

Refinement and Reduction in the Production of Genetically Modified Mice: Sixth Report of the BVAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement

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Summary — This paper introduces the sixth report of the BVAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement (JWGR), which addresses reduction and refinement in the generation, management and care of genetically modified (GM) mice. The report provides a comprehensive review of the potential for reduction and refinement at all stages of the generation and maintenance of GM animals. The issues covered include scientific, procedural and practical issues. The need for good training in all of these areas is emphasised. The report concludes with a section on the need to develop comprehensive criteria for welfare assessments for GM mice and a preliminary list of criteria is provided. The full report has been published in *Laboratory Animals*.⁴

Key words: genetic manipulation, GM, reduction, refinement, welfare assessment.

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Introduction

The BVAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement (JWGR) was set up in 1989, to define ways husbandry and procedures could be refined to reduce suffering and improve animal welfare, and to publish and disseminate information to develop “best practice”. The JWGR works through a series of individual working groups set up to address specific issues. Each working group comprises experts in the field under discussion, including scientists from academia, industry and animal welfare organisations, veterinarians, animal technicians and others with relevant experience.

Topics that have been addressed and published to date are: blood sampling (1), rabbit, mouse, and bird husbandry (2–4), the administration of substances (5), dog husbandry and care (6), and telemetry (7). The report described in this presentation is the sixth report in the series and applies the principles of both reduction and refinement to the generation, management and care of genetically modified (GM) mice (8). This was chosen as a topic for consideration because:

- of the escalation in numbers of GM mice being generated, maintained and used;
- the technology is inherently wasteful of animals;
- there are predicted and unpredicted adverse effects that can cause animal suffering; and
- many of the people involved in this research are new to *in vivo* animal work and are unfamiliar with the broad principles of laboratory animal care and welfare, and with the Three Rs.

The full report has been published in *Laboratory Animals*, so only a very brief summary of its content is given here. The members of the GM Working Group are listed in (8).

It is hoped that by raising the issues, options and opportunities for reduction and refinement, current best practice for GM mice will be disseminated and greater attention will be focused on the animal welfare implications associated with transgenic technologies.

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Content of the Report

The report provides a comprehensive review of the potential for reduction and refinement at all stages of the generation and maintenance of GM animals. The issues covered include scientific, procedural and practical issues. The need for good training in all of these areas is emphasised.

With respect to the scientific issues, the Working Group focused on the two most commonly used methods of producing GM mice, namely pronuclear microinjection and gene targeting in embryonic stem (ES) cells. The importance of careful consideration of all the factors affecting the design, expression and transmission of the transgene is discussed, including the choice of animal and technique, the design of the construct and the ES cell culture. A key objective is to improve the efficiency of the genetic modification process so as to reduce animal wastage, and hence, the overall number of animals used. The most important point, however, is to always check that the desired GM animal is not already available elsewhere.

In reviewing procedures, the Working Group has taken a comprehensive approach, encompassing all of the procedures involved, including superovulation, vasectomy, embryo transfer, ovary transplant, rederivation and phenotype assessment and the collection of biopsies for genotyping and identification. Each of these procedures is examined in turn; potential problems are identified and recommendations for "best practice" proposed. For example, potential problems associated with superovulation are suffering resulting from intraperitoneal injection of hormones and injury to young females during mating. The aim must be to maximise egg production (thus minimising the numbers of females used) whilst minimising harm to these females. Refinements in this respect require careful attention to injection technique, optimisation of the size and weight of females, careful monitoring for injury and careful selection and monitoring of stud males. Similarly, for embryo transfer, the goal is to optimise embryo transfer and survival and as a consequence reduce the numbers of animals used in their generation. The importance of considering the strain, weight, oestrus cycle and number of embryos produced are therefore all discussed. The advantages and disadvantages of using vasectomised versus sterile males are reviewed, and methods of tissue sampling and biopsy are discussed at length, the emphasis being on the need to use and/or develop minimally invasive techniques.

Many of the procedures require surgery, and the report therefore stresses the importance of good surgical technique, appropriate anaesthesia, analgesia and pain management, and pre-operative and post-operative care.

The practical issues addressed relate to good husbandry and care and include:

- resource issues: the need for adequate space, time, funding;
- isolators and individually ventilated cages;
- transport: numbers, stress, cryopreservation;
- specialist care;
- breeding; and
- euthanasia.

Recommendations to develop "best practices" in all of these areas have been made by drawing on published material and on the practical experience of the Working Group members.

Genetic manipulation can result in morphological, physiological, biochemical and behavioural abnormalities that can cause animals pain, suffering and distress even if the phenotypic effects are subtle. Therefore, careful assessment and monitoring of animals is essential, and the report concludes with a section on welfare assessments for GM mice. There is a need to develop detailed criteria in this respect, and a preliminary list was set out by the Working Group as follows:

- developmental abnormalities;
- reproductive performance;
- pre/post weaning mortality;
- behaviour;
- suckling ability;
- immune status;
- litter size;
- neurology;
- morphology;
- growth rates;
- clinical signs;
- microbiological status;
- clinical biochemistry;
- post mortem and histopathology; and
- movement/posture.

It is stressed that this list of criteria needed further development.

Finally, good training in all these areas is vitally important, not only with respect to specialist

knowledge and skills, and competence in these, but also with regard to developing the right attitude — that mice matter. These animals are not just expendable scientific tools. Ensuring that people have the necessary motivation to look for improvements is crucial to implementing all Three Rs. Since genetic modification is such a fast moving field, there is also a need for regular update and review of training needs and practices.

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