oxidised DNA levels in wildland firefighters enrolled in actual fire combat. Understanding wildfire fighting-related health impacts is essential for developing effective preventive measures and ensuring the safety and well-being of these essential first responders.

Our study aims to close this gap and provide insights into the biological mechanisms and potential health outcomes of occupational exposure for wildland firefighters. To achieve our goal, a group of Northern Portuguese wildland firefighters were studied at two distinct moments of their activity - before the wildfire season (Phase I, baseline levels) and during the wildfire season after combating a real wildfire event (Phase II, post-exposure levels). Paired wildland firefighters' samples were evaluated for primary DNA damage, oxidative DNA damage, and γ H2AX. Their associations with urinary hydroxylated metabolites of PAHs (OHPAHs) and metal(loids) levels were assessed. The relationship between these biological endpoints and both socio-demographic and work-related variables, collected through questionnaires, was also explored.

2. Methods

2.1. Study population

The present study was conducted in accordance with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the University of Porto (reference number 92/CEUP/2020).

Fifty-nine wildland firefighters ($n = 59$) were recruited from five fire stations located in the northern region of Portugal, between 2021 and 2023. A pre-post study design was used, with each participant serving as their own control at two different time points: Phase I (before wildfire season, late spring) and Phase II (after combating a fire event, during wildfire season). Before their enrolment, firefighters were briefed on the study's aims, potential risks, data confidentiality, and their right to withdraw. A signed consent was required from subjects who agreed to participate. Data on sociodemographic characteristics (e.g., sex, age), medical history (e.g., chronic diseases), lifestyle (e.g., smoking habits), and occupational details (e.g., years of service) were obtained via a self-reported questionnaire applied during Phase I (before wildfire season). Additionally, after combating wildfire firefighters completed a brief survey to report details on exposure duration (in hours), wildfire event duration (in hours), and any acute symptoms experienced during fire combat.

2.2. Biological sampling

Biological samples, namely blood and urine, were collected in both phases. Before the wildfire season (Phase I) samples were collected during the firefighters' shift (morning period) in a clean and designated area at the fire station. For Phase II, post-fire samples were collected during the morning period, after returning to the fire station from wildfire suppression activities (combat/post-combat). The samples were collected within a similar timeframe for all participants.

2.2.1. Urine sampling collection

Participants were instructed to provide a spot urine sample in a 100 mL sterile plastic container. Urine samples were immediately coded, transported to the laboratory under cold conditions (4 °C) and stored at -20 °C for further analysis.

2.2.2. Blood sampling collection

Peripheral venous blood samples were collected via venipuncture into dipotassium ethylenediaminetetraacetic acid (EDTA)-coated tubes (Vacuette® Tube K2EDTA). After collection, blood samples were coded, maintained at 4 °C, and transported to the laboratory to be processed. Upon arrival, two aliquots with 200 μ L of whole blood were immediately stored at -80 °C (slow freezing procedure) for subsequent comet assay analysis (both alkaline and modified-enzyme version). Peripheral blood mononuclear cells (PBMC) were isolated under aseptic conditions for γ H2AX evaluation. Briefly, each blood sample was diluted with sterile 0.9 % NaCl at a 1:1 ratio. The diluted blood was carefully layered into

pre-labelled tubes containing sterile Lymphoprep™ density gradient medium at room temperature, following manufacturer instructions. After centrifugation for 20 min at 900–1200 g the PBMC ring layer was carefully removed, transferred to another tube containing 6 mL of cold 0.9 % sterile NaCl and centrifuged at 480 g for 10 min. After discarding the supernatant, the pellet was gently resuspended in the remaining volume washed again with 6 mL of cold sterile 0.9 % NaCl and centrifuged at 480 g for 10 min. The supernatant was discarded and the resuspended PBMC were slowly cryopreserved in a solution of cold sterile 90 % fetal bovine serum (FBS) and 10 % dimethyl sulfoxide (DMSO) cryopreservation solution at -80 °C and kept stored for further analysis.

2.3. Urinalysis of OHPAHs metabolites and metal(loids)

Urinary PAHs metabolites, namely, 1-hydroxynaphthalene (1-OHNaph), 1-hydroxyacenaphthene (1-OHAce), 2-hydroxyfluorene (2-OHFlu), 1-hydroxyphenanthrene (1-OHPhen), and 1-hydroxypyrene (1-OHPyr) were quantified by high-performance liquid chromatography (HPLC) with fluorescence detection, as described by Barros et al. (2024).

The metal(loids) [i.e. lithium (Li), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), rubidium (Rb), strontium (Sr), molybdenum (Mo), cadmium (Cd), antimony (Sb), cesium (Cs), barium (Ba), mercury (Hg), thallium (Tl) and lead (Pb)] were analysed by inductively coupled plasma mass spectrometry (ICP-MS) according protocol described by Paiva et al. (2024a) and Azevedo et al. (2023). Concentrations of OHPAHs and metal(loids) were normalised through urinary creatinine levels (μ mol/mol creatinine).

2.4. Medium-throughput comet assay (12-gel comet assay)

2.4.1. Assay controls

Assay controls for standard alkaline comet assay and the enzyme-modified comet assay were prepared using a pooled sample of blood from four non-smoking healthy volunteers. Aliquots of untreated blood cells were used as baseline controls for the electrophoretic runs. For the enzyme-modified comet assay, blood cells were treated with 1.5 mM potassium bromate (KBrO₃) – to induce DNA oxidation - in PBS (pH 7.4) for 1 h at 37 °C. After preparation, assay controls were cryopreserved, after a slow freezing process, at -80 °C for subsequent use in the comet assay, alongside participants' samples (Møller et al., 2020a).

2.4.2. Alkaline comet assay procedure

Primary DNA damage was evaluated by comet assay following Singh et al. (1988) with some minor modifications described by Esteves et al. (2020). In short, firefighters' whole blood samples and assay-controls (untreated and KBrO₃-treated) were rapidly thawed on ice. Afterwards, 5 μ L of blood was gently resuspended in 0.6 % (w/v) of low melting point agarose (Sigma Aldrich Company, Merck KGaA, Darmstadt, Germany) and 5 μ L of this cell suspension was dropped onto a frosted glass slide pre-coated with 1 % (w/v) of normal melting point agarose (Lonza, Basel, Switzerland) forming a mini-gel. After solidification (for 5 min at 4 °C) slides were immersed in a Coplin jar containing ice-cold freshly prepared lysis solution (2.5 M NaCl, 100 mM Na₂EDTA, 10 mM Tris-base, and 250 mM NaOH, pH 10) supplemented immediately before use with 1 % (v/v) Triton X-100, for 1h, protected from light, at 4 °C. Subsequently, slides were immersed in ice-cold alkaline electrophoresis solution (200 mM Na₂EDTA, 10 M NaOH, pH 13) for 30 min, protected from light, at 4 °C, for DNA unwinding. Slides were then transferred to an electrophoresis tank (Axygen, UK) with an ice-cold electrophoresis solution. Electrophoresis followed for 20 min at approximately 1 V/cm under cold conditions (4 °C). Mini-gels were neutralised with ice-cold PBS (10 min) and washed with cold deionised H₂O (10 min), dehydrated and fixed with ethanol 70 % (15 min) and 96 % (15 min) and let to dry overnight, at room temperature. For scoring, dried slides were rehydrated with Tris-EDTA (TE) buffer solution (10

mM Tris-HCl and 1 mM EDTA; pH 7.5–8.0) at room temperature for 20 min; slides were then stained with SYBRTM Gold (InvitrogenTM, Waltham, MA, USA) diluted in TE buffer at room temperature for 30 min and then washed twice in distilled water and left to dry at room temperature. Microscopic analyses were performed blindly by the same reader on a fluorescence microscope (Motic BA410E) equipped with Comet Assay IV (Instem) a semi-automated image analysis software used for image capture and analysis. A total of 150 cells (75 per mini-gel) were scored for each subject (and assay controls) and the percentage of DNA in the comet tail (TDNA%) was used to evaluate the DNA damage at the cell level. The coefficient of variation for untreated assay controls was approximately 30 % (A.R. Collins et al., 2014).

2.4.3. Enzyme-modified comet assay (FPG)

Oxidative DNA damage was evaluated via comet assay following Teixeira-Gomes et al. (2020) and others (Costa et al., 2011). In brief, after lysis, slides were washed 3 times (5 min each) with ice-cold buffer F (40 mM HEPES, 100 mM KCl, 0.5 mM Na₂EDTA, 0.2 mg/ml BSA, pH 8). After assembling the 12-Gel Comet Assay UnitTM (Severn Biotech Ltd., Worcestershire, United Kingdom), 30 μ L of formamidopyrimidine DNA glycosylase enzyme (FPG) solution (8000 units/mL), used according to manufacturers' instructions, acquired from New England Biolabs®, Ipswich, MA, USA) or 30 μ L buffer F were added to each well covering the mini-gel. Incubation was done for 30 min at 37 °C. The following steps were performed according to the comet assay described in previous studies (Esteves et al., 2020). The percentage of DNA in the comet tail (TDNA%) was the DNA damage parameter chosen to evaluate comet formation. Net FPG-sensitive sites, for each subject, were calculated by subtracting the TDNA% value obtained for buffer and from the value obtained for FPG. The coefficient of variation of TDNA% KBRO3-treated assay controls, for net FPG measurements, across the different experiments was 21 % (Esteves et al., 2020; Møller et al., 2020b).

2.5. H2AX phosphorylation (γ H2AX)

2.5.1. Assay controls

γ H2AX assay controls were prepared under aseptic conditions using a pool of PBMC from four non-smoking healthy volunteers. As described herein, lymphocytes were isolated using a sterile LymphoprepTM density gradient, following the manufacturer's protocol. PBMC were then subjected to treatment with bleomycin (20 μ L/mL) for 4 h at 37 °C to serve as a positive control for DNA DSBs induction. Untreated PBMC were used as negative controls. Aliquots containing approximately 1×10^6 cells were resuspended in 1 mL of sterile freezing medium (50 % FBS, 40 % RPMI-1640 and 10 % DMSO) and slowly cryopreserved at –80 °C until further analysis.

2.5.2. γ H2AX analysis

The γ H2AX analysis was performed based on the protocols described by Sánchez-Flores et al. (2015), Tanaka et al. (2009) and Watters et al. (2009). In brief, cryopreserved PBMC samples were thawed at a 37 °C water bath for approximately 60 s (Barcelo et al., 2018). Following thawing, cells were resuspended in 5 mL of culture medium (RPMI-1640 with L-Glutamine, supplemented with 15 % heat-inactivated fetal bovine serum and 1 % Penicillin-Streptomycin solution). The cell suspensions were centrifuged at 810 g for 10 min, and the supernatant was discarded. Cells were fixed with 1 mL of cold, filtered 1 % p-formaldehyde for 15 min at 4 °C, and followed by centrifugation at 1300g for 5 min (4 °C). The supernatant was removed, and cells were permeabilised with 1 mL of cold 70 % ethanol (–20 °C) and stored overnight at 4 °C. On the following day, cells suspensions were centrifuged at 1300 g for 5 min at 21 °C. After centrifugation, the supernatant was aspirated, and the cell pellet was washed with filtered phosphate-buffered solution (PBS) (pH 7.4) with 1 % BSA. Cells were again centrifuged for 5 min at 1300 g at 21 °C. Subsequently, cells were incubated with 100 μ L of 1:100 Alexa FluorTM 488 anti-phospho-histone H2A.X (Ser139) monoclonal antibody

(Invitrogen 53-9865-82) diluted in filtered PBS 7.4 with 1 % bovine serum albumin (BSA) for 15 min at room temperature in the dark. After this period of incubation, cells were washed with 1 mL of filtered PBS (pH 7.4) containing 1 % BSA and then centrifuged for 5 min at 1300 g at 21 °C. The supernatant was removed, and cells were resuspended in 250 μ L of PBS (pH 7.4) containing 0.1 mg/mL of RNase A and 40 μ g/mL of propidium iodide (PI) and incubated for 30 min in the dark at room temperature.

The analysis by flow cytometry was performed in a GUAVA EasyCyte 8HT (Luminex Corporation, USA) flow cytometer, equipped with a 488 nm excitation laser. Data were analysed using Guava® easyCyteTM software. The PBMC population was gated based on side scatter (SSC) for complexity and forward scatter (FSC) for size. Approximately 20,000 event gates were acquired for each sample. The analysis assessed the signals in Red-B Fluorescence (Red-B-Hlin) and Green-B Fluorescence (GRN-B-HLog) to identify cells marked with propidium iodide (PI) and anti- γ H2AX, respectively. Each sample was analysed in duplicate, and the result for each sample was calculated as the mean of both duplicates. To obtain the percentage of γ H2AX, the following equation was used: “number of PBMC positively marked with antibody γ H2AX and PI/total number of PBMC positively marked with PI” x 100.

2.6. Statistical analysis

Statistical analyses were conducted using IBM SPSS Statistics 29.0 for Windows. Descriptive statistics were used to summarise the data, with continuous variables reported as means $\pm$ standard deviations (SD), and categorical variables presented as frequencies and percentages. The normality of the data was assessed using the Kolmogorov-Smirnov test and visual analysis of graphical dispersion plots. Distribution departed from normality and therefore nonparametric tests were considered for statistical analysis. Univariate analysis was conducted to investigate possible associations between genotoxicity biomarkers i.e. primary DNA damage, oxidative DNA damage, and γ H2AX, and exposure-related factors i.e., urinary metal(loid)s, urinary OHPAHs, and self-questionnaire retrieved variables (e.g., exposure duration (hours), acute symptomatology experienced during firefighting, wildfire duration). The effect of age, sex and smoking habits was also investigated. Participants were classified as non-current smokers if they had never smoked or had quit smoking for more than one year. Those who were currently smoking or had quit smoking for one year or less, were classified as current smokers. Statistical differences among independent groups were tested using the Man-Whitney *U* Test (two groups) and Kruskal-Wallis test (more than two groups). Bonferroni adjustment was used for multiple comparisons. The Wilcoxon test was employed to assess the differences in biomarkers between paired samples collected before and during the wildfire season. Correlations between continuous variables were analysed by Spearman's rank correlation test. Frequency ratios (FR) with 95 % CI were calculated to assess the relative change in the studied endpoints (i.e., primary DNA damage, oxidative DNA damage and γ H2AX) between pre- and post-exposure.

Effect biomarkers that exhibited statistically significant differences after wildfire exposure were further analysed. Linear regression analysis was conducted to identify statistically significant associations between exposure factors and variations in effect biomarker levels across the two study phases (Pre-exposure – Phase I; Post-exposure – Phase II). To assess the relative change in biomarker levels, we first log-transformed the dependent variables for both phases (Phase I – Pre-exposure; Phase II – Post-exposure) and then calculated the difference in the log-transformed values [$\log(\text{Post-exposure}) - \log(\text{Pre-exposure})$] to obtain the variation in respective biomarker [$\Delta = \log(\text{Post-exposure}) - \log(\text{Pre-exposure})$]. This step allowed the calculation of the FR by exponentiating the beta coefficients (e^{β}) from the linear regression analysis. A statistical significance level of 0.05 was considered.

3. Results

The general characteristics of the study population ($n = 59$) are summarised in Table 1. The wildland firefighters had a mean age of 35.5 ± 9.0 years (range: 20.0–55.0) and were predominantly males (78 %). In terms of smoking habits, 32 (54.2 %) were current smokers and 27 (45.8 %) were non-current smokers, with all former smokers ($n = 12$) reporting that they had quit for more than one year (data not shown). Also, on average, our group, had 15.9 ± 8.9 years of service as wildland firefighters, ranging from 3 up to 34 years.

Data retrieved from the short survey on Phase II, showed that most of the reported wildland fires (49.2 %) lasted for 12 h or less, 23.7 % were between 48 and 72 h, 20.3 % persisted for more than 72 h, and a smaller proportion (6.8 %) lasted between 12 and 48 h.

When categorising exposure duration to active wildfire as reported by wildland firefighters: 40.7 % reported being exposed during fire-combat activities for 1 h to 3 h, 30.5 % for 3–12 h, and 28.8 % for more than 12 h. As for the symptoms, among others, the most reported symptomatology was eye and respiratory irritation (22.0 %), difficulty breathing (20.3 %), and headaches (11.9 %) (Table 1).

Regarding effect biomarkers, our data showed that participation in wildland firefighting significantly increased the levels of primary DNA damage and oxidative DNA damage by 22 % (95 % CI: 1.11–1.35; $p = 0.03$) and 23 % (95 % CI: 1.04–1.45; $p = 0.04$), respectively (Fig. 1). No statistically significant differences in γ H2AX (%) levels were observed after fire intervention (FR = 0.91, 95 % CI: 0.83–1.22; $p > 0.05$).

Univariate analysis showed significant differences between Phase I and Phase II levels of urinary markers (1-OHNaph+1-OHAce, 1-OHPy, Ni and As and Cs), as well as γ H2AX and comet assay endpoints (Table S1 and Table S2, respectively - supplementary material). No significant effects from self-reported variables (age, sex, smoking) or other studied covariates (i.e., BMI, smoking habits, physical exercise, diet and years of service as a firefighter) were observed on the studied endpoints in Phase I (Table S3 - supplementary material). Fig. 2 shows

Table 1
General characteristics of the study population ($n = 59$).

Study participant characteristics		Wildland Firefighters ($n = 59$)
Sociodemographic	Age (years) ^a	35.5 ± 9.0 (20.0–55.0)
	Sex	
	Male, n (%)	46 (78.0 %)
	Female, n (%)	13 (22.0 %)
Lifestyle-related	Smoking habits	
	Non-current smoker, n (%)	27 (45.8 %)
	Current smoker, n (%)	32 (54.2 %)
Occupational-related	Years of service ^a	15.9 ± 8.9 (3.0–34.0)
Active wildfire exposure	Fire duration	
	≤ 12 h, n (%)	29 (49.2 %)
	> 12–48 h, n (%)	4 (6.8 %)
	> 48–72 h, n (%)	14 (23.7 %)
	> 72 h, n (%)	12 (20.3 %)
	Exposure duration (hours)	
	1–3 h, n (%)	24 (40.7 %)
	> 3–12 h, n (%)	18 (30.5 %)
	> 12 h, n (%)	17 (28.8 %)
	Reported symptoms ^b	
	Eyes & respiratory irritation, n (%)	13 (22.0 %)
	Breath difficulty, n (%)	12 (20.3 %)
	Headaches, n (%)	7 (11.9 %)

^a Mean $\pm$ Standard deviation (min-max).

^b Symptomatology during fire suppression activities.

the significant positive correlations found between post-exposure levels of genotoxicity biomarkers and exposure biomarkers, respectively; post-exposure levels of γ H2AX (%) were significantly correlated with urinary 2-OHFlu levels assessed after fire-combat ($r = 0.30$; $p = 0.03$) (Fig. 2a). Post-exposure levels of oxidative DNA damage were slightly significantly correlated with post-exposure levels of urinary 2-OHFlu ($r = 0.26$ $p = 0.05$) (Fig. 2b) and significantly correlated with urinary Cs ($r = 0.42$; $p = 0.01$) (Fig. 2c). Moreover, a significant positive correlation was found between levels of primary DNA damage and oxidative DNA damage measured after firefighting exposure ($r = 0.34$; $p = 0.01$), confirming the association between these two endpoints (results not shown).

When exploring the influence of exposure biomarkers on the variation of effect biomarkers [$\Delta = \log(\text{Post-exposure}) - \log(\text{Pre-exposure})$, FR], our data showed that per each unit increase of urinary Cs ($\mu\text{g/L}$), the risk of Δ primary DNA damage and Δ oxidative DNA damage increased by 4 % [FR = 1.04 (1.01–1.06); $p < 0.05$] and 3 % [FR = 1.03 (1.01–1.05); $p < 0.05$], respectively (Table S4 supplementary material). In addition, our data showed that per each unit increase of 1-OHFlu ($\mu\text{mol/mol creatinine}$), there was a 20 % higher risk of Δ oxidative DNA damage [FR = 1.20 (1.09–1.32); $p < 0.01$] (Table S4 supplementary material). No association between other urinary OHPAHs or metal (loid)s, on Δ primary DNA damage and Δ oxidative DNA damage was observed in this study. The described results on Δ effect biomarkers were maintained when adjusted for sex, age, and smoking status (Tables S5–S7, supplementary material).

Significant associations were found between post-exposure levels of genotoxicity biomarkers and the prevalence of symptomatology reported by firefighters during wildfire combat (Phase II). Wildland firefighters who reported breathing difficulties during firefighting (20.3 %) had significantly higher levels of primary DNA damage than those who did not (23.31 ± 2.62 vs. 16.01 ± 0.98 , respectively; $p = 0.01$) (Fig. 3), with this association remaining after adjusting for smoking habits (data not shown). Although oxidative DNA damage was also higher in firefighters who reported breathing difficulties during firefighting activities (16.87 ± 1.61 vs. 13.14 ± 1.17 , $p = 0.02$), this significance disappeared after controlling for smoking habits; a similar trend was observed for γ H2AX levels (1.42 ± 0.41 vs. 1.02 ± 0.19), though the difference was not statistically significant (data not shown).

Firefighters reporting participating in a fire event lasting for more than 72 h presented higher Δ oxidative DNA damage than those reporting a fire event lasting for less than 12 h (0.71 ± 0.14 vs 0.31 ± 0.15 , respectively; $p = 0.02$) (data not shown).

Regarding the duration of fire combat, wildland firefighters who reported being in the field between 3 and 12 h exhibited significantly increased levels of primary DNA damage compared to those engaged less than 3 h (21.07 ± 2.37 vs. 14.30 ± 1.11 ; $p = 0.01$) (Fig. 4).

4. Discussion

With the global upsurge in wildland fires, preventing occupational health effects on wildland firefighters has become an urgent and critical priority of public concern (Stephens et al., 2013; Westerling et al., 2006). Despite firefighting being classified as carcinogenic to humans, very few studies have investigated the biological impacts of occupational exposure on wildland firefighters (IARC, 2023), particularly the effects induced by exposure during real wildfires. Our study aimed to evaluate the genotoxic effects experienced by wildland firefighters at different stages of occupational exposure, before (Phase I) and during wildland fire season (Phase II). We observed a statistically significant increase in both primary DNA damage and oxidative DNA damage levels after firefighting activities, compared to baseline levels (Phase I). Based on our knowledge, this is the first study longitudinally assessing primary DNA damage and oxidative DNA damage among wildland firefighters exposed to real wildfire events. Other studies carried out in real-combat scenarios had a cross-sectional design (Abreu et al., 2017; Oliveira et al., 2020). Abreu et al. (2017) reported increased levels of both primary and

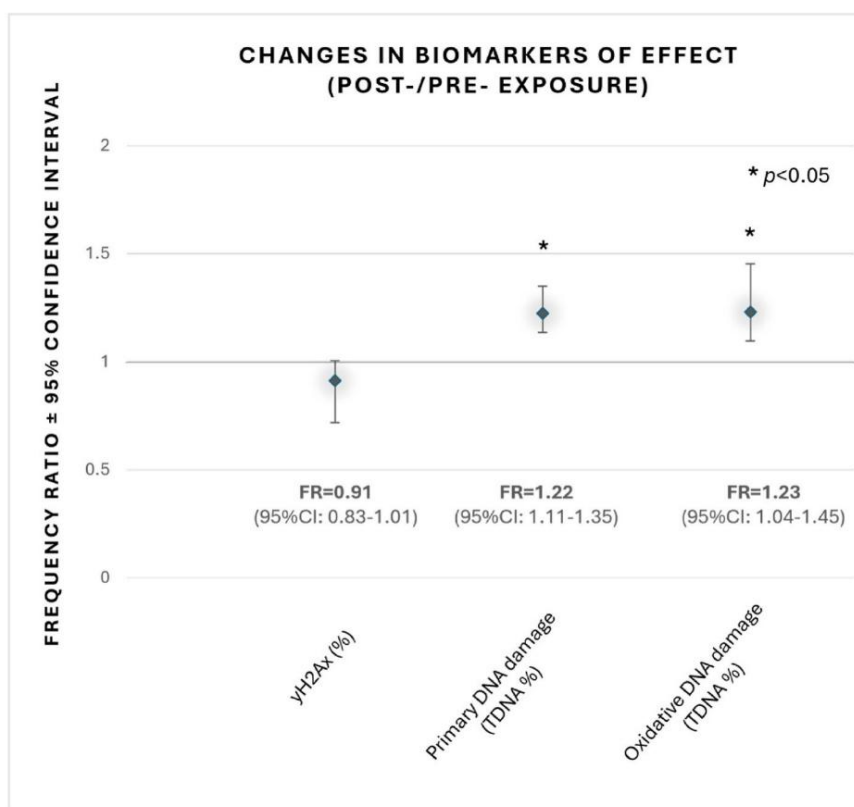


Fig. 1. Frequency ratios (FR) and respective 95 % confidence interval, showing changes in effect biomarkers after firefighting occupational exposure (post-/pre-exposure). *Statistical significance is indicated by FR values significantly different from 1 (no effect), with p-values <0.05.

oxidative DNA damage (measured by comet assay) in a group of Portuguese wildland firefighters ($n = 60$) compared to controls from the general population ($n = 63$). However, only primary DNA damage reached statistical significance, being 76 % higher than controls (Abreu et al., 2017). Another study conducted by Oliveira et al. (2020) found significantly higher levels of oxidative DNA damage in a group of non-smoking wildland firefighters ($n = 48$), directly involved in firefighting activities within 48h before sample collection, compared to firefighters with no recent exposure ($n = 93$); results for primary DNA damage were inconclusive (Oliveira et al., 2020).

Consistent with our findings some authors have also observed increased levels of other oxidative stress markers associated with wildland firefighting exposure (Gaughan et al., 2014; Wu et al., 2020). Gaughan et al. (2014), for example, found significantly elevated levels of urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker of oxidative DNA damage, in U.S. male firefighters recently exposed to wildland firefighting ($n = 20$) compared to those with no recent exposure ($n = 18$). In addition, Wu et al. (2020) found higher levels of 8-isoprostane, a marker of lipid peroxidation, in the exhaled breath condensate of U.S. wildland firefighters ($n = 12$) during prescribed burns, when compared to levels assessed in non-burn days.

Our data also aligns with reports from studies enrolling structural firefighters (Andersen et al., 2018a, 2018b). Andersen et al. (2018a) investigated the association between firefighting and levels of DNA damage (primary and oxidative DNA damage) in a cross-over study involving 53 firefighter conscripts from Denmark undergoing firefighter structural training. The authors found that firefighting activities significantly increased the levels of oxidative DNA damage in PBMC by 8.0 % (95 % CI: 0.02–15.9), but no association was observed for primary DNA damage (Andersen et al., 2018a). However, a significant increase in primary DNA damage levels was found after exercises involving wood

pallets fuel, compared to exercises using mixed fuels (i.e., wood, foam mattresses, and electrical cords) (Andersen et al., 2018a). In a subsequent study also conducted by Andersen et al. (2018b) authors followed a group of 22 male structural professional firefighters (Copenhagen, Denmark) before and after a 24-h shift. No association was found between work-shift exposure and primary and oxidised DNA damage levels. It is important to note, that firefighters used a self-contained breathing apparatus (SCBA) which protects structural firefighters from inhaling contaminants (Andersen et al., 2018b). Insufficient smoke-exposure gradient and sampling-time challenges were some of the other limitations presented by Andersen et al. (2018b) that could have contributed to the observed results. It is important to note that, generally the use of SCBA is not feasible in a wildland firefighting context, either because of its weight or the limited duration of air supply (Esteves et al., 2022). Inhalation of wood smoke has been demonstrated to adversely influence human health (Augusto et al., 2020). In a controlled intervention trial, Ferguson et al. (2016) investigated the effects of wood smoke inhalation by exposing a group of participants from general population ($n = 10$) to wood smoke for 1h and found significantly higher levels of 8-isoprostane (marker of oxidative stress) in the exhaled breath condensate compared to control conditions (filtered air) ($p < 0.05$) (Ferguson et al., 2016).

Regarding our analysis of γ H2AX, no significant differences were observed following wildland firefighting exposure. To date, no studies have assessed this endpoint to monitor occupational exposure in wildland firefighters. However, Jasra et al. (2022) reported a significant accumulation of γ H2AX foci in lymphoblasts (GM03798) exposed *in vitro* to aerosolised dust originated from the World Trade Centre fires (WTC-PM), characterised to contain high concentrations of PAHs. Given the exposure of first responders, mostly firefighters, to high levels of WTC-PM, the authors suggested that the dust exposure might have

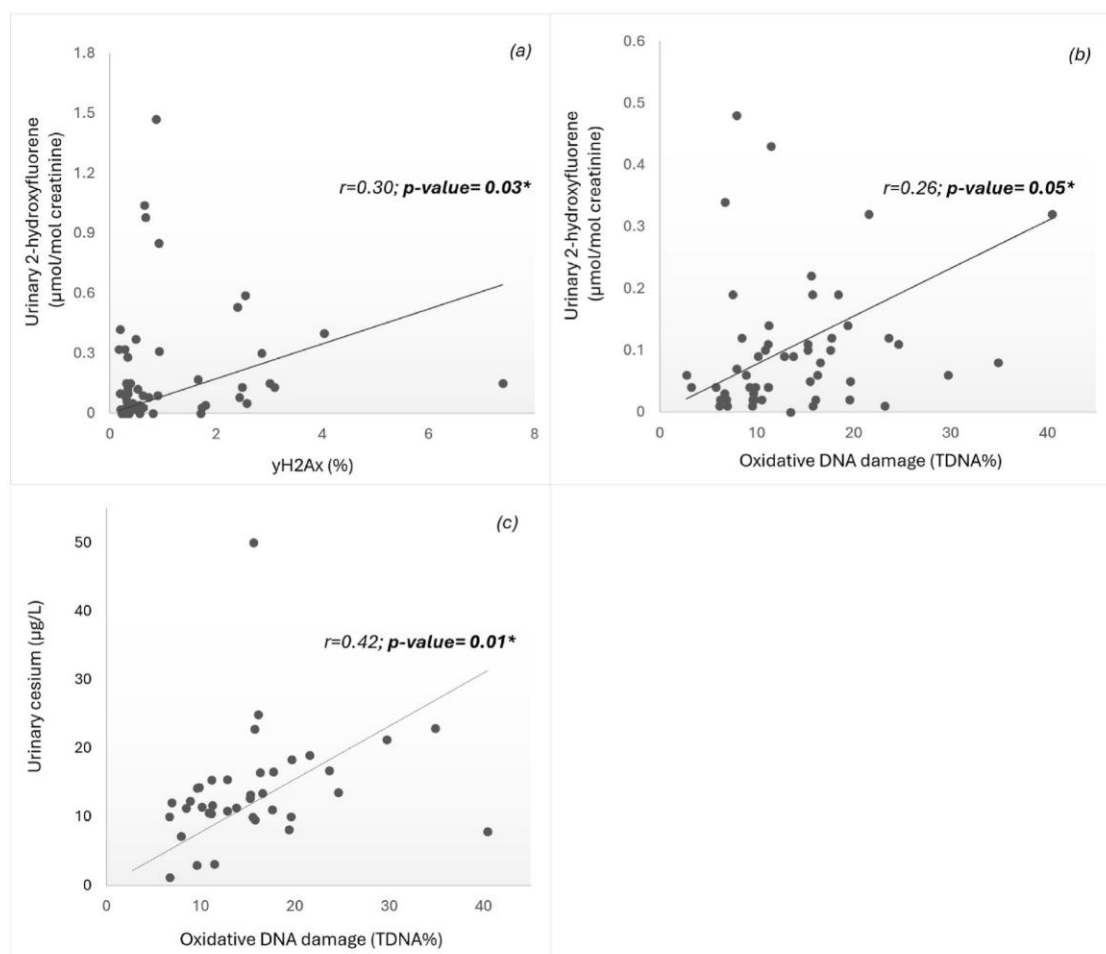


Fig. 2. – Scatter plots illustrating statistically significant correlations between post-exposure levels of exposure and effect biomarkers. *Indicate the statistical significance of FR, with $p\text{-values} \leq 0.05$.

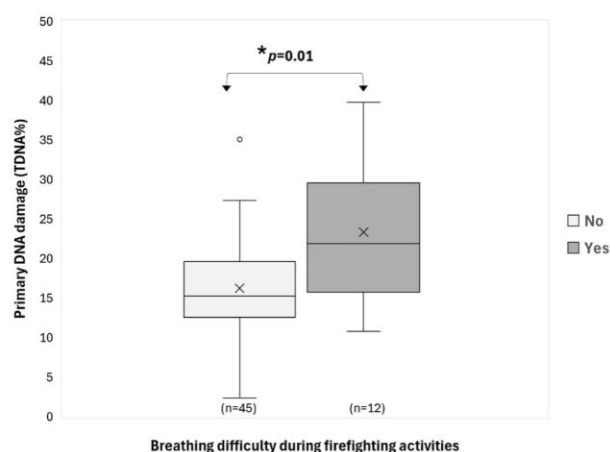


Fig. 3. – Post-exposure levels of primary DNA damage levels (TDNA%) in firefighters with and without breathing difficulties during firefighting activities. *Statistically significant difference $p < 0.05$. Statistical significance maintained when adjusted for smoking.

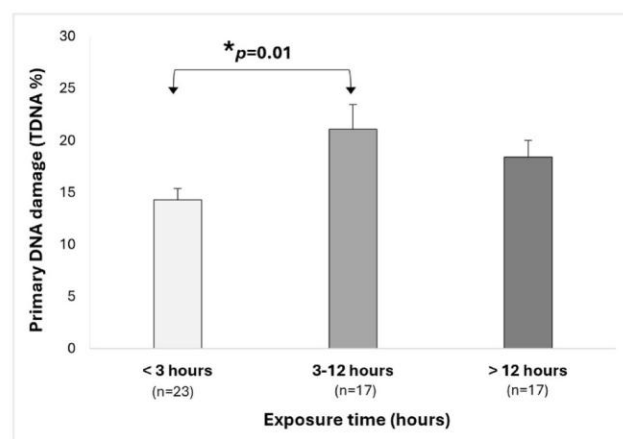


Fig. 4. Post-exposure levels of primary DNA damage (TDNA %) according to the number of hours spent in firefighting activities. * Statistically significant difference $p < 0.05$.

induced γH2AX formation. The phosphorylation of H2AX, forming γH2AX, is an early event in DNA damage response. It occurs rapidly after DNA DSB as part of the cellular mechanism to signal the activation of DNA repair pathways (Sánchez-Flores et al., 2015). Once the damage is

repaired, γ H2AX is dephosphorylated or removed, making it undetectable. However, if γ H2AX persists after repair, it may indicate ongoing genomic instability (Podhorecka et al., 2010). The use of γ H2AX holds great potential for environmental and occupational monitoring, allowing early detection and prevention of potential long-term adverse health outcomes (Laffon et al., 2021). Some studies, for example, have identified other DNA alterations associated with firefighting occupations. For instance, Zhou et al. (2019) reported significant differences in DNA methylation patterns between experienced firefighters and new recruits. These methylation alterations were found to correlate with cancer-related pathways, suggesting that such epigenetic modifications may contribute to increased cancer risk in firefighters. Similarly, Goodrich et al. (2022) verified that firefighting was associated with changes in DNA methylation, further supporting the hypothesis that occupational hazards may induce long-term genetic modifications. Given the role of epigenetic modifications in the development of various diseases, including cancer (Quaid et al., 2024), monitoring these changes, along with biomarkers of DNA damage and repair, could be crucial for identifying occupational risks in wildland firefighters.

In accordance with previous studies (Paiva et al., 2024b), all urinary OHPAH levels in this study were increased after exposure to woodsmoke. The urinary OHPAHs with the highest concentrations after firefighting exposure were 1-OHNaph+1-OHAc, followed by 2-OHFlu, 1-OHPhen and 1-OHPyr. The concentrations of 1OHNaph+1OHAc in this study were higher than those reported in previous studies conducted on firefighters exposed to wildfire emissions (Oliveira et al., 2020). Regarding 2-OHFlu and 1-OHPhen, levels verified in this study were similar to those found in previous studies focused on Portuguese wildland firefighters (Oliveira et al., 2016, 2017, 2020). 1-OHPyr was the PAH metabolite found at the lowest concentration, which is consistent with other studies that assessed urinary OHPAHs in firefighters exposed to woodsmoke and verified that 1-OHPyr is one of the OHPAHs detected at lower concentrations (Adetona et al., 2017; Oliveira et al., 2020; Li et al., 2016).

Concerning the relationship between genotoxicity endpoints and urinary OHPAHs, we found that levels of γ H2AX and oxidative DNA damage were positively correlated with urinary 2-OHFlu levels, correspondingly. In addition, we observed a significant contribution of urinary 2-OHFlu for the observed variation (post/pre-exposure) of oxidative DNA damage. Previous studies have also explored correlations between genotoxicity biomarkers and firefighting exposure to PAHs (Andersen et al., 2018a, 2018b; Oliveira et al., 2020). Similarly to our results, Oliveira et al. (2020), observed a positive correlation between oxidative DNA damage and urinary 2-OHFlu levels among two groups of Portuguese wildland firefighters (smokers vs non-smokers) exposed to forest fires. In the Andersen et al. (2018a) study, a positive correlation was found between primary DNA damage levels and urinary levels of 1-OHPyr. Furthermore, both primary and oxidative DNA damage were positively associated with total PAHs levels retrieved from skin wipes (Andersen et al., 2018a). In our study, we found a significant positive association between primary and oxidative DNA damage and the levels of Cs (133Cs) excreted in urine. According to our understanding, this is the first study correlating blood genotoxic/oxidative biomarkers with wildfire smoke-derived metals among wildland firefighters enrolled in real scenarios of fire suppression activities. Up to date, information available on health effects and toxicity of Cs in humans is limited (Yermishev, 2023). Previous studies have shown that stable Cs compounds are able to induce genomic instability, namely, chromosomal aberrations in cultured human primary cells (Ghosh et al., 1993) and micronuclei in mouse bone marrow cells *in vivo* (Santos-Mello et al., 1999). Additionally, a recent study in rats found that stable Cs had pronounced damaging effects on skeletal and cardiac striated muscle tissue, linking it to adverse cardiovascular outcomes (Yermishev, 2023).

In the environment Cs is naturally present only in one stable form - the isotope 133 Cs (non-radioactive). The lithological formations in the Northern region of Portugal - where our study was conducted - is

predominantly characterised by granite (pegmatite formation) and schist (Agroconsultores, 1991; Meireles, 2011), where Cs-bearing minerals may occur in different concentrations (Williams, 2004). We hypothesise that wildland burning may promote the release of this element into the environment (Al-Malki, 2009) thereby increasing the risk of exposure. In fact, a study conducted by Wolfe et al. (2004) found higher Cs (133Cs) concentrations in the urine of firefighters enrolled in wildfire combat when compared to NHANES III reference values (Third National Health and Nutrition Examination Survey) (Paschal et al., 1998). Paiva et al. (2024a) characterised the impact of real-life wildland firefighting operations on urinary levels of pollutant metal(loid)s among Northern Portuguese firefighters, and found significant higher post-exposure urinary Cs levels, when compared to baseline levels. Nevertheless, more studies are needed to confirm our results, to better understand the impact of wildland burning on the release and bioavailability of metal(loid)s, and their effects on human health and to facilitate the comparison of urinary metal(loid)s concentrations among wildland firefighters. When examining the link between wildland firefighters' symptomatology during fire combat and genotoxicity biomarkers, we found that those who reported breathing difficulties had significantly higher levels of primary DNA damage compared to those who did not. Although not statistically significant, a similar trend was also observed for oxidative DNA damage and γ H2AX. During active fire combat usually, firefighters do not wear adequate respiratory protection, furthermore, they continue to be exposed after combat, exposing them to high concentrations of pollutants in both gaseous and particulate form (Esteves et al., 2024). Consequently, reported breathing difficulty during fire combat may reflect greater inhalation of smoke contaminants, explaining the results obtained.

Our results exploring the association of work-related variables, gathered at Phase II, after the fire-event combat, have shown a significant positive influence of fire exposure and event duration on DNA damage endpoints.

Longer-lasting wildfires may release higher amounts of pollutants due to prolonged biomass combustion, leading to increased pollutant accumulation in the atmosphere and greater exposure. Kganyago et al. (2021) for example, observed that larger burned areas were associated with higher concentrations of smoke pollutants (i.e., PM and black carbon). Furthermore, prolonged exposure to smoke pollutants during long-lasting wildfires may occur through several factors, including the permanence of wildland firefighters near the affected area, their geographical proximity to the wildfire event (residential area or fire station), or ongoing exposure to contaminated personal protective equipment (PPE)/firefighting tools/vehicles, in the fire station. These factors may simultaneously contribute to increased risk of exposure to smoke pollutants and, consequently, enhance biological damage.

Despite our findings, our study has some limitations. Since data on social-demographic and work-related factors were collected by self-administered questionnaires, information bias may have occurred, inclusively regarding the precise number of hours spent on wildland firefighting activities and regarding the smoking status (e.g., recall bias or social desirability bias). Further studies should assess cotinine levels to accurately determine tobacco exposure. Additionally, it is important to consider the metabolism and excretion rates of certain combustion-derived products (Bader et al., 2021), which may lead to an underestimation of exposure levels due to the time interval between the exposure event and biological sample collection. Moreover, further studies are needed to confirm our results.

Nonetheless, our study uses a pre/post-study design, where participants serve as their own control, reducing the risk of potential confounders. This longitudinal analysis includes repeated measurements at different time points of the occupational exposure, enrolling the same individuals engaged in real-wildfire scenarios, *in situ*, providing more accurate, close-to-reality, insights into potential causal associations between exposure and observed outcomes.

In occupational hygiene, once a health risk is identified, the primary

mitigation measure is to eliminate, reduce, or substitute the source of exposure, thereby minimising its presence and lowering the associated risk (Schoket, 1999). However, for wildland firefighters, this may constitute a challenge. Therefore, the proper use of PPE, shift-rotation, and decontamination practices, and comprehensive training on these procedures, are of extreme importance to reduce contact with wildland smoke contaminants (Esteves et al., 2024). In this regard, our data seems to suggest the importance of rotative shifts during fire combat, rest areas protected from fire contaminants and effective decontamination procedures. Moreover, adopting a healthy lifestyle is essential for improving overall health and mitigating potential health effects of firefighting occupational exposure. Healthy behaviours include maintaining a balanced diet, rich in antioxidants to support cellular redox balance, engaging in regular physical exercise, practicing good sleep hygiene, monitoring mental/emotional health and avoiding risky behaviours such as tobacco and alcohol consumption (IARC, 2023).

