Project Overview

What's in the Smoke from Prescribed Fire?

Project Context & Background

The goal of this project is to measure polycyclic aromatic hydrocarbons (PAHs) in fine particulate matter generated during ignition and smoldering stages of prescribed burns in northern Arizona.

Scientists are interested in the organic composition of fine particulate matter because of its potential impact on health, climate, and visibility.

Several studies have examined PAH levels in wood smoke during residential wood burning, but only a few have examined particle-phase PAHs generated during prescribed burns.

Who is This Project For?

This project is best suited for chemistry majors interested in environmental, analytical, or atmospheric chemistry, as well as students who want to learn more about gas chromatography/mass spectrometry (GC/MS) as an analytical tool.

What Will I Do?

In this project, you will analyze PAH concentrations in $PM_{2.5}$ from three different prescribed burns using GC/MS. Prescribed burns are analyzed during the ignition and smoldering phases.

Before (above) and after (below) prescribed fire

Canned Research Project 2

What Will I Learn About?

- Role of prescribed fire in forest restoration

- $PM_{2.5}$ and PAHs – what they are and why they're important

- Soxhlet extraction and Kuderna-Danish concentration

- Use of surrogates & internal standards in GC/MS

Note: Some familiarity with Excel is required.

To Learn More

Fine, P. M.; Cass, G. R.; Simoneit, B. R. T. Chemical characterization of fine particle emissions from fireplace combustion of woods grown in the Northeastern U.S. *Environ. Sci. Technol.* **2001**, *35*, 2665–2675.

Hays, M. D.; Geron, C. D.; Linna, K. J.; Smith, N. D.; Schauer, J. J. Speciation of Gas-Phase and Fine Particle Emissions from Burning of Foliar Fuels. *Environ. Sci. Technol.* **2002**, *36*, 2281–2295.

Lee, S.; Baumann, K., Schauer, J. J.; Sheesley, R. J.; Naeher, L. P.; Meinardi, S.; Blake, D. R.; Edgerton, E. S.; Russell, A. G.; Clements, M. Gaseous and particulate emissions from prescribed burning in Georgia. *Environ. Sci. Technol.* **2005,** *39,* 9049–9056.

Schauer, J. J.; Kleeman, M. J.; Cass, G. R.; Simoneit, B. R. T. Measurement of emissions from air pollution sources. 3. C1–C29 organic compounds from fireplace combustion of wood. *Environ. Sci. Technol.* **2001**, *35*, 1716–1728.

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Canned Research Project 2
Polycyclic Aromatic Hydrocarbons in Wood Smoke from Prescribed Fire

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PROJECT INTRODUCTION

The fire-suppression policies of the past century have left national forests in an unhealthy state characterized by dense undergrowth and heavy fuel loads. Such conditions have increased the likelihood of catastrophic wildfire. To reduce this threat, federal, tribal, and state land managers have agreed to increase their use of prescribed (or intentional) fire to restore wildland ecosystems to their pre-1900 condition. Along with the benefits of prescribed fire, however, are risks associated with fire-generated pollutants. Foremost among these is an expected increase in fine particulate matter or $PM_{2.5}$, airborne particles with aerodynamic diameters of ≤ 2.5 μm. Such particles are small enough to reach the alveoli in the lungs and have been linked to pulmonary disease, morbidity, and even death.

The health effects of $PM_{2.5}$ are related not only to their size but also to their composition. For example, $PM_{2.5}$ generated during residential wood burning (RWB) of both hard and soft woods has been shown to contain polycyclic aromatic hydrocarbons (PAHs), species known for their mutagenic and carcinogenic effects. The PAH levels in wood smoke from prescribed fire, however, are not as well understood. Prescribed fire is different in several ways from RWB. Most notably, prescribed fire consumes primarily pine needles and small diameter branches, not logs. Logs are more resinous than needles, and this resin content may result in higher PAH production and mutagenicity. Moreover, prescribed fires are low-intensity burns with both smoldering and flaming stages; smoldering favors PAH production, whereas flaming favors more complete combustion into volatile organics, carbon dioxide, and water. Because of these differences, it is important to examine the PAH content of wood smoke generated during prescribed fire.

