More is Less: Reducing Animal Use by Raising Awareness of the Principles of Efficient Study Design and Analysis

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Summary — Good experimental design and the appropriate use of statistical tests form the corner stone of high-quality scientific research. This is especially important when the experiments involve the use of laboratory animals, to ensure that their use is appropriate and that the minimum number of animals will be used that will provide data which are sufficiently statistically-sound to meet the objectives of the study. One way to raise awareness of the importance of efficient study design and analysis is to provide training courses. This paper reports the views of participants at two such training schools, with reference to why they felt that attendance was necessary and how effective they felt the experience had been. The implications of the responses are discussed, and considerations for future training events are noted.

Key words: *animal experiment, experimental design, questionnaire, reduction, statistics, Three Rs, training school.*

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Introduction

In their ground-breaking book, *The Principles of Humane Experimental Technique*, Russell and Burch observed that "One general way in which great reduction may occur is by the right choice of strategies in the planning and performance of all lines of research" (1). By this, they were referring to the need for scientists to think through the entire process of scientific investigation, from the initial clear definition of the value and scientific objective of the proposed study to the way the findings would be applied. This planning must extend to the choice of an experimental strategy that avoids the use of animals whenever possible. If animals have to be used, provided that the potential outcomes are justified, the method adopted must have the least possible impact on their well-being, and must require the minimum number of animals necessary to achieve the desired scientific outcome. Considerations which need to take place at this stage include: the choice of animal model (species and strain), particularly with regard to its ability to accurately represent the biological feature(s) of interest; the neurophysiological sensitivity of the model (i.e. the extent to which it is likely to feel pain or discomfort as a consequence of the procedure being conducted); the facilities available for care and use in a manner appropriate to the biological and behavioural needs of the model; and the ability of the model to provide the scientific data needed, by using the fewest animals possible. In turn, these considerations inform the choice of study design. Minimising the number of animals required also involves paying attention to minimising diversity among individuals in the population, itself a function of genotype (use of inbred or F1 hybrid strains) and phenotype; control of the latter involves attention to the husbandry of animals and the way in which the scientific procedure is to be conducted. The adoption of these measures precedes the application of mathematical procedures to establish the smallest number of animals required to provide clear statistical evidence about whether or not an effect exists.

Scientists have normally received training and have achieved expertise in their area of academic interest, but they may not always be well-informed about the current thinking on experimental design and statistics, or the opportunities offered by emerging technologies which may have a direct impact on the Three Rs; evidence for this is offered by Festing (2). One example is the continuing widespread use of outbred strains of rodents, when the use of inbred strains would require fewer animals, and would provide data of better quality and/or of greater scientific relevance.

In April 2001, the FRAME Reduction Committee (FRC) held a meeting together with the Laboratory Animal Science Association (LASA) Alternatives Section, entitled: *Scientists, Statisticians, and Ethical Review Process (ERP) Members and Trainers: Optimising Animal Use by Enhanced Teamwork* (3). One purpose of this meeting was to explore ways in which statisticians and researchers could work toward reducing the numbers of animals used in experiments, by improving the efficiency of experimental designs. During this meeting, it

emerged that, not only did many scientists lack the skills necessary for appropriate experimental design, but that there was a shortage of statisticians with sufficient understanding of biology to be able to provide adequate support to the biomedical research community in the UK. Thus, there was a clear need for biologists and statisticians to become better acquainted with each other's technical and scientific fields. At the subsequent FRC symposium: *Reduction — Current status and future prospects*, consideration was given to ways in which this shortfall of expertise could be addressed (3).

Training Schools

In the UK, one opportunity for raising awareness of issues surrounding experimental design arises during compulsory training programmes for applicants for Personal and Project Licences. Unfortunately, the time available is usually very limited, and there is little opportunity to do more than raise awareness of the importance of the subject. A solution to this problem (which is not unique to the UK) is to ensure the availability of training materials, such as the text by Festing *et al.*: *The Design of Animal Experiments* (4). However, full appreciation of such principles is greatly facilitated by discourse, and there is likely to be benefit in courses specifically addressing these sensitive topics.

In November 2006, the University of Kuopio, Finland, organised a training school entitled: *Experimental Design and Statistical Methods in Biomedical Experimentation*. The school ran for a full week, and funding was provided by the European Cooperation in the field of Scientific and Technical Research (COST). COST was founded in 1971 to promote coordination of nationally-funded research at a European level. One of the "activity centres" of COST — Action B-24 — is particularly concerned with increasing the knowledge necessary to sustain the ethical and scientific validity of laboratory animal research in Europe, by application of the Three Rs (5). This Action currently has a membership of 24 countries (Box 1).

