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For professionals in the fields of laboratory animal care and use

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Beyond the Three Rs

For a long while after the Three Rs were first proposed by Russell and Burch, anti-vivisectionists rejected the concept, on the grounds that experiments on living vertebrates which cause them pain, suffering, distress or lasting harm, were ethically unacceptable and scientifically unnecessary, so there was no point in reducing, refining or replacing them. In recent decades, however, some organisations, such as the BUAV and PETA, have moved tentatively into the middle ground, and have made positive contributions toward the Three Rs, without comprising their fundamental beliefs.

The ultimate goal of Russell and Burch themselves was *replacement*, which they said, "is always a satisfactory answer", with *reduction* and *refinement* merely being steps along the way. That was also the position of the founders of FRAME, the Fund for the *Replacement* of Animals in Medical Experiments, although the charity has made many contributions in support of the other two Rs, since its foundation in 1969.

This issue of *ATLA* contains two important articles about the future of the Three Rs, as well as the latest in an important series of outstanding exposures of the insurmountable limits of laboratory animals as models of humans.

In the *PiLAS* supplement, Craig Redmond argues the case for replacing the Three Rs with One R (*Replacement*),[1] but goes further in saying that only what Russell and Burch defined as *absolute replacement* (where "animals are not required at all at any stage") should be considered acceptable, since *relative replacement* can still involve suffering, as in the use of invertebrates, less-sentient vertebrates, or cells and tissues taken from protected animals and used *in vitro* or *ex vivo*.

Michael Balls goes further in his *ATLA* Comment,[2] proposing that "the time has come to plan for a future where the Three Rs will have served their purpose, animal experimentation will have been consigned to history, and humane biomedical science in research, testing and education will have become the norm, for the benefit of humans and animals alike".

Finally, the article by Jarrod Bailey in this *ATLA* issue, on monkey-based research,[3] demonstrates that major molecular differences, revealed by comparative genomics and molecular biology, underlie inter-species phenotypic disparities. The collective effects of these differences are striking, extensive and widespread, and show that the superficial similarity between human and monkey genetic sequences is of little benefit for biomedical research. Therefore, the extrapolation of biomedical data from monkeys to humans is highly unreliable, and the use of monkeys must be considered of questionable value, particularly given the breadth and potential of alternative methods of enquiry that are currently available to scientists.

Comments on these articles, for possible publication in *PiLAS*, would be welcomed.

[1] Redmond, C. (2014). 'One R' is the new 'Three Rs'. *ATLA* **42**, P50–P52.
[2] Balls, M. (2014). Animal experimentation and alternatives: Time to say goodbye to the Three Rs and hello to humanity? *ATLA* **42**, 327–333.
[3] Bailey, J. (2014). Monkey-based research on human disease: The implications of genetic differences. *ATLA* **42**, 287–317.

CONTENTS

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DISCUSSION

‘One R’ is the new ‘Three Rs’

Craig Redmond

The Lush Prize, which rewards initiatives to end animal testing, believes that more attention needs to be given to the ‘One R’ of absolute replacement, and that research methods that exploit animals in any way (including tissues and cells) should not be considered as ‘alternatives’

Introduction

Replacement of animal experiments is one of the Three R concepts (alongside *Reduction* and *Refinement*) first put forward by Russell and Burch in 1959.[1] However, this can be either *absolute replacement* (i.e. methods that do not involve animals or animal tissues) or *relative replacement* (e.g. methods that use only cells or tissues of animals *in vitro* or *ex vivo*).

It has become accepted by many in the research community that some uses of animals can be classed as ‘alternatives’. In particular, the use of whole animals of species thought to either not experience pain or to have a lower level of sentience (e.g. fruit flies, nematodes and zebrafish), or of animal parts (including tissues, embryos, sera and cells). The use of these methods is reinforced by regulatory bodies, making it more difficult to reach a time when no animal use will occur in scientific research. André Ménache, of Antidote Europe, believes that in the region of 80% of ‘alternatives’ validated by ECVAM (the European Union Reference Laboratory for alternatives to animal testing) still use animals or animal tissues (*personal communication*, 04.09.13).

