

What's in store for animal research in the EU?

Researchers should have little to fear from the EU's new animal-welfare directive, but the menace is in the minutiae

In October 2003, the European Commission (EC) proposed a new programme for assessing the safety of old and new chemicals, called REACH (Registration, Evaluation and Authorisation of Chemicals). Among other things, REACH necessitates more toxicity and carcinogenicity tests, and once adopted it will inevitably dictate more experiments on animals. Add to that the increasing need for mice in disease-model and gene-knockout studies in biomedical research, and for nonhuman primates in neurobiology and vaccine development, and the trend is clear: more research animals will be needed if industry and academia are to fulfil the public's expectations of improved health.

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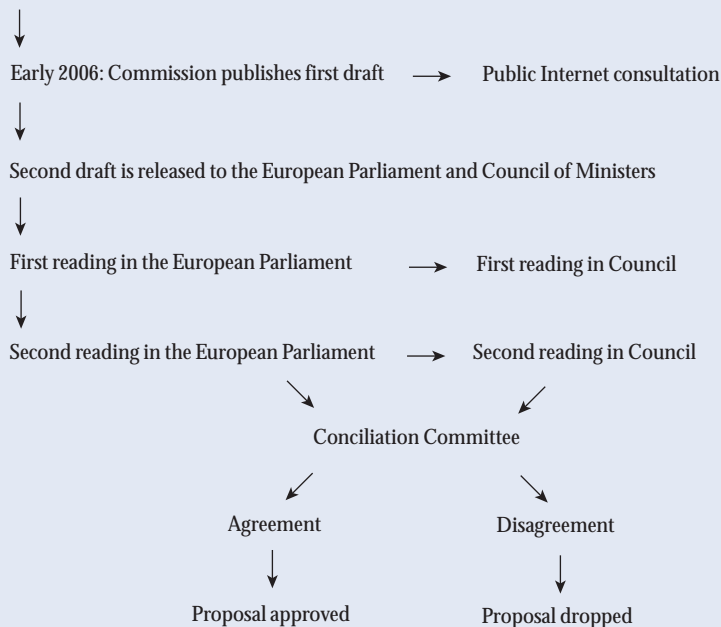
As Alastair Kent, Director of the Genetic Interest Group (London, UK), noted, "research is a human and a humane response to suffering; not using animals would send the message to sufferers that their loss of life quality is not serious enough to justify animal experiments [on their disease]." However, the long-overdue revision of the EC's directive on the protection of animals used for experimental and other scientific purposes (EEC, 1986) seems to be progressing with the blissful ignorance of the academic research community. If this continues, scientific considerations will probably play a minor role in the final rounds of the revision process.

THE DIRECTIVE REVISION PROCESS

The first draft of the amended directive is likely to appear early in 2006. Its basis will be the answers already given to a 'thought starter' issued to the Technical Expert Working Group (TEWG) in 2003.

The process will unfold along the following lines:

Mid to late 2005: Lead EC Directorate-General (DG) starts consultation between DGs



Few doubt that the directive, originally issued in 1986 to achieve a uniformly high standard for the use and care of animals in research across the EU, is a bit outdated. Furthermore, there is no question that the underlying aim of the directive is the replacement, reduction and refinement of animal experimentation (Matthiessen *et al*, 2003), also known as the three Rs (Russel & Burch, 1959). But research and the use of

animals have taken some quantum leaps in the meantime, which is precisely why particular care is needed to ensure that the revision takes full account of new techniques and knowledge. The fact that some particularly strict national regulations—including those of non-EU-member Switzerland—may act as a precedent for the revision has not escaped the attention of those who are pushing for reasonable and balanced

changes. And as each EU member state is obliged to fulfil and enforce the conditions set out in the directive through national laws and regulations, it has the potential to affect biomedical research across the whole of Europe. Some countries, notably the UK and the Netherlands, already have stricter regulations than the directive requires, but not in any particularly consistent way; indeed, variation between countries is what the new directive seeks to prevent.

