We thank both referees for their thoughtful reviews of our work, which have significantly improved the clarity of the manuscript. Below, we address each referee comment and highlight specific changes made to the manuscript. Our author changes are in blue, and the enumerated referee comments are in black.

Anonymous Referee #1

Received and published: 20 October 2019

This manuscript presents comprehensive molecular characterization of smoldering woodsmoke aerosols generated from controlled burning of different hardwood samples. Organic aerosol compositions were correlated with cell toxicity and visible light absorption measurements to probe their potential health and climate effects. While this manuscript is overall well-written, there are several major concerns regarding the study design that need to be addressed before publication.

(1) The selection of cell type and biological endpoint for toxicity testing does not seem to be relevant to the major route of exposure for woodsmoke aerosol exposure. The authors stated that for the cell toxicity bioassay, human epidermal keratinocytes (i.e., skin cells) were used to determine the toxicity of WSA, and the protein content from envelope formation was used as a quantitative biomarker from toxicant exposures. However, compared to dermal contact, inhalation should be more relevant. Use of pulmonary cell models to measure cytotoxicity (cell death or cell proliferation) would be more appropriate for this type of research. The authors should provide clear justifications for the usage of cell type and biological endpoint for toxicity testing.

We appreciate the intent of this recommendation, which is to provide a better approximation to reality. Keratinocytes are found normally in the upper respiratory tract (oropharynx, laryngeal pharynx), throughout the oral cavity and are seen in the trachea as a result of insult (e.g., chronic smoke inhalation); thus, they are relevant to inhalation exposure. Furthermore, keratinocytes have been shown to be a useful model for responses common to epithelial cells (Rhim, 1989; Neugebauer et al., 1996; Rogers et al., 2001).

It should be kept in mind that the respiratory tract is populated by about 40 different cell types, all with different properties. Except for white blood cells, none can be serially cultured in their normal state. Cell lines are available derived from the respiratory tract, but these are generally neoplastic and considerably deviated from normal. The commonly used BEAS-2B cell line, for example, is aneuploid and was derived from human bronchial epithelial cells by transformation with an adenovirus-SV40 hybrid virus (Reddel et al., 1988).

The human SIK line that we use is minimally deviated from normal (one chromosomal aberration (Rice et al., 1993)). The response we are using exploits a property of keratinocytes that is part of their normal terminal differentiation program occurring as the cells become permeable to trypan blue dye (Green, 1977). Moreover, the lung is not the only target tissue of air pollutants. Pollutants are taken up into the blood stream and distributed throughout the body with various target cells including keratinocytes of the esophagus and even epidermis. No assay will successfully reproduce the *in vivo* environment, but the one we use is as good as or better than others. Please note that the

Ah receptor assay does not use cells from the respiratory tract but is an excellent and relevant assay nevertheless.

We have added the following justification to Section 2.4:

"*Keratinocytes can be found naturally in the upper respiratory tract, oral cavity, and formed in the trachea as a response to air pollution, cigarette smoke, and woodsmoke inhalation exposure (Plamenac et al., 1979b; Plamenac et al., 1979a; Lee et al., 1994; Moran-Mendoza et al., 2008). Moreover, they have been shown to be a useful model for responses common to epithelial cells (Rhim, 1989; Neugebauer et al., 1996; Rogers et al., 2001).*"

(2) As discussed in section 3.1 (Line 188-201), the authors provided an exposure scenario, stating that a human adult would take ~2175 breaths to reach the cell toxicity EC50 value of Red Oak smoldering woodsmoke aerosols. Again, since epidermal keratinocytes (i.e., skin cells) were used to determine cell toxicity by measuring the protein content from envelope formation, the EC50 values derived from this assay are not appropriate parameters for an exposure assessment through inhalation. The authors should limit the inhalation-related discussion based on their EC50 values.

We have addressed this concern in (1) – it has been demonstrated in the literature that keratinocytes are valid models for comparing inhalation exposure. Although a single cell type cannot display the sensitivities of the numerous cell types that are exposed in the lung and elsewhere in the body, it permits calculating a benchmark suggestive of potential toxicity. We only offer a back-of-the-envelope calculation showing that the measured values are relevant. Extrapolating values based on "lung" cells are not necessarily more relevant to *in vivo* sensitivity. In view of all uncertainties, the estimate provided in the manuscript remains useful.

We added the following to Section 3.1., to offer perspective for extrapolating *in vitro* values:

"Although no assay will successfully reproduce the in vivo *environment, i.e., exposure to approximately 40 different cell types in the respiratory tract, distribution of toxicants throughout the bloodstream to major organs, and either deactivation or activation by metabolism, these (and other)* in vitro *results are useful as an estimation of risk."*

(3) The toxicity bioassay was quantified colorimetrically at the wavelength of 495 nm. This wavelength overlaps with the visible brown carbon absorption. Did the author measure the absorption from aerosol sample extracts to account for the potential interference from brown carbon absorption at this wavelength?

The envelope assay involves isolating these protein structures by washing them in SDS solution before measuring their protein content. Thus, the smoke extracts are separated from the envelopes, so no interference occurs from their color.

We added the following clarification to Section 2.4:
"*As the protein structures of SIK cultures exposed to WSA were isolated during the washing process, no interference from WSA absorption occurs in the colorimetric analysis*."

(4) Line 158: What were the positive controls used in the cell toxicity bioassay to simulate the maximum activity?

We added the following information to Section 2.4:
"*Ionophore X5375, which is known to induce envelope formation in the large majority of cells (Rice and Green, 1979), was used as the positive control*."

(5) Line 245: The cell toxicity measured by the protein content from envelope formation was not a direct measure of cell death. How did the authors determine whether the WSA extracts kill cells?

We have stated that envelope formation is actually a measure of cell death, since it occurs when the cells become permeabilized:

Line 143: "*The cross-linking can be induced by permeabilization of the cells, permitting a rise in cytosolic calcium ions that activates the membrane-bound transglutaminase TGM1* ***and results in cell death***."

(6) The rationale for measuring AhR and ER activity from woodsmoke aerosol exposure should be strengthen. What are the underlying hypotheses?

We added the following to the Introduction to strengthen our hypotheses:

"*We measured the total cellular toxicity effects of smoldering WSA. In addition, the Ah receptor (AhR) and estrogen receptor (ER) are known to play critical regulatory roles in mediating the biological and toxicological effects of diverse environmental chemicals. As wood combustion produces PAH-like compounds and phenolic compounds, classes of chemicals that are known to activate the AhR and/or ER signaling pathways (Denison and Nagy, 2003; Krüger et al., 2008; Stejskalova et al., 2011; Li et al., 2012; Cipolletti et al., 2018), we measured the AhR and ER activities of smoldering WSA"*

(7) Throughout the manuscript, a lot of discussions between molecular composition and biological endpoints (e.g., AhR activity) were based on correlations. The authors should be careful about drawing conclusions from this relationship since correlation does not imply causation. Validation work should be carried out.

We agree that care should be taken when drawing conclusions from correlations. It is unreasonable, however, to avoid reaching some conclusions altogether from a correlation study. We have been careful to discuss findings in terms of what is known and what is speculative (e.g., "***potential chromophores***" (Line 69); "***it is not clear which compounds specifically effect*** *(sic)* ***cell toxicity***" (Line 346)) and we discussed at length how some of our findings are unexpected or may be coincidental. Here are examples of explicit caveats.

a. Line 323: "*A positive effect means that the signal of the peak directly …and a negative effect means anti-correlation,* ***neither of which is necessarily causative***." (this sentence is followed by direct examples of why correlation is not necessarily causation in our work)

b. Line 320: "*The occurrence of structural isomers is a limitation that may **confound the statistics** in this work…"*

c. Line 388: "*Even so, many of the correlations are not easily understood and **could be due to coincidence or driven by unassigned isomers***."

We mentioned that the correlation portion of this work "*identified multiple targets for further toxicological testing*." Validation work should be done in future studies but is outside the scope of the current study.

(8) For the brown carbon measurement, why did the author measure the visible light only (400-700 nm), but not the full range for tropospheric-relevant radiation (300-700 nm)? Note that most brown carbon constituents absorb light at near-UV wavelengths (300-400 nm) and to a lesser extent visible light.

We measured a broad UV-vis spectrum in the range of 200 – 800 nm, but only use the visible spectra to correlate with the HRMS data. We fully understand that BrC typically have high absorption at UV wavelengths and the corresponding author has previously published papers on BrC showing the full absorption spectra. However, this manuscript is focused only on understanding the molecular identities of the "brown" chromophores, i.e., "visible light absorption" is in the title and all of our MAC values are labeled as MACvis. As BrC is a mixture, there are many compounds in the aerosol that do not absorb light – in fact, it has been documented that the chromophoric fraction in some BrC is rather low but the chromophores are potent (Laskin et al., 2014). Doing the correlations with the inclusion of the UV portion of the absorption is expected to dilute the strength of the correlations to the visible-light-absorbing chromophores.

We added this sentence of clarification to Section 2.3. "*As this work is focused on visible-light-absorbing chromophores, the UV portion of the brown carbon absorption was not examined*."

(9) Line 380-385: What fractions of sinapaldehyde and coniferaldehyde converted to their enol forms in the extract solutions?

Sinapaldehyde and coniferaldehyde don't have accessible enol forms. In general, enol formation is highly unfavorable at atmospherically relevant temperatures (i.e., equilibrium is highly shifted toward keto form, e.g., (Keeffe et al., 1990), or organic chemistry textbooks), and for conjugated compounds like sinapaldehyde and coniferaldehyde, where a hypothetical enol formation removes aromaticity, this likelihood will be further reduced to zero. Our point is that with vanillydene acetone, this tautomerization is possible due to its neighboring sigma carbon. We made the following changes (underlined) to be more clear.

"*…but vanillylidene acetone can achieve <u>some</u> additional resonance with its keto-enol tautomerization (which tends not to happen in the ring because the aromatic phenol is highly favored) <u>while sinapaldehyde and coniferaldehyde cannot</u>*."

(10) Line 386: The authors could check on the pKa of these detected products and compare that to the pH of your solution.

We will clarify our discussion of the pH effects on line 386. The phenolates in question either occur either as isolated compounds in solution or as a member of a charge-transfer pair. As the pH of our solution is roughly 5, the free phenolate form of, say, coniferaldehyde (pKa ~ 8, Ragnar, Lindgren, and Nilvebrant, 2008) would be expected to be roughly 0.1% of the phenol form if equilibrium is reached in solution. However, as a member of a charge-transfer pair, the acid/base dissociation equilibrium is less important. A charge transfer pair can exist at a wide variety of pH because of the localized effects. Importantly, the pH-dependence of charge transfer pair to produce chromophores differs by their chemical structure. For example, the pH dependence of the chromophore from phenol + old yellow enzyme is strong for chlorophenol but weak for methoxybenzaldehyde (Abramovitz and Massey, 1976). Because of these differences, we stated in our conclusion that we "*hypothesize that the mechanism of action is through charge transfer reactions to form phenolates*."

We have now inserted the solution pH value and revised the text in question (revisions underlined) to be more clear:

"*~~It could be that~~ Our solution may not be sufficiently basic (pH ~ 5) for the deprotonated form of some phenols to substantially exist in equilibrium (e.g., pKa for sinapaldehyde is 8.2, coniferaldehyde is 7.98, vanillin is 7.4, and bivanillin is 6.16, (Ragnar et al., 2000); however, localized charge-transfer interactions may facilitate the deprotonation of phenols at pH much lower than their pKa (Abramovitz and Massey, 1976).*"

(11) Line 423-425: Does the formation of phenolate occur only in the aqueous solution?

No, phenolates can be observed in aerosols as well (Phillips et al., 2017; Lin et al., 2017) as most aerosols have some water on them. Furthermore, on aerosols, there may not be just one pH but a distribution of pHs throughout the phase-separated pockets so the phenols can exist separately from phenolates.