Founded in 2012, the Lush Prize rewards global initiatives to end animal testing, particularly in the area of toxicology. A total of £250,000 is shared annually between five prize categories covering science, training, young researchers, lobbying and public awareness. Lush Prize believes that more attention needs to be given to the ‘One R’ of *absolute replacement*, and that research methods that exploit animals in any way (including tissues and cells) should not be considered as ‘alternatives’.

‘Alternatives’ that still exploit animals

The United States Department of Agriculture refers to ‘alternatives’ as “a term that has different meanings to different people, and this difference largely depends on which side of the issue one is found”.[2] So, for example, animal researchers might use *relative replacement* methods in addition to their use of animals (or look to refine existing animal tests), whereas abolitionists see ‘alternatives’ in terms of *absolute replacement*. Other examples of where the use of the term ‘*replacement*’ serves to reinforce the idea that *relative replacement* is routinely acceptable are:

- where 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”.[3] The words ‘non-sentient’, ‘conscious’ and ‘vertebrates’ ensure that the use of invertebrates and species considered as ‘lower organisms’ continues to be accepted.
- when, in its current “step-by-step approach to an alternatives search”, the Johns Hopkins University Center for Alternatives to Animal Testing (CAAT) suggests that, in addition to cell culture, tissue culture, models, simulations, etc., researchers “might look for a non-mammalian animal model — fish or invertebrates, for example — that would still give you the data you need”.[4]

Perhaps as a direct result of this widespread inherent acceptability of *relative replacement* alternatives, researchers at the University of British Columbia, looking into people’s acceptance of the use of particular species in laboratories, found that species such as fish and invertebrates “are typically rated below mammals, and, as such, are often considered an appropriate replacement for mammals in research”.[5]

The philosopher Joel Marks notes that “developing alternatives to the use of animals can mean simply using a different animal” and considers that “the characterisation of the other animal […] as ‘lower’

on a phylogenetic 'scale' is arbitrary and disputed. The alternatives movement is therefore at risk of becoming a bait-and-switch con".[6] By this, Marks means that 'alternatives' are advertised as one thing (i.e. *absolute replacement*), but often turn out to be something completely different (i.e. simply the use of another species).

Some examples of *relative replacement* alternatives are:

- *Invertebrates*: The horseshoe crab, *Limulus polyphemus*, is used in the *Limulus* amoebocyte lysate (LAL) assay. This method replaces the rabbit pyrogen test for the detection of endotoxin in, for example, hepatitis B vaccines.[7] The rabbit test involves injecting the test substance into a marginal vein of the ear of each of three rabbits.[8] However, the LAL assay uses blood cells from the horseshoe crab, with up to 30% mortality resulting from the bleeding procedure.[9]
- *Fish*: Zebrafish (*Danio rerio*) are widely used in research, including research on genetics, cancer and, increasingly, toxicology. The maintenance costs of zebrafish are less than one thousandth of the costs of maintaining mice,[10] and they can produce 100–300 eggs per week, making their embryos useful for high-throughput screening.[11] It is widely acknowledged that fish can feel pain,[12] with as much evidence for this as there is for birds and mammals.[13] Other studies have shown that they have conscious awareness.[14]
- *Tissues*: Hundreds of thousands of animals are bred and killed each year in Britain alone, solely to provide tissues for research.[15] Human tissue is to be preferred, due to species differences, yet animal tissue is often used on the grounds of cost and availability.[16] Human tissue can be obtained from patients during diagnosis, removed as 'waste' during surgical operations, placentas or 'afterbirth', or tissues obtained after death.[15] People can voluntarily donate blood or other tissue for transplantation or research, or their organs or bodies after death. Human tissue removed from the body in the course of disease diagnosis or treatment is the main source.[17] However, although upwards of 600,000 residual surgical tissues are generated each year in England and Wales, only a tiny fraction of them are made available to researchers.[18]