Triggered by an 'own-initiative' report commissioned by Member of the European Parliament (MEP) Jill Evans in 2002, the revision process has been grinding slowly forward in typical EU style, and still has a long way to go (see sidebar titled 'The Directive revision process'). In 2003, a Technical Expert Working Group (TEWG; see the sidebar titled 'Invitees to participate in the Technical Expert Working Group') responded to a long and detailed list of questions about the changes in the directive—a so-called 'thought starter'. Their answers form the basis of the revision, and although broadly speaking they are sensible, the menace is in the minutiae: incremental, almost bureaucratic, changes that have yet to be decided. Proponents of the revision argue that research in most of Europe has nothing to fear from it. But, according to Simon Festing, Executive Director of the Research Defence Society (London, UK), "a minor point can contain a land mine." For instance, a redefinition of experimental re-use of animals, a transportation ban on some animals and the limitation on the use of nonhuman primates to the captive-bred F_2 generation (two generations distant from wild animals) could seriously affect biological and pharmaceutical research. Other possible amendments, such as the inclusion of fetal and embryonic forms and certain invertebrates, and compulsory cost-benefit analyses for all experiments, would not necessarily constrain research, but would certainly increase the administrative burden on researchers.

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Experimental re-use, for example, is allowed at present under the condition that "an animal shall not be used more than

INVITEES TO PARTICIPATE IN THE TECHNICAL EXPERT WORKING GROUP

- Member States
- Acceding countries
- Council of Europe
- Eurogroup for Animal Welfare
- EuropaBio
- European Chemical Industry Council
- European Coalition to End Animal Experiments
- European Federation for Laboratory Animal Breeders
- European Federation for Laboratory Animal Science
- European Federation for Laboratory Animal Technicians
- European Federation for Pharmaceutical Industries Association
- European Federation for Primatology
- European Science Foundation
- European Society for Laboratory Animal Veterinarians
- International Federation for Animal Health
- International Society for Applied Ethology

The Commission invited Member States, Acceding Countries and European organizations from industry, science and academia and from the area of animal welfare to nominate scientific-technical experts for participation in the process.

once in experiments entailing severe pain, distress or equivalent suffering." However, as Festing observed, "if even minor interventions count [in the revised directive], that could lead to a huge increase in the number of animals used, and the administration involved," thus counteracting an important aim of the directive. Possibly even more serious is the restriction of the use of nonhuman primates to the captive-bred F_2 generation, which would "pretty much wipe out primate research [in Europe] because we do not have enough capacity to breed the numbers of F_2 required," according to Festing.

The use of nonhuman primates in basic neurophysiology, behavioural research and vaccine development is growing, not diminishing. Reverse vaccinology—working systematically from genomic screens, through antigens, to physiological immune responses—is a promising new technology for producing new vaccines faster and more efficiently than the current trial-and-error method. Testing new vaccines increasingly requires nonhuman primates for assaying the cellular response to the antigen in question.

A humanized mouse may be an acceptable model for polio, but this approach simply will not work in the case of hepatitis and HIV: only primates show the necessary response. However, it is some compensation that to test every new batch of today's DNA vaccines on animals is unnecessary, given the consistency of production and the ease of assaying for the correct sequence.

Few researchers would think of trying to do such work in the Netherlands, where, as Ronald Bontrop, Director of the Biomedical Primate Research Centre in Rijswijk, pointed out, "legislation threatens soon to impose a total ban on the use of certain non-human primates." In 2004, the country passed a law banning invasive experimentation on great ape species, with the result, according to Bontrop, "that many experiments moved to the USA, Japan and possibly Africa." It hardly seems like the right socio-political climate in which to discuss plans for a European primate-breeding centre, but that is exactly what the research community says is urgently needed. The concept has already been supported by the TEWG for the revision of the Directive (TEWG, 2003). They responded to the recommendation from the Scientific Committee on Animal Health and Animal Welfare of the EC's Directorate-General for Health and Consumer Protection that only F_2 generation animals should be used in research. Experts believe that it would take Europe at least ten years to breed sufficient numbers of F_2 animals to avoid a serious impact on research.

