

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

125360

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology
OND IO *Paul C. Brown 7-28-10*

BLA: 125360

Submission date: 7/2/2009 (receipt date)

Drug: Highly Purified Botulinum neurotoxin type A, free from complexing proteins

Sponsor: Merz Pharmaceuticals GmbH

Indication: treatment of cervical dystonia and blepharospasm in adults

Reviewing Division: Division of Neurology Products

Introductory Comments:

The pharmacology/toxicology reviewer and supervisor both recommend that this BLA be approved from a nonclinical perspective. The reviewer recommends that a pre/postnatal study be conducted as a postmarketing requirement. The supervisor agrees and also recommends that a juvenile animal toxicology study to support clinical trials in the pediatric population for treatment of upper and lower extremity spasticity be conducted as a postmarketing requirement.

Reproductive toxicity:

The applicant conducted nonclinical studies on the effects of their product on fertility and embryofetal development. The product was embryotoxic in rats and increased abortions in rabbits when given at doses higher than the maximum recommended human dose. A pre/postnatal toxicity study was not conducted. Pregnancy category C is appropriate.

Conclusions:

I concur with the Division pharm/tox conclusion that this application can be approved. I agree with the wording for the nonclinical sections of the labeling as proposed in the supervisory memo including deletion of section 13.2. I note that some evidence of systemic exposure has been observed for this product and that pre/postnatal studies have been conducted for other similar products. I understand that a pre/postnatal study and a juvenile animal study may already be underway or completed. I concur that these studies can be submitted after marketing approval.

MEMORANDUM

**DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration**

**Division of Neurology Products (HFD-120)
Center for Drug Evaluation and Research**

Date: July 27, 2010

From: Lois M. Freed, Ph.D. *LMF 7/28/2010*
Supervisory Pharmacologist

Subject: BLA STN 125360 (XEOMIN; incobotulinumtoxin A; NT201), submitted July 1, 2009

The sponsor has submitted BLA 125360 to support marketing of XEOMIN (incobotulinumtoxin A) for treatment of cervical dystonia and blepharospasm in adults. XEOMIN is a botulinum neurotoxin type A product, produced from *Clostridium botulinum* serotype A and free of complexing proteins. A limited battery of nonclinical studies was required, due in part to the nature of the product. The nonclinical studies conducted to assess the safety of XEOMIN consist primarily of general IM toxicology studies (13-week, 9-month) in cynomolgus monkey, an IM fertility and early embryonic development study in New Zealand White rabbit, and embryo-fetal development studies in Sprague-Dawley rat and New Zealand White rabbit; no genetic toxicology or carcinogenicity studies were conducted. PK/ADME/TK studies were not conducted since the product is so toxic that detectable systemic exposure would be lethal.

The nonclinical studies have been reviewed by Dr. Wilcox (Pharmacology/Toxicology BLA Review and Evaluation, BLA 125360, Barbara J. Wilcox, Ph.D., July 19, 2010). Based on her review, Dr. Wilcox finds the nonclinical studies adequate to support approval, but recommends a post-marketing requirement (PMR) for a pre- and post-natal development study. (b) (4)

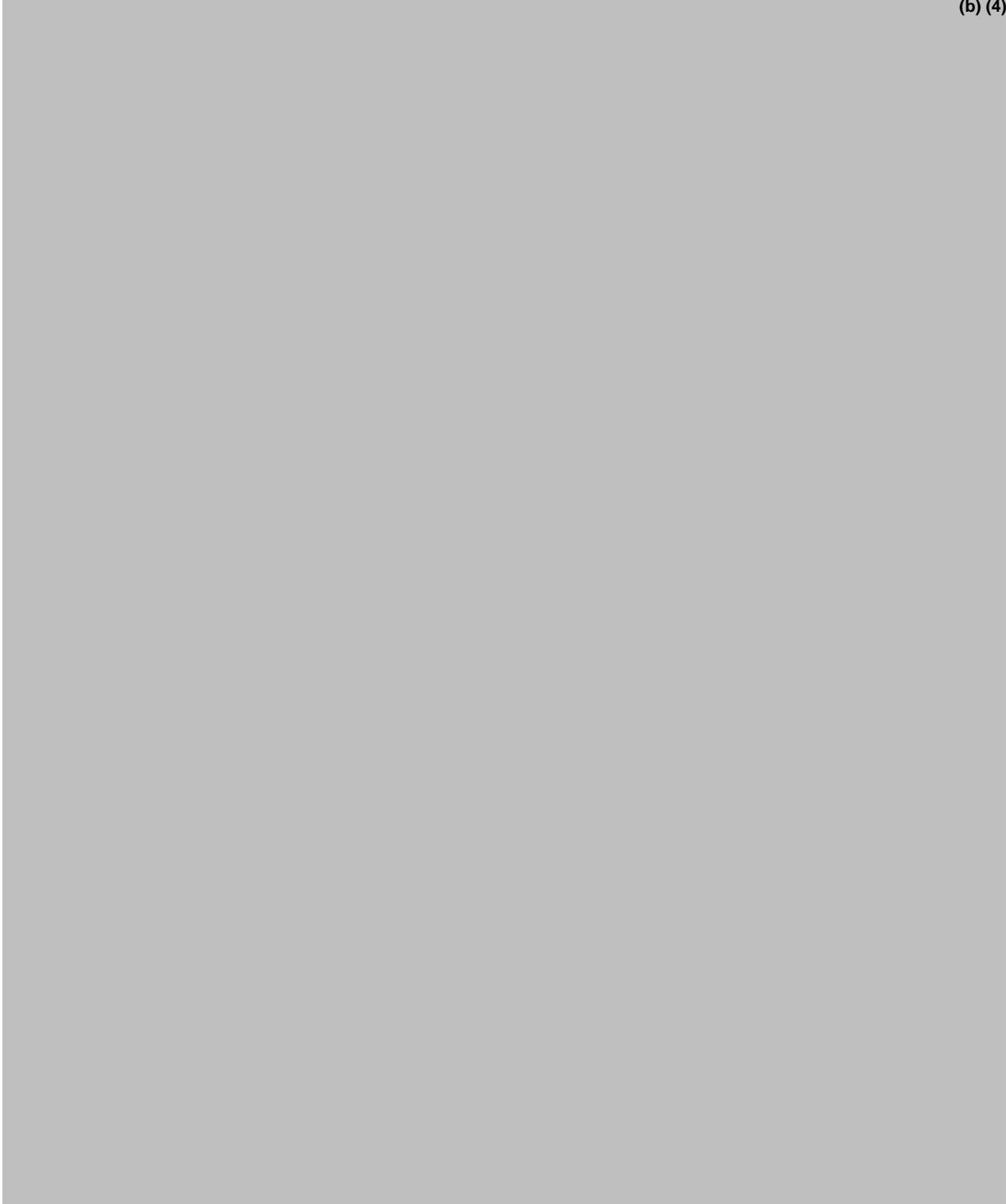
I concur with this conclusion and recommendation. I would also recommend a PMR for a juvenile animal toxicology study to support clinical trials of XEOMIN in the pediatric population for treatment of upper and lower extremity spasticity (*cf.* clinical post-marketing commitments).

Language for the PMRs is provided in separate documents (*cf.* XEOMIN PMR/PMC Development Templates).

[Note added: the sponsor was asked to provide milestone dates for the nonclinical PMRs. In the response, the sponsor noted that a juvenile animal toxicology study is ongoing and a pre- and post-natal development study has been completed.]

Recommended labeling: the following language is recommended, taking into consideration Dr. Wilcox's recommendations and those provided by Dr. Fisher (DNP).

(b) (4)



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**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number: 125360
Supporting document: 0000
Applicant's letter date: 7/1/2009
CDER stamp date: 7/2/2009
PDUFA Date: 7/30/2010
Product: Highly Purified Botulinum neurotoxin type A, free from complexing proteins
Indication: Cervical dystonia and Blepharospasm
Applicant: Merz Pharmaceuticals GmbH
Review Division: Division of Neurology Products
Reviewer: Barbara J. Wilcox, Ph.D.
Supervisor: Lois M. Freed, Ph.D.
Division Director: Russell G. Katz, M.D.
Project Manager: Vandna Kishore, R.Ph.

Disclaimer:

Except as specifically identified, all data and information discussed below and necessary for approval of BLA 125360 are owned by Merz or are data for which Merz has obtained a written right of reference. Any data or information described or referenced below from a previously approved application that Merz does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of BLA 125360. Comparisons with other botulinum toxin products are not relevant. The effects of products other than NT201 were not taken into consideration in determining the approvability of this application.

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1. Executive Summary

1.1. Recommendations

1.1.1. Approvability

Based on the nonclinical data provided, BLA STN# 125360/0 is approvable for the proposed indications. The toxicities of NT201 demonstrated in the nonclinical studies were consistent with the expected effects of the known pharmacologic activity of the product. The clinical patient population can be selected and/or monitored appropriately to avoid unreasonable risk.

1.1.2. Additional nonclinical comments

No pre/post-natal study was included in this application. (b) (4)

This deficiency should not preclude approval at this time, but it is recommended that a pre/post-natal developmental toxicology study be conducted.

In the 9-month study in cynomolgus monkeys (Study#AA41667), atrophy of the contralateral untreated muscle, characterized histologically as reduced and variable myofiber size, increased fibrosis and increased fatty infiltration, was observed, although of lesser magnitude than in the injected muscle. These findings are consistent with systemic spread of the toxin. They were not observed after the 6-month recovery period. In addition, a small but dose-related decrease in absolute and relative heart weight was observed in the 2 highest dose groups in study AA41667. No histological correlations were noted. The biological significance of the reduced heart weight is not clear since no adverse effects were observed in functional, in-life monitoring for the duration of the study. However, the magnitude of the reduced heart weight relative to body weight was smaller than the reductions in absolute heart weight. Therefore, the reductions in total body weight relative to control may account for some of the change in absolute heart weight.

1.1.3. Labeling

(b) (4)

(b) (4) Pregnancy Category C is recommended.

1.2. Brief discussion of nonclinical findings

NT201 (incobotulinumtoxinA, Xeomin) for injection is a neuromuscular blocking agent. The toxin is a large molecular weight protein (ca. 150,000 Daltons) that is synthesized by *Clostridium botulinum*. Botulinum toxin acts at peripheral cholinergic nerve endings to inhibit acetylcholine release at the neuromuscular junction resulting in chemical denervation of the treated muscle. This denervation results in a localized reduction of muscle activity (paresis or paralysis). Dose levels are expressed in units where one unit is the amount of toxin established as the LD₅₀ after intraperitoneal administration in mice.

The doses are normally administered intramuscularly (i.m.) in small volumes in order to avoid systemic exposure. However, as the dose or the volume injected is increased, the toxin may spread to adjacent muscles via diffusion along fascial planes or small amounts may escape into the systemic circulation. As systemic exposure increases, signs of toxicity appear including generalized muscle weakness, autonomic dysfunction, respiratory failure and death.

Due to the extreme toxicity of the molecule, standard pharmacokinetic analyses are not feasible. Exposure is estimated using pharmacodynamic markers such as muscle strength and muscle electrical activity.

The pharmacology and toxicology of NT201 was evaluated in a series of nonclinical studies in rats, mice, rabbits and nonhuman primates. The pivotal studies included safety pharmacology, single and repeat-dose toxicology (with the longest duration of 9 months in non-human primates), fertility, and embryofetal developmental toxicology in rats and rabbits, and local tolerance for eye irritation. The route of administration for all pivotal studies was intramuscular injection (except eye irritation where NT201 was instilled directly into the eye) and doses were given as units of activity (U)/kg. All effects observed were consistent with expected results of the known pharmacological activity of botulinum toxin, including atrophy of the treated muscle accompanied by impaired locomotion and reduced body weight gain generally correlating with reduced food intake which became severe at the higher doses.

In the 9-month monkey study (Study #AA41667), signs of atrophy were observed in skeletal muscle remote to the treated muscle, although of lesser magnitude than those observed in the treated muscle. This finding is consistent with systemic exposure to the toxin. In addition, a small, but dose-related decrease in mean heart weight (absolute and relative) was observed in the treated groups. The biological relevance of this finding is not clear but should not be ignored since parasympathetic nervous system is cholinergic and can be affected by botulinum toxin activity. No in-life cardiac functional or histopathological correlates were noted.

2. Drug Information

2.1. Drug:

- 2.1.1. CAS registry number (optional): N/A
- 2.1.2. Generic name: incobotulinumtoxinA
- 2.1.3. Code name: (b) (4) for drug substance, NT201 for drug product
- 2.1.4. Chemical name: Highly purified Botulinum neurotoxin type A, free from complexing proteins
- 2.1.5. Molecular formula/molecular weight: 150 kDa
- 2.1.6. Pharmacological class: neurotoxin, peripheral muscle relaxant
- 2.1.7. Structure:

2.2. Relevant IND/s, NDA/s, and DMF/s: IND 12128 (cervical dystonia), IND 100163(blepharospasm) (b) (4),

2.3. Clinical formulation:

2.3.1. Drug formulation: Xeomin is to be marketed in 2 strengths: 50 and 100 Units. The excipients will be sucrose and human serum albumin. The drug will be presented as a lyophilized powder and will be reconstituted with commercially available sterile 0.9% NaCl. The formulations are summarized in the tables below, provided by the sponsor.

Table 3.2.P.1-1: Composition of drug product NT 201

Ingredient	Weight per Vial (nominal composition)	Function	Specification
Active ingredient:			
(b) (4)l (Clostridium Botulinum Neurotoxin Type A (150 kDa), free of complexing proteins)	(b) (4) 100 LD ₅₀ units	Active ingredient	Ph. Eur. ¹ In house specification ²
Excipients:			
Sucrose	4.7 mg	(b) (4)r	NF ¹
Human serum albumin	1.0 mg	(b) (4)n r	USP ¹
(b) (4)			

Table 3.2.P.1-1: Composition of the drug product NT201-50 Units

Ingredient	Weight per Vial (nominal composition)	Function	Specification
Active ingredient:			
(b) (4) (Clostridium Botulinum Neurotoxin Type A (150 kDa), free of complexing proteins)	(b) (4) 50 LD ₅₀ units	Active ingredient	Ph. Eur. ¹ In house specification ²
Excipients:			
Sucrose	4.7 mg	(b) (4)	NF ¹
Human serum albumin	1.0 mg	(b) (4)	USP ^{1,3}
(b) (4)			

Standards:

¹ Ph. Eur.	European Pharmacopoeia
USP	United States Pharmacopoeia
NF	national Formulary (current compendia are used)

² In house specification see section 3.2.S.4.1 *Specification*

³ Human serum albumin is supplied as Albumin (Human) 25% solution which is an FDA approved product (BLA 102366)

2.3.2. Comments on novel excipients: N/A

2.3.3. Comments on impurities/degradants: N/A

2.4. Proposed clinical population and dosing: Cervical dystonia and blepharospasm

2.5. Regulatory background: N/A

3. Studies submitted within this submission:

3.1. Studies reviewed within this submission:

Study Number	Study Title	Page Number
442/015	Comparative combined efficacy and cardiovascular risk assessment of NT201 and BOTOX after single intramuscular administration (gluteus medius) in the telemetered conscious cynomolgus monkey	10
(b) (4) (2003)	Effects of NT101 on patch clamp responses of human ether-a-gogo related gene product (hERG) channels expressed in CHO cells	10
026TOX97	Determination of the biological activity of NT201 in the mouse lethality assay	19
058TOX97	Determination of the activity of botulinum toxin in the mouse lethality assay	21
(b) (4) 11252/1/98	Comparative systemic toxicity of NT201 in mice following intravenous administration	9 and 13
16464/03	Acute toxicity study of NT201 and BOTOX in mice by intramuscular injection	16
21110	Acute eye irritation/corrosion test of NT201 in rabbits	68
11373/98	Examination of NT101 on haemolytic properties in human blood (<i>in vitro</i>)	67

422/016	NT-201: 13 week toxicity study with repeated intramuscular administrations in the cynomolgus monkey	22
(b) 11125/1/98	Determination of the biological activity of NT201 in the regional paralysis test in CD-1 mice	26
AA41667	NT201: 9-month toxicity study with repeated intramuscular administrations in the cynomolgus monkey followed by a 6-month treatment-free period	33
422/017	NT201- Dose range-finding study by the intramuscular route in the non-pregnant rabbit.	31
422/018	NT201- Embryo toxicity study by the intramuscular route in the rabbit (Segment II)	54
AA62003	NT201-Dose range-finding study by the intramuscular route in the pregnant rat	49
AA42649	NT201-Embrototoxicity study by the intra muscular route in the rat (Segment II)	61
442/019	Fertility study by the intramuscular route in the rabbit	45
AA62151	NT201-Dose range-finding post-weaning juvenile toxicity study in the rat	43
(b) (4) 12444/99	Comparative antigenicity of three preparations of botulinum toxin in the rabbit	71
10929/97	Comparative antigenicity of BOTOX and NT201 in the rabbit	69

3.2. Studies not reviewed within this submission:

(b) (4)

3.3. Previous reviews referenced:

None.

4. Pharmacology

NT201 consists of a recombinant preparation of botulinum toxin Type A, a neurotoxin classified as a muscle relaxant with ATC-Code M03AX01 that selectively inhibits acetylcholine release from cholinergic nerve terminals. The toxin is synthesized as a single-chain polypeptide with a molecular weight of approximately 150 kD. After secretion, the protein is proteolytically cleaved into 2 subunits (one 50 kD and the other 100 kD). These subunits are designated the heavy and light chains, which are joined by a disulfide bond to form the mature molecule. NT201 refers to the drug product; (b) (4) refers to the drug substance.

4.1. Primary pharmacology

Botulinum toxin Type A is produced and secreted by a specific strain of the bacterium *Clostridium botulinum*. It is secreted as part of a high molecular weight protein complex formed by several hemagglutinins. Other commercially available preparations of the toxin contain the additional complexing proteins. NT201 has been further purified such that the complexing proteins are no longer present, thus potentially reducing the immunogenic potential.

The toxin binds to cell surface docking sites (sometimes referred to in the literature as the BTX receptor) on the presynaptic terminal of cholinergic neurons. The docking sites have been characterized as GT1b, GD1b and Gq1b gangliosides. The toxin is then taken up into the cell via endocytosis where the endopeptidase domain is

translocated into the cytosol. This endopeptidase selectively cleaves the protein, SNAP-25, which is a necessary component in the process of neurotransmitter release.

Within 4 to 10 days, compensatory sprouting from the poisoned nerve terminal begins with proliferation of acetylcholine receptors on the post-synaptic nerve terminals. The collateral sprouting leads to what is referred to as electromyographic jitter. After approximately 8 weeks, poisoned nerve endings begin to recover and re-establish functional motor endplates. Once the toxin has been taken up, normal muscle function can be restored only through this regeneration process.

4.2. Secondary pharmacology N/A

The NT201 is highly specific for pre-synaptic cholinergic terminals and all effects observed in the nonclinical studies were consistent with the known pharmacological activity of botulinum toxin.

4.3. Safety pharmacology

Study # (b) (4) 11252/1/98

Title: Comparative systemic toxicity of NT201 in mice following intravenous administration

This study was intended as both a general toxicology study and a CNS safety pharmacology study. The information pertinent to CNS safety pharmacology is reviewed here. Additional review is included in the Toxicology section of this review.

Testing Facility:

(b) (4)

Date of study initiation: 6/11/1998

GLP compliance: Yes

Lot #: Batch (lot#) 0

Methods:

Animals: male CD-1 mice, 10 per group

Dosing: 0, 9, 20, 30, 45 or 68 U/kg by IV injection.

Observations: Movements of each mouse were recorded for a period of 10 minutes at 24, 48 and 72 hours post-dosing. Clinical signs were assessed daily.

Results:

- All observations occurred in a dose-related manner for onset, magnitude and duration and are expected effects of the biological activity of botulinum toxin.
- Mortality occurred in 2 of 10 mice in the 45 U/kg group on test day 5 and all high-dose animals died on test days 3-4.
- No effects on mobility were noted for the 9 U/kg group.

- For the 20 U/kg group, reductions in movement relative to control began at 48 hours after dosing. Slight pilo-erection was noted in all animals in this group on SD3 and 5.
- In the dose groups receiving 30 U/kg or higher, observations included reduced mobility, ataxia, ptosis, pilo-erection, lacrimation, mydriasis, dyspnea, decreased body temperature, muscle weakness and paralysis.

•••

Study #: (b) (4); (2003)

Title: Effects of (b) (4) on patch clamp responses of human ether-a-gogo related gene product (hERG) channels expressed in CHO cells.

Performing Laboratory: Merz Pharmaceuticals GmbH
 GLP compliance: No.