The philosophy behind the training school was not only to present the basic principles of experimental design and analysis, but also to facilitate contact with tutors, through which students would be encouraged to seek advice or to critically assess experiments with which they were already involved (the timetable for this training school is shown in Appendix 1). In addition, students were provided with a trial version of Minitab and were presented with a number of projects which they were required to address as groups. Two tutorial sessions were held to give students an opportunity to work through exercises with this software. The training school was attended by 43 students (there were almost twice as many applicants), the majority of whom were either postgraduate or post-doctoral researchers. Feedback from the students during and immediately after the training school indicated that they had found the entire event very helpful.

Following the success of this event, COST agreed to fund a second training school, which was held at The University of Manchester, UK, in January 2008. This training school was organised by the FRAME Reduction Steering Committee (FRSC), and adopted a similar format, although it lasted for only four days. The programme was more structured than Kuopio, and although there was less formal opportunity for the students to address their own study design problems, both the students and the tutors were accommodated at the same hotel, so that informal interaction in evenings was possible (the timetable for this event is shown in Appendix 2). Like its predecessor, the Manchester training school was advertised on websites, and by word of mouth. There were 72 applicants, of whom 40 were accepted as participants, 29 of them from outside the UK. The course employed many of the same tutors as that at Kuopio, but was set within the UK regulatory climate, and focused more closely on experimental design and statistics. Informal comments by students at the end of this course, and feedback solicited immediately afterwards, indicated that, in general, they had found the course helpful and were enthusiastic about the organisation of similar courses run in the future.

Methods

The benefits of training schools of this type are not likely to be immediately apparent to those who

Box 1: COST Member Countries party to Action B-24 — Laboratory animal science and welfare

Austria	Belgium	Croatia	Czech Republic
Denmark	Estonia	Finland	France
Germany	Greece	Hungary	Ireland
Israel	Italy	Lithuania	Malta
Netherlands	Norway	Portugal	Spain
Sweden	Switzerland	Turkey	United Kingdom

attend them, so, in December 2008, a questionnaire was circulated to those who had participated in the two schools. The questionnaire (Appendix 3) asked the students to reflect on their learning experience in relation to course expectations. The responses were transferred to a spreadsheet, and a search was made for associations between categories of responses for the different questions, based on course attended, position, prior statistical training received, the reason for attending, whether or not feedback was offered at the home establishment, whether the course improved knowledge, and whether there were opportunities to pass this on. None of the associations were statistically significant, perhaps reflecting the relatively small sample size. Despite this, a number of qualitative findings emerged which provide indications about the success and demand for such training, and which will help the effectiveness of future training schools to be improved.

Findings

Eighty three questionnaires were sent out (43 to Kuopio attendees and 40 to Manchester attendees), of which 30 (11 and 19, respectively) were returned; 13 of the questionnaires sent to Kuopio registrants were returned undelivered, presumably because the attendees concerned had moved. Therefore, the response rate was 43%. The positions held by the respondents at the time of attending the training schools are shown in Table 1. Most of them were postgraduate students in academia, and four worked in government and commercial laboratories. Responses were received from respondents in 13 different European countries: Austria, Croatia, Finland, Greece, Hungary, Italy, Norway, Portugal, Spain, Switzerland, The Netherlands, Turkey and the UK.

Table 1: Position held by respondents at the time of attendance at the training school

	No. of respondents		
Position	**Kuopio school**	**Manchester school**	**Total**
Postgraduate	4	10	14
Postdoctoral	3	6	9
Supervisor	1	1	2
Project Lead	0	1	1
Other	3	3	6

'Other' categories were: laboratory co-ordinator and teacher, head of department, animal welfare officer, professor, laboratory animal veterinarian, director.

Table 2: Amount of training in experimental design and statistics that respondents felt they had received prior to attending the training school

	No. of respondents		
Amount of training	**Kuopio school**	**Manchester school**	**Total**
None	1	1	2
Very small	5	7	12
Small–medium	3	11	14
Medium–large	2	0	2
Very large	0	0	0

Most of the respondents expressed enthusiasm for the training school they had attended, and indicated that they would recommend attendance to others. One of the participants reported that she might recommend the school to others, and another would not recommend it because of a perception that there was not enough focus on statistics and too much about the Three Rs. However, the same respondent noted that such a "course is definitely needed".