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Ultimately, nonhuman primates are good research subjects because of their close phylogenetic relationship with humans. The chimpanzee is the only species, apart from humans, that is susceptible to infection with human hepatitis viruses; there is no alternative model to study vaccine strategies for these diseases. The same is true of HIV. Furthermore, Old-World monkeys infected with simian immunodeficiency virus are vital research subjects for identifying antigenic epitopes and protection mechanisms that can contribute to developing a vaccine against HIV. "In short, the immune system of



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monkeys mimics the human immune system in some critically important ways that mice cannot substitute," Bontrop said. Furthermore, research on primates will be vital for making vaccines against tuberculosis and malaria and for studying multiple drug resistance. It will also have an important role in research on degenerative diseases, xenotransplantation, gene therapy and conservation biology.

Less controversial is research involving large mammals that have been used in agriculture for millennia. However, that does not grant them a special place in the legislation for research, some of which promises to alleviate the serious over-demand for transplant organs in humans. According to Eckhard Wolf, Head of the Institute of Molecular Animal Breeding and Biotechnology (Munich, Germany), a German kidney transplant patient can wait up to six years for a suitable organ, and one in five patients on the waiting list for a heart transplant dies. Humanizing pigs for the purpose of organ transplantation offers a hope of overcoming this shortage until better methods become available. This research is entering a new level of refinement with the use of lentiviral gene transfer: a technique that works with much higher efficiency (100%) than DNA microinjection followed by reproductive cloning, according to Wolf. His group has already used lentiviral transfer to create cattle that express antibodies against human tumours.

Top of the list of animals in the service of biomedical research are mice, the use of which has increased dramatically in recent years. Large projects studying thousands of genes—such as the European N-ethyl-N-nitrosourea (ENU) mouse mutagenesis screens—require huge numbers of mice, and contribute immensely to understanding the genetic basis of normal development and physiology as well as disease. Using 34,000 animals and the technique of chemical mutagenesis, laboratories in Munich, Germany, and Harwell, UK, have so far identified 1,000 gene mutants that cause phenotypes from deafness to defects in bone development. Much of this work involved the use of several screening methods on single animals. In one case, multiple screens quickly and efficiently revealed 18 new phenotypes in a single mutant line, explained Martin Hrabé de Angelis, Head of the European Mutant Mouse Archive, and Director of the Institute of Experimental Genetics at the GSF Research Centre for Environment and Health, near Munich, Germany. He is particularly unsettled by the effect that the redefinition of re-use could have on such a project, but the concept of the 'three Ss' bothers him even more. "Legislation against higher grades of suffering, *per se*, encourages the 'Step-by-Step-to-Stop' process driven by radical activists who wish to ban all animal research," the Munich researcher remarked. The systematic phenotyping schemes developed in the large-scale pan-European project Eumorphia are already experimental refine-

ments, which allow better standardization of experiments and the construction of a comprehensive body of information with which to assess and cater for animals' welfare.

Although the public generally accepts research using rodents, that acceptance depends on two main conditions: that the research benefits society, and that the animals' welfare is taken into account. Attention to animal welfare has a long history in Europe, starting with the 1876 Cruelty to Animals Act in the UK. Today, the Association for Assessment and Accreditation of Laboratory Animal Care International, a global organization, inspects and issues accreditation certificates to industry and academic facilities. The Federation of European Laboratory Animal Science Associations provides training courses in animal housing and handling. And research projects, such as the intergovernmental framework for European Cooperation in the field of Scientific and Technical research, are constantly increasing the knowledge necessary for the ethically and scientifically defensible use of animals.

But the fact remains that experimental animals "suffer harm, give no consent, ultimately die, and accrue no compensatory benefit from being part of an experiment," as Anna Olsson from the Institute for Molecular and Cell Biology in Porto, Portugal, pointed out. Ultimately, "scientists have a lot to gain in credibility if they behave proactively in terms of regulation, and are open about what they do," according to Olsson. She warns of overselling animal research with arguments based on a moral duty to help humanity, and urges researchers to be honest about welfare problems and not to conceal profit as a component of the argument—for example, in pharmaceutical research. It would also help if scientists communicated more about how their research is assessed. "Science has some of the strictest quality control mechanisms of any profession, which are exercised at the level of funding and publication," observed Mark Matfield, Director of the European Biomedical Research Association, London, UK, and as Bontrop added, "scientists have not been good enough at explaining what they have achieved [with animal research] to the public."