Methods:

Cells: CHO-K1 cells

Test article: (b) (4) with 0.1% HSA, 10,000 U per ml (50 X greater than the highest expected injected concentration for clinical use.

Results: No adverse effects were observed under the conditions of this study.

(b) (4) (10,000 U/ml) had no effect on hERG tail currents at -20 mV (99.79% ± 3.65 % of control (n=6).

Conclusions:

The results indicate that (b) (4) at 10,000 U/ml had no effect on hERG tail currents at -20 mV. Therefore, adverse interaction of (b) (4) with hERG channels appears to be unlikely. (Note: (b) (4) is the active drug substance that is formulated into NT201.)

•••

Study #442/015

Title: Comparative combined efficacy and cardiovascular risk assessment of NT201 and BOTOX after single intramuscular administration (gluteus medius) in the telemetered conscious cynomolgus monkey

Testing Facility: (b) (4)
 GLP compliance: Yes
 Date of study initiation: 11/20/03
 Lot #: NT201 lot#020902, BOTOX lot# C0855 C2
 Vehicle/formulation: sterile 0.9% saline containing 1mg/ml HSA

Methods:

Animals: Cynomolgus monkeys, 12 males, age 28 to 39 months, 6 received NT201, 5 received BOTOX

Doses: 16 U/kg, injected IM as a single dose

Regimen: Administered i.m. into the left gluteus medius muscle

Study design:

Telemetry equipment was surgically implanted approximately 4 to 6 weeks prior to initiation of dosing. Animals were then assigned to two treatment groups to receive either NT201 or BOTOX. The following tables, provided by the sponsor, summarize the basic study design.

Group/ Treatment	Dose level(1) (LDU/kg/administration)	Dose volume (ml/kg/administration)	Dose concentration (LDU/ml) (1)
1 NT 201	16	0.2	80
2 Botox®	16	0.2	80

(1) 1 Unit (LDU) corresponds to the LD 50 in the mouse (weighing about 20 g) by the intraperitoneal route

Animals received one injection of vehicle into the right gluteus medius muscle and one injection of the assigned test article into the left gluteus medius muscle. The dose chosen corresponds to the high dose used in the 13-week toxicology study performed in cynomolgus monkeys.

Study day	Study phase
Day -6	Pre-test period of session 1
Day -4	Pre-test period of session 2
Day 0	Treatment day of session 1
Day 2	Treatment day of session 2
Days 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91 and 252	Study follow-up of session 1
Days 9, 16, 23, 30, 37, 44, 51, 58, 65, 72, 79, 86, 93 and 254	Study follow-up of session 2

Study day	Study phase	Time point analysed*
-6 and -4	Pre-test	-1 h, -30 min, 15 min, 30 min, 1, 2, 3, 4, 6 and 12 h
0 and 2	Treatment days	-1 h, -30 min, 15 min, 30 min, 1, 2, 3, 4, 6 and 12 h
7 and 9	Follow-up days	-1 h, -30 min, 15 min, 30 min, 1, 2, 3, 4, 6 and 12 h
14, 16, 21, 23, 28, 30, 35, 37, 42, 44, 49, 51, 56, 58	Follow-up days	3 h**

* Time of treatment was defined as 0 min. The evaluation time points of all study days are based on the time of treatment (study day 0 for session 1 and study day 2 for session 2). The -1 h and -30 min values are related to the time of entrance in the room for treatment and not to the time of treatment.

** Time-point was selected after analysis of the results of pre-test, treatment day and 7 days after treatment.
min: minutes. h: hour(s).

Observations/Results:

- *Mortality/Clinical signs:* No mortality or clinical signs attributable to test article administration were reported.
- *Body weight:* Recorded on SD3 (prior to treatment), every 2 weeks thereafter and on SD94. No significant effects on body weight or body weight change were

noted. The results for the NT201 group are very similar to those of the BOTOX group. All animals gained weight during the course of the study.

- *EMG efficacy evaluation:* Evaluation was carried out for a 24-hour period on one pre-test day and on the day of treatment, then weekly until week 14. An additional evaluation was carried out 36 weeks after treatment. EMG measurements were taken from the treated gluteus medius muscle as well as the contralateral muscle that was treated with control solution. NT201 showed maximal inhibition one week after treatment. Recovery of activity began approximately 9 weeks after injection. Effects of treatment were similar between the two treatment groups.
- *Cardiovascular risk assessment:* Heart rate and ECG monitoring was initiated 2 hours prior to dosing and continued for 24 hours post-injection. Monitoring was again performed one week after treatment for 24 hours followed by weekly measurements from week 3 through week 9. Decreased heart rate was observed 2-3 hour after injection. This change was attributed to the ketamine anesthesia used during treatment. One week post-treatment, markedly increased heart rate was noted that was associated with effects on RR and QTc interval (slight increase) duration relative to pre-test values. These effects were hypothesized to be due to stress of paralysis, and returned to baseline by 4 weeks post-treatment. No adverse effects on PR interval or QRS complex duration were observed. Since botulinum toxin is a large molecule, it is not expected to enter ion channels.

Conclusions:

No significant adverse effect was observed on ECG parameters. Although variations in heart rate, with concomitant small changes in ECG measurements, were noted for animals treated with NT201, no clear effect directly attributable to treatment with the test article was observed. The botulinum toxin molecule is too large to enter ion channels (150 kD) and is not expected to affect the QT interval.

This study is of limited use in evaluating safety of NT201. A single dose level was used for each product. Assessment of additional dose levels would normally be expected for this type of study. (Comparisons of safety among botulinum toxin products are difficult because the unitage of the products are not necessarily equivalent and would need to be established head-to head in a single mouse lethality assay. In this study, it is not clear that adequate equivalence of units was established.)

5. Pharmacokinetics

Direct traditional pharmacokinetic measurement of NT201 is not feasible because only very low (undetectable) levels are normally present in systemic circulation. Should measurable levels of the toxin gain access to the circulation, the animal would not survive. Therefore, pharmacodynamic assays such as the rodent hindlimb paralysis model and the mouse hemidiaphragm test have been developed. EMG assays have also been adapted to assess the activity of botulinum toxin *in vivo*.

Merz has analyzed local spread of NT201 after intra-muscular injection (doses of 2, 4, 16 or 32 U) into the extensor digitorum brevis in human volunteers. Diffusion into

nearby muscles was assessed by EMG measurements of the abductor hallucis and the abductor digitorum quinti over time. In these evaluations, no significant effects of NT201 on muscles adjacent to the injected muscle were detected.

6. General Toxicology

6.1 Single-dose toxicity

Study #11252/1/98

Title: Comparative systemic toxicity of NT201 in mice following intravenous administration

Testing Facility:	[REDACTED] (b) (4)
GLP compliance:	Yes
Study initiation date:	6/11/98
Lot #:	NT201 #SPA 3/1 batch 0 Botox # CGD 007 and 026 Dysport # 130B
Vehicle/formulation:	physiological saline containing 0.1% HSA

Methods:

Animals: CD-1 mouse, male, 60 for each test article (10 per group), aged 28 days

Dosing:

- NT201 and BOTOX: 9, 20, 30, 45, and 68 U/kg
- DYSPORT: 9, 20, 30, 45, and 68 U/kg

Regimen: Intravenous administration, single dose with 14 days observation

Study design: Each animal received a single IV injection followed by 14 days of observation. Dose selection was based upon preliminary studies in which doses between 30 and 152 LDU were used. The table below, provided by the sponsor, summarizes the basic study design.

Administration route i.v.						
Group			Dose level ^a (LDU/kg b.w.)	Animal no.		
NT-201	Botox [®]	Dysport [®]		NT-201	Botox [®]	Dysport [®]
7	1	13	control	61 - 70	1 - 10	121 - 130
8	19	14	9	71 - 80	181 - 190	131 - 140
9	20	15	20	81 - 90	191 - 200	141 - 150
10	2	16	30	91 - 100	11 - 20	151 - 160
11	3	17	45	101 - 110	21 - 30	161 - 170
12	4	18	68	111 - 120	31 - 40	171 - 180

^a factor 1.5 increase; factor 2.25 for the first two dose levels

Observations /Results:

Mortality: Observations were made daily.

- For NT201, 2 of 10 animals in the 45 U/kg died on test day 5, 10 of 10 animals died in the high dose group died on test day 3 or 4.
- Similar results were noted with BOTOX (3 of 10 died in the 45 U/kg group and 10 of 10 in the high dose group by SD5).
- For DYSPORT, no deaths were reported in the 45 U/kg group but 8 of 10 animals died in the high dose group by SD6.

The following table, taken from the study report, summarizes that mortality data among the treatment groups.

Number of deceased animals

Preparation	dose LDU/kg	day														total
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
NT-201	9															0
	20															0
	30															0
	45				2											2
	68		7	2	1											10
Botox®	9															0
	20															0
	30															0
	45			1		2										3
	68		3	6	1											10
Dysport®	9															0
	20															0
	30															0
	45															0
	68		1	3	1		3									8

Clinical signs: Detailed examination of clinical signs was conducted on SD1, 3, 5, 10 and 14. Parameters assessed included ataxia or unusual movements. Appearance of skin and fur, appearance of eyes, nose and mouth, pupil size, respiration, changes in urinary/bowel behavior, body temperature and muscle tone.

- No effects attributable to test article administration were observed in the low dose group of any toxin preparation.
- At 20 U/kg, only pilo-erection was noted for NT201; low body temperature was noted in DYSPORT treated mice.
- At 30 U/kg, additional clinical signs were noted in all animals, although most effects were mild. These signs included impaired mobility and ataxia for the BOTOX and DYSPORT treated groups, as well as midriasis, ptosis, dyspnea, lacrimation, reduced muscle tone. The profiles of dose-related effects were generally similar for all three toxin preparations, with the exception of ptosis and midriasis which were more severe (slight to moderate) for BOTOX and DYSPORT at 30 U/kg than NT201.

Locomotor activity: Recorded at 24, 48 and 72 hours post dosing using computer-assisted mobility assessment equipment. Active and static movements were evaluated and data analyzed separately. See review of mobility data under safety pharmacology, above.

- No effects on mobility were noted for any of the three preparations at the low dose.
- Reduced mobility was noted at 20 U/kg of NT201 beginning 48 hours post-injection.
- At 30 U/kg of NT201, the reduced movement began by 24 hours after dosing.

- For the 45 LDU/kg NT201 dose group, locomotor activity was reduced to 20-25% at 24 hours and by SD3 was estimated to be 10-15% relative to control.

Conclusions:

The sponsor intends the data collected on locomotor activity to meet the requirement for CNS safety pharmacology assessment.

The results were consistent with the expected effects of botulinum toxin and included dose-related reductions in mobility as well as clinical signs attributable to activity on autonomic cholinergic nerve terminals. The no adverse effect dose level (NOAEL) for NT201 under the conditions of this study was 9 U/kg.

As a non-clinical general toxicology study, this study is not adequate. The study design does not include full toxicology endpoint analyses and only includes male animals.

•••

Study #16464/03

Title: Acute toxicity study of NT201 and BOTOX in mice by intramuscular injection

Testing Facility:

[Redacted] (b) (4)

Date of Study Initiation: 2/27/2003

GLP Compliance: Yes.

Lot#: NT201: 21103

BOTOX: CO618

Vehicle/formulation: Sterile saline (0.9% NaCl) containing 1 mg/ml HSA

Methods:

Animals: CD-1 mice, 10/sex/group

Dosing: 0, 5, 50 or 150 U/kg (0.1U, 1U or 3 U total per mouse)

Regimen: single dose

Route of administration: Intramuscular injection in a volume of 0.1 ml (2 X 0.05 ml)

Study design:

The following table, provided by the sponsor, summarizes the basic study design. Clinical signs were recorded prior to dosing and at 5, 15, 30, and 60 minutes post-dose, then at 3, 6, and 24 hours post-dose. After the 24 hour time point, the animals were observed daily through day 14 post-dose. The mice were sacrificed on SD15. Gross changes were recorded and microscopic examination was performed on organs with gross lesions, as necessary.

Group	Toxin	Dose level	Number sex per of animals	Mice nos
1		control	10 ♂	1 - 10
			10 ♀	11 - 20
2	Botox	0.1 U/animal ▲ 5 U/kg b.w.	10 ♂	21 - 30
			10 ♀	31 - 40
3	Botox	1 U/animal ▲ 50 U/kg b.w.	10 ♂	41 - 50
			10 ♀	51 - 60
4	Botox	3 U/animal ▲ 150 U/kg b.w.	10 ♂	61 - 70
			10 ♀	71 - 80
5	NT 201	0.1 U/animal ▲ 5 U/kg b.w.	10 ♂	81 - 90
			10 ♀	91 - 100
6	NT 201	1 U/animal ▲ 50 U/kg b.w.	10 ♂	101 - 110
			10 ♀	111 - 120
7	NT 201	3 U/animal ▲ 150 U/kg b.w.	10 ♂	121 - 130
			10 ♀	131 - 140

Results:

Mortality:

- All of the mice receiving 150 U/kg of NT201 died prior to the 14-day study end point. All were dead by SD7 with no observable difference in time to death between products.
- One animal receiving the mid dose of NT201 died on SD5 without preceding signs of botulism. The relationship to test article for this animal is not clear.

Body weight:

- No effect on body weight was noted for the low dose NT201 group.
- Body weights for the mid and high dose groups were significantly lower than controls on SD4, 8, 11 and 15. The reduction of body weight gain in treated animals was dose-dependent.

Clinical signs:

- No clinical signs attributable to test article administration were noted for either low-dose group.
- Animals receiving the mid-dose of NT201 showed expected effects of toxin activity including reduced mobility, ataxia, reduced muscle tone and ptosis. At the high dose, these clinical signs (at greater severity) and dyspnea were observed. Clinical signs were observable by 3 days post-injection.

Gross necropsy:

- No macroscopic signs attributable to test article were reported for the low and mid dose groups treated with either product.
- Emaciation and dehydration were noted in most (9 of 10 males and 5 -7 of 10 females) animals in the high dose groups of both products.
- Histology was not performed.

The following table, provided by the sponsor, summarizes the clinical observations for this study.

Summarized Results

	(n = 10/sex/group)													
	Group 1 Control		Group 2 5 U/kg b.w.		Botox				NT 201					
					Group 3 50 U/kg b.w.		Group 4 150 U/kg b.w.		Group 5 5 U/kg b.w.		Group 6 50 U/kg b.w.		Group 7 150 U/kg b.w.	
	m	f	m	f	m	f	m	f	m	f	m	f	m	f
reduced motility	none	none	none	none	+/+	+/+	++/+++	++/+++	none	none	+/+	+/+	++/+++	++/+++
					3-6d (10)	3-6d (10)	3-5d (10)	3-6d (8)			3-7d (10)	3-7d (10)	3-7d (10)	3-6d (8)
ataxia	none	none	none	none	+	+	++	+/+	none	none	+/+	+	++	++
					3-5d (10)	3-5d (10)	3-5d (10)	3-6d (8)			3-6d (10)	3-6d (10)	3-7d (10)	3-6d (8)
reduced muscle tone	none	none	none	none	none	none	++	+/+	none	none	+	+	++	+/+
							3-5d (10)	3-6d (7)			4-5d (10)	5d (10)	3-7d (10)	3-6d (8)
dyspnoea	none	none	none	none	none	none	++	+/+	none	none	+		++	+/+
							3-5d (10)	3-6d (7)			4-5d (9)	none	3-7d (10)	3-6d (8)
ptosis	none	none	none	none	none	none	+/+	+/+	none	none	+	+	+/+	+/+
							3-5d (10)	3-6d (7)			4-6d (10)	5-6d (10)	3-7d (10)	3-6d (8)
mortality														
within 6h	0	0	0	0	0	0	0	0	0	0	0	0	0	0
within 24h	0	0	0	0	0	0	0	0	0	0	0	0	0	0
within 7d	0	0	0	0	0	0	10	10	0	0	1	0	8	10
within 14d	0	0	0	0	0	0	10	10	0	0	1	0	10	10

- + slight
- ++ moderate
- +++ severe
- 0' immediately after administration
- m male
- f female
- in brackets: number of animals affected

Conclusions:

This study was performed to compare the lethal doses for NT201 and BOTOX. It was not conducted as a full toxicology study. All animals in the high-dose groups (Groups 4

and 7) died by SD7. All clinical signs observed in animals receiving the mid and high doses of each product were expected results of exposure to botulinum toxin and were clearly dose-related. Signs of toxicity included reduced weight gain, reduced mobility, ataxia, muscle weakness, ptosis and, at the high dose, dyspnea.

The lowest lethal dose was determined to be 1 U/animal for NT201 (50 U/kg), and 3U/animal for BOTOX (150 U/kg).

The NOEL for each product was determined to be 0.1 U/mouse (5 U/kg).

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Study #026TOX97

Title: Determination of the biological activity of NT201 in the mouse lethality assay

Performing Laboratory: (b) (4)
GLP compliance: Compliant with EU GLPs.
Date of study initiation: 7/30/97
Lot #: SPA 3/1-3
Vehicle/formulation: Human serum albumin lot # 24 H 93251

Methods:

Animals: male CD-1 mice, 10/group, 6 dose groups

Doses: 3.91, 4.89, 6.11, 7.64, 9.55, or 11.94 pg/animal

Route/regimen: single dose, intra-peritoneal injection

Group	Dose (pg/animal)	Animal No.
1	3.910	101 - 110
2	4.890	201 - 210
3	6.110	301 - 310
4	7.640	401 - 410
5	9.550	501 - 510
6	11.940	601 - 610

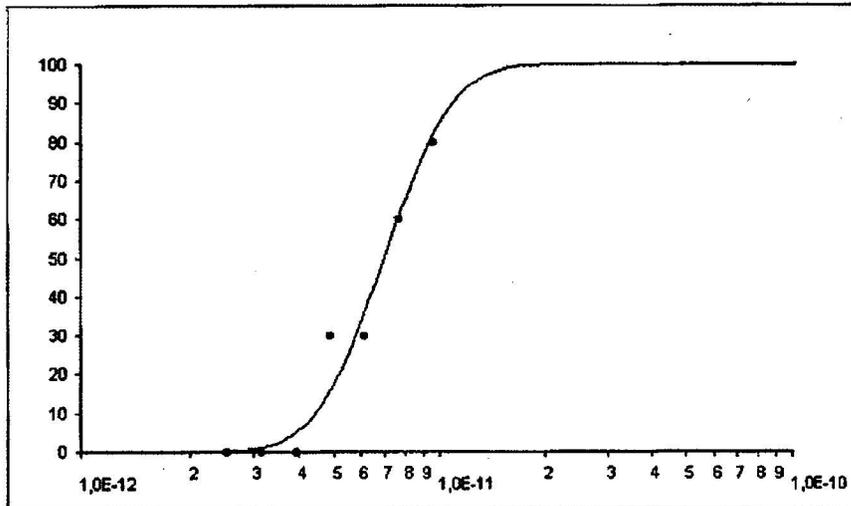
Observations/results:

Mortality: Mortality checks were performed 2 and 5 hours after administration and once daily thereafter for 4 days.

- No deaths were noted on the day of dosing. Deaths occurred 24 to 72 hours after injection.
- The table below, taken from the study report, summarizes the mortality data.

Group (n=10)	Dose (pg/animal)	Mortality (Number of animals found dead) (hours after administration)				
		24 h	48 h	72 h	96 h	Total
1	3.91	0	0	0	0	0
2	4.89	1	3	1	0	5
3	6.11	3	3	1	0	7
4	7.64	6	3	0	0	9
5	9.55	9	1	0	0	10
6	11.94	8	1	0	0	9
Total		27	11	2	0	40

**Mouse Lethality Assay
Botulinum-Toxin**



Conclusions:

The mouse LD₅₀ assay is used to determine the potency and to assign unitage to each lot of NT 201 as is the current standard for botulinum toxin-based products. The conditions used for this assay are standardized including species, sex, strain, number of animals and groups, route and volume of administration, observation period, and testing parameters. The LD₅₀ for this lot of NT 201, as calculated by probit analysis, was 6.9690 pg/animal. Therefore, for this lot, one Unit equals approximately 7 pg.



Study #058 TOX 97

Title: Determination of the biological activity of botulinum toxin in the mouse lethality assay

Performing laboratory:

(b) (4)

GLP compliance:

Compliant with EU GLPs

Date of study initiation:

12/15/97

Lot #:

SPA 3/1-3/4

Vehicle/formulation: Human serum albumin Lot # 24 H 93251

Methods

Animals: male CD-1 mice

Dosing: 2.5 to 9.55 pg/animal

Regimen: single dose administered IP, in a volume of 0.5 ml

Study Design: Group and dose assignments are summarized in the table below, taken from the study report.

