

RESEARCH ARTICLE

# Biomass smoke exposures: toxicology and animal study design

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## Abstract

The International Biomass Smoke Health Effects (IBSHE) conference was convened in Missoula, MT, to define our current knowledge of smoke exposure and the potential health effects. In an effort to ascertain the relative health effects of an exposure to biomass smoke, numerous studies have utilized either animal or in vitro systems. A wide variety of systems that have been employed ranged from more mainstream animal models (i.e., rodents) and transformed cell lines to less common animal (piglets and dogs) and explant models. The Toxicology and Animal Study Design Workgroup at IBSHE was tasked with an analysis of the use of animal models in the assessment of the health effects of biomass smoke exposure. The present article contains a mini-review of models utilized historically, in addition to the adverse health effects assessed, and an overview of the discussion within the breakout session. The most common question that arose in discussions at the IBSHE conference was from local and federal health departments: What level of smoke is unhealthy? The present workgroup determined categories of exposure, common health concerns, and the availability of animal models to answer key health questions.

**Keywords:** *Acute; animal models; biomass; chronic; health effects*

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## Introduction

Exposure to biomass smoke has been epidemiologically associated with pulmonary disease, including asthma and respiratory infection. Despite this evidence, there are several important questions that remain and are difficult to answer without animal studies. These vital questions include the following: How much exposure is unhealthy? What components of biomass smoke are detrimental? What are the mechanisms involved? Historically, a wide variety of animal models has been utilized in biomass smoke studies, including, but not limited to, rodents, pigs, dogs, and sheep. In addition to whole-animal systems, primary cells and transformed cell lines, as well as tissue explants, have been utilized in the health effects assessment of biomass smoke. These various systems have allowed researchers to assess a wide range of health parameters such as cardiovascular

complications, allergy/asthma exacerbation, tumor growth, and general toxicity. In the Toxicology and Animal Study Design Workgroup at the IBSHE conference in Missoula, MT, the use of animal models to better understand the health impact of exposure to biomass smoke was discussed. Specific health parameters, including local and systemic complications, as well as acute versus chronic exposure issues, were discussed, in addition to the myriad systems available that could be utilized to assess the health consequences of exposure to biomass smoke. Prior to any discussion regarding specific animal models, the panel decided to set up parameters and definitions to have a framework for the discussion. This article is a brief summary of models and parameters employed and studied historically in biomass smoke research followed by the discussion in the IBSHE workgroup on animal models.

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## Animal models

A wide range of animal models has been utilized historically in assessing the adverse biological effects of exposures to wood smoke. In addition to the common rodent models (rats and mice) (Barrett et al. 2006; Dubick et al. 2002; Ho and Kou 2002; Huang et al. 2006; Ischiropoulos et al. 1994; Liang et al. 1988; Lin et al. 2000; Matthew et al. 2001; Mumford et al. 1990; Reed et al. 2006; Samuelsen et al. 2008; Tesfaigzi et al. 2002, 2005), other well-known species used in these studies include guinea pigs (Ho and Kou 2000; Hsu et al. 1998a, 1998b, 2000; Hsu and Kou 2001; Kou and Lai 1994; Kou et al. 1995, 1997, 1999; Lai and Kou 1998a, 1998b, 1998c; Lin and Kou 2000; Lin et al. 2001; Wang et al. 1996; Wong et al. 1984) and rabbits (Bhattacharyya et al. 1998; Fick et al. 1984; Loke et al. 1984; Thorning et al. 1982). Other groups have taken advantage of less known species such as pigs/piglets (Jeng et al. 2003; Steinberg et al. 2005), dogs (Brizio-Molteni et al. 1984; Clark et al. 1990; Nieman et al. 1980; Nieman et al. 1989, 1994, 1995), and sheep (Hubbard et al. 1991; Park et al. 2004). While whole-animal exposures are considered closer to the human condition, *in vitro* models have been taken advantage of in particulate exposures. These types of systems include primary cells from rats and mice, as well as transformed cell lines including RAW264.7 (Kubatova et al. 2006; Leonard et al. 2000) and CHO (Hytonen et al. 1983). In addition to the use of cell culture systems, certain studies utilized explants from rats or rabbits to assay wood-smoke particulate effects. While various methods have been employed, a majority of the studies utilized either inhalation or ventilator/respirator techniques of exposure. Utilization of a wide variety of systems has allowed for the assessment of a multitude of parameters that have increased our understanding of the health effects of biomass smoke exposures.

## Parameters assayed

The models listed earlier were used to assay a variety of parameters to assess the effects of exposure to wood smoke or wood-smoke particulates. Some of the pulmonary outcomes that a lot of these studies were aimed at included airway reactivity (Barrett et al. 2006; Bhattacharyya et al. 2004; Hsu and Kou 2001), asthma exacerbation (Samuelsen et al. 2008; Tesfaigzi et al. 2005), pulmonary function or injury (Clark et al. 1990; Dubick et al. 2002; Hubbard et al. 1991; Ischiropoulos et al. 1994; Jeng et al. 2003; Lin et al. 2001; Park et al. 2004; Steinberg et al. 2005; Thorning et al. 1982), effects on surfactant (Jeng et al. 2003; Nieman and Clark 1994), inflammation (Bhattacharyya et al. 2004; Huang et al. 2006), epithelial damage (Bhattacharyya et al. 1998), edema (Lin and Kou 2000; Nieman et al. 1995), apnea (Lin et al. 2000), and antibacterial activity (Fick et al. 1984; Pimenta et al. 2000). Extrapulmonary assessments have included effects on the cardiovascular system (Brizio-Molteni et al. 1984), adjuvant activity (Samuelsen et al. 2008), general toxicity including effects on CYP1A1 and GSH depletion (Iba et al. 2006; Kubatova et al. 2006; Pimenta et al. 2000; Reed et al. 2006), mortality (i.e., via CO) (Matthew et al. 2001), and

