



THE UNIVERSITY OF ARIZONA

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Cancer Research: Updates on the Breast Milk Study and Other Studies

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Women Firefighters' Health and Wellness Conference

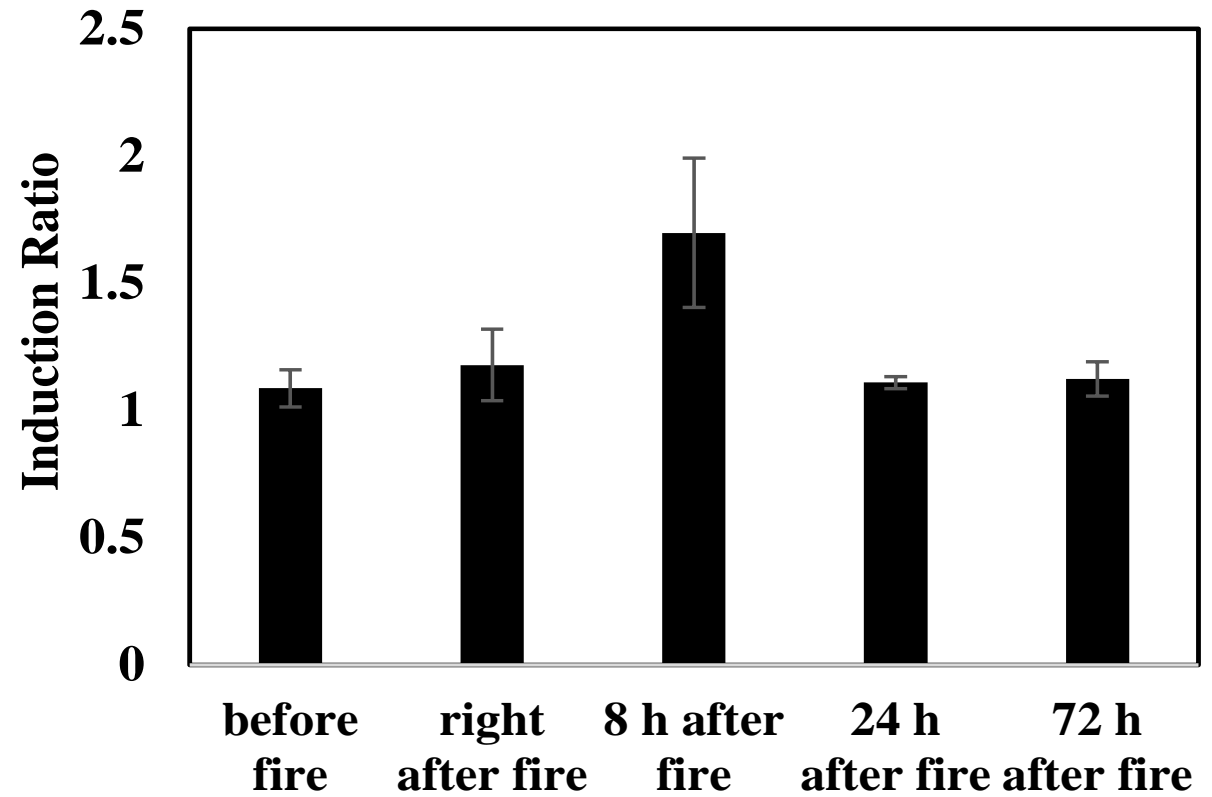
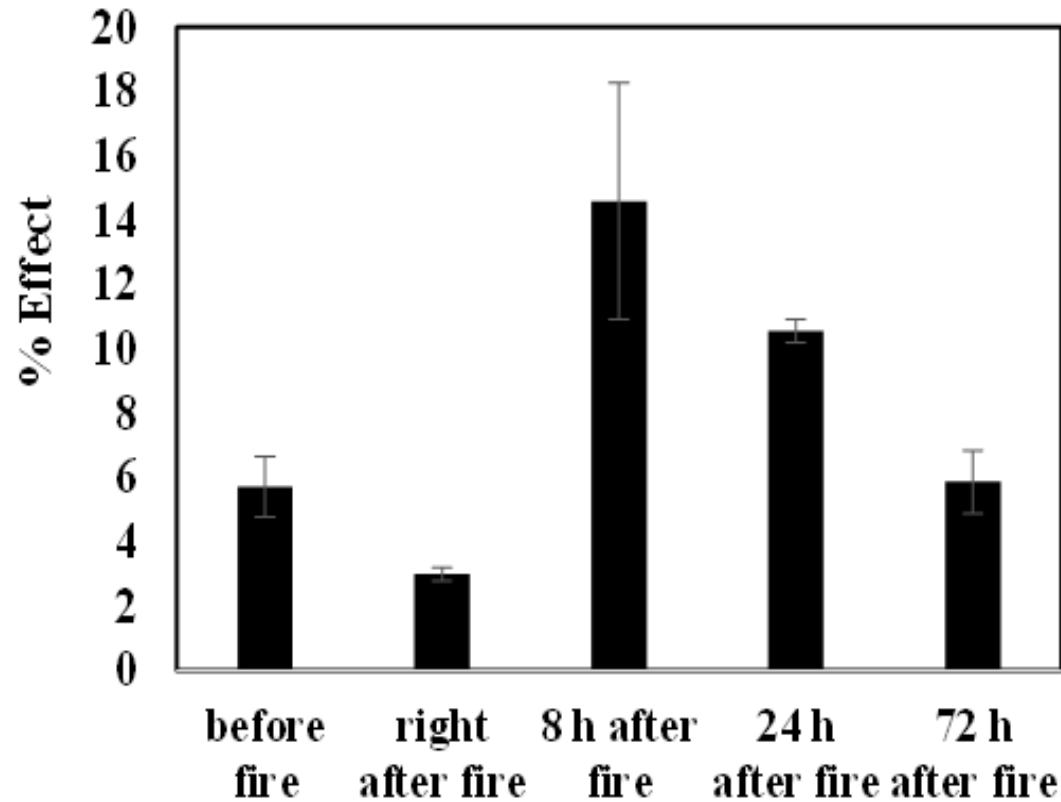
November 8, 2019