Group allocation and dosages

Group	Dose (pg/animal)	Animal No.
1	3.910	101 - 110
2	4.890	201 - 210
3	6.110	301 - 310
4	7.640	401 - 410
5	9.550	501 - 510
6	11.940	601 - 610

Results:

The mortality data observed under the conditions of this study are summarized in the table below, taken from the study report.

Group (n=10)	Dose (pg/animal)	Mortality (Number of animals found dead) (hours after administration)				
		24 h	48 h	72 h	96 h	Total
1	3.91	0	0	0	0	0
2	4.89	1	3	1	0	5
3	6.11	3	3	1	0	7
4	7.64	6	3	0	0	9
5	9.55	9	1	0	0	10
6	11.94	8	1	0	0	9
Total		27	11	2	0	40

Conclusions:

Seven dose levels were administered to groups of male CD-1 mice in a single IP injection followed by daily observation for 4 days. The methods used for this study are standardized as described above under study # 026TOX97. The LD₅₀ for NT 201 lot # SPA 3/1-3/4 is calculated by probit analysis to be 5.37 pg/animal.

6.2 Repeat-dose toxicity

Study #422/016

Title: NT201: 13-week toxicity study with repeated intramuscular administrations in the cynomolgus monkey

Testing Facility:	(b) (4)
Study initiation date:	8/30/ 03
GLP compliance:	Yes
QA report:	Yes
Lot Number:	NT201 lot #020902
Formulation/vehicle:	NaCl 0.9% containing 1 mg/ml HSA

Methods

Animals: Cynomolgus monkey, 3/sex/group

Age/weight: Males: 32-36 months, 2.3 to 3.6 kg

Females: 32 to 43 months, 2.2 to 2.9 kg

Dose levels and regimen: 0, 4, 8 or 16 U of either NT201 (test item 1) or BOTOX (test item 2) via IM injection once every 4 weeks for a total of 4 doses (SD0, 28, 56 and 84)

Rationale for choice of dose: The dose levels were selected taking into account published data on botulinum toxin Type A as well as previous studies conducted with NT201.

Study Design: The table below, taken from the study report, summarizes the basic study design.

Group/Treatment	Dose level ⁽¹⁾ (U/kg/adm)	Dose volume (ml/kg/adm)	Dose concentration (U/ml) ⁽¹⁾	Number of animals	
				Males	Females
1. Control	0	0.2	0	3	3
2. Low dose test item 1	4	0.2	20	3	3
3. Intermediate dose test item 1	8	0.2	40	3	3
4. High dose test item 1	16	0.2	80	3	3
5. Low dose test item 2	4	0.2	20	3	3
6. Intermediate dose test item 2	8	0.2	40	3	3
7. High dose test item 2	16	0.2	80	3	3

adm: administration.

⁽¹⁾ 1 Unit (U) corresponds to the LD 50 in the mouse (of approximately 20 g) by the intraperitoneal route.

Group 1 animals (control) received the vehicle (sterile NaCl 0.9% containing 1 mg/ml Human Serum Albumin).

Observations/Results:

Mortality: Observations were made twice daily

- All animals survived to scheduled sacrifice.

Clinical observations: Observations were recorded daily during treatment-free periods. On treatment days, the animals were observed before dosing, 1 and 4 hours post dosing. Full clinical exams were performed prior to initiation of treatment and at termination. On SD79, clinical observation of calf muscle mass was performed. On week 13, additional blood samples were taken for future immunological testing, if indicated.

- Clinical signs observed included limping, thin appearance and locomotor difficulty. These are expected effects of the test article activity. Limping was observed in a dose-dependent manner beginning on SD29 (within 1 week after the second dose). The locomotor difficulty was noted for the middle and high dose groups only. No effect was noted for the low dose males and the low dose females did not show the limping effect until day 59.
- Local tolerance: Isolated cases of hematoma at the injection site were noted in treated and control animals.

Ophthalmology: Exams performed pre-test and during week 13.

Body weight: Recorded weekly with food consumption estimated daily.

- A dose-related reduction in overall body weight gain is reported. The table below, taken from the study report, summarizes the body weight reduction among treatment groups.

	Overall body weight gain (between days 0 and 91)			
	Males		Females	
Control group	-		+8 %	
Treated groups	Test item 1	Test item 2	Test item 1	Test item 2
Low dose	+4 %	-3 % ⁽¹⁾	-	+4 %
Intermediate dose	-	-4 %	-8 %	-4 %
High dose	-7 % ⁽¹⁾	-11 %	-20 %	-12 %

- no difference

(1) calculated between days 0 and 84

- The weight loss was correlated with an accompanying reduction in food consumption (NT201: -7% for males, -20% for females).

Cardiovascular exams: Performed once prior to study initiation and on SD0, 8, 28 and 84 (weeks 1, 5, and 13) pre-dose and 1 and 4 hours post-dosing. Exams included ECG, systolic, diastolic and mean arterial BP and heart rate (HR).

- No significant, dose-related effects were observed on HR. Small decreases in HR noted in one low-dose female were below the historical control values. For this animal, the decreased HR was correlated with a decreased arterial BP. This was not considered a test article effect because only one low dose animal was affected and that animal showed a similar, slightly decreased HR prior to study initiation.
- No significant effects attributed to the test article were observed on heart rhythm or QT intervals. Small decreases or increases in QT interval were noted sporadically and were generally limited to less than 30 ms. Three animals in the low dose group showed increases in QT interval of +31 to +35 ms. These effects were not seen in the higher dose groups. Therefore, they were not considered to be of biological significance.

Clinical pathology: Hematology, coagulation, clinical chemistry and urinalysis parameters were assessed prior to study initiation, and on SD0, 33, 89.

- No effects on hematology, clinical chemistry or urinalysis parameters attributable to test article were reported.

Necropsy: All animals were sacrificed at least one week after the final dose and full gross examination was conducted.

- Emaciation in 2 high dose female animals was the only gross finding and is related to NT201 exposure.

Organ weights: Selected organs were weighed:

- adrenal glands
- brain
- gastrocnemius muscles (left and right)
- heart
- kidneys
- liver
- ovaries
- pituitary gland
- spleen
- testes
- thymus
- thyroid glands
- uterus
 - Absolute organ weights were similar among all groups. Relative organ weights for brain, adrenals, liver, heart and kidney were higher in the high dose group. This was considered to be the result of the reduced body weight for that dose group.

Histopathology: A full panel of tissues was collected for microscopic analysis. Peer review was performed.

- No microscopic findings related to test article were reported with the exception of skeletal muscle.
- Routine samples of skeletal muscle showed irregular fibers with nuclear crowding in animals in the mid and high dose groups.
- Injection sites and contralateral muscle:
 - Injected calf muscle showed reduced mass for all treated animals on SD79.
 - Mean absolute and relative gastrocnemius muscle weights were dose-dependently lower in treated animals, and were apparently more pronounced in animals receiving test article NT201 (approximately 25%).
 - The mean absolute and relative weights of the contralateral gastrocnemius were larger in the low dose group relative to controls. For the mid and high dose groups, the contralateral muscle weights were lower relative to control in a dose-related trend. Animals given test article NT201 showed a slightly more pronounced effect relative to animals receiving BOTOX.
 - A dose-related increase in diffuse muscle atrophy (rated slight to moderate for the low and mid dose groups, moderate to marked for the high dose groups), nuclear crowding, irregular fiber size and isolated degenerating muscle fibers with inflammatory cell infiltration and slightly increased fibrosis were observed at the site of injection. These effects are expected results of the biological activity of botulinum toxin.
 - For the low dose group, the contralateral gastrocnemius muscle was heavier in the treated animals relative to control. For the mid and high dose groups, the contralateral muscle was smaller relative to control. These findings were considered by the sponsor to be due to weight loss or

secondary to locomotor patterns after the induced weakness in the injected side. However, the findings are consistent with results of toxin pharmacological activity and may be the result of systemic spread of the toxin.

Conclusions:

Clinical signs related to the test article were observed in all dose groups beginning, at least, by the second dose and increasing in incidence, time of onset and severity with increasing dose levels. All effects observed were the expected result of the pharmacological activity of NT201. Female animals appeared to be more affected than males.

Due to the limping and weakness of the injected muscle at all dose levels, no NOEL was established. The NOAEL level was determined to be 4 U/kg due to loss of appetite and body weight in the 8 and 16 U/kg groups for NT201.

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Study #11125/1/98

Title: Determination of the biological activity of NT201 in the regional paralysis test in CD-1 mice

Testing Facility:

(b) (4)

GLP Compliance:

Yes

Date of study initiation:

3/9/1998

Lot number:

NT201 lot#), BOTOX lot# CGC028, DYSPORT lot# 133 F, 142K

Vehicle/formulation:

Sterile physiological saline with 1 mg/ml HSA

Methods:

Animals: male CD-1 mice, approximately 8 weeks old, 5 dose groups, 10 mice per group

Doses: NT201: 0.26 to 0.64 U/animal,
BOTOX: 0.26 to 0.64U/animal,
DYSPORT: 0.64 to 1.56 U/animal

The dose levels for each test article were chosen based on results of a preliminary study that demonstrated a slightly lower potency for DYSPORT relative to NT201 and BOTOX.

Route of administration: Intramuscular injection into the gastrocnemius muscle at a volume of 2 X 0.05ml

Regimen: 3 injections total at intervals of 6 weeks. The second and third doses were administered one week after disappearance of paralysis in all groups.

The tables below, taken from the study report, summarize the basic design of this study.

Number of animals per test substance and dose	NT-201	BOTOX®	DYSPORT®
	Units / animal		
10 ♂	0.262	0.262	0.640
10 ♂	0.328	0.328	0.800
10 ♂	0.410	0.410	1.000
10 ♂	0.512	0.512	1.250
10 ♂	0.641	0.641	1.562

Administration route		
i.m.		
Group	Dose level in units/animal (factor 1.25 increase)	Animal number
NT-201		
1	0.262	1 - 10
2	0.328	11 - 20
3	0.410	21 - 30
4	0.512	31 - 40
5	0.641	41 - 50
BOTOX®		
6	0.262	51 - 60
7	0.328	61 - 70
8	0.410	71 - 80
9	0.512	81 - 90
10	0.641	91 - 100
DYSPORE®		
11	0.640	101 - 110
12	0.800	111 - 120
13	1.000	121 - 130
14	1.250	131 - 140
15	1.562	141 - 150

Observations and results:

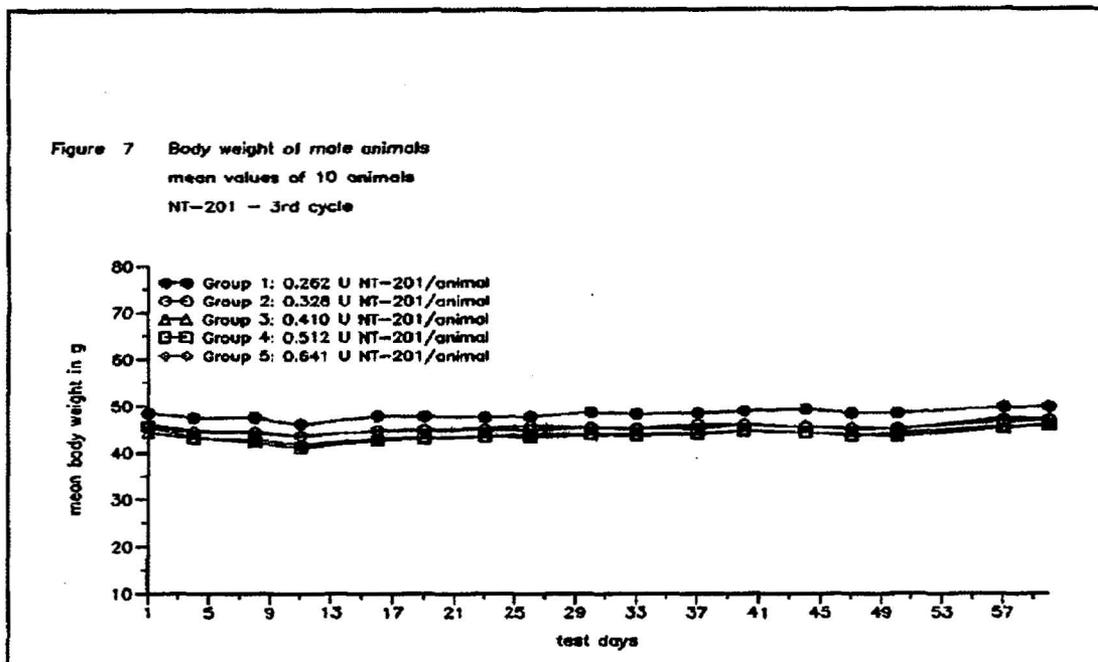
Mortality: Observations were recorded twice daily.

- Three animals were found dead during the treatment cycles. One animal from group 4 (NT201, second highest of 5 doses) was found dead on SD37 of the second cycle. One mouse from group 6 (BOTOX, low dose) was found dead on SD50 of the third cycle and one mouse from group 15 (DYSPORE, high dose)

was found dead on SD23 of the third cycle. The sponsor states that the deaths were not treatment related. However, no cause of death was reported.

Body weight: Data were recorded prior to study initiation and twice weekly thereafter.

- Dose-related weight loss was noted for all three products. For NT201, weight loss of small magnitude (10%) was noted for the two highest dose groups. For BOTOX, only the high dose group showed significant body weight loss (15%). With DYSPORT, a small weight loss (15%) was noted for groups 12-15, with the two highest dose groups showing statistically significant declines on SD12. The following graph taken from the study report illustrates the body weight loss seen during the third cycle for NT201 and BOTOX:



Paralysis: Paralysis was scored on scale of 1+ to 3+ minimal, slight or severe, respectively.

- The degree and duration of paralysis were dose-dependent for all three products.
- Severity and duration of paralysis were greater after the second injection and again after the third injection for all three products.
- The time of paralysis onset was faster with each subsequent injection for all products. The time for maximal effect for each preparation appeared to be similar after the first injection, but varied more with later injections.

Muscular atrophy: Change in diameter of the treated gastrocnemius muscle was compared to the contralateral, control gastrocnemius muscle with a micrometer screw gauge immediately prior to each injection.

- Atrophy of the injected muscle was noted over time for all test preparations, as demonstrated by leg circumference. The effects of the three products were

similar with measurable atrophy observed after the second injection, and achieved statistical significance after the third injection.

Histology of muscle fibers: Animals were sacrificed at the end of the 3rd injection/observation cycle. The gastrocnemius muscle from each side was removed from each animal from the high dose group for each product. Samples from 6 of 10 animals were processed for microscopic examination. Samples from the remaining 4 animals were processed for EM examination, as necessary.

The following table taken from the study report contains a summary of the animals in which the muscle histology was examined:

Group	Test substance / Dose level	Animal No.
5	NT-201 0.641 units/animal	41 - 46 ♂
10	BOTOX® 0.641 units/animal	91 - 96 ♂
15	DYSPORT® 1.562 units/animal	141, 143 - 146 ♂

- Atrophy of muscle fibers and regeneration of muscle fibers was observed with similar severity in the treated muscle of all treatment groups.

Conclusions:

No control group was included in this study. The effects of NT201 were compared to a pre-determined “physiological state of hindlimb usage”.

Under the conditions of this study, repeated intramuscular injection of NT201 in mice produced no remarkable clinical signs other than local paralysis. One death in group 4 occurred on day 37 after the second injection. No cause of death was determined, but was not thought to be related to test article. Transient, dose-dependent reductions in mean body weight after each injection were observed, starting 3 days after the first injection. The time to onset of paralysis was not dose-related, but the degree and duration of paralysis, based on paralysis score, were dose-dependent. The effects of the toxin appeared to be more severe after the second and third injections. At necropsy, leg diameter was significantly reduced at all doses. Upon histological examination of the target muscle at a dose of 0.64 LDU/animal, signs of local atrophy and regeneration of the muscle cells were noted. These changes were accompanied by changes in the diameter of the muscle fibers, and sparse local lympho-histiocytic interstitial infiltration. Given that mean body weight was transiently reduced at all doses of NT201, no NOAEL was established under the conditions of this study.

The usefulness of the results of this study for assessment of general toxicity is questionable for the following reasons:

- Only male animals were evaluated.
- The dose groups did not contain sufficient animal numbers.

- A very limited number of endpoints were assessed.

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Study no.: 422/017

Title: NT201 – Dose-ranging study by the intramuscular route in the non-pregnant rabbit

Testing Facility:	(b) (4)
Date of study initiation:	10/22/2003
GLP compliance:	No
QA statement:	No statement included
Drug, lot #, and % purity:	020902/considered to be 100% pure

Methods:

Doses: 2.5, 3.5, 5, 10, 20 or 40 U/kg

Species/strain: New Zealand White rabbits, female only

Number/sex/group or time point (main study): 3/group

Route: Intramuscular injection once every 14 days (maximum of 3 doses)

Formulation/vehicle: 0.9% sterile saline

Dosing solution analyses/drug stability and homogeneity: Stable at +4°C on ice.

Other analyses were not conducted for this pilot study.

Dose volume/infusion rate: Test article was administered as a bolus injection in a volume of 0.2 ml/kg. The dose was split into 2 equal portions and injected into 2 sites in the left lumbar muscle of each animal.

Satellite groups used for toxicokinetics or recovery: N/A

Age: 17 to 20 weeks old

Weight: 2.7 to 3.3 kg

Unique study design or methodology: The basic study design is summarized in the table below, provided by the sponsor.

Group number	Group description	Dose level ⁽¹⁾ (U/kg/adm.)	Dose volume (ml/kg/adm.)	Dose concentration ⁽¹⁾ (U/ml)
1	Dose 1	2.5	0.2	12.5
2	Dose 2	5	0.2	25
3	Dose 3	10	0.2	50
4	Dose 4	20	0.2	100
5	Dose 5	40	0.2	200
6	Dose 6	3.5	0.2	17.5

adm: administration.

⁽¹⁾ 1 Unit (U) corresponds to the LD 50 in the mouse (of about 20 g) by the intraperitoneal route.

Observations/Results:

Mortality: Checked twice daily

- All three rabbits that received 40 U/kg died or were sacrificed moribund on the third day after the first injection.
- 2 rabbits in the 20 U/kg group were found dead on the morning of the 5th day after the first dose and the third rabbit in this group was sacrificed moribund on the same day.
- One rabbit in the 10 U/kg group was sacrificed moribund on the 7th day after the first dose. The remaining 2 animals in this dose group were sacrificed moribund 3 days after the second dose.
- One animal in the 5 U/kg group was sacrificed moribund 8 days after the 2nd dose. The remaining 2 animals were sacrificed moribund 5 days later (13 days after the second dose).
- No mortality was observed in groups that received doses of 3.5 U/kg or less.

Clinical signs: Observations were recorded daily. On dosing days, all animals were observed prior to dosing as well as 1 and 4 hours after dosing.

- Clinical signs that preceded death included stiff movements or reduced muscle tone, stiff hindlimbs, difficult movements, unsteady gait, slow or difficult breathing.
- The only dose-related clinical observation made in animals that received 3.5 U/kg was a single rabbit that showed stiff movements of the hindlimbs on one day, 5 days after the second dose. No clinical signs related to the test article were observed in the lowest dose group.
- No marked injection site reactions were noted for any dose group. Occasional erythema that persisted for 24 to 48 hours was noted in single animals from the 5, 10 or 40 U/kg groups. Sporadic injection site bruising was noted in several animals from multiple groups as well as injection site edema in one rabbit in the high dose group. No dose relationship was apparent for these injection site observations.

Body weights: Data recorded twice weekly starting one week prior to the first injection.

- All animals in the 40 and 20 U/kg groups underwent severe body weight loss (at least 140 g) during the 3 days prior to death.
- The animals in the 10 U/kg group showed a slight body weight loss over 3 days following the first injection. After the second dose, the 2 remaining rabbits in this group showed a severe weight loss (at least 240 g) over the 4 days post injection.
- Rabbits in the 5 U/kg group showed continuous weight loss throughout the study beginning the 3rd day after the first dose and becoming severe after the second dose.

Food consumption: Measurements were made daily.

Ophthalmoscopy: N/A

ECG: N/A

Hematology: N/A

Clinical chemistry: N/A

Urinalysis: N/A

Gross pathology: Animals found dead or sacrificed moribund were necropsied to determine cause of death, where possible. The surviving animals in groups 1 to 5 were sacrificed 9 days after the third dose. Animals in group 6 were sacrificed 5 days after the 2nd dose.

Organ weights: N/A

Histopathology: No histopathology examinations were conducted for this pilot study.

Toxicokinetics: N/A

Conclusions:

This study was intended to be a pilot study for testing of dose levels to be used in a future embryofetal development study. All dose-related effects noted in this study were expected results of the known pharmacological activity of NT201. No control group was included in the study design. No clinical pathology parameters or histopathology examination was included. The NOAEL under the conditions of this study is 2.5 U/kg.



