A. COVER PAGE

Project Title: Effects of Residual PAH Exposure from Firefighters' Turnout Gear on Plasma MicroRNA Expression	
Grant Number: 1 K01 OH012688-01	**Project/Grant Period:** 9/1/2020 - 12/31/2023
Reporting Period: 1/3/2023 - 12/31/2023	**Requested Budget Period:**
Report Term Frequency: Final	**Date Submitted:**
Principal Investigator Information: Jooyeon Hwang **Email:**	**Recipient Organization:** University of Texas Health Science Center Houston

UTHealth
The University of Texas
Health Science Center at Houston

School of Public Health
Department of Environmental
& Occupational Health Sciences

RPPR Checklist:
PI: Jooyeon Hwang
Reporting Period: 1/3/2023-12/31/2023
Business Office Contact: Martinez Carmen

Formatting Guidelines:

- **Font size**: Must be 11 points or larger. Smaller text in figures, graphs, diagrams and charts is acceptable, as long as it is legible when the page is viewed at 100%. Arial, Georgia, Helvetica, Palatino Linotype fonts are recommended by NIH.
- **Type density**: Must be no more than 15 characters per linear inch (including characters and spaces).
- **Line spacing**: Must be no more than six lines per vertical inch.
- **Margins**: 0.5 inch margins.

Section B. Accomplishments

B.1. What are the major goals of the project?

The overall goal of this project is to assess the residual levels of selected compounds on firefighters' skin and on turnout gear, using exposure variables derived from work practices, and to investigate exposure-associated epigenetic assessment on skin and in the blood of firefighters. During this study, we collected a wide range of biological samples from firefighters and assessed their exposures to fire smoke. Based on this investigation, we explored the association of dermal and plasma polycyclic aromatic hydrocarbons (PAH)-associated multi-omics including miRNAs with PAH on turnout gear and on the skin of firefighters, as well as determine the occupational risk factors from pre- and post-fire-related activities.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2. What was accomplished under these goals?

The major activities accomplished were the laboratory and data analyses, the participant recruitment, and the environmental and biospecimen sample collection in the field. For this study, 74 firefighters (see G.4 Human Subjects) – 68 male and 6 female – have participated from 12 fire departments in Oklahoma and Texas. As this study relies on emergency live fire calls, which are unpredictable, the sampling campaigns at some of the departments have been initiated with baseline measurements. The specific goals were to examine the laboratory and data analyses. For *Aim 1* (Assess the associations between residual levels of PAH on firefighters' turnout gear and on skin with exposure variables derived from work practices), the environmental and biochemical contaminants on gear from pilot samples were tested and the analytical method validated using a Varian 450 Gas Chromatography equipped with a 200 Mass Spectrometry detector. The collected samples from the participants were analyzed using EPA Method 8310. For *Aim 2* (Assess the PAH levels on skin and the PAH-associated miRNA expression on skin and in blood), while we developed an innovative analytical method for deriving miRNA expression from the skin samples, we have been also preparing the serum samples to process miRNA as well as multi-omics studies including epigenetic, transcriptomic, proteomic, metabolomic, and microbiome (see below lists of peer-reviewed manuscripts in B.2.I and B.6). While analyzing this specific aim with miRNA, we successfully identified and quantified 1,325 unique proteins through the proteomic platform. The significant results of prep multi-omics in collected biospecimens showed 45 proteins differential expressions in abundance in response to fire-smoke exposure compared to the baseline. From pathway analysis, we found proteins associated with epithelium development (e.g., RHCG, HEG1, ADAMTSL2) and Alzheimer's disease (SORL1) were significantly increased in response to smoke exposure samples. The following tasks, organized by key outcomes, have been accomplished:

I. Published peer-reviewed manuscripts (*Corresponding author)

1) **Jooyeon Hwang*,** Zongkai Peng, Fares Z Najar, Chao Xu, Robert J Agnew, Xin Xu, Zhibo Yang, Nagib Ahsan*, Urine proteome profile of firefighters with exposure to emergency fire-induced smoke: A pilot study to identify potential carcinogenic effects, Science of the Total Environment, 2024, 172273, DOI: https://doi.org/10.1016/j.scitotenv.2024.172273
2) Jake Mitchell, **Jooyeon Hwang,** Preston Larson, Sumit Mandal, Robert J. Agnew. Preliminary analyses of accumulation of carcinogenic contaminants on retired firefighter ensembles, Journal of Occupational and Environmental Hygiene, 2024 PMID: 38416517 DOI: https://doi.org/10.1080/15459624.2023.2296628
3) **Jooyeon Hwang***, Ngee-Sing Chong, Mengliang Zhang, Robert J. Agnew, Chao Xu, Zhuangjie Li, Xin Xu. Face-to-face with Scorching Wildfire: Potential Toxicant Exposure and the Health Risks of Smoke for Wildland Firefighters at the Wildland-Urban Interface, Lancet Reg Health Am, 2023 Mar 26;21:100482. PMID: 37008196 PMCID: PMC10060103 DOI: 10.1016/j.lana.2023.100482.
4) **Jooyeon Hwang***, Chao Xu, Paul Grunsted, Robert J. Agnew, Tara R. Malone, Shari Clifton, Krista Thompson, Xin Xu. Urinary Metabolites of Polycyclic Aromatic Hydrocarbon in Firefighters: A Systematic Review and Meta-Analysis, Int J Environ Res Public Health, 2022, 19(14):8475, PMID: 35886320 PMCID: PMC9318785 DOI: 10.3390/ijerph19148475
5) **Jooyeon Hwang***, Chao Xu, Robert J. Agnew, Shari Clifton, Tara R. Malone. Health Risk of Structural Firefighters from Exposure to Polycyclic Aromatic Hydrocarbons: A Systematic Review and Meta-Analysis, International Journal of Environmental Research and Public Health, 2021, 18(8), 4209, PMID: 33921138, PMCID: PMC8071552 DOI: https://doi.org/10.3390/ijerph18084209

II. Logistics and required tasks for the study:

With my relocation from the University of Oklahoma Health Sciences Center (OUHSC) to the University of Texas Health Science Center (UTHealth) on January 3, 2023, I have managed the logistical components of the study to ensure an uninterrupted transition.

1) Received approval for NIOSH NCE (4 months)
    - Original study period: 09/01/2020 – 08/31/2023
    - NCE period: 09/01/2023 – 12/31/2023
2) Completed MTA (Material Transfer Agreements) and approved IRB
    - Relocated the collected biospecimens from the OUHSC (previous institution) to the UTHealth (new institution) with cold-chain protocols.
    - Approved OUHSC IRB (#11466) with Dr. Willard M. Freeman from the Oklahoma Clinical and Translational Science Institute (OCTSI) as a PI.
    - Approved UTHealth IRB (#HSC-SPH-23-0085).
3) Participated in a laboratory session on assessing RNA integrity and nucleic acid at the Laboratory for Molecular Biology and Cytometry Research (LMBCR).

III. Coordinate with partnering fire service and medical personnel:

1) Launched *new* partnerships with the Houston Fire Department and the City of Houston for conducting pilot studies at emergency fires and fire academy in Houston, Texas.
2) Presented Research Progress (title: Evaluating Carcinogenic Exposure and Health Effects in Firefighters During Emergency Fires) at the participating fire departments in Oklahoma.
3) Discussed the upcoming studies with metro fire chiefs in Oklahoma and Texas.
4) Expanded study cohorts from the fire departments in Brownsville, San Antonio, Austin, and El Paso in Texas.