Florian Hall - Boston, MA

Fire Service Question

- Are toxic chemicals present in firefighter breast milk before and after a fire response?

Pilot Study



Firefighter breast milk extract *in vitro* analyses: a) AhR response and b) genotoxicity

- Interim recommendation to pump and discard breast milk for 72 hours after a fire

Current Study Objectives

- Collect breast milk from firefighters (n=20) at baseline (1 sample) and at intervals from as soon as possible to 72 hours after a structure fire (5 samples)
- Evaluate brominated fire retardant concentration and toxicity (using AhR assay) in breast milk samples
- Compare to breast milk (1 sample) from non-firefighters

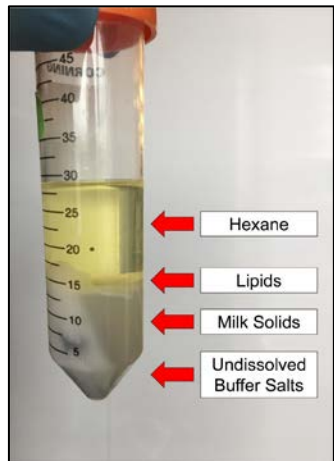
Funding: FEMA EMW-2015-FP00848

Methods - Recruitment & Sample Collection

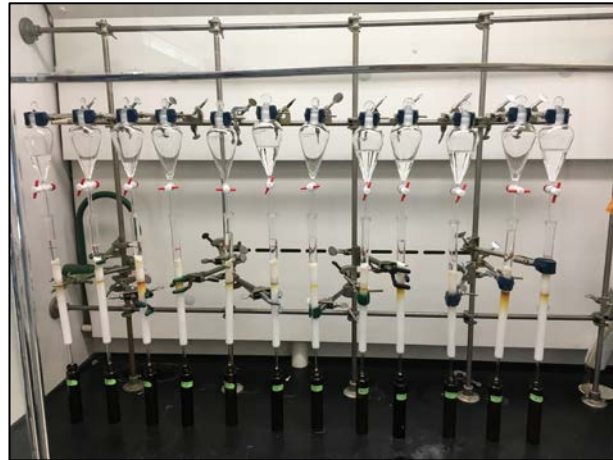
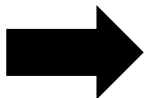
- Subject recruitment
 - Emailed firefighters who completed the NDRI Health and Wellness survey and were willing to be re-contacted
 - Advertised through the Secret List
- Sample Collection (100 ml at each interval)
 - Baseline (with no fire suppression activity for at least 3 days)
 - Post-Fire (immediately after and at 8-10, 24, 48, and 72 hours)
- Baseline and post-fire surveys
- To date: 27 post-fire collections (24 structure, 1 dumpster, 2 training) and 10 non-firefighter baseline samples

