Canadian Council on Animal Care
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Good Animal Practice in Science
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DISCOVERY IN TOXICOLOGY: SERVING SOCIETY THROUGH THE ETHICAL USE OF ANIMALS IN REGULATORY TESTING

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INTRODUCTION

The most visible application of toxicology in society is safety testing for regulatory risk assessment purposes. Unfortunately, this is also the most controversial application of toxicology as these tests require a large number of animals and often cause them pain and distress. While the Canadian public expects the government to protect them from unsafe products, the public supports animal use in science only when mechanisms are in place to minimize pain and distress (MORI, 2000; Canadian Public Health Association, 2001). The Canadian Council on Animal Care (CCAC) acts on behalf of the people of Canada to ensure that the use of animals for research, teaching and testing employs optimal physical and psychological care without compromising scientific integrity. Due to public concern over the potential for animal testing procedures to cause pain and distress, there has been a particular focus to implement the principles of humane experimental technique (Russell & Burch, 1959) in the area of regulatory testing, e.g. replacing animal use protocols with non-animal alternatives, reducing the numbers of animals used, and refining the procedures (the Three Rs).

The Three Rs – Regulatory Testing Methods

Replacement: Animals may be used only if the researcher's best efforts to find a replacement by which to obtain the required information have failed.

Reduction: The fewest animals appropriate to provide valid information and statistical significance should be used.

Refinement: The most humane, least invasive techniques must be used.

The CCAC

The CCAC is the national peer review organization responsible for overseeing the care and use of animals involved in research, teaching and testing throughout Canada. This oversight includes animal use required by regulatory agencies for data submissions supporting the safety of pharmaceuticals, cosmetics, food, biologics, pesticides and chemicals. As part of its mandate, the CCAC has the responsibility to ensure that animals used in protocols for data submissions to regulatory agencies are not being used unnecessarily, and that minimization of pain and distress is a priority.

In 2001, the CCAC, in collaboration with the International Council for Laboratory Animal Science (ICLAS), hosted the *ICLAS/CCAC International Symposium on Regulatory Testing and Animal Welfare* in Québec City, which was attended by 168 representatives from regulatory agencies, industry, academia and the animal welfare movement from 22 countries. A number of recommendations emerged from this Symposium to improve the implementation of the Three Rs in the area of regulatory testing. The 2006-2008 CCAC Fellowship in Animal Policy Development was offered in the area of regulatory testing in order to ascertain the extent to which the Symposium recommendations have been implemented in Canada and abroad, in addition to investigating the obstacles and opportunities for further implementation of the Three Rs in this area. Preliminary findings from the research fellowship suggest that one of the major obstacles to implementing the Three Rs in regulatory testing is the lack of available alternative test methods. Where alternative methods do exist, acceptance of these methods by regulatory authorities is often impeded because they have not undergone international validation or are in various phases of this lengthy process.

APPLICATION OF THE THREE RS IN TESTING

Below are three examples of applications of the Three Rs in regulatory testing. For each of the Three Rs, the instance where testing is required, the currently used in vivo test protocol and the proposed alternative method, which not only incorporates advances in scientific knowledge but replaces, reduces or refines animal use, are described.

1. Replacement: In Vitro Shellfish Toxin Testing

Bivalve molluscs are filter-feeders and readily accumulate toxic compounds which can severely affect the health of those who eat them. To protect consumers, shellfish beds must be regularly monitored for toxins.

Mouse Bioassay

Currently, the standard method to test whether shellfish have been contaminated by marine biotoxins is the mouse bioassay (MBA) (Yasumoto et al., 1995). To perform this test, shellfish from the area being monitored are harvested and homogenized to produce an extract which is then injected into the body cavities of three mice. These mice are then observed to see how long it takes them to die. If the mice die too quickly, the extract is diluted and the process repeated until the mice die within 5-7 minutes. This latency and the dilution factor of the extract are then translated into the concentration of toxin present in the extract. Although the MBA is the internationally accepted standard for shellfish toxin testing, it cannot reveal the exact toxins in the extract and often underestimates their concentration, which can lead to a false negative test. This assay is also highly variable as it is affected by the age, sex and strain of the mice as well as by the pH and salinity of the extract (Park et al., 1986). In addition, this test causes considerable pain and uses a large number of mice.

Jellett Rapid Test

The Jellett Rapid Test is a qualitative test that uses polyclonal paralytic shellfish poison (PSP) toxin antibodies and the principle of lateral flow immuno-chromatography in a strip format (Jellett et al., 2002). Twenty minutes after the shellfish extract is added to the kit, the coloured lines in the indicator window reveal whether the test is positive or negative. This test is very sensitive, does not require extensive training to perform and can be used at the site where the shellfish are harvested. Although not yet globally approved to replace the MBA, it has been incorporated by the United States Food and Drug Administration (US FDA) into the US National Shellfish Sanitation Program. It is currently being used as a screening test for PSP in California where it is projected to decrease animal use by 30% during the months when the incidence of phytoplankton blooms, which cause shellfish contamination, is low (Oshiro et al., 2006).

2. Reduction: In Vitro Potency Testing for Batch Release of Tetanus Vaccine

Because vaccines are derived from virulent microorganisms or toxins, and because they are administered to large sectors of the population, it is important that each batch is tested for purity, safety, and potency.

