

Replacement of animal procedures: alternatives in research, education and testing

Michael Balls

European Centre for the Validation of Alternative Methods (ECVAM), JRC Environment Institute, 21020 Ispra (Va), Italy

Summary

The origins of the concept of *replacement alternatives* in the 1950s, and the impact of societal changes in the 1960s and 1970s, resulting in stricter controls on animal experimentation from the 1980s, based on the Three Rs of Russell and Burch (*reduction, refinement and replacement*), are reviewed. The range of replacement alternative methods, and some of the ethical issues they raise, and progress toward their incorporation into fundamental and applied research, education, and, in particular, toxicity testing, are discussed. It is concluded that much greater effort should be put into overcoming the barriers to the acceptance of replacement alternatives, which currently limit the contributions they have to make toward greater humanity and better biomedical science. Particular emphasis is placed on the need to ensure that the validation of non-animal tests (for their reliability and relevance for specific purposes) is conducted fairly and objectively, and that greater heed is paid to the warning of Russell and Burch about the high fidelity fallacy and the questionable relevance of data provided by animal models for human hazard and risk assessment. Finally, the role of ECVAM in the promotion of valid replacement alternatives, and the opportunities afforded by the Sixth Amendment to the EC Cosmetics Directive, are discussed.

Keywords Animal procedures; cosmetic testing; *in vitro* tests; replacement alternatives; toxicity testing; validation

The concept of replacement

It was at the Universities Federation for Animal Welfare's 1957 *Symposium on Humane Technique in the Laboratory* (UFAW, 1957) that the concept of the Three Rs (*reduction, refinement and replacement*) as a means of removing inhumanity from animal experimentation was first discussed in depth at a public meeting, notably by Charles Hume and William Russell. This Symposium, and

Russell and Burch's book, *The Principles of Humane Experimental Technique* (Russell & Burch 1959, 1992), resulted from an initiative taken by UFAW in 1954, with the advice of a distinguished committee, which met under the chairmanship of Peter Medawar. Russell and Burch defined a *replacement technique* as 'any scientific method employing non-sentient material which may in the history of animal experimentation replace methods which use conscious living vertebrates'. They distinguished between *relative* replacement, in which animals would still be required, but would not be exposed to any distress in the actual experiment, and *absolute* replacement, in which animals would not be required at any stage at all.

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They saw non-recovery experiments on animals under anaesthetic and the use of spinal and decerebrate animals, i.e. animals whose central nervous systems had reliably been made insentient, as examples of *relative* replacements. Another example they discussed was the humane killing of animals to provide cells, tissues and organs for *in vitro* studies.

They recognized four main types of *absolute* replacement: the use (outside the vertebrate body) of metazoan parasites, and of higher plants, microorganisms, and non-living physical and chemical systems. Tissue culture involving human and non-vertebrate tissues was seen as a bridge between relative and absolute replacement.

The concept of replacement has narrowed in the years since 1959, in Great Britain, at least, since experiments on spinal and decerebrate animals, as well as on animals under terminal anaesthesia, which were already controlled by the *Cruelty to Animals Act 1876* (Anon 1876), are now regulated procedures under the *Animals (Scientific Procedures) Act, 1986* (Anon 1986a).

Russell and Burch lamented the lack of a general theory of replacement. They recognized that the development of replacement methods was part of the normal evolution of scientific methodology, often because of insuperable obstacles in the way of the use of animal models, citing, in particular, the case of the introduction of tissue culture techniques into animal virology. However, they saw advances for these scientific and empirical reasons as isolated and haphazard events, and argued that the systematic and rational extension of replacement would result in both greater humanity *and* better science. They saw the search for replacement alternatives as a respectable and laudable scientific activity in its own right.

Changes in attitudes and laws since 1959

Russell and Burch's book had little obvious impact on thinking or practice in the early years after its publication. Nevertheless, significant changes were taking place at

that time, which were eventually to lead to widespread acceptance of the Three Rs principles, and even to their use as the basis of new laws in a number of countries and regions, particularly in Western Europe.

The 1960s saw great progress in relation to human rights, especially in the civil rights of racial minorities and women, which was later to lead to discussions and proposals for action in terms of *animal* rights. Meanwhile, industrial and academic scientific activity expanded very considerably, and developments in molecular and cell biology led to the increased use and greater acceptability of non-animal techniques as fundamental to progress in the biomedical sciences. However, at the same time, expectations of greater safety for human beings and demands for greater protection of the environment were leading to a dramatic expansion of routine toxicity testing in animals, including the introduction of new testing requirements every time a new problem was identified.

For various reasons, the view that there was a middle way in the animal experimentation debate, between total abolition and scientific libertarianism, steadily gained acceptance. All of those who were involved in these developments in any way will have their own lists of the key players and the main events. Apart from the formation of FRAME (Fund for the Replacement of Animals in Medical Experiments) in 1969, specifically to advance Russell and Burch's vision that humanitarian and scientific benefits would accrue from the systematic and rational extension of replacement, my own list would include the following:

1. The publication of David Smyth's excellent survey, *Alternatives to Animal Experiments*, in 1978 (Smyth 1978).
2. The establishment of the FRAME Toxicity Committee in 1979, which has already produced two important reports on the use of animals and alternatives in toxicity testing (Balls *et al.* 1983, 1991).
3. The formation of the Johns Hopkins Center for Alternatives to Animal Testing (CAAT) in Baltimore in 1982 (Frazier & Goldberg 1990).

4. The effectiveness of the collaboration between the British Veterinary Association (BVA), the Committee for the Reform of Animal Experimentation (CRAE) and FRAME, whose proposals (Anon 1983) significantly influenced the policies of the British Government and led to the involvement of this 'Triple Alliance' as advisers to the Government at all stages of the passage of the *Animals (Scientific Procedures) Act 1986*.
5. The establishment in 1983 of the Department of Laboratory Animal Science at the University of Utrecht in the Netherlands. This Department has played a leading role in the many contributions made by Dutch scientists to laboratory animal science in general, and, in particular, to the training of laboratory animal scientists (de Greeve *et al.* 1993, van Zutphen *et al.* 1993).
6. The publication of the US Congress Office of Technology Assessment (OTA) report on *Alternatives to Animal Use in Research, Testing and Education*, in 1986 (Anon 1986b, Balls 1987).
7. The declaration by the EC Commission to the EC Council of Ministers and the European Parliament in 1991 of its intention to establish a European Centre for the Validation of Alternative Methods (ECVAM; Anon 1991).

The OTA accepted Smyth's Three Rs definition of *alternatives*, which had been promoted by FRAME over many years (e.g. Balls 1983):

Alternatives to animal experiments are procedures which can completely replace the need for animal experiments, reduce the numbers of animals required, or diminish the amount of pain or distress suffered by the animals in meeting the essential needs of man and other animals.

This was particularly important, since far too much time has been wasted over the years in semantic discussions on the meaning of 'alternatives', and on whether other terms, such as 'adjunct', 'complementary' or 'substitute', would

be preferable. This debate is now over.

In 1985, the British Government made a Three Rs statement, which was remarkable at the time, when they declared, in a White Paper explaining the basis of their proposed new legislation (Anon 1985), that:

Animal experiments that are unnecessary, use unnecessarily large numbers of animals, or are unnecessarily painful, are indefensible.

