

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
125274

PHARMACOLOGY REVIEW(S)

Comments on BLA 125286

From: A. Jacobs, AD for Pharm/tox
Date: April 17, 2009

AJ
4/17/09

I have reviewed the pharm/tox review and the supervisory memo.

I agree that there are no outstanding pharm/tox issues.

I agree that category C as a pregnancy category is appropriate.

The multiples of the human exposure for doses administered to animals in the proposed labeling are based on another indication for which higher doses are given to humans.

I have discussed these and previous comments with the reviewer and supervisor and they can address the comments as they consider appropriate

**APPEARS THIS WAY
ON ORIGINAL**

Memorandum

To: File

From: Jill C Merrill
Through: Barbara Hill, Supervisor*Jill Merrill 4.16.09*
Barbara Hill 4-16-09

Re:

BLA No.: 125286

Drug: Formerly: Botulinum Type A Toxin-Hemagglutinin Complex
Currently: Abobotulinumtoxin AProduct: Dysport®
Indication: treatment of glabellar lines
Sponsor: Ipsen (Biomeasure Inc., U.S. Agent)
Receipt date: 3-12-08
PDUFA date: 4-13-09

Reviewer's note: The original PDUFA date was extended by 3 months due to the submission of a major amendment.

Background:

The sponsor has been informed that their previously proposed tradename for the glabellar lines indication, Reloxin, is unacceptable. The Agency has decided the best way to manage this drug product is with one tradename, DYSPORT. In addition, it has been decided that the established name will be Abobotulinumtoxin A and not Botulinum Type A Toxin-Hemagglutinin Complex. However, these two drugs are one and the same and the reviews written for Botulinum Type A Toxin – Hemagglutinin Complex stand in support of Abobotulinumtoxin A.

After discussion with DNP, a decision was made to take a joint approval action for DYSPORT for DNP's indication of cervical dystonia and DDDP's indication of glabellar lines. The action is targeted for April 29, 2009. There will be one label (below), one MedGuide and one communication plan for this drug product.

DYSPORT Nonclinical Labeling Issues:

Given that the two indications share the same active ingredient and route of administration, the studies conducted to support the nonclinical aspects of the label are also the same for both indications. However, the maximum recommended human dose (MRHD) of DYSPORT is dependent on the particular indication. For the cervical dystonia indication the MRHD is 1000 Units/ 50 kg and for the glabellar lines indication the MRHD is 50 Units/ 50 kg. Thus the MRHD is 20-fold higher for the cervical

dystonia indication than the glabellar lines indication; therefore for purposes of comparison of the animal exposure to human exposure, the multiples of exposure also differ 20-fold, with the multiple being higher for the glabellar lines indication. It was decided that the best way to convey this information in a single joint label was to base the multiples of exposure on the worst case scenario, i.e., the cervical dystonia indication. Consequently the DDDP Pharmacology/Toxicology proposed label has been modified from the 1-23-09 version. The pharmacology/toxicology portions of the currently proposed joint label appear below.

Pharmacology/Toxicology Proposed Label:

8 USE IN SPECIFIC POPULATIONS

8.1 PREGNANCY: CATEGORY C

DYSPORT produced embryo-fetal toxicity when given to pregnant rats at doses similar to or greater than the maximum recommended human dose (MRHD) of 1000 Units on a body weight (Units/kg) basis.

In an embryo-fetal development study in which pregnant rats received intramuscular injections daily (2.2, 6.6, or 22 Units/kg on gestation days 6 through 17) or intermittently (44 Units/kg on gestation days 6 and 12 only) during organogenesis, increased early embryonic death was observed with both dosing schedules. The no-effect dose for embryo-fetal developmental toxicity was 2.2 Units/kg (one-tenth the MRHD on a body weight basis). Maternal toxicity was seen at 22 and 44 Units/kg. In a pre- and post-natal development study in which female rats received 6 weekly intramuscular injections (4.4, 11.1, 22.2, or 44 Units/kg) beginning on day 6 of gestation and continuing through parturition to weaning, an increase in stillbirths was observed at the highest dose, which was maternally toxic. The no-effect dose for pre- and post-natal developmental toxicity was 22 Units/kg (approximately equal to the MRHD on a body weight basis).

There are no adequate and well-controlled studies in pregnant women. DYSPORT should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

13 NONCLINICAL TOXICOLOGY

13.1 CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY

Carcinogenicity

Studies to evaluate the carcinogenic potential of DYSPORT have not been conducted.

Mutagenicity

Genotoxicity studies have not been conducted for DYSPORET.

Impairment of Fertility

In a fertility and early embryonic development study in rats in which either males (2.9, 7.2, 14.5, or 29 Units/kg) or females (7.4, 19.7, 39.4, or 78.8 Units/kg) received weekly intramuscular injections prior to and after mating, dose-related increases in pre-implantation loss and reduced numbers of corpora lutea were noted in treated females. Failure to mate was observed in males that received the high dose. The no-effect dose for effects on fertility was 7.4 Units/kg in females and 14.5 Units/kg in males (approximately one-half and equal to, respectively, the maximum recommended human dose of 1000 Units on a body weight basis).

APPEARS THIS WAY
ON ORIGINAL



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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

BLA NUMBER:	125286
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	3/12/08
PRODUCT:	Reloxin®
INTENDED CLINICAL POPULATION:	patients with moderate to severe glabellar lines
SPONSOR:	Ipsen (Biomeasure Inc., U.S. Agent)
DOCUMENTS REVIEWED:	electronic documents
REVIEW DIVISION:	Division of Dermatology and Dental Products
PHARM/TOX REVIEWER:	Jill C Merrill <i>Jill Merrill 2-4-09</i>
PHARM/TOX SUPERVISOR:	Barbara A. Hill <i>Barbara Hill 2-4-09</i>
DIVISION DIRECTOR:	Dr Susan Walker
PROJECT MANAGER:	Tamika White

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EXECUTIVE SUMMARY

I. Recommendations

- A. Recommendation on approvability – From a pharmacological/toxicological perspective, Reloxin® is approvable for patients with moderate to severe glabellar lines.
- B. Recommendation for nonclinical studies – The sponsor will be required as a post-marketing commitment to repeat the pivotal rabbit embryofetal study, which is considered inadequate.
- C. Recommendations on labeling – Acceptable with modifications including a change to a Pregnancy Category C. Recommended modifications for labeling are located at the end of this review in the “Overall Conclusions and Recommendations” section.

II. Summary of nonclinical findings

- A. Brief overview of nonclinical findings - The sponsor has submitted studies to satisfy the Division’s request for evaluation of both the local and systemic effects of chronic dosing. This includes an evaluation of the effects of repeat dosing at the neuromuscular junction. Three intramuscular injections of Reloxin at 4-week intervals (dose levels of 0.1 or 2 U/injection) in female rats induced a reduction in fiber size in the gastrocnemius muscle. The related clinical observation was an apparent shrinkage of the injected muscle and reduced locomotory activity. At 13 weeks following the third injection recovery was incomplete, but had completely resolved by 26 weeks post injection.

No systemic toxicity was observed after single (up to 6 U/rat) or repeat (up to 10 U/rat, up to 20 U/rabbit) intramuscular dosing. These studies are reviewed in the appropriate section below, but changes were limited to decrease in body weight gain associated with decreased feed consumption in the first week(s) following the injection. This was accompanied by a reduction in the weight of the injected gluteus muscles which was considered to be related to an exaggerated pharmacological action of the test item. There were no compound-related lesions of the muscle groups distant to the injection sites or of the peripheral or central nervous systems.

Given Reloxin’s structure and mechanism of action, neither genetic toxicology or carcinogenicity studies were required.

The sponsor has submitted all required reproductive and developmental toxicology studies (fertility in rats, embryotoxicity in rats and rabbits, peri/postnatal in rats). Although not statistically significantly different from

controls, treatment with botulinum was associated with an increase in pre-implantation loss during the fertility study conducted in rats. Rabbits treated daily with botulinum toxin during embryofetal development also had an increase (not statistically significant) in pre-implantation loss and a tendency for an increase in incomplete ossification (2nd and 4th sternebrae) relative to controls, which achieved statistical significance when does were treated intermittently. A tendency for an increase in pre-implantation loss was also noted in rats as was a tendency for increases in unossified 6th sternebrae. Botulinum toxin was associated with an increase in stillbirths in rats. The rabbit embryofetal study was considered inadequate due to unexpected deaths at the high dose. The sponsor will be required to repeat the rabbit embryofetal study using a maximum dose of 20 SU/day during organogenesis and a flagellin level consistent with the drug product specification (i.e., —) as a post-marketing commitment. Based on the submitted labeling, the sponsor anticipates a Pregnancy Category B. BOTOX®, a similar botulinum type A toxin marketed by Allergan for glabellar lines (BLA 103,000), currently carries a Pregnancy Category C. Pharmacology/Toxicology recommends a Pregnancy Category C for Reloxin.

- B. Pharmacologic activity - Reloxin® will be administered by intramuscular injection producing inhibition of acetylcholine release and subsequent chemical denervation of the muscles responsible for glabellar frown lines.
- C. Nonclinical safety issues relevant to clinical use – Botulinum toxin type A hemagglutinin complex has an acceptable safety profile when administered at the appropriate dose by intramuscular injection. However, accidental systemic exposure (inadvertent intravenous injection) could cause systemic toxicity. Signs include vision disturbances, dysphagia, followed by descending paralysis, hypotension, and respiratory failure as early as 24 hours after exposure. Treatment involves respiratory support and prompt antitoxin administration. At present (11-19-08) the sponsor is seeking a — flagellin specification for the drug product. The maximum level of flagellin contamination qualified by the reproductive toxicology studies is —%. The maximum level of flagellin qualified by the repeat-dose study to examine the effects at the neuromuscular junction is no more than —. This may be an overestimation of the relative flagellin contamination because flagellin is measured by SDS-PAGE and co-elutes with other small peptides, including nontoxic nonhemagglutinin. Therefore the sponsor will be asked to perform the repeat embryofetal study with drug product containing at least —, 'flagellin' contamination. The pharmacology/toxicology recommendation for the final drug product specification should be no higher than the maximum amount qualified nonclinically (i.e., — % flagellin), but definitely less than —%.

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2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

BLA number: 125286

Review number: 1

Sequence number/date/type of submission: 0000/03-12-08/original submission

Information to sponsor: Yes

Sponsor and/or agent: Ipsen (Biomeasure, Inc., - U.S. Agent)

Manufacturer for drug substance: IPSEN Biopharm Ltd.,
Wrexham Industrial Estate
Ash Road
Wrexham, LL13 9UF, UK

Reviewer name: Jill C Merrill

Division name: Dermatological and Dental Drug Products

HFD #: 540

Review completion date: 1-23-09

Drug:

Trade name: RELOXIN®

Generic name: Botulinum Type A Toxin-Hemagglutinin Complex

Code name: CNT52120

Molecular formula/molecular weight: Botulinum type A neurotoxin is produced by *Clostridium botulinum* as a 150 K Da single polypeptide chain composed of 1296 amino acid residues. After synthesis the neurotoxin is proteolytically cleaved to generate a dichain protein composed of a B chain (100 K Da) and an A chain (50 K Da).

Structure: The primary structure of the *Clostridium botulinum* type A neurotoxin has been deduced by nucleotide sequence analysis. The deduced amino acid sequence exhibits 33% similarity to tetanus toxin with the most highly conserved regions occurring between the N-terminal of the respective heavy chains. Residue Cys⁴³⁰ participates in the interchain disulphide bond but plays no part in the toxicity of the neurotoxin.

Relevant INDs/NDAs/DMFs:

Ipsen: BB IND 10673 Reloxin® (Botulinum Toxin Type A hemagglutinin Complex), glabellar lines

Ipsen: BB IND 7434 Dysport® (Botulinum Toxin Type A hemagglutinin Complex), cervical dystonia

Allergan: BB IND 6432 BOTOX® (Botulinum Toxin Type A), blepharospasm and strabismus

Allergan: BLA 103,000 BOTOX® (Botulinum Toxin Type A), glabellar lines (approved 4-15-02)

Drug class: peripheral muscle relaxant

Intended clinical population: adult patients with moderate to severe glabellar lines

Clinical formulation:

The CNT52120 Drug Product contains the active ingredient *Clostridium botulinum* toxin Type A hemagglutinin complex (CNT52120 BAS) with either 500 LD₅₀ Units/vial (500 U/vial) or 300 LD₅₀ Units/vial (300 U/vial). The preparation is presented as a white lyophilized powder for injection. CNT52120 Drug Product 500 U/vial presentation is reconstituted with 1.0 mL of sodium chloride for injection (0.9% w/v) to yield a solution containing 500 LD₅₀ Units/mL of CNT52120 Drug Product. CNT52120 Drug Product 300 U/vial presentation is reconstituted with 2.5 mL of sodium chloride for injection (0.9% w/v) to yield a solution containing 120 LD₅₀ Units/mL of CNT52120 Drug Product. Both presentations contain an identical excipient composition and differ only in either 3 or 5 ng CNT52120 BAS per vial.

Ingredients	Quantity (Dosage form)		Function
	300 U	500 U	
Active Substance			
CNT52120 BAS	300 LD ₅₀ units	500 LD ₅₀ units	Active Substance
Excipients ^a			
Albumin human	125 µg	125 µg	
Lactose monohydrate	2.5 mg	2.5 mg	

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Route of administration: intramuscular injection

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:

The effect of varying doses of Dysport® on muscle force development (TA/03-977)

A study to investigate and compare the effect of two neuromuscular blocking agents (report # 7/950876)

Clostridium botulinum toxin type A hemagglutinin complex- Single dose intramuscular toxicity study with 12-week follow-up in the rat (— AA40423)

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Clostridium botulinum toxin type A hemagglutinin complex – Sub-chronic toxicity study (6 intramuscular injections at 4-week intervals) in the rat (— AA40095)

Botulinum toxin type A hemagglutinin complex (Dysport®)- Embryotoxicity study by the intramuscular route in the rabbit (— AA28028)

Botulinum toxin type A hemagglutinin complex (Dysport®)- Embryotoxicity study by the intramuscular route in the rat (— AA28029)

Botulinum toxin type A hemagglutinin complex (Dysport®)- Preliminary embryotoxicity study by daily intramuscular injection in the pregnant rabbit (— 434/363 RE)

Botulinum toxin type A hemagglutinin complex (Dysport®)- Preliminary embryotoxicity study by two sequential intramuscular administrations in the pregnant rabbit (— 434/364 RE)

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Botulinum toxin type A hemagglutinin complex (Dysport®)- Preliminary study after two intramuscular administrations in the non-pregnant female rabbit (— 434/359 RE)

Botulinum toxin type A hemagglutinin complex (Dysport®)- 14-Day preliminary study intramuscular administration in the non-pregnant female rabbit (— 434/360 RE)

Botulinum toxin type A hemagglutinin complex (Dysport®)- Preliminary embryotoxicity study by daily intramuscular injection in the pregnant rat (— 434/361 RE)

Botulinum toxin type A hemagglutinin complex (Dysport®)- Preliminary embryotoxicity study by sequential intramuscular administrations in the pregnant rat (— 434/362 RE)

Botulinum toxin type A hemagglutinin complex (Dysport®)- Preliminary study after three intramuscular administrations in the non-pregnant female rat (— 434/358 RE)

Botulinum toxin type A hemagglutinin complex (Dysport®)- 14-Day preliminary study intramuscular administration in the non-pregnant female rabbit (— 434/357 RE)

Clostridium botulinum toxin type A hemagglutinin complex- Single dose intramuscular toxicity study with 12-week follow-up in the rat (AA40423)

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Clostridium botulinum toxin type A hemagglutinin complex- Sub-chronic toxicity study (6 intramuscular injections at 4-week intervals) in the rat (AA40095)

AA38304-D: *Clostridium botulinum* toxin type A hemagglutinin complex – fertility toxicity study by the intramuscular route in the rat (Segment I).

AA38305-D: *Clostridium botulinum* toxin type A hemagglutinin complex – Pre- and post-natal development study by the intramuscular route in the rat (Segment III).

AA42572: Method development program for visualization of neuromuscular junctions

AA60533: Dysport (*Clostridium botulinum* toxin type A hemagglutinin complex) - Three-month intramuscular toxicity study with 13- and 26-week follow-up in the rat.

Studies not reviewed within this submission:

The following studies have been reviewed by Dr. Jill Merrill under BB-IND 10673, SN210:

434/199: Dysport botulinum type A toxin – Single dose administration in the beagle dog

204323: Test to evaluate acute ocular irritation and reversibility in the rabbit

TA/04-1055 A comparison of 500 Unit vials of *C. botulinum* type A toxin hemagglutinin complex prepared with bulk active substance 96/002, VPU/2002/006 and WBAS/001 on muscle force development

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Reviewer's comment: Clostridium botulinum toxin Type A hemagglutinin complex is identified as Reloxin® when used to treat glabellar lines, and is identified as Dysport® when used to treat cervical dystonia. One unit of Reloxin®/Dysport® (U) corresponds to the calculated median lethal intraperitoneal dose (LD₅₀) in mice. The method for performing the assay is specific to Ipsen's product. Due to differences in specific details such as vehicle, dilution scheme, and laboratory protocols for various mouse LD₅₀ assays, units of biological activity of Ipsen's drug product are not interchangeable with units of any other botulinum toxin or any toxin assessed with any other specific assay method.

