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Regulation of animal use in research, testing and teaching: Comparison of New Zealand and European legislation

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FOREWORD

This is the third paper in a series about the use of animals in research, testing and teaching published from time to time by the Ministry of Agriculture and Forestry (MAF) under the auspices of the National Animal Ethics Advisory Committee (NAEAC). The objective is to disseminate to a wider audience articles that appear in academic journals and in the proceedings of conferences, or papers prepared for a particular purpose that are considered to be of interest to a more general readership.

The international dimension of animal use in research, testing and teaching is significant. NAEAC is required to keep abreast of developments in science and technology overseas that may call for change in New Zealand's regulatory framework. And the regulatory regimes of other jurisdictions affect New Zealand's trade.

From time to time comparisons are made between the systems regulating animal use in research, testing and teaching in New Zealand and other countries. Such comparisons are not straightforward. Legislation and its interpretation are complex; and data are not always comparable. The paper by Dr Nicki Cross, Ms Linda Carsons (Principal Adviser, Animal Welfare Directorate, MAF) and Dr David Bayvel (Director Animal Welfare, MAF) explores this issue in respect of the current legislation in New Zealand and the European Union. Their conclusion is that "the experimental procedures and the welfare of the animals utilised during the performance of these experiments, is of a similar, or same, standard in both the EU and NZ".

A poster on this topic was presented at the 7th World Congress on Alternatives and Animal Use in the Life Sciences held in Rome in August/September 2009.

John Martin
Chair, NAEAC

September 2009

NAEAC OCCASIONAL PAPER SERIES

1 *Underreporting of the Three Rs deployment that occurs during the planning of protocols that precedes their submission to animal ethics committees,* D J Mellor, J C Schofield and V M Williams, September 2008

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Regulation of Animal Use in Research, Testing and Teaching: Comparison of New Zealand and European Legislation

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Introduction

The use of live animals in research, testing and teaching (RTT) encompasses a range of investigative, experimental and diagnostic procedures, toxicity or potency testing and animals used in education and training. At the present time, a large number of these procedures performed both within New Zealand (NZ) and internationally require the use of animals to produce accurate and meaningful data. We therefore have an ethical responsibility to ensure that these animals are utilised in a way that has the least detrimental impact on their welfare as is possible.

From time to time, comparisons are made between the regulatory regimes of different jurisdictions. Such comparisons are not straightforward. The relevant legislation is complex; different meanings are assigned to descriptions of, for example, the impact of manipulation on animals; and data are not always collected on a comparable basis. This paper discusses the current legislation applying to the use of animals in RTT in New Zealand and the European Union.

Regulation of Animal Use in RTT in New Zealand

The use of animals in procedures relating to RTT is strictly regulated within New Zealand. Any person or institution that wishes to perform experimental manipulations on animals for the purposes of RTT is required to operate according to an approved code of ethical conduct (in accordance with provisions contained in the New Zealand Animal Welfare Act 1999). This code requires that all projects are scrutinised by an animal ethics committee (AEC) prior to approval for commencement of the study. The AEC consists of a panel who collectively possess a very high level of expertise in a broad range of areas relating to animal health, physiology, husbandry and welfare. All AEC committees must include a veterinarian who is not associated with the organisation, a person nominated by an approved animal welfare organisation and a lay person nominated by local government. The committee members assess the potential benefits and value of each experiment (i.e. the potential benefits for humans or other animals as a result of the data generated by performance of the study) and compare these against the potential harms that may be caused to the animals that are utilised in the experimental procedure (i.e. the amount of pain/suffering experienced by each animal). The committee must consider that the potential benefits of performing each study will outweigh the potential harms, and only then will approval for the study be granted. In addition, all persons performing experimental manipulations within NZ must act in accordance with section 80(2) of the Animal Welfare Act which states that ill or injured animals must, where practicable, receive treatment that alleviates any unreasonable or unnecessary pain or distress. Compliance with this legislation ensures that any pain and distress experienced by animals utilised for experimental procedures performed for purposes of RTT is minimized. Data pertaining to animal use for each experimental procedure are then submitted to MAF, and are published in aggregate on an annual basis by the National Animal Ethics Advisory Committee (NAEAC) (MAF, 2008; Marbrook, 2000; Williams, 2006; NAEAC, 2008).