The *Animals (Scientific Procedures) Act 1986* (Anon 1986a) passed through the British Parliament with very little opposition, and came into force on 1 January 1987. It included many important provisions and, in particular, the following two clauses:

5(5). The Secretary of State shall not grant a project licence unless he is satisfied that the applicant has given adequate consideration to the feasibility of achieving the purpose of the programme to be specified in the licence by means not involving the use of protected animals.

5(4). In determining whether and on what terms to grant a project licence the Secretary of State shall weigh the likely adverse effects on the animals concerned against the benefit likely to accrue as a result of the programme to be specified in the licence.

Meanwhile, in line with the recommendations of the *Council of Europe Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes* (Anon 1986c), the Council of Ministers of the EC had adopted *Directive 86/609/EEC* (Anon 1986d), which also has a Three Rs basis and contains the following major clauses:

7(2). An experiment shall not be performed if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal, is reasonably and practicably available.

7(3). When an experiment has to be performed, the choice of species shall be

carefully considered and, where necessary, explained to the authority. In a choice between experiments, those which use the minimum number of animals, involve animals with the lowest degree of neurophysiological sensitivity, cause the least pain, suffering, distress or lasting harm, and which are most likely to provide satisfactory results, shall be selected.

7(4). All experiments shall be designed to avoid distress and unnecessary pain and suffering to the experimental animals.

12(2). Where it is planned to subject an animal to an experiment in which it will, or may, experience pain which is likely to be prolonged, that experiment must be specifically declared and justified to, or specifically authorized by, the authority. The authority shall take appropriate judicial or administrative action, if it is not satisfied that the experiment is of sufficient importance for meeting the essential needs of man or animal.

Thus, by the mid-1980s, as a result of these developments and of similar happenings in other parts of the world, all concerned in any way with laboratory animal experiments had come to have placed upon them the legal and moral duty to recognize the Three Rs principles, to inform themselves about the potential uses for particular replacement alternatives, to actively support the development of such alternatives, and to accept scientifically validated alternatives as replacements for animal procedures. The main question is, then, what real effects have followed these changes of attitude and changes in laws, and, if they are insufficient, what can be done to improve them in the future?

The replacement alternatives

If a replacement alternative is defined as a method which does not involve the use of a living protected animal in a regulated procedure, then the range of methods includes the following (Balls 1983, Boyd & Smith 1991):

1. The improved storage, exchange and use of information about animal experiments already carried out, so that unnecessary repetition of animal procedures can be avoided.
2. The use of physical and chemical techniques, and of predictions based on the physical and chemical properties of molecules.
3. The use of mathematical and computer models, including:
 - a. modelling of quantitative structure-activity relationships (QSAR), i.e. taking advantage of correlations between molecular structure and biological activity in the prediction of the potential desired and undesired effects of series of related chemicals;
 - b. molecular modelling and the use of computer graphics, e.g. in actively designing drugs and other chemicals for specific purposes;
 - c. modelling of biochemical, physiological, pharmacological, toxicological and behavioural systems and processes.
4. The use of 'lower' organisms' not protected by legislation controlling animal experiments, including invertebrates, plants and microorganisms, e.g. *Limulus* in pyrogenicity testing and bacteria in genotoxicity testing.
5. The use of the early developmental stages of vertebrates before they become protected animals. In the case of the British 1986 Act, this is before half-way through gestation (mammals) or incubation (birds and reptiles), or the stage when independent feeding occurs (amphibians and fish), e.g. early chicken embryos in reproductive toxicity tests.
6. The use of *in vitro* methods, including sub-cellular fractions, short-term maintenance of tissue slices, cell suspensions and perfused organs, and tissue culture proper (cell and organotypic culture), including human tissue culture.
7. Human studies, including the use of human volunteers, post-marketing surveillance and epidemiology, e.g. skin patch testing in humans before marketing and monitoring consumer

response after marketing, as alternatives to the animal testing of cosmetic products.

Ethical issues raised by replacement

As was recently discussed in detail by an Institute of Medical Ethics (IME) Working Party, which considered the ethics of using animals in biomedical research (Boyd & Smith 1991), replacement alternatives do not offer total escape from ethical dilemmas. A few illustrations will suffice.

Better use of information depends on its ready and free availability, but many of the results obtained in experiments on animals are considered by industrial companies to be their private commercial property. Whether 'benefit' as intended in the benefit/suffering equation of the British 1986 Act, should be considered to extend to the benefit represented by the competitive advantage of one company over another, is still a matter for debate and resolution. A sensible compromise would be that data owned by a company should be made publicly available after a certain interval.

The use of physico-chemical techniques and of mathematical and computer modelling involves no special ethical problems, but animal data may be required to establish a model or to validate it. In addition, there is the general issue of whether there is a moral obligation to use a higher fidelity model, i.e. a laboratory animal, in certain circumstances.

The presence of a vertebral column is a convenient way of distinguishing between animals to be offered protection in controls on animal experimentation and those which can be considered acceptable as alternatives. However, it is by no means certain that there is a clear demarcation between the degree of sentience and capacity to suffer of the lower vertebrates (e.g. fish) and the higher invertebrates (e.g. some cephalopod molluscs). For this reason, acting on the principle of giving the animals the benefit of the doubt, the British Government has recently decided to extend the *Animals (Scientific Procedures)*

Act 1986 to include the protection of *Octopus vulgaris* (Anon 1993a).

Similarly, the cut-off points used to distinguish between the early development stages of vertebrates at which the animals concerned can be used as alternatives and the stages at which they became protected, is arbitrary and unsatisfactory—it has no strong scientific basis and therefore cannot be ethically satisfactory. The stage of development at which larval/embryonic/fetal movement first becomes detectable might be a far better cut-off point, since it indicates the presence of an active nervous system and could reliably be fixed for each species.

Living material for vertebrate tissue culture has to be obtained from animals or humans. In the former case, provision of material for culture can be a secondary benefit of the use of an animal for a primary purpose (e.g. for food), but the demanding requirements of the culture system (e.g. sterile technique) can often mean that animals are specifically killed for this purpose. The use of tissues of human origin is fraught with difficulties, including safety and logistical problems, as well as the need to obtain permission from bereaved relatives at the time of death of the potential donor. Using cells originally obtained from aborted human fetuses is another ethical minefield. Nevertheless, there is a growing feeling that more should be done to make human cells and tissues more-readily available, albeit safely and decently, for use in *in vitro* studies. Indeed, such is the importance of species specificity in drug/receptor interactions, the use of human material in *in vitro* studies is now an essential stage in the development of new medicines.

Finally, the use of human patients or on healthy human volunteers involves immense ethical dilemmas, most of which have no totally satisfactory solution. For example, if informed consent must be obtained before any treatment is applied to a human volunteer, how can therapeutic remedies be devised which are needed by sick young children or elderly people with senile dementia?

Animal experiments in fundamental research

It is very difficult to establish the *necessity* of animal experimentation in terms of the likely benefits of much fundamental biomedical research, and thus to justify work which is likely to cause animal suffering, in any more than the most general terms, by which any increase in knowledge is seen as potentially 'beneficial'. The dilemma that this presents to the operation of the British 1986 Act, which requires that likely benefit and likely suffering be assessed and weighted before a proposed programme of research involving animal procedures is licensed, was also discussed in detail by the IME Working Party, and workable schemes for conducting the assessment and weighing were proposed (Boyd & Smith 1991).

