

Some Reflections on the Fourth World  
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World Congresses can be especially valuable when, apart from formal platform presentations, they provide opportunities for discussions during workshop and poster sessions, and ad hoc meetings during refreshment breaks and social events. Also, the wide range of participants present and the variety of topics covered often provide contacts, insights, and overall impressions that are far more important than the facts presented in the more-formal lectures.

Of course, much of this is lost to Congress participants because of the need for parallel sessions and, perhaps more seriously, to those who did not—for one reason or another—attend the Congress at all. That is why the following set of personal recollections has been compiled in the hope that some of them will trigger new ideas or encourage new activities in support of the 3Rs.

The author of each section is indicated. In one or two cases, there are two comments on the same lecture or session. (Michael Balls)

## PLENARY LECTURES

Ray Tennant, Director of the U.S. National Center for Toxicogenomics, recently established at the National Institute of Environmental Health Sciences (NIEHS) site at Research Triangle Park in North Carolina, gave a talk on Toxicogenomics. He described the use of microarray technology, whereby differential gene expression can be measured simultaneously in several thousand genes by using microchips on which are situated probes of complementary cDNA molecules immobilized in predetermined arrays. To determine differences in gene expression, RNA extracted from the test and control samples is converted into DNA, which is labeled. The label incorporated is usually fluorescent, and it is possible to have differently colored spots according to whether there has been a decrease, an increase, or no change in gene expression. Tennant explained the rationale for using the aforementioned technology in toxicology: Any toxic insult that induces a response or change in cells will elicit a change in gene expression. However, he also explained that considerable time will elapse before toxicogenomics will be used routinely in toxicity testing. This is because of the need to overcome technical problems such as data interpretation and lack of reproducibility and to check the relevance of the gene expression patterns detected with respect to normal toxicity endpoints.

Charles DeLisi (Centre for Advanced Genomic Technology, Boston, MA) spoke on the application of computers to understanding human biology. He explained that computational approaches will continue to accelerate, especially to enable scientists to cope with increasing amounts of information that are being obtained more rapidly from activities such as the human genome mapping project and the use of microarrays. DeLisi also described his own work on developing a virtual human model that could be used to predict the effects of external exposures on physiology and disease.

Ian Kimber (Syngenta, Macclesfield, England) gave a comprehensive and clear exposition of the status of alternative methods for skin sensitization testing, focusing on the development, validation, and acceptance of the local lymph node assay (LLNA) as an alternative to the standard guinea pig tests. The LLNA involves less stressful procedures because it measures the initiation, not the expression, of an immune response; also, it uses fewer nonhuman animals.

Having dealt with this example of reduction and refinement, Kimber proceeded to discuss recent advances in the use of *in vitro* models of sensitization that might contribute to the search for replacement alternative methods for this important and irreversible toxicity endpoint. There is now greater understanding of the molecular processes involved in sensitization, including the roles of certain cytokines, and there has been progress in the culturing of human dendritic (antigen presenting) cells.

In the meantime, much can be achieved by using a hierarchical approach to sensitization testing and by using expert systems for identifying structurally alerting features on molecules for protein binding, coupled with the assessment of *in vitro* percutaneous absorption. (Robert Combes)

#### SLIDES SHOWN

The refinement theme of the Congress was to have been augmented by a plenary lecture from Margaret Rose (University of New South Wales, Randwick, Australia). Unfortunately, Rose was unwell and could not give the lecture; therefore, her slides were presented for her. They showed how refinement could influence—sometimes in surprising ways—the scientific data being collected. Handling, restraint, method of euthanasia, social isolation, and type of cage: All are factors that can affect scientific measures such as clearance rates of chemicals and drugs, body weight gain, central nervous system AMP levels, neuropeptide binding, blood pressure, hormone levels, and nociceptive thresholds. Habituation to a procedure was always worthwhile. In conclusion, it was noted that there was substantive evidence for a reciprocal relation between animals' experiences of their environment and their general well-being and the scientific outcome. She also made the point that our understanding of animal well-being needs to be informed by science and that this information would affect our perception of animals and influence public policy. (David Morton)

#### THREE LECTURES IN CONTRAST

The lecture by Jean Fleming (University of Otago, Dunedin, New Zealand) on "Ethical, Cultural and Spiritual Objections to Genetically Modified Organisms—a New Zealand Process and Perspective," was absolutely fascinating and very important. I look forward to reading the full text in the Congress proceed-

ings. Fleming reported on the results of the work of the New Zealand Royal Commission on Genetic Modification, which held hearings over 14 months and received more than 10,000 written submissions that revealed deep distrust of scientists and biotechnology companies and strong objections to interference with nature, reflecting the diverse origins of the current citizens of New Zealand.