Regulation of Animal Use in RTT in the European Union

Within the EU, animal usage is regulated according to the legislation contained in Directive 86/609/EEC (which provides an approximation of the laws, regulations and administrative provisions of the member states regarding the protection of animals used for experimental and other scientific purposes; EEC 1986a). This legislation aims to ensure that any pain and distress caused to animals as a result of performance of procedures in RTT is minimal, therefore removing any potential trade barriers among EU member states due to differing national variations in experimental technique and the welfare of animals utilised (EEC, 1986a; Louhimies 2002, TEWG, 2003; ECVAM, 2006). The legislation contained in this directive does not therefore aim to prevent animals being used in RTT, rather it promotes the reduction of pain and distress using actions such as the use of pain relieving drugs and establishment of humane endpoints wherever possible. The directive also outlines provisions for the implementation of reduction and replacement alternatives to reduce the necessity to use animals in some experimental procedures. All members of the EU must comply with this legislation (ECVAM, 2006). In practice, however, the extent of compliance with the directive may be influenced by cultural considerations and differences in interpretation of the legislation between countries and regions. In addition, other national laws exist in some countries which, in some cases, may place a stricter control on the use of animals than does this EU directive (Autissier, 1997; Cussler et al., 1999).

International Comparisons of Animal Usage in Experimentation

International comparisons of animal use in experimentation using quantitative analysis with the aim of producing accurate and meaningful data is, for a number of reasons, not feasible. Data pertaining to animal use in scientific procedures are collected and are published annually both in NZ and in the EU. However, until 2008 publication of these statistics in the EU was performed every three years making accurate comparisons between the two jurisdictions difficult. In addition, data are collected on a general basis in the EU and are allocated into very broad categories (Cooper and Jennings, 2008). These differences in methodologies used for data collection create difficulties in performing meaningful international comparisons about the number of animals used for specific purposes (Purves, 2000; Cooper and Jennings, 2008).

A number of countries adopt a system where the impact that each experiment is expected to have on the animal's welfare can be assessed (TEWG, 2003; Williams et al., 2006). The "impact" or "severity" of each experiment will be dependent on a variety of environmental and experimental factors which are all considered when allocating each experimental procedure to a specific category. The category to which each procedure is allocated is recorded by each research institution and is relayed to the relevant authorities where it is then published as annual national figures (Bayvel et al., 2008; Williams et al., 2006). Information in relation to the use of animals in RTT is of interest to those persons involved directly within animal related industries (e.g. animal scientists, animal ethics committees and regulators) and to those members of the general public who wish to obtain information and reassurance in regard to the use of animals in RTT (Bayvel et al., 2008; Williams et al., 2006).

A scale used to record the impact of each experiment is utilised in a number of countries including Germany, the Netherlands, Poland, the United Kingdom and New Zealand. New Zealand uses a five point impact classification scale which ranges from an experiment having no, or virtually no, impact to an experiment that causes a significant impact on the welfare of the animals utilised (Mellor and Reid, 1994).

However, the legislation contained within EU directive 86/609/EEC does not, at the present time, state that the use of such a scale is a legal requirement and so no standard classification system currently exists in the EU. Different scales are utilised at present within the EU and different countries choose to employ four, five or six point scales. The use of varied classification scales can mean that accurate comparisons of the impact of animal experimentation between different member states are difficult to achieve (Purves, 2000; Williams et al., 2006) and the revision of Directive 86/609/EEC addresses this issue (see below).

In addition to the existence of differing "impact" scales between countries, there is also a certain amount of subjectivity in allocating each experimental procedure to a severity category (Williams et al., 2006). Classification of the severity of an experimental procedure depends on a number of factors including the time interval spent performing the study (i.e. the amount of time that the animal is experiencing pain), the perceived intensity of pain that the manipulation will inflict (e.g. the brief restraint of an animal compared with the performance of a surgical procedure) and the frequency of manipulation (e.g. the severity of obtaining one blood sample compared with obtaining ten blood samples over a specified time period). In addition, other factors such as the competence of each experimenter and the environment in which the animal is housed will also influence the animal's welfare and the amount of pain and distress it will experience (TEWG, 2003; Williams et al., 2006).