Two of the conclusions of the Working Party were that 'judgements about the likely benefits of particular projects should be made by the scientific community in a dialogue with informed public opinion', and that 'any judgement that the use of animals is necessary should be regarded as one which may change over time and with scientific advance'.

The current situation is very unsatisfactory. Criticism by outsiders is often dismissed by some members of the scientific community as ill-informed and malicious in its intent. Some defenders of animal experimentation argue that even the indefensible must be defended, lest yielding ground in one case should lead to the uncontrolled penetration of criticism into animal-based research in general. Nevertheless, an RSPCA/FRAME survey of the use of non-human primates as laboratory animals in Great Britain during 1984–1988 (Hampson *et al.* 1990) raised a number of major causes for concern, which deserve consideration by those responsible for the operation of the 1986 Act.

The second of the Working Party's points is no less important, for some animal procedures are entrenched and seemingly protected from scrutiny, merely because

they are long-established in powerful institutions. This applies to much of the use of non-human primates in fundamental work on neurophysiology, behaviour and vision (Hampson *et al.* 1990).

It should also be recognized that research studies applied to major disease problems, such as cancer, Alzheimer's disease, etc., do not become justified merely because of the seriousness of the problems they are designed to address. If they are to be allowed to proceed, convincing evidence of a high quality of working hypothesis, adequate experience of the personnel involved, and a sufficient likelihood of a successful outcome, should first be provided. The same requirement for scientific justification should be applied to all research proposals which might lead to suffering in laboratory animals. It is vital that particular attention is paid to proposals to develop and use transgenic animals as models for human disease (Lathe & Mullins 1993). This rapidly-developing field affords great scientific opportunities, but also threatens to greatly increase laboratory animal suffering.

It is worth noting that, as the OTA report pointed out (Anon 1986b), behavioural research represents a rather special case, since it is very likely to require the whole organism, so the use of animal models is unavoidable. However, commenting on the relevant sections of the OTA report's supporting documents, Drewett (1987) concluded that it is possible to be too pessimistic—far more could be done by direct non-invasive studies on humans, rather than by seeking to model every human predicament in animals.

Since 1959, the trend in fundamental biomedical research and in much applied research has continued to be away from the use of the whole animal, largely because of the development of the theories and techniques of molecular and cell biology. Reduction and replacement have rarely been the primary objectives of those taking part in these developments, but they have been secondary benefits. These modern techniques can often be applied to human material, so the problems of species

differences and the uncertainty of animal-human extrapolation can then be avoided.

Animal experiments and education

Animal experimentation for educational purposes is far more acceptable in the USA than in Europe, and a great deal of suffering has undoubtedly been caused to animals by pre-college students in the USA (Orlans 1985, Morton 1987). This is unjustifiable on *any* grounds, be they educational, scientific or ethical (Balls 1987). In Britain, experiments on animals in secondary schools were illegal under the terms of the 1876 Act and continue to be so under the 1986 Act. The main question in Britain is whether undergraduate students should be permitted, let alone required, to carry out regulated procedures on animals.

There is much debate about whether first-degree animal physiologists and pharmacologists can be adequately trained without conducting some experiments on animals, but a powerful case can be made in support of the view that such studies should not be required, but restricted to optional parts of the undergraduate course or to post-graduate courses, which would involve participation in the research programmes of experienced laboratory animal scientists. Much can be now achieved through the use of computer models and the use of non-invasive techniques with human volunteers.

On the whole, the use of *in vitro* systems in undergraduate education appears to be neglected, as is consideration of the ethical issues raised by animal experimentation. Two trends are worth noting. Firstly, there is increasing unwillingness among young people to conduct animal experiments, combined with an increasing concern about the issue, albeit all-too-often based on propaganda received from organizations representative of rather extreme positions. FRAME has tried to address this particular problem, by collaborating with Hobsons Publishing in the production of the most objective discussions of the issues that we could manage to write (Balls *et al.* 1992, Fentem *et al.* 1993).

Secondly, there has been a dramatic fall in the use of regulated animal procedures in undergraduate teaching in the UK, partly because of the high cost of the animals, and partly because of the cost and administrative inconvenience of having to apply for large numbers of personal licences under the 1986 Act.

Ethical aspects of the use of animals in education and training were discussed in detail in *Lives in the Balance* (Boyd & Smith 1991)—the report of the Institute of Medical Ethics (IME) Working Party referred to earlier.

There is, of course, another aspect to this part of the subject, namely the education of the individuals who will be in any way involved in animal experimentation, including animal caretakers, animal welfare officers, scientists and those with administrative responsibilities (de Greeve *et al.* 1993). It is to these individuals that van Zutphen *et al.* (1993) have addressed their recent book, entitled *Principles of Laboratory Animal Science*.

There is another fundamental issue here, namely, whether or not it should be permissible for laboratory animals to be used for training in the application of procedures likely to cause pain or distress. In Britain, such use of animals continues to be illegal under the terms of the 1986 Act, as it was under the 1876 Act. There has been no significant demand for this restriction to be removed, nor is there any evidence that those who work with animals in Britain are less skilled than their equivalents in any other countries or that animal suffering is increased as a consequence.

Animal use in testing

The use of animals in the basic science of toxicology is, on the whole, similar to animal use in other kinds of fundamental biomedical research. Toxicity testing, however, represents a special case, for two main reasons. Firstly, as practised, the induction of adverse effects, and even of considerable suffering, is often integral to the procedure and is therefore unavoidable.

Secondly, the application of such procedures is often required (or at least, perceived to be required) by national and/or international legislation and/or regulatory guidelines. Thus, the application of the Three Rs principles to toxicity testing, as is required by other laws, represents a considerable difficulty for all concerned, be they politicians, regulators, toxicologists, lawyers, or scientists committed to the development of relevant and reliable non-animal tests.

Toxicity testing is also special in other ways. The maintenance of the *status quo* is backed by enormous vested interests—in the regulatory authorities, in industry, in academia, and in contract testing establishments. In addition, the weak scientific basis of many current practices in the dominant 'check-list' approach to testing has repeatedly been questioned, not only by animal welfarists, but also by toxicologists themselves, both independently (e.g. Zbinden 1988, Heywood 1990, McLean 1991, Roe 1991) and through group discussions such as those of the FRAME Toxicity Committee (Balls *et al.* 1983, 1991). That the present unsatisfactory situation is tolerated and progress on scientific grounds, let alone in terms of animal welfare, is so difficult to achieve, testifies to the power and pervasive influence of the toxicity testing industry and its stout defence of the *status quo*, which is based partly on inertia and partly on self-interest.

Those who seek genuine progress toward better safety evaluation through improvement of the scientific basis of toxicity testing, not least through the development of replacement alternative methods, are frequently cautioned 'to be more realistic in identifying attainable targets for the science of toxicology' (e.g. Botham & Purchase 1992). Being realistic means facing up to the barriers to the acceptance of alternatives that must be overcome. These barriers are many and varied, and they have recently been said to include (Clark 1994):

1. *The validation barrier*: the method must be adequately validated using a wide range of chemical types.
2. *The scientific barrier*: the method must be based on good science and must not make extravagant claims that run counter to common sense.
3. *The legislative barrier*: since many toxicological tests are undertaken for legislative purposes, it is pointless to replace them unless the legislation requiring the tests can first be changed.
4. *The development barrier*: some toxicologists may not accept methods unless they have been developed in their own laboratories—the 'not invented here' syndrome.
5. *The psychological barrier*: some traditional scientists may feel their careers to be threatened by the introduction of new methodology and techniques.
6. *The fear of litigation barrier*: manufacturers are required by specific or general laws to make their products as 'safe' as possible, and, in the case of litigation, the plaintiff's lawyers would point to current testing practice as the standard to be met.
7. *The regulatory barrier*: the attitudes of government regulators reflect their burden of responsibility and accountability.

