Refinement and Reduction in Botulinum Toxin Testing

Session 5

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

14 November 2006

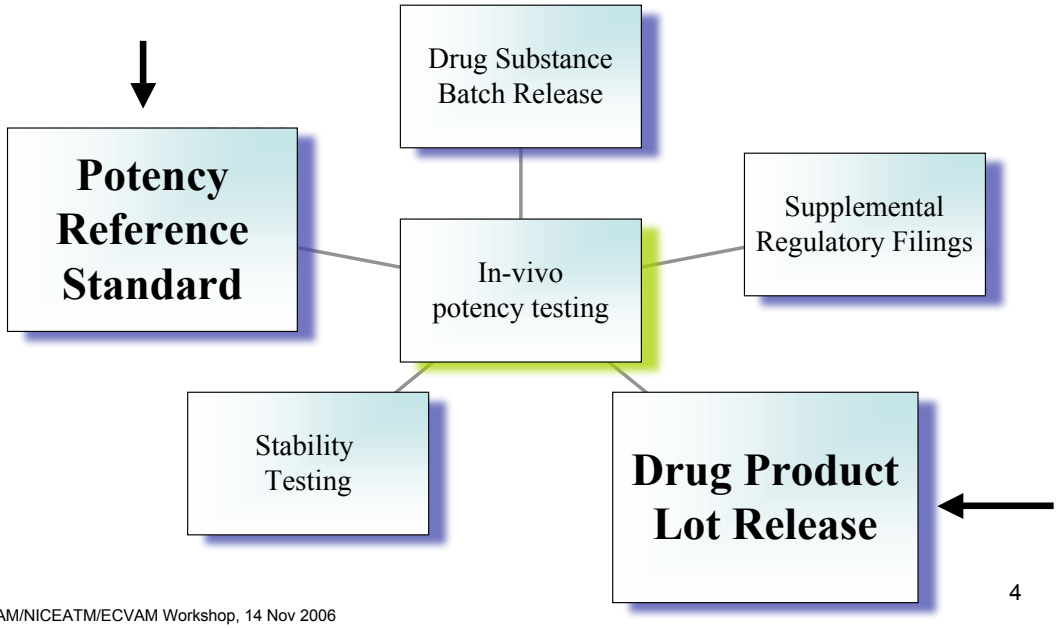
Outline

- Introduction
- Areas for Refinement and Reduction
- Drug Product Lot Release Testing
  - Evolution of Potency Testing
  - Dose Response / Data Analysis
  - Refinement of assay
- Potency Reference Standard Program
- Other Avenues of Reduction
- Future Areas
- Summary

Introduction

- Globally, the LD50 is the required licensed potency test
- Acceptable non-animal tests are under development and are not currently available for implementation
- Recognizing this, in parallel with **R**eplacement of this assay there is focus on **R**eduction and **R**efinement for immediate impact
- Goal: Minimize use of in-vivo testing until 3rd 'R' can be achieved

Areas for Refinement and Reduction

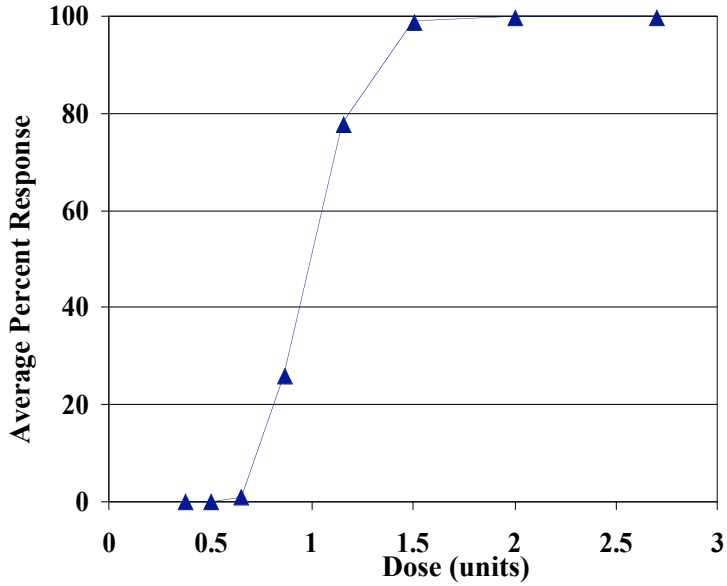


Drug Product Lot Release
(Evolution of Potency Testing)

- Uncorrected Potency
- ✓ Introduction of Potency Reference Standard
- ✓ EP monograph
  - Use of Potency Reference Standard Required
  - 3 alternate assays listed
    - 'Validate with respect to the LD_{50} assay'
- ✓ Refinement of Assay

Progress has been made in Assay Refinement

Drug Product Lot Release
(Dose-Response Overview)