The difficulties in performing accurate comparisons in the extent of animal use in RTT between countries are highlighted when, for example, performing a comparison of statistics relating to animal usage for this purpose in the Netherlands and NZ in 2006/2007. The majority of higher impact experimental procedures performed in both of these two countries are performed for regulatory purposes, in particular for shellfish biotoxin and veterinary vaccine testing. Examination of the data collected in relation to animal usage during this time period suggests that there may be differences in the severity of the experimental manipulations performed between the two countries. In reality, however, similar techniques are used for both shellfish biotoxin and veterinary vaccine testing in both NZ and the Netherlands. The perceived differences in the severity of these tests are therefore attributed to the allocation of these experimental procedures into different categories by the experimental institutions and not to any significant differences in the severity of the actual procedures between the two countries (NAEAC, 2008; Anon, 2006). To add further complexity, information relating to animal use in RTT is often collected at different points during the experimental process in different countries (MAF, 2008). Variations such as these in the methods that are used to report and collect data pertaining to animal experimentation make meaningful and statistically accurate analysis difficult and limit any international comparisons of animal usage to broad comparisons only.

Revision of Directive 86/609/EEC

The recently revised version of Directive 86/609/EEC has been published (EEC 1986b). This revision aims to incorporate knowledge gained from scientific advances since the original issue of this directive (Louhimies, 2002). Issues addressed within the revision include the potential harmonisation of a classification system that can be used across all member states (TEWG, 2003), a reassessment of the procedures that are permissible under the category of RTT and a re-examination of species use, husbandry procedures and the existing legal base for animal usage (EEC, 1986b).

The directive also includes legislation promoting the use of alternative techniques that can replace or reduce the number of animals used in experimental procedures (EEC, 1986b). In addition, the revision has also addressed and clarified a number of ambiguities that exist within the definitions of the current text which have, in some cases, led to misinterpretation of the legislation contained within this directive (Louhimies, 2002). The revision of the directive is not yet legally binding but it is anticipated that the final contents and formulation may be instated as legislation in 2010, following the election of a new European parliament and appointment of a new European Commission in 2009.

Hierarchical Organisation of Legislation in the European Union

Although all member states must comply with the legislation contained in Directive 86/609/EEC it should also be noted that a hierarchy of legislation compliance is utilised within the EU. Legislation can be categorised as either "sectoral" or "horizontal".

Sectoral legislation regulates activities in a specific sector, for example, legislation relating to quality control of vaccines or food safety of shellfish. This legislation will take precedence over "horizontal" legislation, which includes legislation pertaining to animal experiments and multilateral agreements (e.g. Directive 86/609/EEC). In principle, the sectoral legislation in any specific sector encompasses all necessary aspects of the relevant horizontal regulations within its legislation, but in some cases two separate directives may contradict each other.

An example of this can be seen in veterinary vaccine testing. Although all experiments involving animals must be performed according to the legislation contained in Directive 86/609/EEC to minimise pain and distress, veterinary vaccines must also be tested in accordance with requirements outlined in the European Pharmacopoeia to ensure batch to batch consistency and quality control (Cooper and Jennings, 2008). During the performance of some types of experimental testing, it is not always possible to administer pain relieving drugs or to set humane endpoints without potentially decreasing the accuracy of collected data. In a scenario such as this where both Directive 86/609/EEC and the relevant sectoral regulation may contradict each other and experimental accuracy may be compromised as a result of the application of pain relief, priority will be given to the maintenance of experimental accuracy and quality control as regulated by the sectoral legislation (Dennison and Anderson, 2007). In certain cases, this may nullify the necessity to adhere to the requirements stated in Directive 86/609/EEC to use pain reducing agents to minimise pain and distress in experimental animals.

Future Proposed Legislation to Protect Experimental Animals

The European Commission has recently presented an additional proposal to further strengthen the protection of animals used in scientific experiments and to rectify the widening divergence of standards between different European member states. It has been proposed that a new directive be introduced that will revise existing requirements for the use of animals in RTT, taking into account changes in research and advancements in our knowledge of animal welfare since the initial legislation was introduced in 1986. It is anticipated that areas of focus for this new directive will include an increased level of ethical justification for the performance of experimental procedures, determination of minimum standards for the housing, care and acquisition of animals and the introduction of legislation making the use of alternatives to animals compulsory wherever they are available.

