

Oxidative Potential of Aerosol Emitted from Traditional vs. Improved Cookstoves

BY

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ABSTRACT

Fine particulate matter (PM_{2.5}) emitted from combustion processes can partake in cellular redox reactions and induce oxidative stress. This ability for PM_{2.5} to induce oxidative stress is known as its toxicity and is referred to as oxidative potential (OP). This poses a serious health risk for the ~3 billion people globally who continue to rely on biomass burning for their cooking needs as it is a significant source of indoor PM_{2.5}. To combat the problem associated with traditional biomass burning cookstoves, 'improved' biomass burning cookstoves have been developed which have been shown to reduce the mass of PM_{2.5} generated. However, it is unknown whether the overall exposure to toxic chemicals contained in PM_{2.5} is reduced using these cookstoves as the adverse health effects of PM_{2.5} are better linked with OP than mass of PM_{2.5} inhaled. The goals of this study were to determine whether the OP of PM_{2.5} emitted from improved cookstoves was reduced and to determine what effect fuel type has on the OP of PM_{2.5}. To test this, 4 fuel types (2 wood types and 2 charcoal types) were burned in 5 cookstoves (2 traditional and 3 improved) using the simmer-phase of the Water Boiling Test protocol to generate PM_{2.5} samples that were collected on quartz filters for analysis. The dithiothreitol (DTT) assay was employed to determine the OP of the PM_{2.5} and was split into 2 fractions, the water-soluble (WS) and total fraction. The DTT activity values were corrected using two variables, mass ($OP_{\text{Mass}}^{\text{DTT}}$) and volume ($OP_{\text{Volume}}^{\text{DTT}}$). The mass emitted from the 3 improved cookstoves were found to significantly reduce PM_{2.5} compared to the traditional stoves, consistent with previous studies. $OP_{\text{Volume}}^{\text{DTT}}$ was found to be more representative of the overall exposure to toxic PM_{2.5} emitted during a cooking event as it accounts for both intrinsic OP of the PM_{2.5} (toxicity per mass of PM_{2.5}) and the mass emission of PM_{2.5}. The $OP_{\text{Volume}}^{\text{DTT}}$ values were compared for all cooking methods and the wood fuel types were found to have the greatest $OP_{\text{Volume}}^{\text{DTT}}$ for each cookstove. Using the experimentally determined $OP_{\text{Volume}}^{\text{DTT}}$, example mitigation strategies were then suggested. Given the option to only switch cookstove or fuel type from the 3-stone with hardwood cooking method, a greater reduction in $OP_{\text{Volume}}^{\text{DTT}}$ can be achieved by switching from wood to coal (84% reduction) rather than 3-stone to improved stove (62% reduction). WS data was found to vary in its contribution to the total $OP_{\text{Volume}}^{\text{DTT}}$ from less than 15% to just below 75%, suggesting that the chemical composition of components that contributed to the OP of PM_{2.5} is dependent on cooking method.

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TABLE OF ABBREVIATIONS

ACE.....	African Clean Energy One
DIW.....	Deionized Water
DTNB.....	Ellman's reagent
DTT.....	Dithiothreitol
EDTA.....	Ethylenediaminetetraacetic acid
ELF.....	Epithelial lining fluid
GBD.....	Global Burden of Disease
HAP.....	Household air pollution
LMIC.....	Low-middle income countries
OP.....	Oxidative potential
OP ^{DTT}	DTT activity
OP _{Mass} ^{DTT}	Mass-normalized DTT activity
OP _{Volume} ^{DTT}	Volume-normalized DTT activity
PAH.....	Polycyclic aromatic hydrocarbons
PM.....	Particulate matter
PM _{0.1}	Ultrafine Particulate matter
PM _{2.5}	Fine Particulate matter
PM ₁₀	Coarse Particulate matter
PTFE.....	Polytetrafluoroethylene
PQN.....	9,10-phenanthraquinone
ROS.....	Reactive oxygen species
WHO.....	World health organization
WS.....	Water soluble
WS-OP _{Volume} ^{DTT}	Water soluble DTT activity

1. INTRODUCTION

1.1 The Importance of Air Quality for Human Health

Air quality is of great importance as it not only has environmental implications, but also a considerable deleterious effect on human health.¹ The most recent Global Burden of Disease (GBD) report ranks exposure to ambient particulate matter (PM) pollution as the greatest environmental risk factor for premature death.² Moreover, when considered amongst all other risk factors (including non-environmental risks), the GBD ranks ambient PM pollution as the 7th highest early mortality risk factor.³ This out ranks low-density lipoprotein cholesterol, unsafe water, sanitation, alcohol, and drug use.³ Given the importance of PM exposure to human health, it is critical to understand the sources of toxic PM and what chemical components are driving their toxicity. As such, substantial research has been done to characterize the toxicity of PM in numerous outdoor environments.⁴⁻⁶ Despite the push to better understand PM in outdoor environments, little research has been done to characterize the toxicities of PM in indoor environments. Since humans spend around 90% of their lifetime indoors,⁷ it is imperative that the air we breathe indoors be clean. In fact, the same GBD report introduced earlier lists household air pollution (HAP) as the second highest environmental mortality risk factor,³ indicating that indoor air quality is of great significance to human health.

1.2 Characteristics of PM

PM are complex chemical mixtures which can be made up of inorganic matter (e.g. nitrate, sulfate, and transition metals) and carbonaceous matter (both organic and elemental).⁸⁻¹¹ Moreover, the actual make-up of PM depends on a number of factors such as where and how it was generated.^{10,12} For example, higher concentrations of sulfate in PM are observed on the east coast of North America with small concentrations of nitrate while the opposite is true for the west coast.¹¹ This difference in PM composition is due to fact that on the east coast, higher quantities of SO₂ are emitted due to higher concentration of coal burning compared to the west coast.¹³ The SO₂ emitted is then quickly oxidized in the atmosphere to sulfate (SO₄²⁻) and increases the portion of PM made up from SO₄²⁻ on the east coast.¹³

The characterization of PM is also based on physical size, as the aerodynamic equivalent diameter of PM also varies and can fall anywhere within the range of 10^{-9} m to 10^{-5} m.¹⁴ Typically, PM is divided into three distinct categories based on their aerodynamic equivalent diameter: $< 10 \mu\text{m}$ (PM_{10}), $< 2.5 \mu\text{m}$ ($\text{PM}_{2.5}$), and $< 0.1 \mu\text{m}$ ($\text{PM}_{0.1}$).⁸ This distinction in size is important for human health as the size of PM dictates where it is deposited in the body (its endpoint). $\text{PM}_{2.5}$ is one of the most important sizes as it can travel deep into the lungs and deposit in the alveoli where gas exchange occurs. Here they are absorbed by the mucous layer which coats the lungs, known as the epithelial lining fluid (ELF), where components of $\text{PM}_{2.5}$ can react and produce significant toxic effects.^{8,15-17} PM_{10} can also make its way into the lungs, however, due to its size it doesn't travel nearly as deep and typically deposits in the primary bronchi of the lungs.¹⁴ PM which is greater than $10 \mu\text{m}$ is unable to make its way to the lungs and ends up depositing in the nasopharynx after inhalation.¹⁴ Figure 1.2.1 depicts the endpoints of the various sizes of PM.

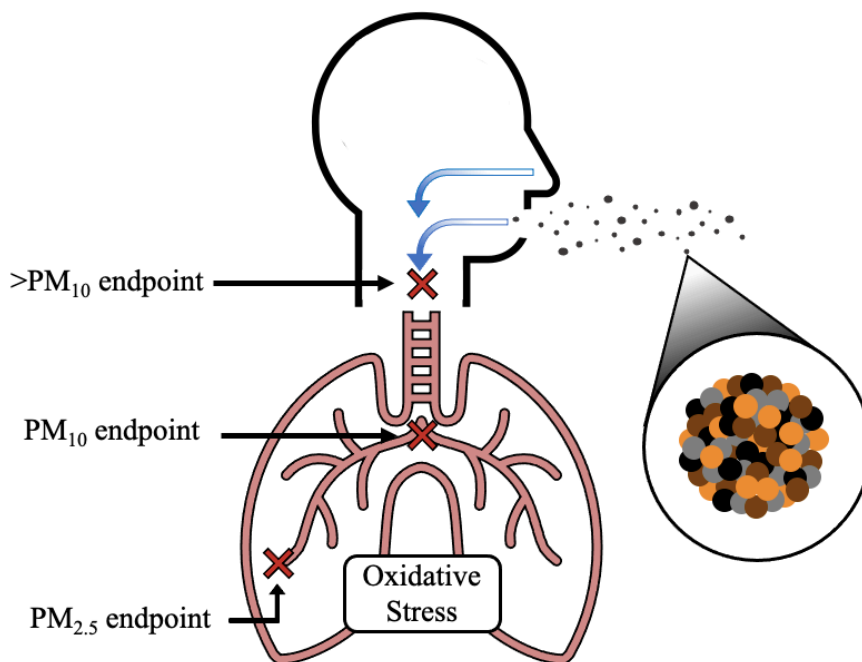


Figure 1.2.1 Endpoints where different sizes of PM deposit after inhalation as well as a cartoon depiction of a magnified PM to illustrate PM being a complex mixture of different species of chemicals.