Statement of Purpose. The goal of this canned research project is to analyze PAH concentrations in $PM_{2.5}$ generated during prescribed fires of the Coconino National Forest near Flagstaff, AZ. The Coconino National Forest is the largest contiguous ponderosa pine forest in North America and is scheduled for intensive restoration efforts through prescribed fire in the next decade. Few studies exist on PAH levels in wood smoke from prescribed fire, and this is one of the first to analyze wood smoke during prescribed burns of the Coconino National Forest.

Method Overview. In this project, you will analyze the PAH content in $PM_{2.5}$ collected during prescribed fires of the Coconino National Forest. $PM_{2.5}$ will be extracted into an organic solvent (dichloromethane) and concentrated to 1 mL. PAH levels in the extract will be analyzed using GC/MS. You will compare the chromatographs and spectra of your samples with selected PAH calibration standards. PAH concentrations will be reported as a mass percent of $PM_{2.5}$ mass (e.g., μg PAH/μg $PM_{2.5} \times 100\%$).

METHODS

Part 1: Previous Work

Research projects are long term and involve many different investigators over several semesters or years. This canned research project is no exception, and you will be joining the project midstream. Previous members of your group have already collected, weighed, extracted, and concentrated the fire-generated $PM_{2.5}$ samples. The 1 mL extracts were stored in the freezer, waiting for you to analyze them by GC/MS.

Thankfully, these group members wrote a Methods section describing their work before they left (Excerpt 1). Additional information is included in their lab notebook (Excerpt 2). Read and analyze these excerpts to better understand the previous work and to guide you in writing your own Methods section.

Excerpt 1 (description of prior work)

Experimental Section

     Prescribed Burns. $PM_{2.5}$ was collected during three prescribed burns conducted by the Peaks Ranger District of the Coconino National Forest Service in October 2007. The prescribed burns took place on three adjacent sites (A–C) of the A1-Mountain region of the Coconino National Forest, a predominantly ponderosa pine (*Pinus ponderosa*) forest in northern Arizona. All fires were broadcast burns and consumed ~100 acres of downed woody material, litter (dead-brown pine needles), and duff (decomposing litter). Each fire was sampled twice, during the ignition/combustion phase (day 1) and during the smoldering phase (day 2).

     $PM_{2.5}$ Collection. During each fire event, ambient air was sampled using a battery-operated $PM_{2.5}$ chemical speciation monitor, equipped with a sharp-cut cyclone to remove particles with aerodynamic diameters >2.5 µm (MetOne SuperSASS, Grants Pass, Oregon). Typical flow rates were 6.5 lpm. Sampling times were 3 h. The monitor collected two $PM_{2.5}$ samples simultaneously, one on a Teflon filter (47 mm), used to measure $PM_{2.5}$ mass, and one on a prebaked (900 ℃, 12 h) quartz-fiber filter (47 mm), used for PAH analysis. One field blank (quartz-fiber) was transported to and from the fire on each day of sampling.

     Mass Analysis. $PM_{2.5}$ mass was determined by weighing the preconditioned Teflon filters (24 h, 25 ℃, 30% relative humidity) before and after exposure. The $PM_{2.5}$ mass on the co-collected quartz-fiber filter was assumed to be the same as the Teflon filter mass (after adjusting for differences in flow rate).

     Extraction and Concentration. Quartz-fiber filters (six exposed filters, six field blanks, and three lab blanks) were extracted in dichloromethane (DCM) using a Soxhlet apparatus, filtered, and concentrated to 1.0 mL in a Kuderna-Danish concentrator according to EPA Method 3540C. Prior to extraction, each filter was spiked with 50 µL nitrobenzene-d_5 (64 mg/L) to measure percent recovery. Extracts were stored in pre-cleaned amber glass vials at -20 ℃ until analyzed.

Excerpt 2 (table of PM$_{2.5}$ masses)

October 30, 2007
PM2.5 masses from three prescribed fires during ignition/flaming (day 1)
and smoldering (day 2). Collection times were 3 h. Field and lab blank
masses were <0.001 mg. Error bars are estimated to be ±0.001 mg.