"*In atmospheric aerosols, phenolates have been suggested to contribute substantially to brown carbon absorption as facilitated by their charge-transfer interactions (Phillips et al., 2017).*"

(12) Line 12 and 86: did the author mean to say "hardwood" here?

No actually, we do mean "***heart***wood." The woody material of a tree can usually be divided into "sapwood" (less dense, living, higher-moisture, outermost portion of a woody stem or branch) and "heartwood" (more dense, dead, drier, inner wood). Usually the sapwood is a lighter color than the heartwood, and is named as such due to the high amount of sap in the cells. Heartwood often comprises the majority of the cross-section and is used to make furniture.

Anonymous Referee #2
Received and published: 2 November 2019

This study investigated the molecular composition, optical and biological properties of woodsmoke aerosols. Correlations between some identified molecular formulas with MAC and bio endpoints were found. Overall, the analyses are sound, and the manuscript is well-written and easy to follow. I recommend that it can be published following some revisions.

(1) I have the same concern as Referee#1 comments (1) and (2) for the selection of cell type. The author should illustrate the rationale to use human epidermal keratinocytes.

Please see the response to Referee #1 comments (1) and (2) above.

Also, why such high passage number (25-35) were being used? Usually the cell passage number shouldn't be higher than 20, otherwise the cell response results can be biased.

We are not aware of any specific limits on passage numbers of cell cultures in general (though they may be advocated for certain assays), but we do understand the concern about passage number. Since neoplastic cell lines evolve in culture, limits are commonly put on passage numbers to assure that the properties of the cells are not evolved far beyond those of the original passage. This is particularly germane if the passage numbers at the beginning of a study are quite different from those at the end. In this case we used passages of a narrow range to avoid such a problem. The SIK line is well characterized through numerous passages and is known to retain essentially normal function at the passage range employed. The cells have a single chromosomal aberration that permits continuous growth while altering their behavior only minimally (Rice et al, 1993). We observed that the cells were similarly sensitive to the measured effects of WSA exposure on envelope formation throughout the range of passages employed.

We added the following justification to Section 2.4:
"*SIK cells have a single chromosomal aberration that permits continuous growth throughout the range of passages employed while altering their behaviour only minimally (Rice et al, 1993)*."

(2) Line 149: cells were incubated with extracts of WSA for 48h. During the 48h incubation in water/acetonitrile solution, cells may not be alive although the author did not find envelope formation in negative control. Did the authors try other cell viability assays to test cell viability? Why the author used such a long incubation time?

The long incubation time was used to increase the sensitivity of the assay when sample amounts were limited (thereby avoiding sample batch effects). Envelope formation is a measure of sufficient cell damage to induce permeabilization, a normal feature of their terminal differentiation (death). We did not test for lesser damage that might permit the cells to survive. Envelope formation is known to depend on transglutaminase activity, which is induced during differentiation. Although the less differentiated cells in the basal layer may lack sufficient transglutaminase to make envelopes, some 90% develop them in the post-confluent stratified cultures assayed (Rice and Green, 1979).

We added the following clarification to Section 2.4:
"*…to increase the assay sensitivity while conserving cell viability*."

(3) Line 263: no correlation between chromophoric species with cell toxicity does not necessarily mean they don't contribute to produce cell toxicity, and vice versa. Indeed, there is a recent paper (Chen et al., es&t 2019) showing significant correlation between DTT activity and water-soluble brown carbon.

We do not mean to imply that there is no correlation between aerosol chromophoric species and toxicity in general. We only reach this conclusion for the chromophores generated in our samples, as there are a vast variety of chromophores in nature that may produce a cell toxicity response (such as PAHs, nitrophenols, quinones, metal-ligand pairs). Chen et al (2019) tested WSOM extracts of ambient aerosols collected from China, where the findings were suggested by the authors to be due to the contributions from PAHs, quinones, or metals that generate ROS through redox cycling or Fenton-like chemistry, which are not studied in this work.

We added the Chen (2019) reference and clarify our statements by making the following changes (underlined):

*"Furthermore, the MACvis values do not correlate to either cell toxicity (**Fig. S5b**, adj. R^2 0.03) or AhR activity (**Fig. S5c**, adj. R^2 -0.06), suggesting that chromophoric species in this work (e.g., specific conjugated compounds, charge transfer complexes, etc.) are not necessarily those producing these effects. In contrast, specific classes of chromophores from atmospheric aerosols in China have been found to have oxidative potential based on a chemical assay, which were suggested to be due to their PAH-like, quinone-like, or metallic constituents (Chen et al., 2019)."*

(Abstract) *"Finally, MAC_{vis} had no correlation with toxicity or receptor signaling, suggesting that key chromophores in this work are not biologically active on the endpoints tested."*

(4) There are a lot of correlation analysis in the manuscript. The authors should also do statistical analysis to show how significant the correlations are.

This is a good suggestion. In general, there is a relationship between the R^2 coefficient and the p value, an indicator of statistical significance. Although strict cutoffs are rarely used by statisticians, $p < 0.05$ is generally accepted as highly significant and for our data, the cutoff of $p = 0.05$ occurs around $R^2 = 0.44$ on average. We have done the significance analysis for all of the statistical fits in the present work using Matlab's correlation coefficient function and found that 90% of the entries in Table 2 had $p < 0.05$, which indicates strong evidence against the null hypothesis. The remainder were primarily borderline cases ($0.05 < p < 0.07$), which indicates weaker evidence. We made the following changes:

a. We added the following text to the caption of Table 2: "*Entries have p-values ≤ 0.05 (90%), or p < 0.1 (100%).*"

b. We added the following text to the Results and Discussion section of the manuscript: "*90% of the correlations shown in **Table 2** have p-values ranging from 0.0002 to 0.05, while 100%*

have $p < 0.1$. This suggests that the correlations have moderate-to-strong evidence against coincidence."

(5) The authors should do some comparisons of their cell toxicity, AhR activity and ER activity results with other previous studies using the same assays. Only looking at the numbers in table 1 cannot get a sense how toxic the WSA are. The authors shall compare their results with other types of aerosols or other toxicants.

Originally, we limited the discussion mainly to aerosols that were derived from woodsmoke; however, we can see the referee's point about the benefit of broader comparisons with other types of aerosols or other toxicants.

While we cannot compare the envelope-formation cell toxicity assay to other works because this is the first time this type of assay has been performed on atmospherically-relevant aerosols, we have added comparisons of AhR activity and ER activity for different size fractions of particulate matter (PM) to the Results and Discussion:

"*Despite dissimilarities in chemical composition, it can be instructive to compare the total AhR and ER activity of smoldering WSA to those measured for ambient particulate matter. Utilizing similar cell-based bioassays, PM_1 collected from rural and urban traffic sites in Switzerland was found to have AhR activity of $0.5 – 2 \times 10^{-6}$ g TCDD EQs/g PM and ER activity of $2 – 23 \times 10^{-9}$ g E2 EQs/g PM (Wenger et al., 2009a; Wenger et al., 2009b). Similarly, PM_{10} collected from downtown Toronto, Canada was found to have AhR activity of $0.04 – 1 \times 10^{-6}$ g TCDD EQs/g PM and ER activity of ~10^{-6} g E2 EQs/g PM (Clemons et al., 1998). PM of various size fractions from different polluted sites in the Czech Republic had AhR activity of $0.001 – 1 \times 10^{-6}$ g TCDD EQs/g PM and ER activity of $0.1 – 20 \times 10^{-9}$ g E2 EQs/g PM (Novák et al., 2014). Organic PM in Wuhan, China was found to have ER activity of ~ $2 – 8 \times 10^{-7}$ g E2 EQs/g, depending on whether the PM was collected on sunny or foggy days (Wang et al., 2004). In comparison, the AhR activities of smoldering WSA in this work are within the range of PM from urban and industrial sites, while the ER activities are similar to some types of urban/industrial PM but a factor of 10^{2-3} lower than others. Wenger et al. (2009) also found the total concentration of AhR agonists to be much higher than concentrations for the traditional AhR agonists such as PCDD/Fs, suggesting a diversity of AhR-active ligands in ambient PM and further supporting the notion that AhR can bind and be activated by a wide range of structurally diverse chemicals in the environment (Denison and Nagy, 2003; DeGroot et al., 2011; Stejskalova et al., 2011).*"

(6) Minor comment: 1. Figure S3, scale the x-axis to 0.1 to 4 mg/mL would be better to present the concentration-dependent curves.

We have changed the scale in the figure as recommended by the reviewer.

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Relationship between the molecular composition, visible light absorption, and health-related properties of smoldering woodsmoke aerosols

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Abstract. Organic aerosols generated from the smoldering combustion of wood critically impact air quality and health for billions of people worldwide; yet, the links between the chemical components and the optical or biological effects of woodsmoke aerosols (WSA) are still poorly understood. In this work, an untargeted analysis of the molecular composition of smoldering WSA, generated in a controlled environment from nine types of heartwood fuels (African Mahogany, Birch, Cherry, Maple, Pine, Poplar, Red Oak, Redwood, and Walnut) identified several hundred compounds using gas chromatography mass spectrometry (GC-MS) and nano-electrospray high-resolution mass spectrometry (HRMS) with tandem multistage mass spectrometry (MS^n). The effects of WSA on cell toxicity, aryl hydrocarbon receptor (AhR)- and estrogen receptor (ER)-dependent gene expression were characterized with cellular assays, and the visible mass absorption coefficients (MAC_{vis}) of WSA were measured with UV-visible spectroscopy. The WSA studied in this work have significant levels of biological and toxicological activity, with exposure levels in both an outdoor and indoor environment similar to or greater than those of other toxicants. A correlation between the HRMS molecular composition and aerosol properties found that phenolic compounds from the oxidative decomposition of lignin are the main drivers of aerosol effects, while the cellulose decomposition products play a secondary role, e.g., levoglucosan is anti-correlated with multiple effects. Polycyclic aromatic hydrocarbons (PAHs) are not expected to form at the combustion temperature in this work, nor were observed above the detection limit; thus, biological and optical properties of the smoldering WSA are not attributed to PAHs. Syringyl compounds tend to correlate with cell toxicity, while the more conjugated molecules (including several compounds assigned to dimers) have higher AhR activity and MAC_{vis}. The negative correlation between cell toxicity and AhR activity suggests that the toxicity of smoldering WSA to cells is not mediated by the AhR. Both mass-normalized biological outcomes have a statistically-significant dependence on the degree of combustion of the wood. In addition, our observations support that the visible light absorption of WSA is at least partially due to charge transfer effects in aerosols, as previously suggested. Finally, MAC_{vis} has no correlation with toxicity or receptor signaling, suggesting that key chromophores in this work are not biologically active on the endpoints tested.

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1. Introduction

The combustion of wood from, for example, residential fireplaces, forest fires, and prescribed burns is a large source of fine particulate matter ($PM_{2.5}$) in the United States and much of the world (Rogge et al., 1998; EPA, 2003; Mazzoleni et al., 2007; Park et al., 2007; Swiston et al., 2008; Zhang et al., 2013), particularly in winter when woodsmoke aerosols (WSA) can account for the majority of organic carbon and up to 90% of $PM_{2.5}$ regionally (Rogge et al., 1998; Gorin et al., 2006; Kleeman et al., 2008; NYSERDA, 2008). In an indoor environment, the incomplete combustion of solid fuels (64 – 95% wood usage in rural India at, but also dung and crops (Menon, 1988)) is the main source of indoor air pollution exposure to roughly 3 billion people worldwide (WHO, 2011). This indoor WSA exposure occurs mostly in developing nations and mostly to women and children, possibly accounting for the highest burden of environmental disease globally (Ezzati and Kammen, 2002; WHO, 2002; Smith and Mehta, 2003).