Extraction and Testing

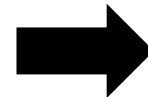
- Compounds isolated from the lipids and proteins in the milk
- Toxicity (AhR bioassay) and contaminants (PBDEs) analyzed



QuEChERS



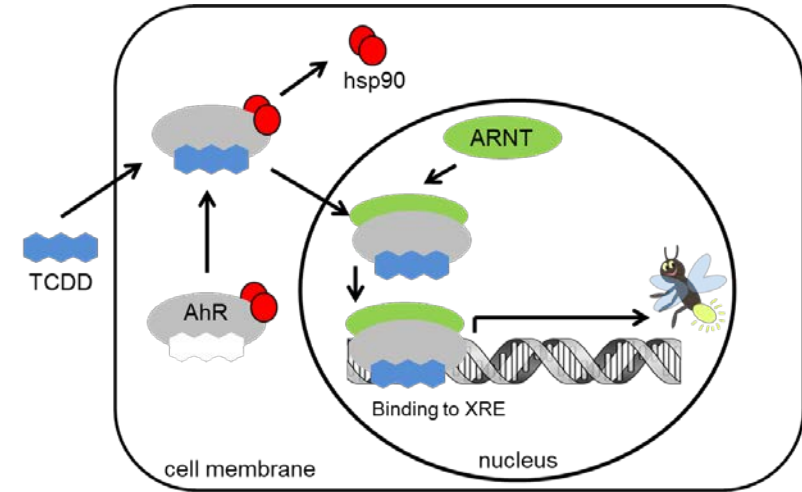
Acidified Silica Cleanup



Gel Permeation
Chromatography



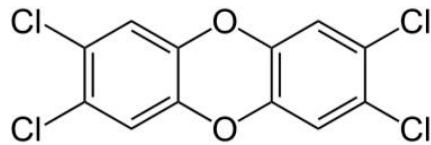
GC-MS/MS



In vitro bioassay

Aryl Hydrocarbon Receptor (AhR)

- AhR regulates response to planar aromatic hydrocarbons such as:
 - dioxins (2,3,7,8-tetrachlorodibenzodioxin used to establish standard curve),
 - polycyclic aromatic hydrocarbons (PAHs),
 - polychlorinated biphenyls (PCBs),
 - polybrominated diphenylethers (PBDEs) and
 - polybrominated dibenzofurans (PBDFs)
- Multiple health consequences
 - Tumor promotion, teratogenesis and immunosuppression



