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

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 converging 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.

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 and guidance on validation requirements for the proposed alternative methods to the LD50 potency test in mice. 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 harmonisation and mutual acceptance criteria for regulatory purposes.

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 and 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 harmonisation and mutual acceptance criteria for regulatory purposes.

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 3Rs 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:
- Afsaps, French Health Products Safety Agency (FR)
- ECVM, European Federation of the Veterinary Medical Centres (IT)
- EDCM, European Directorate for the Quality of Medicines (FR)
- ETF, European Medicines Agency (UK)
- EMA, European Medicines Agency (EU)
- MEB, Mexican Institute of Biotechnology (MX)
- INCB, Indian National Co-ordinating Committee (IN)
- IMB, Irish Medicines Board (IR)
- PEI, Paul Ehrlich Institute (DE)
- BMBF, Federal Ministry of Education and Research (DE)
- Merck Pharmaceuticals (DE)
- Merck Serono (DE)
- MEU, Ministère de l’Éducation et de la Recherche (FD)
- MEB, Mexican Institute of Biotechnology (MX)
- Allergan Inc. (USA)
- Ipsen Biopharm Limited (UK)

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


Federal Institute for Risk Assessment • Thielallee 82-92 • D-14195 Berlin • Tel. + 49 (0) 30 - 184 12 - 0 • Fax + 49 (0) 30 - 184 12 - 47 41 • bfr@bfr.bund.de • www.bfr.bund.de