None of the respondents felt that they had received a large amount of training in experimental design and statistics prior to attending the training school, and only two of them considered they had received a medium to large amount — the role of both of these was related to the oversight of animal welfare. Two students (both postgraduate researchers) reported they had received no previous training; most of the remainder had received a small-medium or a very small amount (Table 2).

When asked to indicate their reason(s) for attending the training school, 18 of the respondents indicated that they wanted to expand on their previous training, and 19 felt that their previous training had been insufficient (Table 3).

The respondents felt that attendance at the training school had improved their research to some degree. Most (47%) thought that their research had improved by either a large amount or by a medium amount (40%; Table 4); 24 respondents (80%) had disseminated the information given at the training school via the methods detailed in Table 5.

It was not possible to distinguish statistically between the responses of participants at the Kuopio and Manchester courses, or between the responses of postgraduate students and postdoctoral researchers.

Beyond these quantifiable data, more general observations can be made from the information provided by respondents, on what they found to be

Table 3: Respondents reasons for attending the training school

Reason	No. of responses*		
	Kuopio school	Manchester school	Total
Felt previous training was insufficient	6	13	19
Expand on previous training	7	11	18
See what was new in the field	1	2	3
Advice on specific problem	2	7	9
Fulfil requirements for compulsory training	0	1	1
Other	2	1	3

**Numbers of responses are not equal to the number of respondents, as some people gave multiple reasons.*

interesting during the training school and what they found most useful afterwards. While the responses were varied, the main themes that reoccurred are summarised in Box 2. In addition, a number of suggestions were made for improving the effectiveness of training. These suggestions included the following, which will be considered when future training schools are in the planning stages:

— allowing for more discussion about the use of statistics tests in specific situations (suggested by two Kuopio attendees);

— including a presentation by a researcher about the planning and performance of an actual animal experiment in practice (How was it planned? What problems arose [and reporting the solutions]? How hard/easy was it, to incorporate the statistical issues presented during the training school?);

— timetabling for less-intensive days;

— making allowances for language difficulties for non-UK people — for example, by providing material beforehand to allow participants to familiarise themselves with key concepts, and providing handouts for all lectures; and

— providing more opportunity for participants to discuss their own experimental and statistical problems.

An anonymised spreadsheet containing more details of the responses to the questionnaire provided by training school attendees, is available on request from Michelle Hudson, at: michelle@frame.org.uk.

Discussion

Delaying the assessment of learning outcomes until students have had opportunities to put their learning into practice, provides a more realistic assessment of the long-term value of training than do responses received immediately after the course has ended. However, such a delay has the disadvantage that student's recollection of the learning environment may be less precise.

Two training schools were held: in Kuopio in November 2006, and in Manchester in January 2008. The questionnaire was distributed in December 2008. The different post-course intervals may have affected the response rates of the attendees.

The training in experimental design and statistical analysis which is offered to young scientists

Table 4: Amount by which respondents felt that the training school had improved their research

Perceived improvement	No. of respondents		
	Kuopio school	Manchester school	Total
Not at all	0	0	0
A limited amount	2	1	3
A medium amount	4	8	12
A large amount	4	10	14
A very large amount	1	0	1

Table 5: Methods by which respondents disseminated the information given at the training school

Method	No. of responses*		
	Kuopio school	Manchester school	Total
Formal presentation	3	2	5
Verbally	5	10	15
Distribution of training school materials	2	4	6
Other	4	4	8

**Numbers of responses are not equal to the number of respondents, as some people gave more than one answer.*

from diverse disciplines is frequently of poor quality, even where standards are traditionally regarded as high (6). The large number of applicants for each course, and the responses to the question on previous training, support the contention that many early career biomedical scientists are not equipped with sufficient knowledge to permit them to efficiently design and conduct experiments involving animals. Over half of the students appreciated this deficiency in their training, because they gave their reason(s) for attending the training school as having insufficient previous training, or having a desire to expand on previous training. The participants were principally early career scientists. However, attendance by senior researchers (including a department head and a professor) shows that the demand for further training goes beyond those just starting their careers. It is also noteworthy that some more-senior responders reported that they had used the knowledge gained from the training school to enhance and/or supplement the information they used when teaching the subject in their own institutions.