5. Conclusion

Despite the obvious challenges that firefighters face, little is known about the biological mechanisms underlying the health outcomes associated with wildland firefighting. Thus, understanding exposure-induced biological mechanisms is crucial for the application of clinical tools in the preliminary identification of potential diseases, including cancer, and for designing measures to prevent disease onset.

Our findings showed that occupational exposure to real wildfire events induced systemic genomic instability, measured by comet assay and γ H2AX, among firefighters. Furthermore, a positive association between effect biomarkers and smoke combustion by-products (PAHs and metals), duration of exposure, wildfire dimension, and firefighters' acute symptomatology, was found. To our knowledge, this is the first longitudinal study investigating the impact of firefighters' occupational exposure to real wildfire events on systemic DNA damage biomarkers. Moreover, this is the first time that γ H2AX was evaluated among wildland firefighters exposed to forest fires.

Our data is of paramount importance to support recommendation measures and policy development vis-à-vis the protection of firefighters' health. The identification of occupational risk factors is crucial for the development of mitigation strategies to minimise exposure risks and adverse health impacts. Future longitudinal studies should focus on the assessment of the impact of firefighting exposures through cancer-related biomarkers, particularly on local-targeted tissues.

CRediT authorship contribution statement

Filipa Esteves: Writing – review & editing, Writing – original draft, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Joana Madureira:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Carla Costa:** Writing – review & editing, Software, Resources, Methodology, Investigation, Formal analysis, Data curation. **Joana Pires:** Software, Methodology, Investigation, Data curation. **Bela Barros:** Methodology, Investigation, Formal analysis, Data curation. **Sara Alves:** Methodology, Investigation, Formal analysis, Data curation. **Josiana Vaz:** Writing – review & editing, Resources, Project administration, Methodology. **Marta Oliveira:** Resources, Methodology, Investigation, Formal analysis, Data curation. **Klara Slezakova:** Writing – review & editing, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Adília Fernandes:** Writing – review & editing, Resources, Project administration, Methodology, Funding acquisition. **Maria do Carmo Pereira:** Writing – review & editing, Project administration, Methodology, Funding acquisition. **Simone Morais:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Vanessa Valdiglesias:** Writing – review & editing, Resources, Methodology, Data

curation. **Stefano Bonassi:** Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **João Paulo Teixeira:** Writing – review & editing, Resources, Project administration, Investigation, Funding acquisition. **Solange Costa:** Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Ethics approval

This work received approval for research ethics by the Accredited Ethics Committee of the University of Porto, Portugal, Report Nr. 92/CEUP/2020, under the project BioFirEx project (PCIF/SSO/0017/2018): “A panel of (bio)markers for the surveillance of firefighter's health and safety”.

Funding

This work received financial support from the project PCIF/SSO/0017/2018 (<https://doi.org/10.54499/PCIF/SSO/0017/2018>) by the Fundação para a Ciência e a Tecnologia (FCT), Ministério da Ciência, Tecnologia e Ensino Superior (MCTES), through national funds. This work is financed by national funds through the FCT within the scope of projects UIDB/04750/2020 (<https://doi.org/10.54499/UIDB/04750/2020>) and LA/P/0064/2020 (<https://doi.org/10.54499/LA/P/0064/2020>). Filipa Esteves was supported by National Funds through FCT, under the Ph.D. fellowship UI/BD/150783/2020. The work of Stefano Bonassi was supported by the Italian Ministry of Health (Ricerca Corrente), Rome, Italy, and by the Competitive Funding for University Research Projects [Finanziamento competitivo di progetti di ricerca di ateneo (FIN/RIC)] San Raffaele University, Rome, Italy.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijheh.2025.114576>.

References

- Abatzoglou, J.T., Williams, A.P., 2016. Impact of anthropogenic climate change on wildfire across western US forests. *Proc. Natl. Acad. Sci. USA*. 113 (42), 11770–11775. <https://doi.org/10.1073/pnas.1607171113>.
- Abreu, A., Costa, C., Pinho e Silva, S., Morais, S., do Carmo Pereira, M., Fernandes, A., Moraes de Andrade, V., Teixeira, J.P., Costa, S., 2017. Wood smoke exposure of Portuguese wildland firefighters: DNA and oxidative damage evaluation. *J. Toxicol. Environ. Health A* 80 (13–15), 596–604. <https://doi.org/10.1080/15287394.2017.1286896>.
- Adetona, O., Simpson, C.D., Li, Z., Sjödin, A., Calafat, A.M., Naeher, L.P., 2017. Hydroxylated polycyclic aromatic hydrocarbons as biomarkers of exposure to wood smoke in wildland firefighters. *J. Expo. Sci. Environ. Epidemiol.* 27 (1), 78–83. <https://doi.org/10.1038/jes.2015.75>.
- Agroconsultores, C., 1991. Carta dos solos, carta do uso actual da terra e carta da aptidão da terra do nordeste de Portugal. Projecto de desenvolvimento rural integrado de Trás-os-Montes, University of Trás-os-Montes and Alto Douro.
- Al-Malki, A.L., 2009. Serum heavy metals and hemoglobin related compounds in Saudi Arabia firefighters. *J. Occup. Med. Toxicol.* 4, 1–6. <https://doi.org/10.1186/1745-6673-4-18>.
- Ali, M.U., Wang, C., Li, Y., Jin, X., Yang, S., Ding, L., Feng, L., Wang, B., Li, P., 2023. Human biomonitoring of heavy metals exposure in different age-and gender-groups based on fish consumption patterns in typical coastal cities of China. *Ecotoxicol. Environ. Saf.* 262, 115316. <https://doi.org/10.1016/j.ecoenv.2023.115316>.
- Andersen, M.H.G., Saber, A.T., Clausen, P.A., Pedersen, J.E., Løhr, M., Keramanizadeh, A., Loft, S., Ebbeløj, N., Hansen, Å.M., Pedersen, P.B., Koponen, I.K., Nørskov, E.-C., Møller, P., Vogel, U., 2018a. Association between polycyclic aromatic hydrocarbon exposure and peripheral blood mononuclear cell DNA damage in human volunteers during fire extinction exercises. *Mutagenesis* 33 (1), 105–115. <https://doi.org/10.1093/mutage/gex021>.
- Andersen, M.H.G., Saber, A.T., Pedersen, J.E., Pedersen, P.B., Clausen, P.A., Løhr, M., Keramanizadeh, A., Loft, S., Ebbeløj, N.E., Hansen, Å.M., Koponen, I.K., Nørskov, E.-C., Vogel, U., Møller, P., 2018b. Assessment of polycyclic aromatic hydrocarbon exposure, lung function, systemic inflammation, and genotoxicity in peripheral blood mononuclear cells from firefighters before and after a work shift. *Environ. Mol. Mutagen.* 59 (6), 539–548. <https://doi.org/10.1002/em.22193>.

- Augusto, S., Ratola, N., Tarín-Carrasco, P., Jiménez-Guerrero, P., Turco, M., Schuhmacher, M., Costa, S., Teixeira, J., Costa, C., 2020. Population exposure to particulate-matter and related mortality due to the Portuguese wildfires in October 2017 driven by storm Ophelia. *Environ. Int.* 144, 106056. <https://doi.org/10.1016/j.envint.2020.106056>.
- Azevedo, R., Gennaro, D., Duro, M., Pinto, E., Almeida, A., 2023. Further evidence on trace element imbalances in haemodialysis patients—paired analysis of blood and serum samples. *Nutrients* 15 (8), 1912. <https://doi.org/10.3390/nu15081912>.
- Bader, M., Bäcker, S., Jäger, T., Webendörfer, S., Van Bortel, G., Van Mieghem, F., Van Weyenbergh, T., 2021. Preparedness as a key factor for human biomonitoring programs after chemical incidents. *J. Expo. Sci. Environ. Epidemiol.* 31 (5), 867–875. <https://doi.org/10.1038/s41370-021-00320-w>.
- Barcelo, H., Faul, J., Crimmins, E., Thyagarajan, B., 2018. A practical cryopreservation and staining protocol for immunophenotyping in population studies. *Curr. Protoc. Cytom.* 84 (1), e35. <https://doi.org/10.1002/cpcy.35>.
- Barros, B., Paiva, A.M., Oliveira, M., Alves, S., Esteves, F., Fernandes, A., Vaz, J., Slezakova, K., Costa, S., Teixeira, J.P., Morais, S., 2024. Baseline data and associations between urinary biomarkers of polycyclic aromatic hydrocarbons, blood pressure, hemogram, and lifestyle among wildland firefighters. *Front. Public Health* 12, 1338435. <https://doi.org/10.3389/fpubh.2024.1338435>.
- Bernstein, D.N., Mahowald, N.M., Hess, P.G., Tilmes, S., Hamilton, D., Connelly, D.S., Krasnoff, R., 2019. 2017–2018 North America Wildfires and Their Impact on Air Quality and Climate. AGU Fall Meet. Abstr.
- Bharti, R., Sharma, R., 2022. Effect of heavy metals: an overview. *Mater. Today Proc.* 51, 880–885. <https://doi.org/10.1016/j.matpr.2021.06.278>.
- Cadet, J., Loft, S., Olinski, R., Evans, M.D., Bialkowski, K., Richard Wagner, J., Dedon, P. C., Moller, P., Greenberg, M.M., Cooke, M.S., 2012. Biologically relevant oxidants and terminology, classification and nomenclature of oxidatively generated damage to nucleobases and 2-deoxyribose in nucleic acids. *Free Radic. Res.* 46 (4), 367–381. <https://doi.org/10.3109/10715762.2012.659248>.
- Cannan, W.J., Pederson, D.S., 2016. Mechanisms and consequences of double-strand DNA break formation in chromatin. *J. Cell. Physiol.* 231 (1), 3–14. <https://doi.org/10.1002/jcp.25048>.
- Casjens, S., Brüning, T., Taeger, D., 2020. Cancer risks of firefighters: a systematic review and meta-analysis of secular trends and region-specific differences. *Int. Arch. Occup. Environ. Health* 1–14. <https://doi.org/10.1007/s00420-020-01539-0>.
- Chen, Y., Pan, Z., Shen, J., Wu, Y., Fang, L., Xu, S., Ma, Y., Zhao, H., Pan, F., 2023. Associations of exposure to blood and urinary heavy metal mixtures with psoriasis risk among US adults: a cross-sectional study. *Sci. Total Environ.* 887, 164133. <https://doi.org/10.1016/j.scitotenv.2023.164133>.
- Collins, A.R., 2004. The comet assay for DNA damage and repair. *Mol. Biotechnol.* 26 (3), 249. <https://doi.org/10.1385/MB:26:3:249>.
- Collins, A., Koppen, G., Valdiglesias, V., Dusinska, M., Kruszewski, M., Møller, P., Rojas, E., Dhawan, A., Benzie, I., Coskun, E., Moretti, M., Speit, G., Bonassi, S., project, C., 2014. The comet assay as a tool for human biomonitoring studies: the ComNet project. *Mutat. Res. Rev. Mutat. Res.* 759, 27–39. <https://doi.org/10.1016/j.mmr.2013.10.001>.
- Collins, A.R., El Yamani, N., Lorenzo, Y., Shaposhnikov, S., Brunborg, G., Azqueta, A., 2014. Controlling variation in the comet assay. *Front. Genet.* 5, 359. <https://doi.org/10.3389/fgene.2014.00359>.
- Costa, S., Esteves, F., 2023. Biomarkers, human health. *Encyclopedia of Toxicology*, fourth ed.
- Costa, S., Pina, C., Coelho, P., Costa, C., Silva, S., Porto, B., Laffon, B., Teixeira, J.P., 2011. Occupational exposure to formaldehyde: genotoxic risk evaluation by comet assay and micronucleus test using human peripheral lymphocytes. *J. Toxicol. Environ. Health A* 74 (15–16), 1040–1051. <https://doi.org/10.1080/15287394.2011.582293>.
- Czerska, M., Mikolajewska, K., Zieliński, M., Gromadzińska, J., Wasowicz, W., 2015. Today's oxidative stress markers. *Med. Pr.* 66 (3). <https://doi.org/10.13075/mp.5893.00137>.
- Danielsen, P.H., Møller, P., Jensen, K.A., Sharma, A.K., Wallin, H.K., Bossi, R., Autrup, H., Mølhave, L., Ravanat, J.-L., Briedé, J.J., Kok, T.M., Loft, S., 2011. Oxidative stress, DNA damage, and inflammation induced by ambient air and wood smoke particulate matter in human A549 and THP-1 cell lines. *Chem. Res. Toxicol.* 24 (2), 168–184. <https://doi.org/10.1021/tx100407m>.
- Decker, J.A., DeBord, D.G., Bernard, B., Dotson, G.S., Halpin, J., Hines, C.J., Kiefer, M., Myers, K., Page, E., Schulte, P., Snawder, J., 2013. Recommendations for biomonitoring of emergency responders: focus on occupational health investigations and occupational health research. *Mil. Med.* 178 (1), 68–75. <https://doi.org/10.7205/milmed-d-12-00173>.
- Esteves, F., Teixeira, E., Amorim, T., Costa, C., Pereira, C., Fraga, S., De Andrade, V.M., Teixeira, J.P., Costa, S., 2017. Assessment of DNA damage in a group of professional dancers during a 10-month dancing season. *J. Toxicol. Environ. Health A* 80 (13–15), 797–804. <https://doi.org/10.1080/15287394.2017.1331599>.
- Esteves, F., Amaro, R., Silva, S., Sánchez-Flores, M., Teixeira, J.P., Costa, C., 2020. The impact of comet assay data normalization in human biomonitoring studies outcomes. *Toxicol. Lett.* 332, 56–64. <https://doi.org/10.1016/j.toxlet.2020.06.024>.
- Esteves, F., Madureira, J., Teixeira, J.P., Costa, S., 2022. The role of biomarkers in the assessment of potential health risks of wildland firefighters' occupational exposure: an overview. In: *Essential Guide to Occupational Exposure*. Nova Science Publishers, Inc. <https://doi.org/10.52305/VGMC6738>.
- Esteves, F., Madureira, J., Teixeira, J.P., Costa, S., 2024. Wildland firefighters: a crucial weapon for forest fire management. Which health risks do they face? In: *Fire Hazards: Socio-Economic and Regional Issues*. Springer, pp. 107–121. https://doi.org/10.1007/978-3-031-50446-4_9.
- Fabian, T.Z., Borgerson, J.L., Gandhi, P.D., Baxter, C.S., Ross, C.S., Lockey, J.E., Dalton, J.M., 2014. Characterization of firefighter smoke exposure. *Fire Technol.* 50 (4), 993–1019. <https://doi.org/10.1007/s10694-011-0212-2>.
- Ferguson, M.D., Semmens, E.O., Dumke, C., Quindry, J.C., Ward, T.J., 2016. Measured pulmonary and systemic markers of inflammation and oxidative stress following wildland firefighter simulations. *J. Occup. Environ. Med.* 58 (4), 407–413. <https://doi.org/10.1097/JOM.0000000000000688>.
- Gaughan, D.M., Siegel, P.D., Hughes, M.D., Chang, C.Y., Law, B.F., Campbell, C.R., Richards, J.C., Kales, S.F., Chertok, M., Kobzik, L., Nguyen, P.-S., O'Donnell, C.R., Kiefer, M., Wagner, G.R., Christiani, D.C., 2014. Arterial stiffness, oxidative stress, and smoke exposure in wildland firefighters. *Am. J. Ind. Med.* 57 (7), 748–756. <https://doi.org/10.1002/ajim.22331>.
- Ghosh, A., Sharma, A., Talukder, G., 1993. Clastogenic effects of caesium chloride on human peripheral blood lymphocytes in vitro. *Toxicol. Vitro* 7 (2), 137–140. [https://doi.org/10.1016/0887-2333\(93\)90123-m](https://doi.org/10.1016/0887-2333(93)90123-m).
- Glass, D.C., Del Monaco, A., Pircher, S., Vander Hoorn, S., Sim, M.R., 2017. Mortality and cancer incidence among male volunteer Australian firefighters. *Occup. Environ. Med.* 74 (9), 628–638. <https://doi.org/10.1136/oemed-2016-104088>.
- Goodrich, J.M., Jung, A.M., Furlong, M.A., Beitel, S., Littau, S., Gulotta, J., Wallentine, D., Burgess, J.L., 2022. Repeat measures of DNA methylation in an inception cohort of firefighters. *Occup. Environ. Med.* 79 (10), 656–663. <https://doi.org/10.1136/oemed-2021-108153>.
- Hwang, J., Xu, C., Grunsted, P., Agnew, R.J., Malone, T.R., Clifton, S., Thompson, K., Xu, X., 2022. Urinary metabolites of polycyclic aromatic hydrocarbons in firefighters: a systematic review and meta-analysis. *Int. J. Environ. Res. Publ. Health* 19 (14), 8475. <https://doi.org/10.3390/ijerph19148475>.
- IARC, 2023. Occupational Exposure as a Firefighter, vol. 132.
- Jasra, S., Giricz, O., Zeig-Owens, R., Pradhan, K., Goldfarb, D.G., Barreto-Galvez, A., Silver, A.J., Chen, J., Sahu, S., Gordon-Mitchell, S., Zeig-Owens, R., Pradhan, K., Goldfarb, D.G., Barreto-Galvez, A., Silver, A.J., Chen, J., Sahu, S., Gordon-Mitchell, S., Choudhary, G.S., Aluri, S., Bhagat, T.D., Shastri, A., Bejan, C.A., Stockton, S.S., Spaulding, T.P., Thiruthuvanantham, V., Goto, H., Gerhardt, J., Haider, S.H., Veerappan, A., Bartenstein, M., Nwankwo, G., Landgren, O., Weiden, M.D., Lekostaj, J., Bender, R., Fletcher, F., Greenberger, L., Ebert, B.L., Steidl, U., Will, B., Nolan, A., Madireddy, A., Savona, M.R., Prezant, D.J., Verma, A., 2022. High burden of clonal hematopoiesis in first responders exposed to the World Trade Center disaster. *Nat. Med.* 28 (3), 468–471. <https://doi.org/10.1038/s41591-022-01708-3>.
- Kamal, A., Cincinelli, A., Martellini, T., Malik, R.N., 2015. A review of PAH exposure from the combustion of biomass fuel and their less surveyed effect on the blood parameters. *Environ. Sci. Pollut. Res.* 22, 4076–4098. <https://doi.org/10.1007/s11356-014-3748-0>.
- Kganyago, M., Govender, K., Shikwambana, L., Sivakumar, V., 2021. Study on blazing wildfires at the outeniqua pass in South Africa during the october/november 2018 period. *Remote Sens. Appl. Soc. Environ.* 21, 100464. <https://doi.org/10.1016/j.rsase.2020.100464>.
- Kim, K.-H., Jahan, S.A., Kabir, E., Brown, R.J., 2013. A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. *Environ. Int.* 60, 71–80. <https://doi.org/10.1016/j.envint.2013.07.019>.
- Laffon, B., Sánchez-Flores, M., Fernández-Bertólez, N., Pásaró, E., Valdiglesias, V., 2021. Applicability of flow cytometry γH2AX assay in population studies: suitability of fresh and frozen whole blood samples. *Arch. Toxicol.* 95 (5), 1843–1851. <https://doi.org/10.1007/s00204-021-03009-z>.
- Lamprecht, M., Greilberger, J., Oetl, K., 2004. Analytical aspects of oxidatively modified substances in sports and exercises. *Nutrition* 20 (7–8), 728–730. <https://doi.org/10.1016/j.nut.2004.04.016>.
- LeMasters, G.K., Genaidy, A.M., Succop, P., Deddens, J., Sobehi, T., Barriera-Viruet, H., Dunning, K., Lockey, J., 2006. Cancer risk among firefighters: a review and meta-analysis of 32 studies. *J. Occup. Environ. Med.* 48 (11), 1189–1202. <https://doi.org/10.1097/01.jom.0000246229.68697.90>.
- Leonard, S.S., Castranova, V., Chen, B.T., Schwegler-Berry, D., Hoover, M., Piacitelli, C., Gaughan, D.M., 2007. Particle size-dependent radical generation from wildland fire smoke. *Toxicol* 236 (1–2), 103–113. <https://doi.org/10.1016/j.tox.2007.04.008>.
- Lewtas, J., 2007. Air pollution combustion emissions: characterization of causative agents and mechanisms associated with cancer, reproductive, and cardiovascular effects. *Mutat. Res. Rev. Mutat. Res.* 636 (1–3), 95–133. <https://doi.org/10.1016/j.mmr.2007.08.003>.
- Li, Z., Trinidad, D., Pittman, E.N., Riley, E.A., Sjodin, A., Dills, R.L., Paulsen, M., Simpson, C.D., 2016. Urinary polycyclic aromatic hydrocarbon metabolites as biomarkers to woodsmoke exposure—results from a controlled exposure study. *J. Expo. Sci. Environ. Epidemiol.* 26 (3), 241–248. <https://doi.org/10.1038/jes.2014.94>.
- Li, Q., Qian, W., Zhang, Y., Hu, L., Chen, S., Xia, Y., 2023. A new wave of innovations within the DNA damage response. *Signal Transduct. Targeted Ther.* 8 (1), 338. <https://doi.org/10.1038/s41392-023-01548-8>.
- Liu, P.-L., Chen, Y.-L., Chen, Y.-H., Lin, S.-J., Kou, Y.R., 2005. Wood smoke extract induces oxidative stress-mediated caspase-independent apoptosis in human lung endothelial cells: role of AIF and EndoG. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 289 (5), L739–L749. <https://doi.org/10.1152/ajplung.00099.2005>.
- Meireles, C.A.P., 2011. Litoestratigrafia do Paleozóico do Sector a Nordeste de Bragança. Trás-os-Montes. Laboratório Nacional de Energia e Geologia (Portugal).
- Mitra, S., Chakraborty, A.J., Tareq, A.M., Emran, T.B., Nainu, F., Khusró, A., Idris, A.M., Khandaker, M.U., Osman, H., Alhumaydhi, F.A., Simal-Gandara, J., 2022. Impact of heavy metals on the environment and human health: novel therapeutic insights to counter the toxicity. *J. King Saud Univ. Sci.* 34 (3), 101865. <https://doi.org/10.1016/j.jksus.2022.101865>.

- Møller, P., Danielsen, P.H., Jantzen, K., Roursgaard, M., Loft, S., 2013. Oxidatively damaged DNA in animals exposed to particles. *Crit. Rev. Toxicol.* 43 (2), 96–118. <https://doi.org/10.3109/10408444.2012.756456>.
- Møller, P., Azqueta, A., Boutet-Robinet, E., Koppen, G., Bonassi, S., Milić, M., Gajski, G., Costa, S., Teixeira, J.P., Pereira, C.P., Dusinska, M., Godschalk, R., Brunborg, G., Gutzkow, K., Giovannelli, L., Cooke, M.S., Richling, E., Laffon, B., Valdiglesias, V., Basaran, N., Del Bo, C., Zegura, B., Novak, M., Stopper, H., Vodicka, P., Vodenkova, S., de Andrade, V.M., Sramkova, M., Gabelova, A., Collins, A., Langie, S. A.S., 2020a. Minimum Information for Reporting on the Comet Assay (MIRCA): recommendations for describing comet assay procedures and results. *Nat. Protoc.* 15 (12), 3817–3826. <https://doi.org/10.1038/s41596-020-0398-1>.
- Møller, P., Muruzabal, D., Bakuradze, T., Richling, E., Bankoglu, E.E., Stopper, H., Langie, S., Azqueta, A., Jensen, A., Scavone, F., Giovannelli, L., Wojewódza, M., Kruszeński, M., Valdiglesias, V., Laffon, B., Costa, C., Costa, S., Teixeira, J.P., Marino, M., Del Bo, C., Riso, P., Shaposhnikov, S., Collins, A., 2020b. Potassium bromate as positive assay control for the Fpg-modified comet assay. *Mutagenesis* 35 (4), 341–348. <https://doi.org/10.1093/mutage/geaa011>.
- Navarro, K.M., Kleinman, M.T., Mackay, C.E., Reinhardt, T.E., Balmes, J.R., Broyles, G. A., Ottmar, R.D., Naher, L.P., Domitrovich, J.W., 2019. Wildland firefighter smoke exposure and risk of lung cancer and cardiovascular disease mortality. *Environ. Res.* 173, 462–468. <https://doi.org/10.1016/j.envres.2019.03.060>.
- Neri, M., Milazzo, D., Ugolini, D., Milic, M., Campolongo, A., Pasqualetti, P., Bonassi, S., 2014. Worldwide interest in the comet assay: a bibliometric study. *Mutagenesis* 30 (1), 155–163. <https://doi.org/10.1093/mutage/geu061>.
- Nikolova, T., Marini, F., Kaina, B., 2017. Genotoxicity testing: comparison of the γ H2AX focus assay with the alkaline and neutral comet assays. *Mutat. Res. Genet. Toxicol. Environ. Mutagen* 822, 10–18. <https://doi.org/10.1016/j.mrgentox.2017.07.004>.
- Nordberg, G.F., 2010. Biomarkers of exposure, effects and susceptibility in humans and their application in studies of interactions among metals in China. *Toxicol. Lett.* 192 (1), 45–49. <https://doi.org/10.1016/j.toxlet.2009.06.859>.
- Oliveira, M., Slezakova, K., Alves, M.J., Fernandes, A., Teixeira, J.P., Delerue-Matos, C., Pereira, M.C., Morais, S., 2016. Firefighters' exposure biomonitoring: impact of firefighting activities on levels of urinary monohydroxyl metabolites. *Int. J. Hyg. Environ. Health* 219 (8), 857–866. <https://doi.org/10.1016/j.ijheh.2016.07.011>.
- Oliveira, M., Slezakova, K., Magalhães, C.P., Fernandes, A., Teixeira, J.P., Delerue-Matos, C., Pereira, M.C., Morais, S., 2017. Individual and cumulative impacts of fire emissions and tobacco consumption on wildland firefighters' total exposure to polycyclic aromatic hydrocarbons. *J. Hazard Mater.* 334, 10–20. <https://doi.org/10.1016/j.jhazmat.2017.03.057>.
- Oliveira, M., Costa, S., Vaz, J., Fernandes, A., Slezakova, K., Delerue-Matos, C., Teixeira, J.P., Pereira, M.C., Morais, S., 2020. Firefighters exposure to fire emissions: impact on levels of biomarkers of exposure to polycyclic aromatic hydrocarbons and genotoxic/oxidative-effects. *J. Hazard Mater.* 383, 121179. <https://doi.org/10.1016/j.jhazmat.2019.121179>.
- Paiva, A.M., Barros, B., Azevedo, R., Oliveira, M., Alves, S., Esteves, F., Fernandes, A., Vaz, J., Alves, M.J., Slezakova, K., Pereira, M.C., Teixeira, J.P., Costa, S., Almeida, A., Morais, S., 2024a. Biomonitoring of firefighters' exposure to priority pollutant metal (loid)s during wildland fire combat missions: impact on urinary levels and health risks. *Sci. Total Environ.* 953, 176105. <https://doi.org/10.1016/j.scitotenv.2024.176105>.
- Paiva, A.M., Barros, B., Oliveira, M., Alves, S., Esteves, F., Fernandes, A., Vaz, J., Slezakova, K., Teixeira, J.P., Costa, S., Morais, S., 2024b. Biomonitoring of polycyclic aromatic hydrocarbons exposure and short-time health effects in wildland firefighters during real-life fire events. *Sci. Total Environ.* 926, 171801. <https://doi.org/10.1016/j.scitotenv.2024.171801>.
- Paschal, D.C., Ting, B.G., Morrow, J.C., Pirkle, J.L., Jackson, R.J., Sampson, E.J., Miller, D.T., Caldwell, K.L., 1998. Trace metals in urine of United States residents: reference range concentrations. *Environ. Res.* 76 (1), 53–59. <https://doi.org/10.1006/enrs.1997.3793>.
- Podhorecka, M., Skladanowski, A., Bozko, P., 2010. H2AX phosphorylation: its role in DNA damage response and cancer therapy. *J. Nucleic Acids* 2010 (1), 920161. <https://doi.org/10.4061/2010/920161>.
- Poetsch, A.R., 2020. The genomics of oxidative DNA damage, repair, and resulting mutagenesis. *Comput. Struct. Biotechnol. J.* 18, 207–219. <https://doi.org/10.1016/j.csbj.2019.12.013>.
- Quaid, M., Goodrich, J.M., Calkins, M.M., Graber, J.M., Urwin, D., Gabriel, J., Caban-Martinez, A., Petroff, R., Grant, C., Beitel, S., Littau, S., Gulotta, J., Wallentine, D., Hughes, J., Burgess, J., 2024. Firefighting, per- and polyfluoroalkyl substances, and DNA methylation of genes associated with prostate cancer risk. *Environ. Mol. Mutagen.* 65 (1–2), 55–66. <https://doi.org/10.1002/em.22589>.
- Radeloff, V.C., Helmers, D.P., Kramer, H.A., Mockrin, M.H., Alexandre, P.M., Bar-Massada, A., Butsic, V., Hawbaker, T.J., Martinuzzi, S., Syphard, A.D., Stewart, S.I., 2018. Rapid growth of the US wildland-urban interface raises wildfire risk. *Proc. Natl. Acad. Sci.* 115 (13), 3314–3319. <https://doi.org/10.1073/pnas.1718850115>.
- Reid, C.E., Brauer, M., Johnston, F.H., Jerrett, M., Balmes, J.R., Elliott, C.T., 2016. Critical review of health impacts of wildfire smoke exposure. *Environ. Health Perspect.* 124 (9), 1334–1343. <https://doi.org/10.1289/ehp.1409277>.
- Sánchez-Flores, M., Páraso, E., Bonassi, S., Laffon, B., Valdiglesias, V., 2015. γ H2AX assay as DNA damage biomarker for human population studies: defining experimental conditions. *Toxicol. Sci.* 144 (2), 406–413. <https://doi.org/10.1093/toxsci/kfv011>.
- Santos-Mello, R., Schmidt, T., Neuhauss, E., Almeida, A., 1999. Induction of micronuclei by CsCl in vivo and in vitro. *Mutation Res./Genet. Toxicol. Environ. Mutagen.* 446 (2), 239–244. [https://doi.org/10.1016/s1383-5718\(99\)00195-3](https://doi.org/10.1016/s1383-5718(99)00195-3).
- Schoket, B., 1999. DNA damage in humans exposed to environmental and dietary polycyclic aromatic hydrocarbons. *Mutat. Res./Fundam. Mol. Mechanisms Mutagen.* 424 (1–2), 143–153. [https://doi.org/10.1016/s0027-5107\(99\)00015-9](https://doi.org/10.1016/s0027-5107(99)00015-9).
- Senoner, T., Dichtl, W., 2019. Oxidative stress in cardiovascular diseases: still a therapeutic target? *Nutrients* 11 (9), 2090. <https://doi.org/10.3390/nu11092090>.
- Singh, N.P., McCoy, M.T., Tice, R.R., Schneider, E.L., 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp. Cell Res.* 175 (1), 184–191. [https://doi.org/10.1016/0014-4827\(88\)90265-0](https://doi.org/10.1016/0014-4827(88)90265-0).
- Stephens, S.L., Agee, J.K., Fule, P.Z., North, M., Romme, W., Swetnam, T., Turner, M.G., 2013. Managing forests and fire in changing climates. *Science* 342 (6154), 41–42. <https://doi.org/10.1126/science.1240294>.
- Tanaka, T., Halicka, D., Traganos, F., Darzynkiewicz, Z., 2009. Cytometric analysis of DNA damage: phosphorylation of histone H2AX as a marker of DNA double-strand breaks (DSBs). In: *Chromatin Protocols*. Springer, pp. 161–168. https://doi.org/10.1007/978-1-59745-190-1_11.
- Teixeira-Gomes, A., Lage, B., Esteves, F., Sousa, A.C., Pastorinho, M.R., Valdiglesias, V., Costa, S., Laffon, B., Teixeira, J.P., 2020. Frailty syndrome, biomarkers and environmental factors—a pilot study. *Toxicol. Lett.* 330, 14–22. <https://doi.org/10.1016/j.toxlet.2020.04.023>.
- Valdiglesias, V., Giunta, S., Fenech, M., Neri, M., Bonassi, S., 2013. γ H2AX as a marker of DNA double strand breaks and genomic instability in human population studies. *Mutat. Res. Rev. Mutat. Res.* 753 (1), 24–40. <https://doi.org/10.1016/j.mmrrev.2013.02.001>.
- Watters, G.P., Smart, D.J., Harvey, J.S., Austin, C.A., 2009. H2AX phosphorylation as a genotoxicity endpoint. *Mutat. Res. Genet. Toxicol. Environ. Mutagen* 679 (1–2), 50–58.
- Westerling, A.L., Hidalgo, H.G., Cayan, D.R., Swetnam, T.W., 2006. Warming and earlier spring increase western US forest wildfire activity. *Science* 313 (5789), 940–943. <https://doi.org/10.1126/science.1128834>.
- Williams, M., 2004. *Toxicological Profile for Cesium*.
- Wolfe, M.I., Mott, J.A., Voorhees, R.E., Sewell, C.M., Paschal, D., Wood, C.M., McKinney, P.E., Redd, S., 2004. Assessment of urinary metals following exposure to a large vegetative fire, New Mexico, 2000. *J. Expo. Sci. Environ. Epidemiol.* 14 (2), 120–128. <https://doi.org/10.1038/sj.jea.7500299>.
- Wu, C.-M., Adetona, A., Song, C., Naeher, L., Adetona, O., 2020. Measuring acute pulmonary responses to occupational wildland fire smoke exposure using exhaled breath condensate. *Arch. Environ. Occup. Health* 75 (2), 65–69. <https://doi.org/10.1080/19338244.2018.1562413>.
- Yermishev, O., 2023. Ecological and toxicological features of the effect of stable cesium on striated muscle tissue (myocardium and skeletal muscles) of mammals. *Regul. Mech. Biosys.* 14 (3), 415–423. <https://doi.org/10.15421/10.15421/022361>.
- Zhang, Y., Lim, S., Sharples, J.J., 2017. Wildfire occurrence patterns in ecoregions of new south wales and Australian capital territory, Australia. *Nat. Hazards* 87, 415–435. <https://doi.org/10.1007/s11069-017-2770-1>.
- Zhou, J., Jenkins, T.G., Jung, A.M., Jeong, K.S., Zhai, J., Jacobs, E.T., Griffin, S., Dearmin-Moore, D., Littau, S., Peate, W., Ellis, N., Lance, P., Yin, C., Burgess, J., 2019. DNA methylation among firefighters. *PLoS One* 14 (3), e0214282. <https://doi.org/10.1371/journal.pone.0214282>.

Supplementary Material

Occupational Exposure to Wildland Firefighting and Its Effects on Systemic DNA Damage

Filipa Esteves^{a,b,c,d}, Joana Madureira^{a,b,c}, Carla Costa^{a,b,c}, Joana Pires^{a,b,c}, Bela Barros^e, Sara Alves^f, Josiana Vaz^{g,h}, Marta Oliveira^e, Klara Slezakovaⁱ, Adília Fernandes^{g,h}, Maria do Carmo Pereira^j, Simone Morais^e, Vanessa Valdiglesias^{j,k}, Stefano Bonassi^{l,m}, João Paulo Teixeira^{a,b,c,*}, Solange Costa^{a,b,c}

^a Environmental Health Department, National Institute of Health, Rua Alexandre Herculano, n° 321, 4000-055 Porto, Portugal

^b EPIUnit - Instituto de Saúde Pública, Universidade do Porto, Rua das Taipas, n° 135, 4050-600 Porto, Portugal

^c Laboratório para a Investigação Integrativa e Translacional em Saúde Populacional (ITR), Universidade do Porto, Rua das Taipas, n° 135, 4050-600 Porto, Portugal

^d Department of Public Health and Forensic Sciences, and Medical School, Faculty of Medicine, University of Porto, Rua Doutor Plácido da Costa, 4200-450 Porto, Portugal

^e REQUIMTE/LAQV-Instituto Superior de Engenharia, Instituto Politécnico do Porto, Dr. António Bernardino de Almeida 431, 4249-015 Porto, Portugal

^f Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

^g Research Centre for Active Living and Wellbeing (LiveWell), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

^h CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

ⁱ LEPABE-ALiCE, Faculdade de Engenharia da Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

^j Universidade da Coruña, Grupo NanoToxGen, Centro Interdisciplinar de Química e Biología - CICA, Departamento de Biología, Facultad de Ciencias, Campus A Zapateira s/n, A Coruña, Spain

^k Instituto de Investigación Biomédica de A Coruña (INIBIC), Complexo Hospitalario Universitario de A Coruña (CHUAC), Sergas, As Xubias, A Coruña 15006, Spain

^l Unit of Clinical and Molecular Epidemiology, IRCCS San Raffaele Roma, 00163 Rome, Italy

^m Department of Human Sciences and Quality of Life Promotion, San Raffaele University, 00166, Rome, Italy

*Corresponding author: João Paulo Teixeira; e-mail address: joao.teixeira@insa.min-saude.pt

Table S1. Descriptive statistics of urinary OHPAHs and metal(loid)s levels in Phase I (before wildfire season – baseline) and Phase II (after fire event – during wildfire season).

Outcome measure	Phase I (Pre-wildfire Season)		Phase II (After the fire event)		P-value
	n	Mean ± SE	n	Mean ± SE	
Urinary OHPAHs					
1-OHNaph+1-OHAce (μmol/mol creatinine)	56	16.15 ± 2.32	56	40.44 ± 6.00	<0.01**
2-OHFlu (μmol/mol creatinine)	56	0.17 ± 0.03	56	0.21 ± 0.04	0.07
1-OHPhen (μmol/mol creatinine)	56	0.07 ± 0.01	56	0.13 ± 0.03	0.05
1-OHPyr (μmol/mol creatinine)	56	0.04 ± 0.01	56	0.06 ± 0.01	<0.01**
Urinary Metal(loid)s					
Li (μg/L)	39	112.96 ± 25.26	39	121.93 ± 20.58	0.21
Co (μg/L)	39	0.50 ± 0.13	39	0.57 ± 0.14	0.05
Ni (μg/L)	39	2.82 ± 0.24	39	3.40 ± 0.28	0.02*
Cu (μg/L)	39	9.10 ± 0.65	39	11.27 ± 1.07	0.04
Zn (μg/L)	39	520.08 ± 65.85	39	579.36 ± 59.03	0.40
As (μg/L)	39	26.61 ± 3.43	39	49.39 ± 11.12	0.03*
Se (μg/L)	39	26.75 ± 2.58	39	24.35 ± 2.43	0.44
Rb (μg/L)	39	2123.15 ± 137.06	39	2332.98 ± 154.16	0.23
Sr (μg/L)	39	137.23 ± 15.51	39	137.60 ± 14.16	0.62
Mo (μg/L)	39	47.44 ± 4.38	39	47.67 ± 4.68	0.83
Cd (μg/L)	39	0.32 ± 0.04	39	0.34 ± 0.05	0.60
Sb (μg/L)	39	0.08 ± 0.01	39	0.11 ± 0.02	0.08
Cs (μg/L)	39	11.06 ± 0.80	39	13.62 ± 1.27	<0.01**
Ba (μg/L)	39	2.33 ± 0.27	39	1.97 ± 0.24	0.23
Hg (μg/L)	39	0.37 ± 0.04	39	0.37 ± 0.05	0.30
Tl (μg/L)	39	0.26 ± 0.03	39	0.26 ± 0.03	0.99
Pb (μg/L)	39	0.94 ± 0.10	39	0.94 ± 0.10	0.82

Bold values represent statistically significant differences within groups ($p < 0.05$); n refers to the number of individuals per group. * $p < 0.05$; ** $p < 0.01$.

Table S2. Descriptive statistics of genotoxicity biomarkers concentrations for primary DNA damage (% TDNA), oxidative DNA damage (% TDNA) and γH2AX (%) levels in Phase I (before wildfire season – baseline) and Phase II (after fire event – during wildfire season).

Outcome measure	Phase I (Pre- wildfire season)		Phase II (After the fire event)		P-value
	n	Mean $\pm$ SE	n	Mean $\pm$ SE	
γH2AX %	50	1.11 $\pm$ 0.30	50	1.01 $\pm$ 0.15	0.61
Comet assay (% TDNA)					
Primary DNA damage	48	14.79 $\pm$ 1.27	48	18.10 $\pm$ 1.14	0.03*
Oxidative DNA damage	48	11.85 $\pm$ 0.85	48	14.59 $\pm$ 1.14	0.04*

Bold values represent statistically significant differences within groups ($p < 0.05$); n refers to the number of individuals per group. * $p < 0.05$

Table S3. Comet assay endpoints and γ H2AX levels (Phase I) within self-reported variables (n=59).