By contrast, I found the lecture by DeLisi to be a chilling experience, as he foresaw a day when human biology would be fully understood and totally open to control and exploitation. At the time, I found myself thinking that the promotion of such goals was totally out of place in a world when the vast majority of our fellow citizens do not have sufficient food to eat or access to clean water to drink and have to live lives ravaged by easily treated diseases. As I write this, these thoughts rise up again at a time when the ridiculous Raelians have claimed to have produced cloned humans and are touting their promise to give us eternal life (but who are the “us” to be?). I was also reminded of the oft-mentioned fourth “R”—responsibility.

Sanity was restored toward the end of the Congress by Kimber whose lecture on hypersensitivity testing was a masterly example of how good fundamental research can lead to new methodology in which the 3Rs can be applied for the benefit of humans and animals alike. (Michael Balls)

### THREE SESSIONS ON REDUCTION

Three sessions at the Congress were devoted largely or solely to reduction. The first was a workshop on “Statistical Reduction Strategies and Using In Vitro Data as Part of Reduction Strategies.”

Only 10 to 15 people attended, but they participated well in the discussion on the two papers presented. The first, by Michael Festing (Medical Research Council Toxicology Unit [MRC], Leicester, England), was titled “Good Experimental Design and Statistics Can Save Animals, But How Can It Be Promoted?” He reviewed the potential scope for reducing animal use as implied in the title and noted that it is difficult to pinpoint exactly who is responsible if published papers use animals inappropriately. Is it the responsibility of the funding organization, the scientist, the ethical review process, the regulators, or the journal editors?

The discussion generally concluded that funding organizations have a vital interest in good quality science but often do not have the appropriate machinery to look at the quality of the published work. In effect, they delegate to journal editors and referees the task of ensuring that the work has been well done. Journals differ in the quality of this refereeing, particularly with regard to statistical methods. Individual scientists have an interest in doing the work well but often are inadequately trained in experimental design and often have no access to statistical advice. Presumably, these deficiencies are the responsibility of the funding organizations who employ the scientists. Ethical reviewers potentially are interested be-

cause poor science leads to unnecessary suffering. However, they may not be well qualified to judge the quality of the science. Journal editors want good quality papers but are in the hands of their referees who, as noted previously, often are inadequately trained scientists. Therefore, it appears that the quality control of scientific experiments using animals may be deficient, with no one really in charge. The quickest way for an animal welfare organization to try to correct the situation probably would be to put pressure on the funding organizations.

The second paper by Raj Chhabra (NIEHS, Research Triangle Park, NC) was on the "Evaluation of Some In-Vitro Tests to Reduce and Replace the Sub-Acute Animal Toxicity Tests." An acute test to set dose levels followed by 90-day and 2-year tests in both rats and mice usually determines the toxic and carcinogenic potential of chemicals. However, the dose levels for the 2-year studies now are gleaned from the literature, and studies are being conducted to see whether in vitro tests could be used to set the dose levels for the 90-day studies. The greatest promise appears to be in the use of the in vitro tests for compounds delivered by the dermal route. Clearly, if this were successful, the elimination of the acute tests would save a useful number of animals and reduce overall suffering.

### Enthusiasm for Parallel Session

A parallel session on "Experimental Design: A Tool to Promote Refinement and Reduction" involved four papers. Although only 15 people attended, they clearly were enthusiastic about the potential of the approach. The first paper by Derek Fry (Home Office, London) on "Reduction by Well-Defined Objectives" made the point that in Europe there is a legal obligation to minimize animal use, and the experience of United Kingdom Home Office inspectors is that the studies often lack clear scientific objectives. Thus, careful consideration of the objectives of the study and how they might be achieved often could lead to a useful reduction, particularly for experiments that are to be repeated. A first step would be to optimize the method rather than just trying it out on a few animals. Even a small change in the definition of a suitable endpoint can bring about a useful reduction. Heavily disguised real examples illustrated the talk.

The second talk by Sylvia Vaughan (Fund for the Replacement of Animals in Medical Experiments [FRAME], Nottingham, England) on "Optimizing Resources by Reduction: The Work of the Frame Reduction Committee," described the work of this committee, which was established in 1998 following an European Centre for the Validation of Alternative Methods (ECVAM) workshop on reduction. Its members have given workshops at various scientific meetings, produced publications, developed a Web-based archive of useful material, and generally raised awareness of the need for reduction, in addition to the refinement and replacement of animal use. So far, however, this raised level of awareness is found

mainly in Europe. Only one of the nine papers in the reduction sessions at the Congress was from an American, and that was concerned mostly with replacement.

Henk van Wilgenberg (Academic Medical Centre, Amsterdam, The Netherlands) talked about "Computer Simulation for Improving the Precision of an Experiment." He and his colleagues have been developing an interactive computer program to assist people in the experimental design, with special reference to determining the optimum sample size. The value of this program will depend on how successfully it is distributed and used in teaching statistics to animal users.