Study no.: AA41667

Title: NT201: 9-month toxicity study with repeated intramuscular administrations in the cynomolgus monkey followed by a 6-month treatment-free period

Testing Facility:

(b) (4)

(b) (4)

Date of study initiation:

6/19/2007

GLP compliance:

Yes

QA statement:

Yes

Lot #:

60707

Methods:

Doses: 0, 4, 8, 12 U/kg/injection

Species/strain: cynomolgus monkey

Number/sex/group (main study): 4

Route: intramuscular injections, once Q 4 weeks for a total of 10 doses

Formulation/vehicle: Sterile 0.9% NaCl containing 1 mg/ml human serum albumin

Dose volume/infusion rate: Bolus injection of 0.2 ml/kg divided between two sites

Satellite groups used for toxicokinetics or recovery: 2/sex/group (control and high dose)

Age: 26 to 30 months old

Weight: Males, 2.2 to 2.6 kg; females, 1.9 to 2.5 kg

Study design: The study design is summarized in the table below, provided by the sponsor.

Group/Treatment	Dose level (U/kg/adm.)	Dose concentration (U/mL)	Number of animals for necropsy at:			
			Terminal kill ⁽¹⁾		Recovery ⁽²⁾	
			Males	Females	Males	Females
1. Control	0	0	4	4	2	2
2. Low dose	4	20	4	4	/	/
3. Intermediate dose	8	40	4	4	/	/
4. High dose	12	60	4	4	2	2

adm.: administration.

/: not applicable.

Dose volume: 0.2 mL/kg/administration.

1 Unit (U) corresponds to the LD 50 in the mouse (of approximately 20 grams) by the intraperitoneal route.

⁽¹⁾: killed 7 or 8 days after the last administration (week 38).

⁽²⁾: killed 6 months after the last administration (week 62).

Observations/Results:

Mortality: Observations were made at least twice daily. No treatment-related mortality was observed.

- One male from group 3 was euthanized early (SD176) due to signs of inflammation and/or infection in the untreated leg. The origin of the infection/inflammation was not determined.

Clinical signs: Observations were recorded daily. Detailed physical examinations were performed once pre-test, weekly during the first 4 study weeks, then monthly for the remaining treatment period and during the recovery period.

- The only test article-related clinical sign was limping associated with the left hindlimb weakness in 2 group 3 males on SD 196.
- The injected muscle (left gastrocnemius) showed atrophy in all treated animals after the third injection on SD 56. Other injection site reactions (tumefaction, hemorrhagic perivenous infiltration, slight hematoma, and/or redness) were observed in all groups including control.

Body weights: Recorded weekly during the pre-test, treatment and recovery periods.

- Statistically significant reductions in body weight gain were observed for females in the low dose group and for both genders in the middle and high dose groups, relative to control.
- A dose-related lower mean terminal body weight was observed in all treated animals relative to control. The difference was statistically significant for the high dose group.

The body weight changes among groups are summarized in the table below, provided by the sponsor.

Group	1		2		3		4	
Dose level (U/kg/adm.)	0		4		8		12	
Sex	M	F	M	F	M	F	M	F
Mean body weight gain (in grams) from days -1 to 258	293	340	308	230*	103 ⁽¹⁾ *	185**	93*	13**
Mean % gain from days -1 to 258	12.14	16.17	12.10	10.47	4.41	8.68	3.86	0.71
Mean body weight gain (in grams) from days 258 to 426	205	375	NA	NA	NA	NA	945	590

NA: not applicable

adm.: administration.

⁽¹⁾: Mean value calculation does not include the premature sacrificed male no. 2324.

* or **: statistically significant differences when compared with control ($p \leq 0.05$ or 0.01 , respectively).

- The effects on body weight were apparently reversed during the recovery period. Body weight gain for the group 4 animals was higher during the recovery period relative to control animals.

Food consumption: Estimated daily.

- No consistent adverse effects on food consumption related to NT201 treatment were observed. Estimations may not have been adequate to reveal small changes in feed intake.

Ophthalmoscopy: Performed once pre-test, during weeks 13, 25 and 37 and at the end of the recovery period (week 62)

- No test article-related effects were observed.

ECG: Performed once pre-test, on SD0 (Day of first dose), then during weeks 13, 25, 37 (before dose administration and 1 and 4 hours after dosing) and at the end of the recovery period.

- No effects attributable to test article exposure were observed.

Blood pressure and heart rate: Measured at the time of ECG testing.

- No consistent effects on blood pressure attributable to NT201 exposure were reported.

Hematology: (Fasted) Blood samples for clinical pathology analyses were performed once pre-test, during weeks 5, 13, 25 and 37 (6 days after administration) and at the end of the recovery period.

- Increased mean neutrophils counts were observed in group 3 and 4 males and all treated female groups relative to control values and group mean baseline values. The effect peaked between weeks 5 and 13. The changes are summarized in the table below, taken from the study report.
- No differences in neutrophil counts were noted at the end of the recovery period.

Mean neutrophil counts (Giga/L) and difference (% in brackets) when compared with controls								
Group	1		2		3		4	
Dose level	0 U/kg/adm.		4 U/kg/adm.		8 U/kg/adm.		12 U/kg/adm.	
Sex	M	F	M	F	M	F	M	F
Pretest	4.908	3.315	3.800 (-23)	5.070 (+53)	4.035 (-18)	4.973 (+50)	5.922 (+21)	5.337 (+61)
Week 5	3.365	4.733	4.720 (+40)	10.085 (+113)	7.755** (+130)	9.493 (+100)	7.682** (+128)	7.760 (+64)
Week 13	4.128	4.117	3.185 (-23)	5.598 (+36)	5.143 (+25)	7.773 (+89)	6.330* (+53)	10.893** (+165)
Week 25	3.768	5.012	2.703 (-28)	7.903 (+58)	6.063 (+61)	7.473 (+49)	5.757 (+53)	7.370 (+47)
Week 37	4.212	6.232	3.728 (-11)	6.140 (-1)	9.480 (+125)	7.630 (+22)	6.675 (+58)	9.423 (+51)

adm.: administration.

- The sponsor considers the changes in neutrophil counts to be transient and not biologically significant. However, although transient and inconsistent relative to control values, the effect appears to have a dose relationship particularly for groups 3 and 4.
- Decreased mean lymphocyte counts were observed for all treated groups during week 5 and in groups 3 and 4 during weeks 13, 25 and 37, relative to control and/or baseline values.
- There was no difference in lymphocyte counts at the end of the recovery period, suggesting that there may have been a treatment-related effect.

The mean lymphocyte counts are summarized in the table below, taken from the study report.

Mean lymphocyte counts (Giga/L) and difference (% in brackets) when compared with controls								
Group	1		2		3		4	
Dose level	0 U/kg/adm.		4 U/kg/adm.		8 U/kg/adm.		12 U/kg/adm.	
Sex	M	F	M	F	M	F	M	F
Pretest	5.965	4.658	4.718 (-21)	4.473 (-4)	4.898 (-18)	3.918 (-16)	4.978 (-17)	3.213* (-31)
Week 5	5.340	4.553	3.473** (-35)	3.083* (-32)	3.453* (-35)	2.903* (-36)	3.975 (-26)	2.430** (-47)
Week 13	6.777	5.000	5.693 (-16)	5.458 (+9)	5.678 (-16)	3.508 (-30)	5.263* (-22)	2.733** (-45)
Week 25	5.433	3.332	5.100 (-6)	4.290 (+29)	3.838 (-29)	2.678 (-20)	3.960 (-27)	2.240* (-33)
Week 37	8.172	5.150	6.080* (-26)	5.653 (+10)	5.770* (-29)	2.745** (-47)	4.100** (-50)	2.173** (-58)

adm.: administration.

* or **: statistically significant differences when compared with control ($p \leq 0.05$ or 0.01 , respectively).

- A transient but dose-related reduction in mean eosinophil counts relative to control and baseline was observed in groups 3 and 4. The sponsor does not consider the changes dose-related. However, the table below, taken from the study report, demonstrates a greater magnitude of reduction from groups 3 and 4 particularly in week 37. A dose relationship can not be ruled out. The reduction in mean eosinophil counts appeared to return to baseline levels by the end of the recovery period; group 4 mean eosinophils count at SD430 was 0.155 for males and 0.365 for females. Due to the small number of animals used for both the main study and recovery period, the biological significance is difficult to judge given the variability of the data.

Mean eosinophil counts (Giga/L) and difference (% in brackets) when compared with controls								
Group	1		2		3		4	
Dose level	0 U/kg/adm.		4 U/kg/adm.		8 U/kg/adm.		12 U/kg/adm.	
Sex	M	F	M	F	M	F	M	F
Pretest	0.402	0.198	0.355 (-12)	0.148 (-25)	0.303 (-25)	0.240 (+21)	0.305 (-24)	0.283 (+43)
Week 5	0.275	0.260	0.318 (+16)	0.133 (-49)	0.198 (-28)	0.160 (-38)	0.292 (+6)	0.145 (-44)
Week 13	0.415	0.308	0.345 (-17)	0.260 (-16)	0.433 (+4)	0.183 (-41)	0.362 (-13)	0.203 (-34)
Week 25	0.218	0.093	0.185 (-15)	0.140 (+50)	0.180 (-17)	0.073 (-22)	0.130 (-40)	0.085 (-9)
Week 37	0.358	0.318	0.323 (-10)	0.220 (-31)	0.150 (-58)	0.165 (-48)	0.150 (-58)	0.090* (-72)

adm.: administration.

*: statistically significant difference when compared with control ($p \leq 0.05$).

This difference was not noted at the end of the treatment-free period.

There were no changes amongst the other parameters.

Clinical chemistry: (Fasted) See above for sampling scheme.

- Group 4 animals showed increased total bilirubin relative to control. However, the difference relative to control was apparent in baseline values prior to administration of NT201. One male in group 4 (2334) showed high bilirubin levels consistently throughout the study (up to 14.7 $\mu\text{mol/L}$), including at baseline and at the end of the recovery period (13.9 $\mu\text{mol/L}$). The increased values for group 4 relative to control were maintained over the course of the study. However, increases in bilirubin levels relative to baseline were noted for all groups. Because the pattern of change was observed in the control group as well as treated groups and the magnitude of change was similar among groups, a relationship to NT201 is questionable. The bilirubin data for the main study are summarized in the table below, taken from the study report.

Mean total bilirubin serum concentration (mcmol/L) and difference (% in brackets) when compared with controls								
Group	1		2		3		4	
Dose level	0 U/kg/adm.		4 U/kg/adm.		8 U/kg/adm.		12 U/kg/adm.	
Sex	M	F	M	F	M	F	M	F
Pretest	2.68	5.48	3.45 (+29)	2.55* (-53)	2.88 (+7)	2.80* (-49)	5.12* (+91)	3.97* (-28)
Week 5	3.63	4.57	4.20 (+16)	3.70 (-19)	4.20 (+16)	5.20 (+14)	6.43** (+77)	7.45* (+63)
Week 13	4.07	4.90	4.38 (+8)	5.05 (+3)	/	4.30 (-12)	7.92** (+9)	8.63** (+76)
Week 25	4.70	6.72	4.53 (-4)	5.25 (-22)	6.13 (+30)	6.43 (-4)	7.70 (+64)	8.18 (+22)
Week 37	4.40	7.48	4.93 (+12)	5.00 (-33)	4.07 (-8)	6.13 (-18)	7.92* (+80)	8.27 (+11)

adm.: administration.

/: no difference

* or **: statistically significant differences when compared with control ($p \leq 0.05$ or 0.01 , respectively).

- A decrease in mean serum glucose concentration was noted for all treated groups in weeks 5, 13, 25 and 37, but not at the end of the recovery period. The sponsor considers this effect as incidental due to the small magnitude.

Urinalysis: (Fasted) Samples were collected on the same schedule as blood sample collection.

- Slightly lower pH was observed for group 4 females on SD90 relative to control that persisted through the recovery period. No other changes in urinary parameters were observed. Because the pH change was isolated to females in one dose group and was of only slight magnitude and was not associated with other signs, this finding was considered incidental.

Gross pathology: Necropsies were performed 7 or 8 days after the final dose for the main study animals and 6 months after the final dose for the recovery animals.

- One male (8U/kg, #2324) was euthanized early (SD176). Gross findings included a lesion on the right (untreated) leg with epidermal ulceration and chronic inflammation of the superficial dermis. The macroscopic findings for this animal also included multiple pale spots on the liver, increased size of iliolumbar and deep inguinal lymph nodes on the right, and increased spleen size. No microscopic correlates were noted for the pale spot on the liver. The skin lesion was correlated with signs of focally extensive inflammation and the enlarged lymph nodes showed increased numbers of germinal centers.
- Enlarged popliteal lymph nodes were observed in several animals in all treated groups. This correlated with increased number and size of lymphoid follicles.

Organ weights:

- Lower weights of the left gastrocnemius (treated) muscle were noted for all treated groups relative to control. This finding is an expected result of NT201 injection into the muscle.
- A dose-related reduced mean absolute and relative weight was noted for the right (untreated) gastrocnemius muscle in treated females. This effect was statistically significant in the high dose females. No change was noted in male animals. This effect could indicate systemic exposure to NT201. The sponsor suggests that the decreased muscle weight is related to the decreased body weight observed in these animals. They also suggest that the reduced muscle weight in the females was secondary to the marked decrease in the weight of the left gastrocnemius muscle (disuse atrophy). The logic of this rationale is not clear. Paresis of one leg would necessitate transfer of additional weight bearing to the contralateral side in order to maintain function. This increased load on the contralateral side would result in an increase in strength and muscle mass rather than disuse atrophy. The loss in muscle weight is summarized in the table below, taken from the study report.

Terminal body weight and organ weights - mean % difference from control group

Group Treatment (U/kg/adm)	2		3		4	
	4		8		12	
Sex	Male	Female	Male	Female	Male	Female
Number of values	4	4	3	4	4	4
Terminal body weight	-3.39	-2.50	-14.48	-5.61	-12.26	-14.26
Left gastrocnemius muscle						
- Absolute	-52.70	-65.90	-63.85	-65.50	-55.18	-63.97
- Relative to BW (%)	-49.68	-65.02	-57.91	-63.66	-49.05	-58.29
Right gastrocnemius muscle						
- Absolute	+10.44	-11.37	-13.19	-15.66	-12.41	-27.16
- Relative to BW (%)	+16.80	-8.90	+1.18	-10.81	-0.37	-15.18

BW: terminal body weight

- A lower mean absolute and relative heart weight was observed for all treated groups of females and high dose males relative to controls. No microscopic correlate was noted. The table below summarizes the mean absolute heart weight for males and females of each group.

Gender	Group 1 (Control)	Group 2	Group 3	Group 4
Male	10.9 ± 0.382	10.500 ± 2.363	9.636 ± 0.599	9.299 ± 1.212
Female	9.649 ± 0.765	8.462 ± 0.645	8.892 ± 0.371	7.643 ± 0.666

The table below summarizes the mean relative heart weight data for males and females of each group. The magnitude of the heart weight reductions relative to total body weight was smaller than that of the absolute heart weight. This may indicate that the reduction in total body weight may have contributed to the changes in heart weight.

Gender	Group 1	Group 2	Group3	Group 4
Male	0.39745 ± 0.023	0.39833 ± 0.040	0.40961 ± 0.017	0.38600 ± 0.048
Female	0.38047 ± 0.029	0.34045 ± 0.015	0.37208 ± 0.026	0.35089 ± 0.018

- Higher mean absolute and relative brain weight was observed in high dose males. No microscopic correlate was noted.
- Recovery: The reduced weight of the treated gastrocnemius muscle persisted through the recovery period. The contralateral, untreated gastrocnemius muscle showed increased weight relative to control at the end of the recovery period. The increased weight of the contralateral untreated muscle observed while the atrophy persisted in the treated muscle is not consistent with the sponsor's stated hypothesis regarding the atrophy and reduced weight of the untreated muscle during the dosing period. If the atrophy observed in the untreated muscle was due to disuse atrophy in response to the weakness in the contralateral treated muscle, then the disuse atrophy of the untreated muscles would have persisted as long as the weakness persisted on the treated side. Therefore, it is more likely that the atrophy consistently noted in the contralateral untreated gastrocnemius muscle is due to systemic spread of the toxin. The terminal body weights and gastrocnemius muscle weights at the end of the recovery period are summarized in the table below, taken from the study report. No differences in heart weight were observed at the end of the recovery period (n=4).

Terminal body weight and muscle weights - mean % difference from control group

Group	4	
Treatment (U/kg/adm)	12	
Sex	Male	Female
Number of values	2	2
Terminal body weight	+26.25	-2.95
Left gastrocnemius muscle		
- Absolute	-46.99	-46.74
- Relative to BW (%)	-56.62	-45.12
Right gastrocnemius muscle		
- Absolute	+24.94	+4.06
- Relative to BW (%)	+1.51	+5.98

BW: terminal body weight

Histopathology:

Adequate Battery: Yes.

Peer review: not stated

- In general, the microscopic findings were expected results of the biological activity of NT201 including: decreased myofiber size of the left gastrocnemius muscle, increased eosinophilia in the injected muscle, increased endomysial and perimysial collagen in the injected muscle at the high dose, increased adipose tissue in the injected muscle. These findings were dose-related, increased in severity with dose and were observed at all dose levels.
- Reduced myofiber size and variability of myofiber size, increased adipose tissue, increased endomysial and perimysial collagen were also observed in the contralateral, untreated gastrocnemius muscle in groups 3 and 4. These findings, although similar to the effects noted in the injected gastrocnemius muscle, were of lower severity.
- A dose-related decreased trabecular bone density of the femur was observed in some treated animals with decreased myofiber size in the soleus muscle (2 of 4 males and 2 of 4 females in the high dose group, 4 of 4 females in the mid dose group, and 1 of 4 males in the low dose group). No mention was made of the side from which the bone samples were obtained, but the findings are consistent with spread of the toxin to adjacent muscles and general weakening of skeletal muscles. The data are summarized in the table below, taken from the study report. There was a clear dose response for these findings in the contralateral gastrocnemius muscle and other skeletal muscle sampled. The sponsor considers these effects to be secondary and not directly related to NT201

exposure. However, because of the dose related nature of the findings, these effects may be indicative of system spread of the toxin.

Treatment-related microscopic findings – right gastrocnemius muscle and skeletal muscle

Group Treatment (U/kg/adm)	1		2		3		4	
	0		4		8		12	
Sex	M	F	M	F	M	F	M	F
No. of animals examined	4	4	4	4	4	4	4	4
right gastrocnemius muscle								
- decreased myofibre size	-	-	-	-	2	1	4	3
Minimal	-	-	-	-	1	1	3	1
Slight	-	-	-	-	-	-	1	2
Moderate	-	-	-	-	1	-	-	-
- variability of myofibre size	-	-	2	4	3	4	4	4
Minimal	-	-	2	4	2	3	2	1
Slight	-	-	-	-	1	1	2	3
skeletal muscle								
- decreased myofibre size	-	-	1	-	2	-	1	1
Minimal	-	-	1	-	2	-	1	1
- variability of myofibre size	-	-	2	1	3	3	4	4
Minimal	-	-	2	1	3	3	4	4

M: male/F: female

-: Observation not recorded in group

- Increased development of splenic white pulp and increased development of lymphoid follicles in most sampled lymph nodes, indicates immune activation and may be explained by development of anti-drug antibody response.
- Recovery: Decreased myofiber size of the injected muscle with moderate to marked increase of endomysial/perimysial collagen (fibrosis) and minimal to slight increase in adipose tissue was observed for all treated animals. Special staining for nestin indicated that the neuromuscular junctions had not returned to normal. Contralateral gastrocnemius muscles did not show significant effects on myofiber size relative to control and no differences in trabecular bone density were observed in treated animals relative to controls.

Immunology: Blood samples were collected once pre-test, during weeks 25 and 37 and at the end of the recovery period; however, results are not included in the study report.

Toxicokinetics: As is true of all botulinum toxin products, the systemic concentration of NT201 is below the level of detection of currently available assays. Therefore, toxicokinetic analyses were not conducted.

Conclusions:

In general, the dose-related effects observed in this study were the expected result of the known pharmacological activity of NT201. No NOAEL was established due to the reduction in weight gain and reduced mean terminal body weight (relative to control) that were observed in all treated groups. In addition, signs consistent with systemic spread of the toxin were observed in groups 3 and 4, including the following:

- Reduced weight and microscopic signs of atrophy of contralateral gastrocnemius muscle and dose-related lower heart weight in groups 3 and 4.
- Decreased myofiber size in the soleus muscle (adjacent to treated muscle) as well as decreased and/or variable myofiber size of the skeletal muscle samples (no indication of which muscle was examined).