		Primary DNA damage (% TDNA)		Oxidative DNA damage (% TDNA)		γ H2AX %	
		n	Mean $\pm$ SE	n	Mean $\pm$ SE	n	Mean $\pm$ SE
Individual characteristics:							
Sex							
	Female	13	10.76 $\pm$ 1.09	13	9.05 $\pm$ 1.46	13	0.68 $\pm$ 0.14
	Male	36	16.01 $\pm$ 1.61	36	12.82 $\pm$ 0.95	36	1.22 $\pm$ 0.38
BMI ^a							
	Normal	20	15.39 $\pm$ 2.42	20	12.76 $\pm$ 1.20	18	1.88 $\pm$ 0.80
	Pre-obesity	24	14.22 $\pm$ 1.61	24	10.97 $\pm$ 1.25	28	0.72 $\pm$ 0.11
	Obesity	5	12.41 $\pm$ 1.66	5	12.12 $\pm$ 2.91	6	0.37 $\pm$ 0.06
Age							
	<32 years	18	15.18 $\pm$ 2.21	18	12.93 $\pm$ 1.38	20	1.19 $\pm$ 0.65
	32-44 years	25	14.90 $\pm$ 1.83	25	10.76 $\pm$ 1.09	24	1.13 $\pm$ 0.33
	>44 years	6	11.77 $\pm$ 2.33	6	12.93 $\pm$ 2.96	8	0.66 $\pm$ 0.16
Lifestyle-related:							
Smoking habits							
	Non-smoker	23	14.49 $\pm$ 1.77	23	14.03 $\pm$ 1.30	21	0.61 $\pm$ 0.09
	Current smoker	26	14.73 $\pm$ 1.81	26	9.86 $\pm$ 0.91	31	1.41 $\pm$ 0.48
Physical activity							
	Occasionally	13	13.34 $\pm$ 2.16	13	9.56 $\pm$ 1.56	16	1.49 $\pm$ 0.80
	Weekly	21	12.43 $\pm$ 1.02	21	13.15 $\pm$ 1.29	20	0.70 $\pm$ 0.14
	Daily	2	37.94 $\pm$ 8.94	2	16.12 $\pm$ 2.17	2	0.47 $\pm$ 0.01
Alcohol daily intake							
	No	37	14.19 $\pm$ 1.31	37	11.99 $\pm$ 0.91	39	0.94 $\pm$ 0.21
	Yes	12	15.93 $\pm$ 3.25	12	11.31 $\pm$ 1.96	13	1.53 $\pm$ 0.99
Occupational-related:							
Years of service ^b							
	<10 years	16	16.67 $\pm$ 2.60	16	13.56 $\pm$ 1.27	18	1.64 $\pm$ 0.81
	≥ 10 - ≤ 20 years	16	12.14 $\pm$ 1.03	16	8.46 $\pm$ 1.12	16	0.69 $\pm$ 0.15
	>20 years	15	16.05 $\pm$ 2.71	15	14.04 $\pm$ 1.69	16	0.85 $\pm$ 0.15
Diet:							
Vegetables consumption							
	Never	4	24.05 $\pm$ 7.21	4	15.30 $\pm$ 1.70	5	2.26 $\pm$ 1.50
	Daily	25	14.75 $\pm$ 1.81	25	11.91 $\pm$ 1.20	24	1.14 $\pm$ 0.54
	Weekly	17	12.72 $\pm$ 1.51	17	11.04 $\pm$ 1.28	19	0.81 $\pm$ 0.16
Tea intake							
	No	30	14.40 $\pm$ 1.52	30	12.21 $\pm$ 1.10	32	1.43 $\pm$ 0.46
	Yes	19	14.95 $\pm$ 2.24	19	11.21 $\pm$ 1.28	20	0.53 $\pm$ 0.08
Coffee intake							
	No	5	12.89 $\pm$ 1.86	5	13.54 $\pm$ 2.60	5	0.78 $\pm$ 0.24
	Yes	44	14.81 $\pm$ 1.39	44	11.63 $\pm$ 0.88	47	1.12 $\pm$ 0.32

^a Body mass index (BMI) established according to WHO categories. ^b Years of service as firefighter. Mann-Whitney U test was used for two-group comparisons, and the Kruskal-Wallis test for more than two groups; No significant differences were found within groups.

Table S4. Frequency Ratios and respective 95% Confidence Intervals (CI) obtained by simple regression analysis within each biological effect endpoints and the respective exposure biomarkers (OHPAHs and metal(loid)s).

	Δ Primary DNA damage	Δ Oxidative DNA damage
Exposure		
Phase I (baseline)	1.00 (ref.)	1.00 (ref.)
Phase II (after wildfire event)	1.22 (1.11-1.35) *	1.23 (1.04-1.45) *
Urinary OHPAHs		
1-OHNaph+1-OHAc (μmol/mol creatinine)	1.00 (0.99-1.01)	0.99 (0.99-1.00)
2-OHFlu (μmol/mol creatinine)	1.11 (1.00-1.22)	1.20 (1.09-1.32) **
1-OHPhen (μmol/mol creatinine)	1.07 (0.87-1.26)	1.13 (0.90-1.41)
1-OHPy (μmol/mol creatinine)	0.80 (0.58-1.11)	0.93 (0.64-1.34)
Urinary Metals		
Li (μg/L)	1.00 (1.00-1.01)	1.00 (0.99-1.00)
Co (μg/L)	1.00 (0.76-1.33)	1.23 (0.87-1.46)
Ni (μg/L)	1.03 (0.90-1.18)	1.08 (0.96-1.22)
Cu (μg/L)	1.01 (0.98-1.05)	0.99 (0.96-1.03)
Zn (μg/L)	1.00 (0.99-1.00)	1.00 (0.99-1.00)
As (μg/L)	0.99 (0.99-1.00)	0.99 (0.99-1.00)
Se (μg/L)	0.99 (0.97-1.01)	0.98 (0.97-1.00)
Rb (μg/L)	1.00 (0.99-1.00)	1.00 (0.99-1.00)
Sr (μg/L)	0.99 (0.99-1.00)	1.00 (0.99-1.00)
Mo (μg/L)	1.00 (0.99-1.01)	1.00 (0.99-1.01)
Cd (μg/L)	0.97 (0.35-2.74)	0.71 (0.28-1.82)
Sb (μg/L)	1.05 (0.70-1.57)	0.97 (0.67-1.41)
Cs (μg/L)	1.04 (1.01-1.06) *	1.03 (1.01-1.05) *
Ba (μg/L)	0.95 (0.79-1.13)	1.14 (0.98-1.33)
Hg (μg/L)	0.70 (0.30-1.63)	0.59 (0.28-1.31)
Tl (μg/L)	1.39 (0.85-2.29)	1.30 (0.82-2.06)
Pb (μg/L)	1.04 (0.65-1.66)	1.09 (0.67-1.58)

Bold values indicate statistically significant associations, * $p < 0.05$; ** $p < 0.01$.

Table S5. Crude and adjusted multivariate analysis for Δ Primary DNA Damage, using urinary cesium as a predictor of primary DNA damage.

	Δ Primary DNA damage (%TDNA)			
	Crude effect		Multivariate analysis^b	
	FR ^a (95% CI)	P-value	FR ^a (95% CI)	P-value
Exposure				
Phase I – Pre-exposure	1.00			
Phase II – Post-exposure	1.22 (1.11-1.35)	0.03*		
Metals in urine				
Cesium ($\mu\text{g/L}$)	1.04 (1.01-1.06)	0.01*	1.03 (1.01-1.06)	0.02*
Sex				
Female	1.00		1.00	
Male	0.83 (0.52-1.35)	0.46	0.71 (0.38-1.33)	0.27
Smoking habits				
Non-smoker	1.00		1.00	
Current Smoker	1.12 (0.72-1.72)	0.60	0.86 (0.49-1.51)	0.59
Age				
≤ 36 years	1.00		1.00	
> 36 years	0.86 (0.56-1.33)	0.49	0.74 (0.42-2.16)	0.30
Model parameters				
P-value (F test)			0.07	
R			0.53	
R-squared			0.28	
Adj. R-squared			0.16	

^a FR, Frequency Ratio; 95% CI, 95% confidence interval.

^b Adjusted for sex, age and smoking habits. Bold values indicate statistically significant associations, * $p < 0.05$; ** $p < 0.01$.

Table S6. Crude and adjusted multivariate analysis for Δ Oxidative DNA Damage, using urinary cesium as a predictor of oxidative DNA damage.

	Δ Oxidative DNA damage (%TDNA)			
	Crude effect		Multivariate analysis ^b	
	FR ^a (95% CI)	P-value	FR ^a (95% CI)	P-value
Exposure				
Phase I – Pre-exposure	1.00			
Phase II – Post-exposure	1.23 (1.04-1.45)	0.04*		
Metal(loid)s in urine				
Cesium ($\mu\text{g/L}$)	1.03 (1.01-1.05)	0.01*	1.03 (1.01-1.06)	0.02*
Sex				
Female	1.000		1.00	
Male	0.78 (0.46-1.33)	0.35	0.63 (0.36-1.11)	0.11
Smoking habits				
Non-smoker	1.00		1.00	
Current Smoker	1.62 (1.03-2.54)	0.04*	1.26 (0.76-2.08)	0.36
Age				
≤ 36 years	1.00		1.00	
> 36 years	0.70 (0.44-1.12)	0.13	0.90 (0.53-1.50)	0.67
Model parameters				
P-value (F test)			0.05*	
R			0.55	
R-squared			0.31	
Adj. R-squared			0.19	

^a FR, Frequency Ratio; 95% CI, 95% confidence interval.

^b Adjusted for sex, age and smoking. Bold values indicate statistically significant associations, * $p < 0.05$; ** $p < 0.01$.

Table S7. Crude and adjusted multivariate analysis for Δ Oxidative DNA Damage, using urinary 2-OHFlu as a predictor of oxidative DNA damage.

	Δ Oxidative DNA damage (%TDNA)			
	Crude effect		Multivariate analysis ^b	
	FR ^a (95% CI)	P-value	FR ^a (95% CI)	P-value
Exposure				
Phase I – Pre-exposure	1.00			
Phase II – Post-exposure	1.23 (1.04-1.45)	0.04*		
Urinary OHPAHs				
2-OHFlu ($\mu\text{mol/mol creatinine}$)	1.20 (1.09-1.32)	<0.01*	1.20 (1.06-1.34)	<0.01*
Sex				
Female	1.000		1.00	
Male	0.78 (0.46-1.33)	0.35	0.74 (0.44-1.23)	0.24
Smoking habits				
Non-smoker	1.00		1.00	
Current Smoker	1.62 (1.03-2.54)	0.04*	0.99 (0.56-1.75)	0.96 [†]
Age				
≤ 36 years	1.00		1.00	
> 36 years	0.70 (0.44-1.12)	0.13	0.78 (0.48-1.28)	0.32
Model parameters				
P-value (F test)			0.01*	
R			0.54	
R-squared			0.29	
Adj. R-squared			0.21	

^a FR, Frequency Ratio; 95% CI, 95% confidence interval.

^b Adjusted for sex, age and smoking habits. [†] discrepancy due to multicollinearity between smoking and 2-OHFlu, resulting from their high correlation. Bold values indicate statistically significant associations, * $p < 0.05$; ** $p < 0.01$.

***B. 3. Micronucleus Assay in Buccal and Urothelial Cells in
Wildfire-Exposed Firefighters (Paper IV)***

*Esteves F., Madureira J., Costa C., Barros B., Alves S., Vaz J., Oliveira M., Slezakova K.,
Fernandes A., Pereira M.C., Morais S., Bonassi S., Teixeira J.P. & Costa S.*

Manuscript submitted for publication

*The PhD candidate was responsible for the conceptualisation and writing of the
manuscript.*

Micronucleus Assay in Buccal and Urothelial Cells in Wildfire-Exposed Firefighters

Filipa Esteves^{a,b,c,d}, Joana Madureira^{a,b,c}, Bela Barros^e, Sara Alves^f, Josiana Vaz^{g,h}, Marta Oliveira^e, Klara Slezakovaⁱ, Adília Fernandes^{g,h}, Maria do Carmo Pereiraⁱ, Simone Morais, Stefano Bonassi^{j,k}, João Paulo Teixeira^{a,b,c*} Solange Costa^{a,b,c}

^a Environmental Health Department, National Institute of Health, Rua Alexandre Herculano, n° 321, 4000-055 Porto, Portugal

^b EPIUnit - Instituto de Saúde Pública, Universidade do Porto, Rua das Taipas, n° 135, 4050-600 Porto, Portugal

^c Laboratório para a Investigação Integrativa e Translacional em Saúde Populacional (ITR), Universidade do Porto, Rua das Taipas, n° 135, 4050-600 Porto, Portugal

^d Department of Public Health and Forensic Sciences, and Medical School, Faculty of Medicine, University of Porto, Rua Doutor Plácido da Costa, 4200-450 Porto, Portugal

^e REQUIMTE/LAQV-Instituto Superior de Engenharia, Instituto Politécnico do Porto, Dr. António Bernardino de Almeida 431, 4249-015 Porto, Portugal

^f Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

^g Research Centre for Active Living and Wellbeing (LiveWell), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

^h CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

ⁱ LEPABE-ALICE, Faculdade de Engenharia da Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

^j Unit of Clinical and Molecular Epidemiology, IRCCS San Raffaele Roma, 00163 Rome, Italy

^k Department of Human Sciences and Quality of Life Promotion, San Raffaele University, 00166, Rome, Italy

*Corresponding author: João Paulo Teixeira; E-mail address: joao.teixeira@insa.min-saude.pt

Abstract

Firefighting is classified as carcinogenic to humans, however mechanistic studies addressing the biological effects of firefighting exposure remain limited. This gap, particularly evident among wildland firefighters, highlights the need for molecular epidemiological studies unveiling these effects. Micronuclei (MN) are early biomarkers of genomic instability widely used in human populations exposed to genotoxic agents, including harmful pollutants. MN assays in non-invasive, locally targeted epithelial tissues, such as buccal cells and urothelial cells, are of particular interest for monitoring occupational exposure in firefighters, as many cancers originate in epithelial tissues. This study aimed to longitudinally evaluate genomic instability (e.g., MN frequency) and other cellular endpoints, in buccal and urothelial cells of a group of wildland firefighters, actively involved in real

wildfire scenarios. Additionally, we sought to assess the contribution of total urinary hydroxylated polycyclic aromatic hydrocarbons (Σ OHPAHs) in the studied endpoints.

A group of 59 wildland firefighters (mean age: 35.5 ± 9.0 years) from five fire stations in northern Portugal were followed at two time-points: before wildfire baseline (Phase I), and during wildfire season when firefighters engaged in wildland fire-combat activities (Phase II). Information on sociodemographic (e.g., age, sex), lifestyle (e.g., smoking), occupational (e.g., years of service) and diet were collected via a self-administered questionnaire. Buccal mucosa cells and urine samples were collected for the application of the Buccal Mucosa Cytome (BMCyt) assay and MN assay in urothelial cells, respectively.

The risk of MN formation in urothelial cells was more than two times higher during the wildfire season compared to baseline levels (FR: 2.13, 95% CI: 1.99–2.27, $p=0.01$). The frequency of binucleated (BN) buccal cells also increased by more than twofold during the wildfire season (FR: 2.20, 95% CI: 1.60–2.99, $p<0.01$). Additionally, although not statistically significant, MN‰ in buccal cells increased by 35% during the wildfire season (FR: 1.35, 95% CI: 0.76–2.40, $p > 0.05$). Urinary Σ OHPAHs exhibited a significant positive association with MN‰ in urothelial cells, with a 4% increase in MN frequency for each unit increase in Σ OHPAHs (FR=1.04, 95% CI: 1.01–1.07, $p < 0.05$).

This study provides the first evaluation of MN frequency in different surrogate tissues of wildland firefighters involved in wildland fire-combat activities, offering novel insights into the mechanistic effects of wildfire exposure on human health. The results highlight the potential of MN assay in urothelial cells as a valuable tool for early detection of occupational DNA damage, with broader implications for improving firefighter safety and public health policies.

Keywords: Micronucleus, chromosomal damage, DNA damage, buccal cells, BMCyt, firefighters, wildfire smoke

1. Introduction

Wildfires are among the most concerning natural disasters due to their potential destructive effects on ecosystems, property, and human lives (1). Wildland firefighters provide vital services to the community, protecting the environment, heritage and human life (2). During their work, wildland firefighters are exposed to various occupational hazards such as heat stress, physical and psychological stressors, and inhalation of smoke pollutants (1). Wildfire smoke contains several harmful pollutants including polycyclic aromatic hydrocarbons

(PAHs), which are known carcinogens (3). PAHs are classified as probable (Group 2A) or possible (Group 2B) human carcinogens by the International Agency for Research on Cancer (IARC) (4). Through the years, epidemiological studies have reported an increased risk of cancer mortality among firefighters (5), however mechanistic studies exploring the underlying biological mechanisms remain limited (5). Molecular epidemiology enables the evaluation of biological effects associated with environmental and occupational exposures (6, 7). Micronuclei (MN) are among the most widely used biomarkers for genomic instability (8). MN arise from chromosome fragments or whole chromosomes that failed to be incorporated into daughter cells nuclei during mitosis, serving as indicators of genotoxicity (9, 10). They reflect structural and numerical chromosomal aberrations and are formed as a consequence of exposure to occupational, environmental and lifestyle genotoxins (11, 12). It is well known that chromosomal damage plays an important role in the aetiology of human cancer (13) and other diseases (14). MN in lymphocytes is a well-established and validated biomarker for the prediction of cancer in humans (15, 16). However, the traditional MN assay in lymphocytes is an invasive method that requires the collection of blood. Therefore, the evaluation of genotoxic endpoints in rapidly dividing epithelial cells appears as a suitable alternative for the assessment of MN (9). Furthermore, the positive significant strong correlation between MN frequency in lymphocytes and MN in exfoliated cells, namely buccal cells, can be interpreted as an indication that MN in such local tissues may also serve as predictive markers for cancer and a potential alternative to this type of assessment (12, 17). The application of MN in these tissues is particularly interesting because up to 90% of all cancers are of epithelial origin (18).

Assessing MN in strategic local-exposed tissues to monitor firefighters' occupational exposure is of utmost pertinence. The most important route for exposure to smoke air-contaminants among wildland firefighters is inhalation (19); the mouth is an organ directly involved in the inhalation of pollutants since humans are also mouth-breathers, and therefore buccal mucosa is directly exposed to airborne contaminants during wildfire combat (20). Thus, buccal mucosa provides a barrier to potential carcinogens (21, 22), being a very sensitive and suitable tissue to monitor early genotoxic events as a result of carcinogens that enter the body via ingestion and inhalation (23). (24-26). MN in buccal cells is an optimal approach to use in wildland firefighters, even more because they are exposed to highly toxic atmospheres in the wildfire scene breathing high concentrations of pollutants. The Buccal Micronucleus Cytome (BMCyt) assay is a minimally invasive cytological and cytogenetic technique for measuring genomic instability (MN and nuclear buds), cell death (condensed chromatin, karyorrhexis, pyknotic and karyolytic cells), proliferative activity (basal cell frequency) and cytokinetic defects (binucleated cells

frequency) (27, 28). The BMCyt assay has been widely applied in human biomonitoring studies to assess environmental and occupational exposure to genotoxic agents (29). This technique is particularly interesting because buccal cells are collected by a minimally invasive procedure.

In addition to the first-local tissue of exposure to air pollutants (buccal cells), it is important to investigate local-excretion tissues, such as the urothelium, which is exposed to carcinogenic metabolites of smoke, including hydroxylated PAHs (OHPAHs). Monitoring genotoxic effects in urothelial cells, through MN assay in urothelial cells, is promising for early detection of cancer, particularly bladder cancer (30), a type of cancer highly prevalent among firefighters (5). Despite IARC has classified firefighting as carcinogenic to humans (5), very few molecular epidemiological studies have investigated the biological effects, at molecular and cellular levels, of firefighting occupational exposure (5), making this gap particularly evident among wildland firefighters (5).

In this line, this work aimed to longitudinally evaluate genomic instability and other cellular endpoints, in buccal and urothelial cells, of a group of wildland firefighters at two distinct time-points of their activity: before and during the wildfire season, when firefighters are actively exposed to fire-combat activities. Additionally, we sought to assess the contribution of total urinary PAHs to the observed changes (Phase II/Phase I) in the studied endpoints. To the best of the authors' knowledge, this is the first study integrating MN assay in urothelial cells to evaluate firefighters' occupational exposure and the first investigating the association between urinary OHPAHs and MN assay endpoints, in wildland firefighters.

2. Material and Methods

2.1. Study population

The study population comprised fifty-nine healthy wildland firefighters from five fire stations located in the northern region of Portugal. Participants were required to be actively engaged in firefighting activities and have at least one year of experience as firefighters. A pre-post study design was used, with participants serving as their controls at two-time-points of their activity: Phase I (baseline) – before the wildfire season, when participants were located at the fire station and had not recently been involved in firefighting activities; Phase II (exposure) – during the wildfire season when firefighters were actively engaged in wildfire combat activities. Data collection occurred over two phases, between 2021 and 2023. All participants signed an informed consent form, after being fully briefed on the study's objectives, procedures for data collection and biological sample collection, confidentiality of data, and their right to withdraw from the study at any time without consequence. In Phase

I, participants completed a comprehensive questionnaire that included questions on sociodemographic characteristics (e.g., sex, age), lifestyle factors (e.g., smoking), occupational details (e.g., years of service) and diet. Biological samples, specifically urine and buccal cells, were collected in both phases of the study.

This study was conducted in accordance with the Declaration of Helsinki and received approval from the Ethics Committee of the University of Porto (reference number 92/CEUP/2020).

2.2. Biological sample collection

In Phase I, before wildfire season, urine and buccal cells were collected at the fire station, during the firefighters' shift (morning period). In Phase II, during participation in wildland fire-combat activities, biological samples were collected at the fire station, when firefighters returned from wildfire-combat activities.

For urine sampling, firefighters were asked to collect a spot urine sample into a 100 mL sterile plastic container. The urine samples were transported to the laboratory under refrigerated conditions (4°C) for processing for the MN assay in urothelial cells, as well as to be stored at -20°C for further analysis of urinary OHPAHs.

The protocol for buccal cell collection, followed methods described by Thomas et al. (18) and Costa et al. (31), with minor modifications. Firefighters were asked to rinse the mouth with tap water to remove unwanted debris. A sample from each cheek was collected using two separate cytobrush (Deltalab) - one for each cheek - which were gently but firmly rotated against the inside of the cheek wall in a circular motion, not touching in mouth or teeth, in a circular motion for 30 seconds. Buccal cells were collected into two 15mL Falcon tubes labelled LC (left cheek) and RC (right cheek), each containing 10 mL Buffer solution (0.01M Tris-HCl, 0.1M EDTA, 0.02M NaCl, pH7). Samples were transported in refrigerated conditions (4°C) to the laboratory processed on the same day for the BMCyt assay.

2.3. Urinary OHPAHs Analysis

Quantification of urinary OHPAHs, in urine samples collected both in Phase I and II, was performed by Barros et al. (32) using solid-phase extraction and high-performance liquid chromatography with a fluorescence detector. Total OHPAHs (Σ OHPAHs) comprised the sum of the following OHPAHs metabolites: 1-hydroxynaphthalene (1-OHNaph), 1-hydroxyacenaphthene (1-OHAce), 2-hydroxyfluorene (2-OHFlu), 1-hydroxyphenanthrene (1-OHPhen) and 1-hydroxypyrene (1-OHPyr). Concentrations of OHPAHs were normalised to creatinine levels ($\mu\text{mol/mol}$ creatinine).

2.4. Buccal Micronucleus Cytome Assay

2.4.1. Exfoliated buccal cells fixation

At the laboratory, exfoliated buccal cells, embedded in Buffer solution, were centrifuged for 10 min at 501 rcf. Supernatant was carefully removed, the cell pellet was resuspended, and cells were centrifuged once again with 10 mL of buccal cell buffer for 10 minutes. This step was repeated one more time. After the third wash, the supernatant was discarded, cell pellet was resuspended in 5 mL of fixative solution ethanol: glacial acetic acid (3:1, v/v) and stored at -20°C to be further processed for the slide preparation for BMCyt assay.

2.4.2. Slide preparation, staining procedure and scoring

The protocol for slide preparation, staining, and scoring was adapted from methods described elsewhere (18, 31). Buccal cell suspension in fixative solution was centrifuged for 10 min at 501 rcf. After discarding the supernatant, the cell pellet of each sample was resuspended, and buccal cells were dropped into clear coded pre-cleaned slides. Slides were able to dry overnight in a horizontal position. In the next day, fixed slides followed the staining process. The air-dried fixed slides were submerged for 1 min each in a Coplin jar with 50% (v/v) and then 20% (v/v) ethanol sequentially, and then into a Coplin jar containing deionised water for 2 min. Slides were immersed in a Coplin jar containing 5 M HCL, for 30 minutes, and then rinsed in running tap water for 3 min. Slides were stained with Schiff's reagent (Merck) in the dark, at room temperature, for 90-120 minutes. Slides were rapidly dipped in deionised water and counterstained for 20 sec in 0.2% (w/v) with Fast Green (Merck). The Feulgen-Fast Green staining method minimizes false-positive results, often observed with non-specific DNA stains. This specificity enhances the reliability and comparability of the results, making it a valuable tool for accurate assessment of chromosomal and nuclear integrity in cytogenetic analyses (28). Slides were immersed 3x in absolute ethanol and let to air dry. Slides were covered with coverslips (Manzel-Gläser), mounted with Entellan®, and kept in the dark to dry until microscope scoring. Stained coded slides were blindly analysed by a single trained operator on a Nikon Eclipse E400 attached to an epi-fluorescence illuminator Nikon C-SHG1 power supply for HG 100W, under 400x magnification. Cells containing MN or other nuclear abnormalities were confirmed under fluorescence microscopy using a G-2A filter. Only cells that were not clumping or overlapping were chosen. Scoring was performed according to criteria proposed by Thomas et al. (18) and Bolognesi et al. (27) criteria. The frequency of basal cells, binucleated cells and cells undergoing death (i.e., condensed chromatin, karyorrhectic cells, karyolytic cells, and pycnotic cells) was determined in 1000 cells, whereas the genomic instability (i.e., MN

frequency) and gene amplification biomarkers, namely nuclear buds (NBUDs), were scored in 2000 differentiated cells (18) per individual.

2.5. Micronucleus assay in urothelial cells

2.5.1. Urothelial cells fixation

Once in the laboratory, urine samples were separated into two 15 mL Falcon tubes and centrifuged at 939 rcf for 20 minutes. The obtained cell pellet was washed twice with 10 mL of 0.9% NaCl and centrifuged at 782 rcf for 10 minutes. The supernatant was carefully removed leaving around 0.5 mL to ensure no cell loss, particularly in urine from males (30). The cell pellet was then resuspended with 5 mL of fixative solution (methanol: glacial acetic, 3:1) and stored at -20°C to be further prepared for MN assay in urothelial cells.

2.5.2. Slide preparation, staining procedure and scoring

Urine cells for micronucleus test were treated according to methods reported by previous studies (30, 33, 34) with slight modifications. Fixed urine-derived cells were centrifuged at 782 rcf for 15 min was conducted. After discarding supernatant, the cell pellet of each sample was resuspended, and urothelial cells were dropped into pre-cleaned slides; urine samples from males typically contain fewer cells, and as a result, require to be concentrated in a smaller volume of fixative solution (34). The cell suspension was dropped on pre-cleaned labelled slides that were left horizontally overnight to dry.

The staining procedure followed the same steps described for exfoliated buccal cells. Scoring criteria were performed as suggested by Thomas et al. (18). Only urothelial cells with typical morphology were analysed, excluding squamous cells from female urine samples.

2.6. Statistical analysis

The software Statistical Package for the Social Sciences (SPSS), version 29 for Windows was used for the statistical analysis of data collected. Descriptive statistics was performed using frequency distribution, central trend and dispersion measures. The results of MN cytome experiments were presented as the frequency of a specific anomaly per 1000 cells (‰), as suggested in the literature (11). The normality of variables was verified through the Kolmogorov-Smirnov Test and the distribution of graphical graphs. The studied endpoints did not follow a normal distribution and therefore non-parametric tests were used. The effect of potential confounders for MN frequencies i.e. age, sex, smoking, consumption of alcohol

(11), and diet (e.g., vegetables, coffee consumption) (20), were studied. Subjects were classified as current smokers or non-smokers (i.e., individuals who reported never having smoked or having quit smoking at least one year prior). Body mass index (BMI) [weight (kg) $\times$ height (m)⁻²] and respective BMI categories (i.e., underweight, normal weight, overweight, and obese) were calculated and classified according to the World Health Organization (35). Differences between independent variables with two groups were checked through the Man-Whitney Test, whereas the Kruskal-Wallis Test (with Bonferroni adjustment) was used for variables with more than two groups. Correlations were checked by the Spearman correlation T-test. For paired samples, differences were tested using the Wilcoxon test. Frequency ratios (FR) (Phase II/Phase I) were calculated for studied endpoints. The association of Σ OHPAHs on the variation (Δ) of studied endpoints was investigated using linear regression analysis [$\Delta = \log(\text{levels Phase II}) - \log(\text{levels Phase I})$]. To interpret the results by ratio, biological endpoints were log-transformed. The level of statistical significance established for α was 5%.

3. Results

The characteristics of the study group are described in Table 1. The study sample involved 59 wildland firefighters, aged between 20.0 and 55.0 years (mean 35.5 ± 9.0), with 46 (78%) males and 13 (22%) females. Regarding the BMI classification, most firefighters (50.8%) were classified as overweight, with a higher prevalence among male firefighters.

Table 1 - General characteristics of the study population (n = 59), stratified by sex.

Study participant characteristics	Male (n=46)	Female (n=13)	Wildland Firefighters (n=59)
Individual characteristics			
Age (years) ^a	35.6 $\pm$ 9.3 (20.0–55.0)	35.1 $\pm$ 8.0 (21.0–45.0)	35.5 $\pm$ 9.00 (20.0–55.0)
Body Mass Index (BMI) (kg m ⁻²) ^a	26.7 $\pm$ 3.3 (19.4–35.9)	26.0 $\pm$ 8.0 (21.2–33.0)	26.5 $\pm$ 3.3 (19.4–35.9)
Normal weight, n (%)	17 (37.0%)	5 (38.5%)	22 (37.3%)
Overweight, n (%)	23 (50.0%)	7 (53.8%)	30 (50.8%)
Obesity, n (%)	6 (13.0%)	1 (7.7%)	7 (11.9%)
Lifestyle-related			
Smoking habits			
Non-current smoker	19 (41.3%)	8 (61.5%)	27 (45.8%)
Current smoker	27 (58.7%)	5 (38.5%)	32 (54.2%)
Occupational-related			
Years of service ^a	16.1 $\pm$ 9.4 (3.0–34.0)	14.8 $\pm$ 6.7 (7.0–28.0)	15.9 $\pm$ 8.9 (3.0–34.0)
Permanent Intervention Teams			
No, n (%)	21 (45.7%)	11 (91.7%)	32 (55.2%)
Yes, n (%)	25 (54.3%)	1 (8.3%)	26 (44.8%)

^a Mean $\pm$ SD (standard deviation), (median, min-max)

Concerning smoking habits, 27 (45.8%) firefighters were non-current smokers, while 32 (54.2%) were current smokers. In terms of occupational characteristics, the firefighters reported a mean of 15.9 ± 8.9 years of service as a firefighter with values ranging from 3.0 to 34.0 years. Among male firefighters, more than half (54.3%) reported being part of a permanent intervention team.

Regarding the variation on the studied biological endpoints within study phases (Phase II /Phase I), buccal BN cells ‰ revealed an FR of 2.20 (95% CI: 1.60–2.99, $p < 0.01$), suggesting that frequencies obtained during the wildfire season were more than double the baseline levels, indicating a substantial increase in cytokinetic defects. Similarly, the frequency of MN in urothelial cells showed a significant increase with an FR of 2.13 (95% CI: 1.99–2.27, $p=0.01$), reflecting a 72% higher risk of genomic instability during the wildfire season compared to baseline levels. Additionally, the frequency of buccal MN increased by 35% during Phase II with an FR of 1.35 (95% CI: 0.76–2.40, $p>0.05$), despite not reaching statistical significance, indicating a trend toward increased genomic instability during the wildfire season.

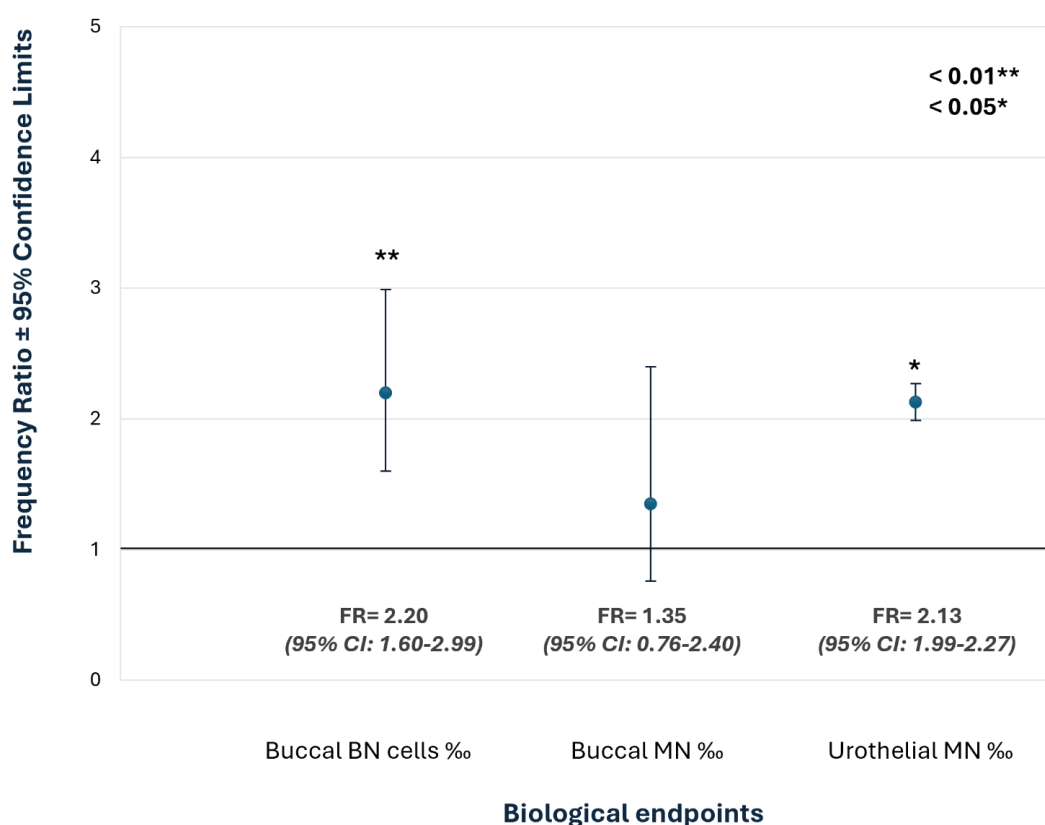


Figure 1 - Frequency ratios (FR) of biological endpoints showing a significant positive variation within study phases (i.e. Buccal BN cells and MN in urothelial cells), along with FR of MN in buccal cells. * and ** indicate the statistical significance of post/pre-exposure ratios, with p -values < 0.05 and < 0.01 , respectively.

In addition, we also aimed to investigate the possible contribution of urinary Σ OHPAHs for the variation Δ observed in the referred effect biomarkers (i.e., BN‰ in buccal cells, MN‰ in urothelial cells and MN‰ in buccal cells) (**Table 3**).

Urinary Σ OHPAHs showed a significant positive association with Δ MN ‰ in urothelial cells (FR = 1.04, 95% CI: 1.01–1.07, $p < 0.05$), indicating a 4% increase in MN frequency per each unit increase in Σ OHPAHs. No significant associations were observed between Σ OHPAHs and Δ BN ‰ in buccal cells (FR = 1.01, 95% CI: 0.99–1.02, $p > 0.05$) or Δ MN ‰ buccal cells (FR = 1.00, 95% CI: 0.99–1.01, $p > 0.05$), suggesting that these biomarkers may be influenced more by other factors unrelated to systemic PAHs absorption.

Table 3 – Frequency ratio and 95% confidence intervals (CI) for variation (Δ) in effect biomarkers from Phase I (baseline) to Phase II (wildfire season). Contribution of urinary Σ OHPAHs levels to the observed variation.

	Δ BN ‰ Buccal cells FR ^a (95% CI)	Δ MN ‰ Urothelial cells FR ^a (95% CI)	Δ MN ‰ Buccal cells FR ^a (95% CI)
Exposure (Phase II)			
No	Ref	Ref	Ref
Yes	2.20 (1.60-2.99) **	2.13 (1.99-2.27) *	1.35 (0.76-2.40)
Urinary PAHs (μ mol/mol creatinine)			
Σ OHPAHs	1.01 (0.99-1.02)	1.04 (1.01-1.07) *	1.00 (0.99-1.01)

^a FR, Frequency Ratio; 95% CI, 95% confidence interval. *Significance level <0.05 ; **Significance level <0.01 .

In this study, we observed no effect of demographic factors (sex, BMI, age), lifestyle factors (smoking habits, alcohol consumption), and diet (consumption of vegetables, fruit, tea or coffee), on studied endpoints (**Table S1** and **S2**, respectively – Supplementary material), but we observed that wildland firefighters with less than 10 years of service (as a firefighter) had significantly lower levels of BN urothelial cells than those with more than 20 years of service (mean $\pm$ S.E., 1.31 ± 0.79 vs. 4.56 ± 1.33 ; $p < 0.01$) (**Table S2** – Supplementary material).

4. Discussion

Micronuclei are considered valuable biomarkers for diagnosing and prognosing various cancers and other diseases (11). The minimal invasiveness of cell collection, low cost, ease of storage, simple slide preparation, and ability to detect genotoxic effects make the MN

assay in epithelial cells, such as buccal mucosa and urothelial cells, particularly well-suited for human biomonitoring studies (12). The buccal mucosa is the first site of contact with hazardous compounds (31), while urothelial cells reflect the final site of exposure to hazardous substances excreted by the body (33).

In the present study, we observed an increase in MN frequency in both urothelial and buccal cells during the wildfire season among wildland firefighters, although statistical significance was only reached for MN in urothelial cells. To date, only two human biomonitoring studies assessed MN frequency among firefighters (28, 36), one focusing on wildland firefighters (20) and the other on structural firefighters (i.e., urban settings) (36). Esteves et al. (28) assessed MN frequency, along with other cellular endpoints, in exfoliated buccal cells of a group of Portuguese wildland firefighters (n=172) during their regular activities at the fire station in the pre-wildfire season. The author found no significant impact of estimated inhaled doses of PM₁₀ and PM_{2.5}, measured both in indoor and outdoor fire stations, on biological endpoints (28). In the study involving structural firefighters, Ray et al. (2005) observed increased MN frequency in buccal cells of Indian municipal firefighters (n=47), compared to a matched control group of office workers (n=40) (36).

In addition to the aforementioned, we observed a significant association between urinary Σ OHPAHs and increased MN frequency in urothelial cells among wildland firefighters. To our knowledge, this is the first biomonitoring study assessing the association of urinary Σ OHPAHs and MN in urothelial cells frequency in firefighters. Indeed, most occupational studies on PAHs exposure and MN frequency have been focused on lymphocytes (37-41), describing a positive effect of PAHs exposure on MN formation. In a meta-analysis, for instance, Wang et al. (37) reported higher MN frequencies in peripheral blood lymphocytes from PAHs-exposed groups (e.g., cook ovens, chimney sweeping) compared to their respective controls. Similarly, Holmén et al. (39), Cao et al. (40) and Leng et al. (41) observed significantly higher MN frequencies in lymphocytes of PAHs-exposed workers (e.g., chimney sweeps, barbecue restaurant and coke oven workers). In addition to human biomonitoring studies, in vitro research has also established a positive link between PAHs exposure to MN formation (42, 43). Zhong et al. (42) detected MN formation in rat lung cells exposed to dibenz[a,h]anthracene, and Takam et al. (43) found increased MN frequencies in human epithelial lung cells (A549) exposed to PAHs than untreated cells.

Given these findings, our study highlights the importance of incorporating urinary Σ OHPAHs as a biomarker for assessing MN formation in urothelial cells, particularly in occupations with high PAH exposure such as firefighting. Occupational exposure to PAHs has been strongly linked to an increased risk of cancer, particularly bladder cancer (5). Therefore, incorporating the MN assay in urothelial cells, in occupational studies involving firefighters

is of utmost interest, particularly because MN frequency in urothelial cells is a potential early cancer risk biomarker. Previous studies have shown significantly higher MN frequencies in urothelial cells of bladder cancer patients, and a positive correlation between MN frequencies and tumour grade, suggesting that MN frequency could serve as both an early detection marker and a predictor of cancer progression (44). Our findings support, from a mechanistic perspective, the existing evidence on the increased cancer risk markers among firefighters, reinforcing the potential of MN frequency as an effective biomarker for occupational health monitoring.

The application of MN assay in epithelial cells, using either urothelial or buccal cells, is of particular interest because it allows the assessment of additional biomarkers beyond genomic instability (18). The results from our study revealed a significant increase in binucleated buccal cells among wildland firefighters during the wildfire season, compared to levels observed before the season (baseline). The literature suggests that an elevated binucleated cell ratio may indicate a high rate of aneuploidy (abnormal chromosome number), which is associated with an increased risk of cancer and neurodegenerative diseases (45, 46). Although the biological meaning of cellular anomalies, other than MN, assessed by BM Cyt, is not yet fully understood, some studies have suggested an association between specific cell types or nuclear anomalies and adverse health outcomes (46, 47). We also found an association between the frequency of urothelial binucleated cells and years of service as a firefighter. Specifically, we observed that wildland firefighters with more than 20 years of service had a significantly higher frequency of urothelial binucleated cells compared to those with less than 10 years of service. These findings suggest that a prolonged career as a wildland firefighter may contribute to increased cellular damage, as reflected in the elevated frequency of binucleated urothelial cells in firefighters with longer service. Binucleated cells in urine cells are considered a marker of genomic instability, resulting from either cytokinesis failure or cytokinesis arrest (48). In a study described herein by Ray et al. (36), the authors also found a significant association between an effect biomarker (i.e. MN frequency in buccal cells) and years of service as a firefighter. Specifically, they observed that firefighters with longer careers (≥ 20 years) had higher MN frequency in buccal cells compared to those with shorter careers (< 20 years) (36). These results highlight the importance of using effect biomarkers to study the impact of cumulative or long-term exposures, such as firefighters' career length, at the cellular and molecular level.

The results of the present study should be interpreted considering some limitations. The voluntary nature of participant recruitment introduces the possibility of selection bias, while self-reported data may be subject to information bias, potentially leading to over- or

underestimation. Additionally, MN in epithelial cells, such as buccal and urothelial cells, may take up to three weeks to peak due to cell turnover from the basal to surface layers (11, 18), which could result in an underestimation of the genotoxic effects.

Despite these limitations, the study offers several strengths. Our study brings novel mechanistic information regarding firefighters' occupational exposure, particularly concerning wildland firefighter exposure to real fire events. It employs a pre-post design in which individuals serve as their controls, improving the reliability of the results. This is the first study investigating the frequency of MN and other cellular endpoints in the urothelial cells of firefighters, regardless of the type of firefighter. Moreover, is the first study assessing MN frequencies in buccal cells of wildland firefighters at two different time-points of their activity (before and during fire season), as well as the first investigating the effect of urinary Σ OHPAHs on MN frequency in urothelial cells, specifically on wildland firefighters.

5. Conclusion

Warmer and drier weather, mostly driven by climate change, have increased the frequency and intensity of wildfires across the globe. As a result, wildfires are expected to engage more firefighters in the coming years, thereby increasing the number of these professionals in firefighting activities, and consequently, their subsequent occupational burden of disease.