The use of fetal calf serum

The move toward the use of *in vitro* cell culture to provide both human and animal cells for alternative methods is a step in the right direction. However, the use of fetal calf serum (FCS) as a cell culture media supplement is unacceptable, in the light of the availability of serum replacements and serum-free culture methods.[19] Blood collected for FCS production is obtained by cardiac puncture, performed by inserting a needle directly into the heart of the non-anaesthetised fetus.[20] According to Jochems *et al.*[20] it is very likely that the fetus is alive at the time of blood collection, and "will experience pain and/or suffering at the moment of heart puncture for blood collection and possibly for a period after that, until it actually dies". The scientific validity of using FCS has been questioned. Risk of contamination is an issue,[21] with the potential presence of viruses, bacteria, mycoplasma, yeast, fungi, immunoglobulins and endotoxins.[20]

Conclusions

These are just a few examples of animal use that some see as 'alternatives'. The use of sentient animals, such as fish and horseshoe crabs, should not be accepted by those working in the field of alternatives to animal testing, despite the entrenched position within the research community and regulatory bodies. Neither should cruel processes such as the collection of FCS be condoned. In addition to greater humanity and greater acceptability, there are a multitude of clear scientific benefits to avoiding the use of animals or animal products.

The Lush Prize promotes the 'One R' of *Replacement* over all of the 'Three Rs', believing that the *true* absolute replacement of animals is essential for ethical and scientific progress.

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3 Balls, M. (1994). Replacement of animal procedures: Alternatives in research, education and testing. *Laboratory Animals* **28**, 193–211.
4 Altweb Project Team (undated). *Search for Alternatives*. Baltimore, MD, USA: CAAT, Johns Hopkins University. Available at: http://altweb.jhsph.edu/resources/searchalt/index.html (Accessed 22.10.14).
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CURRENT DILEMMAS

A Three Rs Perspective on the Mouse Bioassay in Routine Seafood Safety Testing for Algal Biotoxins — 1: *Replacement*

Ian Stewart and Catherine McLeod

Countries that have discontinued routine mouse bioassay testing might gain a market advantage in domestic and export seafood products over countries that continue to use the mouse test

Introduction

The laboratory mouse has been the principal tool for routine testing of seafood — mainly shellfish — for the presence of hazardous concentrations of harmful algal toxins, since regulatory oversight of shellfish fisheries began in the 1920s. If undetected, as often is the case in unregulated fisheries, algal biotoxins can cause serious illness and death in people consuming contaminated shellfish and other seafood. A range of alternative testing techniques that do not rely on the use of live mice are available to the seafood industry and food safety agencies. Some countries — notably Canada, New Zealand, the UK, and now Australia — have discontinued use of the mouse bioassay (MBA) for routine safety testing of shellfish to detect and measure paralytic shellfish toxins and the so-called lipophilic toxins (which includes diarrhetic shellfish toxins), but some other countries seek to maintain access to the mouse test.

Discussions and deliberations at an international level on the credentialing of alternative testing methods occur through the authority of the Codex Alimentarius Commission. Some are frustrated that the Codex forum is the only conduit for implementing the protracted process by which the anachronistic and unethical MBA for routine shellfish safety testing can be consigned to history. Here, we present some strategies that seafood industries in countries which have discontinued use of the MBA might be able to apply, in order to secure market advantages over those in countries that seek to maintain the *status quo*. This, in turn, may put pressure on industries in countries that have as yet failed to adopt alternative *replacement* testing systems, to endorse and refine modern management systems. In a subsequent issue of *PiLAS*, we will discuss the MBA for routine testing of algal biotoxins in seafood from the perspectives of the other Three Rs initiatives: *reduction* and *refinement*.

