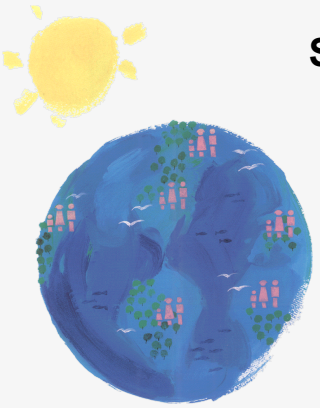
NICEATM — *National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods*

ICCVAM — *Interagency Coordinating Committee on the Validation of Alternative Methods*



Session 5 Panel Discussion Summary: Reduction Alternatives for *In Vivo* Botulinum Testing

Dr. Marlies Halder & Dr. Richard McFarland, Moderators

ICCVAM/NICEATM/ECVAM Scientific Workshop on Alternative Methods to Refine, Reduce, and Replace the Mouse LD_{50} Assay For Botulinum Toxin Testing

November 13 -14, 2006

Crowne Plaza Hotel, Silver Spring, MD

National Toxicology Program | NIEHS | National Institutes of Health

Panel Discussion Question # 1

- **Is it feasible to use the mouse LD_{50} assay to assess the potency of batch production samples of botulinum toxin and use a validated *in vitro* and/or *ex vivo* test method to assess potencies of final production lots? Why, or why not?**
  - It is feasible and practical
    - Need to identify areas where the most animals are used and address them first
    - Regulatory decisions will continue to be made on a case-by-case basis
  - This is done in EU (NIBSC) for the purposes of confirmatory testing
    - Manufacturers provide LD50 - confirmed with alternative at NIBSC
  - Primary responsibility is patient protection
    - Therefore, need assay that measures fully functional molecule
    - Limitations exist for all alternatives
  - Need international harmonization of the LD50 assay with regard to study design
  - Comparability acceptance criteria are not well defined
    - Assigning prospective criteria for acceptance currently very subjective
    - Need statistical approach

Panel Discussion Question # 2

- **Are there validated test method modifications (e.g., use of reference standards) that could be made to the current mouse LD_{50} test method protocol to decrease the number of mice tested?**
  - Modified lot release assay
    - Reduce number of animals used by testing fewer far from the dose response for confirmatory assays (with reference to the well known steepness of the dose-response curve)
  - Potency reference standard program
    - Reduces *in vivo* testing by refinements to:
      - Extend the shelf-life of the working reference standard
      - Improve the efficiency of the qualification program

Panel Discussion Question # 3

- **Should a reference standard always be used in a validation study conducted on the *in vitro* and *ex vivo* methods discussed at this workshop? Why, or why not? Should an international botulinum toxin reference standard be created for this purpose? If yes, how should it be maintained?**
  - It is essential to use a common set of suitable samples in any validation studies and in any studies comparing different assay methods.
    - Inclusion of a set of common samples with known long-term stability and in sufficient quantity for multiple uses is therefore desirable.
    - This allows for comparison between different studies at different times and for continuity/harmonization of assay methods.
  - Use and establishment of an International Standard would contribute towards harmonization
    - However, would be very difficult to implement
    - Product specific standards are still likely to be needed
  - At present, each manufacturer uses own, product specific standard for potency testing
  - Need to develop a central repository with associated standardized methodology

Panel Discussion Question # 4

- **What are the best practices for minimizing the number of animals used?**
  - Tiered testing system (see question #2)
  - Reference standards that could be used in all studies
    - Would minimize the number of replicates needed
  - Consider development of a universal reference standard
  - Developing standardized methodology