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Study no.: AA62151

Title: NT201- Dose range-finding post-weaning juvenile toxicity study in the rat.

Testing Facility:

(b) (4)

Date of study initiation:

Not given

GLP compliance:

No

Lot #:

Not given

Methods

Doses: 0, 10, 20, or 30 U/kg

Species/strain: rat strain not given

Number/sex/group or time point (main study): 5/sex/group

Route: Intramuscular injection (gluteus muscle for groups 1-5, gastrocnemius for group 6)

Weight: not given

Study design:

Groups 1-4 received intramuscular injections of NT201 every 14 days, group 5 was injected every 7 days and group 6 received injections into the gastrocnemius muscle every 14 days. (See the table below, taken from the study report.)

Animals received doses from the age of weaning (3 weeks) up to 8 weeks of age.

Group	Frequency of administration	Injected muscle	Dose level (LDU/kg/adm ⁽¹⁾)	Dose volume (mL/kg)	Dose concentration (LDU/mL ⁽¹⁾)	Number of animals	
						Males	Females
1. Control	Every 14 days	Gluteus	0	0.1	0	5	5
2.	Every 14 days	Gluteus	10	0.1	100	5	5
3.	Every 14 days	Gluteus	20	0.1	200	5	5
4.	Every 14 days	Gluteus	30	0.1	300	5	5
5.	Every 7 days	Gluteus	10	0.1	100	5	5
6.	Every 14 days	Gastrocnemius	20	0.1	200	5	5

adm: administration.

⁽¹⁾ 1 unit (LDU) corresponds to the LD50 in the mouse by the intraperitoneal route.

Group 1 animals received the vehicle (Sterile 0.9 % NaCl containing 1 mg/mL Human Serum Albumin (HSA)).

Observations/Results:

Mortality: Checks were conducted daily. No unscheduled deaths occurred.

Clinical signs: Observations were recorded daily, full clinical examination was conducted weekly.

- Shrinkage of the injected muscle was observed from SD14 on for all treated animals.
- Paresis of the left hind limb was observed on SD34 for 6 of 10 animals that received injections into the left gastrocnemius muscle.

Body weights: Body weights were recorded twice weekly.

- Reduced mean terminal body weight relative to control was observed for females (-19%) and males (-22%) in the 30 U/kg group (group 2). A reduction of 7-15% relative to control was observed for all other treated animals, with the exception of group 5 (10 U/kg every 14 days). In group 5, no difference from control was observed.

Food consumption: Data recorded weekly per cage. A dose-related reduction in mean food consumption was observed for all treated animals. The greatest severity was noted in group 4 (30 U/kg). The mean food consumption for group 4 was 19 to 28% lower than control values.

Hematology: No treatment-related effects were noted.

Clinical chemistry: No treatment-related effects were noted.

Developmental parameters: No effects attributable to test article were noted on the timing of vaginal opening or balano preputial separation.

Gross pathology: Euthanasia and necropsy were conducted on SD35 for males and SD36 for females. The injected muscle (left gastrocnemius or gluteus

muscle) was reported as small relative to control upon gross examination at necropsy.

Organ weights: Absolute and relative weights of the injected left gastrocnemius or gluteus muscles were statistically lower relative to control, as were the contralateral (untreated) muscles in all treated groups.

Histopathology: Not performed.

Conclusions:

This study was conducted as a non-GLP pilot study to determine doses to be used in a future pivotal juvenile toxicology study. The effects of NT201 were expected results of the pharmacological activity of the toxin. A dose-related reduction in size of the injected muscles was noted in all treated groups relative to control. Mean terminal body weights were reduced relative to controls, correlating with a reduction in food consumption. No other effects attributable to NT201 were reported. Microscopic examination of tissues was not conducted. No effects were observed on the limited developmental parameters examined. Systemic exposure is indicated by the reduced body weight relative to controls, as well as the reduced size of the contralateral gastrocnemius or gluteus muscles.

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7. Genetic toxicology

No genetic toxicology studies were conducted for this product.

8. Carcinogenicity

No carcinogenicity studies were conducted for this product.

9. Reproductive and developmental toxicology

9.1 Fertility and early embryonic development

Study # (b) (4) 442/019

Title: Fertility study by the intramuscular route in the rabbit

Performing Laboratory:	(b) (4)
GLP Compliance:	Yes
Date of Study Initiation:	12/22/2003
Lot#:	020902
Vehicle:	0.9% sterile saline containing 1 mg/ml HSA

Methods:

Species: New Zealand White rabbits, 20/sex/group, 16 to 19 weeks old (males) or 16 to 17 weeks old (females)

Doses: 0, 1.25, 2.5 or 3.5 U/kg

Route/Regimen: Intramuscular injection at two-week intervals for a total of 5 doses for males; females received 3 intramuscular injections at two-week intervals. The second dose for both sexes was given on the day prior to the start of mating. The final injection was administered the day prior to necropsy. The drug was injected into the left dorsal muscle on the back of each animal.

Study design:

Cesarean sections were performed on day 14 of gestation. At necropsy, blood samples were taken for possible testing for development of anti-drug antibodies.

Doses were chosen based on results of a preliminary dose ranging study.

The table below, provided by the sponsor, summarizes the basic study design.

Group/ Treatment	Dose level (U [*] /kg/admin.)	Dose volume (ml/kg/day)	Dose concentration (U/ml)
1. Control	0	0.2	0
2. Low dose	1.25	0.2	6.25
3. Intermediate dose	2.50	0.2	12.50
4. High dose	3.50	0.2	17.50

Admin.: administration.

* 1 unit (U) corresponds to the LD50 in the mouse by the intraperitoneal route.

Observations/results:

Mortality: Animals were observed twice daily.

- One high dose male died 8 days after the second injection and a second high dose male was sacrificed moribund on SD10. Signs preceding death included marked decrease in food intake and weight loss (rated severe, greater than 25%). Necropsy did not reveal any treatment-specific effects.

Clinical observations: Observations were made at 1 and 4 hours after dosing. Local cutaneous observations were made at 1, 4, 24 and 48 hours after the first and second doses.

- Slight paralysis of the hindlimbs was noted for 1 high dose male beginning one week after the second injection and another from the high dose group beginning during the last week of the study. A single male from the 2.5 U/kg group showed similar slight paralysis during the last week of the study.
- Local tolerance: there were no observations indicating local irritation following injections at any dose. All groups showed occasional incidences of hematoma at the injection site in animals.

Body weight: For males, body weights were recorded twice weekly. For females, observations were made twice weekly during pre-mating and mating, then on days 0, 6, 9 and 13 of gestation.

- A dose-related reduction in body weight gain was noted for all treated male animals. The reduction was graded severe for the high dose group as this group showed an overall loss of 10% body weight during the course of the study.
- The male intermediate dose group showed an overall weight gain of zero over the course of the study. The low dose male group gained slightly less than control animals, but the difference was not statistically significant.
- A less severe effect on body weight gain was observed for females. The overall mean weight gain of the females over the 14-day pre-mating phase was 2.8, 0.9 and 0.6% in the 1.25, 2.5 and 3.5 U/kg groups compared with 5.6% in the control group. The mean gains of the pregnant females from mating to termination were 6.4, 3.6 and 1.6% in the 1.25, 2.5 and 3.5 U/kg groups compared with 8.9% in the control group. Statistical significance was achieved for all three groups during both phases of the study.

Food Consumption: Recorded twice weekly for males. For females, food intake was recorded twice weekly until mating, then on days 0, 6, 9 and 13 of gestation.

- Body weight effects in males were accompanied by a dose-related decrease in food consumption. The reductions were dose-related in severity, duration and time of onset. Food consumption for the low dose males did not appear to differ significantly from controls.
- Treated females showed a similar reduction in food intake relative to controls beginning in the second week. The differences became more marked during gestation, but were statistically significant for the intermediate and high dose groups only.

Mating performance and fertility:

- Failure to mate was observed in 1, 2, 3 and 2 males in the control, low, intermediate and high dose groups, respectively.
- One female in each of the low and intermediate dose groups failed to copulate. All females in the control and high dose groups mated successfully.
- Of the inseminated females, 1 control, 3 low dose, 1 intermediate dose and 3 high dose animals failed to become pregnant.
- Mean pre-coital interval was slightly longer in the treated groups relative to controls, but the majority of females mated within 4 days. Total pregnancies for each group were 19, 16, 18 and 17 for control, low, mid and high dose groups, respectively. A clear dose-relationship is not apparent.

The table below, taken from the study report, summarizes the mating performance data.

	Group 1 Control, 0 U/kg/admin.	Group 2 Low dose, 1.25 U/kg/admin.	Group 3 Intermed. dose, 2.50 U/kg/admin.	Group 4 High dose, 3.50 U/kg/admin.
NUMBER OF MALES:				
Paired	20	20	20	20
Copulated	19	18	17	18
Induced pregnancy	18	16	16	16
NUMBER OF FEMALES:				
Paired	20	20	20	20
Inseminated	20	19	19	20
Pregnant	19	16	18	17
PRE-COITAL INTERVAL - DAYS				
MEAN	1.60	1.89	1.95	1.95
S.D.	1.31	1.76	2.37	2.26
N	20	19	19	20
COPULATION INDEX (%)	100	95	95	100
FERTILITY INDEX (%)	95	84	95	85

Necropsy: For all animals, a full macroscopic exam was performed at necropsy. For females, ovaries and uteri were removed for examination of pregnancy status, number of corpora lutea, number of live embryos, number of uterine implantations, and the number of resorption sites. Placentae were also examined.

- Females: No treatment related lesions were reported for females except injection site hematoma for a few of the animals. Two of the affected females (one high dose and one mid dose) were not pregnant.
- Males:
 - Premature decedents were necropsied. One showed autolytic organ changes and fluid-filled GI tract, and the other a full stomach and empty intestinal tract. These findings could represent effects on gut motility by NT201. Such an effect would be consistent with blocking of parasympathetic neurotransmission.
 - Mean terminal body weights were lower in all dose groups relative to control.

Organ weights: testes, prostate gland and epididymides, and ovaries were weighed.

- No effects of test article on ovary weight were noted.
- Mean absolute organ weights of testes, epididymides and prostate gland were lower in all treated groups, but statistical significance was achieved for epididymides and prostate only in the high dose group.

Sperm analysis: Sperm counts were made from the left testis. Sperm motility evaluation was made using the left cauda epididymis.

- No effects on sperm count, ratio of motile sperm, or motility parameters relative to control were noted. A reduction in mean sperm count for the low dose group is considered incidental due to the lack of a dose relationship.

Histology: A limited number of tissues were fixed for microscopic examination including pituitary gland, vagina, uterus and ovaries of females with no implantations. For males, the right testis, right epididymis and left caput epididymis were examined microscopically.

- Microscopic examination of the injection sites showed minimal to moderate inflammatory cell infiltration, minimal to marked hemorrhage, and minimal to marked myonecrosis for all groups. Minimal to marked myofiber atrophy occurred only in treated animals.

Litter data:

- The numbers of corpora lutea, pre-implantation loss and the resulting numbers of uterine implantation sites were similar in the treated and control groups.
- The incidence of embryonic resorptions and post-implantation loss were very low (less than 2.5%) in all groups. Mean live litter size was similar in the treated and control groups.

Conclusions:

Results of the study showed a possible increase in failure to mate for the male animals (1, 2, 3, 2 for the control, low, intermediate and high dose groups, respectively). The data include the 2 high dose males who died early. (One male died 8 days after the second dose and the second death occurred 2 days later. Mating was initiated on the day after the second dose. Therefore, these males had 7 and 9 days to mate, respectively.) All other parameters that were analyzed showed no significant effects of test article exposure under the conditions of this study.

9.2 Embryonic Fetal development

Study no.: AA62003

Title: NT201-Dose range-finding study by the intramuscular route in the pregnant rat

Testing Facility:

(b) (4)

Date of study initiation:

9/21/2007

GLP compliance:

No

Lot #:

60506

Methods

Doses: 0, 3, 10 or 30 U/kg

Species/strain: pregnant Sprague/Dawley rats

Number/sex/group or time point (main study): 6/group at study initiation

Route: Intramuscular injection, left gastrocnemius muscle

Formulation/vehicle: 0.9% sterile NaCl containing 1 mg/ml human serum albumin

Dose volume: 0.1 ml/kg

Age: 10 to 13 weeks at mating

Weight: 217 to 239 g

Study design: Animals were injected on GD6, 12 and 19. Cesarean section was conducted on GD20 and litter parameters were recorded. The table below, taken from the study report, summarizes the basic study design. (One female each for the low dose and high dose groups were not pregnant.)

Group/Treatment	Dose level (LDU/kg/adm.) ⁽¹⁾	Dose volume (mL/kg/adm.)	Dose concentration (LDU/mL) ⁽¹⁾	Number of females
1. Control	0	0.1	0	6
2. Low dose	3	0.1	30	6
3. Intermediate dose	10	0.1	100	6
4. High dose	30	0.1	300	6

adm.: administration.

⁽¹⁾ 1 unit (LDU) corresponds to the LD50 in the mouse (approximately 20 g) by the intraperitoneal route.

The group 1 animals received the vehicle (sterile 0.9 % NaCl containing 1 mg/mL Human Serum Albumin (HSA)).

Observations/Results:

Mortality: No unscheduled deaths occurred.

Clinical signs: Observations were recorded daily and on dosing days once prior to and at least once after dosing.

- All animals that received 10 U/kg or greater showed lameness of the left hindlimb at the end of gestation. The lameness was evident by GD18 in the mid dose group and GD16 in the high dose group.

Body weights: Dams were weighed on GD0, 6, 11, 15, 18 and 20.

- The mean body weight of all treated groups was reduced relative to control from GD6-11.
- After GD11, the mean body weight of the low dose group did not differ from control mean, but the reduced body weight gain of the mid and high dose groups persisted through the end of the dosing period. The reduction in body weight gain was most apparent for the high dose group, resulting in mean terminal body weight that was 13% lower than control values.
- The mean control body weight gain was lower than historical control data due to unusually low weight gain in one animal (#2).

Food consumption: Measurements were recorded for GD0-6, 6-11, 11-15, 15-18 and 18-20.

- There were no effects on food consumption observed in the mid or low dose groups relative to control.
- The high dose group showed a slightly reduced food intake relative to control for the period of GD6-15.

Gross pathology: After Cesarean section, dams were euthanized and examined macroscopically. The ovaries and uterus and placentae were removed and examined. One animal each from the low and high dose groups were not pregnant.

- The injected left gastrocnemius muscle appeared small in all treated groups relative to control

Organ weights: Left and right gastrocnemius muscles from each dam were weighed.

- The small appearance of the left gastrocnemius muscle correlated with reduced absolute and relative weight of that muscle for all treated groups (-30, -44 and -35% relative to control for groups 2, 3 and 4, respectively).
- A slight reduction in absolute and relative weight of the contralateral, reduced muscle was observed for the mid and high dose groups relative to control. The sponsor considers this effect on the contralateral gastrocnemius muscle related to the reduced body weight gain noted for these two groups. A similar effect was observed in the 9-month study in monkeys (study #AA41667). In both studies, the effect was dose-related and consistent with systemic spread of NT201.

Litter/pregnancy parameters:

- All pregnant females (6, 5, 6, and 5 for groups 1, 2, 3, and 4, respectively) had viable fetuses.
- No effect was observed on mean numbers of corpora lutea among the treated groups relative to control.
- The mean number of early resorptions was higher in the high dose group relative to control values. (2.6 for group 4, 1.5 for concurrent control group) The sponsor considers this effect to be of questionable biological significance because the difference is attributed to 2 high dose females that had 4 early resorptions each. However, the affected animals represent a large proportion of the small group size (5/group). Similar findings have been noted after treatment with other botulinum toxin products in pregnant rodents. Therefore, a relationship to NT201 exposure should be considered. The Cesarean section data are summarized in the tables below, taken from the study report.

- The number of early resorptions was lower in the low and mid dose groups relative to control. The sponsor states that the concurrent control group had a higher rate of early resorptions relative to their historical control data (10.5% vs. 9.1%, respectively).
- No late resorptions were observed in any group.
- No differences in fetal sex ratios were observed among the treated groups relative to control.
- The fetal weights were slightly reduced in treated groups relative to control (3.8, 3.9 and 4.0 in the low, mid and high dose groups relative to 4.3 in the control group). The values were within the historical control range.
- No fetal abnormalities were observed in any group. A total of 68 (6), 70 (5), 76 (6 and 54 (5) fetuses (litters) were available for evaluation for each of groups 1, 2, 3, and 4, respectively. The fetal observation data are summarized in the table below, taken from the study report.

SUMMARY OF FETAL EXTERNAL OBSERVATIONS

		Group 1 Control 0 LDU/kg/adm.	Group 2 Low dose 3 LDU/kg/adm.	Group 3 Intermed. dose 10 LDU/kg/adm.	Group 4 High dose 30 LDU/kg/adm.
Litters Evaluated	N	6	5	6	5
Fetuses Evaluated	N	68	70	76	54
Live	N	68	70	76	54
Dead	N	0	0	0	0
TOTAL FETAL EXTERNAL OBSERVATIONS	N	0	0	0	0

OBSERVATION CODE: M-MALFORMATION V-VARIATION A-ANOMALY

Conclusion:

Under the conditions of this pilot study, exposure to NT201 by intramuscular injection of the pregnant female on GD6, 12 and 19 resulted in a small increase in early resorptions in the high dose group (30 U/kg) relative to control and the lower dose groups. The sponsor does not consider this finding biologically relevant because the early resorptions occurred in only 2 females in that group. However, with only 5 animals available for evaluation, 2 animals represent a large proportion of the group. Furthermore, early resorptions were observed in embryofetal studies with similar products and systemic exposure is suspected

in other repeat-dose studies with NT201. Therefore, a relationship to systemic exposure of NT201 should be suspected. Under the conditions of this study, the developmental NOAEL is 10 U/kg.

Study # (b) (4) 442/018

Title: NT-201: Embryo toxicity study by the intramuscular route in the rabbit (Segment II)

Performing laboratory:	(b) (4)
GLP Compliance:	Yes
Date of Study Initiation:	12/18/2003
Lot #:	020902
Vehicle:	0.9% NaCl containing 1 mg/ml human serum albumin

Methods:

Animals: Mated female New Zealand White rabbits (22/ group)

Age: 16-19 weeks at mating

Weight: 2.9 to 4.2 kg at mating

Dose levels: 1.25, 2.50 or 5.00 U/kg

Route and Regimen: Animals were injected intramuscularly (left lumbar) on days 6, 18 and 28 of gestation.

Dose justification: The doses were chosen based on results of a preliminary study in non-pregnant rabbits.

Study design: The basic study design is summarized in the table below, taken from the study report.

Group/ treatment	Dose level (U ⁽¹⁾ /kg/adm.)	Dose volume (ml/kg/adm.)	Dose concentration (U/ml)
1. Control	0	0.2	0
2. Low dose	1.25	0.2	6.25
3. Intermediate dose	2.5	0.2	12.50
4. High dose	5.0	0.2	25.00

Observations/Results:

Mortality: Observations were made twice daily.

- One animal in the high dose group was sacrificed on GD24 due to paralyzed hindlimbs. Five additional rabbits from the high dose group were terminated after aborting on days 23 to 29 of gestation.
- No deaths or early sacrifices are reported for the lower dose groups.

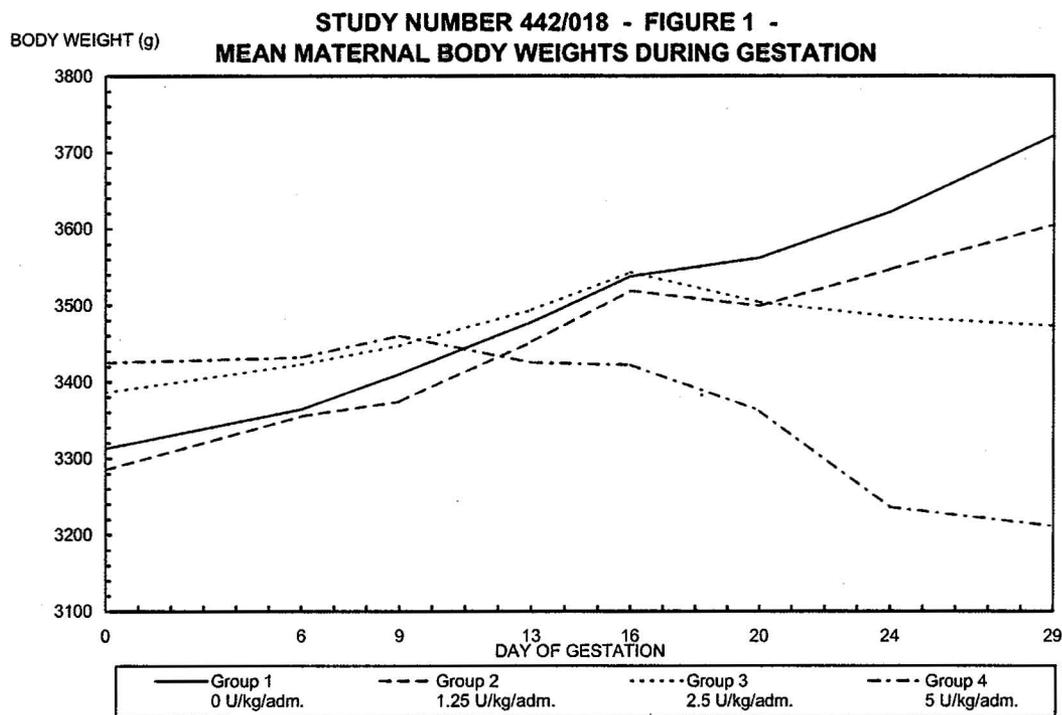
Clinical observations: Observations were made 1 hour prior to and 4 hours after dosing on treatment days (except for the dose on GD28). Local observations were made 1, 4, 24 and 48 hours after the first and second doses.