Robert Shaw (AstraZeneca, Macclesfield, England) talked about "Reduction by Use of Factorial Experimental Design." Many animal experiments are performed repeatedly with only small variations in the treatments. For example, large numbers of potential new drugs may be screened in an animal model by using a standard experimental design in which the only change is the drug. If such designs can be optimized by selecting those conditions that maximize the response to a positive control, it should be possible to reduce the numbers of animals used in each screen without loss of information. Such optimization can be done using factorial experimental designs in which a wide range of conditions are compared in a single experiment. Having found the best conditions, future screens would use those conditions. The designs can be complex, particularly if there are a large number of factors such as age, sex, prior treatment, treatment route, and time after treatment. In such circumstances, advice from a statistician is strongly advised.

### A Workshop, Three Papers, and Discussion

A workshop on "Refinement and Reduction Through Design of Experiments" involved three papers and extensive discussion by an audience of approximately 10 to 15 people.

Ngairé Dennison (Rowett Research Institute, Bucksburn, England) gave a talk on a "Pilot Investigation of the Use of General Anesthesia to Refine the Statutory Mouse Bioassay for Paralytic Shellfish Poison," which was a pleasure to hear. Found in shellfish worldwide, this poison is potentially lethal. Shellfish extracts are injected into mice; if the poison is present, the mice will get convulsions and die. This is not a nice test, but no *in vitro* alternatives have been developed, partly because more than 20 poisons are involved and partly because little work has been done to develop an alternative. However, this pilot study investigated using anesthetized mice and found that the results correlated closely with results obtained using conscious mice. There was even some indication that using anesthetized mice gave better results. The studies are continuing under field conditions.

The next talk, on "Refinement and Reduction Through the Control of Variation," given by Michael Festing (MRC Toxicology Unit), reviewed how sample size could be reduced by controlling variation. In particular, he discussed the wide-

spread use of outbred animals, which many scientists seem to think are useful in research because they are genetically (and therefore phenotypically) variable. However, these scientists also seem to recognize that the use of phenotypically heterogeneous animals leads to experiments that lack statistical power. This results in the ludicrous situation in which scientists state that they want phenotypically variable animals and then select animals of a narrow weight range for their experiments—eliminating some of the phenotypic variation that they said they wanted.

The third paper by Derek Fry (Home Office) discussed the use of seminars and workshops to teach better experimental design. He gave several examples of the type of topic suitable for discussion. In studies where the endpoint is a change in body weight, large numbers of animals may be needed, using a between-animal experimental design in which animals are assigned to different treatment groups. However, if a within-animals design can be used in which each animal has a treatment for a period and then is switched to a different treatment, much smaller numbers can be used to achieve the same level of statistical precision. The important point is to get scientists to think about alternative ways of achieving their objectives in the most humane way.

### Poor Man of the 3Rs

My overall impression is that although there is enormous potential, reduction is the poor man of the 3Rs. There were no papers directly on this theme from anyone in North America, and the three sessions (all linked with “refinement”) were poorly attended. Members of the FRAME Reduction Committee gave more than half of the talks (6/11). The pharmaceutical industry appears to be making some effort to reduce the use of animals, probably because animal research is very expensive. However, academics continue to rely on the inefficient scientific refereeing of published papers to reduce animal use, and there is little incentive for individual scientists to improve the way they conduct research involving animals. (Michael Festing)

## WORKSHOP ON REDUCTION

“The Use of Dogs and Other Non-Rodents in Toxicity Testing” was the subject of a workshop cochaired by Horst Spielmann (German National Centre for the Documentation and Evaluation of Alternatives to Testing in Animals [ZEBET], Berlin, Germany) and Barry Phillips (Research Animals Department, Royal Society for Prevention of Cruelty to Animals [RSPCA], Horsham, England). Current regulatory testing requirements for pharmaceuticals and pesticides in partic-

ular stipulate that a rodent and a nonrodent species should be used. Over the years, the dog has become the second species routinely used; in many cases, regulatory agencies require justification only if the dog is not used, even if there might be very good scientific reasons why the dog is unsuitable. Other nonrodent species used include nonhuman primates and the mini-pig.

Few scientific investigations have been undertaken to investigate the necessity of the data obtained by using the dog for regulatory purposes. Moreover, there have been allegations that dogs are used primarily because they are easy to use and handle and because there is a large database in company and agency files relating to dog use.

Spielmann provided evidence from an analysis of regulatory data on 172 pesticide substances to show that chronic safety studies conducted on dogs do not provide any specific or unique information additional to that obtained from 26-week studies in the same species. This implies that perhaps chronic studies in dogs are scientifically unnecessary for the testing of pesticides.

Phillips described the interim results of a large interlaboratory collaborative study aimed at minimizing and refining the use of dogs for pharmaceutical safety testing, which is being co-organized by a steering group of industrial scientists in collaboration with FRAME and the RSPCA. This has resulted in the identification of several strategies both for making significant improvements in the ways in which dogs are used and for reducing the numbers used. He described in detail one of these strategies, the production of a best practice guide to study design. This guide is being written and includes information on group sizes, the use of control animals, the use of single sexes, and the determination of the maximum tolerated dose.