Botulinum toxin type A is a protein product of *Clostridium botulinum* and is one of seven antigenically distinct serotypes (A to G). It is synthesized as a single-chain polypeptide (~ 150 KDa) that is transformed by a protease into a dichain consisting of a heavy, or B chain (~100 KDa) linked by a disulfide bond to a light, or A chain (~50 KDa); the dichain is the active form. Botulinum toxin is taken up by the nerve terminal when it

binds to synaptic vesicle protein 2 (SV2) that is transiently accessible on the synaptic surface after fusion of the vesicle (Dong et al., 2006). When the vesicle is recycled, botulinum toxin type A acquires access to the cytosol. The A chain, which is a zinc-dependent protease, is then released and subsequently cleaves its target protein, SNAP-25, which is necessary for vesicle fusion. Neurotransmission is thus inhibited. Although Botulinum toxin type A acts preferentially on cholinergic nerve endings, at higher concentrations it can also block release of norepinephrine, serotonin, GABA, glycine, and met-enkephalin (MacKenzie et al., 1982).

Botulinum toxin can undergo retrograde transport in the nerve, but it is not known whether it retains its activity or is metabolized. Studies with ^{125}I -labeled botulinum have demonstrated transynaptic transport at very low levels, but it is not known whether it was the intact protein or its breakdown products that were transported.

Botulinum toxin type A is so potent that the minimum concentration for toxicity at the cellular level has not been established. Botulinum toxin poisoning in humans is usually by the oral route and occurs when food contaminated with *Clostridium botulinum* is ingested. Unidentified transporters in the upper GI tract are thought to facilitate transfer of the toxin to the blood stream. Death occurs in a matter of days by respiratory arrest, presumably by paralysis of the phrenic nerve. Botulinum toxin does not cross the blood brain barrier and does not produce CNS toxicity.

Botulinum toxin is used therapeutically primarily to treat dystonias. It is administered by multiple small injections directly to the affected muscle. Efficacy is long-lasting (weeks to months), but efficacy can eventually diminish if neutralizing antibodies are produced. The observed safety of injected botulinum toxin type A is due to the fact that (a) there is a low probability of accidental systemic injection when botulinum toxin type A is administered to superficial musculature in small amounts by an experienced professional and (b) diffusion out of the tissue is slow so pharmacological effects tend to be local.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: Reloxin® is administered by intramuscular injection and exerts its paralytic action by binding to presynaptic cholinergic nerve terminals at the neuromuscular junction. The SNAP-25 protein is essential to the docking and fusion of synaptic vesicles with the presynaptic membrane, resulting in the release of acetylcholine at the synaptic terminal. The toxin works within the nerve ending to antagonize those events that are triggered by Ca^{+2} which culminate in transmitter release. It does not affect postganglionic cholinergic transmission or postganglionic sympathetic transmission.

Although the various serotypes (i.e., A to G) and subtypes differ in their primary sequences and 3-dimensional structures, they all adhere to the same general scheme in producing blockade of neuromuscular transmission. This scheme includes three main events which are: a) cell surface binding; b) productive internalization; and c) intracellular blockade of exocytosis.

Initially the toxin binds to the presynaptic nerve membrane and undergoes internalization into endosomal-like compartments. This membrane translocation is facilitated by the heavy chain of the toxin. The toxin subsequently undergoes disulfide bond cleavage releasing zinc-endopeptidase light chain of the toxin which targets its enzymatic action on the synaptosome-associated SNAP-25 protein, a presynaptic membrane protein required for fusion of neurotransmitter-containing vesicles, resulting in the inhibition of acetylcholine exocytosis, diminishing the endplate potential and resulting in temporary paralysis of the muscle tissue.

Botulinum toxin has a remarkably long duration of action in animals and humans, the length of which is serotype-specific. Botulinum toxin type A has a duration of action that is measured in weeks-to-months. When transmitter release is blocked, the neuromuscular junction responds as though it has been denervated. Recovery occurs gradually as new nerve terminals sprout and contact is made with the post synaptic motor endplate, a process which takes 6-8 weeks in the experimental animal.

The mechanism(s) by which the light chain is metabolized and/or removed from the nerve ending is also a determinant of duration of action. No one has firmly established how intraneuronal toxin is disposed of: export, diffusion and dilution, cytosolic metabolism, lysosomal metabolism? It is likely that some or all of the toxin is degraded (e.g., proteosome or lysosome cleavage) and thus intact toxin probably does not leave the cell in amounts that would affect neighboring nerve cells.

The sponsor has conducted the following two studies in support of the primary pharmacodynamics.

Title: The effect of varying doses of Dysport on muscle force development (TA/03-977)
Paper format, no raw data

The aim of this study was to determine the effects of varying doses of intramuscular Dysport (0 U/kg, 3 U/kg, 6 U/kg, 12 U/kg, and 18 U/kg) on the muscle force development in both the injected gastrocnemius muscle and the muscles of the contralateral hindlimb of the rat. Muscle force of the triceps surae group (gastrocnemius, plantaris and soleus) was measured before injection and after injection at 2, 24, 48, 72, and 96 hours. Additional measurements were also made at 7, 10, 18, 25, 32, 39, 46, 53, 60, 67, and 74 days post-injection. Body weights were recorded at the same time intervals.

Dysport induced a significant reduction on force generation of the injected hindlimb, but without a dose relationship. The decline in force was 27, 25, 22, and 10% of control values at 3, 6, 12, and 18 U/kg, respectively) and remained at these levels for 7-24 days before force generation began to recover. At 68 days post injection, the force generation by the muscle had recovered to 70, 55, 50 and 41% of the control force (3, 6, 12, and 18 U/kg, respectively). The 3 U group recovered faster than the 18 U group ($p < 0.05$) but there were no differences between the other treatments. The systemic effect of Dysport was evaluated by measuring the force generation in the contralateral hindlimb muscle.

The maximal decline in force, expressed as a percent of the original force values, was 53, 40, 28, and 27% (3, 6, 12, and 18 U, respectively). The reduced force plateaued at ~10 days and started to recover ~20 days post-injection.

Body weight gain was transiently decreased at 3 U/kg throughout the post injection period, whereas 6, 12, and 18 U/kg groups lost body weight during the 10-20- day post injection period. The weight of the gastrocnemius muscle ranged from 16% to 33% of controls in the 3 to 18 U/kg groups, respectively. The weight of the plantaris muscle ranged from 28 to 20%, and the weight of the soleus muscle ranged from 54 to 27% of control in the 3 to 18 U/kg groups, respectively. The weight of the gastrocnemius and soleus muscles in the contralateral limb was decreased mainly at 18 U/kg, around 77% of controls, whereas the weight of the contralateral soleus was not affected.

In conclusion, Dysport had a predictable effect on skeletal muscle mass and force generating ability at all doses. At the higher doses, the effect caused significant change in body mass.

Title: A study to investigate and compare the effect of two neuromuscular blocking agents (report # 7/950876)

The aim of this study was to assess and compare the effects of two neuromuscular blocking agents on glycogen depletion in rats following electrical stimulation of the sciatic nerve.

Dysport and Botox inhibited glycogen depletion in rat tibialis anterior and digitorum longus muscles following electrical stimulation of the sciatic nerve, generally in a dose-dependent manner. The profiles of activity of the two agents were very similar.

When equipotent dose levels of the two test articles were compared no statistically significant differences in the glycogen levels in either the tibialis anterior or digitorum longus muscles were recorded. This indicated that the localized diffusion of the agents between the adjacent muscles was similar.

Drug activity related to proposed indication: Reloxin® will be administered by intramuscular injection producing inhibition of cholinergic neurotransmission and subsequent denervation of the muscles responsible for glabellar frown lines. This will result in localized paralysis of the affected muscles, thereby relaxing the frown lines.

2.6.2.3 Secondary pharmacodynamics

No studies on the secondary pharmacodynamics of Reloxin® have been conducted in animals.

2.6.2.4 Safety pharmacology

Clostridium botulinum toxin type A hemagglutinin complex administration is intended as a local treatment; therefore safety pharmacology studies are not considered appropriate for *Clostridium botulinum* toxin type A hemagglutinin complex.

2.6.2.5 Pharmacodynamic drug interactions

No studies on pharmacodynamic drug interactions of Reloxin® have been conducted in animals.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Not applicable.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Clostridium botulinum toxin type A hemagglutinin complex is an extremely potent neurotoxin injected directly into the targeted muscles wherein it exerts its paralytic action and therefore is considered a localized treatment. *Clostridium botulinum* toxin type A hemagglutinin complex is not expected to be present in the peripheral blood at measurable levels following intramuscular injection at the therapeutic dose. Based upon *Clostridium botulinum* toxin type A hemagglutinin complex high potency, low therapeutic dose, localized action and well published pharmacodynamic properties and mechanism of action, no studies aimed at assessing the pharmacokinetics of *Clostridium botulinum* toxin type A were conducted.

Likewise the sensitivity of most of the existing bioanalytical methods, including bioassay (mouse protection assay), is not sufficient to allow performance of pharmacokinetic studies (including pharmacokinetic, mass balance, distribution, metabolism, etc.) with *Clostridium botulinum* toxin type A hemagglutinin complex.

2.6.4.2 Methods of Analysis

Not applicable.

2.6.4.3 Absorption

Not performed.

2.6.4.4 Distribution

Not performed.

2.6.4.5 Metabolism

Not performed.

2.6.4.6 Excretion

Not performed.

2.6.4.7 Pharmacokinetic drug interactions

Not applicable.

2.6.4.8 Other Pharmacokinetic Studies

Not applicable.

2.6.4.9 Discussion and Conclusions

Not applicable.

2.6.4.10 Tables and figures to include comparative TK summary

Not applicable.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY –N/A**2.6.6 TOXICOLOGY****2.6.6.1 Overall toxicology summary**

General toxicology: Botulinum toxin type A is so potent that the minimum concentration for toxicity at the cellular level has not been established. Botulinum poisoning in humans is usually by the oral route and occurs when food contaminated with *Clostridium botulinum* is ingested. Unidentified transporters in the upper GI tract are thought to facilitate transfer of the toxin to the blood stream. Death occurs in a matter of days by respiratory arrest, presumably by paralysis of the phrenic nerve. Botulinum toxin does not pass the blood brain barrier and does not produce CNS toxicity.

Botulinum toxin type A is used therapeutically primarily to treat dystonias. It is administered by multiple small injections directly to the affected muscle. The current indication, temporary relief of glabellar lines, represents a cosmetic extension of this indication. As such the nonclinical studies have focused on its effects following intramuscular injection.

No systemic toxicity was observed after single (up to 6 U/rat) or repeat (up to 10 U/rat, up to 20 U/rabbit) intramuscular dosing. These studies are reviewed in the appropriate section below, but changes were limited to decrease in body weight gain associated with

decreased feed consumption in the first week(s) following the injection. This was accompanied by a reduction in the weight of the injected gluteus muscles which was considered to be related to an exaggerated pharmacological action of the test item. There were no compound-related lesions of the muscle groups distant to the injection sites or of the peripheral or central nervous systems.

A study conducted in dogs (434/199; reviewed under BB IND 10,673 SN 210 by Dr. Jill C Merrill) showed that *Clostridium botulinum* toxin type A hemagglutinin complex, when administered once by intramuscular (20 U/kg equivalent to approximately 200 U total dose), oral and percutaneous (20 U/kg equivalent to approximately 200 U total dose), routes or by continuous intravenous infusion (10 U/kg/h approximately 100 U/h/animals), did not induce signs of local or systemic toxicity. The results of this study indicate that the rat can be considered as more sensitive to the effects of *Clostridium botulinum* toxin type A hemagglutinin complex than the dog.

Genetic toxicology:

No genotoxicity studies have been conducted with *Clostridium botulinum* toxin type A hemagglutinin complex. *Clostridium botulinum* toxin type A hemagglutinin complex's biological origin, mechanism of action and its nature do not suggest that it interacts directly with DNA or any other chromosomal material and hence this biological product is not considered to have any genotoxic potential (conveyed to sponsor in a letter denying a meeting request, 10-6-06). Therefore, genotoxicity studies were not conducted.

Carcinogenicity:

No carcinogenicity studies have been conducted with *Clostridium botulinum* toxin type A hemagglutinin complex and this is in concurrence with the Division's comments to the sponsor (conveyed to sponsor in a letter denying a meeting request, 10-6-06). Reloxin® is administered by acute, infrequent, localized, intramuscular injections, given approximately every three months and may be given less frequently based on therapeutic response. As stated in the current ICH guidance documents on the subject, neither biological products nor products administered infrequently or for a short duration of exposure require carcinogenicity testing unless there is a cause for concern. As previously stated, Reloxin®'s structure, class, and biological origin do not suggest concern for any carcinogenic potential. Furthermore, the sponsor's extensive post-marketing clinical experience with Dysport has yielded no evidence of carcinogenicity.

Reproductive toxicology:

A complete set of reproductive studies (fertility, embryo-fetal and pre- and postnatal studies) was conducted to detect the potential effects of *Clostridium botulinum* toxin type A hemagglutinin complex on reproduction. Although not statistically significant from controls, treatment with botulinum was associated with an increase in pre-implantation loss during the fertility study conducted in rats. Rabbits treated daily with botulinum toxin during embryofetal development also had an increase (not statistically significant)

in pre-implantation loss and a tendency for an increase in incomplete ossification (2nd and 4th sternebrae) relative to controls, which achieved statistical significance when does were treated intermittently. A tendency for an increase in pre-implantation loss was also noted in rats as was a tendency for increases in unossified 6th sternebrae. An increase in stillbirths was also noted during the pre-natal and post-natal development study. As such, it seems appropriate for Reloxin® to carry Pregnancy Category C labeling.

Special toxicology:

The ocular irritation and reversibility of *Clostridium botulinum* toxin type A hemagglutinin complex was evaluated in the albino rabbit (← 204323, BB-IND 10673, SN210, reviewed by Dr. Jill Merrill). *Clostridium botulinum* toxin type A hemagglutinin complex was administered in saline at the dose-level of 200 U/mL (0.1 mL/animal) into the inferior conjunctival sac of the right eye of three New Zealand albino rabbits. The ocular examinations were performed in the conjunctiva, iris, and cornea, at 1, 24, 48, 72 hours and 7 days after administration. Special attention was paid to ptosis and/or the effects on ocular alignment. Neither ptosis nor any effect on ocular alignment was noted during the study.

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The sponsor has conducted a study in female rats to evaluate the effects of chronic dosing at the nerve terminals. This study consisted of three monthly intramuscular injections at two dose levels (0.1 and 2 U/injection) followed by a 13-week or 26-week recovery period. This treatment regimen induced a reduction of the fiber size in the gastrocnemius muscle and was observed clinically as an apparent shrinkage of the injected muscle and reduced locomotory activity. Most of the fibers had returned to their normal size after a 13-week recovery period. Recovery of the muscle fiber was essentially complete after 26 weeks, with full recovery of locomotory activity at 17 weeks post treatment.

2.6.6.2 Single-dose toxicity

Clostridium botulinum toxin type A hemagglutinin complex- Single dose intramuscular toxicity study with 12-week follow-up in the rat

The objective of this study was to evaluate the appearance and recovery of the histopathological changes in muscles and nerves following a single intramuscular administration of the test item, *Clostridium botulinum* toxin type A hemagglutinin complex to the Sprague-Dawley rat. Animals were injected with the placebo (control group) or with the test item (treated groups) at the dose levels of 2 or 6 units (U) in the left gluteus muscle (25 µL) and the right gluteus muscle received physiological saline (0.9% NaCl). The designated animals (6 animals/sex/group/day) were euthanized on days 7, 30, 60 or 90. Organ/tissues samples were fixed and preserved at necropsy with selected tissues (injection sites, muscles, nerves) examined histopathologically.

Group/Treatment	Dose Level (U/rat)	Dose Volume (μ L)	Dose Concentration (U/ μ L)	Number of Animals for Termination on:							
				Day 7		Day 30		Day 60		Day 90	
				M	F	M	F	M	F	M	F
1. Control	0	25	0	6	6	6	6	6	6	6	6
2. Low Dose	2	25	0.08	6	6	6	6	6	6	6	6
3. High Dose	6	25	0.24	6	6	6	6	6	6	6	6

There were no adverse clinical reactions and no unscheduled deaths. The males and females treated at 6 U showed a transient reduction in body weight gain over the week following treatment (-11% and -19% for males and females, respectively compared with controls). There were no systemic changes noted histopathologically. The only compound-related change was an area of reduced muscle fiber size in the gluteus muscle surrounding the injection site. No change was observed in the muscle treated with the placebo. The reduced fibers were observed earlier at 6 U (7 days after injection) than at 2 U (30 days after injection). The affected area of muscle was greater at 6 U. At the dose of 6 U, between 30 and 90 days after injection, the area affected became progressively smaller. On Day 90, fatty infiltration was visible replacing atrophic fibers. At the dose of 2 U, the extent of infiltration was generally limited and was only present at a low incidence on Day 90 indicating a return to a normal architecture. The muscle weights at the end of the study were similar to the controls at both dose levels, showing a compensatory change. No histopathological lesions in the peripheral nerves (sciatic tibial and brachial nerves and posterior root ganglia examined) were detected under the conditions of this study.

