



Differential exposure and acute health impacts of inhaled solid-fuel emissions from rudimentary and advanced cookstoves in female CD-1 mice

Eugene A. Gibbs-Flournoy^a, M. Ian Gilmour^b, Mark Higuchi^b, James Jetter^c, Ingrid George^c, Lisa Copeland^b, Randy Harrison^b, Virginia C. Moser^b, Janice A. Dye^{b,*}

^a Oak Ridge Institute for Science and Education, Oak Ridge, TN, USA

^b National Health and Environmental Research Laboratory (NHEERL), Office of Research and Development, US Environmental Protection Agency, Research Triangle Park, NC 27711, USA

^c National Risk Management Research Laboratory (NRMRL), Office of Research and Development, US Environmental Protection Agency, Research Triangle Park, NC 27711, USA

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ABSTRACT

Background: There is an urgent need to provide access to cleaner end user energy technologies for the nearly 40% of the world's population who currently depend on rudimentary cooking and heating systems. Advanced cookstoves (CS) are designed to cut emissions and solid-fuel consumption, thus reducing adverse human health and environmental impacts.

Study premise: We hypothesized that, compared to a traditional (Tier 0) three-stone (3-S) fire, acute inhalation of solid-fuel emissions from advanced natural-draft (ND; Tier 2) or forced-draft (FD; Tier 3) stoves would reduce exposure biomarkers and lessen pulmonary and innate immune system health effects in exposed mice.

Results: Across two simulated cooking cycles (duration ~ 3 h), emitted particulate mass concentrations were reduced 80% and 62% by FD and ND stoves, respectively, compared to the 3-S fire; with corresponding decreases in particles visible within murine alveolar macrophages. Emitted carbon monoxide was reduced ~ 90% and ~ 60%, respectively. Only 3-S-fire-exposed mice had increased carboxyhemoglobin levels. Emitted volatile organic compounds were FD << 3-S-fire ≤ ND stove; increased expression of genes involved in xenobiotic metabolism (COX-2, NQO1, CYP1A1) was detected only in ND- and 3-S-fire-exposed mice. Diminished macrophage phagocytosis was observed in the ND group. Lung glutathione was significantly depleted across all CS groups, however the FD group had the most severe, ongoing oxidative stress.

Conclusions: These results are consistent with reports associating exposure to solid fuel stove emissions with modulation of the innate immune system and increased susceptibility to infection. Lower respiratory infections continue to be a leading cause of death in low-income economies. Notably, 3-S-fire-exposed mice were the only group to develop acute lung injury, possibly because they inhaled the highest concentrations of hazardous air toxicants (e.g., 1,3-butadiene, toluene, benzene, acrolein) in association with the greatest number of particles, and particles with the highest % organic carbon. However, no Tier 0–3 ranked CS group was without some untoward health effect indicating that access to still cleaner, ideally renewable, energy technologies for cooking and heating is warranted.

1. Introduction

Air pollution is a global public health problem to which emissions from rudimentary cooking devices contribute significantly. Current appraisals predict that nearly 40% of the world's population use solid-fuels such as wood, coal, charcoal, crop residues, animal dung, and other types of biomass burning for cooking, lighting, and heating (Anenberg et al., 2013). Burning of solid-fuels in simple inefficient

stoves generates harmful emissions that contribute to poor indoor air quality and have detrimental impacts on human health (McCracken et al., 2012; Gordon et al., 2014). On a global basis, exposure to household air pollutants, including cooking-related emissions, have been linked to increases in morbidity and mortality causing an estimated 3.5+ million deaths annually (Lim et al., 2012; Lacey et al., 2017).

Associated acute health effects include respiratory and eye

* Correspondence to: National Health and Environmental Research Laboratory (NHEERL), Office of Research and Development, U.S. Environmental Protection Agency, MD B105-02, 109 T.W. Alexander Drive, Research Triangle Park, NC 27711, USA.

