ZEBET's Activities to Promote Alternative Methods to the LD50 Potency Test in Botulinum Neurotoxin Product Testing

Federal Institute for Risk Assessment (BfR), Center for Alternative Methods to Animal Experiments — ZEBET
Diedersdorfer Weg 1, 12277 Berlin, Germany

Sarah Adler, Barbara Grune, Andreas Luch and Manfred Liebsch

FEDERAL INSTITUTE FOR RISK ASSESSMENT

Medicinal products containing Botulinum NeuroToxin (BoNT) are used for treating a variety of medical disorders, such as cervical dystonia or blepharospasm. Injection of BoNT results in a blockade of acetylcholine release at neuro-muscular nerve endings, leading to muscle relaxation or flaccid paralysis depending on the dosage (Fig 1).

Prior to release for incorporation into final product, each batch of BoNT produced is assayed using the mouse LD50 potency test as outlined in the *European Pharmacopoeia* (EP) 6.0 monograph "Botulinum Toxin Type A for Injection". In addition, ICH guidelines require further potency testing during BoNT production (Fig 2). The end-point of the assay is unfortunately based on measuring lethality rates in mice. Due to the paralysing toxic syndrome of BoNT, the treatment of animals is associated with severe suffering.

Taking into account the obvious animal welfare issues involved, the Center for Alternative Methods to Animal Experiments (ZEBET), which is part of the German Federal Institute for Risk Assessment (BfR), has recently increased its efforts in promoting the implementation of the 3Rs in BoNT potency testing. Thus, in April 2009, ZEBET organised an international Expert Meeting on the issue of alternative methods to replace the LD50 Potency Test for BoNT Testing (Adler *et al.*, in press). The main outcome of this meeting was the establishment of a "BoNT Expert Working Group- BoNT EWG" dedicated to convening several times per year at the BfR with a view to further discussing all aspects of BoNT potency testing. It aims to clarify the regulatory requirements regarding BoNT product testing on an international level, to select the most promising alternative test methods and to pinpoint further steps necessary to succeed with their validation and regulatory acceptance. ZEBET in collaboration with the BfArM (Federal Institute for Drugs and Medical Devices) takes the chair of the BoNT EWG. Members are representatives of national and international competent authorities, validation organizations and manufacturers of BoNT products as well as academia.

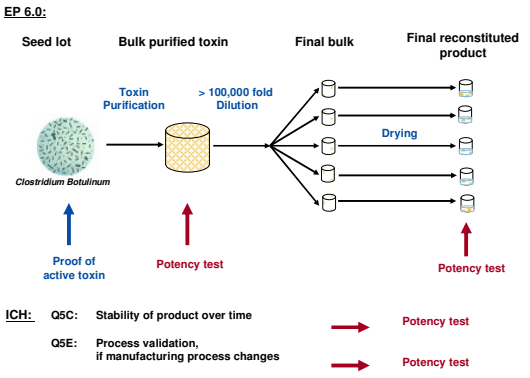

Figure 2: A schematic overview of the BoNT production process, indicating when potency tests are required to meet regulatory requirements. Potency tests are required after the EP for the bulk purified toxin and final reconstituted product. In addition, potency tests have to be performed to demonstrate the product stability over time (ICH Q5C) and for process validation (ICH Q5E). (Adler *et al.*, 2010)

Scope and Objectives of the BoNT EWG

- The goal of the "BoNT Expert Working Group" is to promote the implementation of the 3Rs, i.e. refinement, reduction, and replacement, in BoNT potency testing taking into account the EU Directive 86/609/EEC. The group was established to explore how to overcome current issues in the development, optimisation, validation and regulatory acceptance of alternative methods to the BoNT potency test in mice.
- The objectives of the BoNT EWG include, among others, providing advice and guidance on validation requirements for the proposed alternative methods and defining minimum standards in order to implement the 3Rs in BoNT potency testing. The Group will facilitate replacement, refinement and reduction strategies through reviewing data, reference materials, methods and scientific approaches. Furthermore, it will promote the standardisation of protocols. The Group will also promote awareness and transparency between the stakeholders and regulatory authorities.
- As independent institutions, the German regulatory authorities BfR/ZEBET and BfArM are responsible for the establishment and management of the BoNT EWG. The EWG initially focuses on the situation in Europe in order to speed up the process of the implementation of alternative BoNT potency assays in this region. Moreover, it is the intention that the group will endeavour to work out and to promote a global approach.