Up to now, despite the expectations of many of those who have supported the new legislation in the face of accusations from antivivisectionists that is only a pro-vivisection whitewash, the impact of the new controls on animal experimentation on routine toxicity testing has been very disappointing. For example, the promised effect of the project licensing system of the British 1986 Act, whereby 'every animal experiment must be justified' (Balls 1992a) has not been achieved, especially in relation to acute and chronic toxicity tests on food additives, industrial chemicals and environmental pollution. While there has been a 19.3% fall in animal procedures as a whole, conducted in the UK between 1987 (the first year of application of the 1986 Act) and 1992 (the most recent year for which statistics are available), the number of procedures conducted for non-medicinal safety assessment purposes has risen by

Table 1 Trends in non-medical toxicity test/safety assessment procedures in Great Britain, 1987 and 1992¹

Types of materials tested	Procedures (thousands)		
	1987	1992	% change
Agricultural chemicals	77.5	77.0	-0.6
Industrial chemicals	70.3	91.8	+30.6
Environmental pollutants	28.2	52.2	-84.8
Cosmetics and toiletries	14.5	2.2	-84.8
Household materials	6.9	2.1	-69.6
Alcohol-related	3.7	1.1	-70.3
Food additives	3.3	6.1	+84.8
Tobacco-related	1.3	0.2	-84.6
Other	37.9	19.0	-49.9
Total	243.6	258.6	-6.2
(Total—all procedures)	3,631.4	2,929.3	-19.3

¹Source: *Statistics of Scientific Procedures on Living Animals—Great Britain, 1987 and 1992*. London: HMSO, (Anon 1988a, 1983b)

6.2% (Table 1). There were significant increases in tests on industrial chemicals and food additives, and in connection with environmental pollution. There has been a 7.6% fall in toxicity tests as a whole, but formal LD50/LC50 tests have increased by 37.6% (Table 2). Why have no greater changes been achieved?

The blame cannot all be laid at the door of toxicologists who are content to conduct animal tests in the traditional manner. Those who apply the new animal protection legislation, perhaps too ready to be influenced by their expert toxicologist advisers, have tended to see laws such as the 1986 Act and the 1986 EC Directive as subservient to all the other legislation which requires (or appears to require) conventional check-list testing (Balls 1992a). Also, those seeking replacement alternative tests have carried out too many studies which were poorly focused and/or poorly conducted (Balls 1992b, Flint 1992, Balls & Fentem 1992). However, another serious problem is the rather poor quality of the studies so far carried out in order to evaluate the validity (i.e. the relevance and reliability) of the new methods.

Table 2 Trends in testing in Great Britain, 1987 and 1992¹

Types of procedure	Procedures (thousands)		
	1987	1992	% change
Efficacy evaluation	905.6	704.5	-22.2
Distribution and metabolism	104.1	80.7	-22.5
Nutritional evaluation	40.7	19.6	-51.8
Other non-toxicity tests	2,001.5	1,585.1	-20.8
Toxicity tests (total):	582.3	538.3	-7.6
—acute and sub-acute limit tests	122.5	98.8	-19.3
—formal LD50/LC50 tests	111.3	153.2	+37.6
—acute and sub-acute non-lethal tests	166.1	112.8	-32.1
—tests for clinical signs in eye	5.7	3.4	-41.2
—tests for clinical signs on skin	44.2	20.5	-53.6
—chronic whole-body tests	98.9	110.1	+11.3
—teratogen/mutagen tests	33.6	39.6	-17.8

¹Source: *Statistics of Scientific Procedures on Living Animals—Great Britain, 1987 and 1992*. London: HMSO, (Anon 1988a, 1993b).

Validation

Validation is difficult and the hurdles placed in the path of the replacement alternatives must be high, if mistakes are to be avoided, which could have disastrous consequences and thus delay the achievement of our objectives (Balls 1991a, 1992c). However these hurdles must be fair, especially as the animal tests we are seeking to replace have not themselves been subjected to formal, independent and objective evaluation in terms of their relevance, reliability and applicability to the questions which ought to be being asked in regulatory toxicology.

Validation should be seen as a continuous process, and the principles involved and the correct practices to be followed are still being debated (Balls 1992b, c, Balls *et al.* 1990a). Indeed, validation studies can be classified into a number of types, based on what appear to be their objectives, some of which are more laudable than others (Balls 1992b):

1. *Scientific validation*, which is principally concerned with assessing the relevance and reproducibility of a test or battery of tests for particular purposes, to the satisfaction of our scientific peers.
2. *Political validation*, which involves more laboratories and countries than are scientifically necessary for an interlaboratory study, in an attempt to overcome the 'not-invented-here' syndrome as a block to acceptance.
3. *Commercial validation*, which is aimed at establishing a commercial test to the satisfaction of its potential market.
4. *Screening/adjunct validation*, which is designed to show progressive animal toxicologists how *in vitro* methods can be used before animal studies, to assist in their design (e.g. in dose selection), or alongside them (to provide complementary information).
5. *Replacement validation*, which is for the purpose of convincing regulators that a test, battery or testing strategy could be used in place of the currently-accepted test, without compromising the hazard prediction and risk assessment needed to satisfy regulatory requirements.
6. *In-house validation*, which is widely practised by individual industrial companies, where scientists subject large numbers of candidate compounds to screening tests to the satisfaction of their managers, for the selection of a small number of compounds for further, usually animal, tests. The results of such studies, and even the methods employed, are rarely published in the peer-review literature.
7. *Public relations validation*, usually conducted not by, but on behalf of, industrial companies, in order to convince their animal rightist critics that they are actively seeking alternatives. Meanwhile, the animal testing continues.
8. *Pole vault validation*, which is desired by some regulators, and others with a vested interest in maintaining the *status quo*, to set almost impossible challenges, in order to prevent an *in*

vitro test ever being shown to be acceptable as a replacement for the animal tests to which they have long been accustomed.

Clark (1994) emphasizes the importance of scientific credibility and validation as the main barriers to be overcome along the road to acceptance. By the same token, *scientific* validation is by far the most important type of validation.

One of the greatest problems in planning validation studies is finding *in vivo* data of sufficient quality for use in evaluating the predictive value of the results obtained in *in vitro* tests. This has led to recommendations that an International Reference Chemical Data Bank be established (Balls *et al.* 1990a, b, Purchase 1990), to provide open-access listings of scientifically selected chemicals, backed by first-class toxicological data reviews, safety advice and a source of chemicals of known purity. ECETOC has established a task force for providing chemicals for use in validation studies on alternatives to the Draize eye irritation test (Anon 1988b, Bagley *et al.* 1992).

The high fidelity fallacy as a barrier to acceptance

One of the most fascinating sections of Russell and Burch's book is their discussion on the relative merits of fidelity and discrimination models. Fidelity models, such as are exemplified by the use of rodents and other laboratory mammals in toxicity testing, are used, because, 'in their general physiological and pharmacological properties', they are 'more consistently like us than are other organisms'. Discrimination models, on the other hand, 'reproduce one particular property of the original, in which we happen to be interested'. The use of discrimination models in toxicity testing, for example, is represented by the currently-available *in vitro* systems and other replacement alternatives, which are more suitable for answering a specific question about the mechanism of a toxic effect or toxic response in a particular cell

type than answering the more general question: 'Is this chemical likely to be toxic, in ways which we cannot envisage?'