It is also anticipated that, encompassing principles of replacement reduction and refinement techniques, this new directive will further increase the welfare of animals used in RTT in the EU.

Shellfish Biotoxin and Veterinary Vaccine Testing

Information published by NAEAC outlining animal usage in New Zealand during 2007 show that nearly 80 percent of animals utilised in RTT during this year were classified as experiencing "no" or "little" suffering. Just over eight per cent were classified as having experienced significant (i.e "severe" or "very severe") suffering. The two largest categories for animal usage involving animals that have experienced significant suffering during procedures related to RTT in both the EU and NZ (EC, 2007; NAEAC, 2008) are procedures related to:

1. regulatory testing for shellfish biotoxins; and
2. regulatory testing of veterinary vaccines.

Shellfish Biotoxin Testing

It is compulsory that shellfish are tested for the presence of a number of different biotoxins prior to being made available for sale for human consumption and this is a requirement under the relevant legislation of food safety authorities in both NZ and in the EU. Not all countries perform their own testing of shellfish, and testing is, in general, limited to those countries that have fisheries. A number of countries in the EU therefore contract their testing to other member states (FVO, 2008).

Testing for toxins using mouse bioassays is a widely used procedure in marine biotoxin testing and these tests do have the potential to cause substantial pain and suffering to the animals utilised (ECVAM, 2006; Dennison and Anderson, 2007). In addition, the reliability and accuracy of testing for specific toxins across different laboratories using this procedure has been questioned (BfR; 2005; ECVAM, 2006; Dennison and Anderson, 2007) and therefore, for both ethical and technical reasons, intense efforts are being made to refine and/or reduce reliance on in vivo testing in this area and to advance new replacement technologies (ECVAM, 2006; EFSA, 2008).

To eliminate ultimately the necessity to use animals at all in these tests, focus has been placed on the development of replacement techniques. Methodologies that are currently of interest or are undergoing development to reduce the requirement for live animals include the use of liquid chromatography, mass spectrometry, immunoassays and functional assays (Jellet et al., 2002; McNabb et al., 2005; Turrell et al., 2007).

Reliable alternative testing procedures are, in fact, currently available to test for some classes of toxins. In 2001, the use of a liquid chromatography mass spectrometry (LCMS)-based method (developed by the Cawthron Institute, NZ) was approved as a viable alternative regulatory technique to the mouse bioassay for testing of biotoxins in New Zealand (McNabb et al., 2005). This was the first such approval issued worldwide. The technique has since proved to be faster, more sensitive, and more specific than the mouse bioassay and the introduction of this method in New Zealand has seen a significant reduction in the number of mouse bioassays performed for this purpose. Since its adoption as an approved regulatory technique in 2001, a number of other countries worldwide, including Germany, have also chosen to adopt this methodology for testing of shellfish for regulatory purposes.

The UK is also adopting replacement regulatory techniques and has recently introduced a pre-screening method for the PSP toxin monitoring programme which includes a quantitative high performance liquid chromatography method (Lawrence et al., 2005; Algoet et al., 2007) as a full replacement to the mouse bioassay for the testing of mussels (which constitutes approximately 80 percent of the monitoring programme). These, and other alternative tests, will significantly reduce pain and distress caused to the animals utilised whilst maintaining food safety standards.

Replacement methodologies have not been accepted universally however, and some difficulties have been encountered in encouraging and promoting the use of these techniques (Schiffelers et al., 2007). Problems such as the availability of reference preparations for replacement procedures and in some cases a reluctance by some verification authorities (i.e. food safety and quality control agencies) to accept these new technologies as a safe and accurate method of performing some types of testing have hindered the acceptance and use of these alternative techniques. The difficulties in gaining international acceptance of these replacement techniques can have significant affects on the ability of the countries adopting these technologies to compete economically in an international market and promoting international acceptance of their viability and accuracy is of importance to progress their use on an international scale.