2.6.6.3 Repeat-dose toxicity

Study # 434/358 RE (taken in part from review by Barbara Wilcox)

Title: Botulinum toxin type A hemagglutinin complex (Dysport): Preliminary study after three intramuscular administrations in the non-pregnant female rat

GLP: yes

QA Report: Yes

Animals: Female Sprague-Dawley rats, 5/group

Dosing: 0.1, 1, 3 and 10 SU/animal/dose for 2 doses one week apart; the third dose levels were 20/40/60/80 SU/animal/dose

One week treatment-free period followed dosing

Reviewer's note: One Speywood unit (SU) corresponds to the LD₅₀ in the mouse following intraperitoneal injection.

Observations: Feed consumption (twice weekly), body weight (twice weekly), mortality (at least twice daily), clinical exams (daily), no necropsy performed.

Results:

As no treatment related signs were observed after two intramuscular injections for any dose level, the females were given a third administration with the higher dose levels. No mortality was noted in doses up to 20 SU/animal/administration. All animals showed paralysis of hindlimbs, decreased food intake, marked reductions in body weight gain. The animals receiving doses of 40 SU or above were sacrificed moribund within the week after administration of the third dose.

Although no clinical signs or effects on feed consumption were observed at the dose level of 10 SU/animal, the body weight gain at this level was 70% lower than in control animals. The NOAEL for Dysport under the condition of this study is 3 SU/animal/dose weekly for two weeks.

Reviewer's note: As written, the report incorrectly reports the doses as SU/kg/dose, but they are SU/animal/dose (i.e., 50 µL/animal/dose x 0.4 SU/µL = 20 SU/animal/dose).

Study # 434/357 RE (taken in part from review by Barbara Wilcox)

Title: Botulinum toxin type A hemagglutinin complex (Dysport): 14-day preliminary study by intramuscular administration in the non-pregnant female rat

GLP: yes

QA Report: Yes

Sprague-Dawley rat, female, 5 per group

Dosing: 0, 0.1, 1, 3 or 10 SU/animal/day, IM daily for 14 days followed by 7 day recovery period

Observations: Feed consumption (twice weekly), body weight (twice weekly), mortality (twice daily), clinical exams (daily), no necropsy or histopathology was performed.

Results:

No mortality in any group throughout the study

All 5 females treated with 10 SU/animal/day showed hind limb paralysis, decreased food consumption and marked body weight loss (-20% on study day 14).

No treatment-related clinical adverse effects were observed in animals treated with 3 SU/animal/day or less. However, body weight gain of those treated with 3 SU/animal/day was 37% lower than controls.

The NOAEL for Dysport administered under the conditions of this study (IM, daily) was determined to be 1 SU/animal/dose.

Reviewer's note: As written, the report incorrectly reports the doses as SU/kg/dose, but they are SU/animal/dose (i.e., 50 µL/animal/dose x 0.2 SU/µL = 10 SU/animal/dose).

Study title: *Clostridium botulinum* toxin type A hemagglutinin complex- Sub-chronic toxicity study (6 intramuscular injections at 4-week intervals) in the rat

Key study findings:

Rats treated with 6 intramuscular injections at 4-week intervals of doses of 1, 4, and 12 U had reduced weights of the injected gluteus muscles and those treated with 12 U had reduced terminal body weight related to reduced feed consumption. The observed atrophy in adjacent thigh muscle groups at dose levels of 4 and 12 U was due to local extension of the test item. There was no evidence of recovery of the muscle in the rats treated at 12 U and terminated 1 month after the 5th injection. Recovery was not assessed at 1 and 4 U doses. These changes in the muscles are considered to be related to an exaggerated pharmacological action of the test item. There were no compound-related lesions of the muscle groups distant to the injection sites or of the peripheral or central nervous systems. The high dose level was considered to be the maximum tolerated dose under the conditions of this study in light of the significant reduced bodyweights.

Study no.: AA40095

Volume #, and page #: electronic document (section 4.2.3.2.1)

Conducting laboratory and location:

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Date of study initiation: 12-26-06

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity: Dysport, IB05.001, IB06.005; each flask contained 500 Units of *Clostridium botulinum* toxin type A hemagglutinin complex

No formulation analysis was performed, due to the absence of a suitable analytical method.

Methods

Doses: 0, 1, 4, 12, Units

Species/strain: Sprague-Dawley rats: — OFA(SD)

Number/sex/group or time point (main study): 15/sex/group

Route, formulation, volume: intramuscular, 0.9% NaCl, 50 µL/animal (25 µL in each gluteus muscle)

Satellite groups used for recovery: Groups 2 and 6

Age: ~ 6 weeks

Weight: males: 203 to 251 g; females: 139 to 179 g

Sampling times:

Unique study design or methodology (if any):

b(4)

Group/Treatment	Dose Level (U/rat/month)	Concentration (U/ μ L)	Number of Rats	
			Males	Females
1. Control	0	0.00	15	15
2. Control, recovery	0	0.00	15	15
3. Low Dose	1	0.02	15	15
4. Intermediate Dose	4	0.08	15	15
5. High Dose	12	0.24	15	15
6. High Dose, recovery	12	0.24	15	15

U: one unit (U) corresponds to the LD₅₀ observed in the mouse following intraperitoneal administration.

Groups 1 and 2 (controls) received the placebo (*Clostridium botulinum* toxin type A hemagglutinin complex Placebo in 0.9% NaCl)

Groups 1, 3, 4, and 5 received six administrations (i.e., Days 0, 28, 56, 84, 112 and Day 140) and were euthanized the day after the last administration. Groups 2 and 6 received the first five administrations and were euthanized 4 weeks after the 5th administration.

Rationale for dose selection: dose levels were based on the results of developmental toxicity and dose range-finding studies of up to 21 days in duration in the pregnant rat (study number 43/361, 434/362 and AA28029) and non-pregnant rat (study numbers 434/357 and 434/358) previously performed at — The general condition of the rats treated at doses greater than 1 U/month resulted in decreased body weight gain and associated feed consumption and muscle paralysis at doses greater than 10 U. In all of these studies, animals tended to deteriorate with each administration of *Clostridium botulinum* type A hemagglutinin complex, regardless of the interval between administrations, such that the maximum tolerated dose correlated with the cumulative dose given over the entire treatment period. The high dose level of 12 U/month or 72 U over the 6-month treatment period (or 60 U for the recovery group), is expected to be close to the MTD. The lower dose levels are intended to identify a NOAEL.

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Observations and times:

Mortality: all animals were observed at least twice daily.

Clinical signs: on dosing days animals were observed at least 1 hour before and 1 to 2 hours after administration to detect any abnormalities in appearance, behavior, or other signs of reaction to treatment. On non-dosing days, the animals were observed at least once daily. A full clinical exam was performed weekly.

Body weights: animals were weighed at time of randomization, prior to dosing (day 0) and then once weekly.

Feed consumption: recorded weekly for each cage of animals during treatment and reported as g/animal/day.

Ophthalmoscopy: exam was performed on all animals pre-test and on Groups 1 (control) and 5 (high dose) in Week 20. Using a mydriatic agent examination of the adnexa, optic

media, and fundus was performed by indirect ophthalmoscopy. If necessary a slit lamp was used to investigate abnormalities of the anterior segment of the eye including the lens.

EKG: not performed

Toxicokinetics: not performed as there is no analytical method available.

Hematology: Blood was withdrawn from the retro-orbital sinus while under isoflurane anesthesia from animals fasted at least 15 hours. Samples were collected in tubes containing EDTA for the hematological parameters and in tubes containing trisodium citrate for the coagulation parameters. The following parameters were evaluated: hemoglobin, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, packed cell volume, red blood cell count, mean corpuscular volume, reticulocyte count, platelet count, total white blood cell count, differential white blood cell count, prothrombin time, activated partial thromboplastin time.

Clinical chemistry: Blood was withdrawn from the retro-orbital sinus while under isoflurane anesthesia from animals fasted at least 15 hours. Samples were collected in tubes without anticoagulant for the clinical chemistry parameters: sodium, potassium, calcium, glucose, urea, total cholesterol, total bilirubin, total protein, albumin, globulin (calculated), albumin/globulin ratio (calculated), creatinine, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase.

Urinalysis: urine was collected in individual metabolism cages for ~17 hours from animals deprived of feed and tap water, but receiving 20 mL/kg of tap water by gavage before the beginning of the collection period. The following parameters were evaluated: volume, specific gravity, appearance, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood.

Gross pathology: at the end of the treatment period all animals were fasted overnight, euthanized by carbon dioxide inhalation and exsanguination and subjected to a full necropsy including examination of the following: external surfaces, orifices, cranial cavity, carcass, external surface of the brain and cervical spinal cord, thoracic and abdominal cavities and organs, cervical tissues and organs, injection sites.

Organ weights: adrenal glands, brain, epididymides, gluteus muscles (right and left separately), heart, kidneys, liver, ovaries, pituitary gland, spleen, testes, thymus, thyroid/parathyroids, uterus. Organ weights were expressed as absolute values (g) and relative values (g per 100 g of body weight or brain).

Histopathology: The following organs were collected from all animals. Bolded tissues examined for all groups, other organs examined only for groups 1 and 5 and for animals that died on study. In addition any lesions of the liver, kidney and stomach found at necropsy in the other animals were submitted for histopathological examination: adrenal glands, aorta, bone (femur) and articulation, bone (sternum) with bone marrow, **brain**, bronchi (mainstem), cecum, colon, duodenum, epididymides, eyes, heart, **hindlimb thigh muscle**, ileum, **injection sites**, intercostal muscle, jejunum, kidneys and ureters, larynx, lungs, lymph node (mandibular), lymph node (mesenteric), mammary gland, **muscle (tibial)**, **nerve (brachial)**, **nerve (sciatic)**, **nerve (tibial)**, esophagus, optic nerves, ovaries and oviducts, pancreas, parathyroid glands, peyer's patches, pituitary gland, prostate, rectum, salivary glands (mandibular, parotid, sublingual), seminal vesicles, **skeletal muscle (distant from injection site)**, skin, **spinal cord (cervical, thoracic,**

lumbar), spleen, stomach, testes, thymus, thyroid glands, tongue, trachea, urinary bladder, uterus (horns and cervix), vagina, all gross lesions.

Adequate Battery: yes

Peer review: no

Results

Mortality: Although 4 animals died during the treatment period there were no deaths considered to be related to treatment with the test item. One male (#53) treated at 4 U died on Day 89 as a result of malignant lymphoma. No primary cause of death could be determined for a male (#69) treated at 12 U that was found dead on Day 50, although the animal was found to have pulmonary edema. Likewise, no cause of death could be determined for a female (#169) treated at 12 U found dead on Day 44. One female treated at 1 U died of accidental causes following blood sampling at the end of the treatment period.

The isolated nature of these deaths and the types of lesions found did not suggest an association with the test item.

Clinical signs: Shrinkage of the gluteus muscle, noticed during handling of the rats, was observed in all groups of rats treated with the test article, with severity and rapidity of onset being dose-dependent. All animals treated at 12 U were affected, with visible signs of muscle shrinkage appearing generally after the first injection. At 4 U, muscle shrinkage was noted in some males following the injection and with all males and females affected by the end of the study. Less severe muscle shrinkage was noted in animals treated at 1 U, only becoming evident following the 5th injection.

Body weights: The mean body weights of animals treated at 12 U were statistically significantly lower from Day 35 (one week after the 2nd injection). Consequently the mean overall body weight gain (Day 0 to Day 140) was lower in males (-25%) and females (-33%) treated at 12 U when compared to controls. Terminal body weights (Day 140) were decreased by 15% in both males and females when compared to controls. These differences in terminal body weights were still evident in the animals terminated after the 4-week recovery period (Day 139: -20% and -23% in males and females, compared with controls respectively).

There was no significant effect on body weight at the dose levels of 1 and 4 U.

Feed consumption: Mean feed consumption was reduced in all animals treated at 12 U especially over the first 2 weeks following each treatment (maximum -15% in males during 2nd week after 5th injection; -24% in females during 1st week following the 4th injection when compared with controls).

Ophthalmoscopy: There were no compound related eye lesions.

Hematology: There were no relevant effects of the test item on hematological parameters.

Clinical chemistry: When compared to controls, animals treated at 12 U showed lower mean serum creatinine levels than the control animals at the end of the study (after the 6th treatment: males -12%, females- 16%, or following the recovery period: males -12%, females -14%). This change was most likely related to the reduced muscle mass in the high dose (12 U) rats.

Urinalysis: There were no relevant effects of the test item on urinalysis parameters. The collected volume at the end of the study was statistically significantly lower for high dose (12 U) animals with the 4 week follow-up period as compared to their respective control animals. This was most likely related to lower bodyweights and was not considered to be of toxicological significance.

Gross pathology: There were no deaths considered to be related to the treatment. For all animals euthanized after the 6th injection, the left gluteus muscle appeared small at necropsy in 4/15 females treated at 4 U and in 14/15 males and 15/15 females treated at 12 U. Similarly the right gluteus muscle appeared small in 5/15 females treated at 4 U and in 13/15 males and 15/15 females treated at 12 U. Following recovery, the right and left gluteus muscles of males (15/15) and females (13/15) treated at 12 U appeared small.

Organ weights: For animals euthanized at the end of the treatment period, the mean absolute and relative weights of the gluteus muscles were statistically significantly lower at all dose levels, when compared to controls, with a dose-relationship. No evidence of reversibility was noted in animals treated at 12 U euthanized after the 4-week recovery period.

The following table was taken directly from the study report (section 13.9.2).

**APPEARS THIS WAY
ON ORIGINAL**

Mean differences in gluteus muscle weight compared to respective controls (expressed in %)

Sex	Male				Female			
	3	4	5	6	3	4	5	6
Dose level	1 U	4 U	12 U	12 U (recovery)	1 U	4 U	12 U	12 U (recovery)
Left gluteus muscle								
. absolute	-26	-41	-75	-77	-27	-31	-67	-71
. relative to body	-28	-39	-71	-71	-29	-29	-61	-61
Right gluteus muscle								
. absolute	-34	-39	-73	-81	-35	-41	-72	-76
. relative to body	-36	-37	-69	-76	-36	-40	-67	-68

For high dose animals euthanized at the end of the treatment period, some relative organ weights (both males and females: adrenal glands, brain, heart, kidneys, pituitary gland; males only: spleen, testes, epididymides; females only: liver) were significantly greater than in controls. In the absence of any correlating macroscopic or microscopic pathology, all of them were considered to be due to the significant difference in terminal body weight.

Similarly, recovery animals treated at 12 U were noted to have significantly greater relative organ weights (both males and female: adrenal glands, brain, heart, kidneys, liver, pituitary gland; males only: testes, epididymides; females only: thyroid gland, spleen, ovaries, thymus, uterus). In addition, some mean absolute organ weights were significantly lower than controls for both sexes (heart and spleen), males only (liver and thymus) and females only (brain). In the absence of any correlating macroscopic or microscopic pathology, all of these differences were considered to be due to the significant difference in terminal body weight.

Histopathology: The monthly administration of 1, 4, or 12 U of test item produced dose-related focal atrophy of the muscles at the injection site and to a lesser extent of the adjacent muscles groups. The atrophy was associated with focal fatty infiltration of muscle and focal interstitial fibrosis. Adjacent muscle groups were considered to have been affected as a result of local extension of the test item along fascial planes. Males were more severely affected than females. After the 4-week recovery period there was no clear evidence of resolution or progression of the compound-related changes at the dose level of 12 U. Muscle groups distant to the injection sites were unaffected by treatment.

Conclusion: Rats treated with 6 intramuscular injections at 4-week intervals of doses of 1, 4, and 12 U had reduced weights of the injected gluteus muscles and those treated with

12 U had reduced terminal body weight related to reduced feed consumption. The observed atrophy in adjacent thigh muscle groups at dose levels of 4 and 12 U was due to local extension of the test item. There was no evidence of recovery of the muscle in the rats treated at 12 U and terminated 1 month after the 5th injection. Recovery was not assessed at 1 and 4 U doses. These changes in the muscles are considered to be related to an exaggerated pharmacological action of the test item. There were no compound-related lesions of the muscle groups distant to the injection sites or of the peripheral or central nervous systems. The high dose level was considered to be the maximum tolerated dose under the conditions of this study in light of the significant reduced bodyweights.

Study # 434/359 RE (taken in part from review by Barbara Wilcox)

Title: Botulinum toxin type A hemagglutinin complex (Dysport): 14-Day preliminary study by intramuscular administrations in the non-pregnant female rabbit

GLP: Yes

QA report: yes

Animals: New Zealand White female rabbit (non-pregnant), 3 per group

Dosing: 0.1, 1, 3 or 10 SU/animal/day from study days 0-6.

Reviewer's comment: Although this rabbit range finder was based on preliminary experience with the test substance in rats, there was no mortality or treatment-related signs in any group during the first week of treatment in rabbits. Therefore the doses were changed for the second week.

Dosing: 30, 20, 3 or 10 SU/animal/day from study day 7-13.

Treatment	From Day 0 to Day 6		From Day 7 to Day 13		Dose volume (μ L/animal/day)	Number of Females
	Dose level (SU/animal/day)	Dose concentration (SU/ μ L)	Dose level (SU/animal/day)	Dose concentration (SU/ μ L)		
Control	0	0	0	0	200	3
Low dose	0.1	0.0005	30	0.15	200	3
Intermediate low dose	1	0.005	20	0.1	200	3
Intermediate high dose	3	0.15	3	0.015	200	3
High dose	10	0.05	10	0.05	200	3

Dosed animals were subsequently followed for a 2-week observation period.