Validated Alternative Assays

A wide range of chemical, biological and physical tests and assays have been investigated for the purpose of detecting and quantifying marine biotoxins in shellfish and other seafoods, as potential alternatives to the MBA.[1] Many of these alternative techniques have not progressed beyond the stage of research inquiry, but three liquid chromatographic methods[2-4] and a receptor binding assay (RBA)[5] are now approved official methods for determining harmful concentrations of specific classes of marine algal toxins. Stewart and McLeod[1] recently outlined some of the challenges and constraints in moving from the use of the MBA for routine shellfish safety testing toward modern chemical or RBA analysis. Not least of these are requirements for sophisticated analytical instruments and high-level skills in analytical chemistry needed to run these devices. However, analytical laboratories in many countries now have the capability to provide shellfish safety testing by using chemical methods, either under the aegis of government-managed food safety programmes or as commercial service providers.

Worldwide Regulatory Standards

Requirements for shellfish biotoxin safety testing are determined by standards, guidelines and performance criteria prescribed by the Codex Alimentarius Commission, under the auspices of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO); Codex is also charged with overseeing fair and equitable trade conventions across the food industry. Revision and refinement of performance criteria for biotoxin safety testing is effected by the Codex Committee on Fish and Fishery Products (CCFFP). At the most recent CCFFP meeting in Norway earlier this year, draft performance criteria for the determination of marine

algal biotoxins in molluscs still allow agencies conducting shellfish safety testing to choose between chemical methods or a functional assay, which includes the MBA. The draft criteria[6] state that: "The method selected should be chosen on the basis of practicability, and preference should be given to methods which have applicability for routine use." While it may be the case that this statement merely represents the *status quo*, in practice the ability of alternative chemical and functional tests to formally and globally supplant the MBA will likely rest on the accumulation, over years, of a data set demonstrating that alternative shellfish safety testing is indeed protective of public health to the same broad extent as is the MBA. In this regard, deliberations within the CCFFP continue to advance the ability of seafood industries and food safety agencies to adopt alternative *replacement* testing methods by developing guidelines to address challenging technical issues pertinent to the implementation of non-animal based methods, such as toxicity equivalence factors and other relevant performance criteria.[6]

This is not to suggest, however, that expert opinion is currently of one voice on the need to end the availability of the MBA for shellfish safety testing. National delegates at CCFFP meetings and working groups are divided on the issue. Stewart and McLeod[1] have noted some of the countries that have taken a leadership role in ending use of the MBA for routine shellfish safety testing, particularly Canada, New Zealand and the UK. Ireland was another early adopter of chemical-only testing for routine shellfish monitoring. Dissenting opinion in part centres on the claim that removing access to the MBA would represent an unfair trade advantage in favour of developed countries that have the resources to support the infrastructure and skills required to adopt and run chemical testing regimens. This position in favour of maintaining the *status quo* was expounded in a submission by the Philippines to the 2012 CCFFP meeting, held in Bali, Indonesia. The Philippines supported the position of the USA and Chile that access to the MBA for routine biotoxin monitoring should continue. The Philippines submission[7] notes that: "For almost 30 years now, MBA is continuously protecting millions of Filipinos dependent on shellfish as the cheapest source of protein." Interestingly, the Philippines complements its use of the MBA for shellfish safety testing with a multi-analogue HPLC technique, although this is only performed at a single centre in Manila. However, the MBA itself is performed at several locations across the archipelago.[7]

Trade Barriers: Who Benefits?

We suggest here that the claim for continuing access to the MBA as a routine biotoxin test for seafood safety, because of the potential trade barriers presented by the skills and infrastructure requirements of early 21st century chemical testing, deserves closer scrutiny. We do not dispute the suggestion that the skills and equipment demands of modern chemical testing are not inconsiderable. However, the reciprocal assumption that the MBA represents a low-cost option is not a given. While the disapprobation of *some* scientists and technicians tasked with conducting MBA shellfish safety testing may have had some influence on expert opinion on this matter — author McLeod being one such scientist, who found the assignment objectionable — it is likely that economic considerations have been a more significant determinant of change in countries that have or are in the process of discontinuing use of the mouse test. The experience in Australia may serve to illustrate this point.