1.3 Metric of PM Toxicity

The most commonly used measurement to investigate the health and environmental effects of PM_{2.5} is a mass-based metric.¹⁸ One of the first epidemiological studies to effectively illustrate the negative health effects of PM_{2.5} did so by showing a strong correlation between PM_{2.5} mass concentration (in terms of micrograms of PM_{2.5} per volume of air) in 6 cities in the U.S.A and increased risk ratio for mortality while controlling for other individual risk factors.¹⁹ This study showed a 26% higher mortality rate in the city with the greatest PM_{2.5} concentration when compared to the city with the lowest concentration of PM_{2.5} in the study.¹⁹ This cornerstone study has been replicated worldwide for different populations and across different time periods where similar correlations between PM_{2.5} mass concentration and increased mortality rates were observed.²⁰ This further cements the link between exposure to PM_{2.5} and damaging human health effects.

The problem with using mass-based concentrations for air quality measurements and regulations is that it doesn't provide any information on the composition of the particulate. This viewpoint assumes that all PM_{2.5} have the same effect on human health with the mass concentration of PM_{2.5} in the air being the only concern, rather than where and how the particulate was generated or its chemical composition. However, this limitation of using mass-based metric for air quality is not new, and the problem has been known for over a decade. In 2004, Robert Phalen of the University of California, Irvine published an article stating "PM is the only regulated national criteria air pollutant in the United States that is not specified with respect to chemical composition. Because the measures of PM are typically based on mass as determined gravimetrically, very small particles and their associated chemistries are not represented realistically in the monitoring process."²¹ By continuing to use mass-based concentrations of PM_{2.5} as an air quality metric without taking PM_{2.5} composition into account, it is unlikely the quality of air will be accurately reflected.²¹ This highlights the importance of not only measuring the mass concentration of PM_{2.5}, but also measuring its toxicity.

1.4 Oxidative Stress as a Toxicology Mechanism of PM Exposure

A prevailing toxicological mechanism underlying the negative health effects of PM_{2.5} exposure is oxidative stress, which is the condition where there is an imbalance of

cellular antioxidant and oxidant levels.²² From this point on, when discussing the toxicity of PM_{2.5}, toxicity refers to the ability of PM to induce oxidative stress. Oxidative stress can be induced by PM_{2.5} via two pathways.

The first pathway is thought to occur through oxidizing components contained within PM_{2.5} which react with and deplete antioxidants in the body.²³ Thus, creating an imbalance of cellular oxidants and antioxidants by lowering the amount of antioxidants present in the body. For the second pathway, components contained within PM_{2.5} can catalyze the transfer of electrons from the oxidized antioxidant (described in the first pathway) to oxygen.²³ Then, through redox reactions catalyzed by transition metals, superoxide and other reactive oxygen species (ROS) are generated which can further deplete antioxidant levels.^{23–26} This creates an imbalance by not only reducing the number of cellular antioxidants but at the same time increasing the number of harmful oxidants. These two pathways are visualised as an oxidation cycle of PM in Figure 1.4.1.

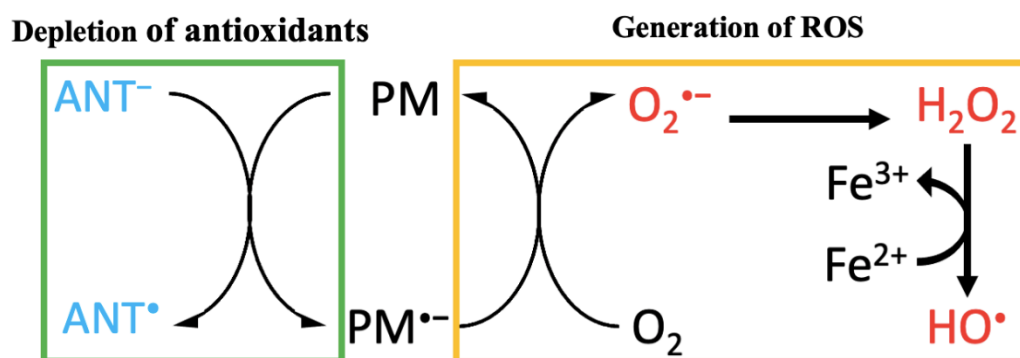


Figure 1.4.1 Pathways for particulate matter (PM) to induce oxidative stress through the redox reaction cycle where an antioxidant (ANT; in blue) is depleted and reactive oxygen species (ROS; in red) are formed.

The ability for PM to generate ROS and induce oxidative stress is known as its oxidative potential (OP). A common ROS product formed in these reactions is the hydroxyl radical (HO•).²⁵ This is believed to be generated through the second pathway where radicals in PM_{2.5} help to catalyze the reduction of oxygen (O₂) to superoxide (O₂•⁻); the superoxide is then converted to hydrogen peroxide (HOOH) which can further react with transition metals such as Fe²⁺ to generate the hydroxyl radicals.²⁷ Both HO• and HOOH can be very damaging to a person's health. Oxidative stress can damage cells and their DNA leading to

apoptosis and inflammation of tissues.^{28,29} When oxidative stress localizes in the lungs, a number of serious health effects can arise such as lipid peroxidation and even cancer.²⁹ Oxidative stress can even exacerbate pre-existing problems such as asthma and fibrosis.²⁹

Recently, epidemiological studies have found that the adverse health effects associated with PM_{2.5} are more strongly linked to the OP of the PM_{2.5} than the actual mass of PM_{2.5} inhaled.^{30,31} This is because the OP of PM_{2.5} changes depending on its composition,³¹ further highlighting the importance of understanding where and how PM_{2.5} was generated since these factors all affect the chemical composition of PM_{2.5}.³²⁻³⁴ Additionally, this suggests that when looking at improving the health effects of indoor polluting cooking, it is more relevant to look at the OP of the aerosol generated rather than the total mass.

As stated earlier, PM can be comprised of many different groups of chemicals, however, only some of the components of PM significantly contribute to the OP.³¹ Since only some components play a major role in the OP of aerosol, this helps to understand why mass doesn't correlate with adverse health effects as well as OP does. An example of this is sulfate; sulfate adds to the total mass of PM_{2.5}, however, it barely contributes to the generation of ROS.³¹ Although the components that give PM_{2.5} the greatest OP will change depending on how it is generated, certain classes of compounds are known to be major contributors of ROS-generation. The two groups of compounds that have the largest contribution to a particulate's OP are organic compounds and certain metals.³⁵ The metals which have had the strongest correlation to OP are Cu, Fe, K, Mn, and Zn, with their water-soluble (WS) forms correlating to toxicity very well.³⁶ As stated earlier, transition metals are believed to be key in catalyzing the reactions that generate ROS,²⁴⁻²⁶ but it has also been observed that organic compounds such as quinones and polycyclic aromatic hydrocarbons (PAH) play a major role in generating ROS for some types of PM_{2.5}.³⁵

1.5 Dithiothreitol Assay as a Measurement of PM Oxidative Potential

One of the most common ways to measure the OP of PM is the dithiothreitol (DTT) assay, which is an acellular assay designed specifically for measuring the OP of aerosol.¹⁸ The assay works by using DTT to imitate a biological antioxidant such as glutathione³⁷, which can transfer electrons to oxygen, reducing it to superoxide, similar to the predicted

biological mechanism.³⁸ When PM_{2.5} is in the presence of DTT, the oxidizing components of the PM_{2.5} can initiate the transfer of electrons from the DTT to oxygen by oxidizing the DTT to its disulfide form.¹⁸ To determine the OP of PM_{2.5} using this assay, the amount of remaining (unoxidized) DTT can be quantified indirectly using a colorimetric analysis of the solution after the addition of Ellman's reagent (dithio-bis-(2-nitrobenzoic acid); DTNB). The DTNB is immediately converted to 2-nitro-5-thiobenzoic acid (TNB), a molecule which absorbs strongly at 412 nm, after reacting with the thiol group of unoxidized DTT.¹⁸ Samples are quantified over time to determine the rate at which the DTT is being oxidized.¹⁸ The rate at which DTT is oxidized is known as the DTT activity and is proportional to the concentration of oxidative components contained in the PM_{2.5}.²³

However, there are limitations with the DTT assay. This assay only acts to measure the depletion of DTT and doesn't measure the generation of ROS,¹⁸ meaning it only captures one side of the redox cycle which contributes to cellular oxidative stress. Additionally, while the assay is performed in physiological conditions of 37 °C and at pH 7.4, the DTT assay only captures the reactions between PM and the thiol group of DTT rather than capture processes which would actually occur in the body,¹⁸ such as the oxidation of antioxidants that do not possess a thiol group (e.g. NADPH),³⁸ or the generation ROS like some cellular assays (e.g. the rat alveolar macrophage assay).³⁹

While there are limitations to this assay, the DTT assay remains one of the best methods to determine the OP of PM due to various reasons. Studies have found that OP^{DTT} is linked to biological markers that indicate oxidative stress and inflammation,^{33,40,41} as well as correlations between OP^{DTT} and negative health effects.³¹ Some studies have found that other common methods of measuring OP cannot be linked to these same negative health effects.^{42,43} The DTT assay is also favoured for how easy and economical it is to operate which is especially important when running replicates of many samples.¹⁸ Additionally, the DTT assay is the most widely used assay in measuring OP of PM amongst different research groups,⁴⁴⁻⁴⁷ in particular PM emitted from biomass burning.⁴⁸ This makes it possible to compare OP of PM emitted from different sources such as gasoline and diesel emissions or even PM from indoor and outdoor sources.

