

## **BOTOX<sup>®</sup> Fact Sheet**

### **What is Botox?**

BOTOX<sup>®</sup> is a drug known most widely for its ability to temporarily smooth out facial wrinkles, though it also has several medical applications. Both its cosmetic and medical applications relate to Botox's ability to block muscle contractions. Its key ingredient is botulinum toxin, the most poisonous substance known, with the capacity to cause muscle paralysis and death in humans and other animals. The same toxin, known as Botulinum Toxin Type A (BTA), is responsible for Botulism food poisoning.

BTA is produced naturally by the bacteria *Clostridium botulinum*. Botox's manufacturer, Allergan, produces the toxin by growing the bacteria in a fermenter. This process is somewhat crude, so the potency of the toxin tends to vary from batch to batch. To standardize the potency of such a powerful toxin, each batch is tested.

### **How is Botox Tested for Potency?**

The standard procedure for assessing the potency of Botox is the LD<sub>50</sub> Test. This test involves giving mice a single injection of the product into their abdominal cavity and seeing if they die within 3-4 days. The mice are first assigned to one of various dose groups. The aim of the test is to estimate the dose that kills 50% of the animals (hence the name “lethal dose 50%”). The LD value is designated as a unit (U) or a Mouse Unit (MU) of potency. The lower the LD<sub>50</sub> value (or the U or MU), the higher the potency. Approximately 100 mice have conventionally been used to test each batch of botulinum toxins products, though Allergan has claimed to The HSUS that the company has significantly reduced this number.

Depending on the dose injected and the potency of the batch being assessed, the test animals experience differing levels of muscular paralysis and impaired vision. The end point of the LD<sub>50</sub> Test is death, usually by suffocation after the respiratory muscle become paralyzed.

### **Is the LD<sub>50</sub> a Good Standard?**

The LD<sub>50</sub> Test is a questionable standard for assessing the potency of botulinum toxin products. Death by paralysis and suffocation, following prolonged respiratory distress, is unacceptable on humane grounds.

Moreover, the LD<sub>50</sub> Test is questionable on technical grounds. LD<sub>50</sub> results for Botulinum toxin potency can vary considerably across laboratories. One study compared results across 11 laboratories to standardize a type A botulinum toxin assay for assessing the toxin in food contaminations; up to 10-fold differences in potency of the same substance were reported. Another study even raises questions about the relevance of LD<sub>50</sub> testing to Botox potency. The researchers used a mouse-based test of localized paralytic activity (mouse hind limb assay, see below) as their standard of comparison. They found that LD<sub>50</sub> values do not accurately predict Botox activity in this more realistic assay. In addition, the LD<sub>50</sub> values (and hence potencies) for Botox were not comparable to those of another form of Botulinum Toxin Type A product (Dysport<sup>®</sup>). The same “units” of Botox and Dysport gave different activities in the more relevant hind limb assay. The authors concluded that the LD<sub>50</sub> assay alone “is not an adequate method for assessing the ... potency of botulinum toxin”.

## What is Botox Used For?

Allergan gained approval from the U.S. Food and Drug Administration (FDA) in 2002 to market Botox to smooth out frown lines on the forehead (so-called glabellar lines between the eyes). The effect lasts from 3 to 6 months. It is sometimes used to treat wrinkles in other facial areas “off-label.” Nearly 2.3 million cosmetic Botox procedures were performed in 2003, a 37% increase from 2002. Once a vial of Botox powder has been mixed with saline, it has a life-span of 4 hours. This has led to the craze of Botox parties in which several people gather for Botox treatments in an effort to eliminate wasting the product.

Botox also is approved by the FDA to treat several medical conditions (its “therapeutic” applications). These include cervical dystonia (involuntary movement and prolonged muscle contraction of the neck), blepharospasm (involuntary forcible closure of the eyelids), strabismus (crossed eyes), hyperhidrosis (excessive sweating), and dynamic muscle contracture in pediatric cerebral palsy patients (an abnormality of motor function usually acquired at a young age). Botox is sometimes used for other medical conditions without specific FDA approval (so-called “off-label” applications which the company cannot advertise).

Botox designated for wrinkle smoothing is marketed as BOTOX<sup>®</sup> Cosmetic, whereas the product destined for medical applications is simply called Botox or Botox Therapeutic.

## Are There Promising Alternatives to the LD<sub>50</sub> Test for Botox?

*Replacement Alternatives:* Several approaches to assessing the potency of Botulinum toxin are potential candidates for replacing the LD<sub>50</sub> Test. While the LD<sub>50</sub> Test dates back to the 1920s, the potential replacements all take advantage of more modern technology and insights into the mechanism by which Botulinum toxin exerts its effects on the body. Perhaps the most promising replacement is the **endopeptidase assay**. Modern research has shown that Botulinum toxin type A (the key ingredient in Botox) exerts its paralyzing effect by disrupting a molecule —SNAP-25— critical in transmitting nerve signals to muscles. The endopeptidase assay evaluates the ability of toxin samples to disrupt this molecule, using the latest tools of molecular biology and immunology. The assay’s developers suggest that it could replace the mouse assay in Botulinum toxin testing.

