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Review

In vitro assessment of the toxicity of bushfire emissions: A review

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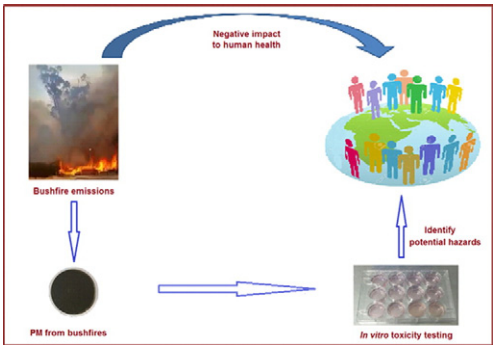
HIGHLIGHTS

- There is a paucity of studies on *in vitro* toxicity of bushfire emissions.
- PM derived from bushfire has been demonstrated to adversely affect cells.
- Experimental factors in *in vitro* toxicity studies of bushfire PM are highly variable.
- Toxicity of whole mixtures of bushfire should be further investigated.
- Effects of fuel types and combustion conditions on bushfire emissions toxicity should be investigated.

GRAPHICAL ABSTRACT



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ABSTRACT

Bushfires produce many toxic pollutants and the smoke has been shown to have negative effects on human health, especially to the respiratory system. Bushfires are predicted to increase in size and frequency, leading to a greater incidence of smoke and impacts. While there are many epidemiological studies of the potential impact on populations, there are few studies using *in vitro* methods to investigate the biological effects of bushfire emissions to better understand its toxicity and significance. This review focused on the literature pertaining to *in vitro* toxicity testing to determine the state of knowledge on current methods and findings on the impacts of bushfire smoke.

There was a considerable variation in the experimental conditions, outcomes and test concentrations used by researchers using *in vitro* methods. Of the studies reviewed, most reported adverse impacts of particulate matter (PM) on cytotoxic and genotoxic responses. Studies on whole smoke were rare. Finer primary particulates from bushfire smoke were generally found to be more toxic than the coarse particulates and the toxicological endpoints of bushfire PM different to ambient PM. However the variation in study designs and experimental conditions made comparisons difficult. This review highlights the need for standard protocols to enable appropriate comparisons between studies to be undertaken including the assessment of physiologically relevant outcomes. Further work is essential to establish the effect of burning different vegetation types and combustion conditions on the toxicity of bushfire emissions to better inform both health and response agencies on the significance of smoke from bushfires.

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1. Introduction

Bushfires, also known as wildfires, vegetation fires or forest fires, along with other types of biomass burning are now considered one of the most significant emission sources of pollutants to the atmosphere (Chen et al., 2007; McMeeking et al., 2009; Vicente et al., 2012). Many studies investigating bushfire emissions have been conducted across the globe, ranging from the countries where bushfires usually occur to countries concerned about long-range transport of pollutants from neighbouring territories (Jalava et al., 2010; Pavagadhi et al., 2013; Sinha et al., 2003; Vicente et al., 2013). Most of these studies have focused on measuring the concentrations of emitted gases and particulate matter (PM) known to have adverse biological effects (Garcia-Hurtado et al., 2014; Junquera et al., 2005; Reisen et al., 2006). There has also been considerable effort devoted to developing pollutant emission factors for individual pollutants for use in models to forecast and predict the potential impact of bushfire smoke on an air-sheds or for population exposure studies (Andreae and Merlet, 2001; Chen et al., 2007; Christian et al., 2003; McMeeking et al., 2009). Other studies have also investigated the effects of bushfire smoke on human health, especially to the respiratory system (Chen et al., 2006; Crabbe, 2012; Henderson and Johnston, 2012; Jalaludin et al., 2000; Johnston et al., 2007; Liu et al., 2014; Morgan et al., 2010). These studies have focused on PM with a number of studies reporting adverse impacts on human health with an increase in the number of hospital admissions during bushfire episodes.

Particulate matter is a major pollutant generated from biomass burning, producing both PM_{10} (particulates with a diameter of less than 10 μm) and $PM_{2.5}$ (particulates with diameter of less than 2.5 μm), where $PM_{2.5}$ accounts for most of the particulate matter generated (Alves et al., 2010a; Bell and Adams, 2008; Garcia-Hurtado et al., 2014). $PM_{2.5}$ has adverse impacts on the human respiratory system and can penetrate deeply into the lungs (Feng et al., 2016; Xing et al., 2016). PM also contains toxicants such as polycyclic aromatic hydrocarbons (PAHs) and metals adsorbed onto its surfaces (Cavanagh et al., 2009; Dieme et al., 2012).

In addition to PM, hundreds of gaseous and volatile chemicals have been identified in vegetation fire smoke (Mobley et al., 1976; Weinhold, 2011). In a study summarizing the emission characteristics of pollutants in biomass burning, Andreae and Merlet (2001) listed more than 90 compounds commonly found in the smoke. Among the compounds are some pollutants that have been demonstrated to have adverse health impacts in epidemiological and *in vivo* experimental studies including carbon monoxide (CO), nitrogen oxide (NOx), sulphur dioxide (SO_2), aldehydes, PAHs and volatile organic compounds (VOCs) (Barboni and Chiaramonti, 2010; Koppmann et al., 2006; Reisen et al., 2011; Sinha et al., 2003).

Although the PM derived from bushfires has been demonstrated to adversely impact human health, it is unclear whether the cause of the impact is the higher concentration of PM *per se*, especially the higher proportion of fine particulates, or if it is a consequence of the changes in chemical composition of bushfire emissions.

Both *in vivo* and *in vitro* approaches have been used to test the toxicity of smoke and PM from bushfire and biomass burning. *In vivo* studies on a range of species (*e.g.* rodents, rabbits, dogs) to determine specific biological end points from exposure to the smoke have been used to predict human toxicity (Dubick et al., 2002; Nieman et al., 1995; Thorning et al., 1982). *In vitro* testing typically involves cultured immortalised cell lines or primary cells (Franzi et al., 2011; Jalava et al., 2012; Leonard et al., 2007; Nakayama Wong et al., 2011; Verma et al., 2009). These *in vitro* approaches have been widely adopted in recent years to identify the potency of inhaled substances (Aufderheide and Scheffler, 2011; Bakand et al., 2006). Owing to the ethical issues associated with *in vivo* toxicity testing, as well as the higher cost and time consuming nature of this work, the use of *in vitro* methods is predicted to increase, particularly the use of cells and tissues derived from humans (U.S. National Research Council, 2007).

With a predicted increase in bushfire frequency, and intensity, due to the effect of climate change (Hughes and Steffan, 2013), there are increasing concerns about the impact of bushfire emissions on population health. There is also a need to understand whether emissions under different combustion conditions and vegetation types results in differing toxicities. The development of *in vitro* toxicity testing is anticipated to meet the demand for a better understanding of the toxic nature of bushfire emissions on human health by making use of different cell types and physiologically relevant outcomes which will serve to inform agencies involved in the prevention and management of human exposures.

To summarize what is already known and to identify the knowledge gaps, we reviewed the literature to explore the current state of knowledge on cells lines, methodological approaches used and the results obtained from *in vitro* studies that have investigated the toxicity of bushfire smoke.

2. Approach and methodology

A literature search was undertaken using various online sources of English journal articles including Google Scholar, ScienceDirect,