Scientific tests on live mammals in Australia — including the MBA for routine shellfish safety testing — must be approved and overseen by a properly constituted ethics committee, and Australian state governments must approve and licence facilities in which such testing is conducted (see http://www.animalethics.org.au/legislation). Laboratories that conduct scientific testing and research are required to apply rigorous standards of hygiene and husbandry, typically involving buildings that allow for the control of environmental variables such as temperature, humidity and lighting. In practice, this usually translates to use of specific pathogen-free animals in facilities staffed by appropriately trained and supervised workers. And the operating costs for such facilities are not insubstantial — costs which are factored into their pricing schedule for service provision. The price per sample for paralytic shellfish toxins by MBA testing at the sole Australian laboratory with ethical clearance to perform the MBA for seafood safety was A$390 (including sample preparation); the charges for qualitative screening (detect/nil detect result provided) and confirmatory testing (quantitative result provided) by liquid chromatography with fluorescence detection methods from the Australian provider are A$85 and A$370, respectively. So the MBA for routine biotoxin monitoring in shellfish is not considered to be a low-cost test in Australia. The compliance cost to Australia's shellfish industry of routine biotoxin monitoring has been a major incentive for changing to a chemical testing programme. An additional motivating factor for the shellfish industry and regulators in Australia was the requirement for a more specific test method that returns results only for the regulated marine toxins and excludes other compounds, such as the cyclic imines, which are not subject to food safety oversight and can cause false-positive results in the MBA. A single commercial analytical laboratory in Australia was awarded the tender, after a committee comprising seafood industry representatives and government food safety agencies considered various options for delivery of this service, including the use of providers in each state, a single national provider, and the use of government, university and private sector laboratories.

Proponents of continuing use of the MBA for routine seafood safety testing assert that restricting access to the test would represent an unfair trade advantage in favour of developed countries with resources to support the skills, equipment and infrastructure requirements of liquid chromatography-based analysis. We suggest that these claims are spurious. We propose, considering the extent that the MBA is a low-cost technique in the hands of agencies in some countries that continue to use the test, that standards of mouse colony breeding and maintenance, husbandry, disease prevention and ethical oversight, must, wholly or in part, be of a lower standard than that seen in the example outlined above of a tightly-regulated dominion like Australia. Therefore, we suggest that countries that conduct the MBA on the cheap, so to speak, are simultaneously cutting corners on animal welfare and potentially experiencing an unfair trade advantage in so doing.

Promoting the 'Ethical Choice' to Consumers

An overwhelming majority of shellfish consumers are likely to be unaware that batch and fishery testing, by any method, for algal biotoxin safety is conducted as a matter of routine. Yet there may be a case for the seafood industry in countries that have relinquished routine MBA testing for algal toxins (e.g. Australia, Canada, Ireland, New Zealand, the UK and some European nations) to take the initiative and actively promote their transition to an ethically unencumbered chemical testing regimen, in order to secure an international market advantage.

Video footage of a bored technician — peering, with stopwatch or electronic timer in hand, at a suffering and terrified mouse in order to ascertain when it has drawn its final asphyxiated breath — will not play well on prime-time television. If and when animal welfare activists decide to campaign on this topic, considerable economic disruption may ensue. The fallout from such a campaign should, of course, be visited disproportionately on seafood products from countries and industries that continue to rely on the MBA, but the potential for some global industry-wide disruption might be realised in the wake of consumer ignorance about the facts of routine monitoring programmes, and the important differences between the MBA and alternative testing methodologies.