- The high dose rabbits showed signs of systemic exposure including reduced activity and unsteady gait beginning approximately 4-10 days after the second injection. These effects generally persisted until sacrifice.
- No clinical signs attributable to test article administration were observed in the low dose group.
- In the mid dose group, reduced fecal output correlating with reduced food intake, was noted later in gestation (generally after GD16).
- No significant local reactions to the injection of the test article were reported. Occasional transient erythema or induration was noted.

Body weight: Data recording on GD0, 6, 9, 13, 16, 20, 24, and 29.

- A dose-related reduction in body weight gain was noted for all treated groups relative to control. The low dose group showed only a slight effect between GD16 and 20. In this group, 15 of 21 animals lost weight compared with 5 of the control animals. Statistically significant reductions in mean terminal body weight relative to controls was achieved for groups 3 and 4 (mid and high dose groups).

The figure and table below, taken from the study report, summarize the mean body weight changes during this study.



		SUMMARY OF GESTATION BODY WEIGHT CHANGES (GRAMS)			
		Group 1 Control 0 U/kg/adm.	Group 2 Low dose 1.25 U/kg/adm.	Group 3 Intermed. dose 2.5 U/kg/adm.	Group 4 High dose 5.0 U/kg/adm.
DAYS 0 TO 6	MEAN	51 d	70	36	8
	S.D.	68	97	94	64
	N	21	21	22	15
DAYS 6 TO 9	MEAN	46 d	18	25	28
	S.D.	40	45	45	42
	N	21	21	22	15
DAYS 9 TO 13	MEAN	69 k	78	47	-35**
	S.D.	47	34	35	69
	N	21	21	22	15
DAYS 13 TO 16	MEAN	60 d	67	50	-3**
	S.D.	59	47	53	52
	N	21	21	22	15
DAYS 16 TO 20	MEAN	24 d	-20*	-39**	-60**
	S.D.	49	51	49	36
	N	21	21	22	15
DAYS 20 TO 24	MEAN	60 d	48	-19**	-126**
	S.D.	61	45	76	61
	N	21	21	22	15
DAYS 24 TO 29	MEAN	100 k	57	-12**	-26**
	S.D.	51	96	72	141
	N	21	21	22	15
DAYS 6 TO 29	MEAN	358 d	249	51**	-222**
	S.D.	159	155	140	161
	N	21	21	22	15

Statistical key: d=Anova/Dunnett test k=Kruskal-Wallis/Dunn test * = p<0.05 ** = p<0.01

Food consumption: Food intake was measured daily from the day of arrival to GD29. Mean consumption was calculated for the intervals 0-6, 6-9, 9-13, 13-16, 16-20, 20-24, and 24-29.

- The body weight changes described above were generally associated with reduced maternal food intake. This finding was dose-related in severity as well as duration. The low dose group did show reduced food intake relative to controls, but the results were not statistically significant.

Necropsy: All surviving animals were sacrificed on day 29 of gestation and examined macroscopically. No treatment-related gross lesions, other than weight loss, were noted for the dams at necropsy.

Pregnancy results:

There was one non-pregnant dam in each group. Due to the treatment-related death and 5 abortions, only 15 pregnant dams were available in the high dose group at the time of Cesarean section. In one high dose dam, parturition initiated spontaneously on the day of scheduled C-section; one pup was cannibalized and 3 died after birth.

- *pregnancy status:* All surviving pregnant dams had viable fetuses (21 of 21 for control, 21 of 21 for low dose, 22 of 22 for the mid dose and 15 of 15 for the high dose group.
- *Pre-Implantation data:* One high dose animal had 10 corpora lutea and 3 implantation sites. A slightly higher rate of pre-implantation loss was noted for the mid and high dose groups. This finding probably represents very early post-implantation loss. The sponsor considers these findings

incidental. However, similar findings have been observed with other botulinum toxin products in similar studies.

Post-implantation data: There were no dead fetuses in any group. Post-implantation loss appeared to be lower in the treated groups relative to control. However, the increased pre-implantation loss (as stated above) most likely represents early post-implantation loss. The mean number of fetuses per dam was lower in the high dose group litters. One high dose female had only one viable fetus.

The table below, provided by the sponsor summarizes the litter data collected.

Study No.: 442018

NT 201 - EMBRYO TOXICITY STUDY BY THE INTRAMUSCULAR ROUTE
IN THE RABBIT (SEGMENT II)

SUMMARY OF CAESAREAN SECTION DATA

		Group 1 Control 0 U/kg/adm.	Group 2 Low dose 1.25 U/kg/adm.	Group 3 Intermed. dose 2.5 U/kg/adm.	Group 4 High dose 5.0 U/kg/adm.
Pregnant	N	21	21	22	15
Dams with no Viable Fetuses	N	0	0	0	0
Dams with Viable Fetuses	N	21	21	22	15
Corpora Lutea	TOTAL	255	252	279	158
No. per animal	MEAN	12.1 d	12.0	12.7	10.5
	S.D.	2.5	2.2	2.2	2.8
Implantation Sites	TOTAL	215	223	233	128
No. per animal	MEAN	10.2 d	10.6	10.6	8.5
	S.D.	2.9	2.6	2.9	2.9
Preimplantation Loss	TOTAL	40	29	46	30
No. per animal	MEAN	1.9 d	1.4	2.1	2.0
	S.D.	1.5	1.7	2.7	2.2
% per animal	MEANZ	16.5 k	12.1	16.0	17.6
	S.D.	14.7	15.7	18.7	20.4
Live Fetuses	TOTAL	192*	208	221	117
No. per animal	MEAN	9.1 d	9.9	10.0	7.8
	S.D.	3.1	2.3	3.0	2.9
Males	TOTAL	97	93	112	58
	MEANZ	49.5 k	42.5	51.1	53.6
	S.D.	16.8	16.3	13.6	24.9
Females	TOTAL	94	115	109	59
	MEANZ	50.5 k	57.5	48.9	46.4
	S.D.	16.8	16.3	13.6	24.9

Statistical key: d=Anova/Dunnett test k=Kruskal-Wallis/Dunn test

* One fetus not sexed in error.

Study No.: 442018

NT 201 - EMBRYO TOXICITY STUDY BY THE INTRAMUSCULAR ROUTE
IN THE RABBIT (SEGMENT II)

SUMMARY OF CAESAREAN SECTION DATA

		Group 1 Control 0 U/kg/adm.	Group 2 Low dose 1.25 U/kg/adm.	Group 3 Intermed. dose 2.5 U/kg/adm.	Group 4 High dose 5.0 U/kg/adm.
Postimplantation Loss No. per animal	TOTAL	23	15	12	10
	MEAN	1.1 k	0.7	0.5	0.7
	S.D.	1.5	0.8	0.7	0.8
% implants per animal	MEANX	12.0 k	6.1	5.6	9.8
	S.D.	16.6	6.4	7.3	17.2
Dead Fetuses No. per animal	TOTAL	0	0	0	0
	MEAN	0.0 k	0.0	0.0	0.0
	S.D.	0.0	0.0	0.0	0.0
% of implants per animal	MEANX	0.0 k	0.0	0.0	0.0
	S.D.	0.0	0.0	0.0	0.0
Resorptions: Early No. per animal	TOTAL	12	7	9	6
	MEAN	0.6 k	0.3	0.4	0.4
	S.D.	1.2	0.5	0.7	0.6
% of implants per animal	MEANX	7.9 k	3.1	4.7	7.4
	S.D.	16.5	4.7	7.5	17.2
Resorptions: Late No. per animal	TOTAL	11	8	3	4
	MEAN	0.5 k	0.4	0.1	0.3
	S.D.	1.0	0.7	0.4	0.6
% of implants per animal	MEANX	4.1 k	2.9	0.9	2.4
	S.D.	7.4	5.1	2.5	5.4

Statistical key: k=Kruskal-Wallis/Dunn test

(1) Includes one dam (no. 4937) sacrificed before term (excluded from calculation in Appendix 7).

Fetal weight: No apparent effect on fetal weight attributable to test article was noted. The tables below, provided by the sponsor, summarize the fetal data.

Study No.: 442018

NT 201 - EMBRYO TOXICITY STUDY BY THE INTRAMUSCULAR ROUTE
IN THE RABBIT (SEGMENT II)

SUMMARY OF CAESAREAN SECTION DATA

		Group 1 Control 0 U/kg/adm.	Group 2 Low dose 1.25 U/kg/adm.	Group 3 Intermed. dose 2.5 U/kg/adm.	Group 4 High dose 5.0 U/kg/adm.
Fetal Body Weight (g)	MEAN	35.3 d	36.2	35.6	35.7
	S.D.	4.7	4.9	5.3	7
	N	21	21	22	7
Male Fetuses	MEAN	35.7 d	35.5	36.3	36.3
	S.D.	5.1	5.7	6.3	7
Female Fetuses	MEAN	34.3 d	36.4	34.7	33.6
	S.D.	6.6	4.8	5.1	6

Statistical key: d=Anova/Dunnett test

The numbers of fetuses (litters) submitted to the different examinations were as follows:

Group	1	2	3	4
<u>External examination</u>	192 (21)	208 (21)	221 (22)	117 (15)
<u>Internal examination</u>				
. thoracic and abdominal cavities only	102 (21)	110 (21)	116 (22)	63 (15)
. thoracic and abdominal cavities and head	90 (21)	98 (21)	105 (22)	54 (14)
<u>Skeletal examination</u>				
. with head	102 (21)	110 (21)	116 (22)	63 (15)
. without head	90 (21)	98 (21)	105 (22)	54 (14)

External examination:

Study No.: 442018

NT 201 - EMBRYO TOXICITY STUDY BY THE INTRAMUSCULAR ROUTE
IN THE RABBIT (SEGMENT II)

SUMMARY OF FETAL EXTERNAL MALFORMATIONS

		Group 1 Control 0 U/kg/adm.	Group 2 Low dose 1.25 U/kg/adm.	Group 3 Intermed. dose 2.5 U/kg/adm.	Group 4 High dose 5.0 U/kg/adm.
Litters Evaluated	N	21	21	22	15
Fetuses Evaluated	N	192	208	221	117
Live	N	192	208	221	117
Dead	N	0	0	0	0
TRUNK					
Litter Incidence	N	1	1	0	0
Fetal Incidence	N	1	1	0	0
GASTROSCHISIS					
Fetal Incidence	N	0	1	0	0
	%	0.0	0.5	0.0	0.0
Litter Incidence	N	0 f	1	0	0
	%	0.0	4.8	0.0	0.0
OMPHALOCELE					
Fetal Incidence	N	1	0	0	0
	%	0.5	0.0	0.0	0.0
Litter Incidence	N	1 f	0	0	0
	%	4.8	0.0	0.0	0.0

Statistical key: f=Chi2/Fisher Exact test

Study No.: 442018

NT 201 - EMBRYO TOXICITY STUDY BY THE INTRAMUSCULAR ROUTE
IN THE RABBIT (SEGMENT II)

SUMMARY OF FETAL EXTERNAL ANOMALIES

		Group 1 Control 0 U/kg/adm.	Group 2 Low dose 1.25 U/kg/adm.	Group 3 Intermed. dose 2.5 U/kg/adm.	Group 4 High dose 5.0 U/kg/ad
Litters Evaluated	N	21	21	22	15
Fetuses Evaluated	N	192	208	221	117
Live	N	192	208	221	117
Dead	N	0	0	0	0
PAW/DIGIT					
Litter Incidence	N	1	0	1	0
Fetal Incidence	N	1	0	1	0
FOREPAW FLEXED					
Fetal Incidence	N	1	0	1	0
	%	0.5	0.0	0.5	0.0
Litter Incidence	N	1 f	0	1	0
	%	4.8	0.0	4.5	0.0

Statistical key: f=Chi2/Fisher Exact test

Visceral examination:

No fetal visceral malformations attributable to test article are reported.

Study No.: 442018

NT 201 - EMBRYO TOXICITY STUDY BY THE INTRAMUSCULAR ROUTE
IN THE RABBIT (SEGMENT II)

SUMMARY OF FETAL VISCERAL MALFORMATIONS

		Group 1 Control 0 U/kg/adm.	Group 2 Low dose 1.25 U/kg/adm.	Group 3 Intermed. dose 2.5 U/kg/adm.	Group 4 High dose 5.0 U/kg/adm.
Litters Evaluated	N	21	21	22	15
Fetuses Evaluated	N	192	208	221	117
Live	N	192	208	221	117
Dead	N	0	0	0	0
TOTAL FETAL VISCERAL MALFORMATIONS	N	0	0	0	0

No statistically significant differences

Study No.: 442018c

NT 201 - EMBRYO TOXICITY STUDY BY THE INTRAMUSCULAR ROUTE
IN THE RABBIT (SEGMENT II)

SUMMARY OF FETAL VISCERAL MALFORMATIONS

		Group 1 Control 0 U/kg/adm.	Group 2 Low dose 1.25 U/kg/adm.	Group 3 Intermed. dose 2.5 U/kg/adm.	Group 4 High dose 5.0 U/KG/adm.
Litters Evaluated	N	21	21	22	14
Fetuses Evaluated	N	90	98	105	54
Live	N	90	98	105	54
Dead	N	0	0	0	0
TOTAL FETAL VISCERAL MALFORMATIONS	N	0	0	0	0

No statistically significant differences

One litter in group 4 (no. 4945) contained only one fetus and was not therefore submitted to this examination.

Fetal Visceral anomalies:

Study No.: 442018

NT 201 - EMBRYO TOXICITY STUDY BY THE INTRAMUSCULAR ROUTE
IN THE RABBIT (SEGMENT II)

SUMMARY OF FETAL VISCERAL ANOMALIES

		Group 1 Control 0 U/kg/adm.	Group 2 Low dose 1.25 U/kg/adm.	Group 3 Intermed. dose 2.5 U/kg/adm.	Group 4 High dose 5.0 U/kg/a
Litters Evaluated	N	21	21	22	15
Fetuses Evaluated	N	192	208	221	117
Live	N	192	208	221	117
Dead	N	0	0	0	0
AORTIC ARCH					
Litter Incidence	N	0	1	0	0
Fetal Incidence	N	0	1	0	0
AORTIC ARCH : DILATED, SLIGHT					
Fetal Incidence	N	0	1	0	0
	%	0.0	0.5	0.0	0.0
Litter Incidence	N	0	1	0	0
	%	0.0	4.8	0.0	0.0

Statistical key: f=Chi2/Fisher Exact test

Study No.: 442018t

NT 201 - EMBRYO TOXICITY STUDY BY THE INTRAMUSCULAR ROUTE
IN THE RABBIT (SEGMENT II)

SUMMARY OF FETAL VISCERAL ANOMALIES					
		Group 1 Control 0 U/kg/adm.	Group 2 Low dose 1.25 U/kg/adm.	Group 3 Intermed. dose 2.5 U/kg/adm.	Group 4 High dose 5.0 U/KG/adm.
Litters Evaluated	N	21	21	22	14
Fetuses Evaluated	N	90	98	105	54
Live	N	90	98	105	54
Dead	N	0	0	0	0
BRAIN					
Litter Incidence	N	1	0	0	0
Fetal Incidence	N	1	0	0	0
BRAIN : VACUOLE					
Fetal Incidence	N	1	0	0	0
	%	1.1	0.0	0.0	0.0
Litter Incidence	N	1 f	0	0	0
	%	4.8	0.0	0.0	0.0

Statistical key: f=Chi2/Fisher Exact test

Visceral variations: For all treated groups there was a slight increase in the occurrence of absent azygos lobe of the lung. The incidence was small and not clearly dose dependent. Therefore, the biological significance is unclear.

Study No.: 442018

NT 201 - EMBRYO TOXICITY STUDY BY THE INTRAMUSCULAR ROUTE
IN THE RABBIT (SEGMENT II)

SUMMARY OF FETAL VISCERAL VARIATIONS					
		Group 1 Control 0 U/kg/adm.	Group 2 Low dose 1.25 U/kg/adm.	Group 3 Intermed. dose 2.5 U/kg/adm.	Group 4 High dose 5.0 U/kg/adm.
Litters Evaluated	N	21	21	22	15
Fetuses Evaluated	N	192	208	221	117
Live	N	192	208	221	117
Dead	N	0	0	0	0
LUNG					
Litter Incidence	N	3	4	2	2
Fetal Incidence	N	3	6	5	3
LUNG : AZYGOS LOBE ABSENT					
Fetal Incidence	N	3	6	5	3
	%	1.6	2.9	2.3	2.6
Litter Incidence	N	3 f	4	2	2
	%	14.3	19.0	9.1	13.3

Statistical key: f=Chi2/Fisher Exact test

Skeletal examination:

- Skeletal examination revealed one additional malformation in the control group (malformed thoracic vertebrae) and 2 in the low dose group (malformed thoracic vertebrae). No skeletal malformations were observed in the high dose group.
- Relative to control and recent historical data, the incidence of incomplete ossification of the centrum of thoracic vertebrae 5 to 8 was greater in the treated groups. However, the distribution of incidence among litters did not appear to be dose-related.
- Incomplete ossification of the first 4 thoracic vertebrae was slightly higher in treated animals relative to control. The sponsor does not consider this finding related to test article because the incidence in the control group was lower than expected relative to recent historical controls.
- A slight increase in incidence of incomplete ossification of the 2nd and 4th sternbrae relative to control was noted. The values did not differ significantly

from recent historical control mean (15% for the historical control, 13% for the current control group and 18% for the high dose group.)

IN THE HAREBIT (SEGMENT II)

SUMMARY OF FETAL SKELETAL MALFORMATIONS

		Group 1 Control 0 U/kg/adm.	Group 2 Low dose 1.25 U/kg/adm.	Group 3 Intermed. dose 2.5 U/kg/adm.	Group 4 High dose 5.0 U/kg/adm.
Litters Evaluated	N	21	21	22	15
Fetuses Evaluated	N	192	208	221	117
Live	N	192	208	221	117
Dead	N	0	0	0	0
THORACIC VERTEB.					
Litter Incidence	N	1	2	0	0
Fetal Incidence	N	1	2	0	0
VERTEBRA, THORACIC : MALFORMED					
Fetal Incidence	N	1	2	0	0
	X	0.5	1.0	0.0	0.0
Litter Incidence	N	1 f	2	0	0
	X	4.8	9.5	0.0	0.0

Statistical key: f=Chi2/Fisher Exact test

Conclusions:

The dams exhibited the expected dose-related effects of NT201, including reduced weight gain and food intake. The high dose group showed significant maternal toxicity. Five abortions were noted for this group. The middle dose level of 2.5 U/kg was determined to be the MTD for this study due to weight loss and reduced food intake.

There were no indications of embryotoxicity, fetal external or visceral malformations or variations attributable to test article in groups receiving 2.5 U/kg or less. A slight increase in incidence of vertebral incomplete ossification was noted in all treated groups, relative to control. The incidence was small and within the range of recent historical controls, but is similar to the effects of other botulinum toxin products in similar studies.

NOAEL was determined to be 1.25 U/kg.