Dioxin



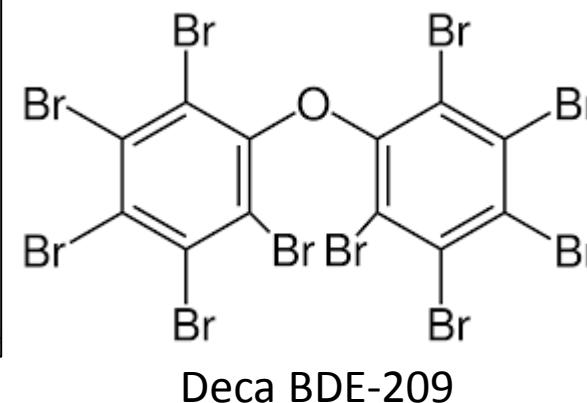
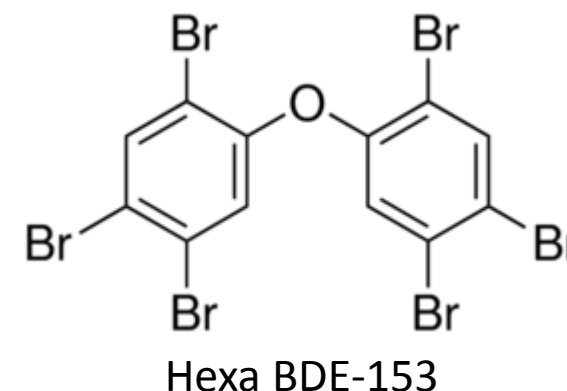
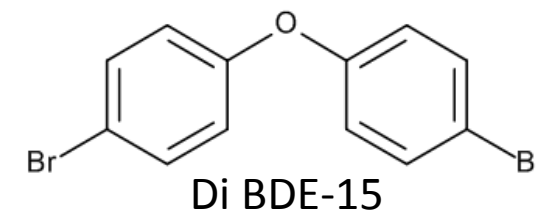
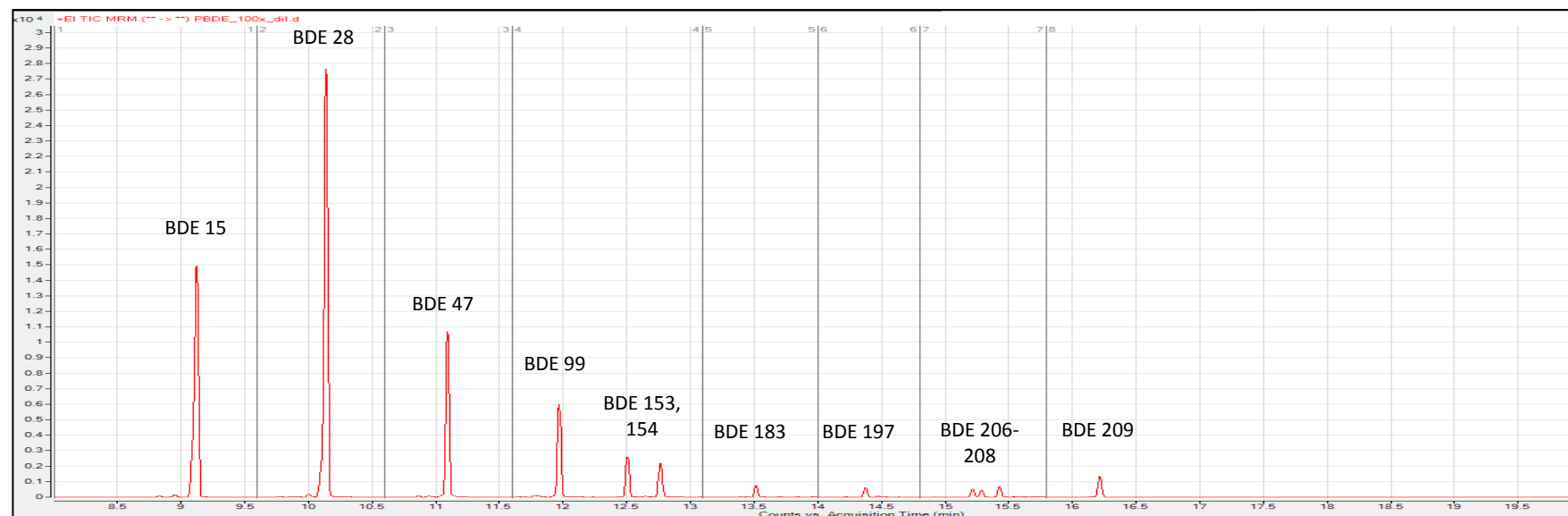
Phenanthrene



Pyrene

Brominated Flame Retardants

- Found in numerous consumer products
- Similar in structure to PCBs, dioxins and furans
- Accumulate in fatty tissues
- PBDE exposure is associated with cancer, neurodevelopmental toxicity and thyroid toxicity