Observations: Mortality (twice daily), clinical signs (daily), body weight (twice weekly), feed consumption (twice weekly).

Two animals euthanized moribund were necropsied. All others euthanized and discarded without further examination

Results:

No clinical signs attributable to test article administration for doses of 0.1 and 1 SU/animal/day for one week and 3 SU/animal/day and 10 SU/animal/day for two weeks. Higher doses showed no clinical signs until study day 16 (3 days after the last injection). At this time, dose related locomotor impairments and muscle atrophy were observed. Reduced locomotor ability was accompanied by reduced feed intake and body weight loss.

Two of three animals receiving 30 SU/animal/day became moribund.

The MTD under the conditions of this study was determined to be 20 SU/animal/day

The NOAEL under the conditions of this study was determined to be 10 SU/animal/day.

Reviewer's note: As written, the report incorrectly reports the doses as SU/kg/dose, but they are SU/animal/dose (i.e., $200 \mu\text{L}/\text{animal}/\text{dose} \times 0.15 \text{ SU}/\mu\text{L} = 30 \text{ SU}/\text{animal}/\text{dose}$).

APPEARS THIS WAY ON ORIGINAL

Study # 434/360 RE (taken in part from review by Barbara Wilcox)

Title: Botulinum toxin type A hemagglutinin complex (Dysport): Preliminary study after 2 intramuscular administrations in the non-pregnant female rabbit

GLP: Yes

QA Report: Yes

Animals: NZW rabbits, non-pregnant female, 3 per group

Dosing: 0, 1, 3, 10 and 30 SU/animal IM on study day 0

Reviewer's comment: Although this rabbit range finder was based on preliminary experience with the test substance in rats, there was no mortality or treatment-related signs in any group after the first administration in rabbits. Therefore the doses were changed for the second administration.

Dosing: 0, 40, 60, 100 and 30 SU/animal IM on study day 7

Treatment	First Administration (Day 0)		Second Administration (Day 7)		Dose volume ($\mu\text{L}/\text{animal}/\text{adm}^*$)	Number of Females
	Dose level (SU/animal/day)	Dose concentration (SU/ μL)	Dose level (SU/animal/adm*)	Dose concentration (SU/ μL)		
Control	0	0	0	0	200	3
Low dose	1	0.005	40	0.2	200	3
Intermediate low dose	3	0.015	60	0.3	200	3
Intermediate high dose	10	0.05	100	0.5	200	3
High dose	30	0.15	30	0.15	200	3

*adm = administration

Dosed animals were subsequently followed for a 2-week observation period.

Observations: Mortality (twice daily), clinical signs (daily), body weight (twice weekly), feed consumption (twice weekly).

Results:

Doses of up to 10 SU/animal showed no significant effects on clinical signs or body weight and feed consumption.

Dose-related adverse effects were observed for 60 and 100 SU/animal leading to moribund condition. Animals receiving 100 SU/animal were sacrificed moribund.

Other signs related to the test article were dose related reduction in feed consumption and loss of body weight in the 60 and 100 SU/animal groups. 60 SU/animal/dose was considered to be the MTD. Doses of 30 and 40 SU/animal/dose were well tolerated, despite a moderate reduction in body weight and feed intake.

Under the conditions of this study, the NOAEL was determined to be 10 SU/animal/dose.

Reviewer's note: As written, the report incorrectly reports the doses as SU/kg/dose, but they are SU/animal/dose (i.e., $200 \mu\text{L}/\text{animal}/\text{dose} \times 0.15 \text{ SU}/\mu\text{L} = 30 \text{ SU}/\text{animal}/\text{dose}$).

2.6.6.4 Genetic toxicology

No genetic toxicology studies were included in the BLA submission.

2.6.6.5 Carcinogenicity

No carcinogenicity studies were included in the BLA submission.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: *Clostridium botulinum* toxin type A hemagglutinin complex – Fertility toxicity study by the intramuscular route in the rat (Segment 1)

Key study findings: Under the conditions of this study, the NOAEL for fertility and general reproductive performance was 5 U/week (males) and 1.5 U/week (females).

Study no.: AA38304

Volume #, and page #: electronic document

Conducting laboratory and location: f

b(4)

Date of study initiation: December 12, 2006

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: Dysport, IB05.001, each vial contained 500 U of *Clostridium botulinum* type A

Methods

Doses: see study design

Species/strain: rats/Sprague-Dawley: — OFA (SD)

Number/sex/group: 10/sex/group

Route, formulation, volume: weekly intramuscular, 0.9% NaCl, 50 µL

b(4)

Reviewer's comment: 50 µL/animal /administration (25 µL in each gluteus muscle) is considered the maximum feasible volume for administration in the gluteus muscle without inducing significant tissue damage, according to the contract laboratory's experience.

Satellite groups used for toxicokinetics: NA

Study design:

Group/Treatment	Males		Females		Dose volume (µL)
	Dose level (U/rat/week)	Concentration (U/µL)	Dose level (U/rat/week)	Concentration (U/µL)	
1. Control (placebo)	0	0	0	0	50
2. Low dose	1.0	0.02	1.5	0.03	50
3. Low intermediate dose	2.5	0.05	4.0	0.08	50
4. High intermediate dose	5.0	0.10	8.0	0.16	50
5. High dose	10.0	0.20	16.0	0.32	50

Males were treated for 4 weeks before mating (from Day 0 of study), throughout mating and up to completion of the 2-week pairing period (i.e., 7 administrations). Females were treated for 2 weeks before mating (from Day 14 of study), throughout mating, and until

Day 7 of gestation (i.e., 4 administrations for females that mated during the first week, up to 6 administrations for those that mated later.) Females that failed to mate received 5 administrations.

Reviewer's comment: The high dose levels, of 10 U/week for males and 16 U/week for females, were selected to give both males and females an equivalent total cumulative dose (70 U for males and 64 U for females). The lower doses were intended to define a NOAEL.

Parameters and endpoints evaluated:

General toxicity: mortality (twice daily), clinical signs (twice daily on dosing days, otherwise once daily), body weight (males: weekly; females: twice weekly during pre-mating and mating periods and on Days 0, 4, 8, 13 of gestation), feed consumption (males and females: weekly during pre-mating and from Days 0 to 8 and from Days 8 to 13 during gestation).

Male Necropsy: (on completion of 2-week pairing period) organ weights (testes, epididymides)

Female Necropsy: (on Day 13 of gestation) ovaries were weighed

Male reproductive parameters: sperm analysis: sperm motility (motile sperm (%), averaged path velocity ($\mu\text{m/s}$), straight line velocity ($\mu\text{m/s}$), curvilinear velocity ($\mu\text{m/s}$), amplitude of lateral head displacement (μm), % straightness, % linearity, sperm concentration (# sperm per testis ($\times 10^6$), # sperm/g weight of testis ($\times 10^6$))

Female reproductive parameters: # corpora lutea, # implantation sites, implantation rate, viable embryos, pre-coital interval (days), copulation index (%), fertility index (%), pre-implantation loss (%), post-implantation loss (%).

Embryo: observed macroscopically for mortality.

Results

Mortality: one female treated at 16 U/week (#197) was found dead on Day 15 during the mating period. The cause of death could not be established at necropsy due to cannibalism. This isolated death was considered incidental. There was no mortality in the other groups of males or females.

Clinical signs: all clinical signs were related to the pharmacological effect of treatment with the test article. Muscular atrophy at the injection sites was observed in all treated groups. Paresis (slight localized muscular paralysis) was observed following the 3rd injection in females treated at 16 U/week and following the 5th injection in males treated at 10 U/week. Atrophy of the gluteus muscles was observed following the 1st injection for the high dose females treated at 16 U/week. This sign was noted during handling of the rats for test article administration the following week (day 7). The same effect was noted following the 2nd injection for males treated at 5 or 10 U/week, following the 3rd

injection for males treated at 2.5 U/week and females treated at 1.5, 4.0 or 8.0 U/week, following the 5th injection for males treated at 1 U/week.

Body weight: There was a dose-related reduction in body weight affecting all 4 dose groups of males and females. When compared to controls, the mean body weight gain of the males was statistically significantly reduced in males from the 1st week at 10 U/week, from the 2nd week at 5 U/week, from the 4th week at 2.5 U/week. The high dose males continuously lost weight on average following the 3rd dose administration until the end of the study. The reduction in terminal body weights of the males (compared to the control group) was 4%, 5%, 10%, and 25% in the males treated at 1, 2.5, 5, and 10 U/week, respectively, and attained statistical significance in the 3 highest dose levels.

The same pattern was observed in females. The mean body weight gain of the females was statistically significantly reduced from the 1st week at 16 U/week, from the 2nd week at 8 and 4 U/week. The females treated at 1.5 U/week were only affected during the first 4 days of gestation. The consequent reduction in the terminal body weights on day 13 of gestation by comparison with the control group was 2%, 9%, 20%, and 36% in the females treated at 1.5, 4, 8, and 16 U/week, respectively, and also attained statistical significance at the 3 highest dose levels.

Feed consumption: There was a slight, dose-related reduction in feed consumption in males and females treated at the high dose and high intermediate dose levels and in the females treated at the low intermediate dose level. The other dose groups were not affected. The feed consumption was reduced from the 1st week of treatment in the females treated at 16 U/week. The reduction was seen also in the males treated at 10 U/week and the females treated at 8 U/week from the 2nd week and in the females treated at 4 U/week from the first week of gestation and attained significance by comparison with the control group.

Toxicokinetics: No analytical method is available.

Necropsy: Subjective observations during necropsy examination confirmed the atrophy of the injected gluteus muscles observed clinically in all males and females in the high dose group, in all females (except # 176 was not observed to have small gluteus muscles at necropsy) and 7/20 males in the high intermediate dose groups and in 7/20 males and 9/20 females in the low intermediate dose group.

Fertility parameters: The copulation index was 100, 95, 100, 90 and 40 % (8/20) in the control, low, low intermediate, high intermediate and high dose groups, respectively. The low copulation index in the high dose group is likely due to the observed paresis.

The mean pre-coital interval was slightly longer in the low dose, the low intermediate dose and the high intermediate dose groups compared with the control group. The mean pre-coital interval for the few high dose pairs that copulated was comparable to that of the control group (2.4 versus 2.15 days in control animals).

One inseminated female in each of the control and high intermediate dose groups and two females in the high dose group failed to become pregnant, thus the fertility index was 95, 100, 100, 94 and 75% (6/8) for the control, low, low intermediate, high intermediate and high dose groups, respectively.

There were no obvious effects of the test item on the reproductive organ weights of the males and females. The absolute testes and epididymis weights were similar in all groups. The differences in the mean relative organ weights were due to the compound-related differences in terminal body weight. The absolute ovary weights of the high dose group were statistically significantly lower than in the control group, but the relative ovary weights remained higher in this group indicating that the difference was due to the lower terminal body weights.

Sperm analysis

There were no effects of treatment on the sperm analysis parameters examined in this study. Mean sperm counts, the percentage of motile sperm and the various motility parameters were comparable in all groups.

Litter data

Pre-implantation data

The mean number of corpora lutea were slightly lower amongst the pregnant females in the high (14) and high intermediate (15) dose groups than in the control (17) group. This reduction was mainly due to low values for one female in the high dose group (#198) and two females in the high intermediate group (#167, 179).

The mean percentage pre-implantation loss was greater in the three highest dose groups than in the control group (11.3, 9.7, 16.7 and 5.9% in low intermediate dose, high intermediate dose, high dose and control group, respectively). The inter group differences were due to considerable individual differences for females within the treated groups. Three high dose females (#183, 192, 198), one high intermediate dose female (#179) and three low intermediate dose females (# 141, 153, 158) each had values $\geq 30\%$ pre-implantation loss.

Reviewer's comment: In the absence of a dose response, the sponsor considers these differences incidental. However, it is difficult to regard this effect as incidental. Possibly with more animals this effect would have become significant and/or showed a dose response.

The resulting mean numbers of uterine implantations were slightly lower in the higher dose groups than in the control group, but nonetheless remained comparable with the historical control mean (15 uterine implants per dam). One dam in the high intermediate dose group had a single viable embryo arising from a single implantation and one corpus luteum.

Post-implantation data

The mean percentage post-implantation loss was not increased by treatment with the test item. One high dose female with mistimed pregnancy and a single implantation site was found to have undergone total resorption; these findings were incidental.

The following table was taken directly from the study report.

**APPEARS THIS WAY
ON ORIGINAL**

Study No.: A438304F
 Clostridium Botulinum toxin type A haemagglutinin complex -
 FERTILITY TOXICITY STUDY BY THE INTRAMUSCULAR ROUTE IN THE RAT
 (SEGMENT I)

SUMMARY OF CAESAREAN SECTION DATA

	Group 1 Control 0 SU/rat/adm.	Group 2 Low dose 1.5 SU/rat/adm.	Group 3 Low inter. dose 4.0 SU/rat/adm.	Group 4 High inter. dose 8.0 SU/rat/adm.	Group 5 High dose 16.0 SU/rat/adm.
Number of dams	19	19	20	17	3
Corpora Lutea per group	321	330	344	248	42
per dam: mean	17 k	17	17	15	14
st. dev.	2	3	3	5	1
Implantations per group	302 f	310	305	224	35
% of Corpora lutea	94.1	93.9	88.7	90.3	83.3
per dam: mean	16 k	16	15	13	12
st. dev.	2	3	4	4	2
Viable Embryos per group	283	297	282	216	31
per dam: mean	15 d	16	14	13	10
st. dev.	2	2	3	4	4
% of Implantations per group	93.7 f	95.8	92.5	96.4	88.6
per dam: mean	94 k	96	92	97	85
st. dev.	11	5	8	5	17
Postimplantation Loss total per group	19 f	13	23	8	4
% of implantations	6.3	4.2	7.5	3.6	11.4
per dam: mean	1 k	1	1	0	1
st. dev.	2	1	1	1	2
No. of dams affected	10	10	13	6	2
Preimplantation Loss per group	19 f	20	39	24	7
% of Corpora lutea	5.9	6.1	11.3	9.7	16.7
per dam: mean	1 k	1	2	2	2
st. dev.	1	1	2	2	2
No. of dams affected	13	11	16	11	3

Statistical key: d=Anova/Dunn test f=Chi2/Fisher EXACT test k=Kruskal-Wallis/Dunn test

Discussion: The high dose level (males: 10 U/week; females 16 U/week) was above the MTD as indicated by the marked reductions in terminal body weight (males: 25%; females: 36%). The high intermediate dose (males: 5 U/week; females: 8 U/week) was the MTD for males (10% terminal body weight reduction) and above it for females (20% terminal body weight reduction). The NOAEL for general toxicity was 1.5 U/week in females and 5 U/week in males. Although 5 U/week was associated with a reduced copulation index (90%), it was not associated with any spermatological effects. It is likely the reduced copulation index relates to some degree of hindlimb insufficiency, or secondary pharmacological effect. Therefore the NOAEL for male fertility is 5 U/week. Although the % pre-implantation loss at 4 U/week (11.3%) was not statistically significantly different from that of controls (5.9%), it is almost twice as much. Therefore the NOAEL for fertility in females is 1.5 U/week.

Embryofetal development

Study # 434/363

Title: Botulinum toxin type A hemagglutinin complex (Dysport): Preliminary embryotoxicity study by daily intramuscular injection in the pregnant rabbit.

GLP: Yes

QA report: yes

Animals: New Zealand White rabbit, 6 per treated group, 5 per control
Dosing: 0, 10 or 20 SU/animal/day (200 µL/animal), IM daily from day 6 to day 19 of gestation (inclusive). These doses were selected based on the results obtained in a preliminary toxicity study in non-pregnant rabbits — study number 434/359), which demonstrated that 20 SU/animal/day was the MTD and that 30 SU/animal/day induced paralysis of the injected muscle leading to premature sacrifice of the animals at this dose level.

b(4)

Observations: Feed consumption (daily), body weight (days 0, 6, 9, 13, 16, 20, 24, and 29 of gestation), mortality (twice daily), clinical exams (daily), pregnancy status, # corpora lutea, # implantations, # and distribution of live fetuses, # and distribution of embryonic/fetal deaths, fetal weights (no histopathology was performed).

Results:

No mortality, but one female at 20 SU/animal/day was sacrificed on day 27 of gestation after aborting.

Marked maternal toxicity at dose level of 20 SU/animal/day (body weight loss and reduced feed consumption), associated with possible embryofetal toxicity (late abortion of one female and increase in pre-implantation loss (19.4% versus 3.8%). Fetal body weight was not adversely effected by treatment. External examination of fetuses did not reveal any obvious treatment-related abnormalities. However, one fetus from a dam

treated at 20 SU/animal/day (#4378) had multiple defects including ancephaly, a facial cleft and ectrodactyly. Five fetuses from a single control dam (#4365) had a domed head.

The NOAEL for Dysport administered under the conditions of this study (IM, daily) was determined to be 10 SU/animal/day.

Study # 434/364 RE

Title: Botulinum toxin type A hemagglutinin complex (Dysport): Preliminary embryotoxicity study by two sequential intramuscular administrations in the pregnant rabbit.