1.6 Toxicity of Indoor Biomass Burning

As discussed previously, despite the overwhelming evidence supporting the importance of indoor air quality for human health, most aerosol toxicity studies continue to focus on outdoor PM sources like traffic emissions. For this reason, the understanding of indoor PM toxicity is still in its infancy.

The severity of HAP and associated respiratory illnesses increase even more when considering poor indoor ventilation since PM and other pollutants can reach over 100 times the World Health Organization's (WHO) recommended safety levels.^{49,50} This makes HAP an even greater concern for households without access to adequate ventilation. Additionally, the WHO estimates that 4.3 million premature deaths are attributed to exposure to HAP yearly with citizens of lower- to middle-income countries (LMIC) typically experiencing worse exposures/impacts.⁴⁹ In some LMIC, such as India, ventilation is even intentionally minimised to conserve energy.⁵¹

Approximately 3 billion people around the world, mostly from LMICs, rely on the burning of biomass (wood, charcoal, plant crops, etc.) for cooking at home.⁵² The pollution generated from incomplete combustion of biomass contains many pollutants, including PM_{2.5}, that are known to have negative health impacts.⁵³ For example, pollution generated from burning wood contains more than 26 different hazardous pollutants.⁵³ Due to the necessity of food preparation, a safer alternative to the traditional means of cooking is required. Traditionally, places such as India and sub-Saharan African countries use clay cookstoves and three stone cookstoves for cooking; but these cookstoves have been well documented to be inefficient in their combustion process and produce high levels of PM_{2.5}.⁵⁴⁻⁵⁶ Figure 1.6.1 illustrates which countries are most effected by HAP from indoor cooking energy.

Due to cultural beliefs and practices in some LMICs, both children and women are disproportionately exposed to HAP as cooking is mainly done by women in the household,⁵⁷ and children end up spending much of their time in and around their houses. As stated earlier, increased exposure to HAP can have negative health impacts, however, for children these impacts can have even more damaging effects that continue to affect them throughout life; in fact, pneumonia is the leading cause of death for children under the age of 5, with over 50% of these pneumonia related deaths being caused by exposures to HAP.⁴⁹ There is

even evidence that being exposed to HAP at a young age could affect cognitive development.⁴⁹

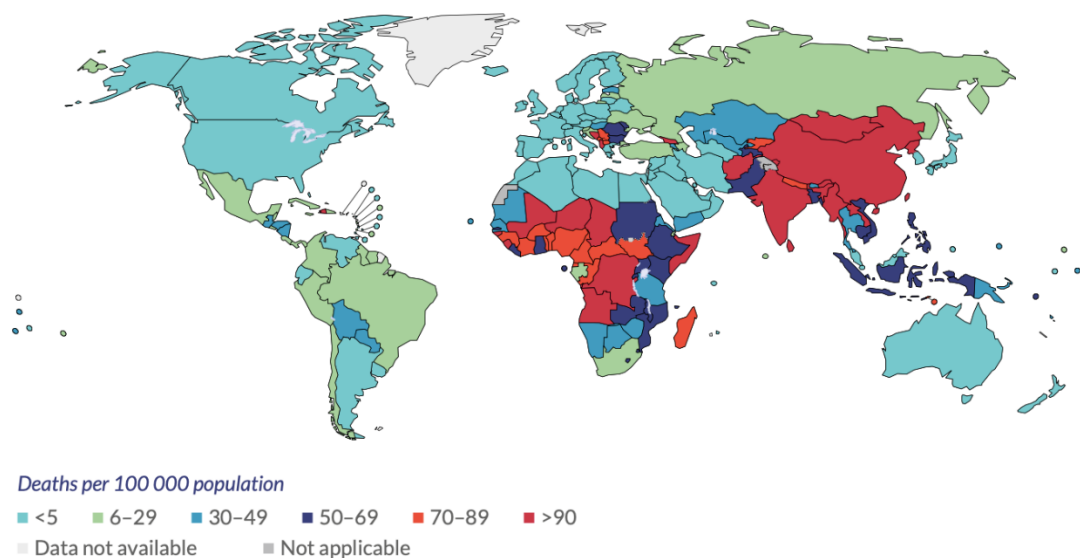


Figure 1.6.1 Map of annual deaths per 100,000 population attributed to household air pollution created from cooking energy (2012). Figure borrowed from the WHO's *Burning Opportunity* Report.⁴⁹

To combat the negative health issues associated with traditional cookstoves, alternative stoves and fuel types have been developed. In fact, it is one of the United Nations Development Programme's missions to have universal access to clean energy cooking by 2030.⁵⁸ Ideally populations using these traditional biomass burning cookstoves would switch to clean alternatives such as electricity, gas, or solar powered cookstoves, however, switching directly to these clean cooking systems is not simple.⁴⁹ This is because there are several limitations to these types of cooking systems such as the cost associated with purchasing the system, maintaining the system, and providing fuel for the cooking system.⁴⁹ In addition, there are cultural preferences associated with these traditional cookstoves preventing households from switching to improved stoves.⁴⁹

One type of cookstove system that still uses the combustion of biomass to provide thermal energy for cooking are the improved biomass cookstoves. These cookstoves work in a similar fashion as the traditional cookstoves where the combustion of solid fuel provides thermal heat for cooking, however, through the use of improved materials and better

optimized designs,⁵⁹ the improved cookstoves are able to burn fuel more efficiently and produce less pollutants like PM_{2.5} (Figure 1.6.2).⁶⁰⁻⁶² Despite studies showing that the total mass of PM_{2.5} decreases with these improved cookstoves, it is unknown whether this reduction of PM_{2.5} mass has a significant effect on reducing toxicity. For example, the total toxicity of aerosol may not be reduced if the emission of components that are toxic remains unchanged or increased (i.e., reduction of non-toxic PM_{2.5} components).

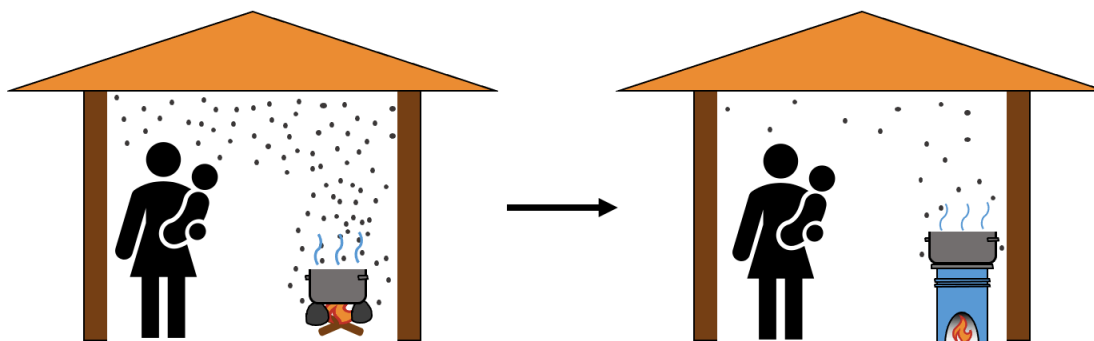


Figure 1.6.2 Cartoon depiction of the reduction of fine particulate matter emitted when a household switches from using a 3-stone traditional cookstove to an improved biomass burning cookstove.

1.7 Research Objectives

A better fundamental understanding of the toxic PM_{2.5} emitted from indoor biomass burning is necessary for effective mitigation of its harmful effects. More specifically, it is important to understand the role of various parameters that effect the emissions of toxic PM_{2.5} components of during the use of cookstoves indoors. To address this, the objective of this research is to determine whether improved biomass cookstoves reduce the toxicity experienced with indoor biomass cooking and when compared with traditional cooking methods. Additionally, alternate fuel types will be tested to determine how switching fuel sources may affect toxicity. This goal of this research is to ultimately improve the quality of life and reduce the health risks associated with cookstove operation for people across the globe who rely on the use of these cookstoves every day.

2. METHODS & MATERIALS

2.1 Burn Events

For this study, five different cookstoves were tested, and for each cookstove, four different fuel sources were used. Of the five cookstoves, two were traditional biomass burning cookstoves (3-stone cookstove and clay cookstove) and three were improved biomass burning cookstoves (African Clean Energy One, EcoZoom Zoom Versa, and EcoZoom Zoom Dura). Of the four fuel types, natural charcoal lumps (Sainte Catherine brand), charcoal briquettes (Royal Oak brand), split hardwood, and softwood pellets (BioGreen brand) were used

The ‘simmer phase’ of the Water Boiling Test protocol was used during each burning event, where PM collection (Section 2.2) begins after the water has reached and cool slightly (within 10 degrees) below its boiling point.⁶³ The simmer phase simulates long cooking methods (e.g., for legumes) that are common throughout the world.⁶³ Throughout the entire PM_{2.5} collection time period, the water was maintained at a simmer (deionized water and fuel were added as necessary). The mass of fuel consumed by the cookstove, the mass of fuel that remained, and the mass of ash left in the stove was recorded after every burn event along with the time spent collecting PM_{2.5} samples.

