Animal Research Review Panel Guideline

Smoke inhalation procedures in rodents

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1. What is a smoke inhalation procedure?

Smoke inhalation procedures are respiratory inhalation models that expose animals to inhaled substances.

There are two approaches:

- Nose-only (also called direct or mainstream) exposure, which employs a purpose-built apparatus in which rodents are tightly and individually restrained while smoke is delivered directly to their nose/head, mimicking direct first-hand smoking.
- Whole-body (indirect or sidestream) exposure, where animals are placed in a chamber into which smoke is introduced however the animals are not restrained.

The species commonly used are mice and sometimes rats.

2. Why are smoke inhalation procedures used?

Smoke inhalation procedures, and specifically those using cigarette smoke, are used to study respiratory conditions such as chronic obstructive pulmonary disease (COPD), emphysema (Ghorani et al., 2017) and lung cancer (Kameyama et al., 2018), as well as the relationship between cigarette smoking and other conditions such as gastrointestinal disease (Fricker et al.)[1]

3. What is the debate about smoke inhalation procedures?

While the importance of studying clinical conditions such as COPD is not in question, the use of cigarette smoke exposure in rodents, and most specifically the use of the nose-only approach, is the subject of debate in the scientific community. This includes concerns about:

- the ethical and welfare implications of smoke inhalation procedures,
- a lack of experimental standardisation in dosing, length and method of exposure,

[1] The adverse health effects linked directly to cigarette smoke include a range of pulmonary diseases (including airway and alveolar damage and lung cancer) and diverse non-pulmonary health effects including age-related macular degeneration, diabetes, cancer, arthritis, inflammation, and impaired immune function. *(2014. The Health Consequences of Smoking: 50 Years of Progress. A Report of the Surgeon General. In: SERVICES, U. S. D. O. H. A. H. (ed.). Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health..*

- variability in response between strains of rodents, and
- inherent limitations of animal models meaning they are only able to mimic some features of disease, making translation of data to the human condition complex (Ghorani et al., 2017, Jones et al., 2017, Tanner and Single, 2020, Fröhlich, 2021).

4. What are the ethical and welfare implications of smoke inhalation procedures?

The ethical and welfare implications of smoke inhalation procedures, which are fundamental requirements for consideration under the *Australian code for the care and use of animals for scientific purposes* (the Code) (NHMRC, 2013 Updated 2021), are contentious in both the scientific and broader community.

Both nose-only and whole-body procedures expose the animals to the direct risk of irritation to the eyes and respiratory tract and of course the chronic development of diseases such as COPD.

Nose-only exposure is considered to have a significant animal welfare impact because of the risk of injury or mortality while undergoing the procedure, demonstrated stress caused by restraint and isolation, and the potential for hypothermia and weight loss (van Eijl et al., 2006).

Whole-body exposure alleviates the impact of restraint stress on the animals but has limitations in that it may expose animals to uptake by other routes than inhalation (e.g oral route via grooming or dermal route) and is less controllable (and therefore less reproducible) (Shu et al., 2017, OECD, 2018). Some whole-body apparatus allows animals to be group housed which alleviates the impact of isolation as well as that of restraint.

A key question being asked is "*can whole body smoke exposure produce a comparable disease model to nose-only exposure*?" Consensus in the literature indicates that similar endpoints can be produced using both approaches, with the primary difference being nose-only exposure increases the severity of changes and requires slightly shorter time frames (Shu et al., 2017, Leberl et al., 2013, Tanner and Single, 2020).

Smoke exposure procedures are also the focus of growing public concern (see for example Humane Research Australia report regarding the procedure "Optimising inhalation research: Transitioning to human-relevant science" October, 2021).

5. What are the alternatives to smoke inhalation procedures?

The Code requires that procedures that avoid the use of animals be sought and implemented wherever possible.

Alternative methods of exposure to the components of cigarette smoke could be considered, for example administration of a cigarette smoke solution intranasally (Ueha et al., 2020). There has been significant development in sophisticated methods to recapitulate *in vivo* human lung conditions with non-animal alternatives applicable to respiratory disease research, including *in vitro* 2D and 3D cultures, *ex vivo* tissue culture, organoids, lung-on-a-chip, precision cut lung slice (PCLS) models and *in silico* and mathematical approaches (Hynes et al., 2020, Fröhlich, 2021). If not used to completely replace animal models, the advances obtained using cellular models and *in silico* techniques should be considered by researchers to reduce the extent of animal use.