GLP: Yes

QA report: yes

Animals: New Zealand White female rabbit, 6 per group

Dosing: 0, 30 or 60 SU/animal/day, IM twice (on day 6 and day 13 of gestation). These doses were selected based on the results obtained in a preliminary toxicity study in rabbits (study number 434/360), which demonstrated that 60 SU/animal/administration was the MTD and that 100 SU/animal/administration induced severe paralysis of the injected muscle leading to premature sacrifice of the females treated at this dose level.

b(4)

Observations: Feed consumption (daily), body weight (days 0, 6, 9, 13, 16, 20, 24, and 29 of gestation), mortality (twice daily), clinical exams (daily), pregnancy status, # corpora lutea, # implantation sites, # and distribution of live fetuses, # and distribution of embryonic/fetal deaths, fetal weights (no histopathology was performed).

Results:

No mortality in any group.

Marked maternal toxicity at dose level of 60 SU/animal/day (paralysis in one animal, body weight loss of 14% relative to control on GD29 and reduced feed consumption in all animals), associated with equivocal embryofetal toxicity (lower mean fetal body weight of 33.6 g versus 37.9 g). External examination of fetuses did not reveal any obvious treatment-related abnormalities.

The dose of 30 SU/animal/day was associated with moderate maternal toxicity (body weight loss of 10% compared to control).

Reviewer's note: In the summary, the doses are incorrectly described as '.....30 and 60 Speywood units (SU)/kg.' This should be 30 and 60 SU/animal.

Study # 434/361 RE

Title: Botulinum toxin type A hemagglutinin complex (Dysport): Preliminary embryotoxicity study by daily intramuscular administration in the pregnant rat.

GLP: Yes

QA Report: Yes

Animals: pregnant rat — OFA.SD. (IOPS Caw), 6 per group b(4)
Dosing: 0, 1, or 3 SU/animal/day, IM daily from day 6 to day 17 of gestation (inclusive)
Observations: Feed consumption (days 0, 6, 11, 15, 18, and 20 of gestation), body weight (on days 0, 6, 11, 15, 18, and 20 of gestation), mortality (twice daily), clinical exams (daily), # corpora lutea, # implantations, necropsy with no histopathology performed, external fetal examination.

Results:

No mortality and no clinical signs in any group. No obvious effect on feed consumption, but body weight gain was lower in both treated groups when compared to controls, reaching -10% and -11% for the treatment period respectively at dose-levels of 1 and 3 U/animal/day. Consequently the resulting terminal mean body weight was lower (3%) in the treated groups compared with control.

There was an increase in post-implantation loss in the 3 SU/animal/day (11.6%) group compared with control (4.6%), due to a higher incidence of early resorptions. This was mainly due to one female. External examination of fetuses did not reveal any obvious treatment-related abnormalities.

Under the test conditions, the dose of 3 SU/animal/day was not high enough to demonstrate a clear maternal response.

Reviewer's note: As written, the report incorrectly reports the doses as SU/kg/dose, but they are SU/animal/dose (i.e., 50 µL/animal/dose x 0.06 SU/µL = 3 SU/animal/dose).

Study # 434/362 RE

Title: Botulinum toxin type A hemagglutinin complex (Dysport): Preliminary embryotoxicity study by sequential intramuscular administrations in the pregnant rat.

GLP: Yes

QA Report: Yes

Animals: pregnant Sprague Dawley rats — OFA.SD.(IOPS Caw), 6 per group b(4)
Dosing: 0, 10 or 20 SU/animal /dose, two IM injections (50 µL/animal), one on day 6 and one on day 13 of gestation
Observations: Feed consumption (on days 0, 6, 11, 15, 18, and 20 of gestation), body weight (on days 0, 6, 11, 15, 18, and 20 of gestation), mortality (twice daily), clinical

exams (daily), # corpora lutea, # implantations, necropsy with no histopathology performed, external fetal examination.

Results:

No mortality in any group.

Muscular atrophy at the injection sites in three females treated at 20 SU/animal/dose 5 days after the second dose. As a consequence there was a reduction in feed consumption and mean maternal body weight. There was a modest increase in pre-implantation loss in both treated groups without dose-effect (19.5% and 20.3% in animals treated at 10 and 20 SU/animal/dose versus 5.4% in controls). A slight increase in post-implantation loss was observed in two out of four animals treated at 10 SU/animal/dose and for one out of six animals treated at 20 SU/animal/dose (22 and 12% versus 7.9% in controls, respectively). These pre- and post-implantation losses resulted in lower litter size in both treated groups. However, the mean fetal body weight was not affected. 20 SU/animal/dose was considered to be above the MTD based on terminal body weight (-24%) and feed consumption. 10 SU/animal/dose resulted in maternal effects on terminal body weight (-15%) and feed consumption, but at a tolerable level.

Reviewer's note: As written, the report incorrectly reports the doses as SU/kg/dose, but they are SU/animal/dose (i.e., 50 µL/animal/dose x 0.4 SU/µL = 20 SU/animal/dose).

Study title: Botulinum toxin type A hemagglutinin complex (Dysport®) – Embryo toxicity study by the intramuscular route in the rabbit (Segment II)

Key study findings: Although this study is considered inadequate due to deaths at the high dose, the low dose of 1.0 SU/day was a NOAEL for both maternal and developmental toxicity. The sponsor will be required to repeat the study with a high dose of 20 SU/day as a post-marketing commitment.

Study no.: AA28028

Volume #, and page #: electronic document

Conducting laboratory and location:

Date of study initiation: August 5, 2005

GLP compliance: Yes

QA reports: yes (x)

Drug, lot #, and % purity: botulinum toxin type A hemagglutinin complex (Dysport®), IB04.015, each flask contained 500 Speywood units

Reviewer's note: One Speywood unit (SU) corresponds to the LD₅₀ in the mouse following intraperitoneal injection.

Methods

Doses: 0, 1, 10, 20 SU/animal/day (dosed daily), and 40 SU/animal/dose (dosed on days 6 and 13 only). Total cumulative doses of 14, 140, 280, and 80 U, respectively.

Species/strain: New Zealand White rabbit, — KBL (NZW) (animal of known bacterial and viral status)

Number/group: 22 mated females/group

Route, formulation, volume: intramuscular injection (right or left biceps femoris in rotation), Dysport® in 0.9% sterile NaCl, 200 µL/injection,

Satellite groups used for toxicokinetics: NA

Study design: All surviving females were euthanized on Day 29 of gestation for examination of their uterine contents.

Group	Dose Level (SU/animal/injection)	Dose volume (µL/animal)	Dose concentration (SU/µL)	Number of females
1. Saline control	0	200	0	22
2. Placebo*	0	200	0	22
3. Low dose	1	200	0.005	22
4. Intermediate dose	10	200	0.05	22
5. High dose	20	200	0.1	11**
6. Intermittent injection	40	200	0.2	22

* placebo formulation – contains similar concentrations of protein and lactose as the formulation given to group 5.

** Only 11 rabbits were allocated to group 5 (20 SU/injection) following mortality during the initial phase of the study.

Frequency and duration:

Groups 1 through 5: once daily from gestation day 6 (G6) to G19, inclusive

Groups 6: on G6 and G13 only

Rationale for dose selection: preliminary studies in the rabbit, in which Dysport® induced muscle paralysis at the injection site following daily or weekly administration, led to reduced body weight gain. These effects were more marked in non-pregnant than in pregnant females. The effects on embryofetal development were similar whether Dysport® was administered daily or by intermittent dosing. Since the MTD by intermittent dosing was only twice that obtained by daily administration it was decided to conduct a single embryo toxicity study in the rabbit using daily dosing at three dose levels and intermittent dosing at one dose level (the MTD) only. Daily intramuscular injection from Days 6 to 19 of gestation was intended to achieve the maximum cumulative exposure of the dams and to potentially expose the embryos on all days of

b(4)

organogenesis. Intermittent dosing on days 6 and 13 of gestation was intended to expose the dams to the highest achievable peak exposures.

Parameters and endpoints evaluated: mortality (twice daily), clinical signs (at least daily), body weights (Days 0, 6, 9, 13, 16, 20, 24, and 29 of gestation), feed consumption (measured daily), pregnancy status, number of corpora lutea, pre-implantation loss (%), post-implantation loss (%), number of implantation sites, number and distribution of live fetuses, number and distribution of embryonic/fetal deaths (classified as early or late), fetal sex, fetal weights, and external, visceral, and skeletal morphology

Results

Mortality: All 11 rabbits given 20 SU/day died or were terminated prematurely for ethical reasons. One rabbit (# 7507) was sacrificed in a moribund condition on Day 19 of gestation and another (#7516) was found dead on Day 20. It was then decided to terminate the remaining rabbits in the group in view of their poor clinical condition. All rabbits in this group had marked body weight loss, losing at least 9.7% of their initial weight during the treatment period, and ate little or no food for several days preceding their death/sacrifice. All had lame limbs (taken to be indicative of muscle paralysis) first appearing during the last four days of the treatment period. The lame limbs were also frequently associated with subdued behavior. The necropsy examination of these rabbits did not reveal any treatment-related lesions other than dark areas on the lungs of 7 dams.

Reviewer's comment: Death in the high dose group (20 SU/day) is at variance with the preliminary study in pregnant rabbits (# 434/363) where 6/6 does survived treatment with 20 SU/day from gestation days 6 through 19 (inclusive).

There was no mortality in the other treated or control groups. One dam (#7530) in the 40 SU intermittent dose group was sacrificed after aborting on Day 24 of gestation. A severe reduction in feed intake and body weight of the aborting female during the treatment period suggests a test article effect, but there was no evidence of paralysis. Dark areas on the lungs were also noted for this female.

Clinical signs: Except for sporadic episodes of reduced fecal output in association with periods of reduced feed intake, there were no treatment-related changes in clinical condition.

Local tolerance: Occasional dams in all groups, including saline control, had hematomas at the injection sites.

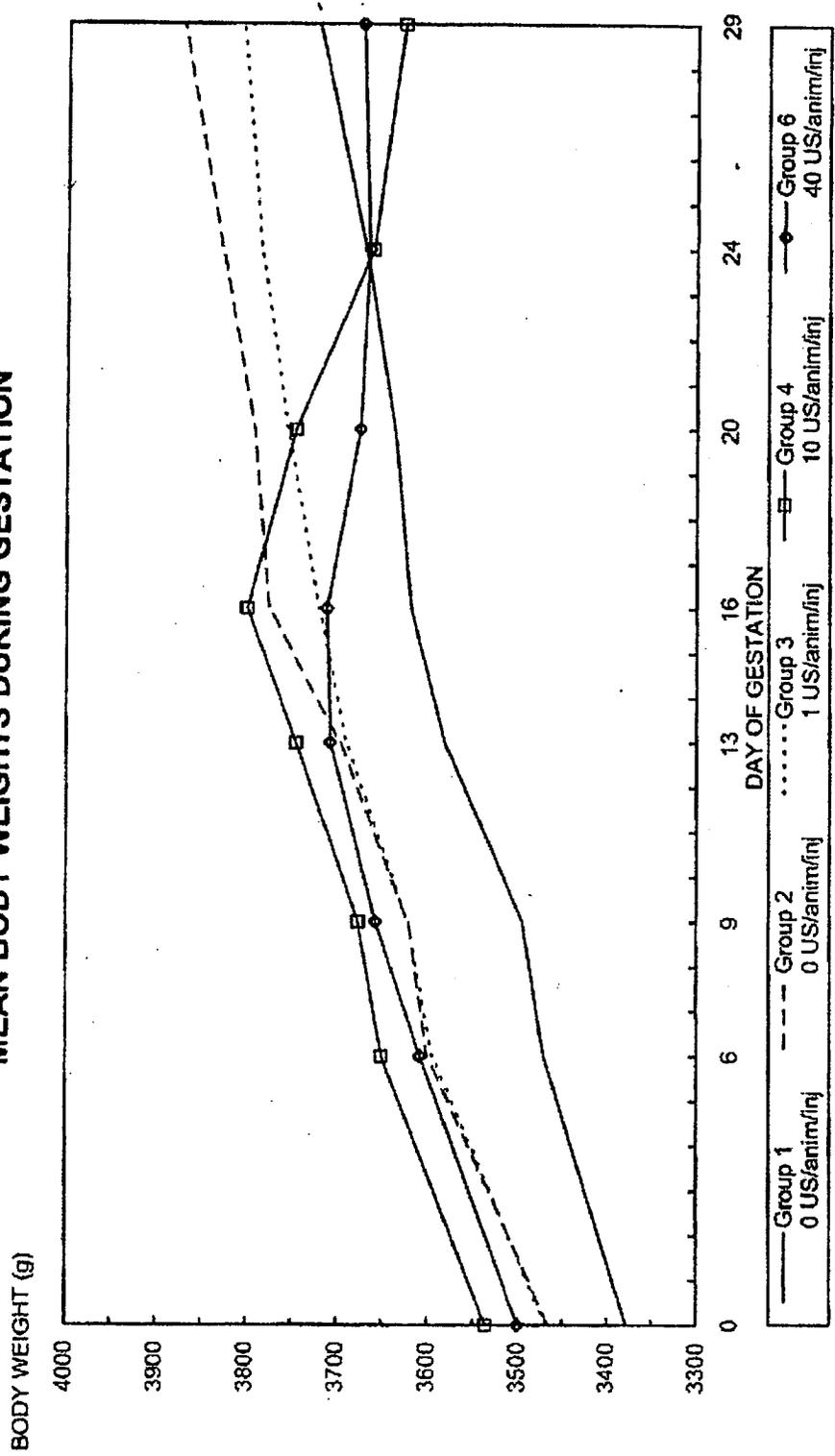
Body weight: The group given 10 SU/day had a reduced body weight gain (-9.6%) compared to control on Day 29 ($p < 0.01$). Two-thirds of the pregnant females in this group lost weight between Days 6 and 29 of gestation and half of the affected females lost at least 3% of their Day 6 body weight. Dams in the 40 SU intermittent group had a weight gain that was only 25% of control ($p < 0.01$). Seven out of 19 pregnant females lost weight between Days 6 and 29 of gestation, with two females (# 7529, 7538) losing

9% and 16% of their Day 6 body weight, respectively. Two rabbits (#7470, 7477) in the 1.0 SU/day group had a marked weight loss over the treatment period (losing 5% and 7% of their initial body weight, respectively). Two pregnant rabbits in the saline control group (#7429, 7439) and one in the placebo group (#7446) also had a slight body weight loss over the treatment period.

The following graph was taken directly from the study report.

**APPEARS THIS WAY
ON ORIGINAL**

STUDY NUMBER AA28028 - FIGURE 1 -
MEAN BODY WEIGHTS DURING GESTATION



One Speywood unit corresponds to the LD₅₀ observed in the mouse following intraperitoneal administration

Feed consumption: Reduced in the 40 SU intermittent dose group following the second dose administration on Day 13 and remained depressed up to Day 29 of gestation. A similar reduction became evident in the group given 10 SU/day after the completion of the treatment period (days 20 to 29; $p < 0.05$ for days 20-24; $p < 0.01$ for days 24-29). Rabbits given 1.0 SU/day consumed the same amount of feed as controls.

Toxicokinetics: not performed

Terminal and necroscopic evaluations:

Necropsy of the dams did not reveal any treatment-related lesions. Occasional dams in both treated and control groups had ovarian cysts, a common finding in pregnant rabbits.

Litter data: One dam in the 40 SU intermittent dose group (#7530) was sacrificed after aborting on Day 24. A severe reduction in both feed consumption and body weight during the treatment period, suggested the event was treatment-related, although there was no evidence of muscle paralysis.

Pregnancy incidence: at least 19 pregnant females at term in all groups. All of the pregnant females submitted to terminal caesarean examination on Day 29 of gestation in the treated and placebo groups had viable fetuses. One saline control dam (# 7440) had one early resorption and no viable fetuses.

Pre-implantation data: Although there was no statistically significant difference in pre-implantation data (# of corpora lutea, # implantation sites, % preimplantation loss) between the treated and control groups, the % preimplantation loss was slightly higher in the 10 SU/day group (12.2%) compared to the saline control (9.4%) and placebo (9.2%).

Post-implantation data: The percentage of early and late resorption and post-implantation loss were not increased by treatment with Dysport®. Although not statistically significant, the mean live litter sizes were marginally superior in groups 3 (10.6) and 4 (9.3) than in the saline control (8.7) or placebo (9.0) groups. There were no dead fetuses in any group.

The following table was taken directly from the study report.

**APPEARS THIS WAY
ON ORIGINAL**

Study No.: A428028

Botulinum toxin type A hemagglutinin complex (Dysport) -
EMBRYO TOXICITY STUDY BY THE INTRAMUSCULAR ROUTE IN THE
RABBIT (SEGMENT II).