IV. Expand the research partnerships to include:

1) State-of-the-art laboratories for multi-omics studies:
    - Human Genetic Center, University of Texas Health Science Center
    - Metabolomics Core, University of Texas MD Anderson
    - Alkek Center for Metagenomics & Microbiome Research, Baylor College of Medicine

- Proteomics Core Facility, Stephenson Life Sciences Research Center, University of Oklahoma

2) Department of Environmental and Public Health Sciences, College of Medicine, University of Cincinnati
3) Department of Earth & Atmospheric Sciences, University of Houston
4) Department of Chemistry, Middle Tennessee State University
5) Department of Chemistry and Biochemistry, California State University at Fullerton
6) Department of Environmental Health and Engineering, Johns Hopkins University
7) Fire and Emergency Services Administration Program at Texas A&M University

V. Articulate further research goals:

Awarded pilot project:

1) Unveiling the Impact of Fire Smoke Neurotoxicants on Firefighters' Mental Health using Whole Shotgun Metagenomics, NIH/NIEHS P30 Gulf Coast Center for Precision Environmental Health (GC-CPEH), **Principal Investigator**, $40,000, 09/01/2024-08/31/2025

Submitted proposals with a larger scope of proposed research:

1) Carcinogenic Exposures at Wildland Fires: Integrated Proteomic and Metagenomic Approach, NIH/NIEHS R01, **Principal Investigator,** Submitted in Cycle III of 2023, Scored 31 (18 percentile)
2) Impact of Inhaled Ambient Fire Smoke on Brain Health in Wildland Firefighters, NIH/NIEHS R01, **Principal Investigator,** Re-submitted in Cycle I of 2024

B.4. What opportunities for training and professional development has the project provided?

The project during the reporting period has provided me with the opportunity to engage and develop my career as follows:

I. Courses/training

1) Rigor and Reproducibility Workshop, Gulf Coast Consortia for Quantitative Biomedical Sciences, October 2023

II. Service

1) Programmatic Panel Member, Toxic Exposure Research Program (TERP), Congressionally Directed Medical Research Programs (CDMRP), Department of Defense, Washington D.C.
2) Ad hoc external reviewer, Intramural research project proposal, Respiratory Health Division, National Institute for Occupational Safety and Health (NIOSH), Morgantown, WV
3) Ad hoc reviewer, NIOSH Safety and Occupational Health Study Section, Washington D.C
4) Ad hoc external expert, NFPA 1989 Standard on Breathing Air Quality for Emergency Services Respiratory Protection Subcommittee (Task Group 4 & 6), Virtual
5) CBRNE Safety Working Group of the City of Houston, Houston Health Department, Houston, TX
6) Academic advocator, The community-based challenges and solutions around advancing sustainability and environmental protection in Houston, Axios Expert Voices roundtable discussion, Houston, TX
7) Ad hoc scientific advisor, Addressing a health concern of a permanent rock crushing facility next to a regional hospital, Harris Health System, Houston, TX

III. Submitted Grants

Through my engagement in the scholarly activities listed above, I have expanded my research base by successfully working with various collaborative teams for research grant proposals.

1) Para-occupational exposure to toxic metals in Mexican-American residential homes in Starr County, Texas, PRIME (Funding for Preliminary Studies for New Investigators), The University of Texas Health Science Center at Houston School of Public Health, **Principal Investigator**
2) Collaborative Research: SCH: Cyber-Physical System of Actionable Prognosis regarding Respiratory Distress and Pulmonary Health Risk for Construction Dust-Exposed Workers, NSF (National Science Foundation), **Collaborative Principal Investigator** (Contact PI: S. Chang)
3) Metaproteomic Exploration of Carcinogenic Exposure Due to Fire Smoke, CPRIT HIHR (Cancer Prevention & Research Institute of Texas High-Impact/High-Risk), **Principal Investigator**
4) Helping low-income households in San Antonio breathe healthier air, McCombs Foundation, **Co-I**

(PI: D. Gimeno)
5) Automatic Airborne Asbestos Detection, Counting, and Sizing Using Machine Learning, CDC/NIOSH R01, **Multi-Principal Investigator** (Contact PI: C. Cai),
6) Characterizing environmental and community factors influencing public health outcomes in the arid Paso del Norte Region, Burroughs Wellcome Fund/Climate and Health Interdisciplinary Award, **Co-I** (PI: A. Gitter)

B.5. How have the results been disseminated to communities of interest?
This study has received great attention from both scientific research and fire service communities. Throughout the reporting period (1/3/2023-12/31/2023), I have shared preliminary or ongoing progress results with both communities of interest, as indicated below:

1) PAH Exposure from Turnout Gear in Firefighters, Translational Research Seminar Series (TRSS), UTHealth at Houston School of Public Health, Brownsville Campus, Brownsville, Texas (Invited speaker)
2) Metro Chiefs & Green Country Chiefs Joint Meeting, Stillwater, Oklahoma (Invited co-speaker)
3) 36th annual US-Korea Conference (UKC 2023), Technical Group Symposium, Medical Science, Pharmaceutical Science, Veterinary Medicine, Physical Education (MPS), Dallas, Texas (Invited speaker)
4) The Effects of Residual Polycyclic Aromatic Hydrocarbons on the Hematological Profile of Firefighters, American Industrial Hygiene Conference & Expo, Phoenix, AZ (Academic advisor for Student Poster presenter, Mingze Zhu). *1st Place Student Poster Awards by 1) Protective Clothing and Equipment Committee and 2) Biological Monitoring Committee
5) Assessment of Firefighters' Health Risks in the Work Environment, Golf Coast American Society of Safety Professionals (ASSP) chapter meeting, Deer Park, Texas (Invited speaker)
6) The Impact of Climate Change on Firefighters' Health, Annual student-led conference Global Issues Summit, Global Studies Academy, Travis High School, Richmond, Texas (Invited speaker)

B.6. What do you plan to do during the next reporting period to accomplish the goals?
This K01 award ended on December 31. 2023. Despite this being the final RPPR, I plan to publish all ten listed peer-reviewed manuscripts in 2024-2025. As a corresponding author (* denoted), I have ensured that all manuscripts feature a robust study design, an up-to-date literature review, and unbiased data analysis with multiple verification steps. All manuscripts also will undergo a comprehensive review by all co-authors and institutional review processes.

1) **Jooyeon Hwang***, Jenny Gipson, Chao Xu, Robert J. Agnew, Willard M. Freeman. Comparison of MicroRNA Profiling in the Skin and Serum of Volunteer and Career Firefighters (Expected Submission: 2024)
2) Mingze Zhu, Robert J. Agnew, Chao Xu, Timothy VanWagoner, Brittany Karfonta, Xin Xu, **Jooyeon Hwang***. Residual Polycyclic Aromatic Hydrocarbons and Firefighters' Hematological Profile, Frontiers in Environmental Health (in review), 2024
3) Paul Grunsted, Chao Xu, Amanda Janitz, Robert J. Agnew, **Jooyeon Hwang***. Understanding of Oklahoman fire incidents using National Fire Incident Reporting System (NFIRS) and the gaps (Expected Submission: 2024)
4) Mingze Zhu, Chao Xu*, **Jooyeon Hwang***. Meta-analysis of Transcriptome Responses Induced by the Carcinogen Benzo[A]Pyrene (Expected Submission: 2024)
5) Yao Jin, **Jooyeon Hwang**, Xin Xu. Metabolomic Profiling of Urinary Changes in Firefighters Pre- and Post-Heavy Load Ascent Exercise (Expected Submission: 2024)
6) Minglun Wang**,** Chao Xu, Robert J. Agnew, Xin Xu, **Jooyeon Hwang***. Plasma Metabolomics for Predicting Metabolic Biomarkers among Firefighters (Expected Submission: 2025)
7) **Jooyeon Hwang***, Nagib Ahsan, Chao Xu, Robert J. Agnew, Xin Xu. Effects of Fire-Smoke Exposure from Emergency Fire Using Serum Proteomic Profiling (Expected Submission: 2025)
8) Minglun Wang**,** Chao Xu, Robert J. Agnew, Xin Xu, **Jooyeon Hwang***. DNA Methylation Changes in Firefighters' Buffy Coat Before and After Emergency Fire Response (Expected Submission: 2025)
9) Chao Xu, Robert J. Agnew, Nagib Ahsan, Xin Xu, **Jooyeon Hwang***. Integrated Transcriptome and Proteome Profiles Revealed the Carcinogenic Effects of Emergency Fire-Induced Smoke Exposure

(Expected Submission: 2025)

10) **Jooyeon Hwang***, Sherri Castle, Bryce Lowery, Susan Sisson, Mike Wimberly, Melissa Van Cain, Chao Xu. Longitudinal Effect of Environmental Exposure to Pollen and Air Pollution on the Well-Being of Children in Oklahoma (Expected Submission: 2025)

Section C. Products

C.1. Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication and monograph) during the reporting period resulting directly from this award?
See B.2.I

C.2. Website(s) or other internet site(s).

