Alternatives to the Mouse LD_{50} Assay For Botulinum Toxin Testing: An ICCVAM/NICEATM/ECVAM Sponsored Workshop

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ICCVAM, NICEATM, and ECVAM co-sponsored a workshop in November 2006 to review the current state-of-the-science and knowledge of alternatives to the mouse LD_{50} assay that is currently used by U.S. and European regulatory agencies to both detect and assess potency of botulinum toxin. Recent research efforts have provided new prospects for test methods that may be faster and more accurate, while also refining (cause less pain and distress), reducing, or replacing animal use. The workshop reviewed the regulatory requirements necessary to protect public health, structural aspects and mechanisms of action of botulinum toxin, and the currently available alternative methods and testing strategies. *In vitro* (e.g., endopeptidase assays, cell-based methods), *ex vivo* (e.g., mouse phrenic nerve-hemidiaphragm assay), and non-lethal *in vivo* (e.g., mouse hind limb assay, mouse abdominal ptosis assay) alternative test methods were considered. Recommended testing strategies incorporate earlier, more humane endpoints. Panel discussions addressed the usefulness and limitations of the alternative methods and testing strategies along with their potential to reduce, refine, and replace the LD_{50} assay. Participants in the workshop identified knowledge gaps and suggested future research initiatives. ILS, Inc. staff supported by NIEHS contract N01-ES 35504.