Preliminary Results

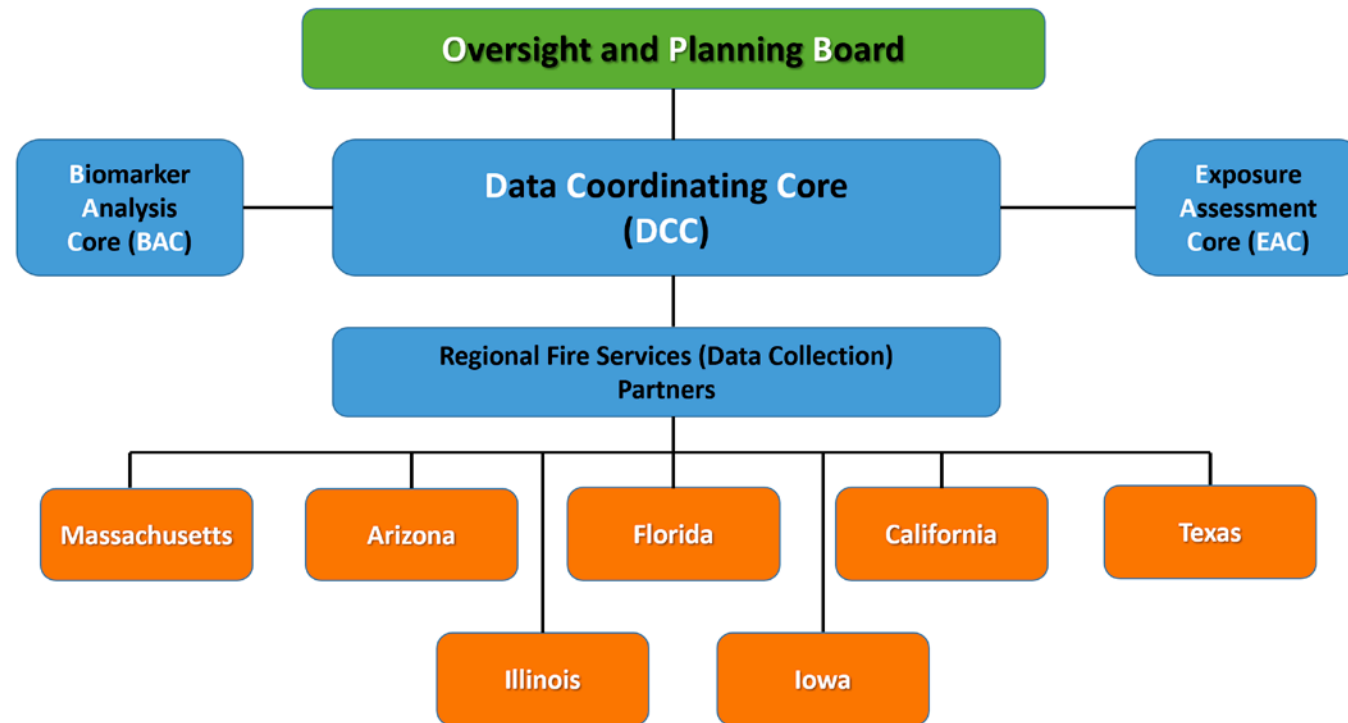
- Results are preliminary at this time and not ready for release outside of the conference

Breast Milk Study Next Steps

- Complete PBDE analysis
- Potentially add genotoxicity or mutagenicity assays
- Explore options for evaluation of additional contaminants
- Formulate recommendations for the fire service
- Prepare results for publication and dissemination

Fire Fighter Cancer Cohort Study (FFCCS)

