

ICCVAM/NICEATM/ECVAM Scientific Workshop on Alternative Methods to Refine, Reduce and Replace the Mouse LD₅₀ Assay For Botulinum Toxin Testing

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A mouse LD₅₀ assay is used by U.S. and European regulatory agencies to detect botulinum toxin in food and environmental samples, and to assess the potency of therapeutic toxin. Recent advances provide new prospects for the development and validation of alternative methods that may be faster, more accurate, and may refine (cause less pain and distress), reduce, and replace animal use. ICCVAM, NICEATM, and ECVAM co-sponsored a public workshop in November 2006 to review the state-of-the-science and current knowledge of alternatives to the mouse LD₅₀ assay. The workshop reviewed public health needs, current regulatory requirements, structural aspects and mechanisms of action of botulinum toxin, and specific alternative methods and approaches for their potential to reduce, refine, and replace the mouse LD₅₀ assay. Specific alternative methods considered included *in vitro* (e.g., endopeptidase assays, cell-based methods), *ex vivo* (e.g., mouse phrenic nerve-hemidiaphragm assay), and *in vivo* test methods (e.g., mouse hind limb assay, mouse abdominal ptosis assay). Alternative approaches considered included evaluation of earlier more humane endpoints. Panel discussion outcomes identified hindrances to further development of alternative methods, including knowledge gaps, and suggested future research initiatives to address them. Panel discussion addressed the current development and validation status of specific alternative methods and approaches, their strengths and limitations, and their potential to reduce, refine, and replace the mouse LD₅₀ assay. Implementation of some of these alternative methods could decrease the number of animals tested and reduce animal pain and suffering. ILS, Inc. staff supported by NIEHS contract N01-ES 35504.

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