SUMMARY OF CAESAREAN SECTION DATA

	Group 1 Control 0 US/anim/inj	Group 2 Placebo 0 US/anim/inj	Group 3 Low dose 1 US/anim/inj	Group 4 Intermed. dose 10 US/anim/inj	Group 5 High dose 20 US/anim/inj	Group 6 Intermit. dose 40 US/anim/inj
Pregnant	N 22	21	22	21	0	19
Dams with no viable fetuses	N 1	0	0	0	0	0
Dams with viable fetuses	N 21	21	22	21	0	19
Corpora Lutea no. per animal	TOTAL 222 MEAN 10.1 d S.D. 3.0	219 10.4 3.1	262 11.9 2.8	232 11.0 2.0	0	209 11.0 2.8
Implantation sites no. per animal	TOTAL 206 MEAN 9.4 d S.D. 3.1	201 9.6 3.1	241 11.0 2.3	206 9.8 2.5	0	194 10.2 3.1
Preimplantation loss no. per animal	TOTAL 16 MEAN 0.7 d S.D. 1.1	18 0.9 1.1	21 1.0 1.0	26 1.2 1.0	0	17 0.9 1.2
% per animal	MEAN% 9.4 k S.D. 14.1	9.2 11.9	7.5 7.3	12.2 11.2		8.1 9.8
Live Fetuses no. per animal	TOTAL 191 MEAN 8.7 d S.D. 3.0	189 9.0 2.9	234 10.6 2.3	196 9.3 2.7	0	178 9.4 2.8
Males	TOTAL 104 MEAN% 54.1 k S.D. 14.7	96 49.7 18.4	125 53.4 15.4	82 42.6 15.2	0	82 46.5 15.2
Females	TOTAL 87 MEAN% 45.9 k S.D. 14.7	93 50.3 18.4	109 46.6 15.4	114 57.4 15.2	0	96 53.5 15.2

Statistical key: d=ANOVA/Dunnnett test k=kruskal-wallis/Dunn test

Offspring:

Fetal weight or sex ratio were not adversely influenced by treatment.

External examination revealed one malformed fetus in each of the placebo, 10 SU/day and 40 SU intermittent dose groups. The malformed fetus in the placebo group (dam #

7448) had marked malrotation of one hindlimb. One fetus in the 10 SU/day group (dam # 7499 fetus #1) had multiple defects including gastroschisis, acaudia, malpositioned kidneys and fused hindlimbs. (This fetus also had skeletal malformations of the vertebrae). One malformed fetus in the intermittent dose group (dam # 7539) had anophthalmia. The distribution of these findings did not suggest an effect of Dysport® on external findings. There were no other fetuses with external abnormalities in any group.

Skeletal examination revealed four malformed fetuses from 3 litters in the 10 SU/day group compared with one in each of the other treated, placebo and saline control groups. Vertebral malformations were found in the above mentioned fetus with multiple external defects (7499-1) and in fetus #11 from dam # 7487. Two fetuses from a single litter (dam #7492, pup # 6, pup # 7) in the 10 SU/day group had a missing first digit of the forepaws (ectrodactyly), which was also noted at 20 SU/day in the preliminary rabbit study (#434/363; dam # 4378). Other than these two fetuses with ectrodactyly, all of the other fetuses with skeletal malformations in all groups had defects of the thoracic and/or lumbar vertebrae.

Reviewer's comment: The loss of the high dose group (20 SU/day) due to early termination makes it difficult to confirm a dose-response for the skeletal malformations noted above.. Given the fact 6/6 does survived treatment with 20 SU/day during the same time period in a preliminary study, it will be necessary to ask the sponsor to repeat the pivotal test with a higher dose to determine if there is a dose response in skeletal malformations.

The incidence of fetuses with incomplete ossification of the 2nd and 4th sternebrae was increased with treatment, being 9%, 8% and 16% at the low, intermediate and intermittent dose versus the saline control (6%). This effect achieved statistical significance in the 40 SU intermittent dose group ($p < 0.05$) and was higher than the recent historical control incidence (9%). This variation (is similar to other low level signals seen in other species (i.e., unossified 6th sternebra in the rat embryotoxicity study; study # AA28029).

The incidences of fetuses with incomplete ossification of the 5th to 8th thoracic vertebral centra were greater in the various treated groups than in the saline control and placebo groups, but observed incidences in the treated groups remained less than the historical control data. For example, the percent fetal incidence of incomplete ossification of the 5th to 8th centra was 0.5, 2.1, 5.6, 3.1, 6.2 in the control, placebo, low dose, intermediate dose, and intermittent dose, respectively, but the historical control ranged from 12.3 to 23.7 % over the 2002/2004 time period. The percent fetal incidence of minor fusion of the sternebra was 0.5, 0.5, 0.9, 1.5, and 2.2 in the control, placebo, low dose, intermediate dose, and intermittent dose, respectively, but ranged from 0.6 to 1.4 % over the 2002/2004 time period.

Visceral examination indicated that one fetus in the 10 SU/day group (7499-1) had malpositioned kidneys. One fetus from the 40 SU intermittent group (7532- 1) had a

displaced pulmonary artery. Occasional fetuses in all groups had no azygos lung lobe, which is a frequent variation in rabbits.

The distribution of malformed fetuses in the various groups was as follows:

Group	1 Saline	2 Placebo	3 1 SU/day	4 10 SU/day	6 40 SU: G6,13
Malformed fetuses (litters)	1	2(2)	1	4(3)	2(2)
- Gastroschisis, acaudia, fused limbs	-	-	-	1	-
- Major defects of thoracic/lumber vertebrae	1	1	1	1 ^a	1
- Malrotated limb	-	1	-	-	-
- Anophthalmia	-	-	-	-	1
- Ectrodactily	-	-	-	2(1)	-

^a excluding above-mentioned fetus with multiple defects

Conclusion

In this study the highest daily dose level of 20 SU/day of Dysport® was above the maximum tolerated dose in the adult female rabbit, as indicated by mortality following reductions in feed consumption and body weight, although it was tolerated by 6/6 does in the preliminary study. The observed clinical signs were indicative of muscle paralysis (i.e., the intended pharmacological activity of the test item).

Daily intramuscular administration of Dysport® to the pregnant rabbit at the dose level of 10 SU/day resulted in significant maternal toxicity characterized by severe persistent reductions in body weight gain (-9.6 % of control) and feed consumption. Although there were no statistically significant indications of embryo toxicity at this dose level, preimplantation loss was 12.2% compared to 9.4% in control animals and skeletal malformations were increased.

Intermittent intramuscular administration of Dysport® on Days 6 and 13 of gestation at the dose level of 40 SU/day resulted in slight maternal toxicity, characterized by reduced body weight gain (25% of control) and feed consumption. The percent fetal incidence of minor fusion of the sternbrae was increased relative to the control, placebo and other treated groups, although it did not achieve statistical significance. However, incomplete ossification of the 2nd and 4th sternbrae in the intermittent group was statistically increased relative to the saline control (p<0.05).

The low dose of 1.0 SU/day was a NOAEL for both maternal and developmental toxicity.

Reviewer's comment: Although skeletal malformations were seen at the highest daily dose (10 SU/day during organogenesis), the study is considered inadequate due to the unexpected deaths at 20 SU/day, which was tolerated during a preliminary study in pregnant rabbits (study # 434/363). The sponsor will be asked to repeat the study using a high dose of 20 SU/day as a post-marketing commitment. Given the inadequacy of this study, the data will not be included in the label.

Study title: Botulinum toxin type A hemagglutinin complex (Dysport®) – Embryo toxicity study by the intramuscular route in the rat (Segment II)

Key study findings: There were no indications of a teratogenic potential of Dysport® in this study. The NOAEL for maternal toxicity was 1.5 SU/rat/day and the NOAEL for developmental toxicity was 0.5 SU/rat/day.

Study no.: AA28029

Volume #, and page #: electronic document

Conducting laboratory and location:

b(4)

Date of study initiation: August 8, 2005

GLP compliance: Yes

QA reports: yes (x)

Drug, lot #, and % purity: botulinum toxin type A hemagglutinin complex (Dysport®), IB04.015, each flask contained 500 Speywood units

Methods

Doses: 0, 0.5, 1.5, 5.0, SU/animal/day, and 10.0 SU intermittent

Reviewer's note: One Speywood unit (SU) corresponds to the LD₅₀ in the mouse following intraperitoneal injection.

Species/strain: Sprague-Dawley rats: OFA.SD. (IOPS Caw)

Number/group: 25 mated females/group

Route, formulation, volume: intramuscular injection, Dysport® in 0.9% sterile NaCl, 50 µL/animal (one administration of 25 µL in each gluteus muscle)

Satellite groups used for toxicokinetics: NA

b(4)

Study design:

Group	Dose Level (SU/animal/injection)	Dose volume (μ L/animal)	Dose concentration (SU/ μ L)	Number of females
1. Control	0	50	0	25
2. Placebo	0	50	0	25
3. Low dose	0.5	50	0.01	25
4. Intermediate dose	1.5	50	0.03	25
5. High dose	5.0	50	0.1	25
6. Intermittent injection	10.0	50	0.2	25

Frequency and duration:

Groups 1 through 5: once daily from gestation day 6 (GD6) to GD17, inclusive

Groups 6: on GD6 and GD12 only

Rationale for dose selection: Based on preliminary studies in the rat, in which Dysport® induced muscle paralysis at the injection site following daily or weekly administration leading to the reduced body weight gain. These effects were more marked in non-pregnant than in pregnant females. The effects on embryofetal development were similar whether Dysport® was administered daily or by intermittent dosing. Because the MTD by the intermittent schedule was only twice that obtained by daily administration, it was decided to conduct a single embryo toxicity study in the rat incorporating daily dosing at three dose levels and intermittent dosing at the MTD only. Daily intramuscular injection from Days 6 to 17 of gestation was intended to achieve the maximum cumulative exposure of the dams and to potentially expose the embryos on all days of organogenesis. Intermittent dosing on days 6 and 12 of gestation was intended to expose the dams to the highest achievable peak exposures.

Parameters and endpoints evaluated: mortality (twice daily), clinical signs (daily), body weights (Days 0, 6, 11, 15, 18, and 20 of gestation), feed consumption, number of corpora lutea, number of implantation sites, number of dead fetuses, number of resorbed fetuses, number of live fetuses, sex ratio of live fetuses, fetal and placental weights, and external, visceral, and skeletal morphology.

Results

Mortality: No mortality in any group.

Clinical signs: No significant clinical reactions at any dose level. Two rats given 1.5 SU/day, 8 given 5 SU/day and 5 given 10 SU by intermittent injection had stained fur

mainly towards the end of gestation. One rat given 5 SU/day (# 109) and another given 10 SU by intermittent injection (# 140) had a thin appearance at the end of gestation.

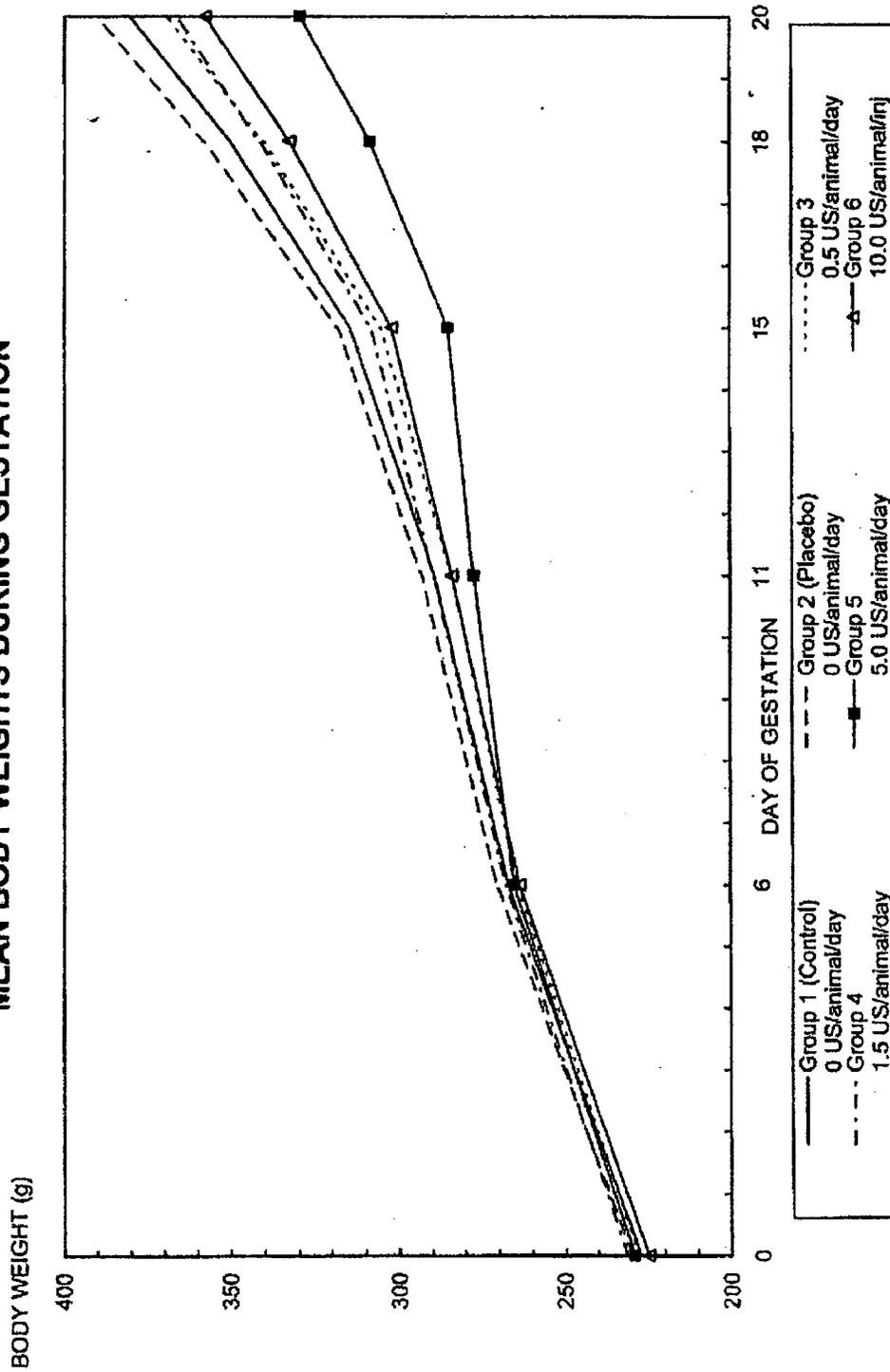
Local tolerance: Occasional rats (all groups) had localized hairloss.

Body weight: Maternal body weight gain was reduced during and after the treatment period in groups treated with daily injections of Dysport® at 1.5 or 5 SU/day and in the group treated with intermittent injections of 10 SU/rat. The 5 SU/day group was most affected, gaining 48% less weight than the saline control group ($p < 0.01$ for body weight gain over all intervals after treatment start). The mean terminal body weight was reduced in this group by 13% with respect to the vehicle control group ($p < 0.01$). The groups treated with 1.5 SU/day or 10 SU intermittently showed a reduced rate of gain over the period of Days 11-15 of gestation ($p < 0.01$, for both groups). However, the mean terminal body weights in these groups were only marginally lower than in the control group (-4 and -6% respectively, not statistically significant). The low dose, treated with 0.5 SU/day, and the placebo group were not noticeably affected.

The following graph was taken directly from the study report.

**APPEARS THIS WAY
ON ORIGINAL**

STUDY NUMBER AA28029 - FIGURE 1 -
MEAN BODY WEIGHTS DURING GESTATION



Feed consumption: Maternal feed consumption was reduced during and after the treatment period in the group given 5 SU/day ($p < 0.05$ or $p < 0.01$ for the various periods). The other treated and placebo groups were not significantly affected.

Toxicokinetics: not performed

Terminal and necroscopic evaluations:

Necropsy of the dams did not reveal any treatment related lesions.

Pregnancy incidence: There were at least 22 pregnant females in all groups. One female in the 10 SU intermittent group (# 140) had a single uterine implantation site with no live fetuses. This is a relatively frequent occurrence in the rat and is not likely treatment-related.

Pre-implantation data: The mean pre-implantation loss was greater in the 1.5 and 5 SU/day group and the 10 SU intermittent group than in control group (not statistically significant). While dosing in this study started on Day 6 of gestation (i.e., after embryonic implantation), very early embryonic deaths (before nidation is complete) may be manifest as pre-implantation losses under the conditions of this study. An early embryo-lethal effect could not be excluded. The mean number of uterine implantation sites was lower in the treated groups than in the control, but this effect was not statistically significant. Pre-implantation data in the 0.5 SU/day/group was comparable to the control.

Post-implantation data: The incidence of early resorptions was marginally greater in the 10 SU intermittent dose group than in the control group (not statistically significant). The incidence of late resorptions was low or zero in all groups. Percentage post-implantation loss was increased in the 10 SU intermittent group (even excluding the dam with no viable fetuses) by comparison with the control group, reflecting the increase in early resorption incidence. There were no dead fetuses in any group. The resulting mean live litter sizes were marginally lower in the four treated groups than in the control group, reflecting the above-mentioned variations in the number of uterine implantations.

Fetal weight and fetal sex ratio was not affected by treatment.

The following two tables were taken directly from the study report.

**APPEARS THIS WAY
ON ORIGINAL**

STUDY NO.: AA28029
 Botulinum toxin type A hemagglutinin complex (Dysport) -
 EMBRYO TOXICITY STUDY BY THE INTRAMUSCULAR ROUTE IN THE
 RAT (SEGMENT II).