Last, Christa Sahlholm (Astra Zeneca, Macclesfield, England) discussed the criteria used to select an appropriate second species for the safety assessment of pharmaceuticals. She emphasized that the application of these criteria can be very complicated and must be approached on a case-by-case basis. Nevertheless, there needs to be more debate and discussion about the use of a second species and more flexibility in the approach and requirements of certain regulatory authorities. (Robert Combes)

## PLATFORM AND WORKSHOP SESSIONS ON REFINEMENT

The platform and workshop sessions on refinement covered topics relating to legislation, housing, training, assessing pain and distress, better methods of data collection with respect to both animal welfare and science, experimental design, and the benefits to animals from research on animals.

The platform session on legislation dealt with the recent revisions to Appendix A of the Council of Europe Convention ETS 123 on the care of animals in the laboratory. The discussion centered around whether they went far enough and how welfare could be improved within institutions. The sessions on novel methods looked at how they could affect welfare and science—often for the benefit of the animals—both in terms of numbers required and level or duration of suffering. These sessions also covered the development and implementation of humane endpoints and techniques such as molecular imaging, echocardiography, implanted telemeters, pain scoring, and the effects of habituation and acclimation of animals. Research benefiting animals had a distinctly antipodean flavor and addressed pest control strategies in which one animal species had to be killed for the sake of others (usually a plant or animal species and a countryside conservation issue) as well as considering the development of cures for animal diseases. The impact of barren housing and inappropriate social interactions on scientific data was an interesting session. It benefited from the contributions of ethologists who have not attended previous world congresses in the series, and some useful criticisms and different research strategies were discussed. This session complemented another on how to measure animal well-being, which also stimulated much interest and discussion.

The first workshop looked at ways of helping ethics committee members assess degrees of suffering and how they might evaluate the management of pain and distress. The second addressed the phenotypic identification of transgenic animals, suggesting that all transgenic lines should have a “passport” that went with the animals and gave details of any adverse effects and their incidence and other information that would enable those receiving animals to better care for them. The third workshop suggested how experiments could be improved so that both reduction and refinement could be achieved—often simultaneously. (David Morton)

#### FOUR PRESENTATIONS ON REPLACEMENT

“On the Acquisition and Use of Human Cells in Research and Testing, Organized by the European Society of Toxicology In Vitro (ESTIV)” was cochaired by Robert Combes (FRAME) and Jan van der Valk (Netherlands Centre for Alternatives, Utrecht, The Netherlands). Four presentations included an introduction to the subject that stressed the scientific and animal welfare advantages of using human cells in preference to animal cell lines. However, there are logistical problems involved, and it was suggested that the way forward is the formation of recognized tissue banks that acquire, process, store, and distribute human cells surplus to medical requirement in an ethical manner, following donation

with informed consent. Several such tissue banks have been, or are being, established in Europe. The formation and operation of one of them, the United Kingdom Human Tissue Bank (Leicester, England) was outlined, and a further talk described lessons learned from establishing and running a tissue bank in Warsaw, Poland.

Several speakers also referred to a recent initiative to establish a European Network of Research Tissue Banks (ENRTB) following discussions at a series of workshops, two of which were organized in conjunction with ECVAM. The ENRTB has been initiated on an informal basis, and a meeting is to take place in 2003 to establish it on a firmer footing. Its principal objective is to bring together those interested in using human cells with those who can acquire and supply such material and also to promote the use of human cells in all areas of biomedical research and testing.

There were several symposia concerned with "Recent Progress in Validating Alternative Test Methods." One described the approaches of ECVAM and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). In addition, Spielmann discussed ZEBET's funding program for developing alternatives, and Combes (FRAME) gave a presentation on the organization, objectives, and progress of the Third FRAME Toxicity Committee. It was noted that since the Third World Congress took place in Bologna, Italy, in 1999, the European Union (EU) and the Organization of Economic and Cooperative Development (OECD) have accepted test guideline protocols for several replacement tests and methods including skin corrosivity, photoirritancy, and percutaneous absorption. How these methods had been validated scientifically and eventually accepted by regulatory agencies was the topic of much debate.

## Serum and Serum Substitutes

A session on "The Use of Serum in Tissue Culture" focused on this important, but often neglected, issue concerning replacement alternatives. Although the widespread use of mammalian cells in tissue culture has contributed substantially to replacing animals in biomedical research and testing, fetal bovine serum or other animal sera usually are added to cell culture media; the collection of such sera raises serious animal welfare concerns. Franz Gruber (Fonds für versuchstierfreie Forschung [FFVFF], Zurich, Switzerland) reported some limited success in research aimed at finding serum substitutes. The research was conducted in the interests of animal welfare and because of the complicated serum; its composition and efficacy can vary from batch to batch, increasing the possibility of variability in results.