Sample No.	Fire(Area)	Date	Event	PM2.5 mass (mg)
S1_IG/F	Rx1(A)	10/13/07	Ign/flam	4.010
S1_SM	Rx1(A)	10/14/07	Smold	2.996
S2_IG/F	Rx2(B)	10/22/07	Ign/flam	4.382
S2_SM	Rx2(B)	10/23/07	Smold	3.094
S3_IG/F	Rx3(C)	10/27/07	Ign/flam	5.205
S3_SM	Rx3(C)	10/28/07	Smold	3.887

STOP Before continuing . . .

Strengthen your understanding of the previous work by doing the following:

- Reread Excerpts 1 and 2. Be sure that you understand the PM$_{2.5}$ mass measurements and the field and lab blank measurements. (You will report these in your paper.)

- Consider Collaboration. This canned research project addresses PAH concentrations in wood smoke particulate. A separate canned research project (*Toxicity of Wood Smoke from Prescribed Fire*) addresses the toxicity of wood smoke particulate from the same three fires. Because PAHs may contribute to toxicity, you might want to collaborate with classmates who have selected the toxicity project, or ask your instructor for information about this corresponding study.

Part 2: Your work

The methods for your canned research project are described below. Note the change in format from Excerpt 1. Excerpt 1 is written as it might appear in a journal article. The methods below are written more like a "to do" list for a lab notebook or lab manual. Your goal will be to rewrite this information for an expert audience in a journal-article format. You will want to describe the materials, instruments, and procedures used to determine PAH concentrations.

A few terms used in this project are defined below:

PAHs   Polycyclic aromatic hydrocarbons. In this work, eight PAHs are targeted.

Analyte   What you analyze in your samples (i.e, the eight PAHs).

CS   In this project, CS is used for "calibration standards". The GC/MS is typically "calibrated" for each analyte you are targeting in a study. In this case, you purchased a mixture of eight PAHs of known concentrations. From this, you will prepare five calibrations standards, varying from least to most concentrated. You will use these standards to a plot a calibration curve for each PAH.

SS   In this project, SS is used for "surrogate standard" (also called a recovery standard). The filters that contain the $PM_{2.5}$ (after $PM_{2.5}$ collection, but before extraction and concentration) are "spiked" with a known amount of the SS. Often the SS is deuterated (D instead of H), assuring that your spike is the only source of this compound in the sample. After spiking, the filter is extracted and concentrated as usual. The GC/MS analysis shows how much SS made it through extraction, concentration, and the GC column. This information is used to estimate the recovery of the unknown PAHs. The SS used is nitrobenzene-d5.

IS   In this project, IS is used for "internal standard" (also known as a reference standard). A known amount of IS is added to all samples (calibrations standards and $PM_{2.5}$ extracts) just before injection into the GC/MS. PAH peaks will be quantified relative to the internal standard peak in each run. The IS used is 1,4-dichlorobenzene.

Note: The abbreviations CS, SS, IS are OK here, in the canned research project, but the full written-out terms should be used in your paper. The abbreviation PAH (or PAHs) is OK to use in your paper, but it should be defined at first use.

Step 1: Prepare stock solutions
Reagents (VWR, research grade, unless otherwise stated)
- dichloromethane (DCM) (solvent)
- PAH mixture (CS, 8 PAHs in 1 mL DCM, each at 2000 μg/mL, MW 128–202 amu) (Ultra Scientific) (see Table 1 for list of PAHs)
- 1,4 dichlorobenzene-d4 (IS) (2000 mg/L in 1 mL DCM) (Ultra Scientific)
- nitrobenzene-d5 (SS) (2000 mg/L in 1 mL DCM) (Ultra Scientific)

Equipment
- variable volume micropipettes (10–100, 100–1000 μL)
- 1 mL glass pipette, pre-cleaned and baked
- volumetric flasks (5, 10, 25, 100 mL), pre-cleaned and baked
- pre-cleaned amber bottles with Teflon caps (10, 20, 25, 40, 125 mL)

Procedure
- prepare CS stock solution (64 mg/L): Take 0.80 mL of PAH mixture (2000 mg/L) and dilute to 25 mL in DCM. Store in 40 mL amber bottle.
- prepare SS stock solution (64 mg/L): Take 0.80 mL of nitrobenzene-d5 (2000 mg/L) and dilute to 25 mL in DCM. Store in 40 mL amber bottle.
- prepare IS stock solution (64 mg/L): Take 0.80 mL of 1,4 dichlorobenzene-d4 (2000 mg/L) and dilute to 25 mL in DCM. Store in 40 mL amber bottle.

