

Stop Botox animal testing!



Botox is the brand name of one of the products containing the nerve poison botulinum toxin. The toxin is used for medical, and increasingly, cosmetic applications. A small injection causes the facial wrinkles disappear for several months. But the price for this short term "beauty" is paid for by the suffering and death of thousands of mice.

Each batch is tested before it reaches consumers. A sample is injected into the abdomen of mice. The mice suffer paralysis, impaired vision and respiratory distress. After three or four days of suffering they die from suffocation.

An estimated 100,000 to 300,000 mice are subjected worldwide to this cruel death every year. Animal-free testing methods are already available, but it is in the hands of the manufacturers if and when they are used.

The Doctors Against Animal Experiments are running a campaign "Stop Botox animal testing" to inform the public, to put pressure on the manufacturers and authorities to speed up the validation and implementation of animal-free methods and to remove the mouse assay in the EU legislation.

What is Botox?

"Botox" is a brand name of a product which is manufactured using the bacteria poison botulinum toxin. Because of its frequent use in the mass media the name "Botox" has been established in the public's mind as a generic term for the injectable anti-wrinkle treatment with the bacteria toxin.

Botulinum toxin is produced by the bacterium *Clostridium botulinum* which can occur in contaminated meat tins. It is the most powerful currently known poison. Inconceivable small amounts, a hundred thousandth milligram, can kill a person.

It blocks the signal transmission from nerve to muscle. The muscle gets paralyzed. A poisoned person dies of suffocation after the respiratory muscles become paralyzed. If highly diluted these properties can be used for the treatment of several medical conditions, including cervical dystonia, torticollis, blepharospasm, hyperhidrosis, strabismus and migraine.

Injecting Botox in facial muscles paralyzes them and causes the facial skin to smooth out. This effect lasts two to six months. That's how long it takes the body to build new nerve endings.

How is Botox tested on animals?

Because the toxin is potentially highly dangerous, it must be much diluted prior to application as a drug for humans. The manufacture process can cause variations in potency. Therefore, each batch is tested for its potency before marketing.

The standard procedure is a classic LD 50 test. Groups of mice are injected with different doses of Botox in the abdomen. The LD50 test causes substantial pain and suffering for the mice. The animals undergo paralysis, impaired vision and respiratory distress. After three or four days of suffering they finally die from suffocation.

For the testing of chemicals, modified LD50 test protocols have been accepted for several years. These tests require fewer animals and are apparently less painful. However, even these small improvements are not accepted for testing Botox. The classical LD50 test continues to be performed which requires death as the endpoint.

Apart from the batch testing for each newly registered product or if changes in the production process are occurring so called stability tests are performed. These tests are

conducted in the first five years after the formal registration and require particularly large numbers of animals

Approximately 100 mice are being used to test each batch of botulinum toxin products (1), 100,000 to 300,000 worldwide every year. And with the growing popularity of botox-type products, the number is increasing. (2).

Which "alternatives" are available?



There are a number of "alternatives" to the current animal test that have been developed, some of which are just refinement methods, which still involve the killing of animals but apparently with less pain and suffering. From the animal welfare point of view any experiment causing suffering to animals is unacceptable.

- Local muscle paralysis: The toxin is injected under the skin of one hind leg of mice and the effects assessed. This is a refinement test that causes a lot less suffering than the LD50 test. For each preparation 32 mice are used. This method was validated by the competent authority in the UK, the National Institute for Biological Standards and Controls (NIBSC) and has been routinely used for several years (3).

- Phrenic nerve-diaphragm preparation: A mouse or a rat is killed to remove their diaphragm including the phrenic nerve. Electric stimulation of the nerve causes the muscle to twitch. The application of varying concentrations of botulinum toxin causes paralysis and thus less muscle twitching. This method is being developed at the Medical School in Hannover, Germany, commissioned by manufacturer Merz.

- Intercostal muscle preparation: Strips of intercostal muscles from rats who have been killed are used. The nerves are electrically stimulated and the muscle response measured after application of botulinum toxin. Six

preparations can be made from one rat (3). This method is developed by the company Ipsen.

- SNAP-25 Endopeptidase Activity Assay: This molecular biological test measures the specific cleavage of the synthetic protein SNAP-25 by botulinum toxin. It is cheap and takes only four hours which is much faster than the mouse assay. It was developed by the UK National Control Laboratory NIBSC which has used it for many years, saving the lives of at least 5,000 mice every year (4). Since 2005 the European Pharmacopoeia allows the use of the Endopeptidase Activity Assay for final batch testing.

The test is not a completely animal free test. Antibodies are used which are produced in animals. The NIBSC is improving the test further to avoid the use of antibodies. The American company Allergan is developing a SNAP-25 test which works with a fluorescent detection system instead of antibodies. Ipsen Ltd. is also working on a SNAP-25 assay.

- Several ELISA-Tests have been developed which detect certain parts of the toxin with antibodies.

- Cell based assays: Allergan is working on an assay with murine neuroblastoma cells.

How is Botox regulated?

The European Pharmacopoeia is a kind of menu for the production, labelling, testing and storing of pharmaceutical products in Europe. The regulations are set by an EU authority, the European Directorate for the Quality of Medicines & Health Care (EDQM) in Strasbourg, France.

The European Pharmacopoeia requires that for every batch of botulinum toxin an LD50 test is carried out on mice (5). Since 2005 three "alternative" tests are also allowed if they produce the same results as the LD50 test.

These are:

1. Endopeptidase Assay
2. Mouse phrenic nerve diaphragm preparation
3. Mouse bioassay using paralysis (not death) as endpoint.