●●●

Study #:AA42649

Title: NT201-embryo toxicity study by the intramuscular route in the rat (Segment II)

Testing Facility:

(b) (4)

Date of study initiation:

5/14/2008

GLP compliance:

Yes

Lot #:

60707

Vehicle:

0.9% NaCl with 1mg/ml human serum albumin

Methods:

Doses: 0, 3, 10 or 30 U/kg/week (groups 1, 3, 4 and 5, respectively); 7 U/kg/day (group 2); 2, 6, or 8 U/kg twice/week (groups 6, 7 and 8, respectively)

Species/strain: Presumed pregnant Sprague/Dawley rats

Number/sex/group: 25/group

Age at mating: 10-13 weeks old

Body weight at mating: 190 – 264 g

Route: Intramuscular injection into the left gastrocnemius muscle

Regimen: Groups 3-5: once weekly (GD6, 12, and 19)

Group 2 received injections daily from GD6 to 19

Groups 6-8 received injections twice weekly (GD6, 9, 12, 16 and 19)

Study design:

The basic study design is summarized in the table below, taken from the study report. The dose levels and treatment regimen were chosen based on data from a preliminary, dose-ranging study in pregnant rats (Study #AA62003). On GD20 Cesarean sections were performed, the dams were euthanized and macroscopic examination was conducted.

Group/Treatment	Dose level (LDU/kg/adm.) ⁽¹⁾	Dose concentration (LDU/mL) ⁽¹⁾	Number of females	Days of dosing
1. Control	0	0	25	G6 to G19
2. Daily dosing	7	70	25	G6 to G19
3. OW Low dose	3	30	25	G6, G12 and G19
4. OW Intermediate dose	10	100	25	G6, G12 and G19
5. OW High dose	30	300	25	G6, G12 and G19
6. BW Low dose	2	20	25	G6, G9, G12, G16 and G19
7. BW Intermediate dose	6	60	25	G6, G9, G12, G16 and G19
8. BW High dose	18	180	25	G6, G9, G12, G16 and G19

adm.: administration.

⁽¹⁾ 1 unit (LDU) corresponds to LD50 in the mouse by the intraperitoneal route.

OW: once weekly dosing.

BW: bi-weekly dosing.

The group 1 animals (control) received the vehicle (Sterile 0.9 % NaCl containing 1 mg/mL Human Serum Albumin (HSA)).

Observations/Results:

Mortality (dams): Observations were recorded daily.

- One animal (103) from group 5 (30 U/kg/week) was found dead on GD19. Necropsy with full macroscopic examination was performed; the fetuses were discarded. The cause of death was not identified. The sponsor considers this death

to be incidental since no other deaths occurred. However, this animal is from the high dose group and a relationship to NT201 cannot be ruled out.

Clinical signs (dams): Observations were recorded daily and at least once after each dose.

- All animals in treated groups (except 2 dams in group 3, lowest weekly dose) demonstrated abnormal gait due to paresis of the left hindlimb. This is an expected effect due to the pharmacological activity of NT201. Lameness was dose and duration dependent in incidence and severity, appearing by GD7 in groups 2, 7 and 8. The effect was not observed in the lowest dose group (group 3) until GD12.

Body weight (dams): All dams were weighed on GD0, 6, 11, 15, 18 and 20

- A dose related reduction in mean body weight was observed for groups 2, 5 and 8 relative to control, resulting in mean terminal body weights of -10, -8 and -9%, respectively. No effect on mean body weight was noted in the other treated groups.

Food consumption (dams): Food consumption was measured for each dam at intervals from GD0-6, 6-8, 11-15.

- The reductions in mean terminal body weight were correlated with reduced food consumption, relative to control. The overall mean food consumption values for groups 2, 5 and 8 were 23.2, 25.5, and 25.1 g, respectively, relative to 27.2 g for the control group.

Weight of gastrocnemius muscle (left and right):

- The left (treated) gastrocnemius muscle was reduced in size and weight (absolute and relative) in all treated groups, relative to control. The difference in muscle weight was statistically significant and dose-related (incidence, severity and time of onset) in all groups. This finding is an expected result of NT201 and confirms exposure to the toxin. The magnitude of reduction in muscle weight was -30% for the rats that were treated daily, -34% for those treated once/week, and -38 to -43% for those treated twice weekly.
- The weight (absolute and relative) of the contralateral (untreated) gastrocnemius muscle was consistently reduced relative to control for all treated groups, although the reduction was of lesser magnitude than observed in the treated muscle. The reduction in contralateral muscle weight was dose-related and reached statistical significance for groups 2, 4, 5, and 8.
- The findings are consistent with observations reported in other repeat-dose studies using NT201 and the reduction of weight of the untreated muscle may reflect systemic exposure to the toxin.

Toxicokinetics: N/A

Terminal and necroscopic evaluations-section data (implantation sites, pre- and post-implantation loss, etc.): The Cesarean section data are summarized in the tables below,

taken from the study report. A small increase in the rate of pre-implantation loss was observed in group 8, relative to control (6.9% vs. 8.8% per animal, respectively.)

Study No.: AA42649

NT 201 - EMBRYO TOXICITY STUDY BY THE INTRAMUSCULAR ROUTE IN THE RAT (SEGMENT II).

SUMMARY OF CAESAREAN SECTION DATA

		Group 1 Control 0 LDU/kg/a	Group 2 Daily dos. 7 LDU/kg/a	Group 3 OW Low dose 3 LDU/kg/a	Group 4 OW Int.dose 10 LDU/kg/a	Group 5 OW Hig.dose 30 LDU/kg/a	Group 6 BW Low dose 2 LDU/kg/a	Group 7 BW Int.dose 6 LDU/kg/a	Group 8 BW Hig.dose 18 LDU/kg/a
Pregnant	N	25	24	25	25	24	22	24	25
Dams with no Viable Fetuses	N	0	0	0	0	1	0	0	0
Dams with Viable Fetuses	N	25	24	25	25	23	22	24	25
Corpora Lutea	TOTAL	347	338	348	379	328	304	334	349
No. per animal	MEAN	13.9 d	14.1	13.9	15.2	13.7	13.8	13.9	14.0
	S.D.	2.8	2.3	2.2	2.3	3.7	2.3	2.0	2.7
Implantation Sites	TOTAL	327	317	334	355	309	289	321	319
No. per animal	MEAN	13.1 k	13.2	13.4	14.2	12.9	13.1	13.4	12.8
	S.D.	3.1	2.9	2.9	2.0	3.9	2.2	1.9	2.6
Preimplantation Loss	TOTAL	20	21	14	24	19	15	13	30
No. per animal	MEAN	0.8 d	0.9	0.6	1.0	0.8	0.7	0.5	1.2
	S.D.	0.8	1.3	1.0	1.2	1.1	0.9	0.7	1.2
% per animal	MEAN%	6.9 k	7.3	5.1	6.0	6.5	4.8	3.7	8.8
	S.D.	9.2	13.5	10.4	7.0	10.9	6.0	4.5	8.9
Live Fetuses	TOTAL	282	295	307	322	289	271	304	305
No. per animal	MEAN	11.3 k	12.3	12.3	12.9	12.0	12.3	12.7	12.2
	S.D.	3.2	3.2	3.1	2.5	4.2	2.5	1.6	2.7
Males	TOTAL	150	136	153	165	135	141	146	146
	MEAN%	54.3 k	44.6	48.6	51.5	44.3	52.2	48.5	47.8
	S.D.	15.2	18.7	14.0	13.9	17.7	16.2	13.9	11.3
Females	TOTAL	132	159	154	157	154	129	158	159
	MEAN%	45.7 k	55.4	51.4	48.5	55.7	47.8	51.5	52.2
	S.D.	15.2	18.7	14.0	13.9	17.7	16.2	13.9	11.3

Statistical key: d=Anova/Dunnett test k=Kruskal-Wallis/Dunn test

Study No.: AA42649

NT 201 - EMBRYO TOXICITY STUDY BY THE INTRAMUSCULAR ROUTE IN THE RAT (SEGMENT II).

SUMMARY OF CAESAREAN SECTION DATA

		Group 1 Control 0 LDU/kg/a	Group 2 Daily dos. 7 LDU/kg/a	Group 3 OW Low dose 3 LDU/kg/a	Group 4 OW Int.dose 10 LDU/kg/a	Group 5 OW Hig.dose 30 LDU/kg/a	Group 6 BW Low dose 2 LDU/kg/a	Group 7 BW Int.dose 6 LDU/kg/a	Group 8 BW Hig.dose 18 LDU/kg/a
Postimplantation Loss	TOTAL	45	22	27	33	20	18	17	14
No. per animal	MEAN	1.8 k	0.9	1.1	1.3	0.8	0.8	0.7	0.6
	S.D.	1.9	0.9	0.9	1.2	1.0	1.0	0.9	0.8
% implants per animal	MEAN%	13.7 k	8.9	8.9	9.8	10.4	6.5	5.0	4.8
	S.D.	15.2	13.9	8.1	9.6	20.6	8.2	5.7	7.0
Dead Fetuses	TOTAL	0	0	0	0	0	0	0	0
No. per animal	MEAN	0.0 k	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	S.D.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
% of implants per animal	MEAN%	0.0 k	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	S.D.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Resorptions: Early	TOTAL	43	22	26	32	20	17	17	14
No. per animal	MEAN	1.7 k	0.9	1.0	1.3	0.8	0.8	0.7	0.6
	S.D.	1.9	0.9	0.8	1.1	1.0	1.0	0.9	0.8
% of implants per animal	MEAN%	13.1 k	8.9	8.5	9.4	10.4	6.1	5.0	4.8
	S.D.	14.9	13.9	7.6	8.7	20.6	7.8	5.7	7.0
Resorptions: Late	TOTAL	2	0	1	1	0	1	0	0
No. per animal	MEAN	0.1 k	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	S.D.	0.3	0.0	0.2	0.2	0.0	0.2	0.0	0.0
% of implants per animal	MEAN%	0.6 k	0.0	0.3	0.4	0.0	0.4	0.0	0.0
	S.D.	2.1	0.0	1.5	1.8	0.0	1.9	0.0	0.0

Statistical key: k=Kruskal-Wallis/Dunn test

- A tendency toward slightly reduced fetal body weight was noted for Groups 2, 5 and 8. Slightly reduced fetal body weight has been observed for other botulinum products in similar studies.

SUMMARY OF CAESAREAN SECTION DATA

		Group 1 Control 0 LDU/kg/a	Group 2 Daily dos. 7 LDU/kg/a	Group 3 OW Low dose 3 LDU/kg/a	Group 4 OW Int.dose 10 LDU/kg/a	Group 5 OW Hig.dose 30 LDU/kg/a	Group 6 BW Low dose 2 LDU/kg/a	Group 7 BW Int.dose 6 LDU/kg/a	Group 8 BW Hig.dose 18 LDU/kg/a
Fetal Body Weight (g)	MEAN	4.1 k	3.8*	4.2	4.1	3.9	4.1	4.1	3.8
	S.D.	0.5	0.3	0.4	0.2	0.3	0.5	0.4	0.5
	N	25	24	25	25	23	22	24	25
Male Fetuses	MEAN	4.2 k	4.0*	4.3	4.2	4.0	4.2	4.2	4.0
	S.D.	0.5	0.2	0.4	0.2	0.3	0.5	0.4	0.5
Female Fetuses	MEAN	3.9 k	3.8	4.1	4.0	3.8	3.9	4.0	3.7
	S.D.	0.5	0.3	0.4	0.3	0.3	0.5	0.5	0.5

Statistical key: k=Kruskal-Wallis/Dunn test * = p<0.05

Offspring (malformations, variations, etc.):

The number of fetuses evaluated for external and visceral malformations is summarized in the table below, taken from the study report.

Text Table 1. The numbers of fetuses (litters) submitted to the different examinations were as follows:

Group	1	2	3	4	5	6	7	8
External ex.	282(25)	295(24)	307(25)	322(25)	289(23)	271(22)	304(24)	305(25)
Internal ex.	133(25)	141(23)	148(25)	154(25)	141(23)	130(22)	147(24)	146(25)
Skeletal ex.	149(25)	154(24)	159(25)	168(25)	148(23)	141(22)	157(24)	159(25)

The fetal malformations observed for each group are summarized in the table below, provided by the sponsor.

- No dose-related changes in incidence of malformations were observed among groups. Although there were no malformations in the control group, the malformations listed below were considered incidental due to the low incidence (one animal/groups 4, 5 and 6; 2 animals/group 7) and lack of dose-relationship (none observed in group 8).

Text Table 2. Malformed fetuses

Group	Malformations
Group 4 Dam 77 - fetus 5	Anophthalmia, subdural brain haemorrhage, malformed major blood vessels, absent innominate artery
Group 5 Dam 111 – fetus 1	Micrognathia, absent claw, cleft palate, fused basisphenoid
Group 6 Dam 135 – fetus 9	Malformed major blood vessels, absent innominate artery
Group 7 Dam 164 – fetus 11 Dam 164 – fetus 13	Malformed vertebral column, acaudia Dilated aortic arch and pulmonary artery

- A trend toward increased incidence of skeletal anomalies and variations were observed in high dose groups relative to control. These findings included small changes in incidence of incomplete or lack of ossification of sternbrae, thoracic and sacral vertebrae, skull bones, metacarpals and metatarsals. Although the changes in incidence are small among groups, these findings are consistent with those observed in similar studies with other botulinum toxin products.

The table below summarizes the incidence of selected skeletal variations and anomalies. Data are presented as % of fetuses/group (% litters/group). (Note: The high doses for each dosing regimen are represented by groups 2, 5 and 8.)

Observation	Group 1 0 U/kg	Group 2 7 U/kg (Daily)	Group 3 3 U/kg (Weekly)	Group 4 10 U/kg (Weekly)	Group 5 30 U/kg (Weekly)	Group 6 2 U/kg (Twice weekly)	Group 7 6 U/kg (Twice weekly)	Group 8 18 U/kg (Twice weekly)
Incomplete ossification parietal bone	3.4(16.0)	3.9 (20.8)	5.7(24.0)	1.8(12.0)	6.8(26.1)	5.7 (22.7)	1.3 (8.3)	3.1 (16.0)
Incomplete ossification interparietal bone	7.4(32.0)	22.1(66.7)	16.4(56.0)	6.5(28.0)	21.6(47.8)	18.4(50.0)	10.2(33.3)	18.2(56.0)
Incomplete ossification presphenoid bone	0.7(4.0)	0.0(0.0)	0.6(4.0)	0.0(0.0)	0.7(4.3)	0.0(0.0)	0.0(0.0)	2.5(8.0)
Incomplete ossification hyoid bone	0.0(0.0)	0.6(4.2)	0.6(4.0)	0.0(0.0)	0.7(4.3)	0.0(0.0)	0.6(4.2)	1.3(20.0)
Incomplete ossification 2 nd or 5 th metacarpal	11.4(32.0)	15.6(58.3)	4.4(28.0)	10.7(36.0)	13.5(52.2)	21.3(50.0)	11.5(29.2)	22.0(52.0)
Unossified metatarsal	0.7(4.0)	0.6(4.2)	0.0(0.0)	0.0(0.0)	0.7(4.3)	2.8(4.5)	0.0(0.0)	8.2(12.0)
Incomplete ossification 6 th sternebra	10.7(40.0)	12.3(45.8)	6.3(20.0)	2.4(16.0)	18.2(52.2)	8.5(22.7)	10.2(37.5)	15.1(52.0)
Incomplete ossification 2 nd /4 th sternebrae	12.1(28.0)	11.0(54.2)	3.8(24.0)	5.4(24.0)	12.8(56.5)	11.3(27.3)	9.6(37.5)	19.5(64.0)
Unossified 5 th sternebra	11.4(28.0)	5.2(25.0)	3.1(20.0)	3.6(20.0)	10.8(34.8)	10.6(27.3)	7.6(37.5)	17.0(44.0)
Bipartite ossification, sternebra	0.7(4.0)	3.2(20.8)	0.6(4.0)	0.6(4.0)	2.0(13.0)	0.0(0.0)	3.2(16.7)	1.9(12.0)
Incomplete ossification of vertebral arch, sacral	1.3(4.0)	1.3(8.3)	1.3(4.0)	0.0(0.0)	4.1(17.4)	6.4(13.6)	0.6(4.2)	5.7(8.0)
Unossified sacral vertebra	0.0(0.0)	0.0(0.0)	0.6(4.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	1.9(4.0)
Incomplete ossification, centrum, 1- 4 thoracic vertebrae	5.4(16.0)	3.9(20.8)	1.9(12.0)	1.2(8.0)	5.4(30.4)	1.4(9.1)	4.5(29.2)	5.7(24.0)
Bipartite ossification of centrum, thoracic vertebra	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	3.4(17.4)	0.0(0.0)	0.0(0.0)	1.9(8.0)

Conclusions:

Observations in the dams were consistent with the expected results of the known pharmacological activity of NT201. The NOAEL for maternal toxicity (based on reduced body weight gain) under the conditions of this study is 10 U/kg administered weekly or 6 U/kg administered twice per week.

Fetal effects included tendencies for slightly reduced fetal body weight, slightly reduced implantation sites per animal in groups 5 and 8, a small increase in pre-implantation loss (very early resorption) in group 8 and a slight increase in incomplete ossification or lack of ossification in groups 2, 5 and 8.

9.3 Prenatal and postnatal development

No prenatal and postnatal development studies were included in this license application.

10. Special toxicology studies:

Study no.: (b) (4) 11373/98

Title: Examination of (b) (4) on haemolytic properties in human blood (*in vitro*)

Testing Facility:

(b) (4)

Date of study initiation:

5/18/1998

GLP compliance:

Yes

(b) (4) **Lot #:**

SPA3/2-3/7

Methods:

Test concentrations: 120, 240 or 480 U/ml

Positive control: 0.1% aqueous Na₂CO₃

Vehicle: 10% sucrose containing 1 mg/ml BSA

Blood source: healthy human volunteer (n=3), containing Na-citrate anticoagulant, diluted in 0.9% NaCl, centrifuged and supernatant discarded. Purified erythrocytes were resuspended in 0.9% NaCl.

Procedure: 1 ml of purified erythrocyte solution was mixed with 5 ml of test solution or control solution and incubated for 30 minutes at 37°C. Samples were centrifuged and examined spectroscopy at 540nm. The test sample should not exceed control value by more than 0.03.

Observations/Results:

The results are summarized in the table below, taken from the study report.

	Extinction				
	Volunteer No.	Sample A	Sample B	Sample C	Mean Value
Control (10% sucrose solution; 1 mg/ml BSA)	1	0.004	0.007	0.002	0.0043
	2	0.000	0.001	0.001	0.0007
	3	0.005	0.004	0.003	0.0040
(b) concentration					
120 units/ml	1	0.008	0.006	0.006	0.0067
	2	0.014	0.000	0.012	0.0087
	3	0.003	0.004	0.003	0.0033
240 units/ml	1	0.008	0.012	0.007	0.0090
	2	0.007	0.005	0.010	0.0073
	3	0.007	0.004	0.011	0.0073
480 units/ml	1	0.008	0.012	0.014	0.0113
	2	0.009	0.012	0.010	0.0103
	3	0.006	0.008	0.007	0.0070
Substance for comparison 0.1% Na ₂ CO ₃ *	(n = 2)	1.551	1.625	-	1.588

* conducted in duplicate - (b) historical background data

Conclusion:

Under the conditions of this study, (b) (4) did not show hemolytic potential when mixed with normal human erythrocytes.

Local Tolerance:

Study # (b) (4) 21110

Title: Acute eye irritation/corrosion test of NT201 in rabbits

Testing Facility:

(b) (4)

GLP Compliance:

European and OECD GLP compliant
QA audit complete

Date of Study Initiation:

4/19/2007

Lot#:

60707

Methods:

Animals: Himalayan rabbits

Age/weight: 27-28 months old; 2.5 -2.9 kg

Number/group: 3 male rabbits in one group

Doses: 100 U/animal

Route: Applied to the conjunctival sac of the right eye

Regimen: Single application in a volume of 0.1 ml

Study design: 100 U of NT201 was instilled into the conjunctival sac of the right eye of each animal. 24 hours after administration, the treated eye was rinsed with 0.9% NaCl.

Observations/Results:

Clinical observations: No effects on behavior or food consumption were observed.

Ophthalmology: Exams were conducted at 1, 24, 48 and 72 hours after dosing.

Fluorescein was administered to the eyes at 24 hours to assist in evaluating for potential corneal lesions.

- No signs of eye irritation were noted in the treated eyes relative to the contralateral untreated eye.

Conclusions:

Under the conditions of this study, instillation of 100 U of NT201 appeared to be non-irritating. The recommended high human dose for blepharospasm is (b) (4) for the muscles around each eye.