the adverse effects on tumor/cancer growth (Bhattacharyya et al. 1998; Liang et al. 1988; Mumford et al. 1990). In addition, these parameters were assessed using a variety of techniques including surfactant supplementation (Jeng et al. 2003), footpad injections for adjuvant analysis (Samuelsen et al. 2008), whole-body plethysmography (Wong et al. 1984) for assessment of pulmonary functions, tracheal and rat lung explants (Bhattacharyya et al. 1998; Iba et al. 2006), and histopathological assessments (Hubbard et al. 1991; Tesfaigzi et al. 2002; Thorning et al. 1982). In addition to these parameters, others were discussed in the IBSHE workgroup, as well as the availability of research systems to properly assess the health outcomes of these exposures.

## Breakout session discussion

### *Exposure definitions*

It was agreed that there are two main types of exposure that have come out of the conference to this point: (1) short-term, or episodic; and (2) long-term, or chronic. The term “acute” exposure was discussed but soon dismissed, as it is highly variable and depends on the researchers and individual definitions. Short-term, or episodic, exposures were defined as those experienced in forest fires where communities were exposed to large doses for relatively short time periods ranging from days to weeks. Long-term, or chronic, exposures were defined as those most commonly experienced in Third World countries where cooking is performed at open hearths in the home. Individuals, mostly adult females and children, are exposed to a constant level of biomass smoke on a daily basis.

### *Health effects of exposure*

The most common question that health departments are faced with is whether or not the current levels of biomass smoke are healthy. This is an overly broad and ambiguous question, and to answer it is no simple task. No animal is a perfect model of human health, so different questions regarding the health effects of biomass smoke are best answered with a variety of animal models. For this purpose, the workgroup outlined the known and plausible health effects for each of the exposure conditions defined earlier.

### *Episodic exposures*

Episodic exposures to high levels of biomass smoke can have effects on multiple aspects of human health. Four categories, in no particular order, were discussed in the workgroup in the context of short-term exposures. The first is termed “general irritation.” This category includes everything from eyes to skin to mucous membranes, and presents as inflammation. A second area of health concern includes exacerbation of existing conditions, of which two of the most common are asthma and cardiovascular disease. The third category is the effect of wood smoke on the immune system. This includes both local (pulmonary) and systemic immunity, and may result in either suppression or hypersensitivity. The last category discussed in the breakout session was that of “immediate response” to biomass smoke exposure.

Some members of the workgroup suggested that there may be adverse respiratory and cardiac responses to smoke exposure that are immediate and would not fall under the "preexisting conditions" category number two.

### Chronic exposures

Consistent, perhaps daily, exposure to biomass smoke can have long lasting health effects. This type of exposure is most commonly seen in Third World countries where cooking is performed in the home using an open fire. The session panel came up with four major categories, in no particular order, of health effects of chronic biomass exposure, in addition to two unknown but potential categories. The first group of health effects is the most evident: effects on the pulmonary system. These include chronic obstructive pulmonary disease (COPD), emphysema, respiratory infections, and interstitial lung disease. The second category of health effects following chronic biomass smoke exposure was on systemic immunity, which can include a skewing of an individual's immune response such that people are more susceptible to infection, atopy, or autoimmunity. The third topic revolved around pregnancy and included both prenatal exposures and fetal development. Within this category were several subcategories that included the following: low birth weight, increased infant mortality rate, abnormal organogenesis, and abnormal immune development. The final major category considered the long-term health implications on cardiovascular disease and hypertension as a result of chronic exposure to biomass smoke. There were two additional health consequences that were discussed in the workgroup, and while they are not necessarily supported by epidemiological data, there is a reasonable concern based on similar areas of health and environmental exposures. These two areas of concern were increased risk of cancer and adverse effects on the central nervous system (CNS).

### Conclusions

The workgroup discussed the use of animal models in studying the health effects of biomass smoke exposure. For the sake of availability, convenience, and practicality the members of the workgroup decided to limit discussion to rodent models only. While all agreed the use of primates would be the most applicable animal model for studying human health effects of biomass smoke exposure, it was felt that these studies are impractical. The panel first divided smoke exposure into two main categories of episodic and chronic. The definitions of these distinctions were such that episodic smoke exposures are typified by communities "briefly" inundated by smoke from forest fires on a seasonal basis, whereas chronic exposures to biomass smoke are typified by individuals in Third World countries who cook over open fires in the home. From these two categories of exposure, the panel then defined the health concerns associated with each type. The discussion then focused on the use of animal models to answer documented, as well as potential, questions regarding health effects associated with the different

types of exposure. The group felt that the best way to discuss the effectiveness of animal models was to consider whether or not there is an available model that researchers could utilize to confidently answer health concerns from public or government agencies (i.e., Centers for Disease Control and Prevention, CDC). Members of the session agreed that there are models available for each health concern listed, but the panel members determined whether they were "confident" or "not confident" in each model. The only health concerns where the panel was not confident of obtaining reliable data from animal models were COPD, emphysema, and potential CNS effects of smoke exposure. Therefore the main conclusion of the IBSHE breakout session on the use of animal models in assessing biomass smoke exposure was that there are current models available that would allow researchers to confidently answer the predominant questions regarding health concerns from such exposures.

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