6. What does this debate mean for researchers and Animal Ethics Committees (AECs)?

The scientific literature supports fundamental advances in knowledge of COPD pathophysiology and other diseases having been made with the use of smoke inhalation procedures using cigarette smoke in rodent models, however, the nature of the experiments has generated significant public concern and scrutiny, with the expectation that the value of the research must be demonstrated if it is to be approved by AECs.

The Animal Research Review Panel (the Panel) considers smoke inhalation procedures to have a high negative impact on the welfare of animals due to both the acute and cumulative effects of smoke exposure, and in the case of nose-only exposure, repeated episodes of restraint induced stress.

The Panel recommends that use of the nose-only procedure be approved by exception only, and requires that the procedure be classified as **Procedure Category P7: Major Physiological Challenge** (see Guidance on completing Form L). This means AECs must provide detailed information in their annual reports to their establishments on the approval of these procedures. The information should include measures implemented to reduce the number of projects approved, the number of animals used, and ongoing refinements to the procedures. (See ARRP Policy 5: Annual reporting by Animal Ethics Committees to accredited animal research establishments).

The Panel will monitor the use of the nose-only procedure and if deemed necessary, may recommend that conditions regarding the use of smoke inhalation procedures are placed on an establishment's accreditation. The Panel's ongoing review will include consideration of further regulatory requirements.

AECs must therefore carefully consider applications by researchers to use smoke inhalation procedures, and only approve nose-only procedures by exception. Issues to consider include:

Justification

- Have the researchers ascribed the model and protocols to the particular phenotype of disease they wish to study, with the exact animal model and time points selected according to the question(s) to be addressed?
- If proposing to use nose-only exposure, what is the researchers' justification to use this over alternative exposure methods, noting that a shorter timeframe using the nose-only method is not a reasonable justification.
- Does the AEC require investigators to undertake a pilot study supported by a systematic literature review to confirm they cannot achieve the ascribed minimum required phenotype with alternative exposure methods such as whole-body or intranasal administration?
- If the outcome sought is a reduction in pathological features induced by treatment, can the researchers determine (and report) this by reference to an appropriate control group that does not require marked severity of pathology?
- Has the strain of mouse been considered noting they have different susceptibly to smoke exposure?
- Have the researchers assessed and explained the cumulative impact of the study over the course of the animals life?

Validation

- What rigour is being used to ensure scientific consistency of the exposure protocol (precision and control over smoke delivery?) For example, has the aerosol been calibrated so that dose rate in mg/kg is known with chamber concentration and conditions controlled and adequately maintained? Has this been confirmed prior to animal exposure in the chamber?
- Do researchers have evidence to confirm the model replicates the hallmark lung features of the disease they are studying?
- Are serum cotinine levels in the mice being measured to confirm the relative amount of smoke exposure?

Refinement

- Have the animals undergone training and acclimatisation to the apparatus and procedure?
- Is there monitoring of air temperature and provision of heat for mice during recovery?
- Have the study endpoints and refinements been adapted by the researchers to use the minimum amount of smoke exposure and restraint. For example, once-a-day dosing may reduce the handling and stress compared to multiple interventions in one day and thermoregulation will be less impaired by exposure (Eijl et al 2006). A shorter time frame (12 weeks vs. 6 months (Ghorani et al., 2017) may still induce the required phenotype.
- Are there detailed monitoring protocols for signs of both acute and chronic toxicity and pain, assessing for immediate (during and after smoke exposure) and cumulative harm. This would include assessment for responses consistent with acute pain (as defined by the Mouse Grimace Scale), monitoring of body temperature, body weight, food consumption and hydration status?
- Have minimum requirements been determined for monitoring animals during the procedure noting the limited access to animals when restrained within nose-only exposure apparatus?
- Is there a protocol for administration of pain relief if the animal is in pain or distressed?
- Is there routine application of ophthalmic gel to protect the eyes?

Reporting

- AECs should request an increased frequency above that of regular annual reporting to the AEC on number of mice used, mortality rates, adverse events, investigations and on-going refinement measures.
- AECs should inspect these procedures regularly to ensure adherence to approved projects.