Most of the respondents reported that attendance at a training school had improved their research by a medium to large amount, a view confirmed by their comments with regard to interesting and useful aspects of the course and the general positive feedback received at the end of each training school. However, it is not possible to conclude from these data whether or not the scientific process had been improved, and further investigation would be needed to investigate this. What *is* revealed by the data is an increased awareness of experimental design and statistics. In addition, many respondents disseminated the

Box 2: Aspects of the training school that respondents found most/least interesting, and information they found most/least useful in their research after the training school

Most Interesting
Aspects of good experimental design – 18 respondents (60%)
Group working – 9 respondents (30%)
Analysis of data – 5 respondents (17%)
Exchange of ideas from people all over the world – 4 respondents (13%)
The Three Rs – 5 respondents (17%)
Discussions with statisticians – 3 respondents (10%)

Least Interesting
Minitab practicals – 7 respondents (6 from Manchester) (23%)
Material irrelevant to statistics – 5 respondents (3 from Kuopio) (17%)

Most Useful
Better ability to design a study – 12 respondents (40%)
Better understanding of data analysis – 7 respondents (23%)
Understanding factorial designs – 8 respondents (27%) (all from Manchester)
Ability to make better use of data analysis software – 5 respondents (17%)
Understanding meaning of power and sample size determination – 7 respondents (24%)
Awareness of how to control variability – 3 respondents (10%)

Least Useful
Minitab exercises – 6 respondents (20%)
Presentations which they felt were of little direct relevance to study design – 5 respondents (17%)

information when they returned to their institutions. While the effectiveness of such dissemination cannot be assessed, it is a strong indication that training schools of this nature provide a useful platform for the dissemination and improvement of knowledge about good experimental design and statistical practice in biomedical research.

The different mix of academic backgrounds did not appear to cause difficulties, although language was a problem for some participants. The support given to students whose native language is not English, and the inclusion of different perspectives, could be accommodated by increasing the time spent on group learning, associated with careful attention to group composition. Both of the courses required the students to work in their own time, but there was also a clear appreciation of the opportunity to meet teachers less formally, a process facilitated by the hotel arrangements at Manchester.

The principal difference between the courses held at Kuopio and at Manchester, was the encouragement of the Kuopio students to bring their own study design and analysis problems to a forum attended by the tutors. This appears to have been very popular, and provided a focus for the learning environment. The Manchester course was structured in a more detailed way, and the students appreciated the relevance of more of what they were taught. The selection of teaching methodologies, from purely didactic approaches to enquiry-based learning, is always difficult, but the responses received indicate that training in experimental design must take place within contexts familiar to the participants, and serious consideration should be given to student-driven learning exercises.

Conclusions

It is clear that there is a need to improve the training of biomedical scientists early in their careers, and that current course provisions, at least in the thirteen European countries from which responses were received, is inadequate.

Two training schools were held: in Kuopio, Finland, in November 2006, and in Manchester, UK, in January 2008. Both of the courses had their strengths and their weaknesses. It is likely that the provision of training schools of this type will continue to prove popular with those who wish to enhance their own knowledge, and to share their experiences with colleagues working in different countries and operating within different regulatory and societal contexts.

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Appendix 1: Timetable of the training school on *Experimental Design and Statistical Methods in Biomedical Experimentation*, University of Kuopio, Finland, November 2006

Monday	
09.15–09.20	Introduction to the course
09.20–10.00	Biophilosophy: Models in biology
10.00–10.45	Designs for behavioural studies
11.00–12.00	Non-statistical aspects of design; bias; choice of animals and precision; design of environment; applicability
13.00–13.45	Designs for regulatory safety evaluation of chemicals
14.00–14.45	Practical randomisation; case for *refinement* and *reduction*
15.00–17.00	Group work on participants' own design and statistics problems
Tuesday	
09.15–12.00	How statistics can help when running experiments; basic statistical ideas; signal and noise; randomness and chance; sources of variability; use of graphical tools; determination of sample size; basic principles of design in animal studies; hypothesis; bias; experimental unit; controls; replication; randomisation; independent and dependent variables; blocking; software available for statistics; how to present data
13.00–17.00	Practical exercises on the day's topics
Wednesday	
09.15–12.00	Basic statistical tests; *t*-tests and confidence intervals; one-way ANOVA; two-way ANOVA and factor interactions; interpretation
13.00–15.00	Designs for *refinement* and scoring adverse effects; *refinement*, scoring adverse effect, humane endpoints; Workshop
Thursday	
09.15–12.00	Design and analysis of randomised complete block experiments, repeated measures experiments and cross-over experiments
13.00–17.00	Design and statistics clinic
Friday	
09.15–11.00	Assumptions and alternatives; important assumptions; transformations; non-parametric tests; covariates; multiple comparisons; common errors
12.00–14.00	Practical exercises on the day's topics

Appendix 2: Timetable of the training school on *Experimental Design and Statistical Analysis of Biomedical Experiments*, The University of Manchester, UK, January 2008