*Reduction Alternatives:* Some studies have looked at ways to reduce the number of animals used in the LD<sub>50</sub> Test for Botulinum potency (conventionally 100 animals). Pearce and colleagues assessed Botulinum toxin potency using various numbers of animals, including 100, 50, and 25, and found excellent results for the 50 and 25 animal tests. They concluded that the 25-animal assay, using 5 mice per dose, may be adequate for most laboratory experimentation when only a single determination of the LD<sub>50</sub> is made. Allergan itself, the manufacturer of Botox, may have found similar results, as the company claims to have reduced animal numbers per test by 50% and is preparing to implement another 50% reduction, once approved by the U.S. FDA, though the company has not revealed the actual numbers of animals involved.

*Refinement Alternative:* Various animal-based tests have been proposed as substitutes for the LD<sub>50</sub> Test in assessing Botulinum toxin potency, all of which cause less animal suffering and are more technologically sophisticated than the LD<sub>50</sub> Test. Instead of injecting high doses of toxin to cause wholesale paralysis and death, the refinement alternatives involve administering low doses to assess localized or regional effects. Perhaps the most promising is the **mouse hind limb assay**, in which the toxin is injected into the calf muscle. Complete paralysis of the injected limb results in two days. Researchers have calculated the dose at which 50% of the animals develop a

paralyzed limb; this is labeled the ED (Effective Dose) 50 or the median paralysis unit (MPU). The MPU provides more precise estimates of toxin activity than the LD<sub>50</sub> value. While the refinement alternatives to the LD<sub>50</sub> Test still use animals and cause some distress, the degree of suffering presumably is far less than in the lethal LD<sub>50</sub> test. Compared to the LD<sub>50</sub> Test, the refined procedures are quicker and more relevant to the clinical uses of Botulinum toxin.

### **Who Markets Botox?**

Botox is marketed by Allergan, Incorporated, which describes itself as “a global specialty pharmaceutical company that develops and commercializes innovative products for the eye care, neuromodulator, skin care and other specialty markets.” Allergan made over \$560 million dollars in net Botox sales in 2003. 40% of that (\$225 million) came from Botox Cosmetic. First quarter sales of Botox increased 190% between 2000 and 2004. Net sales in 2003 for all Allergan products reached \$1.75 billion, of which Botox and Botox Cosmetic accounted for 32%.

### **How is Botox Testing Regulated?**

Botox is the only botulinum toxin product approved for cosmetic use in the United States. Little information is available publicly about the types of potency testing, if any, that the FDA requires Allergan to conduct on Botox. Repeated requests by The HSUS to the FDA have yielded little pertinent information. However, given that the LD<sub>50</sub> Test is the international standard for assessing potency of botulinum toxin products, there is little doubt that the FDA holds Allergan to this standard. In light of the technical and humane problems associated with the LD<sub>50</sub> Test, European authorities have identified potential alternative tests that, once validated, would be acceptable as substitutes in assessing botulinum products. These potential alternatives include the endopeptidase assay and a mouse assay using paralysis as the endpoint, such as the hind limb assay (see above).

### **What is HSUS' Perspective on Botox Testing?**

The HSUS believes that it is unacceptable to cause animals to suffer for the sake of marketing a product destined for a cosmetic application—in the case of Botox, smoothing out wrinkles. Moreover, for Botox Cosmetic, each batch of the product is tested on animals, unlike the toxicity testing historically conducted on cosmetics, which was one-time only. In addition, the test used for assessing the potency of Botox batches, the LD<sub>50</sub> Test, has been the most heavily criticized animal test of the past three decades. Many observers thought the LD<sub>50</sub> Test was discontinued when the Organization for Economic Cooperation and Development deleted the test from its guidelines in 2002.

Progress has been made in eliminating the LD<sub>50</sub> Test in assessing the potency of Botox and other Botulinum toxin-based products. What is needed now is for Allergan to develop a carefully planned, well-funded project to bring one or more of the most promising replacement alternative approaches to fruition. In the meantime, Allergan should vigorously pursue and apply refinement and reduction approaches to minimize animal use and suffering in Botox potency testing. The company also should publicly disclose the details of its animal testing of Botox Cosmetic so that consumers can decide whether or not to use the product in light of this testing.

For its part, the FDA should work with Allergan in efforts towards a more humane and technically superior alternative test, and to change its policy on Botox potency testing accordingly.