2.2 PM_{2.5} Sample Collection

The PM_{2.5} samples were collected by drawing air through an inlet located 2 to 4 cm above the cookstove but below the (aluminium) pot of water, at a constant flow rate (18 slpm), which was regulated using a mass flow controller (Alicat Scientific). The inlet was fitted with a PM_{2.5} cyclone (URG) to remove any PM larger than 2.5 microns (e.g., only PM 2.5 microns and smaller were collected). Following the PM_{2.5} cyclone, the flow was split equally into two streams where the PM was collected using two 47 mm filter holders (URG). In each filter holder, two identical 47 mm quarts filters (heat-treated Tissuquartz; Pall Laboratory) were stacked on top of one another where the first filter (front filter) was used to collect any PM_{2.5} from the burning event and the second filter (back filter) was used to capture any gasses which may deposit on the filters. An example set up is pictured in Figure 2.2.1. Time period for PM_{2.5} collection varied for different cookstove and fuel combination (ranging from 15 to 120 minutes), where the time periods were adjusted to

collect sufficient PM mass onto the filter for analysis. After a burning event, each filter was stored in the freezer (-35 °C) for later analysis.



Figure 2.2.1. Particulate matter collection set-up. This labelled set up shows collection of PM_{2.5} using the African Clean Energy One cookstove following the Water Boiling Test protocol.

2.3 Sample Preparation

Using a sterile punch (0.126, 0.283, 1, or 1.5 cm²), a portion of the sample collected on the quartz filter was removed and added to a sterile vial containing a known volume (15 – 50 mL) of 18 MΩ cm⁻¹ deionized water (DIW). Punch area and DIW volume for extraction varied based on the rate the sample oxidized the DTT (DTT activity); a smaller punch area and larger DIW volume were used for some samples so that the DTT didn't deplete after only collecting 2-3 points of data whereas a larger punch area and smaller DIW volume were used for some samples so that the OP exceeded that of the control (water sample, further explained in section 2.6). The sample vial was then sonicated for 60 min. Enough solution was then removed so that only 2.45 mL of sonicated solution remains in the tube containing the filter punch. This was used to determine the total DTT activity of the sample (e.g., the sample solution contains both insoluble and soluble PM components). The solution that was removed from the vial was then filtered using a 0.45 μm pore size polytetrafluoroethylene (PTFE) syringe filter (VWR) to leave only the WS fraction of the sample. A 2.45 mL aliquot of the filtered solution was then added to a separate sterile vial.

Both sample vials (total and WS PM components) were used to determine the DTT activity using the corresponding assay (section 2.4). Note that the method of determining total and WS DTT activity follows the protocol described by Gao et al.²³

2.4 DTT Assay

The DTT assay procedure follows those outlined in Fang et al.⁴⁵ and Gao et al.²³ Briefly, to each solution containing the PM_{2.5} sample (described in section 2.3), 0.7 mL of 0.5 M potassium phosphate buffer (K-buffer) at pH 7.4 was added. K-buffer was prepared by mixing 65.6 g of potassium dihydrogen phosphate (KH₂PO₄; > 99.5%, VWR) and 118.8 g of di-potassium hydrogen phosphate (K₂HPO₄; 98%, VWR) in 1 L of DIW, where trace metals were removed using a chromatography column containing Chelax-100 resin (Sigma Aldrich). For blank and positive controls, 2.45 mL of DIW and 0.3 μM solution of 9,10-phenanthraquinone (PQN; > 99%, Sigma Aldrich) was added to 0.7 mL of 0.5M K-buffer, respectively. Each of the samples were then incubated at 37 °C to mimic physiological conditions.

To each incubation vial, 0.35 mL of 1 mM DTT (98%, Alfa Aesar) was added to initiate the decay of DTT due to its reaction with the sample. At 5 different reaction times over the course of 40 to 46 min, 100 μL of the mixture was removed and added to vial containing 1 mL trichloroacetic acid (1% w/v TCA; prepared by dissolving 10 g of TCA (> 99%, Sigma Aldrich) in 1 L of DIW) to quench the reaction so that the DTT no longer reacts with the sample.

The concentration of unreacted DTT remaining in a quenched reaction vial was quantified by adding 2 mL of 0.08 M Tris buffer (>99.8%, VWR) containing 4 mM ethylenediaminetetraacetic acid (EDTA; >99.4% Sigma Aldrich) at pH 8.9 and 0.5 mL of 0.2 mM DTNB (99%, Alfa Aesar). Under the higher pH conditions, the DTNB reacts with the unreacted DTT to form a product, 2-nitro-5-thiobenzoic acid, which absorbed strongly at 412 nm, which was detected using absorption spectroscopy. Given that 2-nitro-5-thiobenzoic acid do not absorb at 700 nm, the absorbance value at this wavelength were used as a baseline correction. Two separate absorption spectrometers were used, the Cary 100 UV-Vis (Agilent) and another absorption spectrometer that consists of liquid wavelength capillary cell (10 cm, World Prevision Instrument), a deuterium tungsten light

source (DT-Mini-2, Ocean Optics), and a multi-wavelength detector (USB4000, Ocean Optics).

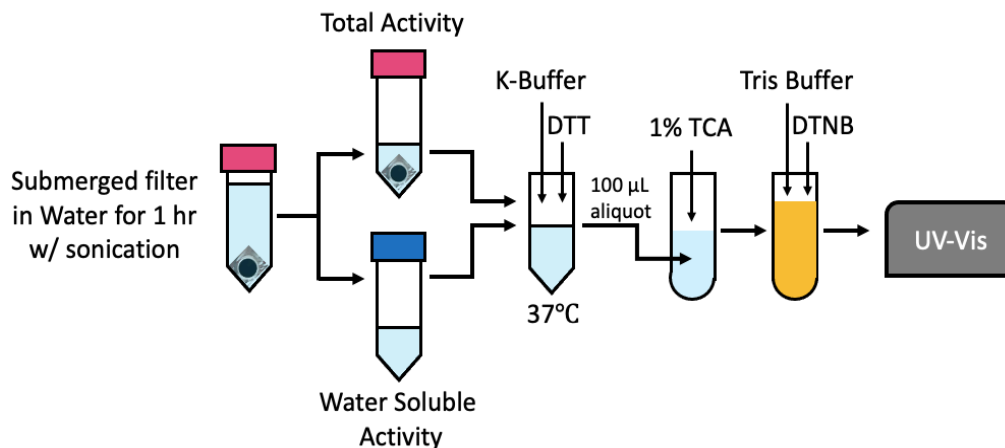


Figure 2.4.1. Flow diagram depicting the steps taken to run the DTT assay.

2.5 Calibration of DTT Quantification

A calibration curve was made routinely to determine DTT concentrations from absorbance values using several standards ranging in concentrations from 0 – 4 μM DTT. To create these standards, different volumes of DTT were added to vials containing 0.7 mL K-buffer and enough DIW to achieve total volume of 3.5 mL. A 100 μL aliquot of the solution was then added to a vial containing 1 mL of TCA (1% w/v), with 2 mL of 0.08 M Tris buffer containing 4 mM EDTA at pH 8.9 and 0.5 mL of 0.2 mM DTNB being added immediately after mixing. After allowing the DTNB to fully react with the DTT, the absorbance values for each DTT concentration were taken and used to create the calibration curve.

2.6 Determining Oxidative Potential (OP)

For the PM samples, the corresponding decay rate of DTT (pmol min^{-1}) was used to determine its oxidative potential. Here, the DTT decay rates are adjusted by subtracting the average DTT decay rate of the blanks, as DTT itself is an unstable compound and this subtraction accounts for its decay in absence of any PM components. Following the procedure of Fang et al.,⁴⁵ the corrected decay rate is then adjusted to account for dilutions

from the incubation vial (3.5 mL), sample volume (2.45 mL), and extraction volume (10 mL), to representing the DTT decay rate of the sample in the incubation vial, expressed as either the sample volume-normalized DTT activity (OP_{Volume}^{DTT} ; $\text{pmol min}^{-1} \mu\text{g}^{-1}$), or sample mass-normalized DTT activity (OP_{Mass}^{DTT} ; $\text{pmol min}^{-1} \text{m}^{-3}$).

3. RESULTS AND DISCUSSION

A total of 5 cookstoves were tested, each using 4 different fuel types, resulting in a total of 20 unique cooking methods tested during this study. For each cooking method, three replicate sets of data were quantified, each replicate representing filters collected from a separate burn event.

3.1 Reduction in PM_{2.5} Emission by Mass

To mitigate the risk associated with cooking using biomass burning cookstoves, improved cookstoves were designed with the goal of creating more efficient cookstoves that produced less PM_{2.5} than the traditional cookstoves by mass. To test whether these cookstoves are successful in producing less PM_{2.5}, the average PM_{2.5} mass loading for each cookstove using all fuel types was calculated (Figure 3.1.1). The PM_{2.5} mass loading represents the mass of a PM_{2.5} sample deposited on a quartz filter, corrected for the volume of air that passed through the filter. To determine the volume of air that passed through the filter, the flow rate was multiplied by the duration of the cooking event.

