BioSentinel Pharmaceuticals

University Research Park
510 Charmany Drive, Suite 259
Madison, WI 53719
Telephone: 1-608-441-8172
Fax: 1-608-441-8173

April 10, 2011

Dr. William S. Stokes
Director of NICEATM
NIEHS
79 West Alexander Drive
P.O. Box 122233
MD EC-17
Research Triangle, NC 27709

Re: Submission of the BoTest™, BoTest™ Matrix, and BoCell™ Botulinum Neurotoxin Activity assays for cross-laboratory validation studies by ICCVAM and NICEATM.

Dear Dr. Stokes,

BioSentinel Pharmaceuticals Inc (BioSentinel) has developed a suite of assays for the detection and quantification of botulinum neurotoxins (BoNTs). This suite includes the *in vitro* BoTest™ and BoTest™ Matrix assays and the cell-based assay BoCell™. We submit these test methods to ICCVAM and NICEATM because these animal-free methods offer sensitivity on par with current animal-testing methods but with much higher throughput and accuracy. With proper validation, we believe these methods will meet regulatory requirements and provide a means to detect and quantify BoNT in a wide range of applications. The rationale for recommending these assays for cross-laboratory validation and support is as follows:

The need for sensitive, high throughput methods for BoNT detection that do not require animals.

- BoNT testing is expanding with the identification of new clinical indications for BoNT and the US Federal Government's stated concern for BoNTs use as a biological weapon (ICCVAM, 2008; Executive Order, 2009).
- Pharmaceutical manufacturing of BoNT-based therapies and cosmetics accounts for an estimated 600,000 animal deaths per year (Adler, 2010).
- Development of new BoNT-based therapies and treatments for botulism, both for natural occurrences and for biodefense, is hampered by the lack of high throughput methods to detect BoNT (ICCVAM, 2008; Larsen, 2009).
- No commercially available test method exists that offers the sensitivity required to replace animal methods.
- No commercially available test methods are compatible with complex samples such as food, environment, clinical, and pharmaceutical samples.

BioSentinel Pharmaceuticals
University Research Park
510 Charmany Drive, Suite 259
Madison, WI 53719
Telephone: 1-608-441-8172
Fax: 1-608-441-8173

- No animal-free test method for the detection of BoNT exists that has been approved by regulatory agencies or validated by ICCVAM.

The BoTest, BoTest™ Matrix, and BoCell™ Assays meet the demands for animal-free methods of BoNT detection.

- BioSentinel's assays address many of the needs and concerns identified by ICCVAM for animal-free testing of BoNT-containing samples (ICCVAM, 2006).
- Our suite of assays can reduce or eliminate animal use from BoNT-testing procedures.
- The assays can be used alone or in combination to meet the needs of specific applications.
- All of the test methods detect and quantify BoNT activity. The BoTest™ and BoTest™ Matrix Assays detect BoNT proteolytic activity. The BoCell™ indirectly or directly detects BoNT cell binding and uptake, translocation, and proteolytic activities.
- The BoTest™ and BoTest™ Matrix assay offer picomolar to femtomolar sensitivity. Detection of a single BoNT/A mouse LD_{50} is possible per CDC and pharmaceutical industry requirements (ICCVAM, 2008).
- The BoTest™ Matrix assay is compatible with a wide range of substances including food, blood, serum, water, field, and pharmaceutical samples per CDC, DOI, and FDA requirements (ICCVAM, 2008).
- The intra-lab studies have shown the assays to accurate and precise.
- The assays do not require extensive training. Some tissue culture skills are required for the BoCell™ assay.
- The test methods do not require extensive instrumentation. A fluorescence microplate reader is required for all assays.
- The assays offer cost savings over animal-based methods.
- Our assay is applicable to the needs of various federal agencies (including, but not limited to FDA, CDC, DOJ, DOD, DHS, EPA) as well as pharmaceutical firms.

For these and other reasons, BioSentinel submits the BoTest™, BoTest™ Matrix, and BoCell™ assays for consideration for cross-laboratory validation studies. Supporting documentation for this nomination is provided for each of the test methods.

BioSentinel is requesting that ICCVAM and NICEATM (1) facilitate collaboration with regulatory agencies to develop a validation strategy which could lead to the regulatory acceptance of these methods for the detection and quantification of BoNT contained in suspect substances, the determination of drug product potency, and/or the clinical diagnosis of botulism and (2) to coordinate and provide funding for validation studies as needed.

BioSentinel Pharmaceuticals

University Research Park
510 Charmany Drive, Suite 259
Madison, WI 53719
Telephone: 1-608-441-8172
Fax: 1-608-441-8173

BioSentinel requests to be selected as one of the laboratories chosen for formal validation studies and that BioSentinel act as the laboratory to provide training and technical support to other laboratories chosen to validate this assay.

Sincerely,

Füsûn Naomi Zeytin, CSO, BioSentinel Pharma Inc

Ward Tucker, Research Director, BioSentinel Pharma Inc

BioSentinel Pharmaceuticals
University Research Park
510 Charmany Drive, Suite 259
Madison, WI 53719
Telephone: 1-608-441-8172
Fax: 1-608-441-8173

Literature Cited:

Adler, S., Bicker, G., Bigalke, H., Bishop, C., Blumel, J., Dressler, D., Fitzgerald, J., Gessler, F., Heuschen, H., Kegel, B., Luch, A., Milne, C., Pickett, A., Ratsch, H., Ruhdel, I., Sesardic, D., Stephens, M., Stiens, G., Thornton, P. D., Thurmer, R., Vey, M., Spielmann, H., Grune, B. & Liebsch, M. (2010). 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. *Altern Lab Anim* 38, 315-30.

ICCVAM (2006) Scientific Workshop on Alternative Methods to Refine, Reduce and Replace the Mouse LD_{50} Assay For Botulinum Toxin Testing, Silver Spring, MD.

ICCVAM (2008) ICCVAM-NICEATM/ECVAM Scientific Workshop on Alternative Methods to Refine, Reduce or Replace the Mouse LD_{50} Assay for Botulinum Toxin Testing., NIH Pub. No. 08-6416

Larsen, J. C. (2009). U.S. Army Botulinum Neurotoxin (BoNT) Medical Therapeutics Research Program: Past Accomplishments and Future Directions. *Drug Dev Res* 70, 266-278.

Executive Order 13527 (2009) Establishing Federal Capability for the Timely Provision of Medical Countermeasures Following a Biological Attack