Step 2: Prepare calibration standards (CS1–CS5) and lab blanks (LB1–LB3)
Using the stock solutions, prepare a 5 mL volume of 5 calibrations standards (CS1–CS5). Each standard will contain eight PAHs and the SS, in equal concentrations, varying from the lowest concentration (CS1 = 0.128 mg/L) to the highest (CS5 = 0.384 mg/L). Each standard will also contain the IS (20 μL at 64 mg/L = 0.256 mg/L).

Procedure
- **LB1 (LB2, LB3)(0 mg/L):** 5 mL DCM. Store in freezer.
- **CS1 (0.128 mg/L):** Mix 10 μL PAH stock (@64 mg/L), 10 μL SS stock (@64 mg/L), and 20 μL IS stock (@64 mg/L). Dilute to 5 mL with DCM. Store in freezer.
- **CS2 (0.192 mg/L):** Mix 15 μL PAH stock (@64 mg/L), 15 μL SS stock (@64 mg/L, and 20 μL IS stock (@64 mg/L). Dilute to 5 mL with DCM. Store in freezer.
- **CS3 (0.256 mg/L):** Mix 20 μL PAH stock (@64 mg/L), 20 μL SS stock (@64 mg/L), and 20 μL IS stock (@64 mg/L). Dilute to 5 mL with DCM. Store in freezer.
- **CS4 (0.320 mg/L):** Mix 25 μL PAH stock (@64 mg/L), 25 μL SS stock (@64 mg/L), and 20 μL IS stock (@64 mg/L). Dilute to 5 mL with DCM. Store in freezer.
- **CS5 (0.384 mg/L):** Mix 30 μL PAH stock (@64 mg/L), 30 μL SS stock (@64 mg/L), and 20 μL IS stock (@64 mg/L). Dilute to 5 mL with DCM. Store in freezer.

Step 3: Obtain 5-point calibration curve
Instrumentation
- The GC/MS instrument you will use is a Hewlett Packard 5890 Series II with 5971A mass selective detector with enhanced ChemStation V. A03.00 software. The instrument will be operated in selected ion mode, using splitless injection with a DB-5 capillary GC column (30 m length, 0.25 mm id, and 0.25 μm film thickness). Injector, detector, and transfer line temperatures will be at 280 ℃.
- Program the GC oven temperature as follows: 40 °C (5 min), ramp to 300 °C at 10 °C per min.

Calibration Procedure
1. Do a background run. Be sure the chromatograph looks clean with no evidence of contamination. Bake column overnight if needed.
2. Run a solvent blank. Inject a 1 μL sample of DCM (with solvent override enabled). Chromatograph should look "clean" with no evidence of contamination. (Hint: Use a pre-cooled syringe and store vials on ice to prevent evaporation.)
3. Inject 1 μL of each calibration standard, beginning with the lowest concentration (CS1) and ending with the highest concentration (CS5). (Hint: Use a pre-cooled syringe and store vials on ice to prevent evaporation of DCM.)
4. Collect a chromatograph for each run. Each chromatograph will have 10 peaks (IS, SS, 8 PAHs). You will be able to distinguish the 10 compounds based on their retention times and major fragment ions (Table 1).