Our study confirms the potentiality of the MN assay in urothelial exfoliated cells to be used in the surveillance of occupational groups at higher risk of developing bladder cancer, particularly wildland firefighters. The use of MN assay on local-tissue wildland firefighters monitoring occupational exposures is highly relevant to understanding biological mechanisms that are associated with long-term exposures. This is a useful effect biomarker to evaluate genotoxicity and early cancer risk associated with harmful human exposures.

Decontamination measures to reduce firefighters' exposure to woodsmoke air contaminants should be the overarching aim to implement in this sector. Periodic medical examinations of firefighters are essential for the early detection of adverse health effects.

Funding

This work received financial support from the project PCIF/SSO/0017/2018 (<https://doi.org/10.54499/PCIF/SSO/0017/2018>) by the Fundação para a Ciência e a Tecnologia (FCT), Ministério da Ciência, Tecnologia e Ensino Superior (MCTES), through national funds. This work is financed by national funds through the FCT within the scope of projects UIDB/04750/2020 (<https://doi.org/10.54499/UIDB/04750/2020>) and

LA/P/0064/2020 (<https://doi.org/10.54499/LA/P/0064/2020>). Filipa Esteves was supported by National Funds through FCT, under the Ph.D. fellowship UI/BD/150783/2020. The work of Stefano Bonassi was supported by the Ministry of Health (Ricerca Corrente), Rome, Italy, and by the Competitive Funding for University Research Projects [Finanziamento competitivo di progetti di ricerca di ateneo (FIN/RIC)] San Raffaele University, Rome, Italy.

Ethics Approval

This work received approval for research ethics by the Accredited Ethics Committee of the University of Porto, Portugal, Report Nr. 92/CEUP/2020, under the project BioFirEx project (PCIF/SSO/0017/2018): “A panel of (bio)markers for the surveillance of firefighter's health and safety”.

References

1. Esteves F, Madureira J, Teixeira JP, Costa S. Wildland Firefighters: A Crucial Weapon for Forest Fire Management. Which Health Risks Do They Face? Fire Hazards: Socio-economic and Regional Issues: Springer International Publishing Cham; 2024. p. 107-21.
2. Ramos C, Minghelli B. Prevalence and factors associated with poor respiratory function among firefighters exposed to wildfire smoke. *International Journal of Environmental Research and Public Health*. 2022;19(14):8492.
3. IARC. Painting, firefighting, and shiftwork: IARC Press, International Agency for Research on Cancer; 2010.
4. Jameson CW. Polycyclic aromatic hydrocarbons and associated occupational exposures. 2021.
5. International Agency for Research on Cancer (IARC). Occupational Exposure as a Firefighter. 2023. 1-730 p.
6. Bonassi S, Ugolini D, Kirsch-Volders M, Strömberg U, Vermeulen R, Tucker JD. Human population studies with cytogenetic biomarkers: review of the literature and future perspectives. *Environmental and molecular mutagenesis*. 2005;45(2-3):258-70.
7. Bennett DA, Waters MD. Applying biomarker research. *Environmental Health Perspectives*. 2000;108(9):907-10.
8. Fenech M, Kirsch-Volders M, Natarajan A, Surrallés J, Crott J, Parry J, et al. Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. *Mutagenesis*. 2011;26(1):125-32.

9. Fenech M. Cytokinesis-block micronucleus cytome assay. *Nature protocols*. 2007;2(5):1084-104.
10. Norppa H, Falck GC-M. What do human micronuclei contain? *Mutagenesis*. 2003;18(3):221-33.
11. Nersesyan A, Kundi M, Fenech M, Stopper H, da Silva J, Bolognesi C, et al. Recommendations and quality criteria for micronucleus studies with humans. *Mutation Research/Reviews in Mutation Research*. 2022;789:108410.
12. Bonassi S, Coskun E, Ceppi M, Lando C, Bolognesi C, Burgaz S, et al. The HUMAN MicroNucleus project on exfoliated buccal cells (HUMNXL): The role of life-style, host factors, occupational exposures, health status, and assay protocol. *Mutation Research/Reviews in Mutation Research*. 2011;728(3):88-97.
13. Boffetta P, van der Hel O, Norppa H, Fabianova E, Fucic A, Gundy S, et al. Chromosomal aberrations and cancer risk: results of a cohort study from Central Europe. *American journal of epidemiology*. 2007;165(1):36-43.
14. Fenech M, Holland N, Kirsch-Volders M, Knudsen LE, Wagner K-H, Stopper H, et al. Micronuclei and disease—Report of HUMN project workshop at Rennes 2019 EEMGS conference. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*. 2020;850:503133.
15. Bonassi S, El-Zein R, Bolognesi C, Fenech M. Micronuclei frequency in peripheral blood lymphocytes and cancer risk: evidence from human studies. *Mutagenesis*. 2011;26(1):93-100.
16. Bonassi S, Znaor A, Ceppi M, Lando C, Chang WP, Holland N, et al. An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans. *Carcinogenesis*. 2007;28(3):625-31.
17. Ceppi M, Biasotti B, Fenech M, Bonassi S. Human population studies with the exfoliated buccal micronucleus assay: statistical and epidemiological issues. *Mutation Research/Reviews in Mutation Research*. 2010;705(1):11-9.
18. Thomas P, Holland N, Bolognesi C, Kirsch-Volders M, Bonassi S, Zeiger E, et al. Buccal micronucleus cytome assay. *Nature protocols*. 2009;4(6):825.
19. Esteves F, Madureira J, Teixeira JP, Costa S. The Role of Biomarkers in the Assessment of Potential Health Risks of Wildland Firefighters' Occupational Exposure: An Overview. In: Marta Madalena Marques de Oliveira SBM, Francisca Pinto Lisboa Martins Rodrigues, editor. "Essential Guide to Occupational Exposure". Safety and Risk in Society. New York, United States: Nova Science Publishers, Inc.; 2022.
20. Esteves F, Madureira J, Teixeira JP, S C. The firefighters on the frontline of forest fires – preliminary characterisation of Bio4FOX study population. In: Viegas X RL, editor. *Advances in Forest Fire Research* Universidade de Coimbra; 2022. p. 1330-4.

21. Vondracek M, Xi Z, Larsson P, Baker V, Mace K, Pfeifer A, et al. Cytochrome P450 expression and related metabolism in human buccal mucosa. *Carcinogenesis*. 2001;22(3):481-8.
22. Spivack SD, Hurteau GJ, Jain R, Kumar SV, Aldous KM, Gierthy JF, et al. Gene-environment interaction signatures by quantitative mRNA profiling in exfoliated buccal mucosal cells. *Cancer research*. 2004;64(18):6805-13.
23. Panico A, Grassi T, Bagordo F, Idolo A, Serio F, Tumolo MR, et al. Micronucleus frequency in exfoliated buccal cells of children living in an industrialized area of Apulia (Italy). *International journal of environmental research and public health*. 2020;17(4):1208.
24. Holland N, Bolognesi C, Kirsch-Volders M, Bonassi S, Zeiger E, Knasmueller S, et al. The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage: the HUMN project perspective on current status and knowledge gaps. *Mutation Research/Reviews in Mutation Research*. 2008;659(1-2):93-108.
25. Cairns J. Mutation selection and the natural history of cancer. *Nature*. 1975;255(5505):197-200.
26. Rosin MP. The use of the micronucleus test on exfoliated cells to identify anti-clastogenic action in humans: a biological marker for the efficacy of chemopreventive agents. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*. 1992;267(2):265-76.
27. Bolognesi C, Knasmueller S, Nersesyan A, Thomas P, Fenech M. The HUMNxl scoring criteria for different cell types and nuclear anomalies in the buccal micronucleus cytome assay—An update and expanded photogallery. *Mutation Research/Reviews in Mutation Research*. 2013;753(2):100-13.
28. Esteves F, Slezakova K, Madureira J, Vaz J, Fernandes A, Morais S, et al. Firefighters' Occupational Exposure in Preparation for Wildfire Season: Addressing Biological Impact. *Toxics*. 2024;12(3):201.
29. Bolognesi C, Fenech M. Micronucleus assay in human cells: lymphocytes and buccal cells. *Genotoxicity Assessment: Methods and Protocols*. 2013:191-207.
30. Fortin F, Anghel T, Brochu P, Lemieux N. Optimizing urothelial cell preparation for the human urinary micronucleus assay. *Toxicology in vitro*. 2010;24(6):1821-7.
31. Costa S, Brandão F, Coelho M, Costa C, Coelho P, Silva S, et al. Micronucleus frequencies in lymphocytes and buccal cells in formaldehyde exposed workers. *WIT Transactions on Biomedicine and Health*. 2013;16:83-94.
32. Barros B, Paiva AM, Oliveira M, Alves S, Esteves F, Fernandes A, et al. Baseline data and associations between urinary biomarkers of polycyclic aromatic hydrocarbons, blood pressure, hemogram, and lifestyle among wildland firefighters. *Frontiers in Public Health*. 2024;12:1338435.

33. Espinoza F, Silverman D, Kogevinas M, Creus A, Fernández F, García-Closas M, et al. Micronuclei assessment in the urothelial cells of women using hair dyes and its modulation by genetic polymorphisms. *Cancer letters*. 2008;263(2):259-66.
34. Hoyos-Giraldo L, Escobar-Hoyos L, Saavedra-Trujillo D, Reyes-Carvajal I, Muñoz A, Londoño-Velasco E, et al. Gene-specific promoter methylation is associated with micronuclei frequency in urothelial cells from individuals exposed to organic solvents and paints. *Journal of Exposure Science & Environmental Epidemiology*. 2016;26(3):257-62.
35. World Health Organization W. Body mass index - BMI: World Health Organization; 2022 [Available from: <https://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi>.
36. Ray MR, Basu C, Mukherjee S, Roychowdhury S, Lahiri T. Micronucleus frequencies and nuclear anomalies in exfoliated buccal epithelial cells of firefighters. *International Journal of Human Genetics*. 2005;5(1):45-8.
37. Wang Y, Yang H, Li L, Wang H, Xia X, Zhang C. Biomarkers of chromosomal damage in peripheral blood lymphocytes induced by polycyclic aromatic hydrocarbons: a meta-analysis. *International archives of occupational and environmental health*. 2012;85:13-25.
38. Sram RJ, Svecova V, Rossnerova A. Systematic review of the use of the lymphocyte cytokinesis-block micronucleus assay to measure DNA damage induced by exposure to polycyclic aromatic hydrocarbons. *Mutation Research/Reviews in Mutation Research*. 2016;770:162-9.
39. Holmen A, Karlsson A, Bratt I, Raihle G, Högstedt B. Increased frequency of micronuclei in lymphocytes of Swedish chimney sweeps. *International archives of occupational and environmental health*. 1994;66:185-7.
40. Cao Y, Xi J, Tang C, Yang Z, Liu W, You X, et al. PIG-A gene mutation as a genotoxicity biomaker in polycyclic aromatic hydrocarbon-exposed barbecue workers. *Genes and environment*. 2021;43:1-9.
41. Leng S, Dai Y, Niu Y, Pan Z, Li X, Cheng J, et al. Effects of genetic polymorphisms of metabolic enzymes on cytokinesis-block micronucleus in peripheral blood lymphocyte among coke-oven workers. *Cancer Epidemiology Biomarkers & Prevention*. 2004;13(10):1631-9.
42. Zhong B, Gu Z, Stewart J, Ong T. Micronucleus formation induced by three polycyclic aromatic hydrocarbons in rat bone marrow and spleen erythrocytes following intratracheal instillation. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*. 1995;326(2):147-53.
43. Takam P, Schäffer A, Laovitthayangoon S, Charemtantanakul W, Sillapawattana P. Toxic effect of polycyclic aromatic hydrocarbons (PAHs) on co-culture model of human

alveolar epithelial cells (A549) and macrophages (THP-1). *Environmental Sciences Europe*. 2024;36(1):176.

44. Espinoza F, Cecchini L, Morote J, Marcos R, Pastor S. Micronuclei frequency in urothelial cells of bladder cancer patients, as a biomarker of prognosis. *Environmental and molecular mutagenesis*. 2019;60(2):168-73.

45. Migliore L, Coppede F, Fenech M, Thomas P. Association of micronucleus frequency with neurodegenerative diseases. *Mutagenesis*. 2011;26(1):85-92.

46. Thomas P, Hecker J, Faunt J, Fenech M. Buccal micronucleus cytome biomarkers may be associated with Alzheimer's disease. *Mutagenesis*. 2007;22(6):371-9.

47. Thomas P, Harvey S, Gruner T, Fenech M. The buccal cytome and micronucleus frequency is substantially altered in Down's syndrome and normal ageing compared to young healthy controls. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*. 2008;638(1-2):37-47.

48. Nersesyan A, Kundi M, Fenech M, Bolognesi C, Misik M, Wultsch G, et al. Micronucleus assay with urine derived cells (UDC): a review of its application in human studies investigating genotoxin exposure and bladder cancer risk. *Mutation Research/Reviews in Mutation Research*. 2014;762:37-51.

Supplementary Material - Paper IV

Table S1 - BMCyt assay endpoints within groups of studied self-reported variables (n=59).

	DNA damage		Cell Death				Cytokinetic Defects	Proliferation Activity
	MN‰	NBUD‰	KYL‰	CC ‰	KHC‰	PIC‰	BN‰	Basal ‰
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Individual characteristics:								
<i>Sex (n)</i>								
Female (13)	0.31±0.12	0.00±0.00	0.62±0.46	0.15±0.15	0.08±0.08	0.08±0.08	1.15±0.37	12.00±2.71
Male (45)	0.42±0.09	0.03±0.02	1.40±0.61	0.00±0.00	0.24±0.11	0.09±0.05	0.82±0.23	14.13±1.88
<i>BMI (n)</i>								
Normal (22)	0.25±0.08	0.00±0.00	1.68±0.98	0.00±0.00	0.09±0.06	0.05±0.04	0.55±0.19	15.45±2.99
Pre-obesity (30)	0.46±0.12	0.02±0.02	0.76±0.48	0.07±0.07	0.17±0.09	1.10±0.35	1.10±0.35	13.28±2.10
Obesity (7)	0.57±0.23	0.14±0.14	1.71±1.71	0.00±0.00	0.71±0.56	1.14±0.40	1.14±0.40	9.57±2.61
<i>Age (n)</i>								
<32 years (21)	0.28±0.09	0.05±0.05	1.24±0.78	1.24±0.78	0.19±0.09	0.10±0.46	1.52±0.46	10.48±2.00
32-44 years (29)	0.36±0.10	0.02±0.02	0.24±0.21	0.07±0.07	0.21±0.14	0.59±0.18	0.59±0.18	14.79±2.42
>44 years (8)	0.81±0.27	0.00±0.00	4.75±2.48	0.00±0.00	0.25±0.25	0.38±0.18	0.38±0.18	17.88±4.97
Lifestyle characterisation:								
<i>Smoking habits (n)</i>								
Never-smoker (14)	0.50±0.18	0.04±0.04	2.57±1.46	0.00±0.00	0.00±0.00	0.07±0.07	0.43±0.17	13.29±3.14
Current smoker (32)	0.33±0.08	0.00±0.00	1.09±0.58	0.00±0.00	0.34±0.15	0.06±0.04	1.16±0.34	13.47±1.75
Ex-smoker (12)	0.46±0.19	0.08±0.08	0.00±0.00	0.17±0.17	0.08±0.08	0.08±0.08	0.75±0.22	14.58±5.06
<i>Alcohol daily intake (n)</i>								
Yes (15)	0.44±0.09	0.01±0.01	1.63±0.64	0.00±0.00	0.14±0.06	0.09±0.06	0.88±0.24	14.44±1.91
No (43)	0.27±0.08	0.07±0.07	0.07±0.07	0.13±0.13	0.40±0.27	0.07±0.07	0.93±0.34	11.40±2.66
Occupational-related:								
<i>Years of service (as a firefighter)</i>								
<10 years of service (19)	0.55±0.15	0.08±0.06	0.95±0.69	0.00±0.00	0.16±0.09	0.00±0.00	1.32±0.46	10.00±2.15
≥10 - 20 years of service (19)	0.34±0.13	0.00±0.00	0.79±0.63	0.11±0.10	0.32±0.22	0.11±0.07	0.68±0.30	13.21±2.38
>20 years of service (18)	0.33±0.09	0.00±0.00	2.11±1.21	0.00±0.00	0.17±0.12	0.17±0.12	0.67±0.26	18.22±3.63
<i>Permanent Intervention Teams (n)</i>								
No (31)	0.42±0.11	0.00±0.00	2.26±0.86	0.06±0.06	0.29±0.15	0.13±0.08	0.90±0.20	15.84±2.10
Yes (26)	0.38±0.10	0.06±0.04	0.04±0.04	0.00±0.00	0.12±0.06	0.05±0.04	0.92±0.38	11.54±2.38
Diet:								
<i>Vegetable consumption (n)</i>								
Never (5)	0.40±0.24	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.60±0.40	11.40±5.43
Daily (29)	0.36±0.10	0.03±0.03	2.17±0.91	0.07±0.07	0.31±0.16	0.07±0.05	0.66±0.14	15.14±2.66
Weekly (20)	0.45±0.14	0.02±0.02	0.35±0.30	0.00±0.00	0.15±0.08	0.10±0.10	1.25±0.47	11.60±1.86
<i>Fruit intake (n)</i>								
No (2)	0.50±0.50	0.50±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.50±0.50
Yes (56)	0.39±0.07	0.03±0.02	1.27±0.49	0.04±0.04	0.21±0.09	0.09±0.05	0.93±0.20	14.13±1.60
<i>Tea intake (n)</i>								
No (34)	0.39±0.10	0.01±0.01	1.29±0.69	0.00±0.00	0.21±0.12	0.09±0.05	1.06±0.32	12.65±1.85
Yes (24)	0.39±0.10	0.04±0.04	1.13±0.65	0.08±0.08	0.21±0.10	0.08±0.08	0.67±0.15	15.08±2.79
<i>Coffee intake (n)</i>								
No (5)	0.60±0.29	0.00±0.00	0.20±0.20	0.00±0.00	0.20±0.20	0.00±0.00	1.00±0.45	10.20±3.99
Yes (53)	0.38±0.07	0.03±0.02	1.32±0.52	0.04±0.04	0.21±0.09	0.09±0.05	0.89±0.21	13.98±1.69

Kruskal-Wallis (independent variables with three groups) and Mann-Whitney (independent variables with two groups) test; n refers to the number of individuals per group.

Table S2 - MN assay endpoints within groups of studied self-reported variables (n=59).

	DNA damage		Cell Death				Cytokinetic Defects	Proliferation Activity
	MN‰	NBUD‰	KYL‰	CC ‰	KHC‰	PIC‰	BN‰	Basal ‰
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Individual characteristics:								
<i>Sex (n)</i>								
Female (11)	0.27±0.10	0.04±0.04	0.00±0.00	0.00±0.00	0.18±0.18	0.00±0.00	1.45±0.47	18.27±6.50
Male (39)	0.29±0.12	0.04±0.03	0.18±0.08	0.00±0.00	0.18±0.10	0.03±0.03	2.72±0.68	14.00±2.25
<i>BMI (n)</i>								
Normal (20)	0.12±0.05	0.00±0.00	0.10±0.10	0.00±0.00	0.00±0.00	2.20±0.76	2.20±0.76	11.65±1.64
Pre-obesity (26)	0.44±0.17	0.08±0.04	0.19±0.10	0.00±0.00	0.35±0.16	2.92±0.87	2.92±0.87	14.58±3.24
Obesity (4)	0.13±0.13	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.50±0.28	0.50±0.29	33.75±15.34
<i>Age (n)</i>								
<32 years (20)	0.17±0.07	0.00±0.00	0.10±0.07	0.00±0.00	0.20±0.16	1.35±0.64	1.35±0.64	18.60±4.93
32-44 years (22)	0.27±0.16	0.07±0.05	0.23±0.13	0.00±0.00	0.14±0.10	2.09±0.54	2.09±0.54	13.50±2.18
>44 years (8)	0.62±0.35	0.06±0.06	0.00±0.00	0.00±0.00	0.25±0.25	6.12±2.35	6.12±2.35	9.75±2.48
Lifestyle characterisation:								
<i>Smoking habits (n)</i>								
Never-smoker (13)	0.35±0.23	0.04±0.04	0.08±0.08	0.00±0.00	0.15±0.15	0.00±0.00	1.77±0.75	9.61±1.74
Current smoker (26)	0.25±0.14	0.04±0.04	0.15±0.09	0.00±0.00	0.27±0.14	0.04±0.04	3.19±0.95	13.96±3.07
Ex-smoker (11)	0.32±0.10	0.05±0.05	0.18±0.18	0.00±0.00	0.00±0.00	0.00±0.00	1.45±0.47	23.54±6.39
<i>Alcohol daily intake (n)</i>								
No (36)	0.28±0.10	0.03±0.03	0.11±0.07	0.00±0.00	0.17±0.10	0.00±0.00	2.67±0.71	12.36±2.12
Yes (14)	0.32±0.22	0.07±0.05	0.21±0.15	0.00±0.00	0.21±0.15	0.07±0.07	1.86±0.68	21.57±5.64
Occupational-related:								
<i>Years of service (as a firefighter (n)</i>								
<10 years (16)	0.19±0.08	0.00±0.00	0.06±0.06	0.00±0.00	0.25±0.19	0.00±0.00	1.31±0.79	16.75±4.55
≥10 - 20 years (17)	0.12±0.05	0.03±0.03	0.12±0.08	0.00±0.00	0.12±0.12	0.06±0.06	1.53±0.42	12.47±2.78
> 20 years (16)	0.59±0.27	0.09±0.07	0.25±0.17	0.00±0.00	0.19±0.14	0.00±0.00	4.56±1.33*	11.75±1.82
<i>Permanent Intervention Teams (n)</i>								
No (28)	0.30±0.13	0.02±0.02	0.11±0.06	0.00±0.00	0.09±0.09	0.04±0.04	2.68±0.81	13.53±3.01
Yes (22)	0.27±0.14	0.02±0.02	0.18±0.12	0.00±0.00	0.09±0.09	2.14±0.71	2.14±0.71	16.73±3.39
Diet:								
<i>Vegetable consumption (n)</i>								
Never (4)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.25±0.25	0.00±0.00	1.50±0.87	10.75±1.70
Daily (25)	0.26±0.06	0.02±0.02	0.20±0.12	0.00±0.00	0.08±0.08	0.00±0.00	3.00±0.92	17.72±3.31
Weekly (17)	0.06±0.04	0.00±0.00	0.12±0.08	0.00±0.00	0.18±0.18	0.06±0.06	0.94±0.28	12.82±4.29
<i>Fruit intake (n)</i>								
No (3)	0.00±0.00	0.00±0.00	0.33±0.33	0.00±0.00	0.00±0.00	0.00±0.00	1.00±0.58	8.33±1.85
Yes (47)	0.31±0.10	0.04±0.02	0.13±0.06	0.00±0.00	0.19±0.09	0.02±0.02	2.53±0.58	15.36±2.37
<i>Tea intake (n)</i>								
No (30)	0.37±0.15	0.05±0.04	0.03±0.03	0.00±0.00	1.93±0.55	0.00±0.00	1.93±0.55	12.37±1.64
Yes (20)	0.17±0.05	0.02±0.02	0.30±0.15	0.00±0.00	3.20±1.08	0.05±0.05	3.20±1.08	18.80±4.99
<i>Coffee intake (n)</i>								
No (6)	0.17±0.10	0.00±0.00	0.17±0.17	0.00±0.00	0.50±0.50	0.00±0.0	0.50±0.34	28.00±12.18
Yes (44)	0.31±0.11	0.04±0.03	0.14±0.07	0.00±0.00	0.14±0.07	0.02±0.02	2.70±0.61	13.16±1.88

Kruskal-Wallis (independent variables with three groups) and Mann-Whitney (independent variables with two groups) test; Multiple comparisons were performed according to Bonferroni adjustment; bold values represent statistically significant differences within groups ($p<0.05$); n refers to the number of individuals per each group.

CHAPTER V.

General Discussion

A. SUMMARY OF MAIN FINDINGS

This discussion section synthesises the main findings presented in Chapter IV, which include data resulting from the baseline characterisation of firefighters' occupational exposure at fire stations (Chapter IV, Section A.1.), as well as from the evaluation of pre- vs post-exposure changes in biological mechanisms triggered by wildland firefighting (Chapter IV, Section B.1., B.2. And B.3.). Together, these insights contribute to a broad understanding of the occupational health risks faced by wildland firefighters and therefore, to the creation of a list of recommendations to safeguard the health and safety of these professionals.

A. Characterisation of firefighters' occupational exposure at fire stations, before the wildfire season (baseline)

Before the wildfire season, firefighters perform a range preparatory activities at the fire station, such as maintenance of equipment and vehicles to ensure preparedness for the wildfire season (1). During these activities, firefighters may be exposed to harmful pollutants, including PM_{2.5} and PM₁₀ (1, 2). Considering the time that these professionals spend at the fire station during the off-season, the inhaled doses of PM₁₀ and PM_{2.5} among firefighters were estimated, along with their association with effect endpoints measured through the BMCyt assay. In this preliminary characterisation, it was observed that indoor PM₁₀ and PM_{2.5} concentrations in fire stations were below the levels set in Portuguese Law No.138-G/2021 (PM₁₀=50 µg·m⁻³ and PM_{2.5}=25 µg·m⁻³) for indoor air quality in public buildings (3). The outdoor PM₁₀ and PM_{2.5} levels were in accordance with the recommendations proposed by WHO (45 and 15 µg·m⁻³, correspondingly) (4). No association between inhaled PM doses and MN frequency in buccal cells was found. In this first study, the influence of self-reported variables on biological endpoints was also explored. The elevated cell death endpoints (e.g., karyolytic cells) among full-time firefighters in Permanent Intervention Teams highlighted the potential cumulative impact of their regular exposure to various occupational stressors. While no association was found between MN frequency and age, gender or smoking, significant differences were observed in other cellular endpoints and some self-reported variables. For instance, pre-obese firefighters had increased cell death markers, aligning with previous findings that link higher BMI to cytogenetic damage (5, 6). Dietary factors also emerged as important key factors to cellular maintenance: regular vegetable consumption was associated with reduced cell death

endpoints. These findings underscore the importance of a healthy lifestyle, to reduce the burden of genotoxic risks among firefighters. Results obtained in this study allowed for the characterisation of wildland firefighters' occupational exposure at fire stations, before the wildfire season, as a respective baseline.

B. Investigating the exposure-induced biological mechanisms associated with wildland firefighting occupational exposure

→ Inflammatory Response

The inhalation of wildfire smoke has been previously linked to an inflammatory response (7), which is a key mechanism associated with the increased risk of several diseases such as cardiovascular (8, 9), respiratory (10), and cancer (10-14). Thus, the second objective of Bio4FOX was to explore the influence of firefighting occupational exposure on the acute inflammatory response through the assessment of pro-inflammatory cytokines. A significant increase in both serum IL-6 and serum IL-8 levels was observed following wildfire combat, whereas no significant differences were found for TNF- α (pre- vs post-exposure). A positive association was found between the exposure duration (hours) and IL-8 levels. A significant association between urinary 2-OHFlu levels and increased IL-8 levels was also found, suggesting IL-8 could serve as a potential biomarker for monitoring firefighting occupational exposure. Additionally, hs-CRP - a clinical biomarker – was used to investigate the levels of acute inflammatory responses particularly related to cardiovascular risk, strongly associated with this occupation (16). The mean plasma hs-CRP levels were above 3.0 mg/L in both study phases, indicating a high risk for cardiovascular disease (17-19). To the best of our knowledge, this was the first study assessing hs-CRP among wildland firefighters enrolled in fire combat in real wildfire scenarios. Our findings support the association between firefighting and increased cardiovascular risk, consistent with epidemiological studies that report a high incidence of cardiovascular events/diseases (e.g., heart stroke, arrhythmias) among firefighters (16).

→ DNA Damage

In the third manuscript, the impact of wildland firefighting on genotoxicity biomarkers was investigated, specifically assessing primary DNA damage, oxidative DNA damage, and γ H2AX. The evaluation of these biomarkers was particularly relevant given that no previous longitudinal studies have assessed primary DNA damage, oxidative DNA damage or γ H2AX

among wildland firefighters exposed to real wildfire events. Our findings revealed a significant increase in both primary and oxidative DNA damage following fire combat activities, consistent with the results of previous cross-sectional studies (20, 21). However, no significant differences were observed in γ H2Ax levels (pre- vs post-exposure). The contribution of urinary PAHs and urinary metal(loid)s to the observed changes (post- vs baseline levels) in primary and oxidative DNA damage was also investigated. A significant contribution of urinary 2-OHFlu, a PAH metabolite, to oxidative DNA damage was observed, a finding that aligns with a cross-sectional study conducted by Oliveira et al. (22). Additionally, a positive correlation was found between urinary 2-OHFlu and γ H2AX. Results obtained in this third study also showed that post-exposure levels of urinary Cs significantly correlated with increased levels of primary and oxidative DNA damage. Although the data on Cs toxicity in humans is limited, *in vivo* studies have shown that Cs could induce genotoxicity (23), damaging effects on skeletal and cardiac striated muscle tissues (24). Findings obtained suggest that exposure to metals present in wildfire smoke pollutants could also be linked to an increased risk of adverse cardiovascular outcomes. So far, this was the first study investigating the impact of wildfire smoke-derived metals on blood genotoxicity or oxidative biomarkers. Finally, we observed a correlation between the duration of wildfire combat activities and primary DNA damage. Additionally, firefighters reporting breathing difficulties during firefighting activities exhibited higher primary DNA damage levels compared to those who did not report such issues, suggesting that respiratory strain during firefighting may negatively impact genotoxic outcomes. Thus, results from this third study suggest that both primary DNA and oxidative DNA damage may serve as potential biomarkers for monitoring wildland firefighters' occupational exposure.

→ *Cytogenetic alterations*

Occupational exposure as a firefighter is classified as carcinogenic, though the underlying mechanisms remain poorly understood, particularly in wildland firefighters. MN is considered one of the most valuable biomarkers for assessing chromosomal damage, being considered a potential early cancer risk biomarker (25-27). Their assessment of minimally invasive biological matrices is particularly useful for human biomonitoring studies. Thus, the MN frequency in local-exposed tissues was studied, specifically in buccal cells (due to their direct exposure to pollutants) and urothelial cells (as the final site of exposure where important smoke pollutant metabolites are excreted). Results obtained showed an increase in MN frequency in both buccal and urothelial cells during the wildfire season, although a significant increase was only found in urothelial cells. In addition, we observed a significant positive contribution of total urinary OHPAH for MN frequency in urothelial cells. To our

knowledge, this was the first time that the frequency of MN in urothelial cells has been used to monitor the occupational exposure of wildland firefighters. These findings suggest that MN frequency in urothelial cells may serve as a useful biomarker for monitoring genetic effects associated with wildland firefighting exposure. The findings obtained in this study, are of great importance, as MN formation in urothelial cells has been linked to an increased risk of bladder cancer in exposed populations (28), and, in turn, firefighters have been shown to have an elevated risk of developing bladder cancer (11). Thus, results obtained demonstrate that MN in urothelial cells could serve as an effective tool for monitoring the health of wildland firefighters, especially considering the non-invasive nature of the sampling procedure. Moreover, the application of MN assays in epithelial cells allows for the evaluation of other cellular abnormalities (29); in this study, it was observed an increase in BN cells in buccal cells following firefighting exposure, as well as an elevated BN frequency in urothelial cells among firefighters with more years of service, compared with those with fewer years. BN cells have been associated with an increased risk of cancer and neurodegenerative diseases (30, 31). Therefore, analysing additional endpoints, beyond MN frequency, could provide further insights into the biological mechanisms underlying firefighters' occupational exposure and its health implications.

B. INTEGRATED DISCUSSION

This PhD study contributes with original data on the biological mechanisms associated with wildland firefighters' occupational exposure. It characterises firefighters' exposure both during the Pre-fire season, at fire stations, and following exposure to real wildfire events, assessing a range of biomarkers related to inflammation, genotoxicity/oxidative stress, and genetic/chromosomal instability. Additionally, the study investigates the impact of harmful occupational pollutants on these effect biomarkers.

Before the wildfire season, outdoor and indoor PM₁₀ and PM_{2.5} concentrations in fire stations were below the respective regulatory thresholds, with no significant correlation observed between inhaled doses to PM₁₀ and PM_{2.5} and BMCyt assay endpoints. However, the long-term, cumulative exposure to these pollutants, even at lower concentrations, should not be neglected, as it may contribute to potential adverse health effects, particularly given the extended periods firefighters spend in these work environments (11). Therefore, it is important to have a set of measures to guarantee a healthy building environment, such as efficient ventilation systems and decontamination procedures (PPE, equipment/vehicles) to avoid cross-contamination within spaces. Regular

air quality monitoring is also crucial to guarantee safe exposure levels and protect firefighters' health.

During Phase II, the biological impact of firefighting occupational exposure to real scenarios of wildfire-combat activities was investigated. The significantly higher levels of serum IL-6 and IL-8 after wildfire combat activities indicate an acute biological response potentially driven by occupational stressors, including exposure to wildfire smoke pollutants (32). These findings are consistent with previous studies that also observed increased levels of serum IL-6 and IL-8 pro-inflammatory cytokines after exposure to wildland firefighting activities (11, 33, 34). Additionally, the consistently elevated plasma hs-CRP levels in both study phases support the potential heightened cardiovascular risk among firefighters (11), suggesting its potential as a clinical biomarker for monitoring cardiovascular risk in this occupational group.

Primary DNA damage and oxidative DNA damage were significantly associated with firefighting occupational exposure, consolidating our hypothesis regarding the adverse biological impact of firefighting on DNA. Firefighters face multiple risk factors during their activities that may contribute to DNA damage, such as exposure to harmful pollutants (many of which are carcinogenic), high levels of physical and mental stress, and elevated temperatures (11). The increased levels of oxidative DNA damage might be associated with DNA lesions caused by oxidative stress due to the imbalance between the body's antioxidant defence systems and ROS production, including free radicals, associated with smoke pollutants, and physical exertion related to the nature of this occupation.

Cytogenetic alterations in tissues associated with primary exposure routes to pollutants were evaluated via MN assay. MN assay is an early-cancer risk biomarker, so, the evaluation in non-invasive matrices can be of paramount importance by either contributing to the research of the biological mechanisms underlining cancer in this occupation or exploring the applicability of new tools for clinical surveillance of wildland firefighters. The significantly higher frequency of MN observed in urothelial cells during the wildfire season provides strong evidence of genetic damage, emphasising the potential increased risk of cancer associated with firefighting (11). An increase in MN frequency in buccal cells was observed, although it did not reach statistical significance. This may be explained by the use of PPE, such as cotton bandanas, which can partially mitigate exposure. MN formation in urothelial cells likely reflects cumulative systemic exposure via multiple pathways, including nasal and oral inhalation, accidental ingestion, and dermal absorption of carcinogenic and genotoxic substances. These findings may suggest the critical importance of effective PPE and the need for strategies to address the diverse exposure routes and associated risks encountered by wildland firefighters.

Regarding the association between studied endpoints and urinary OHPAH, our results show that urinary 2-OHFlu levels were significantly associated with oxidative DNA damage levels measured in blood. The positive correlation between urinary 2-OHFlu levels and γ H2AX further supports the link between PAHs-exposure and DNA damage, in this case, double-strand breaks. This type of DNA damage, when not properly repaired, has been linked with genomic instability and carcinogenesis processes (35). In addition, urinary 2-OHFlu was also associated with increased serum IL-8 levels, which is also known to be triggered by oxidative stress and exposure to wildfire pollutants (11). It also suggests that exposure to some PAHs, such as fluorene, may play a significant role in driving inflammation and oxidative stress which is consistent with previous findings (21).

The association between wildfire smoke-released PAHs and effect biomarkers was also further confirmed by the significant positive association of MN frequency in urothelial cells with urinary total OHPAHs. The implications of this result are far-reaching, emphasising the contribution of PAHs to adverse health effects, and reinforcing its potential role in carcinogenic compounds (11, 36). Urothelial cells, as part of the urinary system, are in contact with PAH metabolites since they are concentrated in urine before excretion. As a result, these metabolites can induce MN formation in these cells; this finding supports the evaluation of IARC, regarding the carcinogenicity of firefighting occupational exposure based on evidence for bladder cancer in firefighters (11). Thus, monitoring MN in urothelial cells may serve as an important and sensitive tool to assess and track firefighters' occupational exposure to hazardous pollutants. Additionally, the potential biological effects of occupational exposure to other wildfire smoke pollutants, such as metals, should be further explored, as findings obtained in Bio4FOX study, suggest cesium may contribute to DNA damage.

The longer a firefighter is exposed to hazardous conditions, the greater the burden of adverse health effects such as inflammation and DNA damage, increasing the risk for long-term consequences (11). Among existing occupations, this is an evident challenge in wildland firefighters, where the professionals may spend several hours, even consecutive days or weeks, in the wildfire scene (15). To minimise cumulative exposure and its adverse effects, it is crucial to adequate work-shift patterns to ensure firefighters have sufficient time for rest and recovery between periods of intensive exposure.

In Bio4FOX study, it was possible to observe that, before wildfire season, members from Permanent Intervention Teams (full-time, contracted firefighters) exhibited higher cellular damage, as well as higher serum IL-8 levels. The full-time nature of their work, characterised by potential continuous firefighters' occupational exposures to harmful contaminants, high commitment and stressful nature of their work may justify the elevated biological impact,

compared with other colleagues, and volunteers. These findings reinforce the need for monitoring and targeted health interventions for full-time firefighters. Implementing strategies to minimize occupational risks, including regular health check-ups, protective measures, and adjustments to work shifts, is essential.

It is important to recognise that firefighter health is influenced not only by occupational exposure but also by lifestyle factors that can either exacerbate or mitigate the biological effects induced by their work environment. Therefore, the adoption of a healthy lifestyle is critical for safeguarding firefighters' health. Some of the findings addressed in Bio4FOX confirmed that a diet rich in vegetables could have potential benefits for cellular health. Given the elevated risk of oxidative stress associated with this occupation, a diet enriched with antioxidants may provide additional protective effects. Conversely, it was observed that a higher BMI was associated with increased cellular damage (condensed chromatin), suggesting that overweight or obesity may exacerbate the biological impacts of firefighting. These results underline the importance of maintaining a healthy lifestyle in the maintenance of firefighters' overall health and well-being. Adopting these strategies not only contributes to the mitigation of adverse effects but also improves the wildland firefighters' physical preparation required for the highly demanding nature of their work.

Overall, the data produced within this PhD study provide new insights into how wildland firefighters' occupational exposures trigger a cascade of biological processes that may contribute to adverse health outcomes. Some of the potential underlying biological mechanisms were identified. The increased inflammatory response and elevated DNA damage levels act as early indicators of potential health risks, while the elevated frequency of micronuclei in urothelial cells further highlights the potential carcinogenic risk of exposure to pollutants during wildland firefighting. The cumulative effect of these exposures over time, coupled with individual risk factors such as obesity and poor diet, may contribute to the development of chronic diseases including cancer. The findings of this study emphasise the need for targeted interventions to mitigate the health risks associated with wildland firefighting. Surveillance health programmes comprising a panel of biomarkers, including the ones identified by the present study, might be of utmost relevance for assessing firefighters' health and enabling the early detection of potential adverse health risks. Moreover, public health interventions should prioritise the mitigation of exposure through the implementation of measures/policies in the sector.

The Bio4FOX study has strengths and limitations, some of which have already been discussed previously in Chapter IV. However, some of the main points are summarised below.

Strengths

The research presented in this thesis provides novel and original insights into the assessment of wildland firefighters' occupational exposure, offering mechanistic data that bridges a critical scientific gap in understanding the adverse health effects among wildland firefighters. Contrary to most studies, this work considered exposure to real wildfire events contributing to more realistic data. Furthermore, this thesis provides a range of biological data, including inflammation, genotoxicity, and genetic instability in wildland firefighters, which adds to a broad understanding of the health risks faced by this group. The identification of specific harmful pollutants, such as PAHs, associated with DNA damage biomarkers, offers important insights into the pollutants linked to the observed health risks. By accounting for lifestyle, occupational, environmental, and health-related factors, this study ensured a more robust and accurate evaluation of the impact of firefighting exposure on biomarker levels. Importantly, the pre- and post-exposure study design, where participants served as their own controls, also strengthens the study's robustness by providing temporal insights into the relationship between exposure and outcome. In sum, the results of this thesis allowed for the formulation of a comprehensive list of recommendations, including the need for regular monitoring and preventive measures to reduce exposure to harmful pollutants, which is of great relevance to improving firefighter health and safety.

Limitations

Some of the limitations include self-reported information (e.g., health history, symptoms, lifestyle factors) collected through questionnaires, which may be subject to recall bias. The time window between exposure and biological sampling may have contributed to an underestimation of exposure levels. Additionally, the results for effect biomarkers may have been underestimated due to the challenges in capturing the peak effect for each biomarker, given the dynamic nature of biomarker responses. In future studies, a more frequent or repeated sampling strategy may provide more precise data on biomarker fluctuations during peak exposure periods. The unpredictable nature of wildfires and the transient nature of volunteer firefighters limited the ability to track a larger number of firefighters in the wildfire season. Firefighters may be exposed to various other risk factors, making it difficult to attribute observed biomarker changes solely to firefighting exposure. These factors should be considered when interpreting the findings. Despite these limitations, this thesis provides valuable insights into the health risks faced by wildland firefighters, which could inform future research and health interventions aimed at protecting their well-being.

C. LIST OF RECOMMENDATIONS

Benjamin Franklin, the founder of the first volunteer fire brigade in the USA, once stated “*An ounce of prevention is worth a pound of cure*” (37). This principle resonates universally, highlighting the critical importance of protecting firefighters from occupational health risks. It also emphasises that reducing these risks is a shared responsibility that involves multiple parties, parts or stakeholders.

Based on our findings, a set of guidelines listed below was developed to provide practical recommendations for mitigating the identified risks. These guidelines published in Esteves et al. (15), are intended for firefighters, fire chiefs, and supervisors, as well as, for health and safety professionals, policymakers, regulatory authorities, and researchers, ensuring a comprehensive approach to improving occupational safety and health of wildland firefighters. This approach aims to enhance accessibility and ensure clear understanding among local stakeholders and practitioners in the field of firefighter health and safety.