Antigenicity

Study # 10929/97

Title: Comparative antigenicity of BOTOX and NT201 in the rabbit

GLP compliance:

Yes

Performing Laboratory:

(b) (4)

Date of study initiation:

1/14/98

Lot #: NT201 lot # SPA 3/1-3/4 (1st pilot formulation), BOTOX lot # CGC 007

Vehicle/formulation: 0.9% saline

Methods

Animals: rabbit, NZW, females, aged 65 days, 2.2-3.1 kg

Dose/regimen: 25 LDU of either BOTOX or NT201 per animal

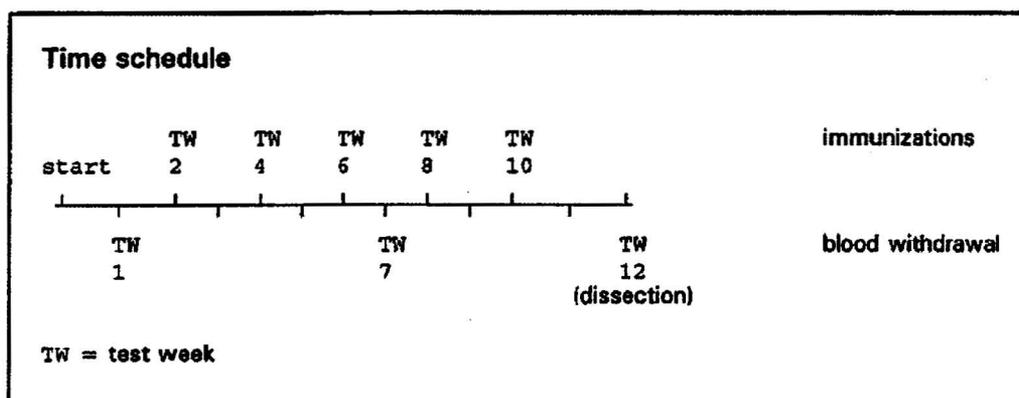
Route of administration: intracutaneous injection, 4 sites per animal at each administration, total volume of 0.4 ml at each administration, total of 5 administrations

Study Design: The test articles were injected intracutaneously in a total of five applications. Total study duration was 10 or 11 weeks (not clear from the protocol).

Group	Test substance	Dose level (units/animal) by intracutaneous injection	Animal number
1	BOTOX	25	1 - 8
2	NT-201		9 - 20

A pre-test phase was conducted to evaluate tolerability of the proposed dose by injection of 25 U intracutaneously in 4 animals, followed by 2 weeks observation. The results of this pre-test were used for dose selection in the main study.

The main study phase consisted of test article injection intracutaneously every two weeks in a volume of 100 µl. The following graphic, taken from the study report, summarizes the dosing regimen.



Observations/Results:

Mortality: All animals injected with BOTOX survived to scheduled sacrifice. Two animals that received NT201 died on test days 45 and 48 (12 days after the 3rd injection).

Clinical observations:

- For the BOTOX group, the first signs of paralysis were noted in one of 8 animals, 2 days after the second injection. Similar signs presented in all animals on the subsequent days. Signs of paralysis decreased and disappeared in 6 of 8 animals after the 3rd or 4th injection.
- For the NT201 group, the first signs of paralysis (reduced mobility and ataxia) were noted 7 days after the first injection, in 2 of 12 animals. Five days after the second injection, signs were present in all animals in this group. Severity of signs appeared to be somewhat more severe in the NT201 animals relative to the BOTOX-treated animals.
- Systemic signs included dyspnea, reduced body temperature, reduced respiration and were observed in all 12 animals.

Body weight: Animals receiving NT201 lost weight. The animals that died early were described as dehydrated and emaciated. An initial increase in weight was noted, followed by a small reduction in both the BOTOX and NT201 groups. Following the third injection for both groups, body weight began to increase slightly.

Antibody induction: No results.

Necropsy: All animals were sacrificed on day 78, 2 weeks after the final immunization. No gross changes were noted for either treatment group, except reduced mass of the injected muscle.

Histopathology: Tissue was retrieved for limited histopathology evaluation (if necessary).

- Kidney
- Cervical lymph node
- Mesenteric lymph node
- Spleen

Histology was not performed, except for injection sites. Mild inflammatory changes were observed for both groups, with minimal to mild immune cell infiltration.

Conclusions:

Although this study was intended as an antigenicity study, no immunogenicity data were included in this report.

The value of this study to support safety of NT201 is questionable.

•••

Study # (b) (4) 12444/99

Title: Comparative antigenicity of three preparations of botulinum toxin A in the rabbit

Testing Facility:

(b) (4)
(b) (4)

GLP Compliance:

Yes.

Date of Study Initiation:

Lot #: NT201- 8/09/02 and 01001

BOTOX - CS239, C213 and C227

DYSPORE - 209A

Methods:

Animals: Rabbit (New Zealand White), female

Number/groups: 20/group

Age/weight: approximately 3.5 months old/2.8 – 3.7 g

Doses: NT201- 16 and 25 U/animal

BOTOX- 16 and 25 U/animal

DYSPORE - 40 and 20 U/animal

Route of administration: Intracutaneous injection

Regimen: 4 injections/immunization cycle

Study design: The doses were established based on the results of earlier studies (11252/1/98 and 10486/1/97). *Note: Study 11252/1/98 is a mouse study in which NT201 was administered i.v., and study 10486/1/97 can not be located in the BLA submission.*

Thus, the rationale for choice of dose levels in the current study can not be verified.

The basic study design is summarized in the table below, taken from the study report. (TW=test week) NT201 and BOTOX were injected once during TW 0, 2, 4, 6, 8, 13, 21, 23 and 33. DYSPORT was injected once in TW0, 2, 4, 6, 8 and 13.

Group	TW	Test substance	Dose level (LDU/animal, i.c.)	Animal no.
1	0 - 23	NT 201	16	1 - 20
	33		25	
2	0 - 23	Botox®	16	21 - 40
	33		25	
3	0 - 8	Dysport®	40	41 - 60
	13		20	

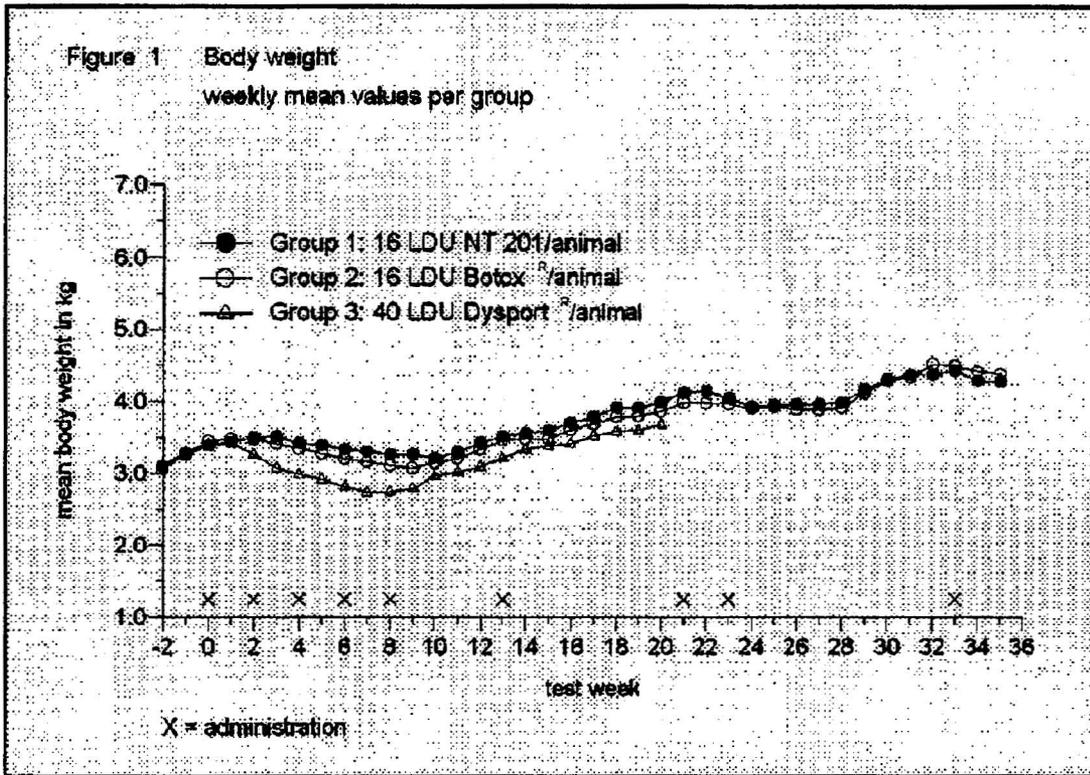
- The 6th injection was delayed until week 13 for NT201 and BOTOX in order to allow the DYSPORT animals to recover sufficiently. At that time, the DYSPORT dose was reduced from 40U/animals to 20U/animal due to marked body weight loss. DYSPORT treatment was stopped in TW 21 due to development of anti-drug antibodies 2 weeks after the 6th injection.

Observations/Results:

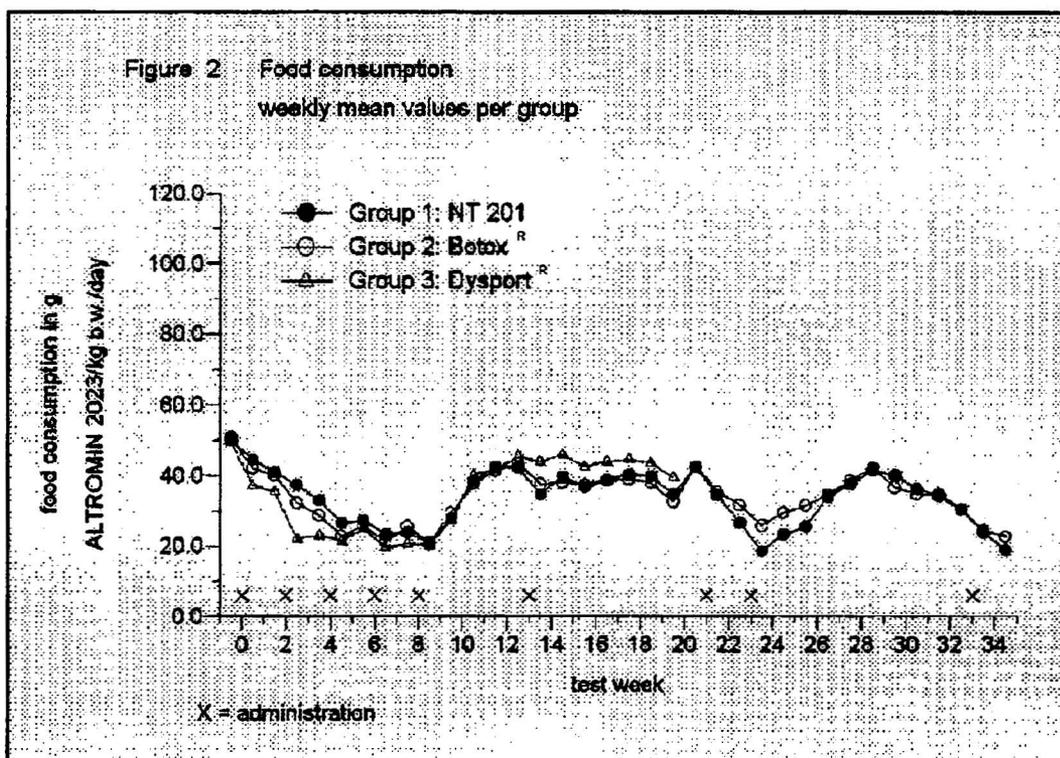
Mortality: No unscheduled deaths occurred.

Body weight: Recorded prior to initiation of dosing and weekly thereafter.

- For NT201-treated animals, in general, mean body weight was slightly reduced beginning after the 3rd injection and persisting through the dosing period. During the longer intervals between doses, body weight gain was noted but was subsequently reduced after the next dose. The body weight data are summarized in the figure below, taken from the study report. (Similar trends were observed for all 3 products.)



- *Food consumption:* Recorded daily
 - For NT201 (as well as BOTOX and DYSPORT), the reductions in body weight were correlated with reduced food intake. Food consumption data are summarized in the figure below, taken from the study report.



Clinical observations:

- Impaired locomotion was observed in all groups. Two of 20 in the NT201 group, 7 of 20 in the BOTOX group, 20 of 20 in the DYSPORT group.

Injection site reactions: Inflammation at the injection site was observed for BOTOX (15 of 16 animals) and NT201 (16 of 20 animals). No local changes were noted for any animals that received DYSPORT.

Immunogenicity: Samples were collected from groups 1 and 2 during weeks 7, 12, 15, and 25. Group 3 was sampled during weeks 7, 12 and 15. The final samples were collected during week 21 for group 3 and week 36 for groups 1 and 2.

- No anti-drug antibody development data were found in this study report.

Conclusions:

Comparisons of NT201 activity to other products are not relevant for the purpose of this review. The value of this study to support safety of XEOMIN is questionable as no immunogenicity data were included in the study report and the justification for the choice of dose levels cannot be verified.

11. Integrated summary and safety evaluation:

NT201 consists of a recombinant preparation of botulinum toxin Type A, a neurotoxin classified as a muscle relaxant that selectively inhibits acetylcholine release from cholinergic nerve terminals. The clinical route of administration is typically

intramuscular (i.m.) injection in very small amounts in order to minimize the potential for systemic and local spread of the toxin. Due to the extreme toxicity of botulinum toxin, standard pharmacokinetic analyses are not considered feasible. Systemic spread of the toxin (below the level of detection by current assays) is reflected in atrophy of muscles distant to the injection site and autonomic effects such as reduced sweating and midriasis. At very high i.m. doses, systemic levels (still below the level of detection) can become sufficient to cause respiratory distress and failure.

The pharmacology and toxicology of NT201 was evaluated in a series of nonclinical studies in rats, mice, rabbits and nonhuman primates. The pivotal studies included safety pharmacology, single and repeat-dose toxicology (with the longest duration of 9 months in non-human primates), fertility, embryofetal developmental toxicology in rats and rabbits, and local tolerance for eye irritation. The route of administration for all pivotal studies was intramuscular injection (except eye irritation where NT201 was instilled directly into the eye) and doses were given as units of activity (U)/kg.

In general, effects observed in all studies were consistent with expected results of the known pharmacological activity of botulinum toxin including atrophy of the treated muscle accompanied by impaired locomotion and reduced body weight gain generally correlating with reduced food intake, which became severe at the higher doses.

Safety Pharmacology: Safety pharmacology studies conducted with NT201 included cardiovascular and behavioral assessments as well as a standard hERG assay. No comprehensive assessment of respiratory function was conducted. No adverse effects related to NT201 were observed in safety pharmacology studies other than the expected results of the known pharmacological activity of botulinum toxin.

General toxicology:

Single-dose toxicology studies were conducted in CD-1 mice with doses up to 150 U/kg. These studies were designed as LD₅₀ assays to establish unitage or to identify and compare lethal doses of NT201 to other botulinum products. However, comparisons of NT201 activity to other botulinum toxin products are not relevant and the activities of products other than NT201 were not considered in this review. No unexpected adverse effects were observed in single-dose studies.

Repeat-dose toxicity studies of varying duration conducted with NT201 included the following:

- A 13-week study in cynomolgus monkeys with doses up to 16 U/kg administered monthly (4 doses)
- A study in CD-1 mice with doses up to 0.64 U/mouse administered at 6 week interval for a total of 3 doses
- A 9-month study with a 6-month recovery in cynomolgus monkeys with doses up to 12 U/kg administered every 4 weeks (10 doses)
- A pilot dose-ranging study in juvenile rats with doses up to 30 U/kg administered every 7 or 14 days (5 or 3 doses)
- A pilot dose ranging study in non-pregnant rabbits with doses up to 40 U/kg administered at 14-day intervals (3 doses)

The dose-related effects were generally limited to the results of the known pharmacological activity of NT201. These findings included atrophy of the injected muscle (up to 50-60% reduction in muscle weight, relative to control) with associated locomotor impairment and reduced body weight gain that was generally correlated with reduced food intake. In several studies, evidence of systemic spread of the toxin was observed. For example, in the 9-month study in cynomolgus monkeys, atrophy of muscles distant to the injected muscles was observed and, in a mouse study (11252/1/98), ptosis, reduced body temperature, pilo-erection, mydriasis, lacrimation and respiratory failure were observed at the high doses.

In the 9-month study, a small but dose-related reduction in mean heart weight (absolute and relative) was observed for all treated groups. Although there were no in-life or microscopic correlates for the reduced organ weight, this finding is of some concern due to the dose-related nature. No differences in heart weight were noted at the end of the recovery period.

Reproductive and developmental toxicology:

Fertility:

The effects of NT201 on fertility were assessed in rabbits. Other than the adverse effects due to the known pharmacological activity of NT201, results showed a possible increase in failure to mate in treated male animals (1, 2, 3, 2 for the control, low, intermediate and high dose groups, respectively). The data include the 2 (of 20) high dose males who died early. A small increase in pre-coital interval was also noted. All other parameters that were analyzed showed no significant effects of test article under the conditions of this study.

Embryofetal development: The effects of NT201 on embryofetal development when administered during the period of organogenesis were evaluated in rabbits and rats. In general, the dams displayed the muscle atrophy and paresis and reduced body weight gain, consistent with NT201 pharmacological activity.

The apparently dose-related effects on pregnancy and litter parameters were small, but were consistent with findings observed in embryofetal studies with other botulinum products. These effects included:

- A small increase in early resorptions in a pilot study in pregnant rats that received 30 U/kg 3 times during organogenesis.
- A small increase in pre-implantation loss (very early resorption) in the group that received 18 U/kg twice per week in the pivotal rat embryofetal study.
- In rabbits, a small reduction in the number of implantation sites per animal was observed, consistent with pre-implantation loss (10.2 in the control group, 8.5 in the high dose group).
- A trend toward slightly reduced fetal weight in rats and rabbits.
- In rabbits, a small reduction in the number of live fetuses per animal was noted (9.1 in the control group, 7.8 in the high dose group).

- An increased rate of abortions in rabbits (5 of 22 dams aborted on GD23-29).
- A small increase in the incidence of incomplete or lack of ossification, particularly in vertebrae and sternebrae, was observed relative to control.

Pregnancy category C is recommended due to the early resorptions and the trends observed in adverse fetal effects.

- No pre and postnatal developmental toxicology study was conducted with NT201. The sponsor's rationale included the following points:
 - Because the botulinum toxin molecule is large (150 kD), it is not expected to cross the placental barrier.
 - Systemic exposure to Xeomin is very low.
 - Results of embryofetal development studies show not adverse fetal effects (Sponsor's opinion).
 - The exposure to Xeomin in the milk of lactating dams should be minimal (maximum of 0.03 pg/injection).

The rationale is not sufficient to justify not conducting a nonclinical pre-and post-natal development study. Although the systemic exposure after administration of Xeomin is below the level of detection, this molecule is highly toxic. Adverse systemic effects were noted in multiple nonclinical studies and dose related adverse effects were observed in the embryofetal development studies including reduced fetal weight and delays in ossification. Although it is generally believed that most molecules larger than 1000 Da will not pass the placental barrier, data specific to Xeomin have not been provided. In addition, adverse effects on fetal development can occur without direct fetal exposure. Therefore, a nonclinical pre- and post-natal development study should be conducted.

The absence of this study should not preclude approval at this time, but a post-marketing requirement for conducting a pre- and postnatal developmental toxicity study is recommended.

Special toxicology: Special toxicology studies included an *in vitro* assessment of the hemolytic potential of NT201 in human blood and an assessment of the potential for eye irritation in rabbits. No hemolytic potential or irritation was noted. Special toxicology also included two antigenicity studies comparing NT201 with other marketed botulinum toxin products. These studies, as well as early single dose studies that compared activity among the three products, were conducted early in product development before the sponsor was aware that such comparisons would not provide useful data for potential approval of NT201.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 125360 Applicant: Merz

Stamp Date: 7/1/2009

Drug Name: Xeomin, NT-201 NDA/BLA Type:

On initial overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?		X	No pre/postnatal toxicology study has been submitted. The requirement for this study was discussed at 2 separate meetings including the pre-BLA meeting. The sponsor was asked to include justification for not conducting a prenatal and postnatal development study with the BLA. Adequacy of the justification will be a review issue.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).			N/A
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?	X		The route of administration for clinical use is I.M. Nonclinical studies using the I.M. route are provided. Additional studies using oral and I.V. routes were also conducted but are not relevant to the intended clinical use.
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	X ¹		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?			N/A

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA/BLA or Supplement**

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?		X ²	
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X ³		
11	Has the applicant addressed any abuse potential issues in the submission?		X ⁴	
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?		X ⁵	

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? ___ Yes ___

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

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Notes:

1. Each pivotal study report contains appropriate documentation of GLP compliance.
2. Not applicable to a BLA. The proposed labeling will require revision.
3. No impurity issues have been identified at this time.
4. Not applicable to a BLA.
5. Not applicable to a BLA

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Barbara J. Welby

 Reviewing Pharmacologist

8/5/09

 Date

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA/BLA or Supplement**

Ari M. Lind
Team Leader/Supervisor

8/5/09
Date