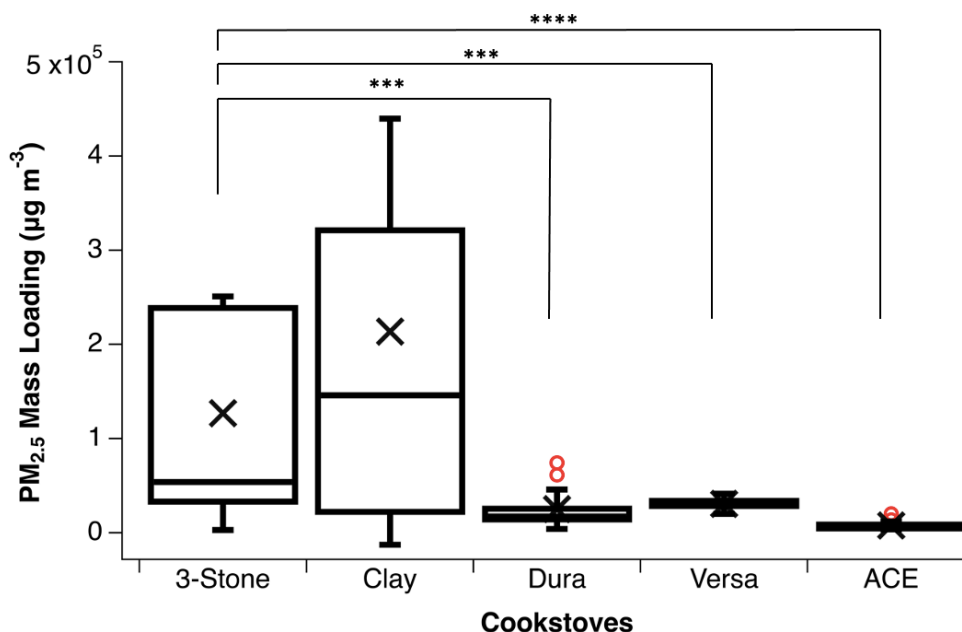


Figure 3.1.1: Average PM_{2.5} mass loading for each cookstove across all fuel types. ‘Whiskers’ represent ± 1 standard deviation, circles represent outliers, and \times represents the mean. Significance was calculated using the Tukey test with ‘p-values’ represented by: * < 0.05, ** < 0.01, *** < 0.001, **** < 0.0001.

As shown in Figure 3.1.1, it was found that both the 3-stone cookstove and the clay cookstove emitted significantly more PM_{2.5} than the improved cookstoves by mass. Of the two traditional cookstoves, the clay cookstove had the greatest average mass loading (214,000 µg m⁻³) which is 1.69 times greater than 3-stone stove (127,000 µg m⁻³). The greatest separation in average PM_{2.5} mass loading for two cookstoves was between the clay stove and the African Clean Energy One stove (ACE; 8,160 µg m⁻³), where the clay stove had a 26.2 times greater emission than the ACE. The smallest separation between traditional and improved cookstove was between the 3-stone stove and the Versa (31,000 µg m⁻³), where the 3-stone stove had a 4.1 times greater emission than the Versa.

Figure 3.1.1 only depicts the 3-stone stove having a greater emission that is statistically significant; however, this is only done to visually simplify the figure. The data with respective p values for other cookstoves can be found in the appendix (Table A1 to A2). The 3-stone stove is of most interest as it is the most common traditional cooking method used due to its simple construction.⁶¹ Additionally, in previous studies that have looked at emissions from cookstoves, the 3-stone stove is typically used to represent traditional cooking with few studies looking at clay cookstoves.⁶⁰⁻⁶²

The ability to compare mass loading data to other studies is very limited because of differences in how mass emissions are measured and types of cookstoves used. However, from a qualitative perspective, this data does agree with previous studies which have looked at PM_{2.5} emissions, as these studies have also found that PM_{2.5} emissions were reduced using the improved cookstoves.^{60-62,64} This demonstrates that the improved cookstoves used in this study are effective in their goal of reducing PM_{2.5} emissions.

3.2 Aerosol Oxidative Potential Metric by Mass

While the improved cookstoves may have been successful in reducing the emissions, this may not be enough to reduce the toxicity and negative health effects associated with the exposure to PM_{2.5} emitted during a cooking event. As stated earlier, recent epidemiological studies have found the adverse health effects associated with PM_{2.5} are better correlated with the OP of the PM_{2.5} rather than just the mass of PM_{2.5} inhaled.^{30,31} Since PM_{2.5} are complex mixtures of chemicals that vary in composition, which is based on

how $PM_{2.5}$ was generated,^{10,12} it is possible that the OP of the $PM_{2.5}$ emitted from the different burn events will vary. Therefore, not only is it important to look at the emission of $PM_{2.5}$ from the cookstoves, but also the OP of $PM_{2.5}$.

To measure the toxicity of the $PM_{2.5}$ emitted from the cookstoves, the DTT activity of the $PM_{2.5}$ samples were determined and corrected for the mass of $PM_{2.5}$ deposited on the filter punch used for analysis to get the OP_{Mass}^{DTT} . This value represents the DTT activity (OP^{DTT}) associated with $1 \mu g$ of $PM_{2.5}$ (i.e., intrinsic toxicity). Figure 3.2.1 shows the OP_{Mass}^{DTT} of the 5 tested cookstoves using hardwood as a fuel source with the $PM_{2.5}$ emissions for each cookstove using hardwood. As seen in this figure, the OP_{Mass}^{DTT} varied among the cookstoves despite all using the same fuel type. In fact, the OP_{Mass}^{DTT} of the $PM_{2.5}$ emitted from the 3-stone cookstove ($48.1 \text{ pmol min}^{-1} \mu g^{-1}$) was found to be significantly different from both the clay ($146 \text{ pmol min}^{-1} \mu g^{-1}$; $p = 0.0015$) and ACE stoves ($297 \text{ pmol min}^{-1} \mu g^{-1}$; $p = 0.0045$). This suggests that cookstoves influence not only the emissions of the $PM_{2.5}$ but also the intrinsic toxicity of the $PM_{2.5}$ that is emitted.

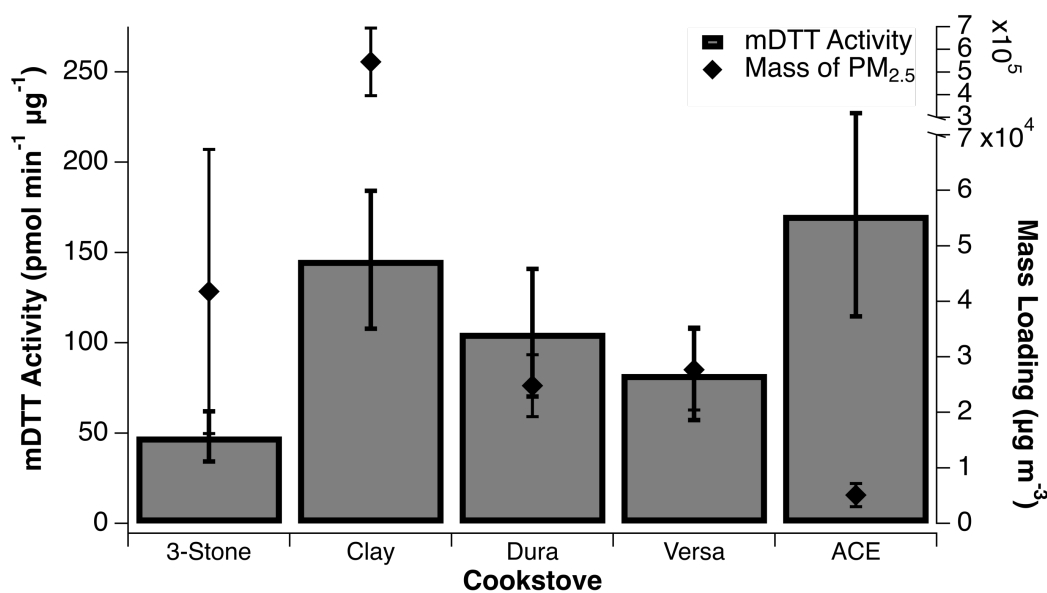


Figure 3.2.1 Average OP_{Mass}^{DTT} ($\text{pmol min}^{-1} \mu\text{g}^{-1}$) and average mass of PM ($\mu\text{g cm}^{-2}$) loaded for each cookstove with hardwood as the fuel source. Error Bars represent ± 1 standard deviation ($n=3$).

While the data in Figure 3.2.1 may be only for cookstoves using hardwood, data was collected and calculated for all cookstove and fuel type combinations. Similar results were also observed for other fuel types where the $OP_{\text{Mass}}^{\text{DTT}}$ varied for each fuel type depending on cookstove used; however, relative $OP_{\text{Mass}}^{\text{DTT}}$ were not the same for each fuel type. Examples of this can be seen in Figure A1. The overall $OP_{\text{Mass}}^{\text{DTT}}$ collected for all cooking methods ranged from $13.4 \text{ pmol min}^{-1} \mu\text{g}^{-1}$ (3-stone with charcoal briquettes) to $413 \text{ pmol min}^{-1} \mu\text{g}^{-1}$ (clay with charcoal clumps), with traditional stoves having an average $OP_{\text{Mass}}^{\text{DTT}}$ of $138 \pm 153 \text{ pmol min}^{-1} \mu\text{g}^{-1}$ and improved cookstoves had $85.1 \pm 78.3 \text{ pmol min}^{-1} \mu\text{g}^{-1}$ for all fuel types. The large error associated with these values is likely due to two factors, the high variability of individual burning events and the differences in cookstoves and fuel types used within each category.

