

# The International Symposium on Regulatory Testing and Animal Welfare: Recommendations on Best Scientific Practices for Acute Systemic Toxicity Testing

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## Introduction

Breakout Group 2 participants addressed approaches to the immediate reduction of animal use and the pain and distress associated with acute systemic toxicity testing. They also discussed programs that could have effects over the long term. The group consisted of individuals (listed at the end of this report) with experience in evaluation of new chemicals and pharmaceuticals, laboratory animal veterinarians, and representatives from regulatory agencies and animal welfare groups. Members had previously reviewed a number of key background references (also listed at the end of the report) that had been selected by the group's leaders before the meeting.

Participants were asked to consider the following questions as part of their general discussion:

1. How can we facilitate the rapid acceptance by industry of the new acute oral toxicity guidelines that were accepted by the Organisation for Economic Co-operation and Development (OECD<sup>1</sup>) at the Joint Meeting prior to the International Council of Laboratory Animal Science (ICLAS<sup>1</sup>)/Canadian Council on Animal Care (CCAC<sup>1</sup>) meeting, and the withdrawal of the old OECD 401 guideline?
  - a. What should the community do to encourage the use of study designs that minimize the number of

- animals needed, both by the users and by the regulatory agencies?
    - b. If training is needed to facilitate the introduction of the new study designs, who will provide the training and who will fund it?
    - c. Does the phase out of the OECD test guideline 401-type design need to be enforced?
    - d. Can the new study designs be applied immediately to acute inhalation studies and/or acute dermal studies?
    - e. Can these study designs be applied to nonrodent studies, should they be?
2. How can we immediately utilize, as much as possible, endpoints that minimize pain and distress in acute systemic toxicity studies?
  - a. Can current humane endpoint guidelines be applied to acute systemic toxicity studies?
  - b. Are these endpoints being applied to acute systemic toxicity studies? Does someone need to enforce the use of humane endpoints; if so, who and how?
  - c. How well do the current nonlethal endpoints in OECD 420 succeed in minimizing pain and distress? Could this example be applied to the other study designs, and if so, what types of validation studies would be necessary?
  - d. Is there a need to develop a specific humane endpoint guideline for acute systemic toxicity studies?
3. How should we implement the proposal to use in vitro cytotoxicity tests to set doses for acute systemic toxicity studies?
  - a. What is being done/should be done to "validate" this proposal? Are human cell lines necessary? Who is or should be responsible? Who is or should be providing funding?
  - b. What are the costs of this proposal, both in time and dollars? Are human cell lines necessary? Who is or should be responsible? Who is or should be providing funding?

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<sup>1</sup>Abbreviations used in this report: ACC, animal care committee; ACT, American College of Toxicology; ECETOC, European Centre for Ecotoxicology and Toxicology of Chemicals; ECVAM, European Centre for Validation of Alternative Methods; EFPIA, European Federation of Pharmaceutical Industries and Associations; EPA, Environmental Protection Agency; FRAME, Fund for Replacement of Animals in Medical Experimentation; ICCVAM, Interagency Coordinating Committee for the Validation of Alternative Methods; ILSI, International Life Sciences Institute; LD<sub>50</sub>, median lethal dose; MEIC, Multicenter Evaluation of In Vitro Cytotoxicity; OECD, Organisation for Economic Co-operation and Development; QSAR, quantitative structure activity relationship; RSPCA, Royal Society for Protection of Cruelty to Animals; SOT, Society of Toxicology.

## Report on Group Discussions

### Current Best Practices

Immediate progress in acute systemic toxicity is possible through the Breakout Group's first recommendation, **use of the three new acute oral toxicity guidelines** approved by

the OECD. Guidelines 420, 423 and 425, available from the OECD, offer major improvements over the previous OECD guideline 401—the median lethal dose ( $LD_{50}$ ) or “classical  $LD_{50}$ .” OECD member countries have agreed to cease accepting data generated using the 401 method if the testing was initiated after December 17, 2002. The difficulty will be in enforcing this statement. There will need to be significant proactive training, particularly in Europe, North America, and Asia, to acquaint toxicologists, regulators, and contract laboratory personnel with the new guidelines and the phase out of 401. Such training is planned in Rome, to be sponsored by the International Life Sciences Institute (ILSI<sup>1</sup>) in 2001. ILSI, the US Environmental Protection Agency (EPA<sup>1</sup>), and the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM<sup>1</sup>) plan to hold a training workshop in the United States in early 2002. There are currently no plans for training in Asia, but the Japanese National Institute of Health Sciences should be encouraged to hold such training.

