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Article

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Editorial

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

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Introduction

Smoking costs the global economy more than $1 trillion a year (USD) and kills annually about 8 million individuals (1,2). This mortality and cost is also accompanied by substantial morbidity that relates not only to that experienced directly by the smoker, but also to those exposed to secondhand 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 tobacco-induced disease and its prediction and genetic susceptibility requires extensive research. Examples of the latter are underway and an excellent example of this is the TRACERx project ()

Since 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 understanding the pathophysiology and pharmacology of ETS-induced disease (3). Decades of research has generated many ETS exposure platforms (3). Given the magnitude of the problem, the varied experimental approaches, and the need for the judicious use of resources (animal-based models), scrutiny is necessary to assess the translational value of current approaches. The goal of this editorial is to provide pragmatic and best practice guidance to authors who seek to publish their ETS research using *in vivo* or *in vitro* models in the British *Journal of Pharmacology*. Our internal audit of articles published in BJP over the past 10 years show……

Our article is not an exhaustive comparative review of ETS models but a distillation of the consensus views of our Editorial board regarding current best practice in this arena. 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 and those describing novel approaches to mitigate against the consequences of smoking. Conversely BJP will not publish studies testing novel smoking/tobacco products aside from studies addressing the negative consequences of smoking on health. No studies sponsored by tobacco manufacturers are considered for publication (ref).

Recommendations regarding *in vivo* tobacco exposure models:

Animal models of ETS: The study of ETS in animals has involved a wide diversity of species and methods. Using sheep, dogs, pigs, rabbits, monkeys, guinea pigs, rats, and mice (3). Additionally, ETS exposure methods, doses and times have been highly variable. Much of the research effort has focused on the pathogenesis of ETS-induced obstructive lung disease (emphysema and chronic bronchitis). Ghorani and others provided a rigorous review of comparative ETS animal models in the study of airway diseases (3). 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 models of disease pathogenesis or therapy, such platforms should embrace the concepts of translation, precision, reproducibility, and vertebrate animal protection (4,5) whilst 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 (3). Several species have been used in models of smoke exposure (e.g. mice, rats guinea pigs, 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 (3). Unless there exists a compelling rationale, rodent models are the preferred animal model for ETS exposure. Further, all studies should address sex as an experimental variable and the humane use of animals as governed by the institutional boards for animal welfare (4,6). Indeed, 13.5% of women in the U.S. smoked, compared to 17.5% of men in 2016 (10). Today, with a much smaller gap between men's and women's smoking rates than in the past, women share a much larger burden of smoking-related disease and death. Female smokers are nearly 22 times more likely to die from chronic obstructive pulmonary disease (COPD), which includes emphysema and chronic bronchitis, compared to women who have never smoked (11).

ETS Delivery and Dosing Techniques: Since ETS contains thousands of chemicals including 43 known carcinogens, modeling animal exposure should encompass smoke exposure of animals with a similar complex composition. 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 levels 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 is formulated by the University of Kentucky (7). When possible, authors should use commonly standardized and accepted approaches in the generation of smoke and justify their approach. Rigor and reproducibility of their approach to smoke and particle generation should also be stated in the methods (4,5).

Current evidence suggests that chronic ETS exposures induces human disease (1,2). Accordingly, the duration and dose of the ETS exposure in animals represents a critical variable in studying pathogenesis. Interestingly, unlike chronic obstructive pulmonary disease 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 (3,9). Conceptually, ETS exposure in animals only 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 rigor, 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 studies, animals studies should involve a ETS exposure for a minimum of three months with a preferred duration of 6 months. Deviations from the 3- or 6-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 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 likely diminishes an animal's stress response and the hyperventilation that can occur with animal restraint models. Unfortunately, the unstrained animal is exposed to ETS can ingest ETS from its topical deposition or after self-cleansing. Further, the actual respirable dose of ETS is unclear. The nose-only approach provides greater precision in dosing with diminished "secondhand" 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. Accordingly, non-exposed animals exposed to similar stress should serve as comparators to understand the effects of ETS exposure.

Since few comparative efficacy studies exist regarding the best practice for ETS delivery to animals, authors should clearly detail their approach and if possible, reference previous comparable studies (4).

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 most valuable in demonstrating the rigor 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 (4,5). In most instances, ETS-exposed animals should be compared with those that are sham-exposed.

Recommendations regarding *in vitro* tobacco exposure models:

In many instances, a reductionist approach is needed 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 human- or animal-derived cell and tissue models. These models have exploited cigarette smoke extract (CSE) or lateral-flow ETS approaches using specialized incubator/delivery systems. 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.