Table 1. Retention times, molecular weights, and major fragment ions for PAHs.

label	PAH	retention time (min)	MW	major ion (*m/z*)
IS	1,4-dichlorobenzene-d_4	12.89	147.00	152
SS	nitrobenzene-d_5	15.05	128.07	128
PAH-1	naphthalene	17.82	128.17	128
PAH-2	acenaphthylene	24.36	152.20	152
PAH-3	acenaphthene	25.15	154.21	154
PAH-4	fluorene	27.29	166.22	166
PAH-5	phenanthrene	31.26	178.23	178
PAH-6	anthracene	31.45	178.23	178
PAH-7	fluoranthene	36.26	202.26	202
PAH-8	pyrene	37.15	202.26	202

Strengthen your understanding of your work thus far by doing the following:

- Check that you understand the following terms:

analyte	internal standard
calibration curve	laboratory blank
calibration standard	surrogate standard

- Using keywords (e.g., GC/MS, internal standard, calibration curve), find journal articles that describe procedures similar to those above. Read the Methods sections of these articles to help you better understand these procedures and to examine ways in which they are described by experts (e.g., consider what details are included or omitted, what subheadings are used, how concentrations are reports).

- Consider how you will refer to the three fire events in your paper. Although "Rx1, Rx2, and Rx3" are OK to use in your notes, you should use more formal language in your paper.

- Go to the "preparing a calibration curve" step of the Data Analysis section below and prepare your calibration curves now. Check that there are no problems with the data before proceeding to the next step in the procedure.

Step 4: Run the field blanks, lab blanks, and samples

You are now ready to measure the PAH content in the fire-generated $PM_{2.5}$ extracts, as well as the quality control samples (lab and field blanks). Recall from Excerpt 1, the $PM_{2.5}$ extracts were concentrated to 1.0 mL in DCM and stored in the freezer in amber glass vials.

1. Remove the vials from the freezer. Let about 100 μL of DCM evaporate, then add 50 μL of IS stock (@64 mg/L) to each vial. Add DCM until the final volume equals 1 mL. *(The final concentration of the IS will be 3.2 mg/L in the $PM_{2.5}$ extracts.)*

2. Inject 1 μL of each sample to obtain the chromatograph. (Be sure to pre-cool each syringe and vial, as described above.) You will run extracts from the exposed filters in triplicate, injecting 3×1 μL samples from each vial. You will run field and lab blank extracts only once, totaling 27 runs (3×6 samples, 1×6 field blanks, and 1×3 lab blanks).

RESULTS

After analyzing the data (see Data Analysis below), you will know (1) the $PM_{2.5}$ mass in each sample (from Excerpt 2 above) and (2) the PAH concentrations in each sample extract. There are many different ways to present these data in your paper. Here are a few points to consider as you prepare your results:

- With only six samples, consider reporting the $PM_{2.5}$ masses in the text rather than in a table or graphic. You can report all six values or two average values, the mass average during ignition/flaming (±stdev) and the mass average during smoldering (±stdev). Note which conditions resulted in higher $PM_{2.5}$ masses.
- Not all eight PAHs will be detectable. Some PAH concentrations will be below the detection limit (<0.1 mg/L). You will need to report this detection limit in your paper, either in the Methods or Results section.
- If most of the eight PAHs are detected in the samples, consider presenting the data in a table. To prevent overwhelming readers with too much data, report only the average value (±stdev) for each PAH during ignition/flaming and smoldering. (Such tables are commonly used in the literature for reporting PAH values.)
- If only a few PAHs are detected in the samples, considering graphing the data (but mention in the text which PAHs were not detected). Limit yourself to no more than two graphs. There are many possible ways to graph the data. One way is by fire (burn 1–3), another is by fire stage (ignition/flaming, smoldering), and a third is by PAH. If possible (i.e., if the graphs are not too crowded), plot individual results for each burn rather than average values. How do you decide which graph is best? Think about the take-home message of your paper. Choose the graph that most clearly conveys this take-home message.

REMEMBER, THESE DATA,

ALTHOUGH BASED ON PRELIMINARY RESULTS,

ARE FICTITIOUS!

HAVE FUN WITH YOUR INTERPRETATIONS,

BUT DON'T TAKE THEM TOO SERIOUSLY!

DISCUSSION

What were your key findings? How can you interpret these results? Consider the following guidelines as you write the Discussion section of your paper.