A pre-emptive approach by the seafood industry to educate consumers about food safety monitoring programmes could deliver appreciable benefits both to industry and its customers. The trends are for increasing consumer concern and awareness of food safety, particularly in well-educated and higher-income demographics.[8] The message of routine monitoring for marine algal toxins should not, in principle, be a difficult sell for the seafood industry, as there is a long history of product safety and public health protection from monitored fisheries, attributable to both the MBA — since monitoring began in the 1920s in the USA — and more recently, from fisheries that have adopted *replacement* alternative testing methods.[1] Marketing strategies to better inform seafood consumers about routine monitoring programmes for algal biotoxins could potentially realise the dual benefits of a) embedding the food safety message that products from monitored fisheries have an excellent consumer protection record, and b) emphasising that products from countries that have discontinued the use of the MBA for routine biotoxin safety testing are not subject to unfavourable animal welfare considerations.

In the event that the routine MBA for detection of algal toxins in shellfish and other seafood becomes the focus of a future public awareness campaign by animal welfare proponent organisations, a pre-emptive information drive by the seafood industry and food safety agencies in countries that have replaced the MBA with alternative testing regimens should effectively insulate local industries against the economic disruption to be expected as a result of such a campaign. The appropriate targets of an animal welfare operation directed at the seafood industry would be fisheries in countries that continue to use the MBA routinely and/or advocate its continued availability. Countries that have discontinued routine MBA testing might well gain a market advantage in domestic and export seafood products, at the expense of imports from countries that continue to use the mouse test.

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THE WISDOM OF RUSSELL AND BURCH

13. Rex Leonard Burch: Humane Scientist and Gentle Man

Michael Balls

Rex Burch played a vital role in collecting background information about practical animal experimentation, and was the first person to use the term 'alternatives' in that context. The senior author of The Principles*, William Russell, greatly valued their collaboration*

Whereas *The Principles of Humane Experimental Technique*[1] reveals a great deal about its first author, it gives us very little insight into the character and contributions of its second author, Rex Burch. As a result, his role in the origins of the Three Rs concept could be underestimated. Happily, that can be avoided, not least because his co-author, William Russell, was solicitous in ensuring that his partner received the credit properly due to him.[2] In addition, a few of us had the privilege of knowing Rex,[3–5] and he recorded his own thoughts in a report produced under contract to ECVAM and published in *ATLA*.[6]

Soon after the UFAW project began, Russell informed Charles Hume that he would need some assistance, and the UFAW Council somewhat reluctantly agreed to the appointment of Burch. The plan was that Burch would "discuss with existing research workers, their techniques and attitudes to modifications, in terms of humaneness, and the idea of replacement techniques which did not require animals".[6] To facilitate this, a letter of introduction was prepared (not without a struggle[2]) and sent to 20 individuals in December 1954. The response was positive, so Burch was able to begin his visits. He carried a tape recorder with him, then sent the interview tapes to Russell, who had their contents transcribed and placed in the records on which their eventual report and *The Principles* would be based.

Russell later commented that, "As fieldwork for our project, Rex travelled widely over Britain, interviewing well over a hundred experimentalists, and every single one of them was cooperative. This shows, I think, not only the goodwill in the British scientific community, but the confidence Rex must have inspired by his friendly approach and his real understanding of the issues. These interviews were, of course, vitally important for our project, but I feel that there was more to them than that. Rex sowed many seeds, and helped to channel the goodwill of scientists into the more systematic approach to humane technique which has produced so many advances in recent years."[4] That was an immense achievement, since it must not be forgotten that, in those days, before the publication of *The Principles* with its introduction of the Three Rs concept, there was virtually no activity in the middle ground between the pro-vivisectionists and the anti-vivisectionists. There were only two positions on animal experimentation — for or against.