E-mail address: dye.janice@epa.gov (J.A. Dye).

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irritation, headache, cough, acute lower respiratory infection and severe pneumonia in children (Smith et al., 2011; Gordon et al., 2014). Longer term exposure is associated with stillbirths (Lakshmi et al., 2013), low birth weight, and preterm births (Malley et al., 2017); as well as increased prevalence of several chronic diseases including chronic obstructive pulmonary disease (COPD), diabetes, hypertension, cardiovascular disease, stroke, lung cancer and other types of neoplasia (IARC, 2010; McCracken et al., 2012; Martin et al., 2014; Gordon et al., 2014; Assad et al., 2015). These health effects are primarily observed in individuals exposed most directly to cookstove (CS) emissions for the longest durations, namely women cooking indoors and their children (Rumchev et al., 2007; Clark et al., 2009). Furthermore, these same emissions contribute to increases in atmospheric black and brown carbon and to greenhouse gases that are predicted to have adverse effects on regional and global climate patterns (Smith, 1994; Anenberg et al., 2013; Laskin et al., 2015). Taking all of these factors together, there is a great need for effective interventions to mitigate the undesirable consequences associated with the use of rudimentary cooking devices.

Serious efforts have been made to lessen the negative impacts of cooking-related biomass burning. One approach has been to develop advanced, more fuel-efficient, hence “cleaner cookstoves” (Kshirsagar and Kalamkar, 2014; Johnson and Chiang, 2015). Advanced CS are intended to reduce emissions and minimize solid-fuel consumption, with the expectation to diminish adverse human health and environmental impacts (e.g., deforestation). Technological advancements for solid-fuel stoves are based chiefly on manipulating, enhancing, or redirecting air flow to maintain optimal oxygen for efficient fuel combustion throughout the cooking cycle (Kshirsagar and Kalamkar, 2014). We have previously developed and used standardized testing to compare fuel efficiencies of CS with differing designs (Jetter and Kariher, 2009). Under laboratory testing conditions (i.e., a 60-min water boiling test), a rudimentary three-stone (3-S) fire emits high levels of carbon monoxide (CO) and particulate matter (PM) and is therefore rated as a *Tier 0* cooking system. Advanced CS emit lower CO and PM concentrations, albeit with varying degrees of success, and thus receive *Tier 1* to *Tier 4* rankings (see Table S1 for metrics used for CS Tiers) (ISO 2011; Jetter et al., 2012; Still et al., 2015).

In addition to CO and PM, solid-fuel combustion generates complex mixtures of volatile and semi-volatile organic compounds (VOCs/SVOCs), and particles with adsorbed SVOCs — the proportions of which relate to the degree of incomplete combustion occurring (Traboulsi et al., 2017). VOCs/SVOCs emitted during relatively incomplete biomass burning include alkanes, alkenes, cyclic hydrocarbons, carbonyls, and assorted polyaromatic hydrocarbon (PAH) species (Brown et al., 1994; Lemieux et al., 2004; Jordan and Seen, 2005; IARC, 2010; Mutlu et al., 2016). On an individual basis, many of these compounds have been studied extensively and are known to be associated with effects such as acute cardiorespiratory irritation, lung and systemic oxidative stress, and cancer (Lemieux et al., 2004; Bates et al., 2015; IARC, 2016; Ye et al., 2017).

A limited number of field and epidemiological studies on health improvements derived from implementing advanced CS usage in homes are beginning to appear in the literature (Li et al., 2016). However, to date, perceived benefits for reducing acute respiratory conditions (e.g., childhood pneumonia) have been inconsistent (Smith et al., 2011; Bruce et al., 2013; Cundale et al., 2017; Mortimer et al., 2017). Equivocal findings appear to relate, in part, to real-world factors such as the poor quality of available solid-fuel, non-ideal operation of advanced CS (Wathore et al., 2017), inconsistent “adoption” of advanced CS despite availability, and stove stacking (i.e., continued use of rudimentary cooking systems along with advanced CS) (Ruiz-Mercado et al., 2013). Although results of field and epidemiologic investigations are highly important, they often lend little information to understanding the mechanistic outcomes of exposure to CS emissions.

