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EDITORIAL

BJP BRITISH PHARMACOLOGICAL SOCIETY

BJP recommendations for publishing research on tobacco smoke and environmental tobacco smoke exposure

1 | INTRODUCTION

Smoking costs the global economy more than 1 trillion USD a year and kills annually about 8 million individuals (National Environmental Health Association, 2022; World Health Organization [WHO], 2022a). This mortality and cost are also accompanied by substantial morbidity; this relates not only to that experienced directly by the smoker but also to those exposed to second-hand, environmental tobacco smoke (ETS), manifesting as significant health consequences. Curiously, not everyone exposed to tobacco smoke develops disease. Understanding the health effects of tobacco smoke, the pathogenesis of the tobacco-induced disease, and its prediction and genetic susceptibility requires extensive research.

Several studies have linked specific genetic predilection to disease pathogenesis induced by cigarette smoking (Quaak et al., 2009). In addition, sex as an important experimental variable must be considered (Stanford et al., 2023). Indeed, 13.5% of women in the United States smoked, compared with 17.5% of men in 2016 (Software ALAEaSUuS, 2016). The WHO estimated that in 2020, 36.7% of the world's men and 7.8% of the world's women were tobacco users, predominantly through smoking (WHO, 2022b). Importantly, women share a much larger burden of smoking-related disease and death than their male counterparts. Female smokers are nearly 22 times more likely to die from chronic obstructive pulmonary disease (**COPD**), which includes emphysema and chronic bronchitis, compared with women who have never smoked (National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, 2014).

Because tobacco smoke exposure can profoundly affect multiple organ systems and induce myriad disease states, models using animal exposures, ex vivo systems or human in vitro cells and tissues can aid in understanding the pathophysiology and pharmacology of ETS-induced disease (Ghorani et al., 2017). Decades of research have generated many ETS exposure platforms (Ghorani et al., 2017). Given the magnitude of the problem, the varied experimental approaches and the need for the judicious use of experimental animals in such research, careful scrutiny is necessary to assess the translational value of current approaches. The goal of this editorial is to provide pragmatic guidance to authors who seek to publish their ETS research, using in vivo or in vitro models, in the *British Journal of Pharmacology (BJP)*.

Abbreviations: ALI, air–liquid interface; COPD, chronic obstructive pulmonary disease; CSE, cigarette smoke extract; ETS, environmental tobacco smoke.

Our internal audit of articles published in BJP over the past 75 years shows that cigarette and tobacco exposure articles published in BJP are well-read. Of the 87 publications, on average, these articles were cited approximately 43 times with an h-index of about 31. Twenty-eight of the 87 articles were published in the period 2013–2022, with 28 citations on average and an h-index of 15. Most notably, nine of the 87 papers were cited more than 100 times (Andre et al., 2009; Benwell et al., 1995; de Jonge & Ulloa, 2007; Förstermann & Li, 2011; Grace et al., 2014; Howard et al., 2003; Liu & Zhao, 2004; Lopez et al., 2003; Valjent et al., 2002). Of particular interest, Armitage et al. (1969) was one of the first to describe a method to expose cats to cigarette smoke to study smoke-induced lung pathology. Although many articles embraced translational research methods by studying cells derived from donors who smoked, there were also a wide variety of animal models of smoke exposure. The lack of a standard approach could induce significant experimental variance that potentially compromises the reproducibility and robustness of the data.

Our article is not an exhaustive comparative review of ETS models but a distillation of the consensus views of our Editorial Board regarding current practice in this research field. It is worth noting at this point that the BJP welcomes articles describing novel models to study the negative consequences of smoking, studies delineating mechanisms of disease progression as indicators of targets for treatments and those describing novel approaches to mitigate the consequences of smoking. Such studies will be considered on scientific merit (which includes ethical justification) and relevance to pharmacology and drug development of new treatments for smoke-induced diseases such as COPD and complications of cardiovascular conditions (BJP, 2023).

2 | RECOMMENDATIONS REGARDING IN VIVO TOBACCO EXPOSURE MODELS

2.1 | Animal models of ETS

The study of ETS in animals has involved a wide diversity of species and methods. Additionally, ETS exposure methods, doses and times have been highly variable. Much of the research effort has focused on the pathogenesis of ETS-induced COPD (emphysema and chronic bronchitis). Ghorani et al. (2017) provided a rigorous review of comparative ETS animal models in the study of airway diseases. Although

some of the BJP recommendations are based on this ETS-induced lung disease, the consequences of ETS on other organ systems should be viewed from the standpoint of inhalational ETS that most closely resembles human exposure.