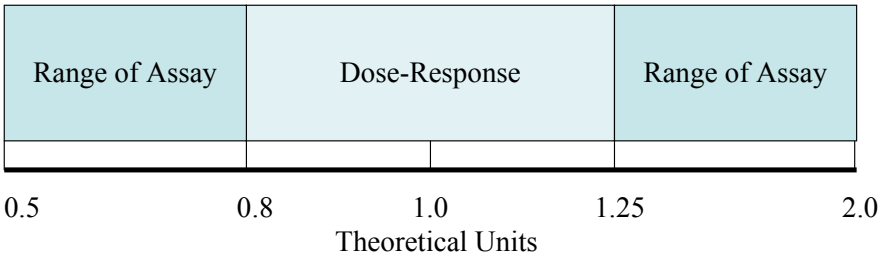


- Very steep dose-response
- Full response over 2 units
- No response under 0.5 units
- Dynamic range less than 4-fold

Note: Most of the dose response falls within 0.8 to 1.25 units

Drug Product Lot Release
(Dose-Response Overview)

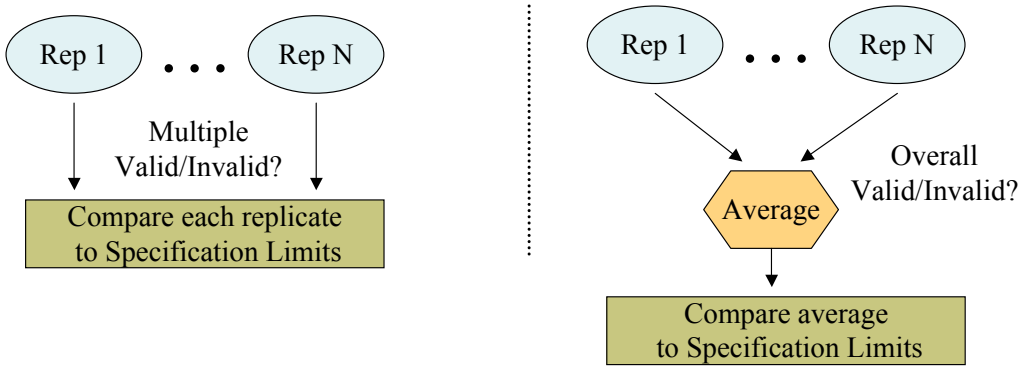
- Question: Since most of the dose-response falls within 0.80 and 1.25 units, why are we testing outside this range?
  - Assay variability
  - Range of Assay
  - Manufacturing variability



Focus testing over a 4-fold range at most to eliminate obtaining redundant information

Drug Product Lot Release
(Reportable Value/Validity/Specification Limits)

- Individual Replicates vs. Average Potency



- Specification = Method + Limits
  - Tighten Limits for average
  - EP Monograph guidance: 80-125%

Drug Product Lot Release
(Refinement of Assay)

- Confirmatory vs. Exploratory Assay
  - Lot release is a confirmatory assay
  - Validated manufacturing process
- Reallocate testing closer to expected dose-response
  - Tighten dilution steps
  - Decrease animals per dose
  - Average reportable value
  - Tighten specification limits

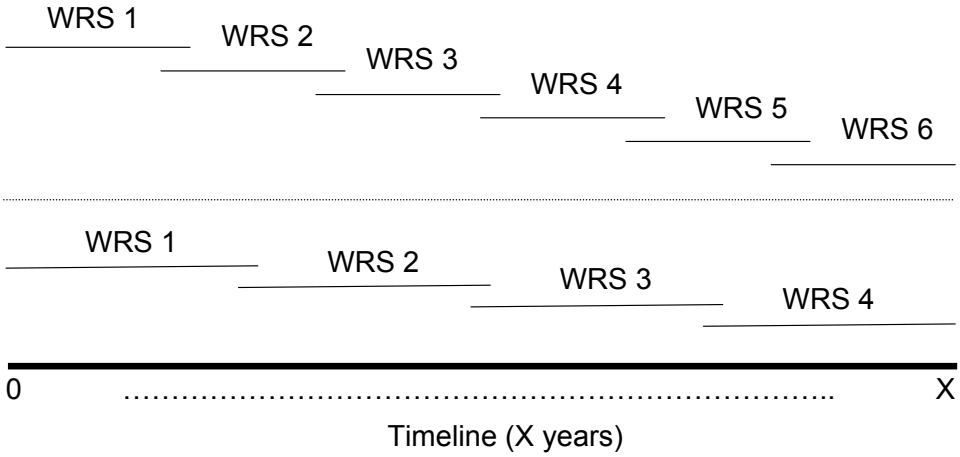
Drug Product Lot Release
(Summary)

- Drug Product has a very steep dose response
- Reduce testing far from dose response for confirmatory assays
- Reduce (N) per dose
- Combine replicates into overall analysis
- Tighten specification limits for new reportable value
- Successful regulatory approvals
  - 50% reduction of in-vivo testing without loss of accuracy or precision

Potency Reference Standard
(Program Overview)