Further improvements can be made by reassessing the role of ethics as integral, rather than parallel, to research. As David Morton, Professor of Biomedical Science and Ethics at the University of Birmingham, UK, commented, "ethics shouldn't be a separate issue for researchers, it should be part and parcel of their daily activity. The three Rs are often a cover-up for scientists; we need more concentration on the intrinsic value of an animal and the role of the researcher as a moral agent." He would prefer the four As: Awareness (of suffering), Assessment, Avoidance and Alleviation. "Always assume that animals will suffer, rather than the reverse," he added. Reduced suffering improves the cost:benefit ratio of an experiment and makes it ethically more defensible, but it also increases the quality of the science, because pain and other forms of suffering can strongly influence physiological responses and hence scientific outcomes.

The better a species is understood, the better its welfare can be addressed. The effect of environmental factors, such as cage enrichment, on phenotype is complex and needs more research. But preliminary findings suggest that they have a significant and unpredictable effect on the results of experiments, according to Steve Brown from the MRC Mammalian Genetics Unit (Harwell, UK), who is the co-ordinator of Eumorphia. In the case of transgenic mice, the need for better phenotyping is now being addressed through large scientific networks across Europe. This is good for standardization, validation and data sharing, all of which contribute to the three Rs. Furthermore, genetic modification of animals is usually both a reduction and a refinement: it often replaces the chemical trauma that was inflicted on pregnant animals over a long period to create a particular defect in the offspring. Conditional mutants even allow the expression of a particular defect to be switched on and off. Less invasive technologies, such as magnetic resonance imaging markers and telemetry for remote monitoring, can also help to refine certain experiments, as can better definitions of humane end-points and improved knowledge of normal and patho-physiological values. In a general sense, greater knowledge minimizes suffering, which in turn improves the quality of experimental data.

Funding bodies and scientific publications can help by requiring scientists to describe in detail the suffering caused to an animal, and how it has been minimized. But nothing can improve the credibility of scientists more than ensuring animal welfare by self-regulation; there is no legislative substitute for good practice enforced in-house. Some researchers who are aware of the directive revision therefore ask why it is necessary if scientists already follow the rules and constantly improve animal use according to the best current scientific knowledge. Overwhelmingly, this is indeed what researchers do, as the tenets of the three Rs and the ethical use of animals do not conflict with sensible research.

What might be at odds with research are amendments born more out of political than scientific considerations. Many groups rightly have a stake in the revision process, and its final stages will rest in the hands of politicians. If researchers want scientific considerations to be included when the discussions become political, they are well advised to identify their nearest MEPs and correspond with them. "Keeping your head down does not work," remarked Festing. Hard-line ani-

mal rights groups will certainly not keep their heads down in the months to come.

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This article is based on a focus meeting of stakeholders held in Madrid, Spain, 18–19 March 2005, and organized by the EMBO Science & Society Programme to discuss the potential impact of the EC directive revision on research using animals.

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doi:10.1038/sj.embor.7400475

Skullduggery

The discovery of an unusual human skeleton has broad implications

When a team of Indonesian and Australian palaeontologists discovered a nearly complete but very strange 18,000-year-old human skeleton in an Indonesian cave in 2003, the find provoked questions about modern human origins. Do these ancient bones belong to a new human species? Are they, as many have claimed, the most important find in hominid palaeontology for decades? Or is this creature—indelibly christened 'the Hobbit' because it is so tiny—simply one of an isolated people who suffered from a deforming malady? The huge stakes in this competitive, caustic debate can be summed up succinctly: money and fame. But Hobbit investigations may eventually have less impact on the study of human evolution than they do on the continuing crusade against the Darwinian account of how life on Earth evolved.

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The bizarre story so far: In 2003, a joint Indonesian–Australian team, digging in Liang Bua cave on the Indonesian island of Flores, found hominid bones and a nearly complete skeleton. The skeleton, designated LB1, was childish tiny, but tooth wear showed the hominid to have been aged about 30 at death. In 2004 at the same site, the team uncovered another mandible and more bones and bone fragments, from a total of eight individuals. Dates inferred indirectly from materials around the finds range from about 94,000 to 12,000 years ago. This suggests that the hominids lived