“Immortalization Versus Redifferentiation of Cell Lines” was discussed in a symposium cochaired by José Castell (Hospital La Fe, Valencia, Spain) and William Smith (U.S. Army Medical Research Institute, Aberdeen Proving Ground, Aberdeen, MD). Immortalization can be a way of overcoming a major limitation in using cells in tissue culture—their tendency to dedifferentiate and have a relatively short life span. Immortalization is achieved by introducing activated oncogenes, sometimes together with other genes of interest.

Castell described successful attempts to induce the redifferentiation of hepatoma cell lines with respect to cytochrome P450 *CYP* isozymes by introducing into the cells the ability to express certain transcription factors for the *CYP* isozymes. The resulting cells proved to be more suitable than primary human hepatocytes and immortalized human hepatocytes for the metabolism of certain drugs. Smith discussed the principal criteria that are important for using skin models comprising primary or immortalized human keratinocytes to investigate the effects of sulphur mustard agents.

Another way of addressing the problems of cell senescence and dedifferentiation is to control apoptosis. Norman Peterson (Johns Hopkins Hospital, Baltimore, MD) presented data to illustrate the use of this approach to generate hybridoma cell lines for producing monoclonal antibodies after they had been transfected with an antiapoptosis gene.

Sandra Coecke (ECVAM, Ispra, Italy) described studies in which a genetically engineered PC12 cell line, induced by using nerve growth factor to develop neuron-like characteristics in culture, was used to show that subchronic treatments with methyl mercury (a known neurotoxicant) induce both necrosis and apoptosis.

A workshop on “Validation of (Quantitative) Structural-Activity Relationship ([Q]SAR) and Other Computational Prediction Models,” chaired by Andrew Worth (ECVAM) and Mark Cronin (Liverpool John Moores University, Liverpool, England) reflected the conclusion from a recent survey on replacement alternatives, conducted by ECVAM, that there is an urgent need for the validation of such systems. This is because the separate and combined application of these approaches offers the most realistic opportunity—short-term and medium-term—for providing useful information on chemicals and chemical products, which will contribute to minimizing substantially the numbers of animals used while allowing the scale of testing to proceed at a rate consistent with the new legislative requirements.

## Two Presentations and Discussion

Worth and Cronin each gave a short presentation on the principles of using (Q)SAR and computational chemistry approaches especially for toxicity predic-

tion and outlined some of the problems inherent in validating such systems. A general discussion followed. A major issue concerns the quality of the data used to generate the models. It was suggested that validation should be undertaken by taking individual models and investigating their relevance and reliability for predicting the toxicity of a specific range of endpoints rather than by attempting to take a large number of models and validating them simultaneously for a wide range of endpoints.

### Long-Term Toxicity Testing

Traditionally, this is a difficult area for methods to be developed, validated, and accepted as complete replacements for regulatory testing. Covering the subject, Sidney Green (Howard University, Washington, DC) and Hiroshi Ono (Hatano Research Institute, Hadano, Kanagawa, Japan) cochaired a symposium in which there were presentations on embryotoxicity, neurotoxicity, and carcinogenicity testing. Spielmann described the recent successful ECVAM prevalidation and validation studies on three *in vitro* tests for embryotoxicity. The three methods involved the use of embryonic stem cells, the micromass assay, and a postimplantation rat whole embryo assay. Spielmann emphasized that whereas predictivity of the individual tests varied from 71% to 80% for a group of substances with varying levels of embryotoxicity, 100% predictivity was achieved by all the tests for strong embryotoxins.

Susan Makris (Office of Pesticide Programs, Environmental Protection Agency [EPA], Washington, DC) described a guideline for developmental neurotoxicity testing (DNT), a relatively recently introduced regulatory requirement by the U.S. EPA for pesticides testing. This guideline is highly controversial, especially with animal protection groups, because it uses large numbers of animals and because there are concerns about the scientific validity of the animal models used. The talk seemed a strange choice in a symposium on alternatives for these types of tests, especially as the EPA only now is beginning to address refinement and reduction issues associated with its DNT protocols.

John French (Laboratory of Molecular Toxicology, NIEHS) described the use of *ras* transgenic and *p53* knockout mouse models for carcinogenicity testing. These mouse strains carry either activated oncogenes (the *ras* model) or inactivated tumor suppressor genes (the *p53* model) in heterozygous forms. This renders them hypersusceptible to carcinogens because the genes can be expressed following their conversion to the homozygous state. It is claimed that tumors arise in these animals earlier than in conventional bioassays and that smaller group sizes need to be used to reliably detect tumorigenicity. Moreover, there have been claims that the *ras* model can detect nongenotoxic carcinogens. Con-

sequently, the models have been investigated as alternatives to the lifetime mouse bioassay.