Multiple factors impact the combustion chemistry, such as the wood composition (lignin/cellulose fractions, lignin H/G/S composition, natural monomers such as resins, waxes, sugars), water fraction, environmental conditions, and other factors, which in turn impact the aerosol composition and effects. During a typical fire, the high-intensity but short initial period of flaming combustion is correlated with CO_2 production and emissions that rise to higher altitudes. In contrast, the low-intensity but long period of smoldering combustion is correlated with CO and other products of incomplete combustion, and emissions that remain closer to the ground (Andreae and Merlet, 2001) where they are more likely inhaled. Smoldering combustion, which produces a higher quantity of particles and most of the organic species in a fire (Einfeld et al., 1991; Yokelson et al., 1997; Bertschi et al., 2003), is responsible for a majority of biomass consumption during prescribed burns, residential wood burning, cigarette smoke, and fires from tropical and temperate forests and deforestation areas (Standley and Simoneit, 1990; Ward et al., 1992; Yokelson et al., 1997; Simoneit et al., 2000; Ohlemiller, 2002; Rabelo et al., 2004). Due to the high toxicant production, the emissions from smoldering combustion are recommended to be used to assess health exposure (Morawska and Zhang, 2002). This work focuses on the organic aerosols generated from smoldering combustion of woods in a controlled laboratory setting.

The significant adverse health effects of WSA on endpoints such as mortality, morbidity, respiratory disease, cancer, among others, are well-documented and are characterized to be as serious, if not more so, than other sources of particulate matter (Lewis et al., 1988; Zelikoff et al., 2002; Boman et al., 2003; Kubátová et al., 2006; Orozco-Levi et al., 2006; Lewtas, 2007; Swiston et al., 2008; Danielsen et al., 2011). For example, organic extracts of particulate matter with high WSA content are approximately 30 times more potent than cigarette smoke condensate in a tumor induction assay (Cupitt et al., 1994). Yet, it is not clear whether the health-related effects of the WSA organics can be attributed to toxicants other than the well-studied polycyclic aromatic hydrocarbons (PAHs), such as the multifunctional aldehydes, ketones, phenols, organic nitrogen, organic acids, etc. (Hedberg et al., 2002) that are also present in WSA with largely unknown biological effects. Previous bulk analyses have shown that the emissions of total particulate matter and PAHs depend on the wood type and burn conditions (Maga and

Chen, 1985; Bølling et al., 2009; Nyström et al., 2017). High-resolution mass spectrometry (HRMS) analyses have offered insight into the complex molecular composition of aerosols from biomass combustion (Smith et al., 2008; Laskin et al., 2009; Lin et al., 2016; Lin et al., 2017; Fleming et al., 2018). However, the links between the chemical origin and macroscopic endpoints are still poorly understood. This work focuses on two critical gaps in knowledge surrounding woodsmoke (Naeher et al., 2007): the fundamental understanding of (1) how different types of woods and burn conditions affect the molecular composition and (2) relationships between the aerosol's properties and its chemical composition.

We measured the total cellular toxicity effects of smoldering WSA. In addition, the Ah receptor (AhR) and estrogen receptor (ER) are known to play critical regulatory roles in mediating the biological and toxicological effects of diverse environmental chemicals. As wood combustion produces PAH-like compounds and phenolic compounds, classes of chemicals that are known to activate the AhR and/or ER signaling pathways (Denison and Nagy, 2003; Krüger et al., 2008; Stejskalova et al., 2011; Li et al., 2012; Cipolletti et al., 2018), we measured the AhR and ER activities of smoldering WSA. Alongside biological effects, we also studied the potential chromophores responsible for the visible light absorption of smoldering WSA. The combustion of wood (and other biomass) is known to produce "brown carbon" aerosols (Andreae and Gelencser, 2006), which absorb light in the troposphere and appear yellow or brown to the eye. Although most of the absorption of WSA and other biomass burning aerosols is due to elemental carbon (soot) in the atmosphere, the organic aerosols such as in smoldering WSA represent a secondary but not insignificant fraction (Kirchstetter and Thatcher, 2012; Washenfelder et al., 2015).

2. Experimental

2.1. Smoldering combustion woodsmoke aerosols

Woodsmoke aerosol (WSA) samples were prepared in a 40 L smoldering combustion chamber (**Fig. 1**) that is fitted with Teflon tubing. The relative humidity (RH) for the combustion was set to ~ 40%, as verified by a membrane humidity probe (Vaisala Inc.), chosen to be consistent with the RH range found in some indoor environments (Salonvaara et al., 2004; Irulegi et al., 2017), where residential wood burning may occur, and with the daytime RH that are correlated with wildfire events (highest burned areas at RH 38 - 42%) (Flannigan and Harrington, 1988; Piñol et al., 1998). Humidified ultra zero air in the chamber was achieved by combining a ~ 1.5 L min^{-1} humid flow through a wet-wall humidifier with a ~ 2.5 L min^{-1} dry flow from a high-pressure cylinder (Airgas UZA: 20 – 22% O_2 in N_2, 2 ppm H_2O, 1 ppm CO_2, 1 ppm CO, 0.1 ppm total hydrocarbons) through flow restrictors.

Heartwood samples of nine types of wood (African Mahogany, Birch, Cherry, Maple, Pine, Poplar, Red Oak, Redwood, and Walnut) were purchased from a lumber store local to Davis, CA and were cut into 4 cm x 2 cm x 1 cm blocks, using a blade cleaned with isopropanol between each use. The subset of wood species selected are a combination of softwoods (Redwood, Pine) and hardwoods that can be found in North American forests (Burns and Honkala, 1990) and often used for firewood or

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for building homes and furniture. Approximately two wood blocks with an average mass of 2.5 g each were used for every burn with a combustion temperature of 330 °C, which produced a thick white smoke from the smoldering process. After 15 minutes of combustion, aerosols were collected from the center of the chamber, approximately 15 – 20 cm above the fuel source, for 25 minutes with a Polyvinylidene fluoride filter (Millipore Sigma, 0.65 μm pore size) and a flow rate of approximately 2 L min^{-1} (the remaining flow is discarded through the vent). An average of 85% by mass of the fuel source was burned. The temperature at the location immediately upstream of aerosol collection was approximately 25 °C. The tubing in contact with aerosols and filter collection apparatus was cleaned between each burn using isopropanol. Multiple (> 3) burns of each wood type were performed, with reproducible results.

The WSA samples were gravimetrically analyzed and, if not used immediately, vacuum-sealed in polyethylene filter holders and stored at –10°C. Prior to analyses, WSA were extracted in solvent using a mixture of 1:1 ultrapure water and acetonitrile with 30 minutes room-temperature agitation on an orbital shaker (IKA Inc.) at 1000 rpm. Each extraction used the required solvent to achieve a mass concentration of 5 mg mL^{-1}, depending on the WSA mass collected, and were diluted for analyses as necessary.

2.2. Molecular composition analysis

Diluted WSA extracts (100 μg mL^{-1}) were analyzed for organic molecular composition using a linear-trap-quadrupole Orbitrap (LTQ-Orbitrap) mass spectrometer (Thermo Corp.) at a mass resolving power of ~ 100,000 m/Δm at m/z 200. The WSA extracts were directly infused into a capillary nano-electrospray ion source (ESI, 50 μm fused-silica capillary tip, 4 kV spray voltage, 275°C capillary temperature, 15 μL min^{-1} flow), and the spectra were taken at a mass range of m/z 50 – 1000 in the positive ion mode. Ionization mechanisms in the positive ion mode ESI include protonation ($[M+H]^+$ ions) and/or sodiation ($[M+Na]^+$ ions). An external 11-point mass calibration in the m/z range of 100 – 600 was performed using a variety of analytical standard solutions (ESI-L tuning mix, amine mixture, and others, (Supelco Inc.)) immediately prior to the MS analysis, such that the mass accuracy is adjusted to be approximately 1 ppm for standard compounds. Insights into molecular structure were obtained using collision induced dissociation (CID) multistage tandem mass spectrometry (MS^n, stages 2 – 4) in the LTQ-Orbitrap for ions that have adequate signal. CID energy was tuned for each mass, so that the normalized precursor ion had 10 – 20% abundance, and neutral or radical losses were analyzed using the Thermo Xcalibur software suite.

The sample mass spectra with signal to noise ratio (S/N) > 3 were processed by subtracting the background mass spectra of the blank filter extracts, deconvoluted with a quadratic fit model and deisotoped using Decon2LS tools (freeware from PNNL), mass corrected with the external calibration curve, and assigned to molecular formulas using a custom Matlab protocol based on heuristic mass filtering rules (Kind and Fiehn, 2007) and Kendrick Mass (KM) defect analysis (Kendrick, 1963; Roach et al., 2011) with KM base of CH_2. Correlations with HRMS peaks are performed using Matlab's linear regression model fitlm. Least-squares fit results with coefficients (R^2) greater than 0.4 having slopes in either direction are reported.

The less polar, higher-volatility components of WSA were extracted using ethyl acetate at 100 μg mL^{-1} and analyzed with gas chromatography mass spectrometry (GC-MS) on an Agilent 6890 GC and 5973 MS detector using an HP-5MS column (30 m x 250 μm x 0.25 μm) and the following temperature program: 50°C (0.5 min hold), 12°C min^{-1} until 260°C (12 min hold). An EPA-610 method for PAHs (US EPA, 2005) and PAH certified calibration standard (TraceCERT, 16 components) were also used to verify if the WSA contained PAHs. The analytes were identified by their electron-ionization fragmentation at 70 eV – the spectra were compared to those from the GC-MS library from the National Institute of Standards and Technology.

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2.3. Mass Absorption Coefficient

The visible light absorption of WSA extracts were measured using a dual beam UV-visible spectrophotometer (UV-1800, Shimadzu Corp.) and 1 cm quartz cuvettes at multiple extract concentrations. The per-gram light absorption of WSA extracts were calculated as a mass absorption coefficient (Moosmüller et al., 2011):

$$MAC(\lambda) = \frac{A(\lambda)*\ln(10)}{C*L} \quad (1)$$

where A(λ) is the wavelength-dependent absorbance of the WSA extract, C is the extract concentration (0.005 g mL^{-1}), and L is the pathlength of the light absorption (1 cm). To account for the wavelength dependence of MAC and compare between the WSA extracts in this study, we used an average MAC that was integrated in the visible region:

$$MAC_{vis} = \frac{1}{700nm-400\ nm} \int_{400\ nm}^{700\ nm} MAC(\lambda)d\lambda \quad (2)$$

The absorption spectra (**Fig. S1**) of the WSA extracts in this study are consistent with other ambient brown carbon spectra, where a featureless tailing absorption is observed into the visible wavelengths. As this work is focused on visible-light-absorbing chromophores, the UV portion of the brown carbon absorption was not examined. MAC_{vis} values determined at multiple extract concentrations varied less than 5% (**Fig. S2**).

2.4. Cell Toxicity Bioassay

Human epidermal keratinocytes were used to determine the toxicity of WSA. Keratinocytes can be found naturally in the upper respiratory tract, oral cavity, and formed in the trachea as a response to air pollution, cigarette smoke, and woodsmoke inhalation exposure (Plamenac et al., 1979b; Plamenac et al., 1979a; Lee et al., 1994; Moran-Mendoza et al., 2008). Moreover, they have been shown to be a useful model for responses common to epithelial cells (Rhim, 1989; Neugebauer et al., 1996; Rogers et al., 2001). When exposed to toxic xenobiotic compounds, keratinocytes form an envelope of isopeptide cross-linked protein at the plasma membrane (Rice and Green, 1979). The cross-linking can be induced by permeabilization of the cells,

permitting a rise in cytosolic calcium ions that activates the membrane-bound transglutaminase TGM1 and results in cell death. The protein content from envelope formation serves as a quantitative biomarker of cell damage from toxic exposures.