List of Recommendations to the Sector

- Fire stations should have a set of standard guidelines that incorporate safe practices and policies to protect firefighters’ safety and health. All staff should be trained in implementing such procedures and most importantly, guarantee that firefighters are aware of the occupational risks that they face and the importance of adopting preventive measures to reduce or eliminate occupational risks.
- Firefighters must know how to correctly use PPE. Equally important is guaranteeing proper maintenance through regular cleaning and inspection of PPE, repairing or replacing it when necessary.
- During fire suppression activities in the field, firefighters should avoid eating, drinking, or smoking with contaminated hands. When necessary, they must do so in a suitable environment with washed hands (e.g., water and soap or cleansing wipes) so that contaminants do not enter via ingestion.
- Decontamination procedures after exposure to fire pollutants are extremely important, including decontamination of firefighting personnel as well as of the contaminated PPE/equipment/vehicles.
- The decontamination of PPE and equipment should be cleaned *in loco* whenever possible; if not, contaminated PPE should be stored in proper containers/bags and transported separately from firefighters i.e., outside the vehicle cab.

- Firefighters should change to clean and dry clothes before returning to the fire station to reduce skin exposure to contaminants, or else take off the undergarment as soon as possible on return to the fire station.
- After fire suppression activities, it is crucial to wash exposed skin areas (e.g., hands, neck, face) with soap and water. When this is not feasible, firefighters may use cleansing wipes on exposed skin parts (e.g., neck and hands) to reduce the number of contaminants (e.g., PAHs).
- It is important to be aware that some particles and other contaminants might remain on the skin, and as long a contaminant is present on the skin more time is available to be absorbed. As such, showering with soap and shampoo should occur as soon as possible when returning to the fire station.
- Personal decontamination should be a priority when returning to the fire station from a fire, thus having available showers in the facilities is crucial.
- Contaminated PPE/clothing must be cleaned/laundered after every fire event, if possible, in a specifically designated area “*contaminated zone*” of the fire station to prevent cross-contamination. During these procedures, it is important to protect areas of exposed skin (e.g., gloves) and airways (e.g., face mask).
- PPE, work tools and vehicles should also be cleaned and decontaminated regularly.
- Construction of fire stations must be designed considering a specific layout (based on the circuit of “*contaminated*” and “*clean*” areas) and proper engineering measures to guarantee a clean airflow within spaces.
- The implementation of efficient ventilation systems must be a concern, particularly in the areas of the fire station where contaminated material is handled.
- The air quality of fire stations should be regularly monitored to guarantee safe exposure levels to air pollutants. Preventing contamination will keep firefighters and other fire station personnel protected from smoke-related contaminants.
- The development and implementation of well-designed fitness programmes that encourage physical activity and a balanced diet (e.g., water, fruits, vegetables, whole grains, and low-fat foods) are critical for the promotion of health and well-being among these individuals.
- Regular health screening should be provided to firefighters to early detect any adverse health events. Understanding the impacts of wildland fires on firefighters’ health can help to design mitigation strategies and efficient policies to protect their health.

D. REFERENCES

1. Bott RC, Kirk KM, Logan MB, Reid DA. Diesel particulate matter and polycyclic aromatic hydrocarbons in fire stations. *Environmental Science: Processes & Impacts*. 2017;19(10):1320-6.
2. Esteves F, Madureira J, Teixeira JP, Costa S. The Role of Biomarkers in the Assessment of Potential Health Risks of Wildland Firefighters' Occupational Exposure: An Overview. In: Marta Madalena Marques de Oliveira SBM, Francisca Pinto Lisboa Martins Rodrigues, editor. "Essential Guide to Occupational Exposure". *Safety and Risk in Society*. New York, United States: Nova Science Publishers, Inc.; 2022.
3. Portaria n.º 138-G/2021, 138-G/2021 (2021).
4. World Health Organization. WHO global air quality guidelines: particulate matter (PM_{2.5} and PM₁₀), ozone, nitrogen dioxide, sulfur dioxide and carbon monoxide: executive summary. 2021.
5. Idolo A, Grassi T, Bagordo F, Panico A, De Giorgi M, Serio F, et al. Micronuclei in exfoliated buccal cells of children living in a cluster area of Salento (Southern Italy) with a high incidence of lung cancer: the IMP. AIR study. *International journal of environmental research and public health*. 2018;15(8):1659.
6. Ernst B, Setayesh T, Nersesyan A, Kundi M, Fenech M, Bolognesi C, et al. Investigations concerning the impact of consumption of hot beverages on acute cytotoxic and genotoxic effects in oral mucosa cells. *Scientific Reports*. 2021;11(1):1-10.
7. Gianniou N, Giannakopoulou C, Dima E, Kardara M, Katsaounou P, Tsakatikas A, et al. Acute effects of smoke exposure on airway and systemic inflammation in forest firefighters. *Journal of asthma and allergy*. 2018;11:81.
8. Guarnier V, Rubio-Ruiz ME. Low-grade systemic inflammation connects aging, metabolic syndrome and cardiovascular disease. *Aging and health-a systems biology perspective*. 2015;40:99-106.
9. Kim S, Lee H, Shin D, Ku B, Oh J, Cho B, et al. Cardiovascular risk in fire academy instructors during live-fire simulation activity. *Annals of Burns and Fire Disasters*. 2018;31(4):313.
10. Orysiak J, Młynarczyk M, Piec R, Jakubiak A. Lifestyle and environmental factors may induce airway and systemic inflammation in firefighters. *Environmental Science and Pollution Research*. 2022;29(49):73741-68.

11. International Agency for Research on Cancer (IARC). Occupational Exposure as a Firefighter. 2023. 1-730 p.
12. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, et al. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*. 2018;9(6):7204.
13. Singh A, Liu C, Putman B, Zeig-Owens R, Hall CB, Schwartz T, et al. Predictors of asthma/COPD overlap in FDNY firefighters with World Trade Center dust exposure: a longitudinal study. *Chest*. 2018;154(6):1301-10.
14. Greten FR, Grivennikov SI. Inflammation and cancer: triggers, mechanisms, and consequences. *Immunity*. 2019;51(1):27-41.
15. Esteves F, Madureira J, Teixeira JP, Costa S. Wildland Firefighters: A Crucial Weapon for Forest Fire Management. Which Health Risks Do They Face? *Fire Hazards: Socio-economic and Regional Issues*: Springer International Publishing Cham; 2024. p. 107-21.
16. Fahy RF. US firefighter fatalities due to sudden cardiac death, 1995-2004: National Fire Protection Association Quincy, MA; 2005.
17. Li Y, Zhong X, Cheng G, Zhao C, Zhang L, Hong Y, et al. Hs-CRP and all-cause, cardiovascular, and cancer mortality risk: a meta-analysis. *Atherosclerosis*. 2017;259:75-82.
18. Ridker PM. C-reactive protein: a simple test to help predict risk of heart attack and stroke. *Circulation*. 2003;108(12):e81-e5.
19. Cushman M, Ballantyne CM, Cooper GR, Pfeiffer C, Grundy SM, Labarthe DR, et al. National Academy of Clinical Biochemistry Laboratory Medicine Practice guidelines: emerging biomarkers for primary prevention of cardiovascular disease. *Clin Chem*. 2009;55:378.
20. Abreu A, Costa C, Pinho e Silva S, Morais S, do Carmo Pereira M, Fernandes A, et al. Wood smoke exposure of Portuguese wildland firefighters: DNA and oxidative damage evaluation. *Journal of Toxicology and Environmental Health, Part A*. 2017;80(13-15):596-604.
21. Oliveira M, Costa S, Vaz J, Fernandes A, Slezakova K, Delerue-Matos C, et al. Firefighters exposure to fire emissions: Impact on levels of biomarkers of exposure to polycyclic aromatic hydrocarbons and genotoxic/oxidative-effects. *Journal of hazardous materials*. 2020;383:121179.

22. Oliveira M, Slezakova K, Alves MJ, Fernandes A, Teixeira JP, Delerue-Matos C, et al. Polycyclic aromatic hydrocarbons at fire stations: firefighters' exposure monitoring and biomonitoring, and assessment of the contribution to total internal dose. *Journal of hazardous materials*. 2017;323:184-94.
23. Santos-Mello R, Schmidt T, Neuhauss E, Almeida A. Induction of micronuclei by CsCl in vivo and in vitro. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*. 1999;446(2):239-44.
24. Yermishev O. Ecological and toxicological features of the effect of stable cesium on striated muscle tissue (myocardium and skeletal muscles) of mammals. *Regulatory Mechanisms in Biosystems*. 2023;14(3):415-23.
25. Bonassi S, El-Zein R, Bolognesi C, Fenech M. Micronuclei frequency in peripheral blood lymphocytes and cancer risk: evidence from human studies. *Mutagenesis*. 2011;26(1):93-100.
26. Bonassi S, Znaor A, Ceppi M, Lando C, Chang WP, Holland N, et al. An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans. *Carcinogenesis*. 2007;28(3):625-31.
27. Espinoza F, Cecchini L, Morote J, Marcos R, Pastor S. Micronuclei frequency in urothelial cells of bladder cancer patients, as a biomarker of prognosis. *Environmental and molecular mutagenesis*. 2019;60(2):168-73.
28. Fortin F, Anghel T, Brochu P, Lemieux N. Optimizing urothelial cell preparation for the human urinary micronucleus assay. *Toxicology in vitro*. 2010;24(6):1821-7.
29. Thomas P, Holland N, Bolognesi C, Kirsch-Volders M, Bonassi S, Zeiger E, et al. Buccal micronucleus cytome assay. *Nature protocols*. 2009;4(6):825.
30. Migliore L, Coppede F, Fenech M, Thomas P. Association of micronucleus frequency with neurodegenerative diseases. *Mutagenesis*. 2011;26(1):85-92.
31. Thomas P, Hecker J, Faunt J, Fenech M. Buccal micronucleus cytome biomarkers may be associated with Alzheimer's disease. *Mutagenesis*. 2007;22(6):371-9.
32. Abbas A. Cellular and molecular immunology. HBJ International Edition. 1991:226-42.
33. Ghio AJ, Soukup JM, Case M, Dailey LA, Richards J, Berntsen J, et al. Exposure to wood smoke particles produces inflammation in healthy volunteers. *Occupational and environmental medicine*. 2012;69(3):170-5.

34. Danielsen PH, Bräuner EV, Barregard L, Sällsten G, Wallin M, Olinski R, et al. Oxidatively damaged DNA and its repair after experimental exposure to wood smoke in healthy humans. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*. 2008;642(1-2):37-42.
35. Bonner WM, Redon CE, Dickey JS, Nakamura AJ, Sedelnikova OA, Solier S, et al. γ H2AX and cancer. *Nature Reviews Cancer*. 2008;8(12):957-67.
36. Jameson CW. Polycyclic aromatic hydrocarbons and associated occupational exposures. 2021.
37. Chiefs IAoF. Fundamentals of fire fighter skills: Jones & Bartlett Learning; 2008

CHAPTER VI.

Final Remarks and Future Perspectives

FINAL REMARKS AND FUTURE PERSPECTIVES

The characterisation of occupational exposure and health risks among Portuguese firefighters is of paramount importance, particularly because Portugal is one of the European countries more affected by forest fires. This study contributes with valuable data to a comprehensive risk assessment of Portuguese wildland firefighters' exposure, enhancing our understanding of the biological mechanisms associated with their exposure to real wildfire combat. By generating a set of breakthrough data, our findings significantly enhance the knowledge of the occupational health risks faced by this workforce.

Nevertheless, further research is needed to confirm our results and expand the generated knowledge. Applying longitudinal assessments to other regions/countries, would allow the comparison of results and contribute to strength the body of evidence regarding wildland firefighters' occupational risks. Cohort studies, particularly those with long-term follow-up, would be important to identify causal relationships between wildland firefighters' occupational exposures and the development of chronic diseases (e.g., cancer, respiratory conditions, and cardiovascular outcomes).

Subgroup analyses examining sex- and age-specific responses, as well as differences between seasonal and different types of firefighters (e.g., volunteers, permanent wildland firefighters), would enable more tailored interventions for different groups. Furthermore, future studies combining biomonitoring with functional health assessments (e.g., lung function) and real-time monitoring tools, such as wearable sensors to monitor environmental (e.g., CO, PM, temperature) and physiological (e.g., heart rate, oxygen saturation, body temperature) parameters would provide more precise and robust data on both the exposure and health outcomes. The resulting data could help design more effective PPE and equipment to better safeguard wildland firefighters from occupational hazards. Moreover, assessing both airborne pollutants (e.g., PM) in fire stations during the wildfire season and biomarkers of effect, would offer valuable insights into the risks wildland firefighters face upon returning to the station, facilitating targeted improvements in decontamination protocols. Another aspect to be considered is the impact of occupational stress and extreme working conditions on firefighters' mental health and its interplay with health risks.

These future directions may contribute to a more comprehensive understanding of the health impacts of firefighting, ultimately informing evidence-based public health strategies to safeguard the health and safety of firefighters. This research, while specific to Portugal, has global implications, particularly given the growing frequency and intensity of wildfires linked to climate change. Addressing these risks through science-based policy development is essential for promoting the health and safety of firefighters worldwide.

Overall, the integrated approach employed in the present PhD study has generated original data that can serve as a basis for future research and policy development. Moreover, it provides practical recommendations that aim to enhance the health and safety of wildland firefighters, reducing occupational risks and protecting both their health and the communities they serve. This set of recommendations has not only a direct societal impact but also aligns with broader public health goals, contributing to the well-being of this crucial workforce and the community, in general.

APPENDICES.

Appendix I – Published Book Chapter entitled “Wildland Firefighters: A Crucial Weapon for Forest Fire Management. Which Health Risks Do They Face”

Wildland Firefighters: A Crucial Weapon for Forest Fire Management. Which Health Risks Do They Face?



Filipa Esteves , Joana Madureira , João Paulo Teixeira ,
and Solange Costa

Abstract Fire when uncontrolled can become a destructive force that risks wildlife, property, and human lives. Global warming has contributed to the increase and severity of wildfires in the last decades, requiring greater political and local authority involvement to protect people. Wildland firefighters are an essential tool for the management of any forest fire. However, they face unique occupational risks characterized by physically demanding tasks, long hours in severe conditions, and exposure to various health risks (e.g., burning, fractures, smoke inhalation), including exposure to carcinogenic pollutants emitted from smoke. These pollutants, including particulate matter, carbon monoxide, nitrogen dioxide, and volatile organic compounds, can cause acute adverse health effects but also long-term effects, such as cancer. Yet, evidence linking wildland firefighters’ occupational exposure and health outcomes is limited. The increasing risk of wildfire occurrences and longer fire seasons highlight the need for occupational studies among these professionals commonly exposed to hazardous pollutants. Scientific evidence has contributed to the establishment of measures related to firefighters’ health promotion. Regular monitoring, surveillance and health promotion activities, innovative firefighting techniques, safer personal protective equipment, and the implementation of written policies and procedures, such as decontaminating fire station equipment and spaces, are crucial to reduce firefighters’ adverse health effects.

Keywords Wildland firefighters · Wildland fires · Climate change · Occupational exposure · Health risks · Air pollutants · Decontamination procedures

F. Esteves (✉) · J. Madureira · J. P. Teixeira · S. Costa
Environmental Health Department, National Institute of Health, Porto, Portugal
e-mail: filipa.esteves@insa.min-saude.pt

EPIUnit—Institute of Public Health, University of Porto, Porto, Portugal

Laboratory for Integrative and Translational Research in Population Health (ITR), Porto, Portugal

F. Esteves

Department of Public Health and Forensic Sciences, and Medical School, Faculty of Medicine,
University of Porto, Porto, Portugal

© The Author(s) 2024

J. Rodrigo-Comino and L. Salvati (eds.), *Fire Hazards: Socio-economic and Regional issues*, https://doi.org/10.1007/978-3-031-50446-4_9

107

1 Wildland Fires: Dealing with a New Era

Fire has always played an important role in shaping ecosystems constituting an essential component of ecological balance (Bond et al., 2005). It may be a useful tool, but, if untamed, may have a severe impact on wildlife habitats, goods, and people's lives. A "wildfire," "forest fire," "bushfire," "wildland fire," or "rural fire" (the term varies within countries) can be defined as an unplanned, uncontrolled, and unpredictable fire event that occurs in nature (Šomšák et al., 2009; CIFFC Glossary Task, and Training Working Group, 2017). The inevitability of global warming, with decreasing precipitation levels and prolonged dry seasons (Esteves et al., 2022a), has contributed to the increase in the number and severity of wildland fires (San-Miguel-Ayanz et al., 2018; Westerling et al., 2006).

As pointed out by the United Nations Intergovernmental Panel on Climate Change, there is a "*greater likelihood of injury, disease, and death due to more intense heat waves and fires*" (Field & Barros, 2014). Furthermore, the rising number of people working and/or living in areas close to forests increases the risk of serious damage to livelihoods or loss of human lives (Hirschberger, 2016; Radeloff et al., 2005). Thus, the more we know about wildfires' impact (e.g., effects on human health, millions lost to the economy, destroyed habitats, air pollution, burnt houses, loss of human lives), the more we must act on, concentrating efforts on prevention and fire response for a balanced and effective forest fire management.

The management of a forest fire per se lay mostly on the intervention of firefighters. In fact, wildland firefighters are considered the backbone for resolving any forest fire occurrence (Beighley & Hyde, 2018; Moreira et al., 2009). Having well-trained and healthy firefighters is crucial for an effective fire response. The predicted increase in wildland fires requires additional efforts from the workforce, and firefighters (Withen, 2015). Although firefighter is considered one of the most dangerous occupations in the world, little is known about the health risks related to their occupational exposure.

2 Wildland Firefighters: A Brief Contextualization

The first fire brigade, called "Vigiles," was created in 24 B.C. under the rule of Emperor Augustus and consisted of approximately 600 enslaved people and conscripts that patrolled the streets of Rome checking for crimes and fires (Butgereit et al., 2014). Later, in 60 A.D., emperor Nero allocated 7.000 freemen to fire prevention, fire-fighting, and building inspection activities (International Association of Fire Chiefs, 2008). After the Great Fire of London (1666) that destroyed nearly 80% of the city, insurance companies formed the first private fire brigades (Alagna, 2003). Over the centuries, firefighting systems were adjusted according to the reality of each country/people and their respective needs.

Currently, there are around 16 million firefighters in the world (91% males and 9% females) mostly volunteers, with the exception of a few countries (e.g., Italy, Spain,

USA, Canada, France, Greece, Israel, and Singapore) (CTIF, 2022). Fire departments may be composed of either professional firefighters (full-time firefighters), volunteer firefighters, or both; in the United States (USA), for example, most fire departments consist of volunteer firefighters (International Association of Fire Chiefs, 2008). Generally, each fire department is responsible for a geographic area guaranteeing a fast response time (International Association of Fire Chiefs, 2008).

The risks posed by wildland firefighting are different from those related to any other type of fire suppression context (e.g., structural firefighting, such as urban). Wildland firefighters may participate in several wildfire occurrences per year and remain on the fire scene for several days/weeks (for multiple shifts with little downtime) in very hostile and unpredictable conditions (Demers et al., 2022).

Wildland firefighters have an enormous physical and psychological workload (Ruby et al., 2002); common tasks include trekking, chainsaw work, and brush removal (IARC, 2010) while carrying a considerable number of heavy equipment and tools (e.g., rakes, axes, shovels, fire hoses) (IARC, 2010; Smith et al., 2001) through remote and difficult terrains. In addition, wildland firefighters face high temperatures, high levels of noise (e.g., sirens, diesel engines) (Broyles et al., 2017), and high levels of stress, particularly when they confront life-risk situations or when they need to deal with injuries or fatalities (Smith et al., 2001). Often beginning at very young ages, most firefighters remain their entire lives in the force, being continually and repeatedly exposed to different hazards including carcinogenic air pollutants (Magnusson & Hultman, 2015). To reduce or restrict their occupational exposure, firefighters are required to use personal protective equipment (PPE) that typically comprises protective clothing, respiratory protection, a helmet, neck shroud, protective hood, gloves, goggles, and boots (Carballo-Leyenda et al., 2018).

The increasing wildfire activity worldwide requires a higher number of wildland firefighters engaged in fire suppression activities (Koopmans et al., 2022). Anticipating that the number of forest fires is expected to increase in several regions of the globe (Flannigan et al., 2006), it is essential to look for appropriate occupational health and safety measures. Even more, the evidence linking health effects to occupational exposure to wildland fire is still limited and prevention measures are seldom mentioned.

3 But... What Exposures Do Wildland Firefighters Face?

The common hazards that wildland firefighters face on the fire line can include burning over/entrapment, heat-related outcomes, injuries, mental stress, fatigue, dehydration, and smoke inhalation (Britton et al., 2013; Koopmans et al., 2022). Firefighters are highly exposed to harmful pollutants in the form of gases and particles (Smith et al., 2001). Wildfire smoke is a complex mixture containing hundreds of gases and particles such as carbon monoxide, nitrogen dioxide, mono- and polycyclic aromatic hydrocarbons (PAHs), aldehydes, and metals (Naeher et al., 2007). Plus, wildland firefighters are also exposed to other pollutants such as diesel exhaust,



Fig. 1 Firefighters' routes of exposure

since they may be working near vehicles on the fire ground, in another emergency occurrence, or even at the fire station (Horn et al., 2022). Hence, it is very difficult to know all the pollutants or the mixture of pollutants that firefighters are exposed to during their activities and the extent of such exposure.

Wildland firefighters are exposed to fire smoke pollutants through different exposure pathways (Fig. 1), namely inhalation, ingestion, and dermal absorption (Ruby et al., 2002). Inhalation is the main exposure pathway for several harmful compounds that are present in smoke (Heus, 2018). The portion of pollutants inhaled is proportional to the volume of air inspired and expired, increasing with physical efforts (Stec et al., 2020). The typical respiratory protection used by wildland firefighters is a cotton bandana which offers very little protection against particulate matter (PM) due to its poor filtration efficiency, and that does not prevent the inhalation of toxic compounds (Austin, 2008; Heus, 2018; Naeher et al., 2007).

Wildland firefighters may also be exposed to fire smoke pollutants through dermal exposure (e.g., PAHS and volatile organic compounds) (Heus, 2018). Previous research has demonstrated that some pollutants may settle in the exposed skin (Rogula-Kozłowska et al., 2020) or even penetrate the firefighting protective ensemble (Barker, 2005) allowing dermal absorption of those contaminants. These exposures may be enhanced by the inadequate use of PPE or by inefficient decontamination procedures after fire suppression activities (Barker, 2005; Demers et al., 2022). In addition, exposure to high temperatures compromises the skin barrier facilitating the dermal absorption of toxic compounds (Heus, 2018; Wingfors et al., 2018). Absorption increases by 400% for every 5 °C increase in skin temperature (Stec et al., 2020).

In addition, wildland firefighters may be exposed to smoke toxicants via ingestion due to the lack of appropriate PPE (unprotected mouth) or even due to inadequate practices such as eating, drinking, and/or smoking with contaminated hands during or after firefighting activities (Heus, 2018). Moreover, contaminants that enter the upper respiratory tract may be carried via saliva into the digestive system (Stec et al., 2020).

Exposure to pollutants occurs not only during firefighting and other fire operations (e.g., prescribed burning) but also in their return to the fire station via diesel exhaust

and contaminated post-fire gear and/or other equipment. The inefficient decontamination procedures of PPE or other equipment used during firefighting also favor cross-contamination in fire stations (e.g., rooms, bedrooms, offices, and garages) compromising the indoor air quality where firefighters spend long periods of their shift (Banks et al., 2021; Oliveira et al., 2017). This may occur due to a lack of knowledge of safety procedures, cultural behaviors, carelessness, or logistical conditions (Magnusson & Hultman, 2015). It should be noted that in some cases, the fire stations are located in old buildings with inefficient ventilation systems that do not prevent or mitigate the spread of pollutants within the indoor spaces (Demers et al., 2022). In this manner, firefighters face a “cocktail” of exposures via multiple routes, leading to potential exposure misclassification that may consequently underestimate firefighters’ health risks. Exposure misclassification may explain some of the inconsistencies seen in the epidemiologic literature (Koopmans et al., 2022; Sparer et al., 2017).

4 What Health Outcomes Do Wildland Firefighters Face?

Firefighters face many risks that can cause immediate or long-term consequences on their health and well-being. World Fire Statistics show that, between 2016 and 2020, an annual mean of almost 70,000 firefighters got injured, and 86 firefighters lose their lives during firefighting occurrences (CTIF, 2022). Cardiac events have been considered an important cause of death among firefighters during fire ground operations (Kales et al., 2003; Koopmans et al., 2022). In the USA, for example, sudden cardiac death (usually heart attacks) continues to be the leading cause of death among firefighters on duty (Fig. 2).

These sudden cardiac events have been associated with different factors such as heat stress, exertion, dehydration, shift work, and stress (Guidotti, 2014), as well as with risk factors such as hypertension, diabetes, cholesterol, and lack of physical fitness (Kales et al., 2007). In addition, mortality rates have been largely associated with aviation-related incidents, entrapments, and vehicle collisions (Butler et al., 2017). The last is a common cause of firefighter mortality worldwide and is often related to excessive speed and the misuse of seat belts (International Association of Fire Chiefs, 2008). Besides these immediate negative health outcomes (e.g., life loss, injuries), the real impact on health is much higher, like an iceberg where we only can see the tip and not the whole bulk.

Firefighters are exposed to a wide range of harmful substances, many of them classified by the International Agency for Research on Cancer (IARC) as known (e.g., benzene, benzo[a]pyrene, formaldehyde, and PM_{2.5}) or probable/possible (e.g., acetaldehyde, benzofuran, ethylbenzene, furan, black carbon, and styrene) human carcinogens (IARC, 2010). There are several studies worldwide indicating an increased risk of different cancers among firefighters (Jalilian et al., 2019; LeMasters et al., 2006; Pukkala et al., 2013) such as melanoma, leukemia (Tsai et al., 2015), colon, rectum, testis, thyroid (Jalilian et al., 2019), laryngeal, and hypopharyngeal

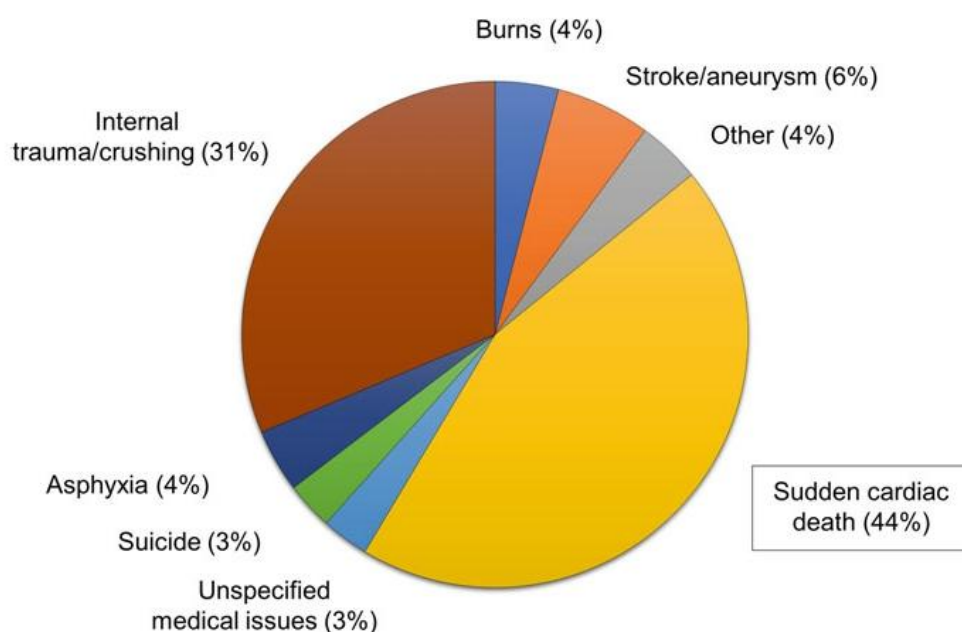


Fig. 2 US firefighters' deaths while on duty: 2021 (Adapted from Fahy et al. [Fahy & Petrillo, 2022])

(Zhao et al., 2020). In this line, in June 2022, occupational exposure as a firefighter was classified by IARC as carcinogenic to humans (Group 1) based on “sufficient” evidence for cancer (bladder and mesothelioma) in humans (Demers et al., 2022).

Not surprisingly wildland firefighting has been associated with other long-term adverse health outcomes such as respiratory (e.g., asthma, chronic obstructive pulmonary disease) (Greven, 2011) and cardiovascular outcomes (e.g., heart attacks, arrhythmias) (Andersen et al., 2017; Fahs et al., 2011; Navarro et al., 2019; Pedersen et al., 2018; Soteriades et al., 2011). The prevalence of self-reported health effects was found to be directly correlated with years of firefighting, and individuals with long careers who have higher chances to develop adverse cardiovascular outcomes (Semmens et al., 2016). According to the National Fire Protection Association, sudden death from a heart event is the most common cause of mortality among firefighters accounting for 40–50% of deaths annually (Fahy, 2005); long-term exposures to cardio-toxic compounds, such as carbon monoxide or PM from fire smoke, have been considered an important factor for vascular diseases (Du et al., 2016).

Other long-term health effects related to occupational firefighting exposure have been described (Jahnke et al., 2018). Previous studies showed that nearly a quarter of US female firefighters' first pregnancy ended in miscarriage (higher than the 10% verified in the general US population) (Jahnke et al., 2018). In addition, the high levels of stress and traumatic situations contribute to firefighters' vulnerability to mental health problems (Walker et al., 2016), leading to substance abuse (Carey et al., 2011), depression (Kimbrel et al., 2011), post-traumatic disorder (Chen et al.,

2007), sleep disturbance (Vargas de Barros et al., 2013), and even suicidal behaviors (Boffa et al., 2017).

5 Better Health for Firefighters: Looking at the Preventive Measures

Benjamin Franklin, founder of the first volunteer fire brigade in the USA, once said “An ounce of prevention is worth a pound of cure” (International Association of Fire Chiefs, 2008). So, the way firefighters can prevent occupational risk in their activity is the responsibility of multiple parts. Compared with some exposures encountered during firefighting, exposures at the fire station may be more easily modified through changes in systems and protocols, representing potential useful intervention targets. Fire stations should have a set of standard guidelines that incorporate safe practices and policies to protect firefighters’ safety and health. All staff should be trained in implementing such procedures and, most importantly, guarantee that firefighters are aware of the occupational risks that they face and the importance of adopting preventive measures to reduce or eliminate occupational risks.

Firefighters must know how to correctly use PPE. Equally important is guaranteeing proper maintenance through regular cleaning and inspection of PPE, repairing or replacing it when necessary. During fire suppression activities in the field, firefighters should avoid eating, drinking, or smoking with unwashed hands (Stec et al., 2020). When necessary, they must do so in a suitable environment with washed hands (e.g., water and soap or cleansing wipes) so that contaminants do not enter via ingestion (Magnusson & Hultman, 2015).

Decontamination procedures after exposure to fire pollutants are extremely important (Fig. 3), including decontamination of firefighting personnel as well as of the contaminated PPE/equipment/vehicles (Magnusson & Hultman, 2015). The decontamination procedure is a sequential set of steps that must be followed to ensure a successful decontamination process.

The contaminated PPE and equipment should be cleaned *in loco* wherever possible; if not possible, contaminated PPE should be stored in proper containers/bags and transported separately from firefighters, i.e., outside the vehicle cab (Stec et al., 2020). Firefighters should change to clean and dry clothes before returning to the fire station to reduce skin exposure to contaminants (Stec et al., 2020), or else take off the undergarment as soon as possible on return to the fire station. After fire suppression activities, it is crucial to wash exposed skin areas (e.g., hands, neck, face) with soap and water. When this is not practical, firefighters may use cleansing wipes on exposed skin parts (e.g., neck and hands) to reduce the number of contaminants (e.g., PAHs) (Fent et al., 2017). However, it is important to be aware that some particles and other contaminants might remain on the skin, and as long as contaminant is present on the skin, more time is available to be absorbed (Fent et al., 2014, 2020;

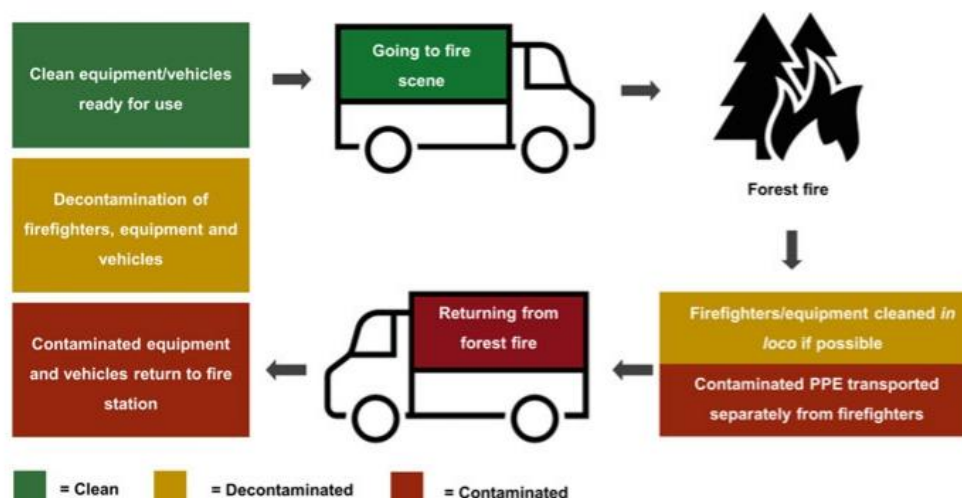


Fig. 3 The (de)contamination cycle (Adapted from Swedish Civil Contingencies Agency [Magnusson & Hultman, 2015])

Keir et al., 2017). As such, showering with soap and shampoo should occur as soon as possible when returning to the fire station.

It is of utmost importance to control the spread of harmful pollutants on return to the fire station through the designation of “contamination zones” and “clean zones.” Personal decontamination should be a priority when returning to the fire station from a fire; thus having available showers in the facilities is crucial. Contaminated PPE/clothing must be cleaned/laundared after every fire event, if possible, in a special designated area “contaminated zone” of the fire station to prevent cross-contamination. During these procedures, it is important to protect areas of exposed skin (e.g., gloves) and airways (e.g., face mask) (Stec et al., 2020). Besides PPE, work tools and vehicles should also be cleaned and decontaminated regularly (Stec et al., 2020).

Construction of fire stations must be designed considering a specific layout (based on the circuit of “contaminated” and “clean” areas) and proper engineering measures to guarantee a clean airflow within spaces. The implementation of efficient ventilation systems must be a concern, particularly in the areas of the fire station where contaminated material is handled. The air quality of fire stations should be regularly monitored to guarantee safe exposure levels to air pollutants (Slezakova et al., 2022). Preventing contamination will keep firefighters and other fire station personnel protected from smoke-related contaminants.

Firefighting is a highly physically demanding activity that requires a good fitness level including aerobic fitness, anaerobic capacity, muscular strength, and endurance. The development and implementation of well-designed fitness programs that encourage physical activity and a balanced diet (e.g., water, fruits, vegetables, whole grains, and low-fat foods) are critical for the promotion of health and well-being among these individuals. In addition, regular health screening should

be provided to firefighters to early detect any adverse health events. Understanding the impacts of wildland fires on firefighters' health can help to design mitigation strategies and efficient policies to protect their health.

6 Taking Care of Firefighters' Health in a New Era of Forest Fires—The Portuguese Reality

There are around 27.000 firefighters in Portugal (81% males and 19% females), distributed by 465 fire stations, of which 63% are volunteers and 37% are professional, with ages comprised mostly between 26 and 50 years and the majority with 12th grade of scholarly (INE 2020). A recent study found that 80% of a group of Portuguese wildland firefighters reported being exposed to smoke, gases, and particles during their activities (Esteves et al., 2022b); this finding is particularly concerning due to the long careers (average of 16 years) and the long periods of work that were described (i.e., the majority claimed to work in firefighter activities more than 10 h/day) (Esteves et al., 2022b).

Portugal is one of the European countries most prone to wildfires (Beighley & Hyde, 2018). On average 3% of Portuguese forests burn annually (Schleussner et al., 2019), corresponding to around 100.000 hectares. However, this trend has been increasing through the years (Schleussner et al., 2019); from 2008 to 2017, for example, the annual burned area varied from 18.000 hectares to 500.000 hectares (Schleussner et al., 2019).

The evident demographic shifting of the population moving from rural to urban areas, the highly fragmented and unmanaged private forested lands, the typically ever-green and drought-resistant vegetation, the high number of human-cause ignitions (negligence or intentional), and climate changes (high temperatures and decreasing precipitation levels) all contribute for the increased risk of forest fires (Beighley & Hyde, 2018; Carmo et al., 2011; Nunes et al., 2005). In December 2019, the European Council recognized that Portugal is one of the European countries most affected by climate change (European Commission, 2019). Portugal has already experienced severe heat waves, storms, and droughts (Schleussner et al., 2019). During the extreme wildfires of 2017, a record of burnt areas was registered in Portugal (500.000 hectares) and 120 human lives were taken (Turco et al., 2019), including firefighters' lives. European Union Joint Research Center (EU-IJR) forecasts an increase in forest fire danger, particularly noticeable in the Southern countries including Portugal (Fig. 4).

On the contrary, the number of Portuguese firefighters (professional and volunteer) has been decreasing through the years registering a drop of 33% in just a decade (Fig. 5) (Beighley & Hyde, 2018; PORDATA, 2020).

Firefighters are crucial human tools in forest fire defense. Thus, efforts should be done to retain experienced firefighters and to attract younger people to this workforce creating attractive remuneration conditions (e.g., higher wages) and improving

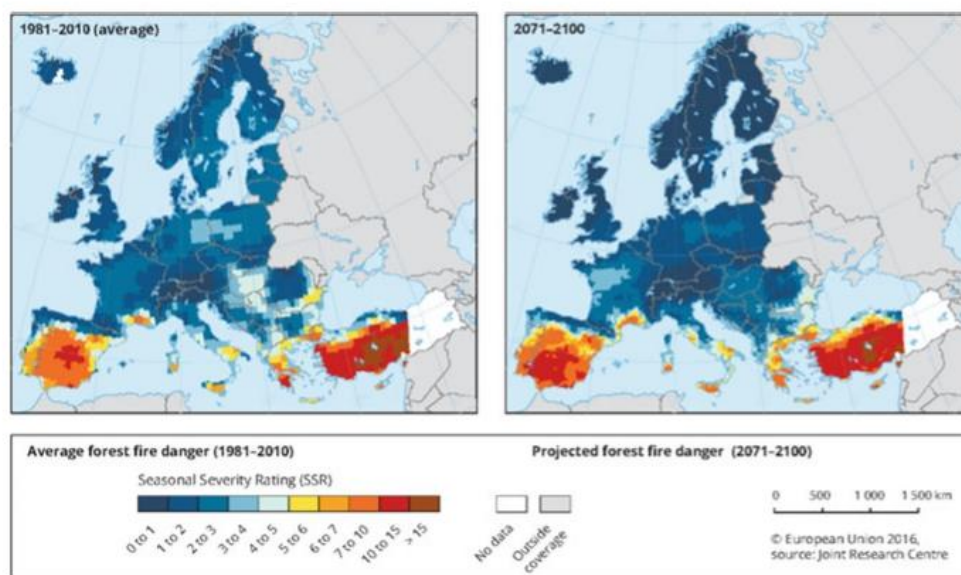


Fig. 4 Historical and future trends of forest fire danger in Europe (Retrieved from EU-IJR [European Commission, 2019])

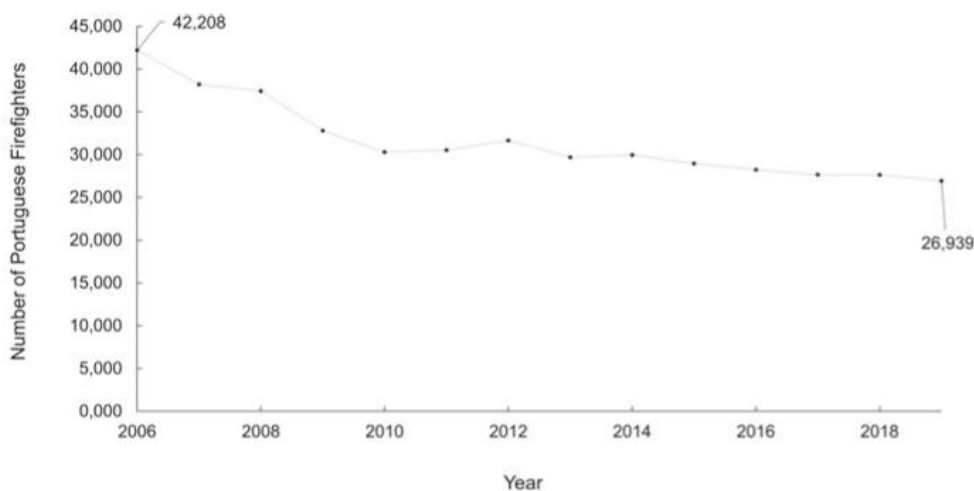


Fig. 5 Number of Portuguese firefighters over the years (Adapted from PORDATA [PORDATA, 2020])

health and safety conditions (Beighley & Hyde, 2018). Surveillance based on monitoring programs (biological and environmental) may be a possible solution to identify potential occupational risks and their impact on firefighters' health.

7 Conclusions and Recommendations

The implementation of a comprehensive set of preventive measures must be a priority to reduce fire occurrences and resulting damages. This is particularly relevant because, during the past decades, we have witnessed a rise in forest fires (i.e., number and severity) driven by unusually high temperatures. Government is a vital stakeholder in better structuring forest policies and providing the resources needed to prevent and deal with forest fires. Prevention efforts across different scales are essential for the success of a forest fire prevention system. Prescribed burning, fuel breaks, water reservoirs, forest access roads, meteorological warnings, and information campaigns to promote the individual responsibility of citizens are examples of some important and effective preventive measures. In parallel, it is crucial to strengthen the fire response structure so that forest fires can be rapidly detected and suppressed at early stages, preventing human and ecological loss/threats. Having healthy and well-prepared firefighters is essential to save human lives and preventing forest fires from escalating. Thus, efforts must be done to implement appropriate practices and policies to promote health and safety in this workforce.