The manufacturers can develop and validate their own alternative assays which are then accepted only for their product. If all three manufacturers have validated the same alternative, then the mouse assay can be removed from the European Pharmacopoeia.

All companies, however, are currently working on different test systems. As long as they are not prepared to cooperate with each other, the LD50 test will remain being seen as the "gold standard" despite its inherent cruelty and unreliability (6).

Botox products are approved as drugs, which is why the EU Cosmetic Directive which bans testing cosmetic products on animals does not apply here. In addition the Directive defines cosmetic products as being applied topically (to the skin and so forth), whereas botox products are injected.

Doctors who use botox-type products for aesthetic purposes are doing this mostly "off label"; this means that the drug has not been approved for this purpose. Depending on which preparation is used patients have to sign an agreement that they are being treated at their own risk with a drug that has not been officially approved for treating wrinkles. Botox Cosmetics®, Vistabel® and Azzalure® are licensed for the temporary treatment of certain wrinkles such as frown lines. All other cosmetic applications are done "off label".

How was Botox discovered?

Botulinum toxin was found in tins of meat which had been contaminated with a particular bacterium. In 1895 the Belgian microbiologist Emile Van Ermengen identified the bacterium *Clostridium botulinum* as the causative agent of the deadly "sausage poison". He called it "botulinum toxin" after the Latin words "botulus" = sausage and "toxin" = poison.

In 1946 botulinum toxin A was purified for the first time. Monkeys were used to test the paralyzing effects. In the 1980's it was used to treat people suffering with cross eyes by injecting the toxin into the eye muscles. More medical indications were soon found. In 1989 Allergan received approval for its drug Oculinum for the treatment of strabism, blepharospasm and other muscle conditions. Later the product was renamed BOTOX. Nowadays more than 50 nerve and muscle diseases can be treated with botulinum toxin.

Since 1992 the toxin has been increasingly used for cosmetic purposes. In 2002 Allergan's BOTOX © COSMETICS received approval in the U.S. (1). Soon the bacteria poison became a lifestyle drug and a worldwide big seller.

Which companies are producing botulinum toxin?

There are 9 distinct types of botulinum toxin, referred to as types A, B, C etc.. Botulinum toxin A is primarily used for medical and cosmetic purposes. Botulinum toxin B is also available as a drug.

Manufacturers of botulinum toxin A products:

Allergan Inc.
P.O. Box 19534
Irvine, CA 92623
USA

BOTOX® is the original product. In 2002 BOTOX® COSMETICS gained approval for cosmetic applications in the U.S. It is manufactured in smaller dosages than BOTOX® which is destined for medical purposes. BOTOX® COSMETICS is marketed in European countries as Vistabel®. Production and animal tests for the European market are carried out in Ireland. For the marketing of its products in Japan and China Allergan has given the license to GlaxoSmithKline.

Ipsen Ltd.
190 Bath Road
SL1 3XE Slough
UK

Dysport® is approved for medical applications only. The animal experiments are carried out at the Wickham Laboratories, UK.

Merz-Pharma GmbH & Co. KGaA
Eckenheimer Landstraße 100
60318 Frankfurt
Germany

Xeomin® is approved for two medical purposes only: the treatment of torticollis and blepharospasmus. Merz plans to obtain a license for cosmetic purposes. The animal tests are conducted at the contract testing lab LPT in Hamburg, Germany.

Galderma Ltd.
Meridien House, 3rd Floor
69 - 71 Clarendon Road
Watford, Hertfordshire
WD17 1DS
UK

Galderma is a joint venture of Nestlé and L'Oreal. The botulinum toxin product Azzalure®

was approved in several European countries for the treatment of frown lines in 2009. It is a formulation of Dyport®, processed for cosmetic applications.

How does the market develop for Botox products?

The human body needs three to six months to rebuild the paralyzed nerve endings. The anti-wrinkle effect wears off and the treatment must be repeated. This is how beauty patients become permanent customers.

Promoted by the mass media the Botox boom has become a huge business with enormous growth rates. In 1993 the global sales of Allergan stood at \$ 25 million. By 2001, this figure had reached \$ 310 million. Its approval for cosmetic use increased the worldwide sales significantly. In 2005 Allergan sold Botox products for \$ 831 million and in 2006 even \$ 1.2 billions (1,7). Ipsen sold Dysport® for 128 million Euros in 2006 (8).

BOTOX® was the second most sold drug in 2005 in the world, with Viagra® being number one (7). In 2005 43% of the BOTOX® products were used for cosmetic applications, 57% for medical purposes (7).

What are the dangers for the consumer?

There have been severe unwanted side-effects after the use of botulinum toxin products both for medical and cosmetic purposes. The toxin can spread from the site of injection to other parts of the body and can cause muscle paralysis. This can result in respiratory distress and difficulties swallowing. More than 600 unwanted side-effects and 28 deaths have been reported worldwide (9). In February 2008 the American Food and Drug Administration (FDA) reported further severe side-effects. Several children with limb spasticity had died after the treatment (10).

What needs to be done?

This topic is not widely known and it is, therefore, important that the European public is educated on this issue. Every member of the public, every cosmetic surgeon or dermatologist needs to know that the anti-wrinkle treatment involves horrific animal suffering. The more Botox is used, the more animals have to suffer. As long as these

animal experiments are still conducted, Botox products should be boycotted.

The aim of the campaign "Stop Botox animal testing" is

- to inform the public about these horrific animal experiments
- to make the manufacturers to use animal-free methods instead of the mouse test
- to remove the mouse test from the EU regulations.

You can help!

- Don't use Botox products
- Spread the word
- Write to the manufacturers asking them to speed up the validation and implementation of animal-free methods

Further information:

www.doctors-against-animal-experiments.org

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