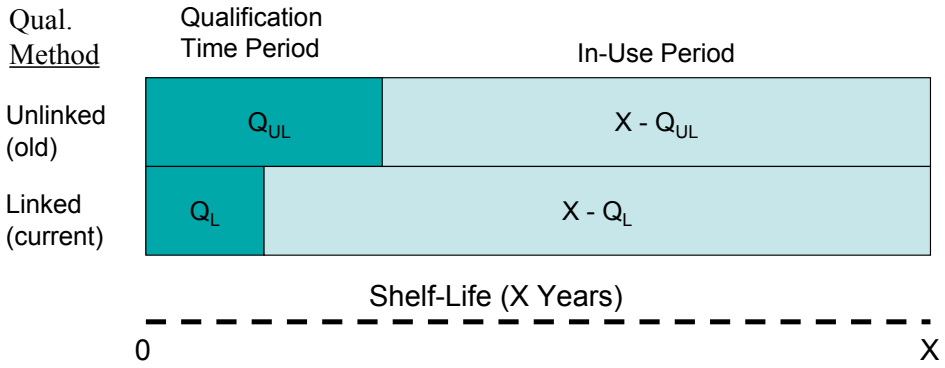
- In-vivo potency testing is required
  - Qualification ←
  - Stability
- Reduction of in-vivo testing by refinements to structure of qualification program
  - Decrease # of working standards
  - Improve efficiency in method to qualify standards

Potency Reference Standard
(Shelf-Life Extension)



- Extending the shelf-life of the Working Reference Standards (WRS) has decreased the need for in-vivo qualification testing by 33%

Potency Reference Standard
(Qualification Procedure Refinements)



- In-use period of working standard is increased
  - Further decreases # of working standards over time
  - Decrease need for in-vivo qualification testing
- Decrease in-vivo testing during qualification

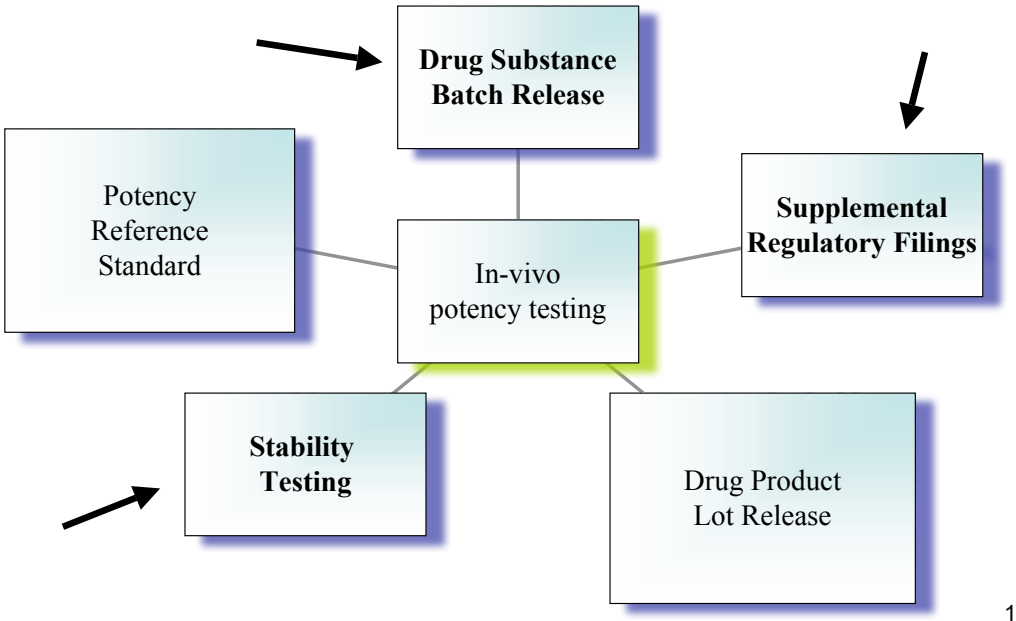
Potency Reference Standard
(Summary)

- Increased shelf life of working potency reference standards
  - 33.3% reduction of in-vivo testing
- Improved qualification procedure for working potency reference standards
  - 25% reduction in in-vivo testing for qualification
  - Decrease qualification time of working standard
    - Increase in-use time of working standard
    - Decreases number of working standards over time
      - » ~20% reduction over time

Other Avenues of Reduction

- **Effect of Drug Product Lot Size**
  - Larger lot sizes effectively reduces amount of in-vivo testing on a per vial basis
  - Increased lot size over time
    - Increased efficiency
- **Identity Test**

Future Areas for Refinement and Reduction



Summary

- **R**eduction and **R**efinement is important until globally approved **R**eplacement Potency Assay available
- Allergan has received approvals for a refined lot release assay
  - Up to a 50% reduction per lot
  - Maintains accuracy/precision
- Potency Reference Standard Program
  - Increased efficiency
- Guidance needed by regulatory agencies on defining areas where further **R**eduction/**R**efinement/**R**eplacement can be obtained