ETS represents a complex mixture of toxicants and particulates that are inhaled or topically deposited. Since 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 (9). 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 are then measured. Although the

approach obviates the need to deliver ETS by lateral flow/aerosol, the pharmacological and physiological relevance of the exposure remains unclear. In most instances, the derivation of the CSE is not standardized and may differ among laboratories thereby impacting on the reproducibility and rigor 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; 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 special circumstances will the BJP consider manuscripts that solely use CSE as a surrogate for ETS exposure

Since ETS exposure likely modulates 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 defense against toxicant exposure, *in vitro* responses to ETS should preferably be studied in fully differentiated epithelial cells (8). In airways of the lung, air-liquid interface (ALI)-differentiated cells have an architecture that is akin to *in vivo* conditions (8). 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 (4,5). Rigor 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 (5). A clear description of the numbers of technical and biological replicates should be provided with a 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 should be provided with considerations of sex as a biological variable.

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-disease. BJP is committed to publishing the highest quality of pharmacological studies that focus on the effects of ETS in human health. To that end we provide guidelines that will enhance the likelihood that authors producing such manuscripts will publish their work in the Journal. We anticipate their studies will meet the rigor,

quality and reproducibility sought by the scientific community that is dedicated to understanding fundamental and translational mechanisms by which ETS exposure impacts on human health.

References:

1. National Environmental Health Association. [(accessed on 15 May 2022)]. Available online: https://www.neha.org/

2. World Health Organization. [(accessed on 15 May 2022)]. Available online: https://www.who.int/

3. Ghorani, V., Boskabady, M.H., Khazdair, M.R. *et al.* Experimental animal models for COPD: a methodological review. *Tob. Induced Dis.* **15,** 25 (2017). https://doi.org/10.1186/s12971-017-0130-2.

4. Lilley E, Stanford SC, Kendall DE, Alexander SPH, Cirino G, Docherty JR, George CH, Insel PA, Izzo AA, Ji Y, Panettieri RA, Sobey CG, Stefanska B, Stephens G, Teixeira M, Ahluwalia A. ARRIVE 2.0 and the British Journal of Pharmacology: Updated guidance for 2020. Br J Pharmacol. 2020 Aug;177(16):3611-3616. doi: 10.1111/bph.15178. Epub 2020 Jul 14. PMID: 32662875; PMCID: PMC7393193.

5. Curtis MJ, Alexander SPH, Cirino G, George CH, Kendall DA, Insel PA, Izzo AA, Ji Y, Panettieri RA, Patel HH, Sobey CG, Stanford SC, Stanley P, Stefanska B, Stephens GJ, Teixeira MM, Vergnolle N, Ahluwalia A. Planning experiments: Updated guidance on experimental design and analysis and their reporting III. Br J Pharmacol. 2022 Aug;179(15):3907-3913. doi: 10.1111/bph.15868. Epub 2022 Jun 7. PMID: 35673806.

6. Karp, N. A., and Reavey, N. Sex bias in preclinical research and an exploration of how to change the status quo. Br J of Pharmacol. 2019; 176: 4107– 4118. doi:10.1111/bph.14539.

7. Leberl M, Kratzer A, Taraseviciene-Stewart L. Tobacco smoke induced COPD/emphysema in the animal model-are we all on the same page? Front Physiol. 2013 May 15;4:91. doi: 10.3389/fphys.2013.00091. PMID: 23720629; PMCID: PMC3654205.

8. Upadhyay S, Palmberg L. Air-Liquid Interface: Relevant In Vitro Models for Investigating Air Pollutant-Induced Pulmonary Toxicity. Toxicol Sci. 2018 Jul 1;164(1):21-30. doi: 10.1093/toxsci/kfy053. PMID: 29534242.

9. Krimmer DI, Oliver BG. What can in vitro models of COPD tell us? Pulm Pharmacol Ther. 2011 Oct;24(5):471-7. doi: 10.1016/j.pupt.2010.12.002. Epub 2010 Dec 21. PMID: 21182977.

10. Centers for Disease Control and Prevention. National Center for Health Statistics. National Health Interview Survey, 2016. Analysis performed by the American Lung Association Epidemiology and Statistics Unit using SPSS software

11. U.S. Department of Health and Human Services. The Health Consequences of Smoking—50 Years of Progress: A Report of the Surgeon General, 2014.