- Determine if $PM_{2.5}$ masses were greater during ignition/flaming (day 1) or smoldering (day 2).

- Consider why some PAHs may be present in greater abundance than others. Hint: consider PAH molecular weight and volatility. PAHs that are too volatile will evaporate and will not stay condensed on the particulate.

- Consider why the weight percent of PAHs is greater during smoldering. What are the products of complete combustion? How might combustion products differ between a flaming and smoldering fire?

- How do your data compare to literature values? Focus on the eight PAHs analyzed in this project. Have other researchers detected any or all of these PAHs in wood smoke? If so, how do their values compare with your values?

  Note: In several papers, the authors report PAHs in wt % organic carbon (OC) rather than wt % $PM_{2.5}$. Convert to wt % $PM_{2.5}$ by multiplying the PAH wt % of OC (mg PAH/mg OC) by the OC wt % of $PM_{2.5}$ (mg OC/mg $PM_{2.5}$) × 100%.

- If you collaborated with classmates who analyzed wood smoke toxicity, what insights (if any) can you gain from their results? Check the toxicity of the PAHs of interest (e.g., search PAHs of interest using the Integrated Risk Information System, online at http://www.epa.gov/iris/).

- What might be some broader implications or applications of your findings?

DATA ANALYSIS

Part 1: Analyzing the Data
This section walks you through the steps needed to convert your raw data to the polished results that you will present in your paper. (See also Part 2, Understanding Calibration Curves, for more information on the formulas used in the calibration curves.)

Before continuing . . .

- Often students first attempt to use calculators to analyze their data. We discourage this approach and recommend that you use a spreadsheet program (Excel) instead. Although the calculator approach may seem easier at first, in the long run, calculators are time consuming and error prone. For example, using a calculator for multistep calculations often leads to rounding errors. Moreover, calculations "by hand" do not provide a track record of your steps. By using a spreadsheet, you have an electronic record of your calculations; the spreadsheet is easily corrected and can serve as a permanent record of your data analysis.

- This canned research project assumes that you have already used Excel and are familiar with using formulas and preparing graphs. If you are a beginner, try using the built-in Excel help or ask a friend or instructor to walk you through the spreadsheets. It will not take long before you realize how much easier it is to use Excel than a calculator!

1. Prepare Calibration Curves

You will use the peak areas in the chromatographs to plot a 5-point calibration curve for each PAH (8 graphs) and the SS (1 graph). The calibration data are included in the Excel file entitled **PAH CalData.xls.** This file is divided into three worksheets. The worksheets are labeled by tabs at the bottom of the Excel page (Calibration Data, Sample Calibrations, and Remaining Calibrations). Clicking on the tabs will move you back and forth among the worksheets.

1.1   Click on the first tab (Calibration Data) to view the data for the five calibration standards.

1.2   Click on the second tab (Sample Calibrations). This worksheet shows you how to use the chromatograph data to plot calibration curves for PAHs 1–4. The data are arranged in six columns:

Run	numbers 1–5, corresponding to the five calibration runs
PAH area	chromatograph data (copied and pasted from Calibration Data)
[IS] mg/L	the concentration of the IS (the same for all calibration runs)
IS area	chromatograph data (copied and pasted from Calibration Data)
x axis	a formula that calculates the values for the *x* axis (to see the formula, click on cell 6E, highlighted in yellow).
y axis	the values for the *y* axis, which are the concentrations of the PAHs in the five runs (copied and pasted from Calibration Data)

Following the columns of data, you will see a scatter graph of the XY data, plotted using Excel. A linear curve (trendline) is fit to the data. The equation for the line and the R-squared value are shown on the graph. *You will need these equations to analyze your authentic samples.*

Your Turn. Click on the third tab (Remaining Calibrations). This worksheet has been set up for you to create the five remaining calibration curves (PAHs 5–8 and SS). To make things easier, the column data have been entered except for the PAH areas and the formula needed to calculate the *x*-axis values. Complete these cells and then plot the curves, fitting each curve with a trendline. (If you are a beginner, you may need to seek help in using Excel.)