### Disappointing Study Results

The results of an international collaborative study, organized by the International Life Science Institute (ILSI) have not been encouraging. No model was shown to be sufficiently robust, reliable, and predictive to be recommended for the previously mentioned use. However, French described more-recent data on the ability of the tests to detect a range of carcinogenic and noncarcinogenic chemicals, including 14 International Agency for Research on Cancer Class 1 human carcinogens thought to be more supportive of their usage (Pritchard, French, Davis, & Haseman, 2003).

Cell transformation was another of the assays for carcinogenicity testing that was included in the ILSI collaborative study. In cell transformation, cultured mammalian cells are induced to develop into a morphologically altered phenotype that has the same characteristics as cells taken from a tumor and grown in culture. This phenomenon of cell transformation has been used to model carcinogenesis *in vitro* and as the basis of an assay for carcinogens. It is possible in some systems to detect promoting and initiating agents (by modeling the two stage process of carcinogenesis) and genotoxic, as well as nongenotoxic, carcinogens. The most widely used transformation assays are based on rodent cell lines. Umeda (Hatano Research Institute) presented data on the use of Balb/C 3T3 and Bhas 42 cell lines to detect a number of tumor promoters and showed that use of the correct medium was crucial to the accuracies of the assays for the correct distinction between carcinogens and noncarcinogens.

### Use of the Comet Assay

The last presentation was given by Ray Tice (Integrated Laboratory Systems, Research Triangle Park, NC) on the use of the so-called comet assay as a method to detect genotoxic carcinogens. Tice was one of the originators of this test in which DNA damage is detected after the DNA is released from exposed cells on a cover slip. Damage, in the form of strand breaks, is measured as the extent of release of the DNA as comet tails, and the assay can be performed under both neutral and alkaline conditions to allow distinction between the induction of single- and double-strand breaks. The test is used widely to provide information as to the mechanism of action of a chemical, but Tice emphasized that

its regulatory usage will require the development of standardized protocols followed by formal validation. (Robert Combes)

## VALIDATION, ACCEPTANCE, AND SADNESS

This was the fourth Congress in the series, and it was interesting to see that there have been significant changes over the years. One of the most significant developments has been in relation to the validation and acceptance of alternative test methods based on growing confidence in the predictive capacity of *in vitro* data for both human and animal effects. At this Congress, for the first time, it appeared to be accepted that when the *in vivo* data are too variable, the more precise *in vitro* data should be used instead.

For me, the major value of the Congress was again the opportunity to meet and discuss with some of the major supporters of the FRAME Research Programme, now in its 20th year. In addition, the research workers who attended from the FRAME Alternatives Laboratory had a chance to meet those involved in the main developments over the years, although it was a pity that their posted presentations, in poorly attended sessions fitted into the lunch breaks, received little attention.

There was a noticeable lack of attendance from the United States, which contributed to the relatively low attendance and speaks volumes about the U.S. attitude to experiments on rats, mice, and birds.

The greatest sadness was a clear statement by a representative of the European Commission's Directorate General for Research that there would be no specific funding for the development, validation, and acceptance of alternative methods in the EU's Sixth Framework Programme (2003–2006). This is despite growing public concern about human health and environmental protection and that increased and urgent focus on replacement alternative tests will be crucial to application of the new policy on the safety of chemicals. (Richard Clothier)

## USE OF ANIMALS IN THE LABORATORY

I took part in three interesting, but contrasting, sessions related to the use of animals in the laboratory and nonanimal methods in testing. The first of these should have been a point-counterpoint session to discuss the question, "Are animal tests inherently valid?" This arose from an OECD workshop on validation, held earlier in the year in Stockholm, Sweden, at which a North American participant said that, unlike nonanimal tests, tests involving laboratory animals did not need to be validated (i.e., independently shown to be relevant and reliable

for their stated purposes) because, being animal tests, they were “inherently valid.” I had agreed to oppose this proposition. Unfortunately, the Congress organizers were unable to find anyone to defend it. As a result, I had to make my presentation with nobody to put the opposite point of view. The session chairman, Leon Bruner (Gillette Medical Evaluation Laboratories, Needham, MA), gave a talk emphasizing the need for the highest possible quality of experimental design and statistical evaluation if validation studies themselves are to be relevant and reliable.

A session on “Policy Aspects of Chemicals Testing and Alternatives,” cochaired with Kathryn Stitzel (Procter & Gamble Company, Cincinnati, OH) was particularly important. It focused on the High Production Volume Challenge in the United States and the Strategy for a Future Chemicals Policy in the EU, both of which seek to address the problem represented by the thousands of chemicals in use before regulations on the testing of new chemicals came into force. I was left feeling rather optimistic that common sense might prevail so that additional animal testing on a large scale can be avoided. I also see this as a great opportunity for the development, validation, and acceptance of replacement alternatives. I am not convinced, however, that there is the political will on either side of the Atlantic to provide the resources necessary for maximizing their potential.