Studies directly assessing health improvements derived from use of

advanced CS in a controlled laboratory environment are lacking. Thus, to generate health effects data on CS emissions, we recently determined the mutagenicity emission factors (revertants/megajoule_{delivered}, rev/MJ_d) of particles emitted during simulated cooking using a three-stone (3-S) fire (Tier 0), a natural-draft (ND, Tier 2), and a forced-draft (FD, Tier 3) stove burning dry red oak (Mutlu et al., 2016). Using organic extracts of emitted PM in the *Salmonella* mutagenicity assay, we found that the mutagenicity emission factor (rev/MJ_d) was reduced by 72.5% and 97.0% by the ND and FD stoves, respectively, compared to the 3-S fire. However, even this reduction resulted in the FD stove having a mutagenicity emission factor (expressed as rev/MJ_{thermal}) that was nonetheless on parity with that of diesel exhaust, a Group 1 (known) human carcinogen (IARC, 2014).

The present study was designed to further assess differential exposure and acute respiratory system effects of inhaled CS emissions. We exposed healthy female outbred (CD-1) mice to emissions from the same stoves and wood used by Mutlu et al. (2016). We compared the results to those of mice inhaling filtered air. Assessments included: (1) characterization of CS emissions, (2) biomarkers of exposure, and (3) health effects related to altered behavior, pulmonary function, innate immunity, and development of lung injury, inflammation, oxidative stress, or upregulation of adaptive genes. We hypothesized that acute inhalation of solid-fuel emissions generated by FD and ND stove technologies would result in lower levels of exposure biomarkers and less pulmonary or systemic health effects compared to that of the 3-S fire. Our approach was also intended to provide mechanistic information as to which of the key components present in solid-fuel emissions elicited or were associated with the above health outcomes.

2. Materials and methods

2.1. Experimental animals

Young adult (8-wk-old) female CD-1 mice (18–21 g) were used to as surrogates of the women using indoor stoves. Mice were purchased from Charles River Laboratories (Raleigh, NC), and housed in groups of four in polycarbonate cages in an AAALAC-accredited, barrier-isolated, animal research facility (21 ± 1 °C, 50 ± 5% relative humidity, and 12:12-h light/dark cycle). Standard mouse chow (Prolab RMH 3000; LabDiet, St. Louis, MO) and water were provided ad libitum. All experimental procedures requiring laboratory animals were pre-approved and performed in accordance with the U.S. EPA NHEERL Institutional Animal Care and Use Committee recommendations.

2.2. Generation of cookstove emissions and exposure protocols

Based on the water boiling test protocol, dried red oak (≤ 6% moisture content on a wet basis) was used as the solid-fuel, and emissions were generated from a FD stove (Philips HD4012), a ND stove (Envirofit G-3000), and a traditional 3-S fire (as described by Jetter et al. (2012), <http://cleancookstoves.org/technology-and-fuels/testing/protocols.html>).

Then, to better link differences in the performance of stoves operated under controlled conditions to health outcomes, mice were exposed only to emissions generated during the cold start-up and high-power phase (in which 5 l of water was heated from ~ 20 °C to the boiling temperature) followed by a 45-min low-power phase (in which the water temperature was maintained at ~ 3 °C below the boiling temperature). The high- and low-power phases simulated a cooking event. Cookstoves were operated as intended by manufacturers with typical fuel and cooking pots, but emissions from actual use in the field may vary due to different operation and use of alternative fuels or cooking vessels. The fire was extinguished after each simulated cooking event, so mice were not exposed to emissions from smoldering wood, although human exposure to smoldering emissions may occur in actual use. After the first simulated cooking event, there was a 15-min

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