2. Determine PAH Concentrations and Weight Percents

The GC/MS analysis of the authentic samples will produce chromatographs that you will use to determine the PAH concentrations in the wood smoke samples. The data are included in the Excel file entitled **PAH Data.xls**. This file is divided into seven worksheets. The worksheets are labeled by tabs at the bottom of the Excel page (field blanks, lab blanks, Rx1, Rx2, Rx3, sample calculation, sample percent recovery). Clicking on the tabs will move you back and forth among the worksheets.

2.1   Click on the first tab (field blanks). Examine the field blank data. Was there any evidence of field contamination? Why or why not?

2.2   Click on the second tab (lab blanks). Examine the lab blank data. Was there any evidence of lab contamination? Why or why not?

2.3   Click on tabs 3, 4, and 5 (Rx1, Rx2, and Rx3). These worksheets contain the GC/MS data from the filter extracts for each fire event (separated into ignition and smoldering). Using Table 1 and the retention times, identify the two PAHs that are above the detection limit. (Only PAH concentrations above the detection limit need to be calculated for any given fire event).

2.4   Click on tab 6 (sample calculation). The calculations for PAH-7 (fluoranthene) have been completed for you. Note that the worksheet is set up to calculate fluoranthene levels in all three burns under both ignition and smoldering conditions. Eleven columns were created for the data (highlighted in yellow). The first five columns include data from other worksheets. For example, for Rx1, columns 1–5 are as follows:

Area(PAH-7)   data from Rx1 (Ignition, runs 1–3; Smoldering, runs 1–3)
Area(IS)   data from Rx1 (Ignition, runs 1–3; Smoldering, runs 1–3)
[IS] mg/L   concentration of internal std (the same for all runs)
RF   response factor (slope from calibration curve for PAH-7)
b   intercept from calibration curve for PAH-7

Column 6 is a formula that calculates the concentration (mg/L = μg/mL) of PAH-7 in the 1 μL sample injected into the GC/MS. The formula is based on the following equation:

$$[\text{PAH-7}] \; = \; \frac{\text{RF} \times \text{Peak area(PAH-7)} \times [\text{IS}]}{\text{Peak area(IS)}} + \text{intercept}$$

where

[PAH-7]	concentration (mg/L = μg/mL) of PAH-7 in sample
RF	response factor (slope) from PAH-7 calibration curve (unitless)
Peak Area(PAH-7)	peak area from chromatograph for PAH-7
[IS]	concentration of internal standard (3.2 mg/L)
Peak Area(IS)	peak area from chromatograph for IS
intercept	y intercept (b) from calibration curve (mg/L)

Column 7 is the PAH mass (in μg) in the sample.

Column 8 is the $PM_{2.5}$ mass (in μg) of the sample (from Excerpt 2).

Column 9 calculates the weight percent of the PAH in the $PM_{2.5}$.

$$\text{wgt \% PAH} = \frac{\text{mass of PAH (}\mu\text{g)}}{\text{mass of PM}_{2.5}\text{ (}\mu\text{g)}} \times 100\%$$

Columns 10 and 11 calculate the average and standard deviation for the three runs.

These calculations are repeated for all three prescribed burns. The data for PAH-7 are summarized in a graph at the bottom of the page. *Note: this graph is not formatted correctly for a journal-quality paper.*

Your Turn. Using the PAH-7 worksheet for guidance, calculate the weight percent of PAH-8 in the six samples. (Hint: You might want to copy the worksheet and paste it into a new worksheet or file.)

3. Calculate Percent Recovery

Click on tab 7 (Sample Percent Recovery). Recall, prior to extraction and concentration, each filter was spiked with 50 μL nitrobenzene-d_5 (@64 mg/L), the surrogate standard. This was extracted into a final volume of 1 mL; hence, the final concentration of SS (assuming 100% recovery) should be

$$\frac{50\ \mu\text{L}}{1} \times \frac{1\ \text{mL}}{1000\ \mu\text{L}} \times \frac{64\ \text{mg}}{\text{L}} \times \frac{1}{1\ \text{mL}} = 3.2\ \frac{\text{mg}}{\text{L}}$$

As shown in columns 1–6, the final concentration of SS in each run is determined the same way you calculated PAH concentrations above.

