Acute toxicity testing of chemicals : opportunities to avoid redundant testing and use alternative approaches / NC3Rs.

■ Skin irritation testing *continued*

When animal testing is required, best practice options such as the initial use of one animal and restriction of testing to the minimum number of animals necessary to achieve the study objectives, as set out in international test guidelines, should be employed.

■ Eye irritation testing

Tiered testing strategies such as those set out by the OECD and GHS should be implemented to reduce *in vivo* eye irritation testing to a minimum.

Positive findings in *ex vivo* assays are accepted by the EU for identification of severe irritants. Two OECD Test Guidelines using isolated eye methods are also available and several US regulatory agencies have indicated their support for these methods for identifying severe eye irritants[2].

When *in vivo* studies are required, best practice options such as the initial use of just one animal and restriction of testing to the minimum number of animals necessary to achieve the study objectives, as set out in international test guidelines, should be employed.

■ Skin sensitisation testing

The local lymph node assay (LLNA) provides a refinement over the guinea pig assays for skin sensitisation, and is the preferred method under REACH and European regulations on plant protection products (91/414/EEC).

Analysis presented in the paper indicates that LLNA performs at least as well as guinea pig assays for predicting the sensitisation potential of formulations. In addition, a recent NICEATM-ICCVAM[3] independent peer review panel has concluded that the LLNA should be considered appropriate for testing pesticide formulations and other products[4]. The LLNA should therefore be accepted globally for sensitisation testing of substances, mixtures and formulated products, apart from cases where there is a scientific basis for exclusion.

National Centre for the Replacement, Refinement and Reduction of Animals in Research

20 Park Crescent
London
W1B 1AL

Tel: 020 7670 5331
Fax: 020 7670 5178
email:enquiries@nc3rs.org.uk

www.nc3rs.org.uk

Printed on stock containing 55% recycled fibre

NC3Rs

National Centre for the Replacement, Refinement and Reduction of Animals in Research

ACUTE TOXICITY TESTING OF CHEMICALS: OPPORTUNITIES TO AVOID REDUNDANT TESTING AND USE ALTERNATIVE APPROACHES

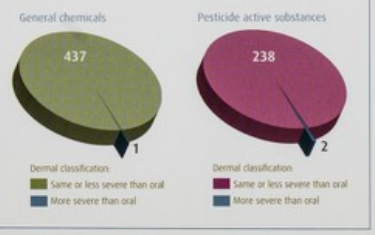
Acute toxicity testing of chemicals: opportunities to avoid redundant testing and use alternative approaches

Experts from the chemical industry, CROs and regulatory authorities, together with the NC3Rs, have published a review paper highlighting opportunities to waive requirements for acute toxicity testing of non-pharmaceutical chemicals. The review focuses on acute oral, dermal and inhalation toxicity, skin and eye irritation and skin sensitisation. Alternative approaches that can replace, reduce or refine the use of animals are also discussed.

The paper, which is published as an open access article in *Critical Reviews in Toxicology*[1], is intended to provide a focused review for the regulatory community to use when considering the need to generate acute toxicity data.

Adoption of best practices as presented in the paper will reduce and refine animal use in acute toxicity testing of chemicals. This leaflet summarises the key points highlighted in the paper.

Analysis of acute toxicity classifications for industrial chemicals and pesticide active substances shows that acute dermal testing rarely provides value for hazard identification or classification and labelling purposes when an acute oral study has been conducted.



General options for waiving of testing

A number of general options for waiving of acute toxicity testing are set out under REACH and other international regulatory frameworks, and the main points, which should be considered as best practice in determining when *in vivo* testing is not required, are summarised in the following table:

GENERAL CASES WHERE ACUTE TOXICITY TESTING SHOULD BE WAIVED
Substance is likely to be corrosive based on pH, physicochemical properties or result of a validated *in vitro* assay
A weight of evidence analysis demonstrates that other information is sufficient for a hazard characterisation
Exposure to the substance is adequately controlled
Substance is not bioavailable via a specific route and possible local effects are adequately characterised
Relevant data on related substances are available allowing read-across
Bridging principles and calculation methods can be applied to classify mixtures of chemicals, based on data available for the ingredients
SPECIFIC CASES FOR WAIVING ACUTE INHALATION TOXICITY TESTING
Particle size is greater than the relevant regulatory cut-off
Vapour pressure is very low (< 0.1 Pa at 20˚C)
Not technically possible to generate a testing atmosphere
Substance in its native form is not inhalable

1 Creton S, Dewhurst IC, Earl LK, Gehen SC, Guest RL, Hotchkiss JA, Indans I, Woolhiser MR, Billington R. (2010) Acute Toxicity Testing of Chemicals: opportunities to avoid redundant testing and use alternative approaches. *Critical Reviews in Toxicology* **40**(1): 50-83

2 http://iccvam.niehs.nih.gov/methods/ocutox/invocutox/ocu_recommend.htm

3 National Toxicology Program Interagency Centre for the Evaluation of Alternative Toxicological Methods – Interagency Coordinating Committee on the Validation of Alternative Methods

4 http://iccvam.niehs.nih.gov/methods/immunotox/llna.htm

Endpoint specific waiving options:

■ Acute oral toxicity testing

When *in vivo* testing is necessary, European regulations require that the test selected should be that expected to cause the least animal pain, suffering, distress and lasting harm. Of the three OECD test methods for acute oral toxicity, the Fixed Dose Procedure (FDP; TG 420) does not employ lethality or impending death as an endpoint. Use of the FDP should therefore be considered where the method will meet scientific and regulatory requirements.

■ Acute dermal toxicity testing

Analysis presented in the paper demonstrates that for pesticide active substances and general chemicals, acute dermal toxicity testing very rarely provides information of value for hazard identification or classification and labelling purposes, when an acute oral study has already been conducted (see pie charts).

These findings suggest that acute dermal toxicity studies should not be performed except in exceptional circumstances, for example where information on absorption, toxicokinetics or mode of action suggests that acute toxicity might be greater by the dermal rather than oral route.

■ Acute inhalation toxicity testing

Generic and endpoint-specific options to avoid inhalation testing of chemicals are highlighted in the table.

If *in vivo* testing is required, the Acute Toxic Class method (ATC; TG 436) uses fewer animals than the LC50 method (TG 403). The ATC method is able to satisfy most regulatory needs, providing a range estimate of the LC50 and supporting classification and labelling, and should be selected in all cases where it is able to meet scientific or regulatory requirements.

■ Skin irritation testing

An *in vitro* test has been validated and accepted within the EU for distinguishing between skin irritants and non-irritant substances. Wider adoption by the OECD is also anticipated in the near future. This test should be used wherever it is accepted and meets regulatory needs.