Acknowledgements This work received financial support from the project PCIF/SSO/0017/2018 by the Fundação para a Ciência e a Tecnologia (FCT), Ministério da Ciência, Tecnologia e Ensino Superior (MCTES) through national funds. Filipa Esteves, the recipient of the PhD grant UI/BD/150783/2020, is supported by FCT and by the European Social Fund (ESF). Joana Madureira, under the grant SFRH/BPD/115112/2016, is supported by FCT and by ESF, through Programa Operacional Capital Humano (POCH). The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support by national funds in the scope of projects UIDB/04750/2020 and LA/P/0064/2020.

References

- Alagna, M. (2003). *The Great Fire of London of 1666*. The Rosen Publishing Group, Inc.
- Andersen, M. H. G., Saber, A. T., Pedersen, P. B., Loft, S., Hansen, Å. M., Koponen, I. K., Pedersen, J. E., Ebbenhøj, N., Nørskov, E.-C., & Clausen, P. A. (2017). Cardiovascular health effects following exposure of human volunteers during fire extinction exercises. *Environmental Health*, 16(1), 1–9.
- Austin, C. (2008). *Wildland Firefighter Health Risks and Respiratory Protection (Report R-572)*. Montreal, Quebec, Canada: IRSST (Institut de recherche Robert-Sauvé en santé et en sécurité du travail).
- Banks, A. P., Thai, P., Engelsman, M., Wang, X., Osorio, A. F., & Mueller, J. F. (2021). Characterising the exposure of Australian firefighters to polycyclic aromatic hydrocarbons generated in simulated compartment fires. *International Journal of Hygiene and Environmental Health*, 231, 113637.
- Barker, R. L. (2005). *A Review of gaps and limitations in test methods for first responder protective clothing and equipment: a final report presented to National Personal Protection Technology Laboratory, National Institute for Occupational Safety and Health (NIOSH)*.

- Beighley, M., & Hyde, A. (2018). Portugal wildfire management in a new era assessing fire risks, resources and reforms. Centro de Estudos Florestais-Instituto Superior de Agronomia/ Universidade de Lisboa.
- Boffa, J. W., Stanley, I. H., Hom, M. A., Norr, A. M., Joiner, T. E., & Schmidt, N. B. (2017). PTSD symptoms and suicidal thoughts and behaviors among firefighters. *Journal of Psychiatric Research*, 84, 277–283.
- Bond, W. J., Woodward, F. I., & Midgley, G. F. (2005). The global distribution of ecosystems in a world without fire. *New Phytologist*, 165(2), 525–538.
- Britton, C., Lynch, C. F., Ramirez, M., Torner, J., Buresh, C., & Peek-Asa, C. (2013). Epidemiology of injuries to wildland firefighters. *The American Journal of Emergency Medicine*, 31(2), 339–345.
- Broyles, G., Butler, C. R., & Kardous, C. A. (2017). Noise exposure among federal wildland fire fighters. *The Journal of the Acoustical Society of America*, 141(2), EL177–EL183.
- Butgereit, L., Moonsamy, S., Thomson, T., Van Zyl, T., & McFerren, G. (2014). *Fire hazard notifications via Satellite, Twitter, Citizen Reports, and Android Apps*.
- Butler, C., Marsh, S., Domitrovich, J. W., & Helmkamp, J. (2017). Wildland firefighter deaths in the United States: A comparison of existing surveillance systems. *Journal of Occupational and Environmental Hygiene*, 14(4), 258–270.
- Carballo-Leyenda, B., Villa, J. G., López-Satué, J., Collado, P. S., & Rodríguez-Marroyo, J. A. (2018). Fractional contribution of wildland firefighters' personal protective equipment on physiological strain. *Frontiers in Physiology*, 9, 1139.
- Carey, M. G., Al-Zaiti, S. S., Dean, G. E., Sessanna, L., & Finnell, D. S. (2011). Sleep problems, depression, substance use, social bonding, and quality of life in professional firefighters. *Journal of Occupational and Environmental Medicine/American College of Occupational and Environmental Medicine*, 53(8), 928.
- Carmo, M., Moreira, F., Casimiro, P., & Vaz, P. (2011). Land use and topography influences on wildfire occurrence in northern Portugal. *Landscape and Urban Planning*, 100(1–2), 169–176.
- Chen, Y.-S., Chen, M.-C., Chou, F.H.-C., Sun, F.-C., Chen, P.-C., Tsai, K.-Y., & Chao, S.-S. (2007). The relationship between quality of life and posttraumatic stress disorder or major depression for firefighters in Kaohsiung, Taiwan. *Quality of Life Research*, 16(8), 1289–1297.
- International Association of Fire Chiefs. (2008). *Fundamentals of fire fighter skills*. Jones & Bartlett Publishers.
- European Commission. (2019). Speech by President von der Leyen in the Plenary of the European Parliament at the debate on the conclusions of the European Council meeting of 12 and 13 December 2019. https://ec.europa.eu/commission/presscorner/detail/en/speech_19_6802
- CTIF. (2022). World Fire Statistics. Center of Fire Statistics, Report n° 27. https://www.ctif.org/sites/default/files/2022-08/CTIF_Report27_ESG.pdf
- Demers, P. A., DeMarini, D. M., Fent, K. W., Glass, D. C., Hansen, J., Adetona, O., Andersen, M. H., Freeman, L. E. B., Caban-Martinez, A. J., & Daniels, R. D. (2022). *Carcinogenicity of occupational exposure as a firefighter*. The Lancet Oncology.
- Du, Y., Xu, X., Chu, M., Guo, Y., & Wang, J. (2016). Air particulate matter and cardiovascular disease: The epidemiological, biomedical and clinical evidence. *Journal of Thoracic Disease*, 8(1), E8.
- Esteves, F., Madureira, J., Teixeira, J. P., & Costa, S. (2022a). The role of biomarkers in the assessment of potential health risks of wildland firefighters' occupational exposure: An overview. In S. B. M. Marta Madalena Marques de Oliveira, Francisca Pinto Lisboa Martins Rodrigues (Ed.), *Essential Guide to Occupational Exposure*. Nova Science Publishers, Inc. <https://doi.org/10.52305/VGMC6738>
- Esteves, F., Madureira, J., Teixeira, J. P., & Costa, S. (2022b). The firefighters on the frontline of forest fires – preliminary characterization of Bio4FOX study population. In R. L. Viegas X (Ed.), *Advances in Forest Fire Research* (pp. 1330–1334). Universidade de Coimbra. https://doi.org/10.14195/978-989-26-2298-9_201

- Fahs, C. A., Yan, H., Ranadive, S., Rossow, L. M., Agiovlasis, S., Echols, G., Smith, D., Horn, G. P., Rowland, T., & Lane, A. (2011). Acute effects of firefighting on arterial stiffness and blood flow. *Vascular Medicine*, 16(2), 113–118.
- Fahy, R., & Petrillo, J. T. (2022). *Firefighter Fatalities in the US in 2021*.
- Fahy, R. F. (2005). US firefighter fatalities due to sudden cardiac death, 1995–2004. National Fire Protection Association Quincy, MA.
- Fent, K. W., Alexander, B., Roberts, J., Robertson, S., Toennis, C., Sammons, D., Bertke, S., Kerber, S., Smith, D., & Horn, G. (2017). Contamination of firefighter personal protective equipment and skin and the effectiveness of decontamination procedures. *Journal of Occupational and Environmental Hygiene*, 14(10), 801–814.
- Fent, K. W., Eisenberg, J., Snawder, J., Sammons, D., Pleil, J. D., Stiegel, M. A., Mueller, C., Horn, G. P., & Dalton, J. (2014). Systemic exposure to PAHs and benzene in firefighters suppressing controlled structure fires. *Annals of Occupational Hygiene*, 58(7), 830–845.
- Fent, K. W., Toennis, C., Sammons, D., Robertson, S., Bertke, S., Calafat, A. M., Pleil, J. D., Wallace, M. A. G., Kerber, S., & Smith, D. (2020). Firefighters' absorption of PAHs and VOCs during controlled residential fires by job assignment and fire attack tactic. *Journal of Exposure Science & Environmental Epidemiology*, 30(2), 338–349.
- Field, C. B., & Barros, V. R. (2014). *Climate change 2014—Impacts, adaptation and vulnerability: Regional aspects*. Cambridge University Press.
- Flannigan, M. D., Amiro, B. D., Logan, K. A., Stocks, B. J., & Wotton, B. M. (2006, July 1). Forest fires and climate change in the 21st century. *Mitigation and Adaptation Strategies for Global Change*, 11(4), 847–859. <https://doi.org/10.1007/s11027-005-9020-7>
- Greven, F. (2011). *Respiratory effects of fire smoke exposure in firefighters and the general population*. Utrecht University.
- Guidotti, T. (2014). *Health risks and occupation as a firefighter*. Medical Advisory Services.
- Heus, R. (2018). *Exposure to smoke. An overview report of the studies to exposure*.
- Hirschberger, P. (2016). Forests ablaze: Causes and effects of global forest fires. WWF. <https://www.wwf.de/fileadmin/fm-wwf/Publikationen-PDF/WWF-Study-Forests-Ablaze.pdf> [Verified 26 March 2020].
- Horn, G. P., Fent, K. W., Kerber, S., & Smith, D. L. (2022). Hierarchy of contamination control in the fire service: Review of exposure control options to reduce cancer risk. *Journal of Occupational and Environmental Hygiene*, 19(9), 538–557.
- IARC. (2010). *Painting, firefighting, and shiftwork* (Vol. 98). IARC Press, International Agency for Research on Cancer.
- Jahnke, S. A., Poston, W. S., Jitnarin, N., & Haddock, C. K. (2018). Maternal and child health among female firefighters in the US. *Maternal and Child Health Journal*, 22(6), 922–931.
- Jalilian, H., Ziaei, M., Weiderpass, E., Rueegg, C. S., Khosravi, Y., & Kjaerheim, K. (2019). Cancer incidence and mortality among firefighters. *International Journal of Cancer*, 145(10), 2639–2646.
- Kales, S. N., Soteriades, E. S., Christophi, C. A., & Christiani, D. C. (2007). Emergency duties and deaths from heart disease among firefighters in the United States. *New England Journal of Medicine*, 356(12), 1207–1215.
- Kales, S. N., Soteriades, E. S., Christoudias, S. G., & Christiani, D. C. (2003). Firefighters and on-duty deaths from coronary heart disease: A case control study. *Environmental Health*, 2(1), 1–13.
- Keir, J. L., Akhtar, U. S., Matschke, D. M., Kirkham, T. L., Chan, H. M., Ayotte, P., White, P. A., & Blais, J. M. (2017). Elevated exposures to polycyclic aromatic hydrocarbons and other organic mutagens in Ottawa firefighters participating in emergency, on-shift fire suppression. *Environmental Science & Technology*, 51(21), 12745–12755.
- Kimbrel, N. A., Steffen, L. E., Meyer, E. C., Kruse, M. I., Knight, J. A., Zimering, R. T., & Gulliver, S. B. (2011). A revised measure of occupational stress for firefighters: Psychometric properties and relationship to posttraumatic stress disorder, depression, and substance abuse. *Psychological Services*, 8(4), 294.

- Koopmans, E., Cornish, K., Fyfe, T. M., Bailey, K., & Pelletier, C. A. (2022). Health risks and mitigation strategies from occupational exposure to wildland fire: A scoping review. *Journal of Occupational Medicine and Toxicology*, 17(1), 1–17.
- LeMasters, G. K., Genaïdy, A. M., Succop, P., Deddens, J., Sobeih, T., Barrera-Viruet, H., Dunning, K., & Lockey, J. (2006). Cancer risk among firefighters: A review and meta-analysis of 32 studies. *Journal of Occupational and Environmental Medicine*, 48(11), 1189–1202.
- Magnusson, S., & Hultman, D. (2015). *Healthy Firefighters-The Skellefteå Model improves the work environment*. Swedish Civil Contingencies Agency.
- Moreira, F., Vaz, P., Catry, F., & Silva, J. S. (2009). Regional variations in wildfire susceptibility of land-cover types in Portugal: Implications for landscape management to minimize fire hazard. *International Journal of Wildland Fire*, 18(5), 563–574.
- Naeher, L. P., Brauer, M., Lipsett, M., Zelikoff, J. T., Simpson, C. D., Koenig, J. Q., & Smith, K. R. (2007). Woodsmoke health effects: A review. *Inhalation Toxicology*, 19(1), 67–106.
- Navarro, K. M., Kleinman, M. T., Mackay, C. E., Reinhardt, T. E., Balmes, J. R., Broyles, G. A., Ottmar, R. D., Naher, L. P., & Domitrovich, J. W. (2019). Wildland firefighter smoke exposure and risk of lung cancer and cardiovascular disease mortality. *Environmental Research*, 173, 462–468.
- Nunes, M., Vasconcelos, M. J., Pereira, J., Dasgupta, N., Alldredge, R. J., & Rego, F. C. (2005). Land cover type and fire in Portugal: Do fires burn land cover selectively? *Landscape Ecology*, 20(6), 661–673.
- Oliveira, M., Slezakova, K., Alves, M. J., Fernandes, A., Teixeira, J. P., Delerue-Matos, C., & do Carmo Pereira, M., & Morais, S. (2017). Polycyclic aromatic hydrocarbons at fire stations: Firefighters' exposure monitoring and biomonitoring, and assessment of the contribution to total internal dose. *Journal of Hazardous Materials*, 323, 184–194.
- Pedersen, J. E., Petersen, K. U., Ebbenhøj, N. E., Bonde, J. P., & Hansen, J. (2018). Incidence of cardiovascular disease in a historical cohort of Danish firefighters. *Occupational and Environmental Medicine*, 75(5), 337–343.
- PORDATA. (2020). Bombeiros: Quantos bombeiros há profissionais ou voluntários? <https://www.pordata.pt/Portugal/Bombeiros-1188>
- Pukkala, E., Jan Ivar, M., Elisabete, W., Kristina, K., Elsebeth, L., Laufey, T., Pär, S., & Paul A. D. (2013). Cancer incidence among firefighters: 45 years of follow-up in five Nordic countries. *Occupational and Environmental Medicine*, oemed-2013–101803.
- Radeloff, V. C., Hammer, R. B., Stewart, S. I., Fried, J. S., Holcomb, S. S., & McKeefry, J. F. (2005). The wildland–urban interface in the United States. *Ecological Applications*, 15(3), 799–805.
- Rogula-Kozłowska, W., Bralewska, K., & Jureczko, I. (2020). BTEXS Concentrations and Exposure Assessment in a Fire Station. *Atmosphere*, 11(5), 470.
- Ruby, B. C., Shriver, T. C., Zderic, T. W., Sharkey, B. J., Burks, C., & Tysk, S. (2002). Total energy expenditure during arduous wildfire suppression. *Medicine and Science in Sports and Exercise*, 34(6), 1048–1054.
- San-Miguel-Ayaz, J., Durrant, T., Boca, R., Libertà, G., Branco, A., De Rigo, D., Ferrari, D., Maianti, P., Artés Vivancos, T., & Costa, H. (2018). Forest Fires in Europe, Middle East and North Africa 2017. Publications Officer of the European Union. <https://doi.org/10.2760/663443>
- Schleussner, C. F., Menke, I., Theokritoff, E., van Maanen, N., & Lanson, A. (2019). Climate impacts in Portugal. In: *Climate Analytics*.
- Semmens, E. O., Domitrovich, J., Conway, K., & Noonan, C. W. (2016). A cross-sectional survey of occupational history as a wildland firefighter and health. *American Journal of Industrial Medicine*, 59(4), 330–335.
- Slezakova, K., Esteves, F., Vaz, J., Alves, M., Madureira, J., Costa, S., Fernandes, A., Teixeira, J., Morais, S., & Pereira, M. (2022). Occupational Exposure of Firefighters in Non-fire Settings. In *Occupational and Environmental Safety and Health IV* (pp. 79–88). Springer.
- Smith, D. L., Manning, T., & Petruzzello, S. J. (2001). Effect of strenuous live-fire drills on cardiovascular and psychological responses of recruit firefighters. *Ergonomics*, 44(3), 244–254.

- Šomšák, L., Dlapa, P., Kollár, J., Kubíček, F., Šimonovič, V., Janitor, A., Kanka, R., & Šimkovic, I. (2009). Fire impact on the secondary pine forest and soil in the Borská nížina lowland (SW Slovakia). *Ekologia (bratislava)*, 28(1), 52–65.
- Soteriades, E. S., Smith, D. L., Tsismenakis, A. J., Baur, D. M., & Kales, S. N. (2011). Cardiovascular disease in US firefighters: A systematic review. *Cardiology in Review*, 19(4), 202–215.
- Sparer, E. H., Prendergast, D., Apell, J. N., Bartzak, M. R., Wagner, G. R., Adamkiewicz, G., Hart, J. E., & Sorensen, G. (2017). Assessment of ambient exposures firefighters encounter while at the fire station: An exploratory study. *Journal of Occupational and Environmental Medicine*, 59(10), 1017.
- Stec, A., Wolffe, T., & Clinton, A. (2020). Minimising firefighters' exposure to toxic fire effluents. Interim Best Practice Report. University of Central Lancashire.
- Tsai, R. J., Luckhaupt, S. E., Schumacher, P., Cress, R. D., Deapen, D. M., & Calvert, G. M. (2015). Risk of cancer among firefighters in California, 1988–2007. *American Journal of Industrial Medicine*, 58(7), 715–729.
- Turco, M., Jerez, S., Augusto, S., Tarín-Carrasco, P., Ratola, N., Jiménez-Guerrero, P., & Trigo, R. M. (2019). Climate drivers of the 2017 devastating fires in Portugal. *Scientific Reports*, 9(1), 1–8.
- Vargas de Barros, V., Martins, L. F., Saitz, R., Bastos, R. R., & Ronzani, T. M. (2013). Mental health conditions, individual and job characteristics and sleep disturbances among firefighters. *Journal of Health Psychology*, 18(3), 350–358.
- Walker, A., McKune, A., Ferguson, S., Pyne, D. B., & Rattray, B. (2016). Chronic occupational exposures can influence the rate of PTSD and depressive disorders in first responders and military personnel. *Extreme Physiology & Medicine*, 5(1), 1–12.
- Westerling, A. L., Hidalgo, H. G., Cayan, D. R., & Swetnam, T. W. (2006). Warming and earlier spring increase western US forest wildfire activity. *Science*, 313(5789), 940–943.
- Wingfors, H., Nyholm, J. R., Magnusson, R., & Wijkmark, C. H. (2018). Impact of fire suit ensembles on firefighter PAH exposures as assessed by skin deposition and urinary biomarkers. *Annals of Work Exposures and Health*, 62(2), 221–231.
- Withen, P. (2015). Climate change and wildland firefighter health and safety. *New Solutions: A Journal of Environmental and Occupational Health Policy*, 24(4), 577–584.
- Zhao, G., Erazo, B., Ronda, E., Brocal, F., & Regidor, E. (2020). Mortality Among Firefighters in Spain: 10 Years of Follow-up. *Annals of Work Exposures and Health*, 64(6), 614–621.

Open Access This chapter is licensed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license and indicate if changes were made.

The images or other third party material in this chapter are included in the chapter's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the chapter's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.



Appendix II – Published Book Chapter entitled “The Role of Biomarkers in the Assessment of Potential Health Risks of Wildland Firefighters’ Occupational Exposure: An Overview”

Reprinted with kind permission of Nova Science Publishers, Inc., In an “Essential Guide to Occupational Exposure”, “The Role of Biomarkers in the Assessment of Potential Health Risks of Wildland Firefighters’ Occupational Exposure: An Overview.”, pp47-79, copyright © 2022. Filipa Esteves, Joana Madureira, João Paulo Teixeira, Solange Costa.

Chapter 3

The Role of Biomarkers in the Assessment of Potential Health Risks of Wildland Firefighters’ Occupational Exposure: An Overview

**Filipa Esteves^{1,2,3,4,*}, Joana Madureira^{1,2,3},
João Paulo Teixeira^{1,2,3} and Solange Costa^{1,2,3}**

¹Environmental Health Department, National Institute of Health, Porto, Portugal

²EPIUnit - Institute of Public Health, University of Porto, Porto, Portugal

³Laboratory for Integrative and Translational Research in Population Health (ITR),
Porto, Portugal

⁴Department of Public Health and Forensic Sciences, and Medical School,
Faculty of Medicine, University of Porto, Porto, Portugal

Abstract

Worldwide, wildfires are among the most common forms of natural disasters. In recent decades, these events have become more frequent and burn more intensely and widely than they have in the past fueled by climate-related changes. The proximity of firefighters to fires exposes them to high levels of toxic compounds, making this occupation one of the most dangerous in the world. The International Agency for Research on Cancer classified occupational exposure as a firefighter as possibly carcinogenic to humans. However, little is known about the biological mechanisms related with wildland firefighters’ occupational exposure. Woodsmoke is a complex mixture of gases and particles with the potential to cause short- and long-term deleterious health effects.

* Corresponding Author’s Email: filipa.esteves3@hotmail.com.

In: An Essential Guide to Occupational Exposure
Editors: Marta Madalena Marques de Oliveira et al.
ISBN: 978-1-68507-819-5
© 2022 Nova Science Publishers, Inc.

Complimentary Contributor Copy

Previous studies have shown that the inhalation of woodsmoke promotes systemic oxidative stress, higher levels of molecular and inflammatory biomarkers and excretion of exposure-related biometabolites. The exposure to smoke has been related to the incidence of cardiorespiratory diseases and with the increased risk of developing site-specific cancers. In addition to inhalation exposure, transdermal absorption has been postulated as an important route of exposure for certain smoke compounds. The integration of exposure data with biological monitoring information is of great importance to predict risks and prevent diseases related to hazardous exposures. This chapter resumes the existing knowledge of the role of biomarkers to assess potential health risks, in particular the most recent findings related to wildland firefighters' biomonitoring. Principal strengths and limitations identified in this field will also be discussed. This overview reveals the need for further research in this area, as well as the importance of public/decision-makers awareness for the health protection of this professionals. Surveillance (bio)monitoring, along with policies and recommendations may constitute an important tool to prevent short- and long-term adverse health effects related to occupational firefighting.

Keywords: biomarkers, wildland firefighters, biomonitoring, occupational exposure, air pollution

Introduction

Global Trends in Wildfires

Fire has always made part of our history. Human ancestors started to use fire intentionally around 1.5 million years ago for hunting or cooking, helping their survival [1]. Fire has different beneficial purposes but also has the strength to destroy ecosystems and communities bringing social, ecological, and economic serious consequences. Wildfires are defined as any type of uncontrolled burning of vegetation [2] that depend on different factors such as temperature, water deficit, soil moisture, vegetation, shrubs, and other potential fuels [3, 4]. Globally, only 4% of the forest fires occurs due to natural causes, e.g., high temperatures, storms, or lightning, being the most part caused by direct (e.g., seasonal burning, slash-and-burn activities, or arson) or indirect human action (e.g., discarded cigarettes or bonfires) [5].

Wildfires' behavior is changeable among the different areas of the globe and is mostly characterized by the amount and layout of vegetation, the

Complimentary Contributor Copy

topography, and the weather conditions [6]. The most affected areas have environmental characteristics that promote/exacerbate wildfire occurrence, such as the coniferous forests of California, the eucalyptus forests of Australia, the African savannahs, the dry forests of South Asia and the Mediterranean region [6]. By the early 20th century, the improvement of firefighting activities together with fire prevention actions, fire suppression policies, and some changes in agricultural practices, contributed to the decrease of the burned area in temperate ecosystems (e.g., temperate forests of Europe) [7, 8]. However, during the late 90's, occurred not only an increase of burned area in tropical forests (i.e., deforestation activities for the expansion of agriculture), but also an increase in the burned area in some temperate forests (e.g., boreal forest in North America and Europe) [7]. In United States, for example, the annually burned area caused by wildfires increased from about 1 hectare (1985-2000) to 2 million hectares (2001-2016) [9]. In Europe about 65.000 wildland fires occurs every year, burning approximately 0.5 million hectares of wildland and forest areas [10]. Most of the affected area, over 85%, is in the European Mediterranean region [10]. With a forest area of 3.2 million hectares Portugal is one of the countries more affected by wildfires [11]; a previous report shown that in 30 years more than 3.8 million hectares were consumed by wildfires [11].

As previously mentioned, weather and climate – temperature, precipitation, atmospheric moisture, wind - are important aspects of wildfire activity. Climate has been warming in the last decades with prolonged periods of dry weather. Subsequently, higher drought periods have increased the likelihood of fire among dry climate forests [12-14]. Climate-mediated changes have been associated to changes in the frequency, size, and/or severity of wildfires [8]. Wildfire activity and this type of phenomenon's are predicted to increase in several regions of the globe due to the progressive increase of temperature and a pronounced decrease in precipitation patterns caused by climate change [15]. The inevitability of global warming and its strong direct or indirect tie to wildfires, arises the importance to develop tools that may help in the wildfire occurrence prediction and combat, as well as protecting firefighters' health.

Wildland Fire: A Major Source of Pollutants Exposure

Wildfires occurrence depends on three elements: heat or an ignition source, oxygen, and fuel (e.g., biomass). Wood, the predominant biomass in forests,

Complimentary Contributor Copy

is mainly composed of two polymers, cellulose, and lignin, and by small amounts of low-molecular-weight compounds such as resins, waxes, sugars, and inorganic salts [16]. During the combustion, pyrolysis occurs, and the polymers break apart producing a variety of solid, liquid, and volatile compounds. The incomplete combustion of forest fuel results in a very complex mixture of gaseous and particulate matter (PM) called smoke [17]. Combustion of biomass proceeds through a sequence of phases: ignition, flaming and smoldering, each with different physical and chemical processes resulting in different emissions [18]. The composition and amount of these emissions are changeable according to different factors e.g., oxygen availability, temperature, and composition of the fuel [18]. Thus, in the initial stage of a wildfire, a complex mixture of products, including volatile organic compounds (VOCs), is released forming a flammable mixture when in contact with air [19]. When this mixture ignites, flaming combustion occurs [19]. Flaming phase (1127°C) is characterized by the production of most of the black (or elemental) carbon particles and by the emission of highly oxidized gases such as carbon dioxide (CO₂), nitrogen oxide (NO_x) and sulphur dioxide (SO₂) [20, 21]. Smoldering (527°C - 727°C) is a slow, low temperature, flameless form of combustion, that yields a substantially higher conversion of fuel to toxic compounds [19]. During this stage, carbon monoxide (CO), amines, nitriles, methyl halides (i.e., CH₃Cl, CH₃Br, and CH₃I), non-methane organic compounds (NMHC), methane (CH₄) and ammonia (NH₃) are released [19, 20, 22, 23]. As smoke interacts with atmosphere some intermediate chemicals are produced, mostly in the form of carbon, hydrogen and oxygen free radicals [24]. In addition to these chemicals, a large quantity of PM (solid or/and liquid particles) is emitted to the air either directly (primary particles) released from biomass combustion or indirectly (secondary particles) by the interaction with other produced pollutants, such as VOCs (e.g., NMHC, oxygenated NMHC, NO_x, alcohols, aldehydes and organic acids) [20, 23, 25]. Although the particles themselves are microscopic sized (0.3-1.6 µm) they rapidly coalesce and become visible [26]. PM₁₀ (aerodynamic diameter of 10 µm or less) and PM_{2.5} (aerodynamic diameter of 2.5 µm or less) are the most described in the literature [25], being PM_{2.5} the ones more frequently associated with forest biomass burning [27]. Differences in the particle size depend on factors namely the type of fuels and the combustion phase [28]. Particles have adsorbent properties being an additional vehicle for the transport/inhalation of toxic combustion compounds such as polycyclic aromatic hydrocarbons (PAHs) [26]. PAHs include

Complimentary Contributor Copy

compounds such pyrene, benzo[a]pyrene, m-chrysene, phenanthrene, among others [29].

Some of the pollutants referred above (e.g., PM, VOCs, PAHs, CO and NO₂) have been linked with adverse health effects among general population [30], being inclusively some of these recognized as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer (IARC) [26]. However, few studies have been focused on the health effects associated with wildland firefighter smoke exposure [31]. The body of work shows that further epidemiological studies are needed to clarify the biological mechanisms related with wildfire smoke exposure among populations, particularly in wildland firefighters.

Firefighters' Occupational Exposure

Worldwide, a high number of people are exposed to wildfire smoke in different situations and settings [32]. Wildland firefighters are at greater risk due to the nature of their work, being considered one of the most dangerous occupations of the world [33]. Wildland firefighters perform high physiological demand activities in hostile environments [16]. Typical tasks include not only the fire attack, as well as long hikings, fire-line construction, chainsaw work and bush removal [26]. Their immediate proximity to fire exposes them to high temperatures and high concentrations of pollutants. Due to the risky nature of their work, they are required to wear proper protective fire-resistant clothing to protect themselves. The personal protective equipment (PPE) used by firefighters typically comprises a structural helmet with eye protection (goggles or shield), masks (e.g., cotton bandana), gloves, flash hood, boots, and fire-resistant clothing. Previous studies have shown that the use of these PPE offers a good protection against smoke compounds [34]. However, there are a general reluctance to wear PPE since it adds physiological demands, heat stress upon the user [26] and difficulty in communication with crew. On the other hand, the development of more heat resistant clothing materials (e.g., polybenzimidazole or aramid polymers) may encourage firefighters to get closer to fire, exposing them to higher levels of combustion products [35]. Firefighting exposure occurs through different routes, namely inhalation, dermal absorption and ingestion [36], being this intensified by the difficult nature of work (high physical demand in extreme conditions). Inhalation is the main absorption route for several toxic chemicals that are present in smoke, such CO, NO₂, SO₂, aldehydes (formaldehyde and

Complimentary Contributor Copy

acrolein), ketones and PAHs [37]. Wildland firefighters are exposed to a wide range of inhaling irritants, but the respiratory protection typically used is not ideal to protect effectively from the exposure to these smoke compounds [38]. The use of a self-contained breathing apparatus (SCBA) - equipment supplied with fresh air - is unfeasible with wildland firefighting activities due to its weight and limited period of service (15-30 minutes) [21, 26, 38]. In some cases, the only respiratory protection of wildland firefighters is a cotton bandana covering nose and mouth [16] that do not prevent toxic compounds e.g., PM, gases, and vapors, from being inhaled.

Dermal exposure is thought to be another important pathway to certain smoke pollutants (e.g., PAHs and VOCs) among firefighters [37]. Previous studies have shown that some compounds may settle and/or condense on equipment or in the exposed skin, allowing the penetration of these substances into the human body [39]. Firefighters' dermal absorption may be enhanced by the inappropriate use of protective equipment, the inadequate PPE cleaning after fire extinction or due to eventual imperfections in the equipment [34]. Chemicals vapors or liquids may penetrate through porous PPE material whereas molecules of chemicals have the capacity to permeate into protective clothing fibers [40]. In addition, dermal absorption could be also affected by other factors; previous studies have shown that higher levels of skin humidity (firefighters' sweating) caused by high temperatures may reduce the skin barrier and facilitate the absorption of toxic substances into the body [34, 37].

Ingestion, in the other hand, is considered the least relevant exposure route since oral absorption mostly occurs through hand-to-mouth contact when hands are not cleaned [37]. Exposure to toxic compounds (e.g., PAHs) may occur when firefighters do not use PPE properly or interrupt their activities to eat, drink and/or smoke with uncleaned hands, favoring the accidental ingestion of particles.

Besides the evident forms of firefighters' exposure to pollutants, in the field, other relevant occupational contamination sources exist. Firefighters are exposed in the return to the fire station and into the building via contaminated vehicles, equipment and PPE. These sources of pollutants can affect the indoor air quality including the "clean" areas such as rooms, bedroom, or offices where firefighters remain during long periods of time [41, 42]. A recent study published by Rogula-Kozłowska et al. [39] verified high levels of VOCs (i.e., benzene, toluene, ethylbenzene, xylene, and styrene) both in the changing room and in the garage of fire station, suggesting that the major sources of indoor air pollution in these settings were contaminated PPE or other equipment used during firefighting. Other growing concern is related with the

Complimentary Contributor Copy

type of raw materials used in some PPE textiles, as a potential source of exposure to perfluoroalkylacids (PFAS) [43]. PFAS are a concern to human health due their persistency and toxicity. Previous studies suggest that populations with increased PFAS concentrations in blood serum have higher vulnerability to a wide range of diseases [43]. Exposure to these substances could occur either by dermal absorption (i.e., skin contact) or through the degradation of equipment and subsequent ingestion or inhalation [43].

Moreover, also the exposures resulting from fire training events or by emissions from service vehicles may contribute to the total exposure of firefighters to hazardous pollutants [44].

Health Risks

Smoke compounds have a negative impact on environment, ecosystems and among populations health. Prior studies have demonstrated a positive association between wildfire smoke exposure and both respiratory morbidity and all-cause mortality [45]. The main symptoms associated with acute exposure to wildfire smoke comprises eye and respiratory irritation, dizziness, headaches, and nausea [46]. Most available studies on long term health effects concerning firefighters are focused on respiratory outcomes [16, 47]. Some works have reported a decline in the lung function capacity of wildland firefighters, that is particularly evident across shifts and between seasons (pre- and post-fire season) [47, 48]. Gaughan et al. [47] for example observed that the increased levels of eosinophilic cationic protein and myeloperoxidase in sputum and nasal lavage fluid were associated with higher postfire respiratory overall symptoms scores (i.e., cough, wheeze, sputum production, shortness of breath or chest tightness, and shortness of breath while walking, as well as other eye, nose, and throat symptoms). These results are in accordance with those obtained by Rothman et al. [49] that also documented a significant increase of respiratory reported symptoms (i.e., cough, phlegm, wheeze, eye and nasal irritation) due to cross-season changes. Moreover, the repeated inhalation of smoke compounds is known to lead to some respiratory diseases, such asthma [50].

Besides respiratory symptoms, there are evidence that firefighters have an increased risk of cardiovascular diseases [31, 51-54]. A previous study from the National Fire Protection Association shown that sudden cardiac events (heart attacks and other heart related deaths) are the leading cause of death among firefighters, being responsible for 40-50% of fatalities each year [55].

Complimentary Contributor Copy

Similarly, Navarro et al., [31] reported that wildland firefighters have an increased risk of cardiovascular mortality due to smoke exposure. High temperatures allied with physically challenging activities leads to a significant elevation of core temperature [56, 57], which may trigger this type of cardiovascular events in susceptible firefighters [58]. The duration of wildland firefighting experience seems to be positively proportional to the prevalence of self-reported health outcomes [59]. Semmens et al. [59] observed that wildland firefighters with more years of experience have higher odds of having adverse cardiovascular outcomes (e.g., hypertension and arrhythmia) than those with less years in the profession. However, it is important to stress that short-term firefighting may also impact cardiovascular system; Fahs et al. [52] found increased arterial stiffness and vasodilation after three hours of firefighting activities.

Several studies have explored the adverse health effects caused by specific combustion products. PM, for example, has been related with several adverse health outcomes [60]. PM enter into the body through inhalation reaching the lungs and bloodstream, causing adverse health effects in the cardiovascular, respiratory, immune and neural system [61]. Both short- and long-term exposure to PM was associated with cardiovascular (PM_{2.5}) and respiratory morbidity (PM_{2.5}, PM₁₀) [25]. PM with smaller diameter is particularly harmful to human health due its higher ability to penetrate the lungs and pass to bloodstream [25, 62]. PM_{2.5} has been linked with wildland firefighters' chronic obstructive pulmonary disease (COPD), asthma and infectious diseases [63], as well as with other important cardiovascular diseases such as myocardial infarction, stroke, and arrhythmias [25, 64]. A previous study verified that outdoor air concentrations of PM₁₀ were strongly correlated with clinic visits due to respiratory illnesses in a community affected by a wildfire [65]. It is important to underline that associations between the exposure to PM₁₀ and adverse health effects have been more evident among susceptible populations as elderly, children, or individuals with pre-existing health conditions [66-68]. However, so far, no report of health effects related to PM₁₀ exposure among wildland firefighters was found in literature.

Beside the non-carcinogenic effects stated herein, other important health effects have been related with firefighters' occupational exposure, including cancer [26].

Carcinogenic Risk

Carcinogenesis is a multistage process that involves modification and mutation to genes that regulate normal cellular function. Human

Complimentary Contributor Copy

carcinogenesis is the outcome of interactions between exogenous and endogenous processes. Although risk factors such as genetic predisposition play an important role in carcinogenesis, a wide range of environmental and occupational factors also contribute to the development of cancer.

Firefighters are exposed to a complex mixture of toxic compounds, many of them carcinogens. Some studies have been comparing the mortality and cancer incidence between firefighters and the general population, showing a significant number of cancer occurrence among firefighters (e.g., digestive, and respiratory cancers) [69, 70]. Some woodsmoke compounds are classified by IARC as known (Group 1) or probable/possible (Group 2) human carcinogens [26]. Group 1 includes smoke compounds such benzene, benzo[a]pyrene, vinyl chloride, formaldehyde, 1, 3-butadiene and polychlorinated bisphenols and PM2.5 [26]. Group 2 comprises acetaldehyde, benzofuran, ethylbenzene, isoprene, furan, black carbon and styrene [26]. In addition, there are other non-smoke related exposures that could also increase the risk of cancer among these professionals, such as PPE and the diesel exhaust from firefighting vehicles [26].

IARC classified occupational firefighting activity as possibly carcinogenic to humans [26] based on evidence of increased risk of testicular cancer, prostate cancer and non-Hodgkin's lymphoma among firefighters (when compared with the general population) [71-73]. During the past decades, different studies have been assessing the carcinogenic risk related with firefighting occupational exposure. A case-control study used California Cancer Risk data (1988-2007) to evaluate the risk of cancer among firefighters and found an increased risk of melanoma and leukemia in these professionals (independently of the stratification by race) [74]. Pukkala et al. [75] examined the patterns of cancer among Nordic firefighters and observed that professionals aged between 30-49 years had a significant increase of skin melanoma and prostate cancer when compared with other age groups. Moreover, a recent and comprehensive meta-analysis reported increased incidence risk for other types of cancers among firefighters, such as colon, rectum, testis, bladder, thyroid and pleura [76]. Furthermore, Zhao et al. [77] also observed an increased risk of mortality from laryngeal and hypopharyngeal cancer among firefighters.

The increasing risk of wildfires episodes and longer fire seasons highlight the need for occupational studies among these professionals that are commonly exposed to hazardous pollutants. Some studies have been assessing the firefighters' occupational exposure to smoke pollutants, which is often challenging due to the volatile environment, demanding characteristics of the

Complimentary Contributor Copy

field, and remote fire locations. Nevertheless, this assessment is crucial to identify the mechanisms of action by which woodsmoke impacts firefighters' health, considering not only the fire suppression operations but also the remaining potential exposure scenarios (e.g., contact with contaminated equipment or clothing).

Monitoring of Wildland Firefighters' Exposure

Exposure assessment plays a central role for the identification, evaluation, and control of occupational risks. Human monitoring is a crucial approach to identify potential signals of exposure to toxic compounds and to predict certain health risks [78]. It comprises a routine evaluation and interpretation of environmental and/or biological parameters to detect possible health risks before the occurrence of the disease [78]. The exposure is usually assessed through the environmental monitoring, where the chemical agents are quantified in environmental samples, as well as through biological monitoring that consists in the measurement of biological endpoints, commonly called biomarkers [79].

Environmental Monitoring

Environmental monitoring is related to the quantification of the pollutant in the occupational environment and aims to estimate the hazard level to which individuals are exposed, comparing measured values with well-established reference values. These measurements could be performed during a single task as well as during an entire day of work exposure. However, monitoring firefighters' exposure during a fire combat is a very challenging task particularly due to instability and characteristics of the environment, namely fire location and the weather conditions. Additionally, environmental monitoring represents only a snapshot of exposure in time, not counting variations over time and not considering other confounder factors that may have a substantial influence on compounds uptake, such as the individual characteristics or other contextual factors (e.g., use of PPE). In this sense, biomonitoring is an important tool that overcomes these types of difficulties.

Biological Monitoring

Biomonitoring has been widely used to provide information regarding the level of exposure to chemicals through different pathways (absorption, distribution, metabolism, and excretion) and exposure routes (e.g., inhalation,

Complimentary Contributor Copy

ingestion, and dermal absorption). Biomonitoring comprises data on individual internal dose resulting from the exposure to a xenobiotic with (bio)markers of effect and or susceptibility. Thus, biological monitoring represents a valuable tool for assessing the potential health effects in exposed groups. The main purpose of biomonitoring is not only to characterize the exposure but suggest preventive measures to mitigate the risk of short- and long-term adverse health outcomes in assessed populations.

By now, there are a limited number of studies focused on biomonitoring of smoke exposure in wildland firefighters [34, 80-83]. Some of these studies comprise the biological and the environmental monitoring, and the collection of data through a questionnaire to obtain information about the exposure history. Up-to date, some studies have used biomonitoring as a tool to assess firefighters' exposure, applying different methods by means of specific biological matrices such as blood, urine, and exhaled air [84].

Biomarkers: A Brief Contextualization

The concept of biomarkers was proposed by the US National Research Council in 1987 citing "xenobiotically-induced variations in cellular or biochemical components or processes, structures or functions that are measurable in a biological system or sample" [85]. Biomarkers are therefore intermediates between exposure and clinical outcomes, representing a pattern that starts with the exposure, and ends with the clinical outcome, the known concept of "continuum of biological events" [85]. These tools are of great interest in occupational studies because they not only signal the exposure to a compound but may also provide data to assess the risk of potential adverse health effects [79]. According to the International Program of Chemical Safety, biomarkers can be categorized as: biomarkers of dose, biomarkers of effect and biomarkers of susceptibility [86].

Biomarkers of dose involve the quantification of exogenous substances and/or metabolites of xenobiotic compounds in different biological matrices. They can be distinguished into two subcategories: internal dose and biological effective dose. A biomarker of internal dose indicates the level of a particular compound through the quantification of its metabolite(s) in a tissue or body fluid (e.g., t, t-muconic acid in urine, lead in blood, etc.) [87]. The use of an internal dose biomarker usually does not provide information about the extension of the interaction between chemical and the critical cellular targets [79]. A biomarker of effective dose on the other hand, is used to assess the biologically active fraction of xenobiotics which was able to interact with

Complimentary Contributor Copy

tissues, cells, organelles, or macromolecules (e.g., DNA adducts, protein adducts) [86].