SUMMARY OF CAESAREAN SECTION DATA

	Group 1 Control 0 US/ann/day	Group 2 placebo 0 US/ann/day	Group 3 Low dose 0.5 US/ann/day	Group 4 Intermed. dose 1.5 US/ann/day	Group 5 High dose 5.0 US/ann/day	Group 6 Intermit. Inj 10.0 US/ann/in
Pregnant	N 24	22	23	22	23	24
Dams with no viable fetuses	N 0	0	0	0	0	1
Dams with viable fetuses	N 24	22	23	22	23	23
Corpora Lutea	TOTAL 351	317	321	299	343	350
No. per animal	MEAN 14.6 d	14.4	14.0	13.6	14.9	14.6
	S.D. 3.9	2.4	3.4	2.9	3.2	3.3
Implantation sites	TOTAL 319	301	292	264	280	300
No. per animal	MEAN 13.3 d	13.7	12.7	12.0	12.2	12.5
	S.D. 3.7	2.2	3.2	3.3	3.5	3.6
Preimplantation Loss	TOTAL 32	16	29	35	64	50
No. per animal	MEAN 1.3 k	0.7	1.3	1.6	2.8	2.1
	S.D. 2.2	1.1	1.2	2.0	3.6	1.8
% per animal	MEAN% 9.1 k	4.7	9.2	11.7	17.6	15.9
	S.D. 14.2	6.9	9.0	15.3	20.5	15.1
Live FETUSES	TOTAL 295	288	265	241	255	265
No. per animal	MEAN 12.3 d	13.1	11.5	11.0	11.1	11.0
	S.D. 4.1	2.4	2.9	3.4	3.3	4.0
Males	TOTAL 155	131	134	117	131	127
	MEAN% 49.7 k	45.3	49.8	48.1	50.6	47.9
	S.D. 19.0	13.5	15.4	12.7	13.0	12.7
Females	TOTAL 140	157	131	124	124	138
	MEAN% 50.3 k	54.7	50.2	51.9	49.4	52.1
	S.D. 19.0	13.5	15.4	12.7	13.0	12.7

Statistical key: G=Anova/Dunnnett test k=kruskal-wallis/Dunn test

Study No.: AA28029 Botulinum toxin type A hemagglutinin complex (Dysport) -
 EMBRYO TOXICITY STUDY BY THE INTRAMUSCULAR ROUTE IN THE
 RAT (SEGMENT II).

SUMMARY OF CAESAREAN SECTION DATA

	Group 1 Control 0 US/ann/day	Group 2 Placebo 0 US/ann/day	Group 3 Low dose 0.5 US/ann/day	Group 4 Intermed. dose 1.5 US/ann/day	Group 5 High dose 5.0 US/ann/day	Group 6 Intermed. Inj 10.0 US/ann/day
Postimplantation Loss No. per animal	24 1.0 k 1.3	13 0.6 0.7	27 1.2 1.0	23 1.0 0.8	25 1.1 0.9	35 1.5 1.4
% of implants per animal	9.0 k 17.2	4.7 5.5	8.8 7.2	9.9 8.8	8.7 7.8	16.4 21.9
Dead Fetuses No. per animal	0 0.0 k 0.0	0 0.0 0.0	0 0.0 0.0	0 0.0 0.0	0 0.0 0.0	0 0.0 0.0
% of implants per animal	0.0 k 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Resorptions: Early No. per animal	23 1.0 k 1.3	13 0.6 0.7	27 1.2 1.0	22 1.0 0.8	25 1.1 0.9	35 1.5 1.4
% of implants per animal	8.8 k 17.3	4.7 5.5	8.8 7.2	9.6 8.8	8.7 7.8	16.4 21.9
Resorptions: Late No. per animal	1 0.0 k 0.2	0 0.0 0.0	0 0.0 0.0	1 0.0 0.2	0 0.0 0.0	0 0.0 0.0
% of implants per animal	0.3 k 1.4	0.0 0.0	0.0 0.0	0.3 1.3	0.0 0.0	0.0 0.0

Statistical key: k=kruskal-wallis/Dunn test

Offspring:

External examination revealed one malformation, a fetus with exencephaly from a 0.5 SU/day dam (# 71). The isolated nature of this finding did not suggest an association

with the test item. There were no other fetuses with external malformations in any group. The only other external findings were one pair of placental twins in each of the control group, the 5 SU/day group and the 10 SU intermittent dose group.

Fixed soft tissue examination revealed one malformed fetus in the control group, two in the 0.5 SU/day group (including the malformed fetus found at external examination, described above) and two in the 10 SU intermittent dose group. The malformed fetus from the control group (dam # 7) and one of the malformed fetuses from the 10 SU intermittent group (dam # 139) had enlarged ventricular chamber(s) of the heart. The other malformed fetus in the 10 SU intermittent group (dam # 132) had unilateral microphthalmia. The fetus with exencephaly in the 0.5 SU/day group (dam # 71) was found to also have bilateral anophthalmia. The other malformed fetus in this group (dam # 75) had a very small left kidney. There were no fetuses with soft tissue malformations in the placebo, 1.5 and 5 SU/day groups.

The incidences of fetuses with soft tissue anomalies and variations did not suggest any adverse effects of the test item. The types of abnormality found were limited to dilated renal pelvis, and dilated and/or convoluted ureters. The incidence of fetuses with dilated renal pelvis was relatively low in the saline control group by comparison with the historical background, but remained comparable between the placebo and treated groups.

Skeletal examination revealed one fetus with cleft palate in each of the placebo and 10 SU intermittent dose groups (dam # 48 and 132). The affected fetus from the treated group was from the same litter as the fetus with microphthalmia.

The incidences of fetuses with unossified 6th sternebra was increased in the high dose (7.5%) and intermittent (8.0%) groups relative to the saline control (4.5%), although this effect was not statistically significant.

Conclusion

Daily intramuscular administration of Dysport® to the pregnant rat at the dose level of 5 SU/rat throughout the embryonic period resulted in maternal toxicity characterized by reduced feed consumption leading to a mean 13% reduction in body weight at term. Although not statistically significant, rats treated with 5 SU/day also had an increase in preimplantation loss and an increase in unossified 6th sternebrae. Although these findings failed to achieve statistical significance, they are similar to those seen in the rabbit embryotoxicity study (study # AA28028) and are therefore likely to be of biological significance.

Intermittent intramuscular administration of Dysport® on days 6 and 12 of gestation at the dose level of 10 SU/rat resulted in marginal maternal toxicity, characterized by a reduced body weight gain (17% less than the saline control) with no corresponding influence on maternal feed consumption. As with the 5 SU/day dose, rats treated intermittently also had an increase in the incidence of early embryonic death and an increase in unossified 6th sternebrae.

Daily intramuscular administration of Dysport® to the pregnant rat at the dose level of 1.5 SU/rat/day was characterized by a slightly reduced body weight gain and an increase in pre-implantation loss.

The NOAEL for maternal toxicity was 1.5 SU/rat/day and the NOAEL for developmental toxicity was 0.5 SU/rat/day.

There were no indications of a teratogenic potential of Dysport® in this study at any dose level.

Prenatal and postnatal development

Study title: Clostridium botulinum toxin type A hemagglutinin complex – pre- and post-natal development study by the intramuscular route in the rat (Segment III)

Key study findings: The dose level of 2.5 U/administration was the maternal toxicity NOAEL for the F0 and the dose of 5 U/administration was the NOAEL for the F1 generation based on an increase in stillbirths at the high dose.

Study no.: AA38305

Volume #, and page #: electronic document

Conducting laboratory and location: ✓

b(4)

Date of study initiation: December 12, 2006

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: botulinum toxin type A hemagglutinin complex (Dysport®), IB05.001, each flask contained 500 Speywood units

Methods

Doses: 0, 1, 2.5, 5 and 10 SU/ F₀ females/week

Reviewer's note: Rats received the dose levels on a weekly basis from Day 6 of gestation until weaning (Day 21 of lactation inclusive). There was a total of 6 dosing days (i.e., on Days 6, 13, 20 of gestation and on Days 6, 13 and 20 of lactation) resulting in a cumulative dose of 6, 15, 30 and 60 SU/animal.

Species/strain: rats/Sprague-Dawley: ✓ JFA (SD)

Number/sex/group: 25 mated females/group

Route, formulation, volume: intramuscular, Dysport® in 0.9% sterile saline, 50 µL/animal (one administration of 25 µL in each gluteus muscle)

Satellite groups used for toxicokinetics: NA

b(4)

Study design: Only F0 generation females were treated. Selected F1 offspring (one male and one female pup from each litter) were maintained untreated for monitoring of post-weaning development, behavioral tests and mating to form a second generation.

Group	Dose Level (SU/animal/injection)	Dose volume (μ L/animal)	Dose concentration (SU/ μ L)
1. Control	0	50	0
2. Low dose	0.5	50	0.02
3. Low intermediate dose	2.5	50	0.05
4. High intermediate dose	5.0	50	0.1
5. High dose	10.0	50	0.2

Parameters and endpoints evaluated: morbidity/mortality (twice daily), clinical signs (twice daily on dosing days and daily during nondosing period), body weight (F₀: gestation days 0, 6, 11, 15, 18, and 20 and lactation days 1, 4, 7, 10, 14, 17 and 21; F₁ males: weekly; F₁ females: weekly during pre-mating and mating periods and on Days 0, 4, 8, and 13 of gestation), feed consumption (F₀ only, days 0, 6, 11, 15, 18, 20 of gestation and days 1, 4, 7, 10, 14, and 21 of lactation); pregnancy and parturition (F₀ females only): duration of gestation, abnormalities of delivery, nesting or nursing behavior, number of implantation sites; litter data (F₀ → F₁ animals): number of live pups, sex of pups, pup weights, external abnormalities, F₁ post-weaning development, behavioral tests, mating; F₁ pregnancy status: #of corpora lutea, # and type of uterine implantations.

Results

F₀ in-life: There were no unscheduled deaths. The only treatment-related effect was apparent shrinkage (atrophy) of the injected gluteus muscle. Most F₀ rats treated with 2.5, 5 and 10 U showed small gluteus muscles after the 2nd injection (cumulative dose of 5, 10, and 20 U, respectively). All F₀ rats treated with 10 SU showed small gluteus muscles after the 3rd injection (cumulative dose of 30 U). All F₀ rats treated with 2.5 and 5 U showed small gluteus muscles after the 5th injection (cumulative dose of 12.5 and 25 U, respectively).

There was a dose-related reduction in body weight gain affecting the two highest doses (5 and 10 U). Body weight gain was statistically significantly reduced from Day 6 of gestation at 10 U. The same trend was noted from Day 11 of gestation at 5 U. The body weight gain through gestation was statistically significantly lower at 10 U (+89.9 g, $p < 0.01$) and slightly lower at 5 U (+113 g) compared with control (+121.5 g). The mean body weight at the end of gestation was 8.5% lower ($p < 0.01$) at 10 U and 3 % lower in the 5 U group by comparison with the control group. The corresponding difference on the day after parturition was -12% ($p < 0.01$) at 10 U and -5% ($p < 0.05$) at 5 U. During the first 4 days of lactation, the body weight gains in the 10 U and 5 U groups remained statistically and significantly lower than in the control group ($p < 0.01$ and $p < 0.05$,

respectively). During the last 2 weeks of lactation, the dams treated at 5 and 10 U lost more weight than did the control dams (weight loss during this period is normal in the rat). The resulting differences in group mean body weight at weaning with respect to the control group were -8.3% and -15% at 5 and 10 U, respectively. There were no relevant effects of treatment on body weight throughout gestation and lactation in the 1 and 2.5 U groups.

Feed consumption: There were no significant influences of treatment in the F0 females' feed consumption throughout the study (gestation and lactation). Although feed consumption was slightly lower at 10 U during the entire treatment period (from GD 6 to L21) and attained statistical significance ($p < 0.05$) from Days 15 to 18 of gestation, the observed differences were too small to be of biological significance.

Litter data

The mean duration of gestation (~22 days) and the fertility index were similar in all groups. One dam in each of the control, 1 U, 5 U, and 10 U groups failed to give birth following total litter resorption. All of the remaining pregnant females in all groups delivered live pups.

The mean litter size at birth, pup survival up to weaning, and sex ratio on Days 0 and 21 post partum and were comparable in all groups. However, the number of stillbirths increased at the high dose.

The following table was taken directly from the study report.

**APPEARS THIS WAY
ON ORIGINAL**

Study No.: AA38305

CICSTRIDIUM BOTULINUM TOXIN TYPE A HAEMAGGLUTININ COMPLEX -PRE- AND POST-NATAL DEVELOPMENT STUDY BY THE INTRAMUSCULAR ROUTE IN THE RAT (SEGMENT III).

SUMMARY OF DELIVERY AND LITTER DATA

	Group 1 Control 0 SU/rat/adm.	Group 2 Low dose 1.0 SU/rat/adm.	Group 3 Low inter. dose 2.5 SU/rat/adm.	Group 4 High inter. dose 5.0 SU/rat/adm.	Group 5 High dose 10.0 SU/rat/adm.
Females on Study	N 25	25	25	25	25
Females Mated	N 25 f %	25 100.0	25 100.0	25 100.0	25 100.0
Females Pregnant:	N 23 f %	24 96.0	25 100.0	23 92.0	25 100.0
Female Fertility Index	N 22 f %	23 95.8	25 100.0	22 95.7	24 96.0
Females with Liveborn Gestation Index	N 22 f %	23 95.8	25 100.0	22 95.7	24 96.0
Females Completing Delivery	N 22 f %	23 95.8	25 100.0	22 95.7	24 96.0
with Stillborn Pups	N 1 f %	2 8.7	25 100.0	1 4.0	3 12.5
with all Stillborn	N 0 f %	0 0.0	0 0.0	0 0.0	0 0.0
Litters with Liveborn, But no Pups Alive	N 0 f %	0 0.0	0 0.0	0 0.0	0 0.0
day 4	N 0 f %	0 0.0	0 0.0	0 0.0	0 0.0
day 21	N 0 f %	0 0.0	0 0.0	0 0.0	0 0.0
Duration of Gestation	MEAN 22.0 d S.D. 0.4 N 22	22.0 0.0 23	22.1 0.4 25	22.0 0.3 22	22.0 0.2 24

Statistical key: d=Anova/Dunnnett test f=Chi2/Fisher Exact test

Pup weights at birth (recorded on Day 1 post-partum) were marginally lower in the 10 U (mean of 7.1 g) than in the control group (mean of 7.4 g). Although pup growth up to weaning was then essentially similar in all groups, the initial difference in mean pup weight at 10 U remained evident through to weaning (-7.3% $p < 0.05$ on Day 7 for male and female pups). This marginally reduced pup weight in the 10 U group was consistent with the lower body weight of the dams. There were no adverse effects on pup weight at the lower dose levels.

There was no adverse effect of treatment on physical and functional development of the pups, as assessed by the day of pinna unfolding, day of incisor eruption, day of eye opening and the presence of the surface righting, gripping, auditory and pupil reflexes.

F₀ necropsy:

Necropsy of F0 females did not reveal any compound-related lesions.

There were no compound-related macroscopic findings among the pups euthanized during lactation, at weaning or among pups found dead. Occasional pups in all groups, including the control, had dilatation of one or both renal pelves. One pup from a dam treated with 10 U had a missing hindlimb.

F1 Generation

There was one F1 generation death, but it was not considered to be compound-related. A male (#190) from the 2.5 U group was found dead on Day 64 and necropsy revealed dark areas on the lungs. There were no clinical signs in the F1 generation considered to be related to maternal treatment.

F₁ physical development:

Body weight profiles of the F1 males and females before mating and of the F1 females during gestation were comparable in all groups. The mean age of sexual maturation (as measured by age at which balano preputial skinfold cleavage occurred in F1 males and at which vaginal opening occurred in F1 females) was comparable in all groups.

F₁ behavioral evaluation:

There was no evidence of any adverse influence of maternal treatment with the test item on learning capacity, memory and motor activity of the F1 animals, as measured by the water maze test. There was no evidence of an adverse effect of maternal treatment on activity and exploratory behavior of the F1 generation, as assessed by activity and positioning in the open field. There was a minor decrease in ambulatory activity with a corresponding minor increase in inactivity for one female (#300) in the 1 U group and 3

females (#302, 310, 316) in the 2.5 U group compared with the control group which attained statistical significance for the group mean. As a consequence, the total distance traveled was slightly lower for these females compared with the control animals. However, these differences were not observed in the two highest groups and were therefore considered incidental.

F₁ reproduction:

There was no evidence of an adverse effect of treatment of the F0 females on the mating performance of the F1 offspring, nor was there an adverse effect of treatment on fertility, with most females becoming pregnant across the treatment groups (24 out of 25 inseminated females for control, 24/24 for 1 U, 23/24 for 2.5 U, 23/25 for 5 U and 23/25 for 10 U). The pregnancy incidence of F1 females was not affected by the treatment of F0 females. All pregnant females had viable embryos with the exception of one in the 1 U group, but this isolated case was considered incidental. The mean number of corpora lutea, implantations per dam and percentage post-implantation loss were comparable in all groups. The percentage of pre-implantation loss was slightly higher in the 1 U group than in the control group, but distribution of the data (2 females with a loss of approximately 50%) did not suggest an effect of F0 maternal treatment.

F₁ necropsy: Necropsy examination of F1 animals did not reveal any relevant lesions. Incidental macroscopic findings included one male in the 2.5 U group with renal pelvic dilatation and one female in the same group with alopecia on forelimbs.

Conclusion

Weekly intramuscular administration of clostridium botulinum toxin type A hemagglutinin complex to pregnant Sprague-Dawley rats at dose levels of 1, 2.5, 5, and 10 U/week from Day 6 of gestation until weaning (Day 21 of lactation) divided between the gluteus muscles resulted in an increase in stillbirths at the high dose level. Slight maternal (F0) toxicity, characterized by reduced body weight gain during gestation and lactation, was observed at the two highest dose levels of 5 and 10 U/week. Clinical signs associated with the expected pharmacological action of the test item (i.e., muscle shrinkage at the injection site) were observed from the dose levels of 2.5 U/week and above.