Briefly, cells were cultured and assayed as follows: spontaneously immortalized keratinocytes (SIK, passages 25-35) were serially cultivated in a 2:1 mixture of Dulbecco-Vogt Eagle's and Ham's F-12 media supplemented with fetal bovine serum (FBS) (5%), hydrocortisone (0.4 µg/ml), epidermal growth factor (10 ng/ml), insulin (5 µg/ml), transferrin (5 µg/ml) and 0.18 mM adenine (Allen-Hoffmann and Rheinwald, 1984; Rice et al., 1993). SIK cultures have a single chromosomal aberration that permits continuous growth throughout the range of passages employed while altering their behaviour only minimally (Rice et al, 1993). After reaching confluence in 24 well plates, the cell cultures were incubated in a medium lacking epidermal growth factor for two days. Cultures were then incubated for 48 h with extracts of WSA (5 mg/mL in 1:1 v/v water:acetonitrile, 15 – 240 µL) in the concentration range of 0.23 to 3.75 mg/mL in a total well volume of 320 µL, to increase the assay sensitivity while conserving cell viability. After incubation, the medium was discarded, and the cultures were further incubated for 24 h in an aqueous solution of 2% sodium dodecyl sulfate, 20 mM dithiothreitol and 50 mM Tris buffer (pH 8) at 37ºC. The envelopes from each well were isolated by centrifugation, rinsed 3 times in 0.1 % sodium dodecyl sulfate and assayed for relative protein content with bicinchoninic acid, quantified colorimetrically at a wavelength of 495 nm (Smith et al., 1985). As the protein structures of SIK cells exposed to WSA were isolated during the washing process, no interference from WSA absorption occurs in the colorimetric analysis. WSA extracts were assayed at each concentration in triplicate. Blank controls (extracted blank filter) and solvent controls did not result in envelope formation, nor inhibition of envelope formation. Ionophore X5375, which is known to induce envelope formation in the large majority of cells (Rice and Green, 1979), was used as the positive control.

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The half maximal effective concentration (EC_{50}) of the WSA extract that induced a cell toxicity response halfway between the baseline and the maximum activity, was obtained using concentration-response curves fitted in Origin software with adjusted $R^2 > 0.94$. The EC_{50} uncertainties were propagated from fit uncertainties and the standard deviations of triplicate analyses. Individual concentration-response curves and fits for the WSA extracts are shown in **Figure S3**.

2.4. Aryl hydrocarbon Receptor (AhR) and Estrogen Receptor (ER) bioassays

Screening of WSA extracts for AhR- or ER-active compounds were carried out using recombinant mouse hepatoma (H1L6.1c2) cells containing a stably integrated AhR-responsive luciferase reporter gene plasmid (pGudLuc6.1) and human breast carcinoma (VM7Luc4E2) cells containing a stably integrated ER-responsive luciferase reporter gene plasmid (pGudLuc7ere), as previously described (He et al., 2014; Brennan et al., 2016). Briefly, for AhR analysis, H1L6.1c2 cells in growth medium (alpha minimal essential media (aMEM) containing 10% FBS) were plated (75,000 cells/well) into white, clear-bottomed 96-well tissue culture plates and incubated at 37°C for 24 h prior to the addition of WSA extracts. For ER analysis, VM7Luc4E2 cells were switched from maintenance medium (aMEM containing 10% FBS) to estrogen-stripped

medium (phenol red-free aMEM containing 10% charcoal-stripped FBS), incubated for 3 days at 37°C before plating into white, clear-bottomed 96-well tissue culture plates at a density of 75,000 cells/well and further incubated at 37 °C for 24 h prior to chemical addition. Cells were incubated with 1 µL of: DMSO solvent (1% (v/v)), the reference standard dissolved in DMSO (2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) for H1L6.1.c2 cells and 17β-estradiol (E2) for VM7Luc4E2 cells), acetonitrile solvent, or WSA extract for 24 h.

After incubation, cells were visually inspected for signs of toxicity, rinsed twice with phosphate-buffered saline, followed by the addition of Promega cell lysis buffer, and shaking for 20 min at room temperature to allow complete cell lysis. Luciferase activity in each well was measured by using an Orion microplate luminometer as previously described (He et al., 2014). Luciferase activity was corrected for background activity in DMSO-/acetonitrile-treated cells and values (in relative light units (RLUs)) expressed as a percent of the luciferase activity obtained with the maximally inducing concentration of TCDD or E2. All incubations and analyses were performed in triplicate. TCDD bioanalytical equivalents (BEQs) for AhR active samples were determined by comparison of the luciferase activity of the sample extract to that in a TCDD concentration response curve, as described elsewhere (Baston and Denison, 2011), and shown in **Figure S4**. The relative magnitude of AhR and ER induction by WSA (5 x 10^{-6} g of original material in each plate well) was normalized to the equivalent level of AhR and ER luciferase gene induction from the TCDD and ER concentration response curves, respectively, on a g/g basis. The uncertainties in AhR and ER relative activity were propagated from the uncertainties in the triplicate determinations and the reference calibration concentration-response curves.

3. Results and Discussion

3.1. Biological and optical properties of WSA

Table 1 shows cell toxicity, AhR activity, ER activity and MAC_{vis} results for the nine types of WSA extracts studied in this work, normalized by the mass of the original aerosol material. The results provide a clear indication that this type of complex mixture can affect macromolecular targets in cells. An important question is how relevant are the findings with smoldering WSA to health effects from actual exposures. Although no assay will successfully reproduce the *in vivo* environment, i.e., exposure to approximately 40 different cell types in the respiratory tract, distribution of toxicants throughout the bloodstream to major organs, and either deactivation or activation by metabolism, these (and other) *in vitro* results are useful as an estimation of risk. Considering toxicity in an outdoor environment such as a wildfire event, $PM_{2.5}$ concentrations in excess of 300 µg/m^3 can be observed in surrounding areas (Wu et al., 2006; Wegesser et al., 2009) and up to 2.7 mg/m^3 near the burn site (Adetona et al., 2011). As much of the aerosol comes from the first and second smoldering phases (Alves et al., 2011), a realistic exposure to smoldering WSA in cities surrounding a fire can be 100 µg/m^3. Assuming a typical pulmonary tidal volume of 500 mL for adults (Beardsell et al., 2009), and the cell toxicity EC_{50} of Red Oak smoldering WSA, we estimate that a human adult would take ~ 2175 breaths to reach the EC_{50} value. As a normal respiratory rate for an adult at rest is 12 breaths

per minute (Carey et al., 2005), the EC_{50} limit is reached after 3 h of woodsmoke exposure, yet residents near fires can be exposed to the smoke for many days (Wegesser et al., 2009). The acute exposure health risk of residential wood burning in an indoor environment is more severe. $PM_{2.5}$ concentrations around cookstoves are high (e.g., 1.8 mg/m^3) (Chowdhury et al., 2012), such that the EC_{50} limit is reached in ~ 120 breaths or 10 minutes, a highly plausible exposure duration that occurs almost daily for some people. The cell toxicity results are consistent with epidemiological and toxicological evidence for negative human health impacts of WSA ((Naeher et al., 2007), and references therein). In light of previous findings that smoldering combustion at medium temperature produces particles that are more toxic than those from high-temperature combustion or complete combustion ((Bølling et al., 2012), and references therein), the results from this work suggest that the smoldering fraction of WSA could significantly contribute to the overall WSA health impacts.

The interpretation of AhR activity of WSA is more complex. AhR (Denison and Heath-Pagliuso, 1998) is a ligand-activated transcription factor that mediates the biochemical and toxic effects of various well-recognized environmental toxicants, such as dioxins (Mandal, 2005; Denison et al., 2011) and PAHs (Machala et al., 2001; Barron et al., 2004; Billiard et al., 2006), but also a wide variety of other structurally-diverse chemicals (Denison and Nagy, 2003; Nguyen and Bradfield, 2007; Petkov et al., 2010). However, AhR-dependent (i.e. dioxin-like) toxicity appears to be mediated only by dioxin-like chemicals (e.g., polyhalogenated dibenzo-p-dioxins, dibenzofurans and biphenyls) and not all AhR agonists, the majority of which are metabolically inactivated. Determination of the presence of toxic AhR ligands in a sample requires extensive sample workup to isolate dioxin-like chemicals from the large number of nontoxic AhR active chemicals. Thus, the AhR bioassay results for WSA in this work provide a measure of the overall ability of the extract to activate the AhR and AhR-dependent gene expression and does not provide a direct measure of AhR-dependent toxic potency. However, they do not rule out the presence of toxic AhR ligands; further sample isolation and analytical analysis would be needed to confirm the presence of dioxin-like chemicals in the WSA samples. The relatively high levels of AhR active chemicals observed in the WSA samples tested here could potentially lead to adverse effects via the ability of these compounds to stimulate AhR-dependent expression of gene products such as cytochrome P4501A1, which can contribute to the metabolic activation of PAHs into their mutagenic form, as well as contribute to increased oxidative stress as a result of the production of reactive oxygen species as a byproduct of CYP1A1 metabolism of endogenous and exogenous chemicals (Park et al., 1996; Nebert et al., 200, 2004). Our results are also fairly consistent with the previous observation that WSA has a relative carcinogenesis potency of $1 - 6 \times 10^{-4}$ compared to pure benzo(a)pyrene (Lewtas, 2007), another AhR ligand with well-recognized adverse health effects, although the lack of information about the specific wood fuels and burn conditions used in that study do not allow direct comparisons to the WSA results presented here.

The ER activity assay results demonstrate the presence of relatively low levels of endocrine active chemicals (EACs) or low potency EACs in WSA to which inhalation exposure could occur. WSA exposure risk would need to take into account a variety of aspects, including the ambient dose and the physicochemical characteristics and metabolic stability of the responsible EACs.

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In terms of ecological risk, the concentration of WSA chemicals from partitioning into water would be expected to be low, and thus it would be unlikely to produce endocrine effects in wildlife from this route. In terms of exposure risk to humans, the comparison is challenging because the concentrations of known EACs are not as widely measured in atmospheric aerosols as the "usual suspects" like PAHs, and many EACs still remain to be identified. Additionally, the ability of a chemical to act as an EAC in vitro does not address whether it can produce adverse endocrine-related health effects in vivo (i.e. whether it acts as an endocrine disruptor chemical (EDC)). In an indoor environment the WSA concentration can be higher than outdoors, but the exposure from using cookstoves is not as sustained over many hours or days such as that which occurs with a wildfire, and is not nearly as continual as with exposure to ER-active chemicals from consumer or industrial products. It's possible that risks from WSA exposure may be comparable to other EACs/EDCs of concern in some situations.

Despite dissimilarities in chemical composition, it can be instructive to compare the total AhR and ER activity of smoldering WSA to those measured for ambient particulate matter. Utilizing similar cell-based bioassays, PM_1 collected from rural and urban traffic sites in Switzerland was found to have AhR activity of 0.5 – 2 x 10^{-6} g TCDD EQs/g PM and ER activity of 2 – 23 x 10^{-9} g E2 EQs/g PM (Wenger et al., 2009a; Wenger et al., 2009b). Similarly, PM_{10} collected from downtown Toronto, Canada was found to have AhR activity of 0.04 – 1 x 10^{-6} g TCDD EQs/g PM and ER activity of ~10^{-6} g E2 EQs/g PM (Clemons et al., 1998). PM of various size fractions from different polluted sites in the Czech Republic had AhR activity of 0.001 – 1 x 10^{-6} g TCDD EQs/g PM and ER activity of 0.1 – 20 x 10^{-9} g E2 EQs/g PM (Novák et al., 2014). Organic PM in Wuhan, China was found to have ER activity of ~ 2 – 8 x 10^{-7} g E2 EQs/g, depending on whether the PM was collected on sunny or foggy days (Wang et al., 2004). In comparison, the AhR activities of smoldering WSA in this work are within the range of PM from urban and industrial sites, while the ER activities are similar to some types of urban/industrial PM but a factor of 10^{2-3} lower than others. Wenger et al. (2009) also found the total concentration of AhR agonists to be much higher than concentrations for the traditional AhR agonists such as PCDD/Fs, suggesting a diversity of AhR-active ligands in ambient PM and further supporting the notion that AhR can bind and be activated by a wide range of structurally diverse chemicals in the environment (Denison and Nagy, 2003; DeGroot et al., 2011; Stejskalova et al., 2011). Furthermore, it has been reported that $PM_{2.5}$ exposure can affect sperm development in humans (Wu et al., 2017; Lao et al., 2018), particularly in men who were exposed to wintertime air pollution where WSA is high (Selevan et al., 2000), however, whether ER- and/or AhR-active chemicals present in smoldering WSA could contribute to these endocrine effects remains an open question.