As seen in the Shiraiwa *et al.* review article,⁴⁷ the intrinsic toxicity of $\text{PM}_{2.5}$ can vary substantially based on where and how they are generated. Verma *et al.* found that biomass burning organic aerosols had an average $OP_{\text{Mass}}^{\text{DTT}}$ of $151 \pm 20 \text{ pmol min}^{-1} \mu\text{g}^{-1}$,³⁸ which is within the range of the observed values in this study and close to the average $OP_{\text{Mass}}^{\text{DTT}}$ of the traditional stoves (7.9% difference). For reference, ambient $\text{PM}_{2.5}$, which is comprised of $\text{PM}_{2.5}$ from various sources, typically has an $OP_{\text{Mass}}^{\text{DTT}}$ of $10 - 70 \text{ pmol min}^{-1} \mu\text{g}^{-1}$,³⁸ whereas typical $OP_{\text{Mass}}^{\text{DTT}}$ values for $\text{PM}_{2.5}$ emitted from diesel engine exhaust, an anthropogenic $\text{PM}_{2.5}$ source that has been well-correlated with adverse health effects, is $41.4 \pm 13.8 \text{ pmol min}^{-1} \mu\text{g}^{-1}$.⁶⁵

It is interesting to note that despite having the lowest $\text{PM}_{2.5}$ emission for the hardwood burn events, the ACE, which is an improved cookstove, had the greatest $OP_{\text{Mass}}^{\text{DTT}}$. This indicates that while the $\text{PM}_{2.5}$ emitted is reduced, a higher fraction of this emitted $\text{PM}_{2.5}$ can induce oxidative stress, further highlighting that characterizing either just the emissions of $\text{PM}_{2.5}$ mass from cookstoves or the intrinsic toxicity of the $\text{PM}_{2.5}$ emitted is not sufficient, where a combination of the two, which represents the overall exposure to toxic components from $\text{PM}_{2.5}$ emitted from cookstoves, is a more representative health metric.

3.3 Aerosol Oxidative Potential Metric by Volume

To look at the OP associated with the overall exposure to toxic chemicals in $\text{PM}_{2.5}$, the DTT activity for each of the samples was corrected for the volume of air that passed

through the filter punch (OP_{Volume}^{DTT}). This value represents the OP^{DTT} associated with 1 m^{-3} of air, which can also be thought of as the OP^{DTT} associated with the volume of air inhaled. This metric describes the overall exposure better than OP_{Mass}^{DTT} since it accounts for both the concentration of $PM_{2.5}$ in the air ($PM_{2.5}$ emission) and the intrinsic toxicity of the particulate matter in the volume of air. Figure 3.3.1 shows the average OP_{Volume}^{DTT} of each of the cookstoves with hardwood as the fuel source.

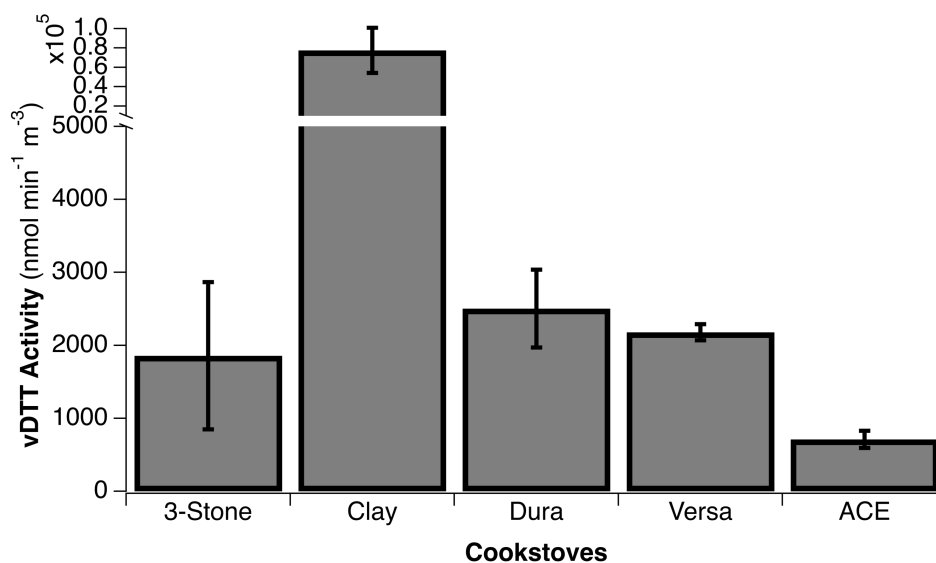


Figure 3.3.1 Average OP_{Volume}^{DTT} (nmol min⁻¹ m⁻³) for each cookstove with hardwood as the fuel source. Error Bars represent ± 1 standard deviation ($n=3$).

From a qualitative perspective, Figures 3.2.1 and 3.3.1 are very similar with each of the cookstoves having similar relative OP^{DTT} values compared to one another with one exception where ACE stove has the lowest OP_{Volume}^{DTT} ($711\text{ nmol min}^{-1}\text{ m}^{-3}$) for the hardwood samples despite having the greatest OP_{Mass}^{DTT} for the hardwood samples. The ACE stove with hardwood had a 3.55 times greater OP_{Mass}^{DTT} than the 3-stone with hardwood; however, the 3-stone had a 2.61 times greater OP_{Volume}^{DTT} than the ACE. This data suggests that despite having greater intrinsic toxicity associated with the $PM_{2.5}$ emitted from the ACE stove, $PM_{2.5}$ emissions were low enough to offset the overall toxicity associated an exposure during a cooking process.

As stated earlier, the goal of this study was to determine the overall toxicity associated with exposure to $PM_{2.5}$ emitted from cooking processes. Since OP_{Volume}^{DTT} appears

to account for overall exposure better than $OP_{\text{Mass}}^{\text{DTT}}$, for the rest of the results, only the $OP_{\text{Volume}}^{\text{DTT}}$ will be discussed.

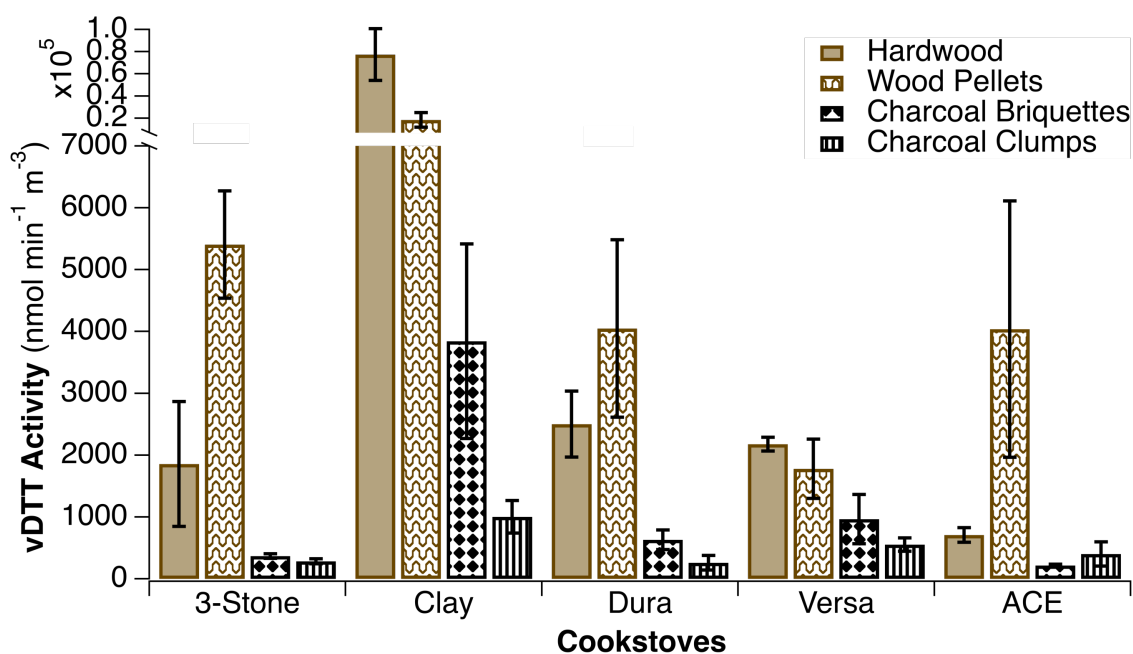


Figure 3.3.2 Average total $OP_{\text{Volume}}^{\text{DTT}}$ (nmol min⁻¹ m⁻³) for each cooking method. Error bars represent ± 1 standard deviation ($n=3$).

Figure 3.3.2 displays all the average $OP_{\text{Volume}}^{\text{DTT}}$ values for each of the cookstove and fuel type combinations (cooking methods) where it is apparent that $OP_{\text{Volume}}^{\text{DTT}}$ varies drastically between different cooking methods. The average $OP_{\text{Volume}}^{\text{DTT}}$ for the cooking methods tested ranged from 215.2 nmol min⁻¹ m⁻³ (ACE stove with charcoal briquettes) to 77,474.1 nmol min⁻¹ m⁻³ (clay stove with hardwood). The average $OP_{\text{Volume}}^{\text{DTT}}$ of all the cooking methods combined is $5,910 \pm 16,890$ nmol min⁻¹ m⁻³; however, as the large standard deviation of that value suggests, $OP_{\text{Volume}}^{\text{DTT}}$ for each cooking method varies drastically, and as such, each cooking method must be examined individually. For comparison, Vreeland *et al.* found that ambient PM_{2.5} emitted around Bangalore, India had an average $OP_{\text{Volume}}^{\text{DTT}}$ of 0.79 ± 0.13 nmol min⁻¹ m⁻³ (range: 0.66 – 1.03 nmol min⁻¹ m⁻³).⁵ The lowest average $OP_{\text{Volume}}^{\text{DTT}}$ from the tested cooking methods was still approximately 209 times greater than the highest recorded $OP_{\text{Volume}}^{\text{DTT}}$ from Vreeland *et al.* and the cooking method with the greatest $OP_{\text{Volume}}^{\text{DTT}}$ was 75,217 times greater.⁵ This shows just how dangerous the PM_{2.5}

emitted from these stoves are even when using an improved cooking method, especially when considering that ambient PM is 7th highest early mortality risk factor.³ The same study found that PM_{2.5} emitted from burning roadside trash had OP_{Volume}^{DTT} ranging from 89 – 3,510 nmol min⁻¹ m⁻³ which is comparable to some of the cooking methods tested,⁵ however, in general, the cookstoves reached higher OP_{Volume}^{DTT} than even these roadside burning events.

