Comment on “The Botulinum Neurotoxin LD$_{50}$ 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$_{50}$ 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.*

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.*

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$_{50}$ for an alternative assay.

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2 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 \( \text{LD}_{50} \) method. These requirements generally are different from those applied to other animal assay replacements (Worth and Balls, 2002). The mouse \( \text{LD}_{50} \) 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 \( \text{LD}_{50} \) assay, as validated by all the manufacturers, measures all four properties of BoNT; binding to the neuromuscular junction (NMJ) receptors, endocytic 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 \( \text{LD}_{50} \) 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 \( \text{LD}_{50} \) 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**


In order to have access to the most accurate state of affairs, the animals undergo testing of BoNt products annually. Of course, with the public and to publish their exact records on how many made to share their complete data on animal experimentation. I Ipsen and the other manufacturers of BoNt products should be informed “…the FDA granted marketing authorisation for Dysport™ … for the treatment of cervical dystonia and also for aesthetic medicine.” Further the report states, “Azzalure® received the collective green light … in aesthetic use for the treatment of frown lines.” It is further stated that Dysport® is marketed for aesthetic indications by Medics in the US and in Europe by Galderma, under the Azzalure® brand, and that the group has granted Galderma the exclusive right to develop, promote, and distribute its botulinum toxin type A for aesthetic indications in Europe and certain other territories (Ipsen, 2009).

According to the manufacturers themselves, cosmetic uses account for around one half of all applications. Allergan stated in 2005, “Presently, Botox® is approved for 20 indications in more than 75 countries, with an estimated 57 percent of sales relating to therapeutic uses and 43 percent to aesthetic use” (Allergan, 2005).

The “off-label” use of BoNt products cannot be denied. In Switzerland, for example, it is en vogue for many people to use their lunch hour to visit a beauty clinic to get a beauty treatment with a botulinum toxin product. It is highly doubtful that severe psychological reasons cause people to visit a beauty clinic. Therefore, it is incorrect of Dr. Pickett to claim that botulinum toxin is applied solely for medical indications.

However, independent of whether BoNt products are used for cosmetic or medical indications, it is unsustainable that the manufacturers are not committed strongly enough to support the deletion of the LD₉₀ test from the European Pharmacopoeia by validating one of the available and allowed alternative tests. For the animals used in the cruel and scientifically unreliable LD₉₀ test, it does not matter whether the product tested on them will be used for medical or for aesthetic reasons. The suffering experienced by the mice is always the same: they die over the course of a couple of days by asphyxiation. Additionally, extremely cruel procedures have been filmed undercover in Ipsen’s contract laboratory in Wickham, Hampshire (http://www.arzneimittel-telegramm.de/zeit/0709_a.php3).

Dr. Pickett claims that he wants to give an accurate update on the current situation and is of the opinion that the estimated number of animals used for BoNT testing is incorrect. However, in his comment any figure, number, or detail on animal use which might prove estimated animal numbers wrong is completely missing. Only the manufacturers know the exact numbers of animals who die in the LD₉₀ test for BoNT products. Ipsen and the other manufacturers of BoNT products should be made to share their complete data on animal experimentation with the public and to publish their exact records on how many animals undergo testing of BoNT products annually. Of course, in order to have access to the most accurate state of affairs, the manufacturers also have to be open to independent audits at any time without notice.

In his statement, Dr. Pickett further appeals to the fact that the LD₉₀ test is regarded as the “gold standard.” However, it is a matter of fact that the LD₉₀ test is criticised by scientists, not only because of its cruelty, but also for its lack of scientific reliability (Hartung, 2009). Further, this animal-based assay has never been validated and is thus regarded unproven as “gold standard.”

The European Pharmacopoeia clearly allows three alternatives which are preferable to the conventional mouse assay in terms of animal welfare subject to validation (European Pharmacopoeia, 2006). Dr. Pickett states, that the Snap-25 endopeptidase assay, which is one of the allowed alternatives developed by the National Institute for Biological Standards and Controls in the United Kingdom, “… has never been accepted for product quality testing and release by any worldwide regulatory authority.” Here, Ipsen and the other manufacturers of BoNT products are asked to submit data on what exactly they have undertaken to validate this test or any other alternative test for their products and to make their validation study, aiming at the regulatory acceptance of the Snap 25-assay or another test, publicly available.

Apart from the severe animal welfare problem BoNT testing is causing, it is irresponsible of the manufacturers to also accept that people are subjected to a risk. Several adverse effects and deaths due to BoNT products have been reported (FDA, 2008; arznei-telegramm, 2007). This shows that the LD₉₀ test is not able to ensure safety for patients or persons undergoing BoNT treatments for aesthetic reasons.

As long as the manufacturers are not willing to intensively promote the deletion of the LD₉₀ test on mice, a ban on the use of BoNT products for cosmetic use is urgently needed while on a scientific and political level the mandatory application of an animal-free method for BoNT testing must be driven forward.

References
As long as our current science is not capable of producing botox in a humane way, there can only be one conclusion: we have to discontinue the use of botox for non-life-threatening indications until our science and culture are capable of producing it in a humane way.

I can accept the argument in the comment of Dr. Pickett that a substance injected into the skin is not a classical cosmetic.

The claim of Dr. Pickett – that botox is only being given to people who are about to develop a serious depression because of their wrinkles – is unacceptable. None of my colleagues in the field of dermatology, and much less those working in walk-in botox clinics, ever seriously assess the severity of an upcoming depression caused by wrinkles. And even if they did, I am sure that most people would gladly declare to be somewhat depressed because of their wrinkles – as a matter of fact, I am too!

And yes, character does matter in this subject. We should expect from people of today’s culture to decline asking mice to suffer so severely to relieve them from a few wrinkles for a short time.

The principle stating that we should not use technology we are not yet able to manage in a proper way should also apply to other fields, e.g., the REACH program. Of course it would be nice to know more about the toxicity of old chemicals. But as long as we are not able to test them in a humane manner, without harming and killing millions of animals, and in spite of the fact that we do not know how many humans will ever profit from this research, we should wait until we are able to test with animal-free methods.

Our science and culture are neither ready for botox nor for REACH.

Further information

For clarification, excerpts of the relevant texts pertaining to the above discussion from Directive 76/768/EEC and the European Pharmacopoeia are transposed here.