As with any animal model of disease pathogenesis or therapy, such platforms should embrace the concepts of translation, precision, reproducibility and vertebrate animal protection (Curtis et al., 2022; Lilley et al., 2020) while simultaneously recognizing the limitations of any chosen method. Experimental parameters that should be considered include smoke source, species, duration and dose of exposure, method of exposure and specific outcomes (Ghorani et al., 2017). Several species have been used in models of smoke exposure, including mice, rats, rabbits, guinea pigs and non-human primates. To date, no animal model has been identified as an ideal species to use for simulation of the consequences of ETS-induced disease in humans. Regarding airways diseases, rodent ETS models offer advantages of a small body size, short reproduction cycles and genomic similarities to humans and the opportunity for genetic manipulation. Limitations of rodent models include the lack of extensive bronchial branching, intratracheal cilia and a bronchial circulation plus the paucity of submucosal glands in the airways. Dogs, pigs and non-human primate models have advantages over rodent models as their size and anatomy are more akin to humans. However, the ability to genetically alter the animals and the long reproductive cycle and husbandry costs limit their utility (Ghorani et al., 2017). Substantial ethical issues abound in the use of protected species for cigarette and smoke exposure which requires investigators to seek alternative approaches. Unless there exists a compelling rationale, rodent models are the preferred animal model for ETS exposure. Further, all studies should address sex and age as experimental variables and the humane use of animals as governed by the institutional boards for animal welfare and other relevant regulatory bodies having oversight of work with experimental animals (Stanford et al., 2023).

2.2 | ETS delivery and dosing techniques

Because ETS contains thousands of chemicals including 43 known carcinogens, modelling animal exposure should encompass smoke exposure of animals with a similar complex composition (Prieux et al., 2020). The use of 'standard' cigarettes for the exposure source can enhance the reproducibility of the work by others. Typically, standardized research-grade cigarettes deliver a specified dose of total suspended particles/total particulate matter and units/volume for nicotine and carbon monoxide generation. Standard cigarettes may be filtered or unfiltered to mimic real world exposures. A common research cigarette used in ETS exposure models in the United States has been formulated by the University of Kentucky (Leberl et al., 2013); globally, research cigarettes are also available from several other sources (Roemer et al., 2012). When possible, authors should use commonly standardized and accepted approaches in the generation of smoke and justify their approach. Rigour and reproducibility of their approach to smoke and particle generation should also be stated in the methods (Curtis et al., 2022; Lilley et al., 2020).

It is now accepted that chronic ETS exposure induces human disease (National Environmental Health Association, 2022; WHO, 2022a). Accordingly, the duration and dose of the ETS exposure in animals represents a critical variable in studying pathogenesis. Interestingly, unlike COPD in which some patients' disease progresses despite smoking cessation, animal exposure to ETS typically induces mild emphysema that does not worsen after cessation of ETS exposure (Ghorani et al., 2017; Krimmer & Oliver, 2011). Conceptually, ETS exposure in animals mimics some but not all aspects of ETS-induced disease in humans. To date, there is little consensus or standards on the dose and duration of ETS that should be used in animal studies. For reproducibility and rigour, authors must detail the smoke source and the dose (puffs or total smoke exposure) and duration by day, week and month. Additionally, given the chronic nature of ETS consequences on human health, animals studies should involve an intermittent ETS exposure for a minimum of 3 months with a preferred duration of 6 months, balancing study time with cost of husbandry and so on (Ghorani et al., 2017). Deviations from the minimum of 3-month protocols should be justified in the methods.

As with variability in the approaches to dosing and duration of ETS exposure in animals, the mode of exposure used for smoking studies also varies. Current methods use whole body exposure in unrestrained animals or head/nose-only inhalational systems with restraints. Advantages and limitations exist for both methods. Whole body exposure in unrestrained animals is likely to diminish an animal's stress response and the hyperventilation that can occur with animal restraint models. Unfortunately, the unrestrained animal can ingest ETS from its topical deposition or after self-cleansing. Furthermore, the actual respirable dose of ETS is unclear. The nose-only approach provides greater precision in dosing with diminished 'second-hand' ETS exposure, by avoidance of topical ingestion. However, the repetitive stress on the restrained animal may complicate the interpretation of the data. Undoubtedly, the restrained animals will express a stress response (Panin et al., 2014; Perhach & Barry, 1970). Accordingly, non-exposed animals that experience similar stress should serve as comparators to understand the effects of ETS exposure.