Burch's success in gaining the confidence of research scientists undoubtedly stemmed from his own considerable experience in laboratory work, even though he was only 28 years old when he joined the UFAW project. He put it like this,[6] in his own words: "In the early part of the Second World War, I worked in the North Riding Laboratory of Pathology and Public Health at Scarborough in North Yorkshire. One of my duties was to test milk for tubercle bacilli, and I had a constant stock of 200 guinea-pigs for this purpose. After their intraperitoneal injection of a centrifuged deposit of the milk sample (two guinea-pigs were used for each sample, one being killed after three weeks, the other after six weeks), their inguinal glands were examined for tubercle bacilli. My concern, before taking charge of this work, was about the humane killing. I was shown the method — a short, sharp blow to the nape of the neck with a specially made wooden instrument, which certainly caused instant death. I was made to practice on dead animals, and my first live guinea-pig did die instantly. From here I joined the army, serving in the Middle East and still using guinea-pigs for this diagnostic work. After the war, I read medicine at Guy's Hospital Medical School in London (UK), but had to abort my studies and find paid work. I was offered work in the research department of Boots Pure Drug Company, where a large variety of laboratory animals were used. My appointment was that of an assistant in tropical medicine. At an early stage, disease broke out in a large mouse unit which supplied the various laboratories, and all of the mice had to be destroyed. I suggested that there should be a diagnostic laboratory for all of the laboratory animals, in order to provide early warning of disease. Within a matter of days, the Head of Research instructed me to set up such a unit. This work gave me wide experience in animal husbandry and experimental procedures, because I became deeply involved with work in the breeding units and with all those working experimentally in different disciplines. After a few years, I resigned from my appointment to set up my own research unit and breeding unit, in Huntingdonshire. My bank manager soon made me aware that there was a Professor Alastair Worden, only a few miles away, carrying out what he thought was similar work to my own. I soon made my acquaintance with Alastair Worden and, at our first meeting, he invited me to carry out histological and microbiological work for him on a casual basis. In the course of what seemed a comparatively short time, he told me that UFAW was appointing a very talented zoologist from Oxford University, Dr William Russell, to carry out a laboratory animal survey." Worden knew that Russell needed an assistant, and suggested that Burch should contact Hume about the possibility of an appointment.

It is clear that, in 1954, Burch had greater, and wider, experience in practical laboratory work than his senior partner. Nevertheless, Burch's crucial role in the project was not recognised at first, even by UFAW's President, Edward Hindle FRS, who refused to support his application for membership of the Institute of Biology. Happily, largely as a result of Russell's vigorous support, Burch became an MIBiol in mid-1959, at the time of the publication of *The Principles*. He was elected a Fellow of the Institute in 1987.[2]

For many years, there was confusion about when the term 'alternatives' was first used in relation to experiments on living animals. For a long while, the credit was given to Terry Hegarty, a FRAME Trustee and the son of FRAME's Founder Chairman, Dorothy Hegarty. However, on a visit to the Russell Archive at the University of Nottingham, I discovered that the fact is that it was *Burch himself* who first used the term 'alternatives' in the context of the Three Rs — in October 1954, even before he had joined William Russell to work on the UFAW project! The story is as follows.[2] Russell produced a draft of the letter of introduction to be sent to heads of university departments and research institutions, which was amended by Burch. In a sentence in which Russell wrote, "Mr. Burch is on a fact-finding mission, and with the information we hope to be able to suggest improvements in routine methods of research…", Burch crossed out "improvements", and suggested, as indicated in a footnote, that "some possible alternatives" should be used instead. Russell rejected that suggestion, crossed out "to suggest possible alternatives to techniques used at present" and suggested that "to produce a review of progress in the development of humane techniques" should be used. His position was supported by Charles Hume, Head of UFAW, who said, "The only thing that worries me a little is the reference to suggesting possible alternatives. Of course we do hope to do this, but if we say so bluntly I fear there may be a counter action — 'Who are you to tell me how to do my job?', I would suggest deleting the line 'to suggest …at present' and substituting something of this sort: 'to present a review of progress in the development of humane techniques'."

