nature genetics

The '3Is' of animal experimentation

Animal experimentation in scientific research is a good thing: important, increasing and often irreplaceable. Careful experimental design and reporting are at least as important as attention to welfare in ensuring that the knowledge we gain justifies using live animals as experimental tools.

We have previously argued for the necessity of animal experimentation and the duty to explain the work to the public, despite the difficulty of doing so while treating animal experimentation as a problem in need of regulatory reduction (*Nat. Genet.* **38**, 497–498, 2006). We now note that many experiments may be wasting human and animal lives because the focus on reduction in animal experiments can lead to unreliable results from underpowered experiments on too few animals.

In our opinion, the classic review by Sean Scott and colleagues (*Amyotroph. Lateral Scler.* **9**, 4–15, 2008) should be required reading for anyone designing an animal experiment. It shows the danger of publishing positive results from underpowered studies on noisy experimental systems. By systematically investigating the standard model for familial motor neuron disease—the transgenic mouse bearing the human $SOD1^{G93A}$ variant—this group showed that transgene copy number, non–amyotrophic lateral sclerosis (ALS) cause of death and litter clustering contributed to noise in mean survival time and that any two small groups of animals always had a very high probability of showing differential survival of the previously publishable magnitude *without any drug treatment*. Rather than the previous publication mode of 5–10 mice per group, they used their knowledge of the sources of experimental variation to redesign the standard assay with 24 mice per group (saving 6 animals per group by same-gender litter matching and including equal numbers of males and females in case of a gender-specific drug effect). They were then able to repeat over 50 published studies of drug trials, often with twice the number of mice used in the combined preceding studies and with 90% power to detect the published effects. None of the drugs showed the published effect. These authors systematically investigated welfare issues relevant to experimental outcome, finding that basic, clean and specific pathogen–free housing made no difference to mean survival time. They also developed a surrogate endpoint for complete paralysis (wherein a mouse on its side takes >30 s to right itself) to prevent distress in mice with advanced disease.

It has been difficult to arrive at international standards for animal research because of regional variations in attitudes and legislation, but there is broad agreement on principles and practices for humane and scientifically appropriate treatment of animals (http://cioms.ch/publications/guidelines/1985_texts_of_guidelines.htm). Still, it has been possible to export best practice from one region to another. The UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs; www.nc3rs.org.uk) is an effective agency with broad support from scientists, funders, veterinarians and pharmaceutical companies, in spite of its quaint name (somewhat redolent of Orwellian doublespeak—in contrast, the US government has a plain Office of Laboratory Animal Welfare; http://grants.nih.gov/grants/olaw/olaw.htm). The name reflects a 1959 UK Home Office policy (3Rs) that has consistently influenced that nation's approach to legal and ethical protection for animal research subjects, and the current NC3Rs and its collaborators have been able to develop reporting guidelines to encourage best practice. We have adopted these guidelines in our Guide to Authors (http://www.nature.com/authors/policies/experimental.html), in which the *Nature* journals insist upon authors applying the widely accepted Animals in Research: Reporting *In Vivo* Experiments (ARRIVE) guidelines for reporting animal research (*PLoS Biol.* **8**, e1000412, 2010). In the form of a checklist, these guidelines are easy to follow and apply, and statistical issues, such as those discussed above, are front and foremost. However, one point, item 18c, may come as a surprise, as the recommendation is slanted in an entirely negative direction that may be unfamiliar to experimenters outside the UK:

"Describe any implications of your experimental methods or findings for the replacement, refinement, or reduction (the 3Rs) of the use of animals in research."

In the interests of both good experimental design and continuing to explain to the public why animal research is useful and necessary, we emphasize that this duty to report scientific implications is also a duty to note any implications of your experiments for the importance, irreplaceability or, indeed, increase in animal experimentation. The privilege to know comes with a duty to explain. ■