From: Frank Gessler
Date: Fri, 10 Mar 2006 12:00:41 +0100
To: <niceatm@niehs.nih.gov>
Subject: Comments on Federal Register January 27, 2006: Vol. 71, No. 18, page 4603; Botox-workshop

Dear Dr. Stokes,

in reply to the nomination to hold a workshop on alternative methods to replace the mouse LD50 assay for Botulinum Toxin Potency testing, published in the Federal Register (January 27, 2006: Vol. 71, No. 18, page 4603), I would like to submit the attached files:

GesslerCov.pdf Cover letter of the submission
gessler.pdf Comments on the workshop

Thank you very much,
Best Regards

Frank Gessler

_____

Frank Gessler, Dr. med. vet.
Institute of Agronomy and Animal Production in the Tropics
University of Goettingen
Germany

Institut für angewandte Biotechnologie der Tropen an der Georg-August-Universität Göttingen

Institute for Applied Biotechnology in the Tropics at the Georg August University Goettingen

Dr. Wiiliam S. Stokes
Director NICEATM
NIEHS
P.O. Box 12233
MD EC-17
Research Triangle Park, NC 27709
USA

by e-mail niceatm@niehs.nih.gov

Göttingen, March 9th, 2006

Federal Register January 27, 2006: Vol. 71, No. 18, page 4603
Comments on nomination of Workshop on Alternative Methods to replace the mouse LD_{50} assay for Botulinum toxin potency testing

Dear Dr. Stokes,

in-process control and batch release testing of therapeutic/cosmetic preparations of Botulinum neurotoxins (BoNT) show the need for refining or replacing the currently used mouse bioassay. Potency testing of the toxins, however, is not limited to this application. The biological activity of the toxins needs to be quantified in various clinical samples as well as in food/feed and environmental matrices. For more than ten years the Institute for Applied Biotechnology in the Tropics has focused on the lab detection and quantification of Botulinum neurotoxins type A to F: During the recent years approx. 3000 samples per year have been submitted for BoNT detection and have been examined in the mouse bioassay. Serological in vitro assays were successfully developed, established and include an immunoaffinity column and a magnetic beads assay for BoNT/C and D.

Most current in vitro methods for potency testing are limited to the quantification of the biological activity of the light chain of the toxins. We are about to focus our research efforts on the development of BoNT potency tests, e.g. a cell culture based assay, which

Kellnerweg 6, D-37077 Göttingen

Telefon: (05 51) 39 33 96
Telefax: (05 51) 39 34 08
e-mail: hboehne@gwdg.de
IBAN DE72260500010044301885

Konto Nr. 44 301 885
Sparkasse Göttingen
BLZ 260 500 01
BIC NOLADE21 GO

Sitz des Vereins
Birkenweg 2a
D-37120 Bovenden
VAT Nr. DE 115 312 786

Vereinsregister
Amtsgericht
Göttingen 1668

should allow for the quantification of the BoNT biological activity of the heavy and light chain as well as for the detection of neutralizing antibodies.

For your information I have attached my short CV and my list of publications on Botulinum neurotoxins and neurotoxin detection.

I would appreciate if you will consider the comments, which you will also find attached.

Sincerely Yours,

Frank Gessler, Dr. med. vet,
Institute for Applied Biotechnology in the Tropics
University of Göttingen
Kellnerweg 6
37077 Göttingen
Germany
phone +49 551 393393
fax +49 551 393408
e-mail fgessle@gwdg.de

IBT

Institut für angewandte
Biotechnologie der Tropen
an der Georg-August-Universität
Göttingen

Institute for Applied
Biotechnology in the Tropics
at the Georg August University
Goettingen

Comments on the

Nomination to hold a workshop on Alternative Methods to replace the mouse LD_{50} assay for Botulinum toxin potency testing issued by NICEATM

Federal Register January 27, 2006: Vol. 71, No. 18, page 4603

ad (1) Information on development and/or validation activities

The Institute for Applied Biotechnology is currently involved in two developments for botulinum neurotoxin potency testing: One method aimes to quantify the peptide cleavage activity in all liquid laser desorption ionization, the second approach is a cell culture based assay, which measures the biological activity of the neurotoxins as a whole (heavy and light chain).

ad (2) Comments on the appropriateness and priority of a workshop

Currently several approaches for BoNT potency testing are under development, which have the potential to replace or at least refine the mouse bioassay. Urgent action is needed to identify the most promising techniques and the applications for them. R&D activities, in-process control and batch release testing of BoNT in therapy and cosmetics do not necessarily need the same methods as testing of clinical, food or environmental samples. However, alternative methods suitable for a variety of applications would merit the validation work with BoNT products, but with detection/diagnostic evaluations of other sample matrices as well. To conclude, a workshop would offer the unique opportunity to move forward in refining/replacing the mouse bioassay and should be given high priority.

ad (4) Submission of data from mouse LD_{50} botulinum potency testing

With approx. 3000 mouse bioassays per year, the Institute for Applied Biotechnology has gained experience in BoNT potency testing with human, veterinary, food and environmental samples, but also with BoNT preparations of various purities (culture supernatant, toxin complex, 150 kD toxin) and of almost all types (A to F). A

considerable effort is needed to carefully check and analyse the data. Thus the evaluation and the raw data can not be supplied by March, 13th, but would be available at a later stage.