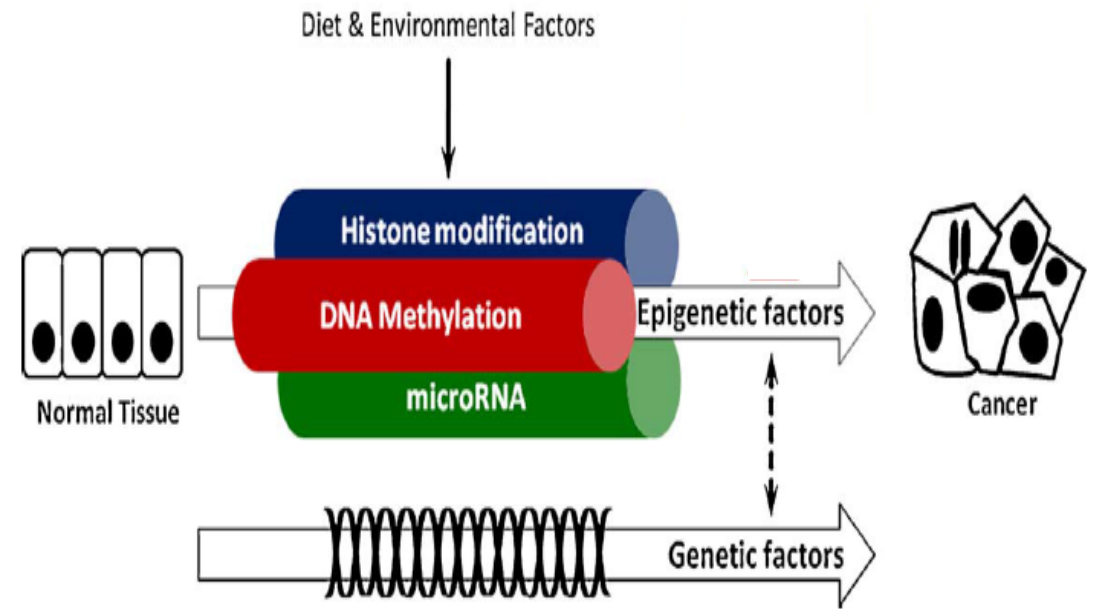
Goal: Establish a large prospective multicenter study focused on carcinogenic exposures and effects



Funding: FEMA AFG EMW-2015-FP-00213 & EMW-2017-FP-00860

Epigenetic Changes

- Change in gene expression without changes in DNA sequence
- Profound roles in carcinogenesis
- DNA hypermethylation silences tumor suppressor genes
- microRNA: small molecules that control gene expression
 - Can act on oncogenes or tumor suppressor genes



Link et al. Biochem Pharmacol 2010;80:1771-92.

DNA Methylation Pathway Analysis

Disease annotation	p-value	# genes	Hub genes
Abdominal cancer	5.1e-18	88	STAT3, TP63, TP73, FOXO1, PML, DAXX, RUNX2, INSR, PCNA
Colon tumor	5.9e-09	44	STAT3, TP63, TP73, FOXO1, DAXX, RUNX2, INSR, PCNA
Skin cancer	2.9e-07	51	STAT3, TP63, PML, DAXX, RUNX2, INSR
Lung tumor	6.6e-07	49	INSR, PCNA, STAT3, TP63, TP73

Zhou et al. *PLoS One*. 2019 Mar 26;14(3):e0214282.