Progress in many other countries has focused on reducing the number of animals used in each test and refinement of the mouse bioassay to reduce the impact on the animals. The European Centre for the Validation of Alternative Methods (ECVAM) published a report in 2006 discussing refinement, reduction and replacement techniques and suggests ways to lessen the requirements for animal testing in this area. Similar reports have also been made by other organisations including a joint report by the Food and Agriculture Organisation of the United Nations, Intergovernmental Oceanographic Commission of Food Safety Authority (EFSA) panel on contaminants in the food chain (2008). Representatives from the food safety authorities from both the EU and NZ have been involved in the ECVAM and CODEX working groups.

As a result of the activities of groups such as these mentioned above, advancements in methodologies that refine the way animals are utilised in these experiments have already been achieved in some areas of biotoxin testing. The UK has introduced modifications to existing techniques that reduce the number of mice required to test for Paralytic Shellfish Poison (PSP) (currently performed using two mice per sample, rather than three, as was previously required) and a reduction in the duration of the assay (Dennison and Anderson, 2007).

Introduction of new methodologies, including PSP testing under general (non-recovery) anaesthesia, are also being progressed with the aim of significantly reducing the amount of pain and distress each animal will experience during performance of this procedure (Holtrop et al., 2006). The introduction of humane endpoints for Diarrhoeic Shellfish Poisoning (DSP) toxin testing has also recently been made compulsory in the UK (Dennison and Anderson, 2007).

Veterinary Vaccine Testing

Veterinary vaccines are ultimately used to protect animals, but, as they are of a biological origin, it is legally required to test them for batch to batch consistency before they are made available for sale (Cooper and Jennings, 2008; OIE, 2008). A large number of experimental animals are utilised during this testing process for purposes of quality control (Cussler et al., 1999).

Vaccine manufacturers and regulatory bodies are located both in NZ and in several EU countries. Animal usage in this area of RTT is high, largely due to the use of potency tests, which require a greater number of animals per test than other batch tests and frequently require that the end points of testing are severe clinical signs and death (Cooper and Jennings, 2008).

In addition, the use of challenge assays is relatively common in the testing of veterinary vaccines. These tests involve direct infection of the animals with the disease agents and the associated potential to cause severe pain and distress. Due to the potential for potency tests and challenge assays to adversely affect the welfare of an animal, should they be deemed necessary, humane endpoints should be pre-determined wherever possible and adhered to should an animal succumb to infection from which it is not expected to recover (Cussler et al., 1999).

The development of fish vaccines is necessary to ensure the health of production stock in fisheries but the welfare of fish that are utilised during the testing of the vaccines may be an area of particular concern. These tests are performed using a similar experimental technique to that used for mammals, but due to a lack of reliable serological markers in fish, testing is commonly performed using challenge assays. It is required that all procedures related to the testing of veterinary vaccines in fish adhere to the legislation contained in Directive 86/609/EEC. However, the lack of knowledge in identifying clinical signs in infected fish may result in some fish suffering significantly or dying prior to humane intervention being possible (Johansen et al., 2005).

Moves are being made to encourage revision and refinement of the methodologies that are currently used in veterinary vaccine testing. Suggestions for improving animal welfare in this area include accurate collection of data pertaining to animal usage, wider acceptance and further progression of the development of novel methodologies that utilise fewer animals, increased sharing of information between vaccine manufacturers and consistent application of humane endpoints where possible (Cooper and Jennings, 2008).

Promotion of the Three Rs

New Zealand adheres to the Three Rs of Russell and Burch (1959) and encourages "replacement, reduction and refinement" in all experimentation utilising animals. The Three Rs are applied in the order stated above in that proposed experimentation utilising animals is "replaced" with other alternative methods that do not utilise animals, wherever possible. If other alternative methodologies are not available, then the number of animals used for each experimental procedures are "reduced" using appropriate existing technologies and/or performing alternative statistical analytical techniques, whilst ensuring that the validity and accuracy of the data obtained is maintained. If, following consideration of the first two Rs, the experiment is still to be performed using animals then all procedures are "refined" to ensure that they are carried out in a manner that causes the minimum amount of pain and distress.