1) "Potential Toxicant Exposure and Health Risks of Smoke for Wildland Firefighters" Southwest Center for Occupational and Environmental Health (SWCOEH), Written by Matthew Pedersen, Published April 20. https://sph.uth.edu/research/centers/swcoeh/news/potential-toxicant-exposure-and-health-risks-of-smoke-for-wildland-firefighters
2) "Does Bad Air Cause Cancer? How to Protect Yourself from Wildfire Smoke in the Air" Survivornet, Written by Liz Lane, Published June 8. https://www.survivornet.com/articles/bad-air-quality-wildfire-smoke-cancer-protect-yourself/
3) "Fairfield Raising Money to Support two Firefighters now Fighting Cancer" CBS News Sacramento, Reported by Sade Browne, Published July 29. https://www.cbsnews.com/sacramento/news/fairfield-raising-money-to-support-two-firefighters-now-fighting-cancer/
4) "SWCOEH Industrial Hygienists partner with Houston Fire Department for Neurotoxicant Study", Written by Matthew Pedersen, Published Jan 23. https://sph.uth.edu/research/centers/swcoeh/news/swcoeh-industrial-hygienists-partner-with-houston-fire-department-for-neurotoxicant-study

C.3. Technologies or techniques.
We have developed an innovative analytical method for deriving miRNA expression from skin samples. This will be highlighted as one of the key features in our upcoming manuscript (Title: Comparison of MicroRNA Profiling in the Skin and Serum of Volunteer and Career Firefighters, Expected Submission: 2024)

C.4. Inventions, patent applications, and/or licenses.
Have inventions, patent applications and/or licenses resulted from the award during this reporting period?
No

C.5. Other products and resource sharing.
I prepared slides and handout materials for each of my presentations (see B.5).

Section D. Participants

D.1. What individuals have worked on the project?
I joined the University of Texas Health Science Center at Houston on January 3, 2023, where my primary role is Associate Professor in the Department of Epidemiology, Human Genetics and Environmental Sciences. Additionally, I hold the position of Deputy Director at the Southwest Center for Occupational and Environmental Health, one of the NIOSH ERC.

PI: Jooyeon Hwang (9 Calendar months equivalent to 75% effort)
All other contributors (listed as an author in B.2.I and B.6) have worked less than a month per year on this project during the reporting period.

D.2.a. Level of Effort
Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the

Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?
Not Applicable.

D.2.b. New Senior/Key Personnel
Are there, or will there be, new senior/key personnel?
Not Applicable.

D.2.c. Changes in Other Support
Has there been a change in the active other support of senior/key personnel since the last reporting period?
No

D.2.d. New Other Significant Contributors
Are there, or will there be, new other significant contributors?
Not Applicable.

Section E. Impact

E.2 What is the impact on physical, institutional, or information resources that form infrastructure?
An R01 proposal was built on the exposure-associated multi-omics research acquired during this career development K01 award, and leverages preliminary data acquired in that research project. Based on extensive deliberations with omics specialists on the study team, I have assembled a highly interdisciplinary research team (see B.2.IV. Expand the research partnerships) that will collaborate to ensure the success of upcoming studies. The team will use omics approaches including epigenetics, transcriptomics, metabolomics, proteomic and metagenomic profiling, and state-of-the-art integrated omics (e.g., proteome-metagenome) analyses to identify the chronic, adverse effects of exposure to fire smoke on firefighters' health, targeting cancer.