To facilitate the adoption of the guidelines, the results of the OECD working groups on the new guidelines, as well as the report of the ICCVAM Peer Review of the revised up-and-down method (guideline 425), should be published. The working group for the up-and-down method has made several presentations on this method and is planning to publish their work in 2002. The biomathematical basis of the revised acute toxic class method (guideline 423) has been published. The working group for the fixed dose procedure (guideline 420) is encouraged to publish their work as well.

The OECD working groups are also encouraged to present their work at national and international meetings of toxicologists such as those of the Society of Toxicology (SOT<sup>1</sup>), the American College of Toxicology (ACT<sup>1</sup>), and the Asia Alliance of Toxicology Societies, as well as toxicology meetings in Australia and New Zealand. Guideline 425 will be mentioned at the Toxicology Forum meeting in the United States in July 2001 and will be addressed at a workshop at the 4th World Congress on Alternatives in 2002.

The reports and publications should focus on when acute oral toxicity data are needed, how the data can be used, and the new methods for performing the tests. They should also include a discussion of the value of the new methods not only in reducing animal use and, in the case of Guideline 420, reducing distress but also in their increased statistical robustness. Presentations should also stress the regulatory acceptance of the new methods and the phase out of the 401 method.

A second important source for immediate progress, Breakout Group 2 participants' second recommendation, is **the use of in vitro data to set starting doses for any acute oral toxicity test**. Several papers have described this approach, and the ICCVAM Workshop on Acute Oral Toxicity recommended its immediate adoption in the fall of 2000. This approach provides a good estimate of the  $LD_{50}$  for approximately 70% of the compounds, and it should be used whenever there are not good data on similar compounds to

help in dose setting. Publication of guidelines for this approach is needed as well as training on the use of the data.

Publications and presentations should emphasize the benefits of the approach. All three guidelines perform better when the starting dose is close to the actual  $LD_{50}$ , supporting the use of the in vitro method to help determine starting doses. In addition, the use of the in vitro method can save test material by minimizing animal use and can reduce the duration of the in vivo test both by reducing the number of dosing steps needed and by reducing animal use. It is also possible to generate some mechanistic data from in vitro tests. The EPA and ICCVAM are funding further studies to evaluate this approach, and the European Centre for Validation of Alternative Methods (ECVAM<sup>1</sup>) also has funds for similar studies.

In countries with active animal care committees (ACCs<sup>1</sup>), the information on the adoption of the three new guidelines, the phase out of the 401 guideline, and the recommendations to use in vitro data to set starting doses should be proactively communicated to all ACCs by the appropriate agencies and groups (e.g., Scientists Center for Animal Welfare) that provide training to members of these committees. The ACCs have the authority to enforce the use of alternative methods, and because all OECD countries have now adopted the alternative methods, there should be little justification for the use of older methods. In addition, contract laboratories should request and receive enough information on test materials to set adequate starting doses and, in the case of guideline 425, estimate the slope of the dose response curve.

The third recommendation is to **consider immediately applying the changes in study design incorporated in the three new acute oral toxicity guidelines to the guidelines for acute dermal and acute inhalation studies**. Although it may not be possible or practical to conduct these studies using all three approaches, it should be possible to modify these guidelines to incorporate sequential testing and range finding techniques. A group of scientific experts in this area should be assembled to determine the feasibility of this approach, statistical considerations, reduced use of animals, and improvements in the quality of the data obtained from the tests. Some of this work is already in progress in Europe, and further work could be done through the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC<sup>1</sup>), ECVAM, SOT, ACT, and other funding agencies at the national level.

The fourth recommendation is to **evaluate critically the use of nonrodent species, particularly dogs and primates, in pharmaceutical testing**. A coalition of the Fund for Replacement of Animals in Medical Experimentation (FRAME<sup>1</sup>), the Royal Society for Protection of Cruelty to Animals (RSPCA<sup>1</sup>), and the European Federation of Pharmaceutical Industries and Associations (EFPIA<sup>1</sup>) is currently evaluating approaches to minimize dog use in preclinical toxicology. The coalition is considering establishing a vehicle effects database for canine studies, defining best practices in several aspects of study design, and

critically evaluating the need for terminal 3- to 6-mo studies. At a minimum, the choice of the second species should be based on an understanding of the pharmacology of the test substance, data sharing, and assessing the need for a particular study, rather than on policy consideration or “expected practice.”