Russell and Burch warn us of the high fidelity fallacy and of the danger of expecting discrimination in particular circumstances from models which show high fidelity in other, more general, terms. They point out that the fidelity of mammals as models for man is greatly overestimated, and conclude that the assumption that 'mammals are always the best models' for man 'is maintained with special stubbornness in some special fields (such as that of toxicity testing)'. They go on to say that the most important consequence of the high-fidelity myth is that it 'ignores all the advantages of *correlation*', whereby 'the responses of two utterly different systems may be correlated with perfect regularity', despite other differences between them. The argument about fidelity, discrimination and correlation test systems is still going on today, e.g. in the pages of recent issues of *ATLA* (Balls 1992b, Flint 1992). What Russell and Burch said about the emotional weight acquired by the high fidelity fallacy when the demands of public health and safety are involved, remains as true today as it did in the 1950s!

Thus, the demand for realism can justifiably be made of those who advocate the continued reliance of biomedical research and testing on high-fidelity laboratory animal models, just as it is required of those who promote the development and use of more-discriminating replacement alternatives.

This is not just a philosophical issue, for those who are involved in the planning of validation studies have to consider whether it is right to use laboratory animal test data as the 'true' standard against which the performance of potential replacement alternatives should be judged, given our knowledge of the limited usefulness of the animal tests and of the predictions they provide. Most of us would probably agree that it is *not right*, but that there is no other way that would gain the approval of animal-test toxicologists and regulators.

The role of ECVAM

The European Centre for the Validation of Alternative Methods (ECVAM) has recently been set up as part of the Environment Institute of the EC Joint Research Centre (JRC), at Ispra, near Lake Maggiore, in Italy, as a result of a Communication from the Commission to the Council and the European Parliament in October 1991 (Anon 1991).

This Communication pointed to the requirement in *Article 23 of Directive 86/609/EEC* that:

The Commission and Member States should encourage research into the development and validation of alternative techniques, which could provide the same level of information as that obtained in experiments using animals, but which involve fewer animals or which entail less painful procedures, and shall take such other steps as they consider appropriate to encourage research in this field. The Commission and Member States shall monitor trends in experimental methods.

The Communication spelled out ECVAM's duties in the following terms. ECVAM was to be set up:

1. To coordinate the validation of alternative test methods at Community level. This will involve the specification of test protocols, the organization of ring-test exercises, the choice of chemicals to be used in these tests, and the analysis and evaluation of the results, etc.
2. To act as a focal point for the exchange of information on the development of alternative test methods.
3. To set up, maintain and manage a data base on alternative procedures, with associated user services.
4. To promote dialogue between legislators, industrial companies, biomedical scientists, consumer organizations and animal welfare groups, with a view to the development, validation and international recognition of alternative test methods.

The Communication went on to specify that suitably-qualified staff would be recruited for ECVAM, and that ECVAM would interact and collaborate with other parts of the JRC, but would have its own Scientific Advisory Committee, composed of individuals from the Member States, industry, the academic world and animal welfare organizations. In addition to the representatives of the 12 Member States, the Commission subsequently appointed to this Committee members selected from nominations made, by invitation, by COLIPA, ECETOC, EFPIA, ERGATT* and EUROGROUP for Animal Welfare.

The Head of ECVAM reports directly to a small Management Team, which includes representatives of the JRC and of DGXI*, the section of the Commission responsible for the implementation and administration of the provisions of *Directive 86/609/EEC*. To date, 3 meetings of the ECVAM Scientific Advisory Committee have taken place at Ispra, and the ECVAM Management Team has had 2 meetings. At its first meeting, the Scientific Advisory Committee re-defined the main goals of ECVAM, as follows:

ECVAM will promote the scientific and regulatory acceptance of alternative methods which are of importance to the biosciences and which reduce, refine or replace the use of laboratory animals.

Abbreviations not identified in the text: COLIPA: the European Cosmetic, Toiletry and Perfumery Association; DG: Directorate General of the Commission of the EC/EU—there are 23 of these departments of the Commission, plus a number of other services, such as the Consumer Policy Service (CPS); DGXI: the DG responsible for the Environment, Nuclear Safety and Civil Protection; DGXII: the DG responsible for Science, Research and Development and for the Commission's Joint Research Centre (JRC); EC: European Community or European Communities, now to be known as the European Union (EU); EEC: European Economic Community; ECETOC: the European Chemical Industry Ecology and Toxicology Centre; EFPIA: the European Federation of Pharmaceutical Industries Associations; ERGATT: the European Research Group for Alternatives in Toxicity Testing.

This emphasizes that ECVAM will not focus its attention merely on regulatory toxicity tests. An ambitious programme of activities is already under way. In addition, because the work of ECVAM will be of importance to other services of the Commission, an *Inter-DG Alternatives and Validation Discussion Group* is to be set up. Similarly, because not all the interested parties could possibly be represented on its Scientific Advisory Committee, ECVAM expects to establish a wide network of contacts with appropriate individuals and organizations, not only in Europe, but throughout the world.

ECVAM information services

ECVAM is already benefiting from collaboration with the Environmental Informatics Unit (another unit of the Environment Institute at Ispra) and from data banks which have previously been supported by DGXI, namely, the Galileo Data Bank, located at Pisa, Italy (which has an emphasis on data produced by *in vitro* toxicity tests), and the *INVITTOX* data bank, located at Nottingham, UK (which has an emphasis on *in vitro* test methods). ECVAM will also be collaborating with *ATLA (Alternatives to Animal Experiments)*, so that news of the Centre's activities and the availability of ECVAM's information services can be widely distributed within the scientific, industrial and animal welfare communities. We also anticipate having a close working relationship with the new European Chemicals Bureau, which is also being established at Ispra.

ECVAM workshops, task forces and symposia

One of ECVAM's first priorities must be to become well-informed about the current state-of-the-art of non-animal test development in relation to particular types of chemicals, types of products and potential toxic hazards. This will involve consultation, not least with DGXII, since we see a natural evolution from basic research, through pre-normative studies to pre-validation exercises, as a prelude to

formal, and rather costly, interlaboratory validation programmes.

The aim of the ECVAM Workshop series is to provide state-of-the-art reviews on selected areas of practical *in vitro* toxicology, pharmacology and biologicals and biomaterials testing. Particular emphasis will be placed on making recommendations about what further steps should be taken, in terms of development, pre-validation or formal validation, in order to facilitate the emergence of relevant and reliable procedures for introduction into regulatory testing, as screens, complementary methods or replacements for the currently-accepted animal procedures.