Our dissemination strategy aims to raise awareness of the significant role fire smoke plays in fire service communities. The team and I have presented the study's objectives, research progress, and conducted Q&A sessions at least three times throughout the study – at baseline, post-fire events, and follow-up discussions – during various meetings with firefighters and fire chiefs. One of our key messages to the firefighter communities emphasizes the importance of adhering to recommended practices to maintain their gear and respirators in good condition as they heavily rely on the personal protective equipment (PPE) practices.

E.4. What dollar amount of the award's budget is being spent in foreign country(ies)?
Nothing to Report.

Section F. Changes

F.2 Actual or anticipated challenges or delays and actions or plans to resolve them
Given the world-wide pandemic raging at the beginning of this project, I requested a No-Cost Extension (NCE) to complete this field-based study according to our progress. NIOSH approved a 4-month extension (08/30/2023-12/31/2023). At the beginning of the pandemic, we conducted limited monitoring in the field, especially with respect to the biospecimen (blood/dermal) collection from the study cohort, given that it is composed of front-line responders. As the pandemic waned, we exceeded our goal in recruiting study participants. So far, we have collected over 800 environmental and biospecimen samples from 74 enrolled firefighters for this study.

F.3.a Human Subjects
No Change.

F.3.b Vertebrate Animals
Not Applicable.

F.3.c Biohazards

Not Applicable.

F.3.d Select Agents
Not Applicable.

Section G. Special Reporting Requirements

G.1. Special Notice of Award and Funding Opportunity Announcement Reporting Requirements
None

G.4.b Inclusion Enrollment Report
As stated, we exceeded our goal in recruiting study participants. We recruited >4 times as many participants as initially planned (n=18), and enrollment is still ongoing. These participants will be followed up in the longitudinal study. Please see attached Enrollment Report Table.

G.5 Human Subjects Education Requirement
Are there personnel on this project who are or will be newly involved in the design or conduct of human subjects research?
No

G.6. Human Embryonic Stem Cells (hESCs)
Does this project involve human embryonic stem cells?
No

G.9. Foreign Component
Has significant scientific activity for this grant been performed outside of the United States, whether or not grant funds were expended?
No foreign component.

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?
No

H. Budget

H.1 Budget Form
Please see the attached form, which shows that $5,734.98 allocated for this study remained unspent.

I. Outcomes of the Award

Project: Effects of residual PAH exposure from firefighters' turnout gear on plasma microRNA expression
Contact PI: Jooyeon Hwang
Organization: University of Texas Health Science Center

Refer to the peer-reviewed publications in Section B.2.I for this study's outcomes. The progression of this study, from addressing reviewer comments to analyzing bioinformatic data, has its results compiled in Section B.6, with direct findings itemized as follows:

1) **Jooyeon Hwang***, Jenny Gipson, Chao Xu, Robert J. Agnew, Willard M. Freeman. Comparison of MicroRNA Profiling in the Skin and Serum of Volunteer and Career Firefighters
2) Mingze Zhu, Robert J. Agnew, Chao Xu, Timothy VanWagoner, Brittany Karfonta, Xin Xu, **Jooyeon Hwang***. Residual Polycyclic Aromatic Hydrocarbons and Firefighters' Hematological Profile
3) Paul Grunsted, Chao Xu, Amanda Janitz, Robert J. Agnew, **Jooyeon Hwang***. Understanding of Oklahoman fire incidents using National Fire Incident Reporting System (NFIRS) and the gaps
4) Mingze Zhu, Chao Xu*, **Jooyeon Hwang***. Meta-analysis of Transcriptome Responses Induced by the Carcinogen Benzo[A]Pyrene

5) Yao Jin, **Jooyeon Hwang**, Xin Xu. Metabolomic Profiling of Urinary Changes in Firefighters Pre- and Post-Heavy Load Ascent Exercise
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7) **Jooyeon Hwang***, Nagib Ahsan, Chao Xu, Robert J. Agnew, Xin Xu. Effects of Fire-Smoke Exposure from Emergency Fire Using Serum Proteomic Profiling
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