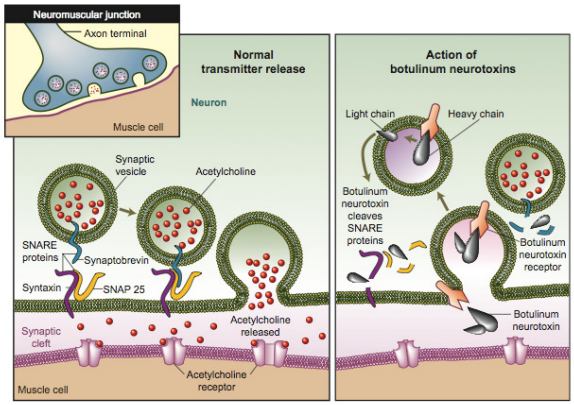

Figure 1: The mechanism of toxicity of BoNT comprises the four stages of binding, internalisation, translocation and proteolytic cleavage of a substrate, which results in inhibition of synaptic exocytotoxic transmitter release.
http://www.drknp.com/wp-content/uploads/2010/04/Picture-1.png (accessed 12.8.2010)

Constitution of the BoNT EWG

- The BoNT EWG comprises experts from European regulatory authorities, 3Rs and validation institutions, manufacturers and scientists. Membership and chairmanship is determined by the EWG member panel itself.
- Experts from overseas are invited as observers. Depending on the topics dealt with, additional experts and stakeholders will be invited to join meetings of the BoNT EWG on a case-by-case basis.

Deliverables of the BoNT EWG

- **Review research and scientific approaches of 3R methods for their application to BoNT potency testing including identification of deficiencies**
- **Provide recommendations on validation requirements for alternative methods for BoNT potency testing**
- **Identify strategies for the implementation of the 3Rs in the BoNT production process**
- **Promote harmonisation and mutual acceptance criteria for regulatory purposes**

Member Institutions of the BoNT EWG

Chairs:
BfR, Federal Institute for Risk Assessment / ZEBET (DE)
BfArM, Federal Institute for Drugs and Medical Devices (DE)

Members:
Afssaps, French Health Products Safety Agency (FR)
European Commission: Joint Research Centre / ECVAM (IT)
EDQM, European Directorate for the Quality of Medicines (FR)
NIBSC, National Institute for Biological Standards and Control (UK)
FDA, Food and Drug Administration (USA)
IMB, Irish Medicines Board (IR)
PEI, Paul Ehrlich Institute (DE)
Hannover Medical School (DE)
Wickham laboratories (UK)
Free University Berlin (DE)
Merz Pharmaceuticals (DE)
Allergan Inc. (USA)
Ipsen Biopharm Limited (UK)

Observers:
BMBF, Federal Ministry of Education and Research (DE)
Home Office, Animal Scientific Procedures Inspectorate (UK)

Reference: Adler S, Bicker G, Bigalke H, Bishop C, Blümel J, Dressler D, Fitzgerald J, Gessler F, Heuschen H, Kegel B, Luch A, Milne C, Pickett A, Ratsch H, Ruhdel I, Sesardic T, Stephens M, Stiens G, Thornton PD, Thürmer R, Vey M, Spielmann H, Grune B and Liebsch M. The Current Scientific and Legal Status of Alternative Methods to the LD50 Test for Botulinum Neurotoxin Potency Testing. The Report and Recommendations of a ZEBET Expert Meeting. *ATLA* **38**: 1-15 (2010)