The relative biological effects of WSA from different woods is a novel insight from this study to the best of our knowledge. From **Table 1**, it appears that the WSA extracts that are highly active in the AhR bioassay do not necessarily kill cells, and vice versa. In fact, we found that the cell toxicity and AhR activity of woodsmoke WSA were negatively correlated with a fairly strong adjusted R^2 coefficient of 0.74 (**Fig. S5a**). This suggests that the smoldering WSA analyzed in these bioassays contain compounds whose cytotoxic activity is not AhR mediated, but may be classified among the myriad other AhR active chemicals that have been identified (Denison and Nagy, 2003; Galati and O'brien, 2004; Nguyen and Bradfield, 2007; DeGroot

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et al., 2011). We found that the biological effects of WSA have a moderate ($R^2 > 0.5$) relationship with the percent by mass of the wood fuel that has been burned in a set duration of time (**Fig. 2**), which may be related to the lignin content and solvent-soluble monomers in the fuel (White, 2007). As all WSA effects have been normalized by mass, this indicates that the biological properties may be related to the degree of oxidation/degradation of the woods during the smoldering process. That said, we found no relationship between aerosol properties and the O:C ratio of the WSA compounds. This may be because even oxidative processes can fragment molecules in the wood in addition to functionalizing certain molecules with oxygenated moieties. This interesting relationship should be further explored with more focused studies.

The MAC values for different WSA in this work are within range of organic-rich brown carbon (Updyke et al., 2012) and particulate matter from a smoldering fire (Patterson and McMahon, 1984), but lower than ambient brown carbon likely because ~ 90% of that absorption comes from elemental carbon (Washenfelder et al., 2015). The MAC for the extracts in our study may also be lower than those extracted in less-polar solvents such as acetone (Chen and Bond, 2010). Within the limited fuel types employed in this work, there does not appear to be an obvious correlation between MAC_{vis} and wood hardness or percent of wood burned by weight ($R^2 \sim 0.08$) – suggesting visible light absorption of the aerosols may depend on the more specific chemistry to form chromophoric constituents in the combustion. Furthermore, the MAC_{vis} values do not correlate to either cell toxicity (**Fig. S5b**, adj. R^2 0.03) or AhR activity (**Fig. S5c**, adj. R^2 -0.06), suggesting that chromophoric species in this work (e.g., specific conjugated compounds, charge transfer complexes, etc.) are not necessarily those producing these effects. In contrast, specific classes of chromophores from atmospheric aerosols in China have been found to have oxidative potential based on a chemical assay, which were suggested to be due to their PAH-like, quinone-like, or metallic constituents (Chen et al., 2019).

3.2. Molecular composition of WSA and correlations to aerosol properties

The chemical composition of aerosols from the oxygen-poor smoldering combustion or oxygen-free pyrolysis of lignocellulose material such as wood has been the subject of extensive study (Edye and Richards, 1991; Simoneit et al., 1993; McKenzie et al., 1994; Ingemarsson et al., 1998; Simoneit et al., 2000; Oros and Simoneit, 2001a, b; Simoneit, 2002; Hosoya et al., 2007b; Nunes et al., 2010), with many of the previous works focusing on the mid- to low-polarity constituents that can be analyzed by GC-MS. Low-intensity combustion abundantly forms phenolic derivatives with hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units from the breakdown of lignin. Furan derivatives (e.g., furfurals), sugar anhydrides (e.g., levoglucosan, mannosan, galactosan), and other products are formed from the combustion of cellulose under a variety of conditions (Shafizadeh and Fu, 1973; Simoneit et al., 1999). PAHs are not expected to be formed at the 330 °C combustion temperature in this work (Rhee and Bratzler, 1968; Sharma and Hajaligol, 2003), and indeed the GC-MS analysis using ethyl acetate solvent confirmed that PAHs were not observed above the detection limit (**Table S1**). If formed, PAHs might have ended up in tar-like material as opposed to the aerosols that were collected and tested. Thus, the biological activity and light absorption of the smoldering WSA in this work are not likely due to PAHs. However, we observed molecular formulas that could be assigned

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to PAH-like compounds (**Fig. 4**) from lignin decomposition. The GC-MS analysis identified sugar anhydrides, phenolic compounds, and alkane derivatives in the mid- to low-polarity fraction of the smoldering WSA (**Fig. S6**, **Table S1**), consistent with previous reports.

Figure 3 shows the positive ion mode HRMS spectra of WSA extracts, highlighting the more abundant constituents in the polar, lower-volatility fraction. MS^n analyses support that the majority of smoldering WSA compounds are phenolic species (e.g., CH_3OH losses, H_2O losses, and phenyl ionic fragments) that have a variety of carbonyl, alcohol, alkenyl, acid, and other moieties. There are 300 – 400 peaks in each spectrum that are both over the limit of detection and that can be assigned to a molecular formula within 2 ppm mass accuracy. There are more than 700 unique peaks observed in all samples.

Figure 4 shows the chemical structures of select observed compounds. The compound with the highest abundance in most samples was sinapaldehyde ($C_{11}H_{12}O_4$, **Fig. 3** green), except for the coniferous softwoods (Pine/Redwood) where coniferaldehyde ($C_{10}H_{10}O_3$, **Fig. 3** blue) was the highest peak. This is consistent with the fact that coniferyl alcohol is the main polymer building block of softwood lignin, while sinapyl alcohol (to a higher extent) and coniferyl alcohol are both important building blocks of hardwood lignin (Graglia et al., 2015). We did not observe high abundance of hydroxyphenyl (H) derivatives, (e.g., coumaraldehyde is ~ 25 times less abundant than sinapaldehyde on average) which is consistent with the fact that coumaryl alcohol is the dominant lignin building block in grasses instead of wood (Himmelsbach and Barton, 1980). Levoglucosan (and its isomeric sugar anhydrides, $C_6H_{10}O_5$, **Fig. 3** magenta) was also abundantly observed in HRMS, with the largest signal in Redwood WSA, alongside its decomposition products such as furfural, hydroxymethylfurfural, tetrahydrofuran derivatives, and others (Hosoya et al., 2007a; Lin et al., 2009).

High-molecular weight compounds that were observed are tentatively assigned to dimers from phenolic G and S units building blocks with various linkages (**Fig. 4,** bottom) according to previous observations (Goñi and Hedges, 1992; van der Hage et al., 1994; Guillén and Ibargoitia, 1999; Christensen et al., 2017), mechanistic feasibility (Beste, 2014), and MS^n evidence where available. Radical chemistry of the lignin formation and combustion is probabilistic, such that the diversity of isomers increases with molecular mass, so there are likely multiple structures possible for each larger molecular formula. For example, $C_{14}H_{14}O_4$ is assigned to 5,5'-diguaiacol based on its $C_7H_8O_2$ guaiacol neutral loss fragment but concurrent losses of CO and C_2-C_3 fragments indicate the presence of other species with the same elemental makeup. Many of the proposed dimer assignments can be rationalized with linkages that are already present in the wood itself (e.g., β-O-4, β-β, β-5, 5-5', (Watts et al., 2011)), suggesting that simple depolymerization of the lignin plays an important role in forming aerosol-phase species, similarly to pyrolysis. Attempts to perform MS^n on most high-molecular-weight peaks were inconclusive due to the higher density of accurate-mass peaks at each m/z that provided a challenge for their isolation by the ion trap. Chemical assignments without associated MS^n study in this work should be treated as purely speculative. A representative MS^2 spectrum and proposed fragmentation loss pathways are shown in **Figure S7**. The full list of common ions, regardless of signal or correlations, is shown in **Table S2**.

We then performed linear least-squares correlations of the accurate-mass m/z peaks with the aerosol properties of cell toxicity, AhR activity, and visible light absorption (MAC_{vis}) for each WSA sample. A potential concern is that HRMS signals in complex mixtures can suffer from matrix effects so that the signal is not necessarily representative of concentration; however, we found the HRMS signals are strongly correlated with GC-MS signals (e.g., sinapaldehyde $[M+H]^+$ m/z = 209.081, GC retention time 16.6 minutes, has adj. $R^2 = 0.81$, **Fig. S8**), suggesting that the matrix is similar enough between the different samples that correlations using HRMS are likely meaningful. Peaks are required to be present in 8 out of 9 samples for the statistical analysis. **Table 2** shows the correlation results, the m/z and neutral molecular formulas, neutral or radical loss fragments derived from CID, and proposed assignments for select peaks based on the MS^2 evidence when available, literature previously cited in this article, and guidance from GC-MS observations (**Table S1**). 90% of the correlations shown in **Table 2** have p-values ranging from 0.0002 to 0.05, while 100% have $p < 0.1$. This suggests that the correlations have moderate-to-strong evidence against coincidence.

The occurrence of structural isomers is a limitation that may confound the statistics in this work, especially if those isomeric compounds have opposing effects on bioactivity or light absorption. With those caveats in mind, we observed a number of moderate-to-strong correlations with exact m/z peaks, either with a positive or negative effect, for the three aerosol properties examined. A positive effect means that the signal of the peak directly correlates (higher signal = lower cell toxicity EC_{50}, higher AhR activity, higher MAC_{vis}) and a negative effect means anti-correlation, neither of which is necessarily causative. For example, levoglucosan was found to be anti-correlated with MAC_{vis} (**Fig. 5**, $R^2 = 0.75$) and we interpret this to mean that lignin derivatives are more likely responsible for visible light absorption because of their highly-conjugated structures versus cellulose derivatives (**Fig. 4**), so when a wood has more cellulose content (producing more levoglucosan), the MAC_{vis} decreases. Also, for example, we found the m/z of vanillin to be somewhat correlated with AhR activity ($R^2 = 0.45$), and even though vanillin itself is not AhR active, Bartonkova and Dvorak (2018) found that the complex mixture of vanilla is highly AhR active, postulating that the AhR activity of the vanilla mixture is caused by minor constituent(s) (< 10%). Similarly, our correlation of vanillin with AhR activity may be due to vanillin's interaction or co-formation with other constituents in the WSA extract.