During 1993–94, ECVAM is inviting small groups of up to 15 experts to attend workshops on the following topics:

- the uses of cultured hepatocytes
- *in vitro* phototoxicity testing
- *in vitro* neurotoxicity testing
- *in vitro* corrosivity testing
- *in vitro* teratogenicity testing
- *in vitro* tests for dermal penetration
- *in vitro* tests for nephrotoxicity
- *in vitro* tests for respiratory toxicity
- *in vitro* tests for acute toxicity and their use in the classification and labelling of chemicals
- *in vitro* testing of surfactants and surfactant-based products
- alternatives in ecotoxicology
- a reassessment of the Amden validation principles (Balls *et al.* 1990a)
- vaccine potency testing *in vitro*
- *in vitro* tests and the quality control of hormones

The reports of ECVAM workshops will be published in *ATLA*, and by mid-1995 an extensive set of reviews and recommendations should be available, for use in guiding ECVAM's research and validation policies in the immediate future. Our strategy also involves the setting up of *ECVAM Task Forces* to focus on the achievement of narrowly-defined, specific goals. A number of task forces are already active, on selecting chemicals known to be phototoxic, for use in a forthcoming international validation study; on ways of

overcoming the scientific, legal, ethical, logistic and safety problems which currently limit the use of human tissues in *in vitro* studies; on the integrated use of QSAR and *in vitro* studies; on statistical comparisons of *in vivo* and *in vitro* data; and on the conflicting requirements of various EC directives. We see task forces as ways of implementing the recommendations of workshops in the planning of validation studies and of seeking acceptance of the outcome of successful validation studies.

ECVAM Symposia will deal with wider issues, and at present we have only one symposium under consideration—a revisitation of the principles of validation—to mark the opening of the new ECVAM building, in October 1994.

External and internal ECVAM projects

ECVAM will not merely be an information centre, but will have its own laboratories, which will be used for the development of new test methods, for participation in pre-validation studies and in formal validation programmes, and for training courses. This was welcomed by the ECVAM Scientific Advisory Committee at its first meeting.

We hope to have five laboratory-based teams by 1990, in addition to our statistical and information services. However, it is clear that ECVAM would never be able to provide expertise in all the different types of tests and areas of pharmacotoxicology for which the validation of alternative tests and test batteries will be necessary in the years to come. Collaboration with academic and industrial alternatives research laboratories in the Member States, and elsewhere, will therefore be essential. Such collaboration is already under way, and we are building on the investment made in the past by DGXI and DGXII. Thus, in 1993, in addition to our external contracts for information services, ECVAM will be funding pre-validation studies on *in vitro* phototoxicology, and *in vitro* neurotoxicology, on the relationship between *in vitro* cytotoxicology and acute lethal potency, on the further development of the ERGATT/CFM Integrated *In Vitro* Toxicity Testing Scheme (ECITTS, Walum

et al. 1992), and on the metabolizing capacity of permanent cell lines. In addition, ECVAM will be involved in four international interlaboratory validation studies, which are already under way or are shortly about to begin. These are:

1. A UK/EEC study on alternatives to the Draize eye irritancy test.
2. An EEC/BRIDGE study on the measuring inhibition of gap junction intercellular communication *in vitro* as a means of identifying tumour promoters.
3. A COLIPA/EEC study on *in vitro* tests for photoirritants.
4. A study on *in vitro* tests for skin corrosivity.

Practical work has already begun within ECVAM's temporary laboratories, since a number of experienced research workers and students have asked to be able to come to work with us. ECVAM will shortly be appointing two pharmacotoxicologists and a statistician, and hopes to build up a full-time staff of 30 by 1998.

Training programmes

Training will be a very important part of ECVAM's activities, if it is to succeed in its mission and contribute positively to the orderly development, validation and acceptance of alternative methods. We anticipate that ECVAM will participate in training programmes in two main ways.

First, we will want to contribute to general training programmes which emphasize the Three Rs approach to the proper regulation and supervision of laboratory animal procedures, as will be required in the Member States in compliance with *Directive 86/609/EEC*. It will be important that there is an international dimension to such courses, at least in terms of discussion of their purpose, scope and content. However, our main commitment will be to training in the principles and practice of validation, taking into account the many factors which must be considered when the reliability and relevance of a new method for a particular purpose or purposes are being assessed and challenged, e.g. in a blind trial. This will involve encouragement in

others of a high degree of realism, coupled with a determination to overcome the barriers to the acceptance of non-animal alternative procedures, which undoubtedly exist. The importance that these activities are conducted at the Community level will be emphasized, in view of the requirements of *Directive 86/609/EEC* and of other directives which require toxicity testing and/or safety assessment. Courses will also be run, in collaboration with other laboratories and organizations, to contribute to the general training of *in vitro* pharmacotoxicologists and others or to develop competence in the application of specific test protocols.

Other support to the Commission

ECVAM is not only concerned with replacement for any alternatives, but also with providing, at the request of DGXI, support in relation to the parts of *Directive 86/609/EEC* related to reduction and refinement. This support will include advice on the gathering and interpretation of statistical information on laboratory animal use in the Member States.

The sixth amendment to the EC Cosmetics Directive

Although only a relatively small number of animals are used each year specifically for testing cosmetic ingredients and products (Table 1), for more than a decade, the cosmetic industry has been singled out as a target for criticism by activists opposed to the use of laboratory animals in toxicity tests. This is partly because cosmetic products are perceived by some as 'unnecessary' luxuries, unlike the 'necessary' products of the pharmaceutical industry, where some use of animals is accepted as unavoidable, and partly because cosmetic manufacturers and retailers have a relatively high public profile. As a result, some cosmetic companies have ceased testing their products in animals and/or have tried to use ingredients not tested in animals at all or not tested after a certain date. Meanwhile, many of the leading cosmetic companies have invested heavily in the search for relevant and reliable

non-animal tests, either in their own laboratories, or via donations to organizations such as CAAT and FRAME. There has also been increasing public pressure to ban cosmetic tests on animals, but legislators are also mindful of public expectation that the workplace will be a safe environment and that products sold to consumers will be safe for them to use.

On 14 June 1993, the Council of Ministers approved a Sixth Amendment to *Directive 76/768/EEC*, known as the Cosmetics Directive (Anon 1976, 1993c). This Amendment has immense implications for animal testing and replacement alternatives in general, as well as for the cosmetics industry, in particular. Its approval follows nearly three years of intense discussion within the Commission, in the European Parliament and in the Member States as well as in the industry and the animal welfare community.

The *Preamble* to the Sixth Amendment includes the following background statements:

Whereas assessment of the safety of use of the ingredients employed in cosmetics and of the final product should take account of the requirements of *Directive 86/609/EEC*, which concerns the protection of animals used for experimental and other scientific purposes, and in particular Article 7 thereof; Whereas testing on animals or ingredients or combinations of ingredients should be banned as from 1 January 1998; whereas, however, that date should be postponed where alternative methods of testing have not been scientifically validated; whereas the Commission should submit a report on progress made with regard to such methods. . . .

Three sections of the amended Directive are particularly important:

Article 3(i)(para2): If there has been insufficient progress in developing satisfactory methods to replace animal testing, and in particular, in those cases where alternative methods of testing, despite all reasonable endeavours, have not been scientifically

validated as offering an equivalent level of protection for the consumer, taking into account OECD toxicity test guidelines, the Commission shall, by 1 January 1997, submit draft measures to postpone the date of implementation of this provision, for a sufficient period, and in any case for no less than two years, in accordance with the procedure laid down in Article 10. Before submitting such measures, the Commission will consult the Scientific Committee on Cosmetology.