One of the most striking results from our data was the difference that fuel type had on the OP_{Volume}^{DTT} for each cookstove. As seen in Figure 3.3.2, the wood fuel types (hardwood and softwood pellets) had the greatest average OP_{Volume}^{DTT} for each cookstove while the charcoal fuel types (charcoal briquettes and charcoal clumps) had the lowest average OP_{Volume}^{DTT} . In some cases, the wood types were only 1.8 times greater than the coal (hardwood and charcoal clumps in the ACE stove) but in other cases they were up to 77.3 times greater (hardwood and charcoal clumps in the clay stove). Since charcoal is a processed version of wood, this suggests that the chemicals that give wood its higher OP_{Volume}^{DTT} are lost in the production of charcoal. This could possibly be through the decomposition/loss of certain chemicals or possibly even the conversion of organic compounds to less reactive compounds due to the charcoal production process.

One of the objectives for characterizing the OP of PM_{2.5} emitted from different cooking methods is to suggest effective strategies to best reduce the toxicity associated with biomass burning cooking. As stated before, the hardwood and 3-stone cookstove combination (average OP_{Volume}^{DTT} of 1860 ± 1010 nmol min⁻¹ m⁻³) is the most common and accessible traditional cooking method. From the perspective of the populations using this cooking method, the following mitigation strategies are proposed based on the findings of our study. The best way to reduce exposure to toxic PM_{2.5} is to switch to the ACE stove with charcoal briquettes (215 ± 21 nmol min⁻¹ m⁻³). This would give an 88.4% reduction in OP_{Volume}^{DTT} and is a significant reduction ($p = 0.00047$). However, this is an ideal scenario where households would be willing and economically able to convert to both a new type of fuel and cookstove. Instead, a more realistic mitigation strategy may be the recommendation of switching either fuel type or cookstove. In this case, the best way to reduce exposure to toxic PM_{2.5} is to switch from using the 3-stone stove with hardwood to using 3-stone stove with charcoal clumps (average OP_{Volume}^{DTT} of 285 ± 21 nmol min⁻¹ m⁻³), which is an 85%

reduction in exposure to toxic $PM_{2.5}$ and is a significant reduction ($p = 0.0025$). Switching cookstove type while continuing to use the same fuel type provides households using the 3-stone with hardwood cooking method only one option. This is because the 3-stone stove already has the 2nd lowest OP_{Volume}^{DTT} for hardwood. The only option in this scenario is to switch to the ACE stove, which provides a significant reduction ($p = 0.026$) of OP_{Volume}^{DTT} but is only reduced by 62%. Since the only cookstove that reduced OP_{Volume}^{DTT} was the ACE, this data also suggests that switching from traditional to improved cookstoves doesn't always lead to a reduction in exposure to toxic $PM_{2.5}$.

3.4 Oxidative Potential from Water-Soluble and Insoluble Components

In addition to looking at the $PM_{2.5}$ emissions, OP_{Mass}^{DTT} , and OP_{Volume}^{DTT} for each cooking method, the contributions of the WS and insoluble fraction to the total OP_{Volume}^{DTT} . While many studies which look at the OP of $PM_{2.5}$ only look at the WS fraction, it is not only the soluble fraction that contribute to the OP but the insoluble fraction as well.⁶⁶

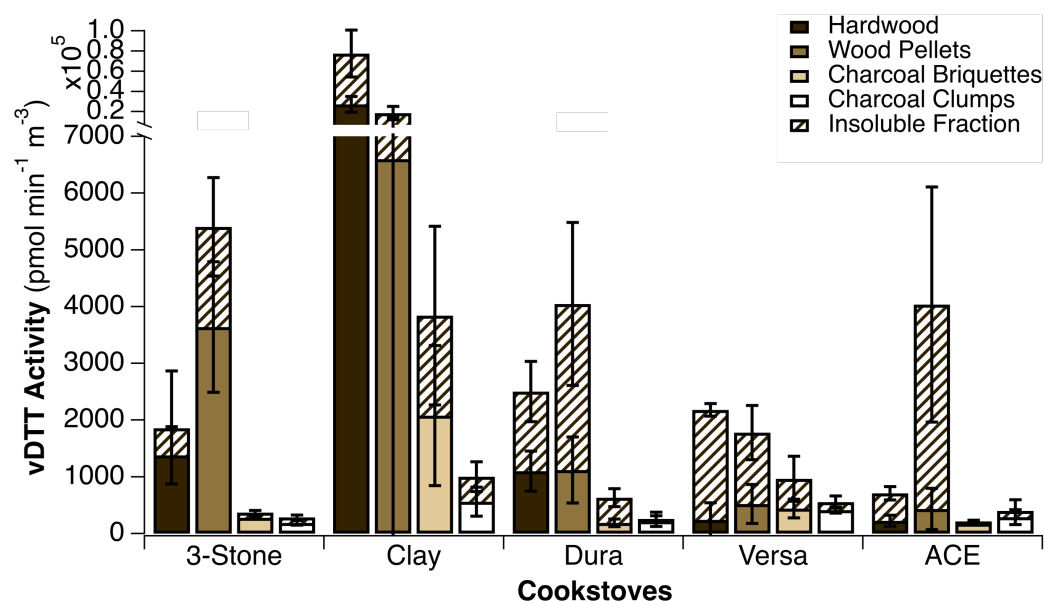


Figure 3.4.1 Average WS and total OP_{Volume}^{DTT} (nmol min⁻¹ m⁻³) for each cooking method. Error bars represent ± 1 standard deviation ($n=3$).

Figure 3.4.1 depicts the portion of OP_{Volume}^{DTT} that comes from WS and insoluble $PM_{2.5}$ components. The portion of WS *versus* insoluble fraction can vary tremendously with

some cooking methods (e.g. wood pellets with ACE) showing WS fraction account for less than 15% of the total OP_{Volume}^{DTT} (i.e., 85% of the total OP_{Volume}^{DTT} is contributed by the insoluble components) and in some cases (e.g., hardwood, 3-stone) about 75% of the total OP_{Volume}^{DTT} is contributed by the WS components (i.e., ~25% is due to insoluble components). The variability in the ratio of insoluble- OP_{Volume}^{DTT} to WS- OP_{Volume}^{DTT} indicates that chemical composition of toxic compound is likely dependent on cooking methods. It is still unknown as to how WS and insoluble fractions interact with the body and which are more serious, but this data could be used to better understand the associated health effects in the future. Additionally, this data could help in better understanding why toxicity differences occur on a per mass basis and could supplement further chemical compositional data in understanding why the intrinsic toxicity of $PM_{2.5}$ changes between not just fuel types, but cookstoves too.

4. CONCLUSION AND FUTURE DIRECTIONS

In summary, the emissions and OP of PM_{2.5} were determined for 20 different cooking methods. The cooking methods used 2 traditional stoves (3-stone and clay) and 3 improved stoves (Dura, Versa, and ACE) with 4 fuel types (hardwood, softwood pellets, charcoal clumps, and charcoal briquettes). The mass of PM_{2.5} emitted is reduced (from 76 – 96%) for improved cookstoves compared to the traditional cookstoves.

The data from the DTT assay were corrected for mass to determine the intrinsic toxicity associated with PM_{2.5}. The results from this varied, even between different cookstoves using the same fuel. In some cases, the PM_{2.5} emitted from improved cookstoves had a high intrinsic toxicity but a low emission. This suggested that OP_{Volume}^{DTT} is a better metric for representing the toxicity associated with exposure during cooking (i.e., both PM_{2.5} emissions and intrinsic toxicity are important).

When looking at the OP_{Volume}^{DTT} data, it was found that for each cookstove that the wood fuel types had the 2 greatest average OP_{Volume}^{DTT} for the PM_{2.5} emitted while the charcoal fuel types had the lowest. Two example mitigation strategies were then provided for households using the 3-stone cookstove with hardwood as it is the most common and accessible cooking method.⁶¹ To see the greatest reduction in toxicity a person using the 3-stone with hardwood method should switch to the ACE with charcoal briquettes as it provides the greatest reduction in toxicity (88% reduction). This strategy seemed too ideal though as there are cultural and financial limitations to switching cooking methods and so a mitigation strategy that only switches the fuel type or cooking method, not both, may have a greater effect. In this case, someone using the 3-stone with hardwood method should switch to using charcoal clumps with the 3-stone stove as it gives an 84% reduction in OP_{Volume}^{DTT} , in comparison to switching to the ACE with hardwood which only gives a 62% reduction in OP_{Volume}^{DTT} . These results suggest that switching fuel source can have as big of a reduction in toxicity as switching cookstoves, if not, better. By providing this data populations can make more informed decisions when choosing an alternative cooking method. Additionally, by providing multiple mitigation strategies, the households using these cookstoves have more options available to them that may have less of a financial impact or effect on cultural cooking practices. These households would then be more likely to switch their cooking method and reduce their exposure to toxic PM_{2.5}.