The cell toxicity correlations appears to be mainly driven by C_{11} compounds, some of which are syringyl derivatives. We found a positive effect of sinapaldehyde and sinapyl alcohol on cell toxicity, and possibly for related compounds such as $C_{11}H_{14}O_5$ that is tentatively assigned to be sinapyl hydroperoxide. The hydroperoxide assignment is based on the facile H_2O loss (MacMillan and Murphy, 1995) with lack of HCOOH, and a feasible mechanism of formation, e.g., C-O cleavage of sinapyl alcohol in a heated oxidative environment to form the R radical (Beste, 2014) that can add O_2 and then form the ROOH through reaction with HO_2 or other RH (Atkinson, 2000). This is somewhat consistent with sinapaldehyde's strong effect on inhibiting the growth of bacterial cells, while other phenolic compounds like vanillin and syringaldehyde had no effect (Figueiredo et al., 2008). Similarly syringaldehyde and vanillin had no effect in our cell toxicity assay. The potential toxicity of monolignols like

sinapyl alcohol has been noted in plants (Whetten and Sederoff, 1995), and while we would expect that coniferyl alcohol may have a similar effect, no correlation was observed. Among other factors, this could be due to isomeric compounds present at the coniferyl alcohol m/z, including an acid (HCOOH loss) and aldehyde (CO loss), compared to the sinapyl alcohol peak that did not have these interferences. Another positively correlated molecular formula is $C_{14}H_{16}O_2$ (R^2 = 0.74) that appears to belong to compounds with quinone, furfuryl alcohol, and other substructures in the WSA based on fragmentation patterns so it is not clear which compounds specifically affect cell toxicity. Quinones can be cytotoxic or cytoprotective depending on structure (Bolton and Dunlap, 2016) and furfuryl alcohol can be mildly immunotoxic (Franko et al., 2011); therefore, the correlation has mechanistic plausibility. We found that 5-hydroxymethylfurfural (HMF), an important decomposition product of cellulose, was anticorrelated with cell toxicity (R^2 = 0.54) for reasons that are unclear. HMF can be biologically active; however, at a dose that would be unfeasibly high for human exposure (~ 200 g based on the oral acute LD_{50} of 2.5 – 3.1 g/kg in rats (Hoydonckx et al., 2000)) and we would expect it to have no effect on cell toxicity in our assay. It is possible that HMF is anti-correlated because of its connection with levoglucosan, which is also anti-correlated but with a weaker relationship (R^2 = 0.31) and consequently not included in **Table 2.** If so, the interpretation would be similar to that for MAC_{vis}, i.e., a higher lignin/cellulose ratio in the wood may increase cell toxicity in the smoldering WSA.

In this study, compounds that correlate with AhR activity tend to have more carbons than the lignin monomers (mainly C_{14}-C_{19}) and more C=C unsaturation. These compounds are either formed in the combustion or exist naturally in wood, as wood dust itself has been shown to be AhR-active (Wilson et al., 2015). The calculated double bond equivalents (rings + π-bonds = DBE, (Badertscher et al., 2001)) are consistent with AhR active compounds having more unsaturation on average. DBEs for the positively-correlated compounds (<DBE> = 8.0, max 11) are notably higher than those that are not correlated (<DBE> = 6.2, max 11) and those that are anti-correlated (<DBE> = 5.6, max 7). This explanation is an oversimplification, as double bonds and even aromaticity do not account for the specific electronic properties or the conformational planarity of the ligands that are key to effective binding with AhR (Denison et al., 2002; Petkov et al., 2010). If the C_{14}-C_{19} compounds are indeed lignin dimers, it is reasonable to postulate that the higher AhR activity is partially due to the ability of the dimers to form PAH-like structures from the radical reactions. For example, $C_{16}H_{14}O_4$ (**Fig 4** bottom right) is assigned to be (at least partially) a phenanthrene derivative generated from β-5 dimerization or radical depolymerization reactions and ring closure (Beste, 2014) and may be potent for induction of AhR – we found a fairly high correlation coefficient (R^2 = 0.65, **Fig. 5**). Similarly, other molecular formulas that can be assigned to a phenanthrene derivatives ($C_{18}H_{16}O_5$ and $C_{18}H_{16}O_6$) are also positively correlated with AhR activity (both R^2 = 0.4).

$C_{14}H_{12}O_3$ (R^2 = 0.57, positive effect for AhR and R^2 = 0.34, negative affect on cell toxicity (not shown on Table 2)) may be related to the naturally-occurring phenol resveratrol which is a dimer of H and dihydroxybenzene with a β-1 linkage. Resveratrol, found in wood in low amounts 0.017 – 0.271 g/kg, (Tyśkiewicz et al., 2019)) and other plant materials, has AhR antagonist activity and may be protective against dioxin (AhR-dependent) toxicity (Casper et al., 1999), consistent with the

negative correlation observed here. The lower signal of this ion in our samples prevented a more-detailed MS^n study, so the assignment is tentative. However, since this molecule has only alcohol groups, the H_2O loss we observed in absence of CH_3OH, CO, or other labile groups does not contradict the assignment. Some smaller compounds were also positively correlated with AhR activity, with the highest correlation for $C_{10}H_{10}O_2$ (R^2 = 0.74), assigned to methoxy cinnamaldehyde based on its relatively-clean MS^2 spectrum. The interaction of methoxy cinnamaldehyde with the AhR-responsive gene product CYP1A1 results in its oxidation into an acid (Hasegawa et al., 2002), and it appears to be mainly protective (Cope, 2019). This would be consistent with the observed anti-correlation of this product with cell toxicity (R^2 = 0.42).

Roughly 90% of the correlations with MAC_{vis} are positive in nature and the positively-correlated compounds are fairly diverse, but many have conjugated structures. The conjugation that is observed for lignin phenols (DBE ~ 5), and even for the observed PAH-like compounds, are insufficient to absorb visible light; however, when relatively-small lignin phenols are deprotonated in solution, they absorb light in the visible range due to the resonance stabilization of the phenolates – the red shift is much more pronounced for phenols with extra conjugation such as sinapaldehyde (Panossian et al., 2001). Our solution is not sufficiently basic (pH ~ 5) for the deprotonated form to substantially exist in equilibrium (e.g., pKa for sinapaldehyde is 8.2, coniferaldehyde is 7.98, vanillin is 7.4, and bivanillin is 6.16, (Ragnar et al., 2000); however, localized charge-transfer interactions may facilitate the deprotonation of phenols at pH much lower than their pKa (Abramovitz and Massey, 1976). It is not clear why sinapaldehyde and coniferaldehyde do not correlate with MAC_{vis}, but vanillylidene acetone with its additional sigma carbon does. Vanillylidene acetone can achieve some additional resonance with its keto-enol tautomerization (which tends not to happen in the ring because the aromatic phenol is highly favored) while sinapaldehyde and coniferaldehyde cannot. Even so, many of the correlations are not easily understood and could be due to coincidence or driven by unassigned isomers. In the ambient, nitrophenols may significantly contribute to wood-burning brown carbon (Mohr et al., 2013; Lin et al., 2017). In this work, these nitrophenols were not observed by GC-MS, so the mechanism of light-absorption is likely different. It is possible that charge-transfer complexes between neighboring OH and C=O in dimers (e.g., $C_{18}H_{18}O_6$, **Fig. 4)** may be chromophoric**,** as suggested previously for brown carbon aerosols (Phillips and Smith, 2014). The phenols may also potentially form intermolecular complexes with proton acceptors such as carbonyls or heterocyclic nitrogen species (e.g., $C_9H_{11}NO_4$ with a pyridine-like (C_5H_5NO) loss fragment), as the aerosols species are in close proximity. While no four-ring PAH-like species that would absorb in the visible range (Samburova et al., 2016) were observed in this work, we can't rule out the possibility that highly-conjugated compounds below the detection limit can contribute to light absorption in combination with other chromophores. For the compounds that are correlated with MAC_{vis}, it is likely that they need to be activated to the ionic form to be chromophoric.

Finally, although composition characterization was not the main focus of this work, we observed some fairly abundant compounds that have not been previously reported. For example, we identified some organic nitrogen compounds as unknown pyridine derivatives (e.g., $C_9H_{11}NO_3$) and nitro or nitrate derivatives (e.g., $C_5H_{10}N_2O_8$), although in general, organic nitrogen

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is a larger fraction of ambient biomass burning aerosols (Laskin et al., 2009; Lin et al., 2016) than in this study. Poplar WSA also has high abundance peaks that have not been previously observed, e.g., corresponding to the molecular formula $C_{20}H_{21}NO_3$ (**Fig. 3**, dark yellow). Due to the high signal, we assigned $C_{20}H_{21}NO_3$ to one or more naturally-occurring alkaloids as opposed to being formed from dimerization processes of phenols (plausible monomers should have higher signals than dimers). However, alkaloids that have been extracted from poplar heartwood with the same molecular formula (Chen-Loung et al., 1976; Cordell et al., 1989) do not appear to be entirely consistent with the MS^2 fragmentation patterns of $C_{20}H_{21}NO_3$ in this work (neutral losses CH_3OH, $C_3H_6O_2$, and $C_3H_9NO_3$), suggesting that this compound may be different than previously-identified alkaloids or modified from an oxidative process in the smoldering combustion. Poplar WSA also have a relatively low EC_{50} value in our cell toxicity study, which is consistent with the higher cell toxicity and genotoxicity response of combustion aerosols from Poplar compared to softwood pellets in residential boilers (Kasurinen et al., 2016). Unfortunately, since other types of woods were not studied by Kasurinen and colleagues (2016), a more thorough comparison can not be made. As the HRMS correlations require the peaks to be present in most samples for adequate statistics, it is not clear whether the properties of the Poplar or other WSA are associated with the uniqueness of their composition rather than commonalities.

4. Conclusions

The composition of smoldering WSA generated in the conditions of this work produced abundant lignin and cellulose oxidation and decomposition products that impact aerosol properties in ways that are not easily predictable. We showed that the components of smoldering WSA have high biological activity that can substantially contribute to the environmental health burden of woodsmoke, and that these health-related effects are not likely PAH-mediated for the smoldering conditions used in this work. The observed bioactivity may be linked to the percent of the fuel that has been burned, and thus to the degree of combustion, with more toxic aerosols formed at earlier stages of the burn. It is possible that the more toxic compounds are eventually degraded during the combustion process. These results underscore the importance of untargeted analyses to move beyond well-studied toxicants when considering organic aerosol properties, as the statistical studies identified multiple targets for further toxicological testing. We found that lignin phenols are correlated with visible light absorption (i.e., levoglucosan is anti-correlated) and hypothesize that the mechanism of action is through charge transfer reactions to form phenolates. In atmospheric aerosols, phenolates have been suggested to contribute substantially to brown carbon absorption as facilitated by their charge-transfer interactions (Phillips et al., 2017). The lignin percent of wood may also drive toxicity effects, but this remains to be explored. Although some mechanistically-probable correlations with bioactivity and light absorption are found in this work, the potential correlations are possibly obscured by isomeric interference at some masses. Future work should add a separation component to the accurate-mass and MS^n analysis of WSA for additional clarity. This information will help understand the contributions of individual components and enhance the value of testing such complex mixtures.

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5. Data Availability

Data are available from the corresponding author upon request.

6. Competing interests

The authors declare no competing financial interests.

7. Author Contributions

TBN and KQN designed the experiments, LKC and KQN carried out the experiments. All authors contributed original data and data analyses. LKC, KQN, and TBN prepared the draft manuscript. All co-authors have reviewed and edited the manuscript.