The fifth recommendation is to **expand the use of humane endpoints in acute toxicity testing to minimize pain and distress**. Wide publication and discussion of the current guidelines are necessary to ensure their appropriate use. In addition, the types of endpoints that can be identified for early euthanasia should be expanded. Thorough retrospective examination of historical data may allow identification or scientific justification for additional endpoints. These studies could be funded by ICCVAM, ECVAM, EPA, the European Union, the American College of Laboratory Animal Medicine Foundation, and other national funding agencies. ACCs should be alerted to the need for careful documentation of clinical signs during acute studies, including the use of improved reporting formats. Regulatory agencies should request these reports as part of the submission of study results. It may be possible to justify earlier endpoints for certain classes of chemicals. At the very least, it should be expected that additional studies on any single chemical would consider use of early predictive endpoints identified in initial acute studies.

Finally, the group recommended carefully considering the use of data from acute toxicity studies and making every effort to **incorporate all relevant endpoints into a single acute toxicology study design**. Oral acute exposure studies are necessary not only for determination of the hazard classification and risk management of chemicals but also because the data generated from these studies are used in setting doses for subsequent repeat dose studies. Acute oral exposure studies are also part of safety pharmacology testing; however, the guidelines for these studies are very different from the guidelines for studies done for chemical safety. At this time, this difference can result in the necessity of performing two separate tests.

Participants recommend developing a single harmonized study design that would meet both needs. This effort may involve developing a way to use data generated by guidelines for hazard classification under the harmonization of classification and labeling principles established by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. Alternatively, it may involve developing a set of common endpoints for both uses. A meeting was suggested to address these specific issues, possibly through SOT and at the 4th World Congress on Alternatives 2002.

## Future Improvements

The need to ensure correct hazard classification and risk management for chemicals in commerce is providing the impetus for efforts to estimate the toxicity of chemicals

based on their chemical structure. Although the use of quantitative structure activity relationship (QSAR<sup>1</sup>) data may be useful for estimation of starting dose, current programs do not support accurate quantitative estimation of acute oral toxicity. When the mechanism of toxicity has been identified, it may be possible to estimate toxicity based on chemical structure. However, in most cases, QSAR as it now exists cannot replace the use of animals for estimation of acute oral toxicity.

In the longer term, current methods for estimation of acute toxicity of chemicals to humans should be reconsidered. The Multicenter Evaluation of In Vitro Cytotoxicity (MEIC<sup>1</sup>) studies revealed that it is possible using in vitro methods to estimate human toxicity for more than 70% of the chemicals studied. These data also indicate that the prediction can be improved when mechanistically based methods are added. The in vitro methods could be used to screen new chemicals for drugs or pesticides.

From the perspective of clinical toxicology, acute death as the only endpoint should be reconsidered. It is important to understand what organ systems will fail first and where to focus treatment. It may be more appropriate to use significant toxicity as the endpoint for human exposure.

Finally, participants considered the development and validation of replacement tests for acute toxicity. Initially, it is necessary to compare in vitro cytotoxicity data with in vivo data for a wide range of chemicals including pesticides and drugs. The chemical classes that were considered adequate for the validation of the acute toxic class, fixed dose, and up-and-down methods should be evaluated as a validation set for future test methods, including the in vitro procedures suggested by the MEIC study. If human toxicity is the most important use of these data, then an increased amount of good quality human data will be needed for evaluation and validation studies. One possible approach is to consider developing in vitro methods that identify chemicals that produce toxicity by certain mechanisms of action. Another possibility is to develop test methods for certain dose concentration ranges. Both of these approaches should be considered.

## Summary and Recommendations

In summary, Breakout Group 2 participants believe there are seven significant steps that could be implemented immediately or in the very near future that would have a major impact on the use of animals in acute toxicity testing and the amount of pain and distress caused by these studies. For the longer term, the primary need is to explore the ability of the in vitro tests identified in the MEIC study to estimate the toxicity of new chemical entities to humans. The following steps are recommended:

1. Results of the OECD working groups on test guidelines 420 and 423 and the report of the ICCVAM peer review of revised test guideline 425 should be published

and presented at national and international meetings of toxicologists.

2. In vitro data should be used to set starting doses for acute oral toxicity test if better data do not exist.
3. ACCs should be informed that the three new guidelines are the current best practices for acute oral toxicity testing. Phase out of TG401 and recommendations to use in vitro data to set starting doses should be communicated.
4. Scientific experts should determine the feasibility of applying the change in study design incorporated in the new oral toxicity guidelines to the guidelines for acute dermal and acute inhalation studies.
5. The effort of the FRAME/RSPCA/EFPIA working group on the need for a second, nonrodent species in pharmaceutical evaluations should be expanded internationally.
6. Retrospective examination of historical data should be used to help identify suitable endpoints for early euthanasia.
7. A single harmonized study design should be developed to meet the needs for acute systemic protocols for both chemical and pharmacology safety testing.

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