Third, I had the privilege of chairing a special lecture by Ingird Newkirk (People for the Ethical Treatment of Animals, Norfolk, VA) on “Bruegel’s Two Monkeys, or Why Finding Alternatives to Animal Tests Matters,” not least because I am a fan of Bruegel (the monkeys in his painting are strangely melancholy and chained in a massive tower) as well as of alternatives. I feel that it is vital that the World Congress series always finds room for such talks, which provide a fundamental philosophical challenge even to those who think they are being progressive by advocating the 3Rs. (Michael Balls)

## ALTERNATIVES IN RELATION TO BIOLOGICALS

Large numbers of animals still are required for the production and quality control of biologicals. During the last decade, several alternatives have been validated, tests have been deleted from the monographs, and more humane endpoints have been incorporated into monographs and guidelines. Despite these achievements and the remaining challenges, only one session and one workshop at the Congress were specifically dedicated to biologicals.

Seven presentations were given in a session on “Vaccines and Biologicals—Application of the Three Rs,” chaired by Coenraad Hendriksen (National Institute of Public Health & the Environment [RIVM], Bilthoven, The Nether-

lands) and Klaus Cussler (Paul Ehrlich Institute, Langen, Germany). Three dealt with general activities, namely, a review of ECVAM's activities on biologicals (Marlies Halder, ECVAM), the efforts undertaken by the Pan American Health Organization to introduce the 3Rs in the quality control of vaccines in developing countries (José Luis di Fabio, Pan American Health Organization, Washington, DC), and the work of the Food and Drug Administration (FDA) office of vaccine research and review (Robin Levis, Center for Biologics Evaluation and Research, FDA, Rockville, MD). The need for international harmonization of requirements for the testing of biologicals was highlighted in a presentation by Lukas Bruckner (Institut für Viruskrankheiten und Immunprophylaxe, Mittelhäusern, Switzerland) on the outcome and recommendations of the ECVAM workshop on rabies vaccines. Two presentations gave examples for alternative methods for the potency testing of bacterial vaccines. Hendriksen presented the successful validation of two serological methods for replacement of the challenge test in the potency testing of human tetanus vaccines, and Larry Elsken (United States Department of Agriculture, Ames, IA) gave an overview on *in vitro* assays based on antigen quantification for the potency testing of leptospiral vaccines. A shift in the current quality control of biologicals from the batch-to-batch approach to the consistency of production concept was the topic of the presentation by Marlies Leenaars (RIVM). She outlined how this concept, which is already in place for well-defined products, could be established for less defined inactivated bacterial vaccines such as tetanus, diphtheria, and pertussis vaccines. Immunochemical, biochemical, physicochemical, and *in vitro* functional tests could be used to monitor the vaccine quality during critical steps in the production. It is hoped that this approach will get sufficient support and funding because it would significantly reduce the numbers of animals used for batch testing of these vaccines.

The use of humane endpoints in the quality control of vaccines was discussed in two sessions dealing with refinement. In the session on "Animal Welfare: Guidelines and Beyond," Cussler spoke about the problems related to the implementation of alternatives into regulatory guidelines, such as those of the European Pharmacopoeia, and outlined how the system could be improved.

"Replacement in Monoclonal Antibody Production" was the topic of a 1-hr workshop. Two speakers from a U.S. research institute and a Belgian pharmaceutical company reported how they had switched from *in vivo* to *in vitro* production. Both these cases clearly showed that *in vitro* production is feasible and meets the requirements of a small-scale production in a research setting as well as the needs for large-scale production in a commercial environment. Unfortunately, some scientists and regulators are still using and permitting *in vivo* production, which is one of the most severe animal procedures. *In vivo* production should be banned immediately in Europe because it is illegal according to animal welfare legislation. (Marlies Halder)

## EDUCATION

University faculty and researchers rightly acknowledge education—the acquisition of knowledge, skills, and attitudes—as a crucially important area in preparing competent professionals. Too often, however, underinvestment and neglect have limited its potential. Humane education, supported by recent developments in technology and in the evolution of ethical thought and educational theory, is one area through which innovation in the life sciences has started to flourish. Individual educators and many university departments already have developed and implemented state-of-the-art multimedia computer assisted learning software for use within practical courses. Other tools such as digital video, mannequins, and simulators have been combined with approaches such as student self-experimentation, clinical work with animal patients, and the use of ethically sourced animal cadavers and tissues as part of a conscious design process to better meet teaching objectives and to cultivate sensitivity and critical thinking.

The preceding alternatives, singly or in combination, have brought about the replacement of harmful animal use in the study of anatomy, physiology, pharmacology, and clinical skills and surgery training in locations across the world. The pedagogical, ethical, social, and economic advantages of alternatives in education have been well documented in the 30 or more published studies showing that alternatives are equal to, or better than, their traditional animal-consumptive counterparts in terms of student learning performance.

The Congress brought together a diverse range of academics, researchers, and campaigners in one of the world's leading events on the 3Rs and innovation in science. "Education," combined with "Information Resources," comprised one of five themes and was explored through the media of speakers, workshops, a point-counterpoint session, posters, and an exhibition room.