A biomarker of effect is defined as a measurable biochemical, physiological, behavioral, or other alteration within an organism that, depending upon the magnitude, can be associated with a possible health impairment or disease [86]. For that reason, they are extensively used in human biomonitoring studies to evaluate the event that occurs at the later end of the continuum between exposure and disease manifestation. Cytogenetic alterations such as chromosomal aberrations or micronuclei frequency are some examples of biomarkers of effect that have been widely used to evaluate the genotoxic potential of hazardous chemicals [88]. However, effect biomarkers are not chemical-specific, and therefore, its analysis should be accompanied by environmental monitoring or/and the measurement of a biomarker of dose [89]. Even so, higher levels of these outcomes in an exposed group (when compared to baseline levels or with an unexposed group) is a clear indicator of chromosomal or genomic instability (genetic events able to cause temporal or permanent genomic alterations).

Biomarkers of susceptibility are commonly used to identify “at-risk” individuals, where this risk may be either inherited (e.g., polymorphisms that may lead to different capacities to detoxify or repair the DNA lesions) or non-genetic indicators (e.g., nutritional status, lifestyle, and age) [86]. These factors are independent of exposure; however, they are useful to identify individuals with more susceptibility to the effect of a hazardous exposure [79]. This type of biomarkers is useful to explain why the same environmental exposure to a specific xenobiotic substance can lead to different levels of biomarkers of exposure and/or biomarkers of effect in different individuals.

Biomarkers of Smoke Exposure

A biomarker of smoke exposure, as any biomarker of dose, should prove adequate sensitivity, specificity, and robustness for its routine analysis given the high number of external variables (e.g., tobacco smoking and charred/smoked item foods) that may be not accurately obtained through self-administered questionnaires [90]. Several compounds found in human biological samples have been proposed as biomarkers of woodsmoke exposure in firefighters for example PAHs metabolites, carboxyhemoglobin (CO), methoxyphenols (lignin) and levoglucosan (cellulose) [91].

Complimentary Contributor Copy

PAHs are largely emitted during combustion processes [29]. Over 100 different PAHs have been identified, from which 16 species have been classified as priority pollutants [93]. PAHs are a major concern, being some classified by IARC as known (i.e., benzo[a]pyrene), probable (e.g., dibenz[a,h]anthracene, dibenzo[a,l]pyrene) or possible human carcinogenic (e.g., chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benz[a]anthracene) [26]. PAHs can be analyzed in different biological matrices, such as blood, urine, and skin [92]. Hydroxylated PAHs metabolites (OH-PAHs) in urine are currently the most used biomarkers in human biomonitoring studies for PAHs exposure [94, 95]; it can be sampled within 24h after exposure [92]. PAHs are metabolized in the liver and mostly excreted through urine mainly as their glucuronide conjugates. Among the existent urinary OH-PAHs metabolites, there are some that are frequently measured, specifically 1-hydroxypyrene (OH-Pyr) and various isomers of hydroxynaphthalene (OH-Nap), hydroxyphenanthrene (OH-Phe), and hydroxyfluorene (OH-Flu) [92]. Urinary OH-Pyr (half-life range from 6 to 32 hours) is the metabolite most widely used for biomonitoring of recent exposures due to the abundance of pyrene in woodsmoke [92]. A recent study from Wingfors et al. [34] analyzed 8 urinary PAHs metabolites among 20 volunteer firefighters and verified that OH-Pyr was the most useful indicator of exposure, with significantly higher levels both at 6h and 20h after smoke exposure. Oliveira et al. [96] found that wildland firefighters engaged in recent fire combat activities had significantly higher levels of post-shift urinary OH-PAHs (1.7-35 times higher) than non-exposed firefighters; the metabolites that contributed the most were OH-Nap, followed by OH-Flu, OH-Phe and OH-Pyr. Additionally, recent studies conducted by Fent et al. [44, 97] identified urinary OH-Phe as a sensitive marker of PAHs exposure in firefighters. Metabolism of some PAHs produces reactive intermediates that can bind to cellular macromolecules (DNA and proteins), generating PAH adducts [98]. A previous study found higher levels of PAH adducts in peripheral blood cells in a group of wildland firefighters exposed to woodsmoke, when compared with the non-exposed controls [99]. The analysis of PAHs and metabolites is commonly performed using the gas chromatography (GC) with tandem mass spectrometry (MS/MS) and high-resolution MS (HRMS), which is optimum for non-target screening of chemical exposures in the environment [100]. In addition to this method, liquid chromatography (LC) coupled both with fluorescence detection and MS/MS has also been widely used in the determination of urinary OH-PAHs [92].

Complimentary Contributor Copy

CO is a major contributor to acute toxicity, when inhaled it combines with hemoglobin in blood and carboxyhemoglobin is formed impairing oxygen transport to vital organs (hypoxia). However, previous authors have stated that carboxyhemoglobin may be only a suitable biomarker of woodsmoke exposure when CO is dominant and exposure is high [91]; Dunn et al., [101] only verified an association when the wildland firefighters' workshift time-weighted average exposure to CO was higher than 5 ppm. In addition, carboxyhemoglobin can be also estimated by chemical analysis of exhaled alveolar breath [102].

Methoxyphenols result from the pyrolysis of the wood polymer lignin. Associations were found between the exposure to woodsmoke among wildland firefighters and the levels of this biomarker in urine [103]. Fernando et al. [104] used a set of methoxyphenols, specifically methyl-, ethyl- and propylsyringol, to determine the effect of woodsmoke exposure among firefighters. These compounds were found to be sensitive markers of smoke exposure since were significantly higher ($p < 0.05$) in 24h post-exposure urine. Similarly, to carboxyhemoglobin and PAHs metabolites, this biomarker is considered useful for woodsmoke high-exposure situations (e.g., firefighters' occupational exposure).

Levoglucosan is an abundant compound in woodsmoke, and it is formed from pyrolysis of cellulose [103]. Some studies [105, 106] have explored the feasibility of this compound as a biomarker of woodsmoke in urine, however there is still limited data. Naeher et al. [106] analyzed urine samples from wildland firefighters before and after their work-shifts and concluded that the utility of urinary levoglucosan as a biomarker of woodsmoke may be limited, since post-shift concentrations were not consistently higher than pre-shift levels, and the cross-shift change was not associated with personal exposure to PM_{2.5}. Nevertheless, the same authors concluded that levoglucosan could still be used to assess woodsmoke exposure, in situations where other sources of levoglucosan (e.g., foods) are scarce or rare [106].

Urine and blood are probably the most frequently used biological matrices to determine firefighters' smoke exposure. However, there other alternatives, such as metabolites in exhaled air. Zhou et al. [107] have determined exhaled carbon monoxide and exhaled nitric oxide using a hand-held battery-operated meter and Nano Coulomb Nitric Oxide Analyzer, respectively. They observed an association between elevated exhaled carbon monoxide and exhaled nitric oxide and increased urinary PAHs metabolites [107]. Other tools have been used in screening strategies to identify metabolites in exhaled air. An electronic nose (e-nose) system, for example, is a technique that allow the

Complimentary Contributor Copy

analysis of VOC profile in exhaled air [108]. Breath monitoring has several advantages because is a simple and non-invasive tool to measure the extent of chemical exposures.

From the Exposure to the Biological Effect

Genotoxicity reflects the ability of an agent to damage the genetic information within a cell which may result in DNA alterations that may lead to malignancies. The exposure to genotoxicants can damage DNA causing a wide range of DNA lesions such as oxidative DNA damage, base loss, DNA cross-links, alkylation damage and DNA adducts [109].

DNA adducts are commonly considered to be a biomarker of biologically effective dose; however, it may also be recognized as a biomarker of effect since adducts are able to induce DNA alterations and consequently increase the risk of cancer development [110]. Some studies have explored the use of PAH-DNA adducts as a biomarker to characterize the exposure of firefighters [91]. The presence of PAH-DNA adducts in human cells have been strongly correlated with an increased risk of different type of cancers [111-114].

Most intermediate chemicals that are formed during biomass combustion are free radicals. Many of them tend to dissipate very fast; however, some persist for up to 20 minutes [21]. Oxidative DNA damage is considered one of the most important types of genetic damage in humans caused by exogenous substances [115]. Gaughan et al. [116] observed a positive association between predictors of arterial stiffness and biomarkers of oxidative stress, specifically of lipid peroxidation (urinary 8-isoprostane) as well of oxidative DNA damage (8-hydroxy-2'-deoxyguanosine). A study conducted in Portugal [117] found higher levels of primary and oxidative DNA damage in blood samples of wildland firefighters exposed to woodsmoke when compared to nonexposed subjects; these biomarkers were analyzed through the comet assay, a commonly used tool in human biomonitoring studies for the evaluation of DNA damage at individual cell level [118]. Similar results were found by Andersen et al. [119] after a 3-day firefighting course. In the same study, authors also found a statistically significant positive correlation between levels of primary DNA damage and both PAHs on skin and OH-Pyr in urine, suggesting that the parameters assessed by comet assay may be a good biomarker for the monitoring of firefighting occupational exposure.

One of the mechanisms of action of woodsmoke exposure also include inflammatory processes. Previous human-controlled interventions revealed

Complimentary Contributor Copy

that after exposure to woodsmoke, individuals demonstrated an increased percentage of pro-inflammatory response [120, 121] in accordance with in-vitro experiments [122-124]. High levels of inflammatory biomarkers, such serum interleukin (IL) -6 and -8, have been linked with smoke exposure from wildfires. Note that, IL-6 mainly plays a role in the acute phase inflammatory responses, whereas IL 8 is overexpressed in chronic inflammatory diseases [125]. Swiston et al. [126] found increased levels of these proinflammatory cytokines in firefighters after a work-shift. Similarly, Hejl et al. [127] also verified significant higher levels of IL-8 at post-work shift among wildland firefighters. Barregard et al. [128] showed that exposure to woodsmoke increased alveolar nitric oxide (a marker of inflammatory response) after 3 hours post-exposure and malondialdehyde levels (a marker of lipid peroxidation) in breath condensate immediately after and 20 hours after exposure. Contrarily, Adetona et al. [129] did not observe any significant cross-shift change in oxidative stress biomarkers (malondialdehyde and 8-oxo-dG) among wildland firefighters exposed to woodsmoke. Nonetheless, the authors found that unadjusted geometric mean of 8-oxo-dG in urine was higher in wildland firefighters than levels reported on general population studies.

Cytogenetic endpoints, such as, chromosomal aberrations, micronuclei, tumor suppressor genes are widely known biomarkers used in occupational studies to detect early effects of environmental genotoxic carcinogens [130]. The relevance of cytogenetic alterations as a cancer risk biomarker have been supported by various epidemiological studies [131, 132]. Bonassi et al. [133] provided evidence that the frequency of micronuclei in peripheral blood lymphocytes is a predictive biomarker of cancer within population of healthy subjects. The analysis of this biomarker with the comet assay could provide information about past versus current exposure, respectively.

Besides the referred genotoxicity biomarkers there are other approaches that may be useful to detect the early effects between the individual and the environment. A recent study for example suggests that telomere length measurements may be a novel indicator of genotoxic stress induced by PM exposure caused by combustion processes both in cell cultures and humans [134].

Complimentary Contributor Copy

Principal Strengths and Limitations

Scientific evidence has contributed for the establishment of measures related with firefighters' health promotion, including the implementation of certain policies and procedures. Some studies have enhanced that hygiene intervention/skin decontamination is highly effective to reduce the exposure. However, there are still gaps in the scientific literature regarding the mechanisms of action of woodsmoke exposure among wildland firefighters' health.

Most part of studies are focused on environmental monitoring in controlled settings; although their findings add valuable information to current knowledge, they do not represent the extreme circumstances of firefighting in real scenarios. Environmental monitoring combined with other assessment tools such as questionnaires and biological monitoring provide a more comprehensive characterization of occupational risks in this sector. Biological monitoring considers factors that affect the relationship between environmental exposures and the uptake of toxic compounds, including the different absorption rates within the subjects, the use of PPE and other personal behaviors. Another advantage of this approach is that biological monitoring can be performed retrospectively; biological samples can be collected after the exposure event (e.g., a wildfire), not interfering with the firefighter activities during fire suppression and not compromising the efficacy of further laboratorial analysis. However, there are some factors that could affect the utility of biomarkers in the measurement of wildland smoke exposure. Woodsmoke is composed by a complex variable mixture of chemicals which depend on different factors such as the type of vegetation and the combustion conditions. Nonetheless, studies have researched individual effects of woodsmoke compounds as stand-alone exposures. For that reason, additional studies are necessary to address health implications related with woodsmoke compounds interactions.

Another limitation of the available literature is related with the few number of studies and the limited sample size – although providing important insight, studies with limited population size are more constrained in giving conclusive information.

Also important in occupational studies is the data collected by questionnaires. It is crucial for the validity of results the inclusion of a comprehensive range of inquiries allowing a better characterization of the worker (e.g., age, cigarette smoking, medical history), the exposure (e.g.,

Complimentary Contributor Copy

shifts, use of PPE, occupational history) and confounding variables (e.g., dietary sources, sources of indoor combustion).

It is important to highlight that, there is a lack of studies evaluating the effects of wildfire smoke exposure at the molecular level namely DNA repair capacity, DNA strand breaks, telomere length and micronucleus. To overcome this gap, future studies should better explore these mechanisms in order to establish possible associations between long-term exposures to woodsmoke and chronic health outcomes.

Conclusion

Biomonitoring is particularly important to address firefighters' occupational exposure, considering not only the internal dose to a certain compound but also the potential biological effects that could occur considering a short- or long-term exposure. Up to date, the existing firefighting occupational studies have been particularly focused in environmental monitoring and biomarkers of internal dose. However, few have analyzed the impact of this exposure on wildland firefighters' health. The study of other complementary genotoxic endpoints, for example, would be of enormous interest to confirm and strengthen these results, namely cytogenetic alterations such as the frequency of micronucleus (a validated cancer risk biomarker). Future works should include biomarkers of dose as well as biomarkers of early genotoxic and oxidative effects to better characterize the association of certain adverse health outcomes in firefighters. Surveillance (bio)monitoring programs are particularly urgent and crucial in those countries that are more severely affected by wildfires. This evidence may help to provide accurate information to support decision making.

Further research on exposure limiting practices, type of PPE material, decontamination of PPE and personal hygiene methods, are needed to define clear methods to reduce firefighters' exposure.

Despite the above, regular monitoring and surveillance activities (e.g., preventive medical checkups), health promotion activities (e.g., adequate use of respiratory equipment, hygiene methods), the introduction of innovative firefighting techniques (e.g., improvements in fire sensors detection, use of CO monitors), better communication and information systems, safer PPE (well-fitting, well designed), and the implementation of written policies and procedures such decontamination of fire station equipment and spaces are crucial. Finally, it is also essential to promote a wise fuel management in

Complimentary Contributor Copy

forests among the population, because reducing the amount of fuel or changing its arrangement before a wildfire can prevent or attenuate severe fire behaviors.

Acknowledgments

This work received financial support by the project PCIF/SSO/0017/2018 by the Fundação para a Ciência e a Tecnologia (FCT), Ministério da Ciência, Tecnologia e Ensino Superior (MCTES) through national funds. Filipa Esteves, recipient of PhD grant UI/BD/150783/2020, is supported by FCT and by the European Social Fund (ESF). Joana Madureira, under the grant SFRH/BPD/115112/2016, is supported by FCT and by ESF, through Programa Operacional Capital Humano (POCH). The authors also acknowledge the Instituto de Saúde Pública da Universidade do Porto (ISPUP).

References

- [1] Gowlett JA. The discovery of fire by humans: a long and convoluted process. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 2016;371(1696):20150164.
- [2] Jensen D, Reager JT, Zajic B, Rousseau N, Rodell M, Hinkley E. The sensitivity of US wildfire occurrence to pre-season soil moisture conditions across ecosystems. *Environmental research letters*. 2018;13(1):014021.
- [3] Wuebbles DJ, Fahey DW, Hibbard KA, Arnold JR, DeAngelo B, Doherty S, et al. Climate science special report: *Fourth national climate assessment* (NCA4), Volume I. 2017.
- [4] Sungmin O, Hou X, Orth R. Observational evidence of wildfire-promoting soil moisture anomalies. *Scientific reports*. 2020;10(1):1-8.
- [5] Stolton S. *Future fires: perpetuating problems of the past*. WWF International; 2003.
- [6] Hirschberger P. *Forests ablaze: causes and effects of global forest fires*. WWF Deutschland; 2016.
- [7] Mouillot F, Field CB. Fire history and the global carbon budget: a 1 × 1 fire history reconstruction for the 20th century. *Global Change Biology*. 2005;11(3):398-420.
- [8] Flannigan MD, Krawchuk MA, de Groot WJ, Wotton BM, Gowman LM. Implications of changing climate for global wildland fire. *International journal of wildland fire*. 2009;18(5):483-507.
- [9] Avenbuan ON, Zelikoff JT. Woodsmoke and emerging issues. *Current Opinion in Toxicology*. 2020;22:12-8.

Complimentary Contributor Copy

66 Filipa Esteves, Joana Madureira, João Paulo Teixeira et al.

- [10] San-Miguel-Ayanz J, Schulte E, Schmuck G, Camia A, Strobl P, Liberta G, et al. Comprehensive monitoring of wildfires in Europe: the European forest fire information system (EFFIS). Approaches to managing disaster-Assessing hazards, emergencies and disaster impacts: *IntechOpen*; 2012.
- [11] Schmuck G SM AJ, Camia A, Durrant T, Boca R, Liberta` G, Petroliagkis T, Di Leo M, Rodriguez Aseretto R, Boccacci F, Schulte E. Forest Fires in Europe, *Middle East and North Africa* 2013. 2014.
- [12] Littell JS, McKenzie D, Peterson DL, Westerling AL. Climate and wildfire area burned in western US ecoprovinces, 1916–2003. *Ecological Applications*. 2009;19(4):1003-21.
- [13] Littell JS, Oneil EE, McKenzie D, Hicke JA, Lutz JA, Norheim RA, et al. Forest ecosystems, disturbance, and climatic change in Washington State, USA. *Climatic change*. 2010;102(1):129-58.
- [14] Littell JS, Peterson DL, Riley KL, Liu Y, Luce CH. A review of the relationships between drought and forest fire in the United States. *Global change biology*. 2016;22(7):2353-69.
- [15] Flannigan MD, Amiro BD, Logan KA, Stocks BJ, Wotton BM. Forest fires and climate change in the 21 st century. *Mitigation and adaptation strategies for global change*. 2006;11(4):847-59.
- [16] Naeher LP, Brauer M, Lipsett M, Zelikoff JT, Simpson CD, Koenig JQ, et al. Woodsmoke health effects: a review. *Inhalation toxicology*. 2007;19(1):67-106.
- [17] Ward D. Combustion chemistry and smoke. Forest fires: Elsevier; 2001. p. 55-77.
- [18] Koppmann R, Von Czapiewski K, Reid J. A review of biomass burning emissions, part I: gaseous emissions of carbon monoxide, methane, volatile organic compounds, and nitrogen containing compounds. *Atmospheric chemistry and physics discussions*. 2005;5(5):10455-516.
- [19] Andreae MO, Merlet P. Emission of trace gases and aerosols from biomass burning. *Global biogeochemical cycles*. 2001;15(4):955-66.
- [20] Akagi S, Yokelson RJ, Wiedinmyer C, Alvarado M, Reid J, Karl T, et al. Emission factors for open and domestic biomass burning for use in atmospheric models. *Atmospheric Chemistry and Physics*. 2011;11(9):4039-72.
- [21] George B. *Wildland firefighters smoke exposure smoke*. Utah State University; 2013.
- [22] Yokelson RJ, Griffith DW, Ward DE. Open-path Fourier transform infrared studies of large-scale laboratory biomass fires. *Journal of Geophysical Research: Atmospheres*. 1996;101(D15):21067-80.
- [23] Durán S, Reisen F, Rideout K. Evidence review: wildfire smoke and public health risk. BC Centre for Disease Control: Vancouver, BC, Canada. 2014.
- [24] Leonard SS, Castranova V, Chen BT, Schwegler-Berry D, Hoover M, Piacitelli C, et al. Particle size-dependent radical generation from wildland fire smoke. *Toxicology*. 2007;236(1-2):103-13.
- [25] Costa S, Ferreira J, Silveira C, Costa C, Lopes D, Relvas H, et al. Integrating health on air quality assessment—review report on health risks of two major European outdoor air pollutants: PM and NO2. *Journal of Toxicology and Environmental Health, Part B*. 2014;17(6):307-40.

Complimentary Contributor Copy

The Role of Biomarkers in the Assessment of Potential Health Risks ... 67

- [26] IARC. Painting, firefighting, and shiftwork: IARC Press, International Agency for Research on Cancer; 2010.
- [27] Simões Amaral S, Andrade de Carvalho J, Martins Costa MA, Pinheiro C. Particulate matter emission factors for biomass combustion. *Atmosphere*. 2016;7(11):141.
- [28] Eck T, Holben B, Reid J, O'Neill N, Schafer J, Dubovik O, et al. High aerosol optical depth biomass burning events: A comparison of optical properties for different source regions. *Geophysical Research Letters*. 2003;30(20).
- [29] Simoneit BR. Biomass burning—a review of organic tracers for smoke from incomplete combustion. *Applied Geochemistry*. 2002;17(3):129-62.
- [30] Manisalidis I, Stavropoulou E, Stavropoulos A, Bezirtzoglou E. Environmental and health impacts of air pollution: a review. *Frontiers in public health*. 2020;8.
- [31] Navarro KM, Kleinman MT, Mackay CE, Reinhardt TE, Balmes JR, Broyles GA, et al. Wildland firefighter smoke exposure and risk of lung cancer and cardiovascular disease mortality. *Environmental research*. 2019;173:462-8.
- [32] Adetona OT. Occupational exposure to woodsmoke and associated changes in lung function and biomarkers of oxidative stress among wildland firefighters: University of Georgia; 2011.
- [33] Adetona O, Simpson CD, Onstad G, Naeher LP. Exposure of wildland firefighters to carbon monoxide, fine particles, and levoglucosan. *Annals of occupational hygiene*. 2013;57(8):979-91.
- [34] Wingfors H, Nyholm JR, Magnusson R, Wijkmark CH. Impact of fire suit ensembles on firefighter PAH exposures as assessed by skin deposition and urinary biomarkers. *Annals of work exposures and health*. 2018;62(2):221-31.
- [35] Lau C, Anitole K, Hodes C, Lai D, Pfahles-Hutchens A, Seed J. Perfluoroalkyl acids: a review of monitoring and toxicological findings. *Toxicological sciences*. 2007;99(2):366-94.
- [36] Ruby BC, Shriver TC, Zderic TW, Sharkey BJ, Burks C, Tysk S. Total energy expenditure during arduous wildfire suppression. *Medicine and Science in Sports and Exercise*. 2002;34(6):1048-54.
- [37] Heus R. Exposure to smoke. An overview report of the studies to exposure. 2018.
- [38] Austin C. Wildland Firefighter Health Risks and Respiratory Protection (Report R-572). Montreal, Quebec, Canada: IRSST (Institut de recherche Robert-Sauvé en santé et en sécurité du travail). 2008.
- [39] Rogula-Kozłowska W, Bralewska K, Jureczko I. BTEXS Concentrations and Exposure Assessment in a Fire Station. *Atmosphere*. 2020;11(5):470.
- [40] Barker RL. A Review of gaps and limitations in test methods for first responder protective clothing and equipment. National Personal Protection Technology Laboratory, National Institute for Occupational Safety and Health (NIOSH). 2005.
- [41] Oliveira M, Slezakova K, Alves MJ, Fernandes A, Teixeira JP, Delerue-Matos C, et al. Polycyclic aromatic hydrocarbons at fire stations: firefighters' exposure monitoring and biomonitoring, and assessment of the contribution to total internal dose. *Journal of hazardous materials*. 2017;323:184-94.

Complimentary Contributor Copy

68 Filipa Esteves, Joana Madureira, João Paulo Teixeira et al.

- [42] Banks APW, Engelsman M, He C, Wang X, Mueller JF. The occurrence of PAHs and flame-retardants in air and dust from Australian fire stations. *Journal of occupational and environmental hygiene*. 2020;17(2-3):73-84.
- [43] Peaslee GF, Wilkinson JT, McGuinness SR, Tighe M, Caterisano N, Lee S, et al. Another Pathway for Firefighter Exposure to Per-and Polyfluoroalkyl Substances: Firefighter Textiles. *Environmental Science & Technology Letters*. 2020;7(8):594-9.
- [44] Fent KW, Toennis C, Sammons D, Robertson S, Bertke S, Calafat AM, et al. Firefighters' and instructors' absorption of PAHs and benzene during training exercises. *International journal of hygiene and environmental health*. 2019;222(7):991-1000.
- [45] Reid CE, Brauer M, Johnston FH, Jerrett M, Balmes JR, Elliott CT. Critical review of health impacts of wildfire smoke exposure. *Environmental health perspectives*. 2016;124(9):1334-43.
- [46] Reinhardt TE. Smoke exposure at western wildfires: US Department of Agriculture, Forest Service, Pacific Northwest Research Station; 2000.
- [47] Gaughan DM, Cox-Ganser JM, Enright PL, Castellan RM, Wagner GR, Hobbs GR, et al. Acute upper and lower respiratory effects in wildland firefighters. *Journal of occupational and environmental medicine*. 2008;50(9):1019-28.
- [48] Betchley C, Koenig JQ, van Belle G, Checkoway H, Reinhardt T. Pulmonary function and respiratory symptoms in forest firefighters. *American journal of industrial medicine*. 1997;31(5):503-9.
- [49] Rothman N, Ford DP, Baser ME, Hansen JA, O'Toole T, Tockman MS, et al. Pulmonary function and respiratory symptoms in wildland firefighters. *Journal of occupational medicine: official publication of the Industrial Medical Association*. 1991;33(11):1163-7.
- [50] Greven F. Respiratory effects of fire smoke exposure in firefighters and the general population: Utrecht University; 2011.
- [51] Andersen MHG, Saber AT, Pedersen PB, Loft S, Hansen ÅM, Koponen IK, et al. Cardiovascular health effects following exposure of human volunteers during fire extinction exercises. *Environmental Health*. 2017;16(1):1-9.
- [52] Fahs CA, Yan H, Ranadive S, Rossow LM, Agiovlasis S, Echols G, et al. Acute effects of firefighting on arterial stiffness and blood flow. *Vascular medicine*. 2011;16(2):113-8.
- [53] Soteriades ES, Smith DL, Tsismenakis AJ, Baur DM, Kales SN. Cardiovascular disease in US firefighters: a systematic review. *Cardiology in review*. 2011;19(4):202-15.
- [54] Pedersen JE, Petersen KU, Ebbeløj NE, Bonde JP, Hansen J. Incidence of cardiovascular disease in a historical cohort of Danish firefighters. *Occupational and environmental medicine*. 2018;75(5):337-43.
- [55] Fahy RF. US firefighter fatalities due to sudden cardiac death, 1995-2004: National Fire Protection Association Quincy, MA; 2005.
- [56] Smith DL, Manning T, Petruzzello SJ. Effect of strenuous live-fire drills on cardiovascular and psychological responses of recruit firefighters. *Ergonomics*. 2001;44(3):244-54.

Complimentary Contributor Copy

The Role of Biomarkers in the Assessment of Potential Health Risks ... 69

- [57] Barr D, Gregson W, Reilly T. The thermal ergonomics of firefighting reviewed. *Applied ergonomics*. 2010;41(1):161-72.
- [58] Drew-Nord DC, Hong O, Froelicher ES, Berryman P, Lukes E. Cardiovascular risk factors among career firefighters. *AAOHN Journal*. 2009;57(10):415-24.
- [59] Semmens EO, Domitrovich J, Conway K, Noonan CW. A cross-sectional survey of occupational history as a wildland firefighter and health. *American journal of industrial medicine*. 2016;59(4):330-5.
- [60] Kim KH, Kabir E, Kabir S. A review on the human health impact of airborne particulate matter. *Environment international*. 2015;74:136-43.
- [61] Breyse PN, Delfino RJ, Dominici F, Elder AC, Frampton MW, Froines JR, et al. US EPA particulate matter research centers: summary of research results for 2005–2011. *Air Quality, Atmosphere & Health*. 2013;6(2):333-55.
- [62] Scott AF, Reilly CA. *Wood and biomass smoke: addressing human health risks and exposures*. ACS Publications; 2019.
- [63] Cascio WE. Wildland fire smoke and human health. *Science of the total environment*. 2018;624:586-95.
- [64] Du Y, Xu X, Chu M, Guo Y, Wang J. Air particulate matter and cardiovascular disease: the epidemiological, biomedical and clinical evidence. *Journal of thoracic disease*. 2016;8(1):E8.
- [65] Mott JA, Meyer P, Mannino D, Redd SC, Smith EM, Gotway-Crawford C, et al. Wildland forest fire smoke: health effects and intervention evaluation, Hoopa, California, 1999. *Western Journal of Medicine*. 2002;176(3):157.
- [66] Anderson H, Atkinson R, Bremner S, Marston L. Particulate air pollution and hospital admissions for cardiorespiratory diseases: are the elderly at greater risk? *European Respiratory Journal*. 2003;21(40 suppl):39s-46s.
- [67] Anderson H, Atkinson R, Bremner S, Carrington J, Peacock J. Quantitative systematic review of short term associations between ambient air pollution (particulate matter, ozone, nitrogen dioxide, sulphur dioxide and carbon monoxide), and mortality and morbidity. Report to Department of Health revised following first review. 2007.
- [68] Von Klot S, Peters A, Aalto P, Bellander T, Berglind N, D'Ippoliti D, et al. Health Effects of Particles on Susceptible Subpopulations (HEAPSS) Study Group. Ambient air pollution is associated with increased risk of hospital cardiac readmissions of myocardial infarction survivors in five European cities. *Circulation*. 2005;112(20):3073-9.
- [69] Daniels RD, Kubale TL, Yiin JH, Dahm MM, Hales TR, Baris D, et al. Mortality and cancer incidence in a pooled cohort of US firefighters from San Francisco, Chicago and Philadelphia (1950–2009). *Occupational and environmental medicine*. 2014;71(6):388-97.
- [70] Glass D, Del Monaco A, Pircher S, Vander Hoorn S, Sim M. Mortality and cancer incidence at a fire training college. *Occupational medicine*. 2016;66(7):536-42.
- [71] Guidotti TL. Mortality of urban firefighters in Alberta, 1927–1987. *American journal of industrial medicine*. 1993;23(6):921-40.

Complimentary Contributor Copy

70 Filipa Esteves, Joana Madureira, João Paulo Teixeira et al.

- [72] LeMasters GK, Genaidy AM, Succop P, Deddens J, Sobeih T, Barrera-Viruet H, et al. Cancer risk among firefighters: a review and meta-analysis of 32 studies. *Journal of occupational and environmental medicine*. 2006;48(11):1189-202.
- [73] Golka K, Weistenhöfer W. Fire fighters, combustion products, and urothelial cancer. *Journal of Toxicology and Environmental Health, Part B*. 2008;11(1):32-44.
- [74] Tsai RJ, Luckhaupt SE, Schumacher P, Cress RD, Deapen DM, Calvert GM. Risk of cancer among firefighters in California, 1988–2007. *American journal of industrial medicine*. 2015;58(7):715-29.
- [75] Pukkala E, Jan Ivar M, Elisabete W, Kristina K, Elsebeth L, Laufey T, et al. Cancer incidence among firefighters: 45 years of follow-up in five Nordic countries. *Occupational and Environmental Medicine*. 2013;oemed-2013-101803.
- [76] Jalilian H, Ziaei M, Weiderpass E, Rueegg CS, Khosravi Y, Kjaerheim K. Cancer incidence and mortality among firefighters. *International journal of cancer*. 2019;145(10):2639-46.
- [77] Zhao G, Erazo B, Ronda E, Brocal F, Regidor E. Mortality Among Firefighters in Spain: 10 Years of Follow-up. *Annals of work exposures and health*. 2020;64(6):614-21.
- [78] Teixeira JP, Gaspar J, Silva S, Torres J, Silva SN, Azevedo MC, et al. Occupational exposure to styrene: modulation of cytogenetic damage and levels of urinary metabolites of styrene by polymorphisms in genes CYP2E1, EPHX1, GSTM1, GSTT1 and GSTP1. *Toxicology*. 2004;195(2-3):231-42.
- [79] Costa S. Occupational exposure to formaldehyde-genotoxic damage and susceptibility evaluation in anatomical pathology laboratory workers. Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto. 2014.
- [80] Adetona AM, Kyle Martin W, Warren SH, Hanley NM, Adetona O, Zhang J, et al. Urinary mutagenicity and other biomarkers of occupational smoke exposure of wildland firefighters and oxidative stress. *Inhalation toxicology*. 2019;31(2):73-87.
- [81] Oliveira M, Slezakova K, Magalhães CP, Fernandes A, Teixeira JP, Delerue-Matos C, et al. Individual and cumulative impacts of fire emissions and tobacco consumption on wildland firefighters' total exposure to polycyclic aromatic hydrocarbons. *Journal of hazardous materials*. 2017;334:10-20.
- [82] Adetona O, Simpson CD, Li Z, Sjödin A, Calafat AM, Naether LP. Hydroxylated polycyclic aromatic hydrocarbons as biomarkers of exposure to wood smoke in wildland firefighters. *Journal of exposure science & environmental epidemiology*. 2017;27(1):78-83.
- [83] Cherry N, Aklilu YA, Beach J, Britz-McKibbin P, Elbourne R, Galarneau JM, et al. Urinary 1-hydroxypyrene and skin contamination in firefighters deployed to the Fort McMurray fire. *Annals of work exposures and health*. 2019;63(4):448-58.
- [84] WHO. *Human biomonitoring: facts and figures*. WHO Regional Office for Europe, Copenhagen. 2015.
- [85] Henderson R, Hobbie J, Landrigan P, Mattisoti D, Perera F, Pfitraer E, et al. Biological markers in environmental health research. *Environmental Health Perspectives*. 1987;7:3-9.

Complimentary Contributor Copy

The Role of Biomarkers in the Assessment of Potential Health Risks ... 71

- [86] Robinson A. Biomarkers and Risk Assessment: Concepts and Principles: *World Health Organization*; 1993.
- [87] Calafat AM, Ye X, Silva MJ, Kuklenyik Z, Needham LL. Human exposure assessment to environmental chemicals using biomonitoring. *International journal of andrology*. 2006;29(1):166-71.
- [88] Costa S, Brandão F, Coelho M, Costa C, Coelho P, Silva S, et al. Micronucleus frequencies in lymphocytes and buccal cells in formaldehyde exposed workers. *WIT Transactions on Biomedicine and Health*. 2013;16:83-94.
- [89] Ladeira C, Viegas S. Human biomonitoring: an overview on biomarkers and their application in occupational and environmental health. *Biomonitoring*. 2016;3(1):15-24.
- [90] Heo HC, Byun YS, Sohn SH, Jo SM, Park SK, Sakong J. Validity assessment of self-reported smoking status in firefighters using the urine cotinine test. *Annals of occupational and environmental medicine*. 2020;32.
- [91] Simpson CD, Naeher LP. Biological monitoring of wood-smoke exposure. *Inhalation toxicology*. 2010;22(2):99-103.
- [92] Gill B, Britz-McKibbin P. Biomonitoring of smoke exposure in firefighters: a review. *Current Opinion in Environmental Science & Health*. 2020.
- [93] Keith LH. The source of US EPA's sixteen PAH priority pollutants. *Polycyclic Aromatic Compounds*. 2015;35(2-4):147-60.
- [94] Gao P, da Silva E, Hou L, Denslow ND, Xiang P, Ma LQ. Human exposure to polycyclic aromatic hydrocarbons: metabolomics perspective. *Environment international*. 2018;119:466-77.
- [95] Oliveira M, Capelas S, Delerue-Matos C, Morais S. Grill workers exposure to polycyclic aromatic hydrocarbons: levels and excretion profiles of the urinary biomarkers. *International journal of environmental research and public health*. 2021;18(1), 230.
- [96] Oliveira M, Slezakova K, Alves MJ, Fernandes A, Teixeira JP, Delerue-Matos C, et al. Firefighters' exposure biomonitoring: Impact of firefighting activities on levels of urinary monohydroxyl metabolites. *International journal of hygiene and environmental health*. 2016;219(8):857-66.
- [97] Fent KW, Toennis C, Sammons D, Robertson S, Bertke S, Calafat AM, et al. Firefighters' absorption of PAHs and VOCs during controlled residential fires by job assignment and fire attack tactic. *Journal of exposure science & environmental epidemiology*. 2020;30(2):338-49.
- [98] Phillips DH. On the origins and development of the 32P-postlabelling assay for carcinogen-DNA adducts. *Cancer letters*. 2013;334(1):5-9.
- [99] Liou SH, Jacobson-Kram D, Poirier MC, Nguyen D, Strickland PT, Tockman MS. Biological monitoring of fire fighters: sister chromatid exchange and polycyclic aromatic hydrocarbon-DNA adducts in peripheral blood cells. *Cancer research*. 1989;49(17):4929-35.
- [100] Hollender J, Schymanski EL, Singer HP, Ferguson PL. Nontarget screening with high resolution mass spectrometry in the environment: ready to go?: ACS Publications; 2017.

Complimentary Contributor Copy

72 Filipa Esteves, Joana Madureira, João Paulo Teixeira et al.

- [101] Dunn K, Devaux I, Stock A, Mott J, Naeher L. Evaluation of exposure to carbon monoxide during prescribed forest burns. *American Industrial Hygiene Conference and Exposition*; May, 2005; Atlanta, GA2005.
- [102] Cone DC, MacMillan DS, Van Gelder C, Brown DJ, Weir SD, Bogucki S. Noninvasive fireground assessment of carboxyhemoglobin levels in firefighters. *Prehospital Emergency Care*. 2005;9(1):8-13.
- [103] Neitzel R, Naeher L, Paulsen M, Dunn K, Stock A, Simpson C. Biological monitoring of smoke exposure among wildland firefighters: a pilot study comparing urinary methoxyphenols with personal exposures to carbon monoxide, particular matter, and levoglucosan. *Journal of exposure science & environmental epidemiology*. 2009;19(4):349-58.
- [104] Fernando S, Shaw L, Shaw D, Gallea M, VandenEnden L, House R, et al. Evaluation of firefighter exposure to wood smoke during training exercises at burn houses. *Environmental science & technology*. 2016;50(3):1536-43.
- [105] Migliaccio CT, Bergauff MA, Palmer CP, Jessop F, Noonan CW, Ward TJ. Urinary levoglucosan as a biomarker of wood smoke exposure: observations in a mouse model and in children. *Environmental health perspectives*. 2009;117(1):74-9.
- [106] Naeher LP, Barr DB, Adetona O, Simpson CD. Urinary levoglucosan as a biomarker for woodsmoke exposure in wildland firefighters. *International journal of occupational and environmental health*. 2013;19(4):304-10.
- [107] Zhou Y, Liu Y, Sun H, Ma J, Xiao L, Cao L, et al. Associations of urinary polycyclic aromatic hydrocarbon metabolites with fractional exhaled nitric oxide and exhaled carbon monoxide: A cross-sectional study. *Science of the Total Environment*. 2018;618:542-50.
- [108] Scarlata S, Pennazza G, Santonico M, Pedone C, Antonelli Incalzi R. Exhaled breath analysis by electronic nose in respiratory diseases. *Expert review of molecular diagnostics*. 2015;15(7):933-56.
- [109] Ersson C. International validation of the comet assay and a human intervention study: Inst för biovetenskaper och näringslära/Dept of Biosciences and Nutrition; 2011.
- [110] Vineis P, Perera F. DNA adducts as markers of exposure to carcinogens and risk of cancer. *International journal of cancer*. 2000;88(3):325-8.
- [111] Tang D, Kryvenko ON, Wang Y, Jankowski M, Trudeau S, Rundle A, et al. Elevated polycyclic aromatic hydrocarbon–DNA adducts in benign prostate and risk of prostate cancer in African Americans. *Carcinogenesis*. 2013;34(1):113-2.
- [112] Chen SY, Wang LY, Lunn RM, Tsai WY, Lee PH, Lee CS, et al. Polycyclic aromatic hydrocarbon–DNA adducts in liver tissues of hepatocellular carcinoma patients and controls. *International journal of cancer*. 2002;99(1):14-21.
- [113] Gammon MD, Sagiv SK, Eng SM, Shantakumar S, Gaudet MM, Teitelbaum SL, et al. Polycyclic aromatic hydrocarbon–DNA adducts and breast cancer: a pooled analysis. *Archives of Environmental Health: An International Journal*. 2004;59(12):640-9.
- [114] Gunter MJ, Divi RL, Kulldorff M, Vermeulen R, Haverkos KJ, Kuo MM, et al. Leukocyte polycyclic aromatic hydrocarbon–DNA adduct formation and colorectal adenoma. *Carcinogenesis*. 2007;28(7):1426-9.