Because few comparative efficacy studies exist regarding the delivery of ETS to animals, authors should clearly detail their approach and if possible, refer to previous comparable studies (Lilley et al., 2020). Regardless of the models chosen for ETS exposure in animals, specific outcomes of the exposure should be studied and correlated with those of ETS-induced human diseases. Numerous outcomes have been assessed after smoke exposure. These include lung and airway pathology, lung function measurements, organ-specific or systemic inflammation, cardiovascular consequences such as pulmonary hypertension, systemic effects on weight gain and growth and lung radiographic imaging. Each outcome can be informative, but composite approaches are likely to be the most valuable in demonstrating the rigour and reproducibility of the exposure. Accordingly, a clear description of the numbers of technical and biological replicates should be provided with an appropriate statistical analysis (Curtis et al., 2022; Krimmer & Oliver, 2011; Lilley et al., 2020). In most instances, ETS-exposed animals should be compared with those

that are sham exposed. This latter approach provides an important control to help mitigate the influence of restraint.

3 | RECOMMENDATIONS REGARDING IN VITRO TOBACCO EXPOSURE MODELS

3.1 | Replacement of in vivo studies

In many instances, a reductionist approach can be used to understand the effect of ETS on human disease. In vitro and ex vivo models using ETS exposure can address molecular mechanisms and pharmacological outcomes in animal-derived or ideally human cell and tissue models. Such approaches foster consideration of non-animal alternative models. These models have exploited cigarette smoke extract (CSE) or lateral-flow ETS approaches using specialized incubator or delivery systems. Lateral-flow approaches enable the animal to inhale ETS via a passive inhalation and not by a tube with direct delivery into the lungs. Specific challenges exist in the use of ETS exposure of in vitro or ex vivo models. Benefits and limitations exist, and authors should identify these attributes of their models.

3.2 | Alternatives to smoke

ETS represents a complex mixture of toxicants and particulates that are inhaled or topically deposited. Because aerosol delivery of ETS in vitro to cells and tissue is complex and can require sophisticated and costly experimental systems, alternative exposure methods using CSE have been developed (Krimmer & Oliver, 2011). To use CSE, investigators either generate their own moiety or purchase commercially available CSE and then expose cells and/or tissue to the aqueous mixture. Levels of inflammatory mediators or altered cellular function can then be measured.

Although this approach obviates the need to deliver ETS by lateral flow or aerosol, the pharmacological and physiological relevance of this type of exposure remains unclear. In most instances, the derivation of the CSE is not standardized and may differ among laboratories, thereby affecting the reproducibility and rigour of the approach. The aqueous exposure of the cells to CSE is markedly disparate from that of aerosolized delivered ETS exposures and may also complicate the interpretation of the data. CSE represents a fundamentally different formulation from ETS derived from a burning cigarette and, therefore, investigators may underestimate or overestimate cellular responses to the toxicant. Few studies have directly compared CSE to aerosol-delivered ETS in the modulation of in vitro cell or tissue function. Currently, state-of-the-art exposure chambers exist that can reproducibly expose cells or tissue to ETS that is derived from burning cigarettes. These instruments control for lateral-flow rates, puff velocity and frequency and, accordingly, mimic in vivo conditions. Given the availability of refined ETS exposure techniques, authors should avoid the use of CSE in characterizing the effects of ETS exposure using in vitro or ex vivo models. Only under some circumstances (e.g., when CSE is the only possible exposure) will the BJP consider manuscripts that solely use CSE as a surrogate for ETS exposure.

3.3 | Optimal cell systems

Because ETS exposure is likely to modulate the cellular function in a variety of cells in complex tissue and organs, studies should be conducted with functionally relevant cells. In the case of epithelial cells, which in most cases serve as the primary defence against toxicant exposure, in vitro responses to ETS should preferably be studied in fully differentiated epithelial cells (Upadhyay & Palmberg, 2018). In airways of the lung, air–liquid interface (ALI)-differentiated cells have an architecture that is akin to in vivo conditions (Upadhyay & Palmberg, 2018). Submerged airway epithelium cultures typically manifest functions that are fundamentally different from those that are ALI-differentiated. If studying cellular function in culture, authors are encouraged to justify the model and use differentiated cells to model in vivo conditions.