Considering its importance—all future life scientists will have some form of biology education, whereas only a minority of them will subsequently perform animal experiments—the education theme did not have a sufficiently high profile in the Congress. It was, however, of generally high quality. New alternative tools were presented—from a frog dissection program that uses layering and morphing technology to a revolutionary live surgery trainer that employs a specially preserved cadaver and pumping device to effectively simulate bleeding, pulsation, and the vascular tree in training in a range of surgical and microsurgical procedures. Developments in virtual reality for training medical procedures and the creative use of digital video alternatives also were presented. Several instructors spoke about the use and benefits of alternatives at their institutions, and the process of effective implementation was addressed.

A diversity of educational tools from the European Resource Centre on Alternatives and from InterNICHE's Alternatives Loan System were on display in the Exhibition room, and producers of alternatives also were able to demonstrate their

software. This resource was underutilized, however, in part because of the poor layout of the conference center and the choice of rooms.

Presentations about Web-based and other training courses for undergraduate students and laboratory animal researchers and technicians asserted the importance of the acquisition of skills through using alternatives, animal welfare awareness, ethics training, and attitude development. Others exposed the poor quality of much experimental design in research and highlighted the need for researchers to be better trained in the use of statistics for reducing animal use and improving scientific output.

Elsewhere, there was a good representation of papers on ethology. One paper explored the challenging issue of the question of sentience in invertebrates. Another explored the study of the behavior of rats released from the laboratory into a natural setting for 6 months.

A survey of U.S. middle school and high school biology teachers by the Humane Society of the United States provided interesting results on the attitudes of educators and the problems facing the implementation of humane alternative methods. The United States is an anomaly in terms of its profligate animal use in education and the opportunities for children to perform animal experiments. The survey found that 79% of U.S. teachers use dissection to teach biology (primarily for the hands-on aspect), although dissection is either banned or rare in many other countries at this level. Almost all (98%) teachers teach in the same way they were taught themselves, despite widespread innovations in technology and teaching style. Awareness of hands-on and other alternative methods and their efficacy was low.

My own point-counterpoint discussion with a defender of dissection was very illuminating. Despite the questions raised by animal suffering and killing, the Executive Director of the National Association of Biology Teachers (NABT), Wayne Carley, saw no ethical problem with harmful animal use, student attitudes toward animals, and life in general. In a doctrinal approach comparable to “the right to bear arms” whatever the cost to human lives and social well-being, he saw dissection as a “right.” It was also the “best” and “only way.” Carley made no reference to the empirical studies refuting the superiority of dissection and dismissed the practice of all other departments and countries where alternatives are used as inferior to the “American way.” He was against student choice, despite American pride in the first amendment of the Constitution, and dismissed conscientious objection as student laziness. When pushed, however, Carley did state, “We [the NABT] would promote ethically sourced animals.” The NABT should, therefore, amend its policy to accord with its Executive Director’s statement.

Further opposition to freedom of conscience and religion and a general lack of commitment to alternatives was shown by the very few signatures from conference delegates on a simple petition asking Massachusetts legislators to support efforts to pass dissection-choice legislation. Without such legislation,

students can face academic and psychological penalties for requesting alternatives and may suffer trauma during animal practicals—if they already have not been put off entering the life sciences. Those who choose to conscientiously object are certainly not lazy: They care deeply about their education and about animals. They exhibit critical thinking and ethical engagement that science dismisses at its peril. Some have played pivotal roles in the development of alternative tools and approaches—from the Virtual Physiology Series designed at the Institute of Physiology at Marburg, Germany to the Body Donation Program at Tufts University School of Veterinary Medicine, Boston, Massachusetts, which meets 100% of its anatomy and clinical skills cadaver requirements from an ethical source (animal patients who were euthanized for medical reasons and with their guardians' consent).

Overall, the conference was well organized and had a good, quality program. However, the lack of common meeting space limited opportunities for networking that so often provide the most important elements of a conference. In addition, there were problems with the venue—mediocre and over-priced facilities and as cold inside as it was hot outside—leaving many researchers calling for enrichment of their own environment.

The concept of the 3Rs, particularly replacement, needs to be applied to a much greater degree than at present, for the education of young people, for the animals, and for the improvement of quality of research and the reliability of testing. Education can play a major role in facilitating this process. (Nick Jukes)

## CONCLUDING NOTES

The proceedings of the Fourth World Congress on Alternatives and Animal Use in the Life Sciences will be published in 2003 as a supplement to *ATLA (Alternatives to Laboratory Animals)*.

The Fifth World Congress on Alternatives and Animal Use in the Life Sciences will take place in Berlin, Germany, in 2005, when the principal organizer will be Professor Horst Spielmann, ZEBET at the BfR, Diedersdorfer Weg 1, 12277, Berlin, Germany. E-mail: spielmann.zebet@bfr.bund.de (Michael Balls)

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