



## Comment

# Comment on “The Botulinum Neurotoxin LD<sub>50</sub> Test – Problems and Solutions”

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The article from Dr. Bitz (Bitz, 2010), on behalf of the organization Doctors against Animal Experiments Germany, provides only one set of views with regard to the testing of botulinum toxin (BoNT) products, namely that no animal LD<sub>50</sub> testing should be performed. Dr Bitz’ opinion article is based on a series of limited, sometimes erroneous assumptions, leading to a biased report. The present commentary seeks to provide a more accurate update on the current situation from a supplier of a BoNT product and to describe some of those solutions to which Dr. Bitz refers.

Dr. Bitz repeats the incorrect statement that the toxin products are used as “cosmetics”, accusing the manufacturers of exploiting legal loopholes that permit the testing of “cosmetics” on animals. BoNT is not a cosmetic product, however. The definition of a cosmetic is clear in European statutes:

*Cosmetics are substances or preparations intended to be placed in contact with the various external parts of the human body, the teeth and the mucous membranes of the oral cavity with a view to.... These are thus products which consumers use daily and with which they are in direct physical contact.<sup>1</sup>*

In accordance with current legislation, BoNT is a prescription medicine that is injected into the patient at intermittent intervals and therefore clearly is not a cosmetic. Indeed, if the correct and full description of the medical use of BoNT for the treatment of hyperkinetic facial lines is properly reported, then BoNT’s status as an injectable medicine is apparent:

*(Product name) is indicated for the temporary improvement in the appearance of moderate to severe glabellar lines (vertical lines between the eyebrows) seen at frown, in adult patients under 65 years, when the severity of these lines has an important psychological impact on the patient.<sup>2</sup>*

Regrettably, the last part of this indication (emphasis added) is never used in articles discussing BoNT’s aesthetic use. This part defines the medical use of the product, however, and it should not be omitted.

A number of disabling medical indications for BoNT have been approved by authorities around the world, and thousands of patients currently achieve a much improved standard of life as a result of such uses.

Dr. Bitz has bizarrely tried to estimate the numbers of mice used worldwide for BoNT testing using the commercial (financial) turnover as an indicator of product volume. This is a flawed and therefore meaningless calculation for many reasons; comparisons cannot be drawn between different products from different companies, with prices per unit product that vary between countries. Other factors not mentioned by Dr. Bitz, such as batch size, number of batches, different requirements in different countries, and commitments to regulatory authority requirements, all will significantly affect such calculations. Product is not required to be stability-tested for 5 years after registration. These requirements vary between regulatory authorities but generally are limited to one batch produced each year for the approved shelf life of the product. Any guesses on the design of the assays used are also highly speculative, other than information described in the literature to date (Straughan, 2006). The designs alone will be highly specific to each product and not interchangeable between the products. Any attempt at a calculation of worldwide animal usage for such assays therefore is inaccurate and could be highly misleading.

Although Dr. Bitz has clearly pointed out that any substitute potency assay must be validated, as required in the European Pharmacopoeia, she has not tried to explain this. Indeed, to date commentaries on alternative assays have seldom mentioned this critical aspect termed “validation.” What does it mean? No regulatory authority anywhere in the world will grant approval for a company to substitute the LD<sub>50</sub> for an alternative assay,

<sup>1</sup> <http://europa.eu/rapid/pressReleasesAction.do?reference=MEMO/91/5&format=HTML&aged=0&language=EN&guiLanguage=en>, accessed 1 September 2010

<sup>2</sup> [http://www.medicines.org.uk/EMC/medicine/21985/SPC/\[Product name\]](http://www.medicines.org.uk/EMC/medicine/21985/SPC/[Product name]), accessed 1 September 2010



whether animal-based or not, unless they are entirely satisfied that the alternative is valid, meets the requirements laid down in national and international guidelines for validity (International Conference on Harmonisation, 2005; United States Pharmacopoeia, 2010), and correlates/is comparable with the current LD<sub>50</sub> method. These requirements generally are different from those applied to other animal assay replacements (Worth and Balls, 2002). The mouse LD<sub>50</sub> assay is the “gold standard” for any potency measurement of BoNT, and to dethrone such a standard requires an approach that is both scientifically sound and relevant to the specific product being tested.

The mouse LD<sub>50</sub> assay, as validated by all the manufacturers, measures all four properties of BoNT; binding to the neuromuscular junction (NMJ) receptors, endocytotic internalization into the NMJ cytosol, translocation of the active portion of the BoNT molecule (the Light Chain), and final enzymatic cleavage of the relevant target substrate. Replication of each of these properties individually is possible but *in vitro* combinations of even two are rarely achieved and scientifically challenging. *Ex vivo* models, using isolated animal organs, are available which can replicate the LD<sub>50</sub> but are generally impractical to apply routinely and/or require significant numbers of organs to achieve appropriate results. In other words, the current state of BoNT science does not permit a direct replacement at present without the use of animals.

Contrary to statements that have often been made in various publications and commentaries, the SNAP-25 endopeptidase assay method, developed by the UK National Institute for Biological Standards and Control (NIBSC) for specific internal use, has never been accepted for product quality testing and release by any worldwide regulatory authority, since it only measures one property of BoNT. Although used by and adequately established for their own purposes, this method has also not been validated in accordance with the requirements of the European Pharmacopoeia, regulatory authorities worldwide or the ICH

guidelines. Additionally, transfer of this assay method outside of NIBSC would be required in order to demonstrate its validity (inter-laboratory testing) (Sesardic, 2010). Until such data are available and assembled for review by the competent authorities, the assay will remain an internal method only.

All parties involved, manufacturers, scientists and regulators, share Dr. Bitz’ desire for an alternative to the LD<sub>50</sub> potency assay, and are working continuously with this aim, as reported in detail (Adler et al., 2010). Several potential alternative assays have been comprehensively studied and have not met the regulatory criteria. Others are currently under study, and BoNT science continues to deliver new prospects for investigation, despite commentaries to the contrary.

### References

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Dr. Pickett dismisses the cosmetic use of botulinum toxin products out of hand. He criticises “...the incorrect statement that the toxin products are used as cosmetics, accusing the manufacturers of exploiting legal loopholes that permit the testing of cosmetics on animals. BoNT is not a cosmetic product.” Indeed, although used to a high percentage for cosmetic purposes, botulinum toxin (BoNT) products are defined as pharmaceuticals, because they are injected into the body, as explained in the original article (Bitz, 2010). Thus pharmaceutical safety testing is required by law even though the application is often aesthetic.

To comply with the 7<sup>th</sup> Amendment of the Cosmetics Directive, which bans animal testing for cosmetic products and ingredients, politics is obliged to close this gap to prevent animal tests for products that are used extensively for aesthetic reasons.

It is impossible to deny the fact that these products are being used not only for medical but also largely for cosmetic applications. Product names such as Botox<sup>®</sup> Cosmetic suggest that BoNT is used not solely for medical purposes. Also, information published by the manufacturers themselves confirms its use for cosmetic purposes. For example, Allergan in its Annual Report