Article 3(i)(para 3): The Commission shall present an annual report to the European Parliament and the Council on progress in the development, validation and legal acceptance of alternative methods to those involving experiments on animals. That report shall contain precise data on the number and type of experiments relating to cosmetic products carried out on animals. The Member States shall be obliged to collect that information, in addition to collecting statistics as laid down by *Directive 86/609/EEC* on the protection of animals used for experimental and other scientific purposes. The Commission shall in particular ensure the development, validation and legal acceptance of experimental methods which do not use live animals.

Article 6(3): (Member States shall take all measures necessary to ensure that, in the labelling, putting up for sale and advertising of cosmetic products, text, names and trade marks, pictures and figurative or other signs are not used to imply that these products have characteristics which they do not have.) Furthermore, any reference to testing on animals must state clearly whether the tests carried out involve the finished product and/or its ingredients.

The Sixth Amendment provides all concerned with both a challenge and an opportunity. If we are good enough, we will be able to make sound and unquestionable progress toward the provision of relevant and reliable, and therefore acceptable, alternatives, by January 1977. However, the new version of the Directive being a mere product of political negotiations,

might be open to various interpretations. However, it is clear that the intention is not to require an all-or-nothing complete set of acceptable alternatives by 1 January 1997, but a step-by-step, test-by-test, problem-by-problem approach. For example, it would be absurd not to replace the Draize eye irritancy test, if it were not yet possible to replace animal skin sensitization tests. Similarly, it would be absurd not to replace the Draize eye test for surfactant-based products, if the alternative methods concerned could not deal adequately with preservatives. Finally, it would surely be advisable to also take levels of toxic potential into account—not to accept a test for severe irritants, merely because it could not distinguish between mild and moderate irritants, would itself be unacceptable. It should, however, be noted that the cosmetic industry has not hitherto been in favour of a product-by-product approach.

Article 6(3) is particularly interesting, and it has important implications for 'cruelty-free' labelling and other appeals to the sympathies of animal lovers in cosmetic product marketing. It means that:

1. There are no limits in terms of the *time* since animal tests were carried out.
2. There is no exclusion according to the original *purpose* of any such animal testing.
3. There is no exclusion according to the *parties* who conducted the animal test.

Therefore, a 'not tested on animals' label will *not* be permissible, if *any* of the ingredients in a product have been tested *ever*, for *any* purpose, by *anybody*. This will, in particular, make life difficult for supporters of the 'five-year rolling rule', whereby a cosmetic ingredient becomes acceptable for use in a 'cruelty-free' product, five years after it was last tested in animals (Balls 1991b).

Finally, the importance of securing the regulatory acceptance of non-animal tests and testing strategies cannot be overemphasized. The Commission has spelled it out like this in a report to the Council (Anon 1988c):

The Commission recognizes that a critical

stage in the development of an alternative method is the transition from that of a potentially useful procedure to that of a method accepted as part of a regulatory testing system.

In the case of cosmetics testing, this will involve discussions involving various parties, including ECVAM, the EC Consumer Policy Service, the Scientific Committee on Cosmetology, COLIPA and its member associations and leading cosmetic companies, DGXI, the Member States, and, eventually, the OECD.

Concluding remarks

The widespread acceptance of the Three Rs concept, and its inclusion as the basis of new legislation on animal experimentation, must be regarded as one of the great achievements of the 1970s and 1980s. Although the impact of changing attitudes and practices, at least in relation to the replacement of animal procedures, is more marked in education and training and in fundamental research than in applied research, and especially in toxicity testing, there are causes for optimism.

The introduction of non-animal procedures of various kinds has led to a *reduction* in the numbers of animals used in many aspects of toxicity testing and safety assessment, and also to *refinement*, in the sense that such animal tests as are carried out will often involve only mild effects, as highly toxic materials will have been identified at earlier stages of testing. To cite one example, it has now been accepted that chemicals likely to be corrosive to the skin can be identified as such in non-animal procedures, so animal testing is not required. Animal testing for skin irritation may be still required, however, for chemicals not identified as corrosive in the non-animal test. Full *replacement* of the animal test is still to be achieved, but there has been progress.

Gaining the replacement of animal tests is one of the main challenges of the 1990s (Anon 1990). Meanwhile, certain conclusions and recommendations can be made:

1. Research specifically aimed at the systemic and rational extension of replacement alternative models of all kinds should be recognized and supported as being a scientifically respectable and ethically laudable form of activity.
2. Long-term vision should be recognized as being consistent with the achievement of short-term goals. For example, the conclusion of the FRAME Toxicity Committee that 'the replacement of all animal procedures in toxicity testing is a morally desirable and scientifically defensible long-term goal' (Balls *et al.* 1991) should not be seen as inconsistent with moves leading to reduction and refinement, e.g. replacing the LD50 with the Fixed Dose Procedure, reducing the number of animals required in a Draize eye irritancy test, or introducing non-animal methods as pre-screens before animal tests are conducted, or as adjunct methods to be performed alongside animal tests as a means of providing complementary information (Balls & Fentem 1992).
3. Far stricter justification of procedures involving living animals in fundamental biomedical research should be required, in line with the requirement that the benefits and suffering involved in such work must be assessed and weighed. The promise of intangible benefits based on the eventual, but undefined and unpredictable, application of new knowledge, should be judged inadequate, just as it would be if such a weak line of argument were put to a grant-giving body. By the same token, the granting of financial support should not be solely on the basis of scientific credibility, and grant-giving bodies should be more willing than at present to take animal welfare and the available, or need for, replacement alternatives into account.
4. More effort should be put into maximizing and realizing the potential value of non-animal methods in toxicity testing—the FRAME Toxicity Committee alone made 66 specific recommendations

to this end in its second report (Balls *et al.* 1991). However, for this to be achieved, those currently involved in the conduct of regulatory animal tests and in the interpretation and implementation of the data they provide, must be willing to be more realistic about the underlying scientific weakness of such animal tests, and more willing to actively consider the acceptance and adoption of alternative tests, test batteries and testing strategies. In addition, those of us who are committed to the search for replacement alternative tests must strive to be ever more inventive and to set ever higher standards in the development of new non-animal test procedures and in their validation.

5. The adoption of the Sixth Amendment to *Directive 76/768/EEC* should be seized as an opportunity to use the cosmetics testing issue to advance the cause of alternatives as a whole. The time for strident campaigns, simplistic slogans, confrontation and boycotts, should now come to an end. We can have all the changes we want, if we work together and if we are good enough as scientists to produce the relevant and reliable non-animal tests which are needed, and if the regulators are brave enough to look to high-quality and meaningful safety assessments in place of traditional and scientifically-dubious toxicity profiles, as a basis for their decisions.