Since the development and introduction of the LCMS method for testing of shellfish by the Cawthron Institute in 2001, the number of animals being used for this purpose has been significantly reduced in New Zealand. Similarly, the EU is consistently working to develop and encourage the use and acceptance of alternatives to replace the utilisation of animals in experimentation. However at the present time, animals are still used for the majority of testing procedures in both NZ and the EU. The development and acceptance of alternative procedures that lessen the requirement for animal usage is a priority in both jurisdictions (ECVAM, 2006; Cussler et al., 1999; Cooper and Jennings, 2008).

The difficulty of making comparisons in this area is illustrated by reference to Articles 8.3 and 8.4 of the European directive as it now stands.

> Article 8.3 *states that "If anaesthesia is not possible, analgesics or other appropriate methods should be used in order to ensure that pain, suffering, distress or harm are limited and that in any event the animal is not subject to severe pain, distress or suffering".*
>
> However, further examination of this Article provides additional information in relation to this point and Article 8.4 states that *"Provided such action is compatible with the object of the experiment, an anaesthetised animal, which suffers considerable pain once anaesthesia has worn off, shall be treated in good time with pain-relieving means or, if this is not possible, shall be immediately killed by a humane method".*

These provisions provide for the reduction of pain and distress in all cases by use of anaesthetics and analgesics except where administration of these substances would interfere with experimental objects of the assay. In the case that administration of these substances may affect the final interpretation of the results of the study, the legislation contained in Directive 86/609/EEC (encouraging application of pain relief) will be overruled by sectoral legislation, which ensures that quality control, production of accurate data and the maintenance of human health standards will be given priority, even at the expense of the animal's welfare. The use of the word "considerable" in this context (Article 8.4) ensures that studies are able to be performed for regulatory purposes whilst ensuring that the animal's welfare is maintained at the highest level possible.

As noted previously, there are some inconsistencies between the legislation contained in Directive 86/609/EEC and that contained within the relevant sectoral legislation in some sectors. These inconsistencies are currently being addressed during revision of the directive. However, the case remains that the experimental procedures and the welfare of the animals utilised during the performance of these experiments, is of a similar, or same, standard in both the EU and NZ.

Conclusions

The adherence to the Three Rs of animal welfare is encouraged in all procedures utilising animals in RTT and significant advances are being made in developing and validating alternative technologies to reduce the number of animals used for this purpose. However, at the present time, alternatives are not currently available for a number of experimental procedures and some testing may cause significant distress to the animals utilised. Testing performed in both NZ and the EU aims to minimise the amount of pain and distress experienced by these animals at all times, but, due to the necessity to ensure that health standards are maintained, administration of pain relief will not overrule the requirements for experimental reliability. This is the case within both the EU and NZ and no significant differences exist between the procedures used or the amount of pain and suffering that is acceptable in both jurisdictions. It is anticipated that revision of Directive 86/609/EEC will enable meaningful quantitative analysis to be performed concerning the number of animals utilised in RTT by harmonising the classification of experimental procedures on an international level.

Intense emphasis is being placed on the development and use of alternatives to replace the use of animals at the present time. Progression of research and associated increase in knowledge in this area, the development of novel in vitro tests and the gradual lessening of the requirement to utilise animals in these experimental procedures is thought by many to be the most effective direction to proceed to ultimately ensure high levels of animal welfare in this area. Perhaps one of most significant hurdles to overcome in progressing these improved technologies that encompass aspects of replacement, reduction and refinement is the international acceptance of these techniques as a valid alternative to the traditional procedures that utilise animals. Verification authorities (i.e. food safety and quality control agencies) have a requirement to uphold safety standards, even at the expense of the animal's welfare, and difficulties have been encountered in gaining acceptance of alternative techniques that use fewer or no animals. These difficulties can, in turn, affect the ability of some countries to export products and compete economically on an international scale. It is hoped that both the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) and the World Organisation for Animal Health (OIE) will be able to facilitate, at an international level, the regulatory acceptance of scientifically validated non-animal tests (Bayvel, 2008). Increased communication and discussion between authorities and considered targeted research focusing on the development and validation of new technologies will assist in devising alternatives that have less detrimental impact on an animal's welfare whilst ensuring the maintenance of quality standards.

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