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Table 1: *Results for cellular assays and visible light mass absorption coefficient (MAC_{vis}) for WSA extracts. Lower EC_{50} values correspond to higher toxicity. AhR and ER activities are expressed as bioequivalent (BEQ) values of calibrant compounds 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 17β-estradiol (E2), respectively. All aerosol properties reference the mass in grams of original WSA material that were tested. The concentration-response curves for the cell toxicity of different WSA (**Fig. S3)** and receptor activity of TCDD and ER (**Fig. S4)** can be found in the supplement.*

Wood sample	Cell Toxicity EC_{50} (μg)	AhR Activity (TCDD BEQ, g/g)	ER Activity (E2 BEQ, g/g)	MAC_{vis} (cm^2/g)
Afr. Mahogany	250 ± 20% [a]	2.5×10^{-6} ± 12%	3.5×10^{-9} ± 15%	2009 ± 10%
Birch	160 ± 25%	1.9×10^{-7} ± 13%	N.D. [b]	1645 ± 10%
Cherry	208 ± 30%	2.2×10^{-7} ± 12%	3.5×10^{-9} ± 17%	1387 ± 10%
Maple	160 ± 20%	1.3×10^{-6} ± 10%	3.5×10^{-9} ± 10%	1927 ± 10%
Pine	256 ± 20%	4.0×10^{-6} ± 25%	1.8×10^{-9} ± 26%	1477 ± 10%
Poplar	163 ± 40%	7.5×10^{-7} ± 14%	1.5×10^{-9} ± 35%	1557 ± 10%
Red Oak	109 ± 40%	8.1×10^{-8} ± 16%	N.D.	1094 ± 10%
Redwood	320 ± 20%	3.4×10^{-6} ± 10%	3.6×10^{-9} ± 25%	637± 10%
Walnut	320 ± 20%	4.0×10^{-6} ± 25%	1.9×10^{-9} ± 30%	1118 ± 10%

a. *Table values are reported as the mean of repeated trials with uncertainty noted as percent of the mean that has been propagated from multiple sources*
b. *Not detected (N.D.). Values are not significantly different than controls at this sample preparation protocol.*

Table 2: *Peaks observed in at least 8 out of 9 samples with either correlations to aerosol properties or other significance (e.g., high signals). The average normalized signal-to-noise in all samples and the neutral molecular formulas are shown. Entries have p-values ≤ 0.05 (90%), or p < 0.1 (100%).*

Obs. m/z	S/N (%)	Formula				Tox		AhR		MAC		Proposed chemical assignment(s)	CID neutral or radical losses
		C	H	O	N	Sign	R^2	Sign	R^2	Sign	R^2		
127.039	1.3	6	6	3	0	neg	0.54	pos	0.40	---	---	5-hydroxymethyl furfural	H_2O, CO, $C_2H_2O_2$ (CH_2O + CO?)
129.054	0.3	6	8	3	0	neg	0.58	---	---	---	---	---	---
137.059	1.3	8	8	2	0	---	---	---	---	---	---	toluic acid, anisaldehyde	CH_3, H_2O, CO, HCOOH, CH_2O+CO
139.075	0.2	8	10	2	0	---	---	---	---	---	---	methylguaiacol	
141.054	0.3	7	8	3	0	---	---	---	---	pos	0.52	methoxymethyl-furfural, others	CO, CH_4O, CH_2O + CO
143.034	0.8	6	6	4	0	---	---	---	---	---	---	5-Hydroxymethyl-2-furancarboxylic acid	HCOOH, H_2O
143.070	0.2	7	10	3	0	---	---	---	---	pos	0.46	methoxyfuranethanol, hydroxymethyfuranethanol	CH_3OH, H2O
149.059	0.2	9	8	2	0	---	---	---	---	---	---	coumaraldehyde	---
151.039	0.6	8	6	3	0	neg	0.46	pos	0.44	neg	0.47	hydroxyphenylglyoxal	C_2H_2O, CO, H_2O
151.075	0.7	9	10	2	0	---	---	---	---	---	---	methoxyvinylphenol, coumaryl alcohol	CO, CH_3OH, H_2O, C_3H_6, C_3H_4O, $C_2H_4O_2$
153.054	0.8	8	8	3	0	neg	0.47	pos	0.45	---	---	vanillin (minor methoxybenzoic acid)	CO, CH_3OH (minor HCOOH, CH_3OH+CO_2)
153.091	0.2	9	12	2	0	---	---	---	---	pos	0.59	hydroxypropylphenol	H_2O, C_3H_8O
155.070	0.6	8	10	3	0	---	---	---	---	---	---	syringol, hydroxyl-ethylbenzene diol	CH_3OH, CH_3, H_2O, $C_2H_4O_2$
163.039	0.7	9	6	3	0	---	---	---	---	---	---	hydroxycoumarin	CO, CO_2
163.075	0.7	10	10	2	0	neg	0.43	pos	0.72	---	---	methoxy-cinnamaldehyde	CO, CH_3OH, C_2H_2O, C_3H_4O
165.091	0.4	10	12	2	0	---	---	---	---	---	---	eugenol, isoeugenol	H_2O, CH_3OH, C_3H_6
167.070	2.7	9	10	3	0	---	---	neg	0.44	---	---	homovanillin, acetovanillone	CH_3, H_2O, CO, CH_3OH, C_2H_2O, CH_2O_2, $C_2H_4O_2$
169.049	0.4	8	8	4	0	---	---	---	---	---	---	vanillic acid, dihydroxyphenylacetic acid	CO, H_2O, CH_3, HCOOH, C_2H_2O,$C_2H_4O_2$
169.086	0.6	9	12	3	0	---	---	---	---	pos	0.46	homovanillyl alcohol	C_2H_4O, H_2O, CH_3OH
177.054	1.2	10	8	3	0	---	---	---	---	---	---	methoxycoumarin, hydroxymethyl-coumarin	CO, CH_3OH, C_4H_4O, CH_3
177.091	0.3	11	12	2	0	---	---	---	---	pos	0.59	ethylcinnamate	H_2O, C_2H_4, C_2H_2O
179.070	3.0	10	10	3	0	---	---	---	---	---	---	coniferaldehyde, methoxycinammic acid	H_2O, CO, CH_3OH, HCOOH, C_2H_2O, C_3H_4O
179.106	0.1	11	14	2	0	---	---	---	---	pos	0.77	---	CO, C_2H_4O, $C_2H_4O_2$ (CO + CH_3OH?)

181.086	1.3	10	12	3	0	---	---	---	---	pos	0.51	coniferyl alcohol, dihydroconiferald-ehyde, methoxyphenyl-propanoic acid	H_2O, CH_3, CO, C_2H_4, C_2H_2O, C_3H_6, HCOOH, C_2H_6O, C_3H_4O, $C_2H_4O_2$, $C_3H_6O_2$
183.065	1.8	9	10	4	0	---	---	---	---	---	---	syringaldehyde, homovanillic acid	H_2O, CO, HCOOH, CH_3OH, $C_2H_4O_2$
183.101	0.4	10	14	3	0	---	---	---	---	pos	0.76	dihydroconiferyl alcohol, others	---
185.042	1.2	6	10	5	0	---	---	---	---	neg	0.75	levoglucosan, mannosan, galactosan	H_2O
187.075	0.2	12	10	2	0	---	---	---	---	pos	0.47	phenyl furancarbaldehyde	CO,$C_5H_5O_2$
191.070	1.2	11	10	3	0	---	---	---	---	pos	0.64	acetyl coumaraldehyde	CO, H_2O, CH_3, $C_2H_4O_2$
191.106	0.2	12	14	2	0	---	---	---	---	pos	0.49	isoeugenyl acetone	---
193.049	0.6	10	8	4	0	---	---	---	---	neg	0.47	hydroxy(oxopropenyl)benzoi c acid	CH_3, CO, H_2O, HCOOH
193.086	1.4	11	12	3	0	---	---	---	---	pos	0.78	vanillylidene acetone, dimethoxyphenylacrylaldehy de	CH_3OH, H_2O, CO, $C_2H_2O_2$, C_3H_4O, $C_2H_4O_2$, C_2H_4O, $C_3H_4O_3$
195.101	0.7	11	14	3	0	pos	0.55	neg	0.41	pos	0.57	methoxyisoeugenol, vanillylacetone	---
197.080	1.7	10	12	4	0	---	---	---	---	---	---	acetosyringone	C_2H_2O, H_2O, CH_3OH, C_2H_2O+H_2O
197.117	0.1	11	16	3	0	---	---	---	---	pos	0.80	---	---
198.076	0.7	9	11	4	1	---	---	---	---	---	---	pyridine derivative	$C_4H_6O_3$, H_2O, C_5H_5NO
199.060	0.2	9	10	5	0	---	---	---	---	---	---	syringic acid	---
201.091	0.2	13	12	2	0	---	---	---	---	pos	0.63	---	---
205.084	1.1	10	14	3	0	---	---	---	---	---	---	dihydroconiferyl alcohol, unknown aldehyde	CH_3, H_2O, CO, CH_3OH, C_2H_2O, HCOOH, C_3H_4O, $C_2H_2O_2$
207.065	0.7	11	10	4	0	---	---	---	---	---	---	---	H_2O
207.101	0.6	12	14	3	0	---	---	---	---	pos	0.70	acetyleugenol	CH_3OH, $C_2H_4O_2$
209.080	5.8	11	12	4	0	pos	0.49	neg	0.51	---	---	sinapaldehyde (minor dimethoxycinammic acid)	H_2O, CO, CH_3, CH_3OH, C_2H_2O, C_3H_4O, HCOOH, CH_2O+CH_2O
209.117	0.1	12	16	3	0	---	---	---	---	pos	0.88		---
211.096	3.7	11	14	4	0	pos	0.44	neg	0.56	---	---	sinapyl alcohol	H_2O, CH_3OH, CH_2O+CH_2O, $C_3H_6O_2$ (maybe C_3H_4O + H_2O)
219.101	0.8	13	14	3	0	---	---	pos	0.41	---	---	unknown phenols, G-methylfuran (α-2) dimer	CH_3, H_2O, CO, CH_3OH, C_2H_2O, HCOOH, C_3H_4O, $C_2H_2O_2$, $C_3H_6O_2$, C_5H_6O, $C_3H_4O_3$, $C_7H_8O_2$
221.080	0.5	12	12	4	0	---	---	---	---	---	---	acetoconiferaldehyde	CH_3, H_2O, CO, CH_3OH, C_2H_2O, HCOOH,$C_2H_4O_2$
221.117	0.2	13	16	3	0	---	---	---	---	pos	0.94	eugenyl propionate, and another phenol	H_2O, CO, CH_3OH, C_3H_6, $C_3H_6O_2$

223.060	0.4	11	10	5	0	pos	0.49	neg	0.62	---	---	---	---
223.096	0.6	12	14	4	0	pos	0.54	neg	0.53	---	---	tetrahydrofuran derivative	C_4H_8O, $C_5H_{10}O$
225.111	0.2	12	16	4	0	pos	0.57	---	---	pos	0.60	---	---
227.052	1.2	5	10	8	2	neg	0.43	pos	0.50	neg	0.58	unknown nitro or nitrate derivative	H_2O, CH_3OH, CH_5NO_2
227.091	0.3	11	14	5	0	pos	0.69	neg	0.66	---	---	sinapyl hydroperoxide	H_2O
229.084	0.2	14	12	3	0	---	---	pos	0.57	---	---	resveratrol	H_2O
235.096	0.6	13	14	4	0	---	---	---	---	pos	0.56	---	---
237.111	0.3	13	16	4	0	pos	0.52	neg	0.44	pos	0.53	---	---
239.091	0.1	12	14	5	0	pos	0.52	neg	0.48	---	---	---	---
245.080	0.2	14	12	4	0	neg	0.50	pos	0.57	---	---	---	---
247.096	0.5	14	14	4	0	---	---	---	---	---	---	diguaiacol (5,5'), others	H_2O, CH_3, CH_3OH, CO, $C_2H_4O_2$, C_2H_2O, HCOOH, $C_3H_4O_3$, C_3H_4O, $C_3H_6O_2$, $C_6H_6O_2$, $C_7H_8O_2$
247.132	0.2	15	18	3	0	---	---	---	---	pos	0.41	---	---
249.111	0.7	14	16	4	0	pos	0.74	neg	0.60	pos	0.49	furfuryl alcohol deriv., hydroxybenzoquinone derivative ($C_6H_4O_3^+$ fragment ion), others	H_2O, $C_8H_{12}O$, CO, CH_3OH, $C_6H_8O_2$, $C_3H_6O_2$, $C_2H_4O_2$, $C_4H_6O_3$, $C_4H_6O_3$, $C_5H_6O_2$, $C_4H_8O_2$, C_3H_6
251.127	0.1	14	18	4	0	---	---	---	---	pos	0.64	---	---
261.112	0.5	15	16	4	0	---	---	---	---	---	---	G-guaiacol dimer (α-2), others	---
265.106	0.3	14	16	5	0	---	---	---	---	pos	0.46	S-furfurylalcohol dimer (α-2)	---
271.096	0.3	16	14	4	0	neg	0.63	pos	0.65	---	---	G-guaicol (β-5, ring closure)	---
273.112	0.2	16	16	4	0	---	---	---	---	---	---	diguaicol (β-1), G-guaicol (β-5)	
289.106	0.6	16	16	5	0	---	---	---	---	---	---	G-syringol dimer (β-5), others	---
301.107	0.3	17	16	5	0	neg	0.41	pos	0.55	---	---	G-vanillin dimer (β-5)	---
303.122	0.3	17	18	5	0	neg	0.44	pos	0.58	---	---	guaicyl-syringyl dimer (stilbene), others	---
305.138	0.3	17	20	5	0	---	---	---	---	pos	0.67	G-guaiacol dimer (β-O-4)	---
313.107	0.2	18	16	5	0	---	---	pos	0.40	---	---	G-homovanillin dimer (β-5, ring closure), others	---
315.122	0.3	18	18	5	0	---	---	pos	0.50	---	---	G-homovanillin dimer (β-5), others	---
317.138	0.2	18	20	5	0	---	---	pos	0.43	---	---	G-G dimer (α-5)	---
327.122	0.3	19	18	5	0	---	---	---	---	---	---	G-coniferaldehyde dimer (β-1), others	---
329.102	0.1	18	16	6	0	---	---	pos	0.40	---	---	S-vanillin dimer (β-5 ring closure), hydroxy G-	---