Happily, this temporary difficulty did not have a lasting effect on the excellent relationship between

Russell and Burch, which was crucial to the development of the Three Rs concept. However, the publication of *The Principles* was not without controversy, largely because Russell rejected any external attempts to edit his text, but correspondence with Hume again bears witness to his respect for Burch. The original agreement with Methuen, publishers of *The Principles*, was that 80% of any royalties would go to UFAW and 20% would go to Russell, as its author, who would also own the copyright. Russell asked that his 20% should be shared equally with Burch, whereupon Hume impatiently commented that 10% of not very much would not be very much.[2]

Friday 5 August 1994, was a very special day for me, as I went to Sheringham, Norfolk, UK, to meet Rex Burch for the first time. As I said later,[4] "I was totally captivated by his enthusiasm, his genuine excitement about the activities of FRAME and ECVAM, and his kindness. Within a few minutes, I felt as if I had known him for many years, and I knew that I would never forget him for the rest of my life". That meeting had two important practical consequences. Firstly, the ECVAM contract, which led to a fascinating article in *ATLA*, *The Progress of Humane Experimental Technique since 1959: A Personal View*.[6] That is the closest we will ever get to the autobiography he had hoped to write. It tells of his life before the UFAW project, gives his perspective on what went on between 1954 and 1959, and comments briefly on what had happened since the publication of *The Principles*.

Secondly, since Rex was too ill to travel far from home, I went back to Ispra, and arranged with Alan Goldberg that we should organise a workshop on the Three Rs, to be held in Sheringham, and with Russell and Burch as special participants. This was the first and, sadly, the last scientific meeting which the two authors of *The Principles* had attended since 1959. It resulted in a report with 58 far-reaching recommendations,[7] and it was a memorable event of very special significance to Rex Burch, humane scientist and gentle man.

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The Principles of Humane Experimental Technique is now out of print, but the full text can be found at http://altweb.jhsph.edu/pubs/books/humane_exp/het-toc. An abridged version, *The Three Rs and the Humanity Criterion*, by Michael Balls (2009), can be obtained from FRAME.

COMMENT AND FEEDBACK

Re Dewhurst & Ward (2014). The Virtual Pharmacology Lab — A repository of free educational resources to support animal-free pharmacology teaching. *ATLA* **42**, P4–P8.

This is NOT free; each CAL package costs £250 for each practical. I am currently looking into replacing my undergrad wet lab practicals that involve a small number of animals per year, with simulated CALs. I cannot find a FREE replacement for such practicals; it would be marvellous if there were such resources and if someone could direct me to them.

Response from the authors of the article:

About the Virtual Pharmacology Lab (VPL: http:// www.virtualpharmacologylab.com): I think you have misunderstood what the repository is and why it was created. Over the last 25 years, I have worked with physiology and pharmacology colleagues in several universities, to develop a range of computer programs, some of which provide alternatives to practical experiments used in teaching. These programs are described at www.sheffbp.co.uk and, as you point out, sell for £250 each (multiuser educational license). Over the years, one of the frequent comments I receive from users has been that they would like to be able to edit these programs to tailor them to their own teaching needs, by, for example, changing certain drugs, adding new drugs etc. To date, this has not been possible.

The VPL is an attempt to give teachers that flexibility. Eleven of the Sheffield BioScience Programs have been disaggregated, and the individual components (learning objects [LOs]) — traces, visuals, text — have been made freely available. Each program, when disaggregated, releases 100–200 LOs. Teachers can use the individual components in any way they wish, adding their own learning objects as necessary. They thus now have two options. They can freely access the LOs from the repository and re-aggregate as they see fit or, if they prefer to use my version of the re-aggregated LOs (i.e. a full CAL package comprising >100 LOs), they can purchase this as described above.

The hope is that, as teachers develop their own LOs, they will add these to the repository and make them freely available, too. The PiLAS article is describing the VPL repository and how it was created. I hope this helps to clarify the situation.

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