Related to most in vitro pharmacological studies, dose/concentration–response and kinetics of response are critical in the evaluation of the quality of the work (Curtis et al., 2022; Lilley et al., 2020). Rigour and reproducibility are required in showing a dose/concentration and time dependency of the cellular effects after exposure to ETS. The dose or concentration of the exposure should be physiologically and pharmacologically relevant and justified by experimental data or previous studies (Curtis et al., 2022). A clear description of the numbers of technical and biological replicates should be provided with an appropriate statistical analysis. In most instances, ETS-exposed cells, tissue, etc. should be compared with those that are sham exposed. The demographics of the donors from which the cells were harvested, including any medications, should be provided with considerations of sex as a biological variable.

4 | SUMMARY

Smoking and ETS evoke profound global morbidity and mortality. Heterogeneity in human responses exists regarding the health consequences of ETS. Current research efforts have been challenged to understand the pathogenesis of ETS-induced diseases. The BJP is committed to publishing the highest quality pharmacological studies that focus on the effects of ETS in human health. To that end, we regularly provide guidelines that will enhance the likelihood that authors producing such manuscripts will improve the quality of the work published in BJP. We anticipate their studies will meet the rigour, quality and reproducibility sought by the scientific community. Following the guidelines produced by the Editorial Board will improve overall research quality and the impact of the important work published in BJP that is dedicated to understanding fundamental and translational mechanisms by which ETS exposure affects human health.

5 | LIST OF RECOMMENDATIONS

1. For in vivo studies
   a. The provenance and composition of the 'standardized' cigarettes used for smoke inhalation should be detailed and freely available.
   b. Due consideration of age, species and sex must be provided in the experimental protocol and justification.
   c. Full details of exposure rates must be provided and be relevant to the human setting.
2. For in vitro studies
   a. The provenance and composition of the 'standardized' CSE used must be detailed and the source/supply freely available
   b. For primary cell work, relevant cell types should be used and full demographics of the donor provided including age, sex and any known diseases.
   c. Full details of exposure rates must be provided and be relevant to the human setting.

KEYWORDS

chronic obstructive lung disease and cancer, cigarettes, guidelines, requirements, smoking

CONFLICT OF INTEREST STATEMENT

The author declares no conflict of interest.

Reynold A. Panettieri Jr[1]
S. Clare Stanford[2]
Steve Alexander[3]
Giuseppe Cirino[4]
Christopher H. George[5]
Paul A. Insel[6]
Dave Kendall[3]
Yong Ji[7]
Hemal H. Patel[8]
Christopher G. Sobey[9]
Barbara Stefanska[10]
Gary Stephens[11]
Mauro Teixeira[12]
Nathalie Vergnolle[13]
Péter Ferdinandy[14,15]
Amrita Ahluwalia[16]

[1]*Rutgers University, New Brunswick, New Jersey, USA*
[2]*University College London, London, UK*
[3]*University of Nottingham, Nottingham, UK*
[4]*University of Naples Federico II, Naples, Italy*
[5]*Swansea University, Swansea, UK*
[6]*University of California/San Diego, La Jolla, California, USA*
[7]*Nanjing Medical University, Nanjing, China*
[8]*VA San Diego Healthcare System and University of California/San Diego, San Diego, California, USA*
[9]*La Trobe University, Melbourne, Victoria, Australia*
[10]*University of British Columbia, Vancouver, British Columbia, Canada*
[11]*University of Reading, Reading, UK*
[12]*Federal University of Minas Gerais, Belo Horizonte, Brazil*
[13]*INSERM, Paris, France*
[14]*Semmelweis University, Budapest, Hungary*
[15]*Pharmahungary Group, Budapest, Hungary*
[16]*Queen Mary University of London, London, UK*

Correspondence
Reynold A. Panettieri Jr., Rutgers Univesity, CHI Building, 89 French St, Suite 4210, New Brunswick, NJ 08901
Email: rp856@rbhs.rutgers.edu

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