Complimentary Contributor Copy

The Role of Biomarkers in the Assessment of Potential Health Risks ... 73

- [115] Azqueta A, Shaposhnikov S, Collins AR. DNA oxidation: investigating its key role in environmental mutagenesis with the comet assay. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*. 2009;674(1-2):101-8.
- [116] Gaughan DM, Siegel PD, Hughes MD, Chang CY, Law BF, Campbell CR, et al. Arterial stiffness, oxidative stress, and smoke exposure in wildland firefighters. *American journal of industrial medicine*. 2014;57(7):748-56.
- [117] Abreu A, Costa C, Pinho e Silva S, Morais S, do Carmo Pereira M, Fernandes A, et al. Wood smoke exposure of Portuguese wildland firefighters: DNA and oxidative damage evaluation. *Journal of Toxicology and Environmental Health, Part A*. 2017;80(13-15):596-604.
- [118] Neri M, Milazzo D, Ugolini D, Milic M, Campolongo A, Pasqualetti P, et al. Worldwide interest in the comet assay: a bibliometric study. *Mutagenesis*. 2014;30(1):155-63.
- [119] Andersen MHG, Saber AT, Clausen PA, Pedersen JE, Lohr M, Kermanizadeh A, et al. Association between polycyclic aromatic hydrocarbon exposure and peripheral blood mononuclear cell DNA damage in human volunteers during fire extinction exercises. *Mutagenesis*. 2018;33(1):105-15.
- [120] Ghio AJ, Soukup JM, Case M, Dailey LA, Richards J, Berntsen J, et al. Exposure to wood smoke particles produces inflammation in healthy volunteers. *Occupational and environmental medicine*. 2012;69(3):170-5.
- [121] Danielsen PH, Bräuner EV, Barregard L, Sällsten G, Wallin M, Olinski R, et al. Oxidatively damaged DNA and its repair after experimental exposure to wood smoke in healthy humans. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*. 2008;642(1-2):37-42.
- [122] Danielsen PH, Loft S, Kocbach A, Schwarze PE, Møller P. Oxidative damage to DNA and repair induced by Norwegian wood smoke particles in human A549 and THP-1 cell lines. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*. 2009;674(1-2):116-22.
- [123] Danielsen PH, Møller P, Jensen KA, Sharma AK, Wallin H, Bossi R, et al. Oxidative stress, DNA damage, and inflammation induced by ambient air and wood smoke particulate matter in human A549 and THP-1 cell lines. *Chemical research in toxicology*. 2011;24(2):168-84.
- [124] Jalava PI, Happonen MS, Kelz J, Brunner T, Hakulinen P, Mäki-Paakkanen J, et al. In vitro toxicological characterization of particulate emissions from residential biomass heating systems based on old and new technologies. *Atmospheric Environment*. 2012;50:24-35.
- [125] Sproston NR, Ashworth JJ. Role of C-reactive protein at sites of inflammation and infection. *Frontiers in immunology*. 2018;9:754.
- [126] Swiston JR, Davidson W, Attridge S, Li GT, Brauer M, van Eeden SF. Wood smoke exposure induces a pulmonary and systemic inflammatory response in firefighters. *European respiratory journal*. 2008;32(1):129-38.
- [127] Hejl AM, Adetona O, Diaz-Sanchez D, Carter JD, Commodore AA, Rathbun SL, et al. Inflammatory effects of woodsmoke exposure among wildland firefighters working at prescribed burns at the Savannah River Site, SC. *Journal of occupational and environmental hygiene*. 2013;10(4):173-80.

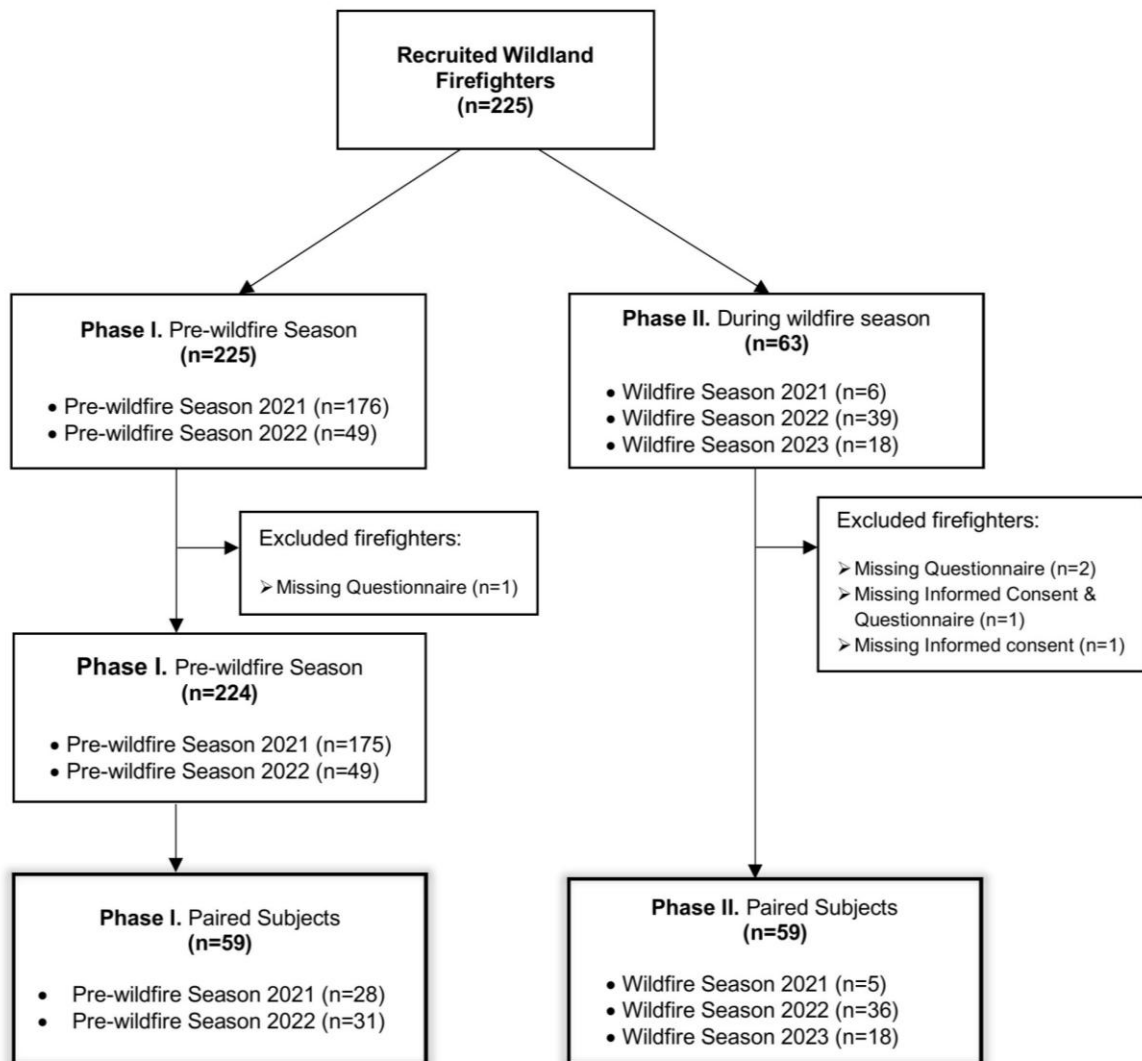
Complimentary Contributor Copy

74 Filipa Esteves, Joana Madureira, João Paulo Teixeira et al.

- [128] Barregard L, Sällsten G, Andersson L, Almstrand AC, Gustafson P, Andersson M, et al. Experimental exposure to wood smoke: effects on airway inflammation and oxidative stress. *Occupational and environmental medicine*. 2008;65(5):319-24.
- [129] Adetona O, Zhang JJ, Hall DB, Wang JS, Vena JE, Naeher LP. Occupational exposure to woodsmoke and oxidative stress in wildland firefighters. *Science of the total environment*. 2013;449:269-75.
- [130] Farmer PB, Emeny J. Biomarkers of carcinogen exposure and early effects. *Nofer Institute of Occupational Medicine*. 2006. p. 163.
- [131] Bonassi S, El-Zein R, Bolognesi C, Fenech M. Micronuclei frequency in peripheral blood lymphocytes and cancer risk: evidence from human studies. *Mutagenesis*. 2011;26(1):93-100.
- [132] Bonassi S, Hagmar L, Strömberg U, Montagud AH, Tinnerberg H, Forni A, et al. Chromosomal aberrations in lymphocytes predict human cancer independently of exposure to carcinogens. *Cancer Research*. 2000;60(6):1619-25.
- [133] Bonassi S, Znaor A, Ceppi M, Lando C, Chang WP, Holland N, et al. An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans. *Carcinogenesis*. 2007;28(3):625-31.
- [134] Ma Y, Bellini N, Scholten RH, Andersen MHG, Vogel U, Saber AT, et al. Effect of combustion-derived particles on genotoxicity and telomere length: A study on human cells and exposed populations. *Toxicology letters*. 2020;322:20-31.

Complimentary Contributor Copy

Appendix III – Flow Chart of the Study Population



Appendix IV – Information to the Participant



INFORMAÇÃO AO PARTICIPANTE

BioFirEx - Um painel de (bio)marcadores para a vigilância da saúde e da segurança do bombeiro

Proponente: REQUIMTE-LAQV do Instituto Superior de Engenharia do Porto

Parceiros: Escola Superior de Saúde do Instituto Politécnico de Bragança (IPB)
 Instituto de Saúde Pública da Universidade do Porto
 Faculdade de Engenharia da Universidade do Porto
 Autoridade Nacional de Proteção Civil
 Unidade Local de Saúde Pública do Nordeste (ULSNE)

Financiamento: Fundação para a Ciência e Tecnologia (referência PCIF/SSO/0017/2018)

OBJETIVO PRINCIPAL: usar uma abordagem multidisciplinar para realizar uma avaliação abrangente da exposição ocupacional dos bombeiros a poluentes gerados durante os incêndios, avaliar os potenciais impactos na saúde e segurança dos operacionais e estabelecer uma lista de recomendações e boas práticas.

POR QUE É QUE A MINHA PARTICIPAÇÃO É IMPORTANTE?

Ao aceitar participar neste estudo está a ajudar-nos a perceber mais e melhor sobre o risco de exposição a diversos poluentes atmosféricos durante o combate a incêndios florestais para a saúde e segurança dos profissionais.

DECIDI PARTICIPAR! O QUE VAI ACONTECER?

O projecto terá a duração de três anos (2020-2022) e é pedida a sua participação nas campanhas de amostragem que decorrerão em 2021 e 2022 em três fases distintas:

Fase I– período pré-exposição;

Fase II– período de exposição a fogos (coordenada por agentes da Autoridade Nacional de Proteção Civil (amostragem em campo) e por investigadores do IPB e ULSNE, já em ambiente de quartel, imediatamente após a participação do bombeiro no combate aos fogos;

Fase III– período de pós exposição.

A sua participação consistirá em:

- Preenchimento de um Questionário (30-45 min) que pretende recolher dados sociodemográficos, clínicos e de exposição ambiental e ocupacional do participante;
- Monitorização pessoal da exposição ocupacional por inalação (amostragem de ar respirável com dispositivo que é colocado na cintura do participante por um período contínuo de 4 horas) e por via dérmica (amostragem na pele com limpeza das principais zonas de contacto: pescoço, rosto e mãos);
- Avaliação física, cardiorespiratória e psicológica por técnicos e médicos da especialidade;
- Recolha de amostras biológicas (sangue, urina e mucosa bucal) por investigadores e profissionais especializados no final do turno de trabalho.

Appendix V – Informative Leaflet

Projeto de investigação

BioFirEx: Um painel de biomarcadores para a vigilância da saúde e da segurança do bombeiro

Este projeto (PCIF/SSO/0017/2018) é suportado pelo orçamento da Fundação para a Ciência e a Tecnologia na sua componente OE e mereceu um parecer favorável da Comissão de Ética da Universidade do Porto.



BioFirEx

FCT Fundação para a Ciência e a Tecnologia

Co-financiado por:

COMPETE 2020

PORTUGAL 2020

UNião Europeia Fundo de Coesão

Objetivos do BioFirEx:

Caracterizar a contaminação dos equipamentos de proteção individual dos bombeiros após o combate a incêndios: identificação dos principais pontos fracos;

Identificar um conjunto de (bio)marcadores apropriados para a vigilância da exposição ocupacional e da saúde e segurança dos operacionais de combate a fogos;

Avaliar os impactos na saúde e segurança dos operacionais;

Estabelecer uma lista de recomendações e boas práticas.

Porque é que a minha participação é importante?

Ao aceitar participar neste estudo está a ajudar-nos a perceber mais e melhor sobre o risco de exposição a diversos poluentes atmosféricos durante o combate a incêndios florestais para a saúde e segurança dos profissionais.

Comité Consultivo:



Participe!

Parceiros:

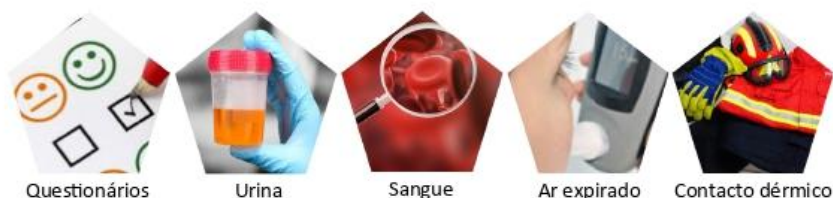




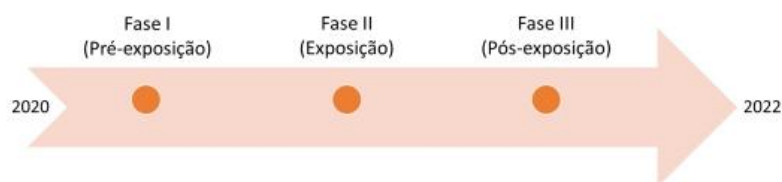
O que vamos fazer?

- Monitorização da exposição ocupacional por inalação e contacto dérmico;
- Avaliação física, cardiorrespiratória e psicológica por profissionais de saúde;
- Avaliação de Biomarcadores de Exposição e de Efeito em amostras biológicas (urina, ar expirado, células da mucosa bucal e sangue).

Como vamos avaliar?



Quando vamos avaliar?



Para podermos comparar os seus dados é necessária a sua participação em todas as fases.

- ◆ A participação no estudo é inteiramente voluntária;
- ◆ Poderá desistir do estudo em qualquer momento;
- ◆ Toda a informação recolhida será guardada em absoluto sigilo e confidencialidade, sendo utilizada apenas para fins de investigação e tratamento estatístico.



Participe!

+INFO

Website: <https://www.isep.ipp.pt/Page/ViewPage/BioFirExPaginaPrincipal>



Adília Fernandes
(adilia@ipb.pt)



João Paulo Teixeira
(joao.teixeira@insa.min-saude.pt)



Maria Carmo Pereira
(mcsp@fe.up.pt)



Simone Morais
(sbm@isep.ipp.pt)
Investigadora Responsável

Appendix VI – Informed Consent.

CONSENTIMENTO INFORMADO, ESCLARECIDO E LIVRE PARA PARTICIPAÇÃO EM ESTUDO DE INVESTIGAÇÃO

(de acordo com a Declaração de Helsínquia¹ e a Convenção de Oviedo²)

Por favor, leia com atenção a seguinte informação. Se achar que algo está incorreto ou não está claro, não hesite em solicitar mais informações. Se concorda com a proposta que lhe foi feita, queira assinar este documento.

Título do Estudo: BioFirEx - Um painel de (bio)marcadores para a vigilância da saúde e da segurança do bombeiro
Projeto PCIF/SSO/0017/2018

Explicação do estudo: Este estudo pretende determinar os níveis de exposição a poluentes durante as actividades de combate a fogos florestais e estimar os principais riscos para a saúde dos operacionais envolvidos. Este projeto tem como principais objetivos: 1) caracterizar a exposição ocupacional aos poluentes gerados durante os incêndios florestais; 2) avaliar os impactos na saúde e segurança dos operacionais; 3) identificar um conjunto de (bio)marcadores apropriados para a vigilância da saúde e segurança dos bombeiros; e por fim 4) estabelecer uma lista de recomendações e boas práticas. O estudo será realizado em três fases distintas: Fase I– período pré-exposição; Fase II– período de exposição a fogos e Fase III– período de pós exposição. Este projeto contempla a participação de bombeiros de diferentes quartéis e operacionais de combate ao fogo de associações florestais e do Grupo de Intervenção de Proteção e Socorro da Guarda Nacional Republicana. Esperamos que o resultado deste estudo contribua para a melhoria das condições de trabalho e qualidade de vida dos operacionais e promova boas práticas de trabalho.

Se concordar, pedimos que:

- Preencha e assine o consentimento informado, livre e esclarecido.
- Permita a colheita de sangue, urina e mucosa bucal (5-10 min) nas três fases de amostragem (Fase I, II e III). As colheitas serão efetuadas no final do turno de trabalho por profissionais habilitados para esta competência, acredita-se que o incomodo seja mínimo.
- Permita a colocação de um equipamento de amostragem portátil na cintura, de modo a não perturbar qualquer actividade que desempenhe, por um período contínuo de 4 horas. A amostragem de ar na Fase II (amostragem em campo) será coordenada por agentes da Autoridade Nacional de Proteção Civil enquanto que as amostragens nas fases I e III (em ambiente de quartel) serão agendadas com investigadores do IPB.
- Permita a recolha de amostras de limpeza de pele nas principais zonas de contacto (pescoço, rosto e mãos) nas fases de amostragem I e II para avaliar a eficácia da protecção dos equipamentos de protecção individual. Investigadores especializados acompanharão todo o processo de amostragem.
- Responda às questões do Questionário sobre saúde, bem-estar, estilos de vida, histórico de exposições ocupacional e ambiental, e ainda que seja avaliado mediante escalas de índice de capacidade para o trabalho, cuidados de saúde física e mental, qualidade do sono e avaliação do stress do bombeiro (30-45 min).

Saiba que

- A participação é voluntária e gratuita. Poderá desistir do estudo a qualquer momento sem qualquer tipo de consequência.
- Toda a informação recolhida será estritamente confidencial e guardada em absoluto sigilo e confidencialidade, como é exigido por lei e será utilizada apenas para fins de investigação. Quer os questionários quer as amostras biológicas serão codificados e tornados anónimos no ato de colheita e deixarão de poder ser relacionados consigo. Apenas o Investigador Principal e investigadores por ele autorizados, sujeitos a sigilo profissional, terão acesso às chaves de codificação.
- O estudo mereceu Parecer favorável da Comissão de Ética da Universidade do Porto.

Muito obrigado pela sua colaboração

¹http://epidemiologia.med.up.pt/pdfs/Helsinq_2013.pdf

²http://portal.arsnorte.min-saude.pt/portal/page/portal/ARSNorte/Comiss%C3%A3o%20de%20%C3%89tica/Ficheiros/Declaracao_Helsinquia_2008.pdf

DECLARAÇÃO DE CONSENTIMENTO INFORMADO E ESCLARECIDO ⁽³⁾

Eu, abaixo assinado, _____ (letras maiúsculas)
confirmo que:

- compreendi a explicação escrita e verbal que me foi dada acerca deste projeto de investigação, tomando conhecimento dos objetivos, métodos, benefícios previsíveis e riscos potenciais, bem como das garantias de confidencialidade previstas para os dados que disponibilizo.
- solicitei todas as informações de que necessitei, sabendo que o esclarecimento é fundamental para uma boa decisão.
- fui informado da possibilidade de livremente recusar a qualquer momento, a minha participação no projecto através do contacto com o investigador responsável abaixo identificado, sem que isso possa ter como efeito qualquer prejuízo na assistência que me é prestada. Sei também que não serei ressarcido de quaisquer despesas decorrentes da participação.

Se terminado este projeto existir ainda alguma das amostras biológicas por mim facultadas, autorizo que sejam conservadas para utilização em estudos futuros devidamente aprovados pela Comissão de Ética da Universidade do Porto? ☐ **SIM** ☐ **NÃO**

Se SIM, pretendo que as amostras sejam tornadas anónimas de forma definitiva? ☐ **SIM** ☐ **NÃO**

Estas são as condições em que decido livremente participar no estudo/investigação

Data: / /

Assinatura:

Investigador(es) responsável(eis):

Adília Fernandes

João Paulo Teixeira

Maria Carmo Pereira

Simone Morais (Investigadora Principal)

Adília Fernandes Tlf: 273 330 950 adilia@ipb.pt Escola Superior de Saúde Avenida D. Afonso V 5300-121 Bragança	João Paulo Teixeira Tlf: 223401141 joao.teixeira@insa.min-saude.pt Instituto Nacional de Saúde Dr Ricardo Jorge Instituto de Saúde Pública da Universidade do Porto, R. de Alexandre Herculano 321, 4000-053 Porto	Maria Carmo Pereira Tlf: 22 508 1590 mesp@fe.up.pt LEPABE, Faculdade de Engenharia da Universidade do Porto R. Dr. Roberto Frias, 4200-465 Porto	Simone Morais Tlf: 228340500 sbm@isep.ipp.pt REQUIMTE, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida 431, 4200-072 Porto
---	---	---	---

³ Feito e assinado em duplicado sendo entregue um exemplar ao participante, juntamente com o documento informativo

Appendix VII – Questionnaire A.



**Um painel de (bio)marcadores para a
vigilância da saúde e da segurança do
bombeiro**

Questionário Individual

Financiamento

Cofinanciado por:



Caro Participante,

Este questionário serve para recolher dados sobre estilos de vida, história ocupacional, exposição ambiental e ocupacional, história clínica e sintomatologia, dados de bem-estar e capacidade de trabalho.

Leia com cuidado todas as perguntas. Quando terminar o preenchimento do questionário verifique, por favor, se respondeu a todas as perguntas.

Muito obrigada pela sua cooperação.

Instruções para o preenchimento do questionário

- Este questionário vai ser submetido a leitura ótica. Por favor use uma esferográfica ou caneta de tinta azul ou preta.
- Para algumas questões apresentamos várias respostas possíveis. Por favor assinale a resposta que melhor se adequa. Assinale apenas uma opção, salvo indicação em contrário.
- Em algumas perguntas pedimos-lhes para especificar um número ou data (por exemplo, “Qual é a sua data de nascimento? - - (dia-mês-ano)”). Por favor escreva os números ou datas no(s) espaço(s) indicado(s).
- Noutras perguntas pedimos-lhe para escrever a resposta em palavras (por exemplo, “Qual é a sua profissão? ”). Por favor escreva de forma clara, se possível em letras maiúsculas e uma letra por cada quadrado.
- Exemplo de preenchimento:

1	2	3	4	5	6	7	8	9	0
A	B	C	D	E	F	G	H		

Não preencha assim



Preencha assim



- No caso de se enganar, coloque um círculo à volta da resposta errada e assinale a resposta correta.



Não



Sim

Entidades Participantes



Data de preenchimento / /
(completar pela equipa de investigação) **dia mês ano**

ID

Questionário A: Caderno de avaliação global

I. DADOS GERAIS

1. Qual é a sua data de nascimento? / /
dia mês ano

2. Género? ☐ Masculino ☐ Feminino

3. Qual é a sua altura? cm Qual é o seu peso? kg

4. Qual é o seu estado civil? ☐ Solteiro/a ☐ Casado/a ou União de facto ☐ Viúvo/a ☐ Separado/a/Divorciado/a

5. Qual o máximo de escolaridade que completou?

- ☐ Nenhum ☐ Bacharelato
☐ Primeiro ciclo do ensino básico (4º ano) ☐ Licenciatura
☐ Segundo ciclo do ensino básico (6º ano) ☐ Doutoramento
☐ Terceiro ciclo do ensino básico (9º ano) ☐ Outro
☐ Ensino secundário (12º ano)

5.1. Com que idade completou? idade em anos

II. SITUAÇÃO PROFISSIONAL

6. Pertence à Guarda Nacional Republicana (GNR)? ☐ Não ☐ Sim

6.1 Se SIM, complete:

Categoria:

Subcategoria:

Posto:

(se respondeu SIM, passe à questão 9)

7. Pertence ao Corpo de Bombeiros? ☐ Não ☐ Sim

7.1 Se SIM, indique a que quadro pertence:

- ☐ Quadro de comando
☐ Quadro ativo
☐ Quadro de reserva
☐ Quadro de honra

8. Se respondeu SIM à questão 7, indique abaixo o Corpo, Carreira e Categoria a que pertence.

8.1 Corpo:

- ☐ Bombeiros profissionais
☐ Bombeiros mistos
☐ Bombeiros voluntários

8.2 Carreira e Categoria:

- | | | |
|---|--|--|
| ☐ Carreira de bombeiro sapador | ☐ Chefe principal | ☐ Subchefe de 1ª classe |
| | ☐ Chefe de 1ª classe | ☐ Subchefe de 2ª classe |
| | ☐ Chefe de 2ª classe | ☐ Bombeiro sapador |
| | ☐ Subchefe principal | |
| <hr/> | <hr/> | <hr/> |
| ☐ Carreira de bombeiro municipal | ☐ Chefe | ☐ Bombeiro de 2ª classe |
| | ☐ Subchefe | ☐ Bombeiro de 3ª classe |
| | ☐ Bombeiro de 1ª classe | |
| <hr/> | <hr/> | <hr/> |
| ☐ Carreira de oficial bombeiro | ☐ Oficial bombeiro superior | ☐ Oficial bombeiro de 2ª |
| | ☐ Oficial bombeiro principal | ☐ Estagiário |
| | ☐ Oficial bombeiro de 1ª | |
| <hr/> | <hr/> | <hr/> |
| ☐ Carreira de bombeiro voluntário | ☐ Chefe | ☐ Bombeiro de 2ª |
| | ☐ Subchefe | ☐ Bombeiro de 3ª |
| | ☐ Bombeiro de 1ª | |
| <hr/> | <hr/> | <hr/> |
| ☐ Carreira de bombeiro especialista | | |

9. Indique, por favor, há quanto tempo é bombeiro/GNR (caso não saiba o nº exato faça a sua melhor estimativa)

anos meses

10. Estime o nº médio de horas em que esteve exposto a um incêndio (florestal/urbano) durante a última semana.

horas

11. Durante a sua participação ativa em incêndios costuma usar equipamento de proteção individual

☐ Não ☐ Sim

11.1 Se SIM, especifique qual/quais:

12. Durante o último ano, teve de faltar ao trabalho devido à sua participação direta no combate aos fogos?

☐ Não ☐ Sim

12.1 Se respondeu SIM à questão anterior, por favor indique quais foram os motivos:

- ☐ Problemas de saúde afetos ao combate a incêndios (mau estar, doença ou ida ao médico)
- ☐ Motivos pessoais
- ☐ Outros



III. CARACTERIZAÇÃO PROFISSIONAL

13. Na sua atividade como Bombeiro/ GNR indique por favor:

13.1 Nome da Corporação/Unidade de Comando:

13.2 Serviço, sector ou unidade:

14. Assinale as funções que desempenha no Corpo de Bombeiros ou na Guarda Nacional Republicana (GNR)

- ☐ Administrativo ☐ Motorista
☐ Comando ☐ Telefonista
☐ Resgate ☐ Equipa de intervenção permanente
☐ Mergulhador ☐ Quarteiro
☐ Socorrista ☐ Outra

(se é GNR passe por favor à questão 20)

15. Quantas horas por dia permanece em média em atividades no Corpo de Bombeiros?

- ☐ menos de 8 horas ☐ entre 8 a 9 horas ☐ mais de 10 horas

16. Para além da atividade no Corpo de Bombeiros tem alguma outra atividade profissional?

- ☐ Não ☐ Sim

16.1. Se respondeu SIM à questão anterior indique qual?

- ☐ Empregado(a) a tempo inteiro
☐ Empregado(a) parcial (pelo menos 15 h semanais)
☐ Empregado(a) menos que o tempo parcial (menos de 15 h semanais)
☐ Trabalhador(a) familiar não remunerada
☐ À procura do 1º emprego
☐ Estudante/em formação profissional
☐ Doméstica/ocupa-se das tarefas do lar
☐ Reformado(a)
☐ Outra situação

17. Se empregado(a): Há quanto tempo está no seu emprego atual? anos meses

18. Se desempregado(a): Há quanto tempo está desempregado(a)? anos meses

19. Qual é a sua profissão atual? No caso de desempregado(a)/doméstica/reformado ou outra, indique a última profissão:

9382



IV. EXPOSIÇÃO OCUPACIONAL E AMBIENTAL

AMBIENTE PROFISSIONAL

20. Na área envolvente ao seu local de trabalho habitual existem indústrias, postos de abastecimento de combustível e/ou pequenos comércio (exemplo: churrasqueiras entre outros) que libertem fumos num raio de 5 a 10 km?

☐ Não ☐ Sim

20.1 Se SIM, especifique qual

21. No passado teve alguma outra profissão em que esteve exposto(a) a algum tipo de agente químico (exemplo: solventes), microbiológico ou físico (exemplo: ruído)?

☐ Não ☐ Sim

21.1. Se SIM,

Idade início	Idade fim	Agente

22. Na sua atividade no Corpo de Bombeiros ou como GNR, a que compostos está exposto(a) e com que frequência? Utiliza habitualmente algum tipo de equipamento de proteção individual (EPI)?

Exposto?	Agente?	Frequência?	Uso de EPI?	Quais?
☐ Não	☐ Fumo, gases, partículas	☐ Diária	☐ Não	☐ Ms ☐ MV ☐ Cp ☐ Lv
☐ Sim		☐ Semanal	☐ Sim	☐ S ☐ Rp ☐ AA
☐ Não	☐ Solventes (ex: combustível)	☐ Diária	☐ Não	☐ Ms ☐ MV ☐ Cp ☐ Lv
☐ Sim		☐ Semanal	☐ Sim	☐ S ☐ Rp ☐ AA
☐ Não	☐ Outro composto químico	☐ Diária	☐ Não	☐ Ms ☐ MV ☐ Cp ☐ Lv
☐ Sim		☐ Semanal	☐ Sim	☐ S ☐ Rp ☐ AA
☐ Não	☐ Radiação (ex: raios-X, beta e gama)	☐ Diária	☐ Não	☐ Ms ☐ MV ☐ Cp ☐ Lv
☐ Sim		☐ Semanal	☐ Sim	☐ S ☐ Rp ☐ AA
☐ Não	☐ Outro tipo de exposição	☐ Diária	☐ Não	☐ Ms ☐ MV ☐ Cp ☐ Lv
☐ Sim		☐ Semanal	☐ Sim	☐ S ☐ Rp ☐ AA

* M - máscara; MV- máscara com viseira; Cp - capacete; Lv- luvas; S - sapatos de proteção; Rp - roupa de proteção; AA - Aparelho de respiração Autônomo.

Se tem outra atividade profissional para além de Bombeiro/GNR, responda ainda à questão seguinte, senão passe à questão 24.



23. No seu local de trabalho, a que compostos está exposto(a) e com que frequência? Utiliza habitualmente algum tipo de equipamento de proteção individual?

Exposto?	Agente?	Frequência?	Uso de EPI?	Quais?
☐ Não ☐ Sim	☐ Solventes (ex: benzeno, metanol, xileno)	☐ Diária ☐ Semanal	☐ Não ☐ Sim	☐ Ms ☐ MV ☐ Cp ☐ Lv ☐ S ☐ Rp ☐ AA
☐ Não ☐ Sim	☐ Partículas (ex: madeiras, farinhas)	☐ Diária ☐ Semanal	☐ Não ☐ Sim	☐ Ms ☐ MV ☐ Cp ☐ Lv ☐ S ☐ Rp ☐ AA
☐ Não ☐ Sim	☐ Pesticidas	☐ Diária ☐ Semanal	☐ Não ☐ Sim	☐ Ms ☐ MV ☐ Cp ☐ Lv ☐ S ☐ Rp ☐ AA
☐ Não ☐ Sim	☐ Outro composto químico 	☐ Diária ☐ Semanal	☐ Não ☐ Sim	☐ Ms ☐ MV ☐ Cp ☐ Lv ☐ S ☐ Rp ☐ AA
☐ Não ☐ Sim	☐ Radiação (ex: raios-X, beta e gama)	☐ Diária ☐ Semanal	☐ Não ☐ Sim	☐ Ms ☐ MV ☐ Cp ☐ Lv ☐ S ☐ Rp ☐ AA
☐ Não ☐ Sim	☐ Outro tipo de exposição relevante 	☐ Diária ☐ Semanal	☐ Não ☐ Sim	☐ Ms ☐ MV ☐ Cp ☐ Lv ☐ S ☐ Rp ☐ AA

* M - máscara; MV- máscara com viseira; Cp - capacete; Lv- luvas; S - sapatos de proteção; Rp - roupa de proteção; AA - Aparelho de respiração Autônomo.

AMBIENTE FAMILIAR

24. Indique por favor qual o nome da sua:

Freguesia:

Cidade:

24.1 Qual a distância aproximada entre a sua residência e o local de trabalho? km

24.2 Contando consigo, qual o nº de ocupantes na sua habitação?

25. Vive na vizinhança (cerca 500 m) de alguma

Fábrica? ☐ Não ☐ Sim Aterro/lixeria? ☐ Não ☐ Sim Zona industrial? ☐ Não ☐ Sim

26. A sua casa encontra-se na vizinhança (cerca de 200 m) de uma área de cultivo (horta, pomar, vinha...) que seja pulverizada com pesticidas?

☐ Não ☐ Sim

27. Utiliza pesticidas em algum dos seguintes locais? Se SIM, com que frequência?

Local	Uso	Frequência	Data aprox. da última aplicação
Casa	☐ Não ☐ Sim	☐ Diária ☐ Semanal ☐ Sazonal	/ / (dd/mm/aaaa)
Jardim/horta	☐ Não ☐ Sim	☐ Diária ☐ Semanal ☐ Sazonal	/ / (dd/mm/aaaa)
Vasos	☐ Não ☐ Sim	☐ Diária ☐ Semanal ☐ Sazonal	/ / (dd/mm/aaaa)
Animais	☐ Não ☐ Sim	☐ Diária ☐ Semanal ☐ Sazonal	/ / (dd/mm/aaaa)

9382



28. Usou algum tipo de aquecimento na última semana? ☐ Não ☐ Sim

28.1 Estime o nº de dias que usou essa fonte de aquecimento nos últimos 7 dias

28.2 Que tipo de aquecimento usou na sua habitação

Fontes elétricas de calor?	☐ Não ☐ Sim	Fontes de calor a gás?	☐ Não ☐ Sim
Aquecimento central?	☐ Não ☐ Sim	Fogão a lenha?	☐ Não ☐ Sim
Lareira aberta?	☐ Não ☐ Sim	Nenhuma das opções?	☐ Não ☐ Sim
Recuperador de calor/salamandra?	☐ Não ☐ Sim		

29. Tem o hábito de acender velas decorativas e/ou incensos em casa? ☐ Não ☐ Sim

29.1 Estime o nº de dias que usou nos últimos 7 dias

30. Durante a última semana, frequentou algum local no qual esteve exposto ao fumo de velas? ☐ Não ☐ Sim

V. ESTILOS DE VIDA

ATIVIDADE FÍSICA

31. Pratica alguma atividade física/desportiva (ex: natação, ginástica, ciclismo, andar de bicicleta, caminhadas)?

☐ Não ☐ Sim

31.1 Com que frequência: ☐ Algumas vezes/ano ☐ Semanalmente dias/semana ☐ Todos os dias

CONSUMO DE ÁLCOOL

32. Consome habitualmente álcool? ☐ Não ☐ Sim (se respondeu NÃO passe para a pergunta 33)

32.1 Que tipo de bebida alcoólica bebe habitualmente? E que quantidade consome em média, por dia?

Vinho ☐ Não ☐ Sim copos/dia*

Cerveja ☐ Não ☐ Sim copos/dia*

Outra ☐ Não ☐ Sim copos/dia*

*Caso não saiba, o nº exato, indicar uma estimativa

CONSUMO DE TABACO

33. Atualmente fuma tabaco? ☐ Não ☐ Sim (se respondeu NÃO passe para a pergunta 34)

33.1. Com que idade começou a fumar? idade em anos

33.2. Quantos cigarros/maços ou mL fuma por dia?

cigarros/dia maços/dia mL/dia

34. Alguma vez fumou? ☐ Não ☐ Sim (se respondeu NÃO passe para a pergunta 35)

34.1. Com que idade começou a fumar? idade em anos

34.2. Com que idade deixou de fumar? idade em anos

34.3. Quantos cigarros/maços ou mL fumava por dia?

cigarros/dia maços/dia mL/dia

35. Nos últimos 7 dias, esteve exposto ao fumo do tabaco? Em que locais e com que frequência?

Em casa:	Nas instalações Corpo bombeiros/GNR:	No local de trabalho:	Em lazer (ex: restaurantes, cafés):
☐ Nunca	☐ Nunca	☐ Nunca	☐ Nunca
☐ Esporadicamente	☐ Esporadicamente	☐ Esporadicamente	☐ Esporadicamente
☐ Diariamente (menos de 1h)	☐ Diariamente (menos de 1h)	☐ Diariamente (menos de 1h)	☐ Diariamente (menos de 1h)
☐ Diariamente (entre 1h e 3h)	☐ Diariamente (entre 1h e 3h)	☐ Diariamente (entre 1h e 3h)	☐ Diariamente (entre 1h e 3h)
☐ Diariamente (mais de 3h)	☐ Diariamente (mais de 3h)	☐ Diariamente (mais de 3h)	☐ Diariamente (mais de 3h)

HÁBITOS ALIMENTARES

36. Por favor complete o quadro seguinte quanto ao consumo semanal, frequência e quantidade dos seguintes produtos alimentares:

	Consumo		Frequência		Quantidade
Chá (chávenas)	☐ Não	☐ Sim	☐ Diária	☐ Semanal	n° chávenas
Café (xícaras)	☐ Não	☐ Sim	☐ Diária	☐ Semanal	n° xícaras
Fruta (peças)	☐ Não	☐ Sim	☐ Diária	☐ Semanal	n° peças
Produtos hortícolas (ex: salada, sopa)	☐ Não	☐ Sim	☐ Diária	☐ Semanal	n° vezes
Produtos fumados (ex: presunto, enchidos)	☐ Não	☐ Sim	☐ Diária	☐ Semanal	n° vezes

36.1. Consumo de peixe e carne (% por semana): (Se não consumiu, coloque 0)

peixe % carne %

37. Tomou nos últimos 12 meses suplementos nutricionais (vitaminas/minerais)? ☐ Não ☐ Sim

37.1 Se SIM, qual(ais) o nome(s) e em que quantidade tomou nos últimos 12 meses?

Nome/marca comercial	Quantidade (n° de embalagens)

38. Por favor assinala o tipo de refeição mais praticado nos últimos 3 dias (selecione uma opção)

☐ assados ☐ guisados ☐ cozidos ☐ grelhados ☐ churrasco



TRANSPORTE

Neste ponto as questões referem-se ao meio de transporte que usa regularmente.

39. Como se desloca habitualmente de sua casa para o seu local de trabalho/Corporação de Bombeiros/Instalações da GNR (assinale todas as opções que usa)?

☐ a pé ☐ automóvel/mota ☐ autocarro ☐ outro

40. Estime o tempo médio de deslocação de casa para o seu local de trabalho (só ida)

☐ menos de 15 minutos ☐ 15 a 30 minutos ☐ mais de 30 minutos

41. Caso se aplique, o veículo no qual se desloca regularmente é movido a:

☐ gasolina ☐ gasóleo ☐ gás ☐ eletricidade ☐ eletricidade e gasolina (híbrido)

VI. DADOS DE SAÚDE

42. As principais exigências da sua atividade de trabalho enquanto membro da Corpo Bombeiros/GNR são:

☐ Mentais ☐ Físicas ☐ Ambas

43. Teve algum problema de saúde relevante no último ano? ☐ Não ☐ Sim

43.1 Se SIM, qual(ais)?

44. E nos últimos 2 meses? ☐ Não ☐ Sim

44.1 Se SIM, qual(ais)?

45. No último ano fez algum tipo de Exame radiológico (Raios X) (exemplo, raio x dentário)? ☐ Não ☐ Sim

45.1 Se SIM, qual(ais)?

46. No último ano foi submetido(a) a alguma intervenção cirúrgica? ☐ Não ☐ Sim

46.1 Se SIM, qual(ais)?

47. No último ano sofreu alguma transfusão sanguínea? ☐ Não ☐ Sim

48. Tomou alguma vacina nos últimos 12 meses? ☐ Não ☐ Sim

48.1 Se SIM, qual(ais)?

49. Para responder às perguntas que se seguem deve considerar o último mês (últimos 30 dias) e responder SIM apenas se os sinais/sintomas não ocorreram no contexto de gripe/constipação

Teve tosse muitas vezes na maioria dos dias? ☐ Não ☐ Sim

Teve expetoração ou "catarro" na maioria dos dias? ☐ Não ☐ Sim

Teve pieira ou sentiu o peito apertado ao respirar? ☐ Não ☐ Sim

Ao realizar as suas atividades normais do dia-a-dia sentiu-se mais cansado que as outras pessoas da sua idade? ☐ Não ☐ Sim

Teve que parar com "falta de ar" ao subir uma ladeira ou um lance de escada? ☐ Não ☐ Sim

9382



50. Atualmente toma pílula contraceptiva ou faz terapêutica hormonal de substituição?

☐ Não aplicável ☐ Não ☐ Sim Há quanto tempo? anos meses

51. Atualmente, toma medicação? ☐ Não ☐ Sim

51.1 Se respondeu que SIM à questão anterior, complete o quadro seguinte (usar MAIÚSCULAS)

Nome medicamento	Posologia	Toma	Prescrito
		☐ Crónica ☐ Temporária	☐ Não ☐ Sim
		☐ Crónica ☐ Temporária	☐ Não ☐ Sim
		☐ Crónica ☐ Temporária	☐ Não ☐ Sim
		☐ Crónica ☐ Temporária	☐ Não ☐ Sim

52. Foi-lhe diagnosticado algum dos seguintes problemas de saúde?

Neoplasias (cancro)? ☐ Não ☐ Sim Respiratórios? ☐ Não ☐ Sim Cardíacos? ☐ Não ☐ Sim

53. E na sua família direta, pais, irmãos e filhos, alguém foi diagnosticado com um dos seguintes problemas de saúde?

Neoplasias (cancro)? ☐ Não ☐ Sim Respiratórios? ☐ Não ☐ Sim Cardíacos? ☐ Não ☐ Sim



Appendix VIII – Questionnaire B.

Questionário B: Participação no combate ao incêndio

Data de preenchimento
(completar por quem está a preencher)
 / /
dia mês ano

ID

Caro participante, por favor, forneça-nos algumas informações relativas à sua participação ativa no combate a fogos.

Obrigada pela sua colaboração1. Tipo de incêndio: ☐ florestal ☐ urbano

2. Local da ocorrência

2.1. Freguesia:

2.2. Concelho:

3. Data de início do incêndio: / /
dia mês ano4. Data da extinção do incêndio: / /
dia mês ano5. Duração do incêndio: :

6. Caso se aplique, qual o tipo de vegetação do local:

7. Quantas horas esteve exposto a este incêndio? horas8. Por favor indique quais os sintomas que teve durante o combate ao fogo
(selecione todas as que se apliquem):☐ irritação dos olhos e vias respiratórias☐ dores de cabeça☐ dificuldade em respirar☐ tonturas e náuseas☐ vômitos☐ nenhum☐ Outro
9. Por favor identifique quais os seus sintomas que teve nas 8 horas após o combate ao fogo
(selecione todas as que se apliquem):☐ irritação dos olhos e vias respiratórias☐ dores de cabeça☐ dificuldade em respirar☐ tonturas e náuseas☐ vômitos☐ nenhum☐ Outro

27720