Immunization-Challenge Procedure

Currently, animals are used at every stage of vaccine development, including the batch-release testing. Many of the potency tests for inactivated vaccines that are currently used are modifications of the immunization-challenge procedure where a large number of animals are inoculated with the vaccine and then exposed to the disease the vaccine is designed to protect against. For potency testing of inactivated vaccines, most monographs in the European Pharmacopoeia specify lethality as the endpoint.

Enzyme-linked Immunosorbant Assay & Toxin Binding Inhibition Test

Two in vitro tests were developed by the National Institute for Biological Standards and Control (UK) as a replacement for the lethal or paralytic challenge procedure in the potency testing of tetanus toxoid vaccines in guinea pigs. The enzyme-linked immunosorbant assay (ELISA) and the toxin binding inhibition (ToBI) test for tetanus vaccines measure antibody concentration in the serum from immunized guinea pigs and compare it to standard serum. Generally, the ELISA and ToBI procedures are more predictive of potency than the in vivo challenge test (Winsnes et al., 1999).

> "An additional advantage of a serological potency test is that a precise value (antitoxin titer) is used instead of a qualitative outcome (death/survival), which increases the amount of data per animal and requires fewer animals without loss of test precision" (Hendriksen et al., 1987).

These methods have been validated by the European Centre for the Validation of Alternative Methods and were adopted by the European Pharmacopoeia Commission in March 2002 (Council of Europe, 2002).

3. Refinement: In Vivo Skin Sensitization Testing

Skin sensitization is a manifestation of chemical allergy. When a sensitizing chemical is applied topically, it may cause a rash, blotching and itching, or even the formation of blisters on the skin at the site of application.

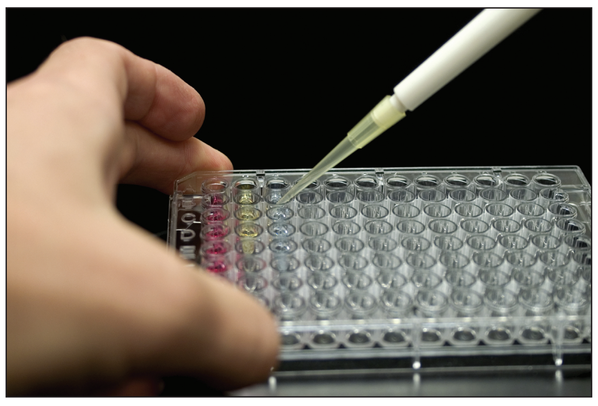
The Guinea Pig Maximization Test

The guinea pig maximization test (GMT) (Magnuson & Kligman, 1969; Nakamura et al., 1994) qualitatively evaluates the irritation and inflammation caused by the application of the test chemical to the shaved flank of guinea pigs. This test has the potential to cause a very painful and distressing cutaneous immune response for test animals.

The Local Lymph Node Assay

The local lymph node assay (LLNA) capitalizes on the underlying biological mechanisms of skin sensitization. When a sensitizing chemical is absorbed by the skin, it is internalized by Langerhans cells in the epidermis which process the allergens and migrate to skin draining lymph nodes where they mature into immunostimulatory dendritic cells (DC). These DCs present the allergen to T lymphocytes in the lymph node which causes them to proliferate, increasing the number of cells able to recognize and respond to the sensitizing chemical. The proliferation is proportional to the dose applied and to the potency of the allergen. The LLNA uses cell proliferation in the lymph nodes as an indicator of skin sensitization (Kimber & Weisenberger, 1989). This is a refinement over the guinea pig maximization test because it does not cause the pain and distress associated with the contact dermatitis elicited by the GMT. This method has undergone international validation and has been incorporated into an Organisation for Economic Cooperation and Development (OECD) test guideline (OECD TG 429).

Toxicologists need to work towards applying new findings in their field to improve both the predictive value of safety toxicology protocols and the implementation of the Three Rs in the area of regulatory testing.



NEXT STEPS

Many other alternative methods exist in addition to those described above. However, not all tests have had the same success in terms of validation and acceptance. Also, it is often the case that a number of alternatives have been developed to replace a single in vivo test, such as the Draize Eye Test, while no alternatives have been developed for other in vivo tests. Many in vivo safety toxicity tests have remained virtually unchanged since their conception and in many cases, these tests are unspecific, slow, costly, and have not been properly validated for their ability to predict toxicity in man. Toxicologists need to work towards applying new findings in their field to improve both the predictive value of safety toxicology protocols and the implementation of the Three Rs in the area of regulatory testing. In particular, scientists need to pursue non-mammalian models and find, and agree on, earlier endpoints for safety toxicology testing. In the interim, there is a need to publicize and promote these efforts, not only to make regulators and industry aware of them, but also to encourage the toxicology community to continue developing alternative test methods (Rollin, 2003). Once alternative methods have been developed, they need to be validated and accepted for regulatory use. This can be achieved by broader communication of these methods through publication in peer-reviewed journals and by improving the dialogue between researchers who develop alternatives, study directors who conduct safety testing, and policy makers (Schiffelers et al., 2005).

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References

Please see handout for a complete list of references.