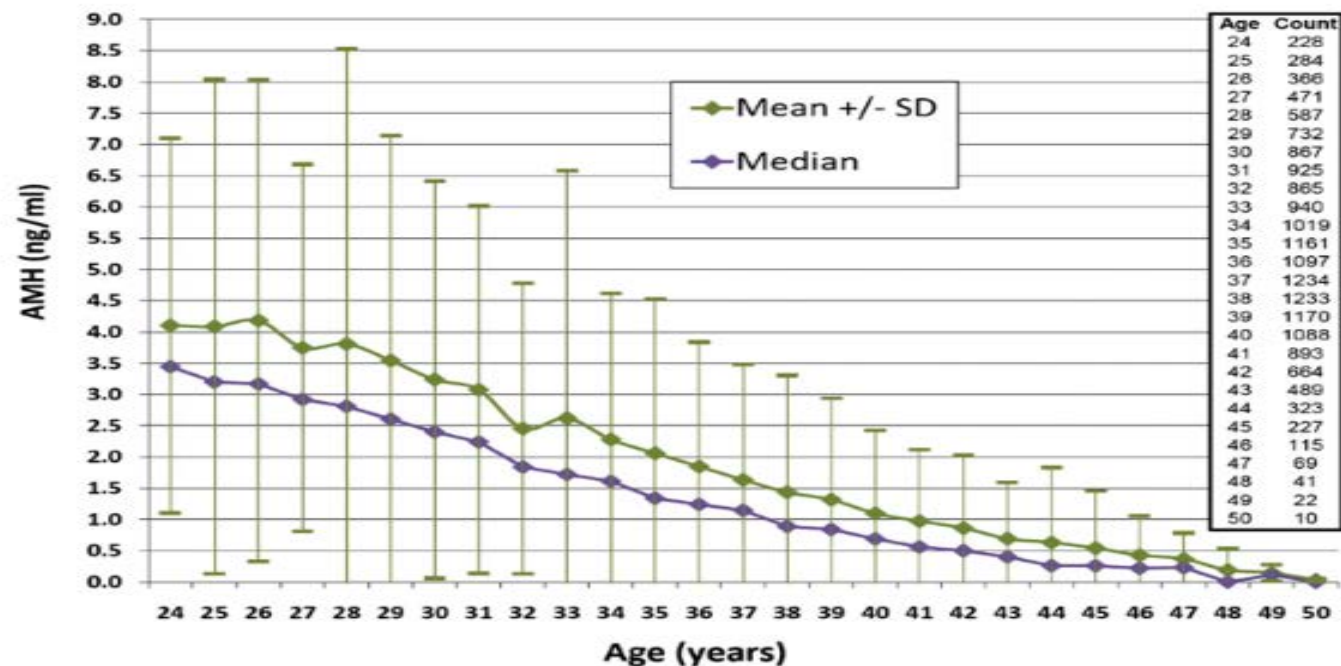
MicroRNA Results

	Incumbents vs. new recruits*				
miRNA Name	FC	95% CI		Role in cancer	Select cancer associations
miR-1260a	0.55	0.43	0.71	Tumor suppressor	Brain (glioblastoma)
miR-548h-5p	0.59	0.51	0.69	Tumor suppressor	Cervical and lung
miR-145-5p	0.44	0.32	0.61	Tumor suppressor	Prostate
miR-4516	0.56	0.48	0.65	Tumor suppressor	Apoptosis of keratinocytes
miR-331-3p	0.60	0.52	0.70	Tumor suppressor	Prostate & colorectal
miR-181a-5p	0.62	0.53	0.72	Tumor suppressor	Lung (Non-small cell)
miR-5010-3p	1.59	1.41	1.81	Oncogene	Colon
miR-374a-5p	1.72	1.40	2.13	Oncogene	Esophageal & gastric
miR-486-3p	3.35	2.59	4.33	Oncogene	Colorectal

*Fold changes of incumbents (n=52) compared to new recruits (n=45), adjusted for age, obesity and ethnicity, male non-smokers only (Jeong et al., *J Occup Environ Med.* 2018;60(5):469-474)

New AMH Study

- Antimüllerian Hormone (AMH) secreted by the granulosa cells and is a clinical marker of ovarian reserve
- AMH decreases with age and can be used to predict early menopause and time to final menstrual period



AMH and Environmental Exposures

- Cigarette smoking is associated with an increased risk for infertility and menopause at an earlier age
- Current smokers have 44% lower AMH values
 - Past and passive smokers may also have lower AMH
- Burning fuel for heating and cooking has also been associated with lower AMH values
- Polycyclic aromatic hydrocarbons cause oocyte destruction in mice
- Little is known about exposures in firefighters in relation to AMH



AMH Study Aims

1. Investigate whether AMH levels are lower in women firefighters compared to women that are not firefighters
2. Investigate whether there is a dose-dependent relationship between years of firefighting and AMH levels
3. Among female firefighters with previously measured PFAS levels and epigenetic (blood microRNA and DNA methylation) markers, investigate whether there is a relationship with AMH

Study Logistics

Spring and Summer 2020:

- Recruit female fire fighters and a comparison group of non-firefighting women
- Participants will be asked to:
 - Complete <30 minute questionnaire related to reproductive history, firefighting history, and environmental exposures
 - Provide and return blood spot to measure AMH



Interested in being involved?

- ✓ Premenopausal, female firefighter
- ✓ Willing to provide a dried blood spot and respond to a questionnaire

Great!

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