Further guidance on matters to consider are in Animal Research Review Panel Guideline 24: Consideration of high impact research projects by Animal Ethics Committees.

Reference documents

2014. The Health Consequences of Smoking: 50 Years of Progress. A Report of the Surgeon General. *In:* SERVICES, U. S. D. O. H. A. H. (ed.). Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health.

FRICKER, M., GOGGINS, B. J., MATEER, S., JONES, B., KIM, R. Y., GELLATLY, S. L., JARNICKI, A. G., POWELL, N., OLIVER, B. G., RADFORD-SMITH, G., TALLEY, N. J., WALKER, M. M., KEELY, S. & HANSBRO, P. M. 2018. Chronic cigarette smoke exposure induces systemic hypoxia that drives intestinal dysfunction. *JCI Insight,* 3**,** e94040.

FRÖHLICH, E. 2021. Replacement Strategies for Animal Studies in Inhalation Testing. *Sci,* 3**,** 45.

GHORANI, V., BOSKABADY, M. H., KHAZDAIR, M. R. & KIANMEHER, M. 2017. Experimental animal models for COPD: a methodological review. *Tobacco Induced Diseases,* 15**,** 25.

HYNES, J., MARSHALL, L., ADCOCK, I., NOVOTNY, T., NIC, M., DIBUSZ, K. & GRIBALDO, L. 2020. Advanced Non-animal Models in Biomedical Research: Respiratory Tract Diseases,. Luxembourg: Publications Office of the European Union.

JONES, B., DONOVAN, C., LIU, G., GOMEZ, H. M., CHIMANKAR, V., HARRISON, C. L., WIEGMAN, C. H., ADCOCK, I. M., KNIGHT, D. A., HIROTA, J. A. & HANSBRO, P. M. 2017. Animal models of COPD: What do they tell us? *Respirology,* 22**,** 21-32.

KAMEYAMA, N., CHUBACHI, S., HEGAB, A. E., YASUDA, H., KAGAWA, S., TSUTSUMI, A., FUKUNAGA, K., SHIMODA, M., KANAI, Y., SOEJIMA, K. & BETSUYAKU, T. 2018. Intermittent Exposure to Cigarette Smoke Increases Lung Tumors and the Severity of Emphysema More than Continuous Exposure. *American Journal of Respiratory Cell and Molecular Biology,* 59**,** 179-188.

LEBERL, M., KRATZER, A. & TARASEVICIENE-STEWART, L. 2013. Tobacco smoke induced COPD/emphysema in the animal model—are we all on the same page? *Frontiers in Physiology,* 4.

NHMRC 2013 Updated 2021. Australian code for the care and use of animals for scientific purposes. 8th ed. Canberra: National Health and Medical Research Council.

OECD 2018. Guidance Document on Acute Inhalation Toxicity Testing. *Series on Testing and Assessment No 39.* Second ed. Paris, France: Organisation for Economic Co-operation and Development.

SHU, J., LI, D., OUYANG, H., HUANG, J., LONG, Z., LIANG, Z., CHEN, Y., CHEN, Y., ZHENG, Q., KUANG, M., TANG, H., WANG, J. & LU, W. 2017. Comparison and evaluation of two different methods to establish the cigarette smoke exposure mouse model of COPD. *Scientific Reports,* 7**,** 15454.

TANNER, L. & SINGLE, A. B. 2020. Animal Models Reflecting Chronic Obstructive Pulmonary Disease and Related Respiratory Disorders: Translating Pre-Clinical Data into Clinical Relevance. *Journal of Innate Immunity,* 12**,** 203-225.

UEHA, R., UEHA, S., KONDO, K., NISHIJIMA, H. & YAMASOBA, T. 2020. Effects of Cigarette Smoke on the Nasal Respiratory and Olfactory Mucosa in Allergic Rhinitis Mice. *Frontiers in Neuroscience,* 14.

VAN EIJL, S., VAN OORSCHOT, R., OLIVIER, B., NIJKAMP, F. P. & BLOKSMA, N. 2006. Stress and hypothermia in mice in a nose-only cigarette smoke exposure system. *Inhalation Toxicology,* 18**,** 911-8.

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