Finally, the WS fraction that made up the OP_{Volume}^{DTT} was calculated. The portion of WS *versus* insoluble fraction was found to vary tremendously with the WS fraction of some cooking methods accounting for less than 15% of the total OP_{Volume}^{DTT} and in some cases about 75% of the OP_{Volume}^{DTT} . This data could help in better understanding why toxicity differences occur on a per mass basis and could also supplement further chemical compositional data. It also further supports that the mass of $PM_{2.5}$ alone does not capture the full picture when looking at the toxicity of $PM_{2.5}$.

This project has numerous opportunities for further directions. The most important ‘next step’ in this project would be the chemical characterization of the $PM_{2.5}$ emitted from the stoves. Having both the elemental analysis data and elemental/organic carbon analysis data could allow for correlating the compositional data with the OP^{DTT} data to determine whether specific elements (e.g., transition metals) or if a specific carbon type (e.g., organic carbon) is driving the OP of the $PM_{2.5}$ emitted from these cookstoves and fuel sources. This data would be useful in understanding how fuel source and cookstove cause changes in $PM_{2.5}$ composition and how they influence toxicity. This information could be useful in future cookstove design and fuel source choice.

In addition to characterization of the $PM_{2.5}$, some improvements that could be done to better improve the understanding of the overall exposure to toxic chemicals could include measuring the OP of $PM_{2.5}$ emitted from the cookstoves after undergoing oxidation. This would be done to mimic possible atmospheric aging that would occur after the $PM_{2.5}$ is emitted and continue to linger in a home. Oxidants such as ozone, nitrous acid, formaldehyde, and nitrogen oxides are all found in indoor environments.⁶⁷ By reacting these common oxidants for varying times with the $PM_{2.5}$ emitted from cookstoves, the changes in $PM_{2.5}$ toxicity with oxidation would be better understood and provide even more information on the overall exposure to toxic $PM_{2.5}$ that occurs after the burning event.

Also, looking at the ratio of CO to CO_2 emitted from the cookstoves would provide an even better understanding between cooking method and $PM_{2.5}$ toxicity. This data would provide information on the combustion efficiency of the cooking methods as complete combustion produces CO_2 and incomplete combustion produces CO.⁶¹ For example, if it was found that for the cooking methods which had lower CO: CO_2 emission, the resulting $PM_{2.5}$ emitted were less toxic, this would suggest that combustion efficiency played a major role in

PM_{2.5} toxicity. Efforts could then be focused on creating even more efficient cookstoves and finding fuel sources that also burned more efficiently.

Hopefully, with the results presented in this study, there will be a greater focus on the reducing the overall exposure toxicity associated with improved biomass burning cookstoves, not just a goal to reduce the mass of PM_{2.5} emitted. This will then hopefully lead to better cookstove designs that significantly reduce the toxicity associated with cooking using these stoves. Additionally, this research provides preliminary results on the effect that fuel source can have on PM_{2.5} toxicity and how it may be a more effective way of reducing exposure to toxic PM_{2.5}. This opens possibilities for testing and developing improved fuel types in addition to improved cookstove types. While there is still a lot of work left to fully understand the intricacies of these cooking devices and the pollutants they emit, our hope is that this research will aid in creating a cleaner burning future.

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APPENDIX

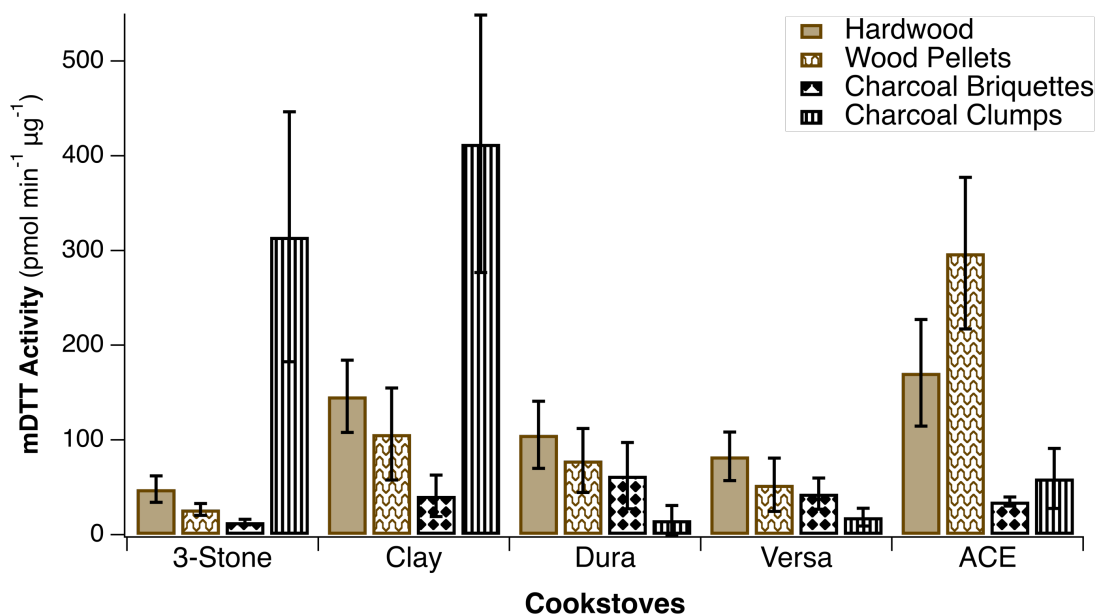


Figure A1: Average total $OP_{\text{Mass}}^{\text{DDT}}$ ($\text{pmol min}^{-1} \mu\text{g}^{-3}$) for each cooking method. Error Bars represent ± 1 standard deviation.

Table A1: Tukey test significance values comparing mass loading of the 3-Stone (3S) stove to the improved stoves at a 95% confidence level.

Stoves	q	q _{0.05}	Accept/Reject H ₀	p
3S vs Dura	6.071	3.75839	Reject	4.58×10^{-4}
3S vs Versa	5.727	3.75839	Reject	9.96×10^{-4}
3S vs ACE	7.439	3.75839	Reject	1.76×10^{-5}
Dura vs ACE	1.099	3.75839	Accept	0.973
Versa vs ACE	1.468	3.75839	Accept	0.939
Dura vs Versa	0.351	3.75839	Accept	0.999

Table A2: Tukey test significance values comparing mass loading of the clay stove to the improved stoves at a 95% confidence level.

Stoves	q	q _{0.05}	Accept/Reject H ₀	p
Clay vs Dura	6.238	3.75839	Reject	3.11×10^{-4}
Clay vs Versa	6.047	3.75839	Reject	4.84×10^{-4}
Clay vs ACE	7.137	3.75839	Reject	3.68×10^{-5}
Dura vs ACE	0.611	3.75839	Accept	0.973
Versa vs ACE	0.816	3.75839	Accept	0.939
Dura vs Versa	0.195	3.75839	Accept	0.999

Table A3: Tukey test significance values comparing $OP_{\text{Mass}}^{\text{DTT}}$ of all 5 stoves using hardwood as the fuel source at a 95% confidence level.

Stoves	q	q _{0.05}	Accept/Reject H ₀	p
3S vs Dura	2.777	4.5736	Accept	0.715
3S vs Versa	1.792	4.5736	Accept	0.342
3S vs ACE	5.936	4.5736	Reject	0.0104
3S vs Clay	4.732	4.5736	Reject	0.0416
Clay vs Dura	1.956	4.5736	Accept	0.650
Clay vs Versa	3.268	4.5736	Accept	0.212
Clay vs ACE	1.203	4.5736	Accept	0.909
Dura vs ACE	3.159	4.5736	Accept	0.237
Dura vs Versa	1.177	4.5736	Accept	0.915
Versa vs ACE	4.554	4.5736	Accept	0.0511

Table A4: Tukey Test comparing the OP_{Volume}^{DTT} of the 3-stone stove with hardwood fuel (3S_{HW}), 3-stone stove with charcoal clumps fuel (3S_{CC}), 3-stone stove with charcoal briquettes (3S_{CB}), ACE stove with charcoal briquettes fuel (ACE_{CB}), and ACE stove with hardwood (ACE_{HW}) at a 95% confidence level.

Cooking Method	q	q _{0.05}	Accept/Reject H ₀	p
3S _{HW} vs ACE _{CB}	8.235	4.45291	Reject	4.74×10^{-4}
3S _{HW} vs 3S _{CC}	6.829	4.45291	Reject	0.00252
3S _{HW} vs 3S _{CB}	6.465	4.45291	Reject	0.00395
3S _{HW} vs ACE _{HW}	4.981	4.45291	Reject	0.0257
ACE _{HW} vs ACE _{CB}	2.483	4.45291	Accept	0.437
ACE _{HW} vs 3S _{CC}	1.848	4.45291	Accept	0.692
ACE _{HW} vs 3S _{CB}	1.483	4.45291	Accept	0.828
3S _{CB} vs ACE _{CB}	0.771	4.45291	Accept	0.981
3S _{CB} vs 3S _{CC}	0.365	4.45291	Accept	0.999
3S _{CC} vs ACE _{CB}	0.349	4.45291	Accept	0.999