Monday	**Why Design?**
09.15–09.45	Introduction to the course, including information on FRAME and COST
09.45–10.25	Introduction to the Three Rs and the link between study design and *reduction*
10.25–11.05	Why design? Ethics, money and time
11.05–11.25	Textbooks, web resources and statistical software
11.40–12.55	Statistics and experimental design; basic design concepts of animal studies; developing hypothesis; measurements and endpoints; samples and populations; descriptive statistics and presentation of data
13.55–14.55	Bias and precision; signal and noise; model selection; hypothesis testing and estimation; confidence intervals; randomisation and replication
14.55–15.25	Simple experiments and statistical tests; robustness of data collection and sample storage
15.40–17.25	*Group Exercise:* Descriptive statistics, tests and data presentation including Minitab demonstration
	Workshop: Reviewing examples of designs and statistics

Tuesday	**Statistical Approaches to Optimising Design**
09.15–10.30	Basic statistical tests; *t*-tests and confidence intervals; one-way ANOVA; two-way ANOVA and factor interactions; interpretation
10.45–11.15	Sample size; power calculations; hypothesis testing; experimental unit; controls; replication; randomisation; independent and dependent variables
11.15–12.30	*Workshop:* Using Minitab for ANOVA and hypothesis testing
13.30–14.30	Limitations of statistical interpretation; assumptions and alternatives; covariates; multiple comparisons; correlation and regression; qualitative data; other modelling approaches
14.30–15.00	Transformations; non-parametric statistical tests and when to use them; common errors
15.15–17.00	*Workshop:* Power and sample size calculation; web-based statistical resources

Wednesday	**Experimental Approaches to Optimising Design**
09.00–09.30	Factorial Design
09.30–10.45	Phenotypic uniformity; non-statistical considerations in study design
11.00–11.45	Genetic uniformity and outbred stock
11.45–13.00	Design and analysis of randomised complete block experiments, repeated measures experiments, cross-over experiments and factorial design
14.00–15.30	*Workshop:* Case studies in optimising experimental design
15.45–17.30	*Group Exercise:* Participants' own design and statistics problems

Thursday	**Designs for Different Approaches**
09.15–11.00	Designs for regulatory evaluation of efficacy and safety; designs for non-regulatory studies
11.00–12.00	*Group Exercise:* Participants' own design and statistics problems
12.00–12.30	*Feedback from Group Exercise:* Question and answer session
12.30	Disperse

Appendix 3: The questionnaire distributed to participants of the two courses

Training for *Reduction* questionnaire

Name: _____

Address: _____

Email: _____

Training Course Attended: _____

(Please note information given above is for correspondence purposes only and will not be published)

Research interests and prior knowledge

1. What is your primary research interest?

2. What was your position at the time of attendance?
   ❑ Postgraduate, ❑ Postdoctoral, ❑ Supervisor, ❑ Project lead, ❑ Other (please specify):

3. How much formal statistical/experimental design training had you received before the Training Course?
   ❑ None, ❑ Very small amount, ❑ Small to medium amount, ❑ Medium to large amount,
   ❑ Very large amount.

Training Course

4. Why did you attend the course?
   ❑ Felt that previous training was insufficient
   ❑ Wanted to expand on previous training
   ❑ Wanted to see what was new in the field
   ❑ Wanted advice on specific problems
   ❑ To fulfil requirements for compulsory training
   ❑ Other (please specify):

5. How did you hear about the course?

6a. What 3 things did you find most interesting?
i. _____
ii. _____
iii. _____

6b. What 3 things did you find least interesting?
i. _____
ii. _____
iii. _____

7a. What 3 things did you find most useful for your work afterwards?
i. _____
ii. _____
iii. _____

7b. What 3 things did you find least useful for your work afterwards?
i. _____
ii. _____
iii. _____

8. Were you required to submit a report about the Training Course to your department/supervisor?
   ❑ Yes, ❑ No

9. Do you feel that the Training Course improved your research?
   ❑ Not at all, ❑ A limited amount, ❑ A medium amount, ❑ A large amount, ❑ A very large amount.

10. Did you pass on any of the knowledge you gained at the Training Course?
❑ No, ❑ Yes

    If Yes, how?
    ❑ Formal presentation, ❑ Verbally, ❑ Electronically, ❑ Written report,
    ❑ Distribution of course materials (e.g. slides, text books), ❑ Other (please specify):

11. Would you recommend the Training Course?
❑ Yes, ❑ No, ❑ Maybe.

12. Any other comments?