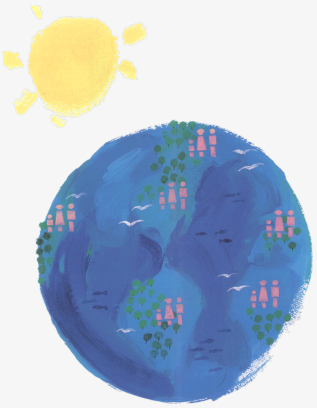
NICEATM — *National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods*

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



Session 2 Panel Discussion: Knowledge Gaps and Research Needs for Botulinum Toxin

Dr. James Keller & Dr. Ram Ramabhadran, Moderators

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

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Crowne Plaza Hotel, Silver Spring, MD

National Toxicology Program | NIEHS | National Institutes of Health | U.S. Department of Health and Human Services

Panel Discussion Question # 1

- **What knowledge gaps in the current understanding of the mechanism of action of botulinum toxin must be addressed to develop non-animal replacement methods for potency testing or detection of botulinum toxin?**
  - The receptors for all botulinum toxin serotypes need to be fully characterized
  - The extent to which toxin potency depends on its intended use needs to be characterized
  - The roles for other proteins in the complex and how they affect potency need to be characterized
  - There is a need to identify ways to demonstrate comparability between products/assays
  - There is a need for standardization in regard to:
    - Reference materials
    - Practices
    - Traceability back to the reference materials
  - There is a need for regulatory agencies to express their expectations in regard to the development of alternatives to the LD50 potency assay
    - Regulators should provide internationally harmonized guidance
  - Although not presently considered by any assay, ways to predict variability in human responsiveness might be useful

Panel Discussion Question # 2

- **To what extent does current research address these knowledge gaps? Does additional effort need to be applied to these areas?**
  - Very little basic research is being supported; most funding in the U.S. devoted to product development for biodefense
  - Funding for research on botulinum toxin in the EU is not coordinated among member countries but should be
  - There is a need to establish an internationally-recognized "standard reference" facility
  - How to bridge from alternative tests to the LD50 test
    - Need to calibrate *in vitro* results in terms of LD50 units
    - Will then need reference standards that were tested in the LD50 test
    - Alternatively, once it has been established that an alternative method is comparable to the LD50 test, can drop the LD50 test and use LD50 equivalent units
    - However, this approach has not been adopted everywhere for diptheria and tetanus toxoids
  - A "functional" assay is the critical standard but thus far, no single alternative assays do not address all "functions"

Panel Discussion Question # 3

- **What research initiatives are necessary to address these knowledge gaps and further characterize mechanisms and modes of action in order to advance the development of non-animal replacement methods for potency testing or detection of botulinum toxin?**
  - Need to evaluate the use of phylogenetically lower species (e.g., daphnia)
  - Recommend funding for a consortium of laboratories to evaluate the different ways of measuring potency and to prioritize future activities
  - Need cell-based assays that mimic presynaptic function
  - Need to characterize the mechanism(s) involved in receptor recognition and the various enzyme substrate
    - internalization/translocation should also be evaluated