												homovanillin dimer (β-5, ring closure), others	
329.138	0.3	19	20	5	0	---	---	pos	0.42	---	---	eugenol-vanillone dimer (5,5'), others	---
331.117	1.0	18	18	6	0	---	---	---	---	pos	0.44	G-vanillin dimer (5,5'), others	---
333.133	0.4	18	20	6	0	---	---	---	---	---	---	S-S dimer (β-1), G-vanillin dimer (β-O-4)	---
345.132	0.2	19	20	6	0	---	---	---	---	pos	0.60	coniferylalcohol-vanillone dimer (5,5'), others	---
357.130	1.3	18	22	6	0	---	---	---	---	---	---	G-syringol dimer or S-guaiacol dimer (β-O-4)	---
359.148	0.2	20	22	6	0	---	---	---	---	pos	0.77	diconiferylalcohol (5,5', β-β, or β-), and others	---
383.146	0.2	20	24	6	0	---	---	---	---	---	---	diconiferylalcohol (β-O-4)	---

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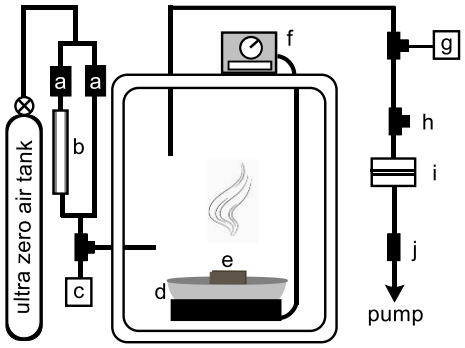


Figure 1: *Simplified diagram of smoldering combustion chamber. Key: (a) pressure flow restrictors, different flow rates each to control humidity; (b) humidifier tube; (c) relative-humidity probe; (d) heating mantle; (e) wood fuel blocks; (f) temperature controller; (g) thermocouple temperature measurement; (h) vent; (i) aerosol filter collection apparatus; (j) vacuum flow restrictor.*

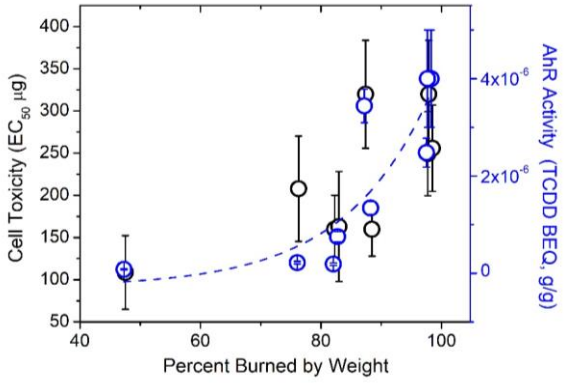


Figure 2: *Relationship between cell toxicity and AhR activity of smoldering WSA extracts with the percent of wood that has been burned at the time of aerosol collection.*

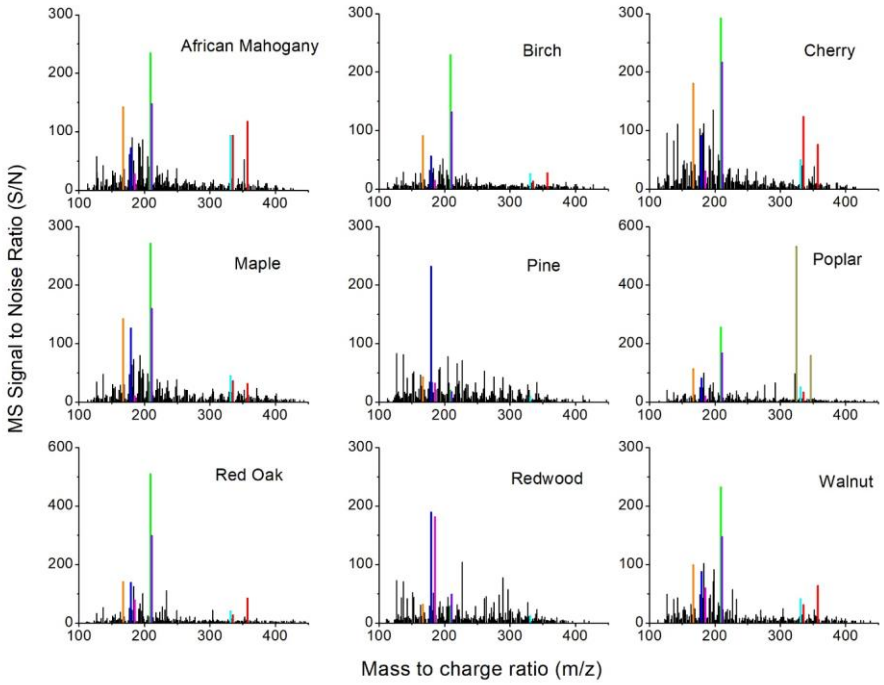
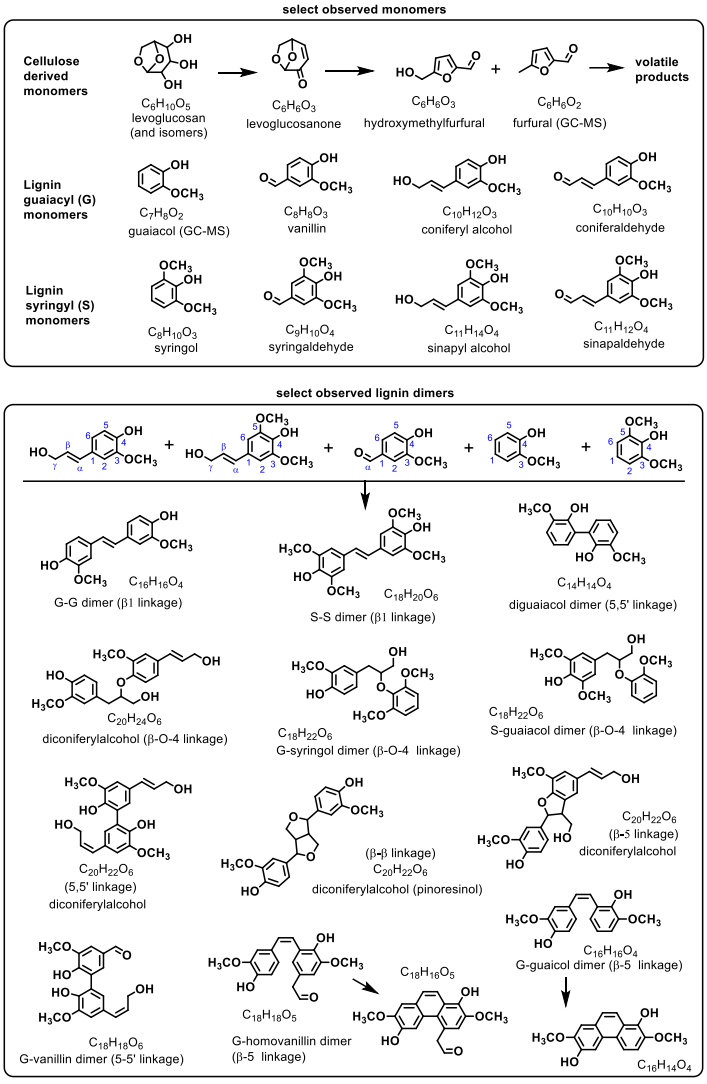


Figure 3: *High resolution mass spectra of WSA extracts in the study, taken at 100 ug/mL concentration. The color key for highlighted peaks with proposed assignments for the major species at each peak is: Orange – $C_9H_{10}O_3$, acetovanillone or homovanillin (protonated); Blue – $C_{10}H_{10}O_3$, coniferyl aldehyde (protonated); Magenta – $C_6H_{10}O_5$, levoglucosan, mannosan, or galactosan (sodiated); Green – $C_{11}H_{12}O_4$, sinapaldehyde (protonated); Cyan – $C_{18}H_{18}O_6$, G-vanillin dimer (protonated); Red – $C_{18}H_{22}O_6$, various S-G dimers (protonated and sodiated); Dark yellow – $C_{20}H_{21}NO_3$, unknown alkaloid (protonated and sodiated)..*



880 **Figure 4:** *Proposed chemical structures of some monomers and dimers observed in this work. More than one structure may be present at each molecular formula.*

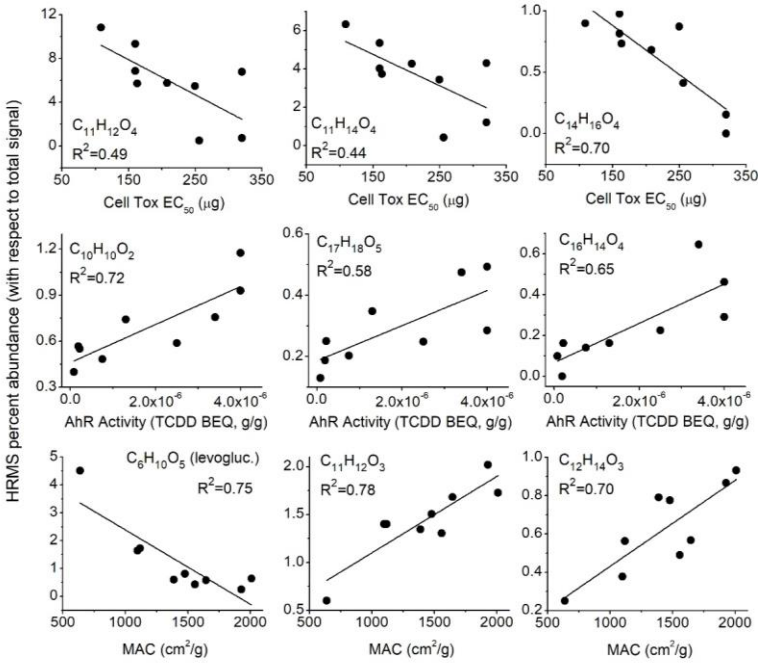


Figure 5: *Select correlations of HRMS peaks to cell toxicity EC_{50}, AhR activity, and MAC_{vis}. Linear fits and correlation coefficients are shown. Proposed chemical identities are listed in Table 1.*

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