References

- Anon (1876) *An Act to Amend the Law Relating to Cruelty to Animals*. London, HMSO: 39 & 40 Vict, CH 77, 8 pp
- Anon (1976) Council Directive 76/768/EEC of 27 July 1976 of the approximation of the laws of the Member States relating to cosmetic products. *Official Journal of the European Communities* **L262**, 169-72
- Anon (1983) *Animal Experimentation in the United Kingdom: Proposals Submitted to the Home Secretary by the BVA, CRAE and FRAME*. Edinburgh: CRAE, 8 pp
- Anon (1985) *Scientific Procedures on Living Animals*. Cmnd 9521. London: HMSO, 25 pp
- Anon (1986a) *Animals (Scientific Procedures) Act 1986*. London: HMSO, 24 pp

- Anon (1986b) *Alternatives to Animal Use in Research, Testing and Education*. Washington, DC: US Congress of Technology Assessment, 441 pp
- Anon (1986c) *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes*. Strasbourg: Council of Europe, 51 pp
- Anon (1986d) Council Directive of 24 November 1986 on the Approximation of Laws, Regulations and Administrative Provisions of the Member States Regarding the Protection of Animals Used for Experimental and Other Purposes. *Official Journal of the European Communities* **L262**, 1–29
- Anon (1988a) *Statistics of Scientific Procedures on Living Animals—Great Britain, 1987*. Cm 515. London: HMSO, 40 pp
- Anon (1988b) *ECETOC Monograph No. 11. Eye Irritation Testing*. Brussels: ECETOC, 64 pp
- Anon (1988c) *Report of the Commission to the Council*. COM (88), final, of 28 April 1988, point 23. Brussels: CEC
- Anon (1990) Replacing animal toxicity tests: the challenge of the 1990s. *Alternatives to Laboratory Animals* **18**, 1–2
- Anon (1991) Establishment of a European Centre for the Validation of Alternative Methods (ECVAM). *Communication from the Commission to the Council and the European Parliament*. Brussels: Commission of the European Communities, 6 pp
- Anon (1993a) Cephalopods. *Alternatives to Laboratory Animals* **21**, 4
- Anon (1993b) *Statistics of Scientific Procedures on Living Animals—Great Britain, 1992*. Cm 2356. London: HMSO, 49 pp
- Anon (1993c) Council Directive 93/35/EEC, amending for the sixth time Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products. *Official Journal of the European Communities* **L151**, 32–6
- Bagley DM, Botham PA, Gardner JR *et al.* (1992) Eye irritation: reference chemicals data bank. *Toxicology in Vitro* **6**, 487–91
- Balls M (1983) Alternatives to animal experimentation. *Alternatives to Laboratory Animals* **11**, 56–62
- Balls M (ed) (1987) The US Congress Office of Technology Assessment Report on *Alternatives to Animal Use in Research, Testing and Education*. *Alternatives to Laboratory Animals* **14**, 289–374
- Balls M (1991a) Why modification of the LD50 will not be enough. *Laboratory Animals* **25**, 198–206
- Balls M (1991b) On establishing the safety of cosmetic ingredients and products produced and/or marketed within the European Economic Community. *Alternatives to Laboratory Animals* **19**, 237–44
- Balls M (1992a) Time to reform toxic tests. *New Scientist* **134** (1819), 31–3
- Balls M (1992b) *In vitro* test validation: high hurdling but not pole vaulting. *Alternatives to Laboratory Animals* **20**, 355–7
- Balls M (1992c) The validation and acceptance of *in vitro* toxicity tests. In: *In Vitro Methods in Toxicology* (Jolles G, ed.). London: Academic Press, pp 511–19
- Balls M, Blaauboer B, Brusick D *et al.* (1990a) Report and recommendations of the CAAT/ERGATT workshop on the validation of toxicity test procedures. *Alternatives to Laboratory Animals* **18**, 313–37
- Balls M, Botham P, Cordier A *et al.* (1990b) Report and recommendations of an international workshop on promotion of the regulatory acceptance of validated non-animal toxicity test procedures. *Alternatives to Laboratory Animals* **18**, 339–44
- Balls M, Bridges J, Southee J (eds) (1991) *Animals and Alternatives in Toxicology: Present State and Future Prospects*. London: Macmillan, 390 pp
- Balls M, Fentem JH (1992) The use of basal cytotoxicity and target organ toxicity tests in hazard identification and risk assessment. *Alternatives to Laboratory Animals* **20**, 368–88
- Balls M, Fentem J, Joint E (1992) *Animal Experiments*. Cambridge: Hobsons, 16 pp
- Balls M, Riddell RJ, Worden AN (eds) (1983) *Animals and Alternatives in Toxicity Testing*. London: Academic Press, 550 pp
- Botham P, Purchase I (1992) Why laboratory rats are here to stay. *New Scientist* **134** (1819), 29–30
- Boyd KM, Smith JA (eds) (1991) *Lives in the Balance: The Ethics of Using Animals in Biomedical Research*. Oxford: Oxford University Press, 352 pp
- Clark DG (1994) Barriers to the acceptance of *in vitro* alternatives. *Toxicology in Vitro*, in press
- Drewett RF (1987) Alternatives to the use of animals in behavioural research. *Alternatives to Laboratory Animals* **14**, 312–17
- Fentem J, Balls M, Robinson P (1993) *Developing Alternatives to Animal Experimentation*. Cambridge: Hobsons, 20 pp
- Flint O (1992) *In vitro* test validation: a house built on sand. *Alternatives to Laboratory Animals* **20**, 196–8
- Frazier JF, Goldberg AM (1990) Alternatives to and reduction of animal use in biomedical research, education and testing. *Alternatives to Laboratory Animals* **18**, 65–74
- de Greeve PCM, Hampson J, van Zutphen LFN (1993) Legislation and animal experimentation. In: *Principles of Laboratory Animal Science* (van Zutphen LFM, Baumans V, Beynen AC, eds). Amsterdam: Elsevier, pp 9–16
- Hampson J, Southee J, Howell D, Balls M (1990) An RSPCA/FAME survey on the use of non-human primates as laboratory animals in Great Britain, 1984–1988. *Alternatives to Laboratory Animals* **17**, 335–400

- Heywood R (1990) Handbook of *in vitro* toxicity testing. *Alternatives to Laboratory Animals* **18**, 354–5
- Lathe R, Mullins JJ (1993) Transgenic animals as models for human disease—report of an EC Study Group. *Transgenic Research* **2**, 286–99
- McLean A (1991) Concluding remarks. In: *Animals and Alternatives in Toxicology: Present Status and Future Prospects* (Balls M, Bridges J, Southee J, eds). London: Macmillan, pp 341–4
- Morton DB (1987) Animal use in education and the alternatives. *Alternatives to Laboratory Animals* **14**, 334–43
- Orlans FB (1985) Science fairs—a sampling of misguided experiments. *Animal Welfare Institute Quarterly* **34**, 108–11
- Purchase IFH (1990) An International Reference Chemical Data Bank would accelerate the development, validation and regulatory acceptance of alternative toxicology tests. *Alternatives to Laboratory Animals* **18**, 345–8
- Roe FJC (1991) Alternatives to Laboratory Animals Volume 18. *Human and Experimental Toxicology* **10**, 296
- Russell WMS, Burch RL (1959) *The Principles of Humane Experimental Technique*. London: Methuen, 238 pp
- Russell WMS, Burch RL (1992) *The Principles of Humane Experimental Technique*. South Mimms: UFAW, 238 pp
- Smyth D (1978) *Alternatives to Animal Experiments*. London: Scholar Press, 218 pp
- UFAW (1957) Symposium on Humane Technique in the Laboratory. *Collected Papers Laboratory Animal Bureau* **6**, 5–81
- Walum E, Balls M, Bianchi V *et al.* (1992) ECITTS: an integrated approach to the application of *in vitro* test systems to the hazard assessment of chemicals. *Alternatives to Laboratory Animals* **20**, 406–28
- Zbinden G (1988) A look behind drug regulatory guidelines. In: *National and International Drug Safety Guidelines* (Adler S, Zbinden G, eds). Zollikon: MTC Verlag, pp 7–19
- van Zutphen LFM, Baumans V, Beynen AC (1993) *Principles of Laboratory Animal Science*. Amsterdam: Elsevier, 389 pp