To determine percent recovery, divide the final SS concentration (column 6) by the original SS concentration (3.2 mg/L, column 7). After multiplying by 100%, the answer is shown in Column 8.

$$\text{Percent recovery} = \frac{\text{final SS} \left(\frac{\text{mg}}{\text{L}}\right)}{\text{original SS} \left(3.2 \ \frac{\text{mg}}{\text{L}}\right)} \times 100\%$$

Your turn. Repeat this calculation for prescribed fires Rx2 and Rx3. Determine (and report) the percent recovery (and standard deviation) averaged over all three fires (18 measurements).

Part 2: Understanding Calibration Curves

Within a range of concentrations (denoted as the linear range), the concentration of a PAH (or SS) is proportional to its integrated peak area. Similarly, the concentration of the internal standard (IS) is proportional to its integrated peak area.

| [PAH] | α | Peak Area (PAH) |
| [IS] | α | Peak Area (IS) |

Due to instrumental variability, a ratio of areas (e.g., area1/area2) is more reproducible than a single absolute area (area1). This is because the analyte molecules are not identically vaporized, carried down the GC column, or detected each time a sample is run. Such processes vary from run to run and are sensitive to changes in temperature, pressure, flow rate, electronics, etc. For example, let's take the following hypothetical duplicate runs (same sample run twice).

Run 1 Peak Area (PAH) = 333,000 counts
Run 2 Peak Area (PAH) = 487,003 counts

A change of 32%! Now consider a hypothetical case in which peak areas for both the PAH and IS are monitored:

Run 1   Peak Area (PAH) = 333,000 counts   Peak Area (IS) = 1,250,000 counts
Run 2   Peak Area (PAH) = 487,003 counts   Peak Area (IS) = 1,828,089 counts

For whatever reason, fewer counts were detected in Run 1. This is true for both the PAH and the IS (in part because the PAH and IS have similar molecular weights and structures). Now let's determine the ratios for each run:

$$\text{Run 1} \quad \frac{\text{Peak Area (PAH)}}{\text{Peak Area (IS)}} = \frac{333{,}000}{1{,}250{,}000} = 0.2664$$

$$\text{Run 2} \quad \frac{\text{Peak Area (PAH)}}{\text{Peak Area (IS)}} = \frac{487{,}003}{1{,}828089} = 0.2664$$

The ratios are the same! The use of an internal standard minimizes the effect of instrument variability from run to run. Thus, in calibration curves, the ratio is used, rather than just the peak area of the PAH. The ratio of the PAH and IS concentrations is proportional to the ratio of their peak areas. Mathematically,

$$\frac{[\text{PAH}]}{[\text{IS}]} \propto \frac{\text{Peak Area (PAH)}}{\text{Peak Area (IS)}}$$

This equation can be rearranged to solve for the concentration of the PAH:

$$[\text{PAH}] \propto \frac{\text{Peak Area (PAH)}}{\text{Peak Area (IS)}} \times [\text{IS}]$$

The proportion sign can be converted to an equal sign by including a proportionality constant, the response factor (RF). With the addition of a y-intercept term (b), the equation becomes the equation for a straight line ($y = mx + b$), where RF equals the slope (m). The y-intercept should equal zero, corresponding to [As] = 0; however, a non-zero value is typically found due to instrument noise.

$$[\text{PAH}] = \text{RF} \times \frac{\text{Peak Area (PAH)}}{\text{Peak Area (IS)}} \times [\text{IS}] + \text{noise}$$

$$y \;=\; m \;\times\; x \;+\; b$$

RF (m) and b can be determined by plotting y (the known PAH calibration standard concentrations) against x (the ratio of PAH and IS peak areas multiplied by the known IS concentration) and then fitting a best fit line to the data points. In turn, RF and b can be used to calculate unknown PAH concentrations (y) in authentic samples:

$$[\text{unknown PAH}] = \text{RF} \times \frac{\text{Peak Area (PAH)} \times [\text{IS}]}{\text{Peak Area (IS)}} + b$$