Reviewer's comment: An increase in preimplantation loss (or early embryonic loss) is a consistent finding, although it did not achieve statistical significance, in both the rodent and nonrodent studies. The significance of the skeletal malformations noted in rabbits is difficult to assess without a higher dose level. The sponsor will be requested to repeat the pivotal embryofetal rabbit study with a high dose of 20 SU/day during organogenesis.

2.6.6.7 Local tolerance

No new local tolerance studies were included in the BLA submission.

2.6.6.8 Special toxicology studies

The sponsor was asked to evaluate the changes and reversibility of any findings in muscles and nerves following repeat intramuscular administration of Clostridium botulinum toxin type A hemagglutinin complex. The literature searched suggested the major lines of approach should be: 1) silver staining for nerve branching and terminal budding; 2) histochemical demonstration of non specific esterase; and 3) immunohistochemistry for acetylcholinesterase. The initial part of method development indicated that silver staining techniques adequately demonstrate nerve fibers (AA42572 Part – 1 – Investigation in normal muscle), but that neither immunohistochemistry or histochemistry represent an accurate reflection of the recovery process due to sampling procedures. Although sampling differences made any assessment of the number of intact NMJs error prone, the sponsor was able to demonstrate damage and subsequent resolution of NMJ on samples of muscles injected with Dysport (AA42572 Part – 2- Investigation to evaluate nestin as an indicator of damage and resolution of NMJ on samples of muscle injected with Dysport (Clostridium botulinum toxin type A hemagglutinin complex).

Nestin is an intermediate filament of 200 kDa which forms the basic scaffolding of the postsynaptic region of the NMJ. Normally the enzymes and proteins associated with the NMJ (e.g. acetylcholinesterase (Borodic et al, 1993) and nestin (Aarimaa et al, 2004) are restricted to the area close to the NMJ, but are known to show a diffuse pattern following damage to the muscle. Staining was reported to return to a more localized pattern as the NMJs became re-established. Thus the sponsor evaluated nestin's ability to serve as an indicator of the damage and resolution of NMJs in samples of gluteus muscles following a single intramuscular injection of 0, 2, or 6 units (U) of Dysport in study AA40423 (a single dose IM toxicity study with a 12-week recovery period). In animals which received 2 U/rat, minimal changes were seen at 7 days. There was a trend for increased diffuse staining at 30 days, which decreased after 90 days to a normal pattern in 3/4 animals and showed only a trace in the fourth. In animals which received 6 U/rat, diffuse nestin staining was present at 7 days and rose to higher levels at 30 days. A slow recovery level of both circular subsarcolemmal and diffuse staining was present in 3/4 rats at 90 days. (The increase in subsarcolemmal distribution of nestin is considered to represent the re-organization of the intermediate filaments). In general, abnormal nestin staining correlated with an histopathological decrease in fiber size. Normal staining of NMJs correlated with only minimal histopathological changes. Muscles fibers at the site of injection were seen to be reduced in size and this change was most obvious at 30 days following an injection of 6 U/rat, but showed a gradual return to normal appearance and mass by 90 days after injection. Those injected with 2 U/rat showed a dose-related lesser effect and a more rapid recovery.

The results from this study (AA40423) showed that immunohistochemical identification of nestin worked well on formalin fixed paraffin sections. The intermediate filament showed diffusion into the fiber, indicating damage to the NMJs, seen at the early time points which correlated with the histological appearance of reduced fiber size. At the later time points there was a return to the normal localization at the NMJ, indicating the

restoration of the NMJ and re-innervation of the fiber. This correlated with the return to more normal sized fibers histologically. Therefore, immunohistochemical identification of nestin is considered to be an appropriate marker of neuromuscular damage and resolution of NMJ structure following damage induced by botulinum toxin.

Study title: Dysport (*Clostridium botulinum* toxin type A hemagglutinin complex)-Three-month intramuscular toxicity study with 13-week and 26-week follow-up in the rat

Key study findings: Three intramuscular injections of Dysport at 4-week intervals, at dose levels of 0.1 or 2 U/injection in female rats induced a reduction of the fiber size in the gastrocnemius muscle. The related clinical observation was an apparent shrinkage of the injected muscle and reduced locomotory activity. Most of the fibers had returned to their normal size after a 13-week recovery period. Recovery of the muscle fiber size was essentially complete after 26 weeks with full recovery of the locomotory activity at 17 weeks post treatment.

Study no.: AA60533

Volume #, and page #: electronic document

Conducting laboratory and location: [redacted]

b(4)

Date of study initiation: 9-27-07

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: *Clostridium botulinum* toxin type A hemagglutinin complex (Dysport®), IB06.005, each flask contained 500 Speywood units

Formulation/vehicle: vehicle: sterile physiological saline (0.9% NaCl)

On day of treatment the test article was diluted with 0.9% NaCl to obtain a 500 U/mL stock solution. From this the test article was prepared to obtain solutions of 0.004 and 0.08 U/μL.

Methods

Doses: 0, 0.1, 2.0 U/rat/injection

Study design: 72 Female Sprague-Dawley rats (~6 weeks of age; 138 to 171 g) were assigned to the following groups and treated by intramuscular injection at 4-week intervals (on Days 0, 27 and 55). Rats received an intramuscular injection of test article in the left gastrocnemius muscle and a similar volume of vehicle was injected in the right muscle.

Reviewer's comment: *The sponsor justifies the use of Sprague-Dawley rats because they are 'one of the rodent species acceptable to regulatory agencies' and because 'background data for the strain are available at the Testing Facility.' However, they do*

not provide justification for using only females. It is acceptable to have conducted this study in female rats only because it is not anticipated that the effects elicited by Reloxin at the NMJ would be different in male and female rats.

Group Number	Dose level (U/rat/injection)	Dose volume (μ L/injection)	Dose concentration (U/ μ L)	Number of females scheduled for termination:		
				2 days after last injection	13 weeks after last injection	26 weeks after last injection
1. Control	0	25	0	8	8	8
2. Low dose	0.1	25	0.004	8	8	8
3. High dose	2.0	25	0.08	8	8	8

Group 1 animals (control) received the placebo formulations (*Clostridium botulinum* toxin type A hemagglutinin complex Placebo) in the left gastrocnemius muscle.

Rationale for dose selection: The low dose of 0.1 U/rat represents a pharmacologically active dose in the rat. The high dose level of 2 U/rat was expected to result in slight toxicity characterized by local lesions of the muscle at the injection site.

Body weight and feed consumption (each cage contained 4 same group females and the feed was assumed to be evenly divided) were measured weekly during the treatment and treatment-free periods. Grip strength was measured on the left hindlimb for all females at the end of Weeks 1, 4, 8, 12, 16, and 20. An open field test was performed for all females during Weeks 2, 4, 8, and 12 (Days 13, 24, 50, and 80 respectively). One animal at a time was placed in the center of a black square PVC arena measuring 40.5 x 40.5 cm and its activity was monitored by a video image analysis system for 3 minutes. The arena was divided into nine equal invisible regions; the time spent by the animal in each type of region (i.e., corner, center or lateral) was recorded. Motor activity was divided into three categories: ambulatory, activity (in which the center of the image moved at more than 7 cm/sec), small movements (including grooming, etc.) and inactivity. The proportion of time spent engaged in each type of activity and the total distance travelled by the rat were calculated.

Two days or 13 weeks after the last injection, 8 females per group were euthanized (CO₂ inhalation) after overnight fasting. The necropsy procedures included examination of: external surfaces, orifices, carcass, cranial cavity, external surface of the brain and spinal cord, thoracic and abdominal cavities and organs, cervical tissues and organs, the injection sites.

The following organs were weighed: adrenal glands, brain, gastrocnemius muscles (left and right), heart, kidneys, liver, lungs, ovaries, pituitary gland, spleen, thymus,

thyroids/parathyroids, uterus. Weights were expressed as absolute values (g) and relative values (g per 100 g of body weight).

The following organs/tissues were sampled, fixed and preserved in 10% neutral buffered formalin for histological examination: brain, muscles, biceps (hindlimb, left and right), muscles, triceps (forelimb, left and right), muscles, gastrocnemius (left and right), muscles, gluteus (left and right), nerve, brachial (left and right), nerve, sciatic (left and right), nerve, tibial (left and right), spinal cord (cervical, thoracic, lumbar).

An immuno-histochemical technique was used for additional histopathological examination to visualize neuromuscular junctions (staining for nestin). Nestin is an intermediate filament of 200 kDa which forms the basic scaffolding of the postsynaptic region of the NMJ. Kang et al. (2007) described the reduction of the local activity of nestin in the muscle 8 to 10 days after an injection of botulinum toxin in rats and mice. Botulinum toxin blocks the liberation of the neurotransmitter from the terminal nerve ending. This loss of activity leads to a loss of integrity of the neuromuscular junction. Nestin is being used to monitor the integrity of the neuromuscular junction and any return to a normal structure following prolonged blockade. Transverse and longitudinal sections of the left gastrocnemius muscle (treated side) from half of the animals euthanized at the end of the treatment period and from all the animals euthanized after the 13 week- and 26-week recovery periods were stained.

Three patterns of nestin immunostaining were observed:

Crescent-shaped sarcolemmal staining represents the normal location of the intermediate filament, delineating the normal NMJ. Circular subsarcolemmal staining surrounding the muscle fiber was seen in two different phases of the study. At the early time points this was considered to be a local 'leakage' of the nestin from the NMJ indicating a loss of integrity of the NMJ. At the later stages this probably represented a relocalisation of the filaments as an early stage of reconstruction of the NMJ. Diffuse staining involving the whole fiber was considered to represent a well established lesion with leakage and diffusion of the nestin throughout the sarcoplasm. The normal appearance of the nestin protein is focal and closely related to the NMJ, represented by a crescent-shaped staining pattern.

Results

Clinical observations:

The only compound-related clinical sign was apparent shrinkage of the Dysport-injected gastrocnemius muscle in all treated groups with a dose related incidence and severity (estimated by group, not by individual animal). The group 3 animals treated at 2 U/injection were affected 3 weeks after the first injection and the muscle shrinkage was still apparent 13 weeks after the last injection. Some animals in group 2 treated with 0.1 U/injection were affected from 4 weeks after the second injection until 3 weeks after the

last injection. Consistent with the shrinkage of the injected muscles, the group 3 animals treated at 2 U/injection showed a slight reduction in body weight starting during the week following the second injection and persisting up to 10 weeks after the last injection.

Body weight:

There was a slight reduction in body weight gain in the high dose group over the week following the second injection (week 4) compared with the control group (80%, $p \leq 0.05$). All of the treated rats continued to gain weight. The mean body weight during the recovery periods was incidentally lower in the high dose group than in the control group by chance associated with the reduced number of rats remaining following the interim necropsies.

Feed consumption:

There were no compound-related effects on feed consumption in any group.

Grip strength:

The grip strength measurements were comparable between the treated and control groups.

Open field test:

There was a compound-related reduction on locomotory activity revealed by the open field test at both doses 3 weeks after each of the second injection with no dose-relationship. This effect was still observed 3 weeks after the last injection. Statistical significance was generally not attained. These results were considered to be a consequence of the gastrocnemius muscle weakness resulting from the intended pharmacological action of Dysport. There was no persisting difference in locomotory activity between treated and control groups after 17 weeks of recovery.

Pathology results 2 days post final treatment:

Microscopic examination revealed lesions restricted to the injected and adjacent muscles. These were composed of a reduction in fiber size (with apparent increased nuclear density) associated with some degeneration and increased connective tissue. Two days after the last injection, the reduction in fiber size, associated with some degeneration and increased connective tissue, was observed with a dose-dependent increase in incidence and severity. Animals treated at 0.1 U/injection showed only minor changes, while animals treated at 2 U/injection showed an obvious reduction in the fiber size two days after the end of treatment. One animal of this group only presented an increased number of adipocytes. All of these findings were considered to be secondary to the pharmacologic activity of the test article.

Two days after the last injection, the nestin immunostaining showed abnormal location of the protein, being circular or diffuse and not only focal, indicating a disorganization of the post-synaptic structures.

There were no peripheral nerve lesions.

Pathology results 13 weeks post final treatment:

There was a tendency towards a decreased severity of the reduced fiber size in both treated groups, indicative of partial recovery. The injected muscle returned to a normal appearance in all but two animals treated at 0.1 U/injection. Associated with this reduction in the fiber size, a minimal to slight adiposis was observed in females treated at 2 U/injection. The amount of connective tissue was similar in the treated and control animals.

The nestin immunostaining revealed a tendency for a decreased incidence and intensity of this abnormal staining observed previously which indicated a partial recovery of the change.

There were no peripheral nerve lesions.

Pathology results 26 weeks post final treatment:

Muscle weight returned to normal in the high dose group. At the third necropsy the recovery of the reduced size of the fibers was considered total with only a few residual fibers. Although the level of adipose tissue was low in the injected muscle, it was still seen in females given 2 U/injection.

There were no abnormal nestin immunostaining patterns seen in the untreated (right) gastrocnemius muscle of the treated animals. Abnormal circular staining was only observed in 1/8 females given 2 U/injection and none of the animals in the 0.1 U/injection group, indicating an almost complete recovery to the normal nestin staining pattern.

The following two summary tables were taken directly from the final study report.

**APPEARS THIS WAY
ON ORIGINAL**

Summary of the findings observed in the left gastrocnemius muscle.									
Time after the last injection	2 days			13 weeks			26 weeks		
Groups	C	L	H	C	L	H	C	L	H
Number examined	8	8	8	8	8	8	8	8	8
Reduced fibre size with increased nuclear density									
<i>Number of animals with the finding</i>	0	5	8	0	2	8	0	0	0
Minimal	-	2	1	-	2	3	-	-	-
Slight	-	3	2	-	-	4	-	-	-
Moderate	-	-	3	-	-	1	-	-	-
Marked	-	-	2	-	-	-	-	-	-
Scattered smaller fibres									
<i>Number of animals with the finding</i>	0	0	0	0	0	0	0	1	3
Present	-	-	-	-	-	-	-	1	3
Muscle fibre degeneration									
<i>Number of animals with the finding</i>	0	2	4	0	0	0	0	0	0
Minimal	-	2	3	-	-	-	-	-	-
Slight	-	-	1	-	-	-	-	-	-
Increased connective tissue									
<i>Number of animals with the finding</i>	0	1	6	4	1	4	6	5	4
Minimal	-	1	6	4	1	4	6	5	4
Increased adipocyte number									
<i>Number of animals with the finding</i>	0	0	1	0	0	6	0	0	6
Minimal	-	-	1	-	-	5	-	-	5
Slight	-	-	-	-	-	1	-	-	1

-: Observation not recorded in group

C: control females

L: Females given 0.1 U/injection

H: Females given 2 U/injection

Summary of the nestin immunostaining results. Left gastrocnemius muscle.									
Time after the last injection	2 days			13 weeks			26 weeks		
Groups	C	L	H	C	L	H	C	L	H
Number examined	4	4	4	8	8	8	8	8	8
Crescent shaped staining (normal NMJ staining pattern)									
<i>Number of animals with the finding</i>	3	3	2	8	6	8	8	8	8
Minimal	2	3	2	5	4	5	4	5	4
Slight	1	-	-	3	2	3	4	3	4
Circular shaped staining (abnormal NMJ staining pattern)									
<i>Number of animals with the finding</i>	0	1	4	0	2	1	0	0	1
Minimal	-	-	1	-	2	1	-	-	1
Slight	-	1	3	-	-	-	-	-	-
Diffuse staining (abnormal NMJ staining pattern)									
<i>Number of animals with the finding</i>	0	2	4	0	0	2	0	0	0
Minimal	-	2	2	-	-	2	-	-	-
Slight	-	-	2	-	-	-	-	-	-

-: Observation not recorded in group

C: control females

L: Females given 0.1 U/injection

H: Females given 2 U/injection

Conclusions:

Three intramuscular injections of Dysport at 4-week intervals, at dose levels of 0.1 or 2 U/injection in female rats induced a reduction of the fiber size in the gastrocnemius muscle. The related clinical observation was an apparent shrinkage of the injected muscle and reduced locomotory activity. Most of the fibers had returned to their normal size after a 13-week recovery period. Recovery of the muscle fiber size was essentially complete after 26 weeks, with full recovery of the locomotory activity at 17 weeks post treatment.

At the end of the treatment staining of the intermediate filament nestin revealed an abnormal pattern of the NMJ at the injection site indicating disruption of the structure post synaptic junction. The integrity of this structure was partially restored after 13 weeks without treatment and returned to a normal pattern after 26 weeks.

The observed changes are consistent with the intended pharmacological action of botulinum toxin. The return to the original size and the reestablishment of the normal nestin pattern indicates that the NMJ has regained a structural and functional integrity after prolonged blockade. Therefore, under the conditions of this study, the effects of repeat treatments with Dysport are reversible and appear to cause no long-term impairment of the NMJs.

2.6.6.9 Discussion and Conclusions

Botulinum poisoning in humans is usually by the oral route and occurs when food contaminated with *Clostridium botulinum* is ingested. In the current indication, botulinum toxin type A is administered by multiple small injections directly into the affected muscle. As such the nonclinical studies have focused on its effects following intramuscular injection. No systemic toxicity was observed after single (up to 6 U/rat) or repeat (up to 10 U/rat, up to 20 U/ rabbit) intramuscular dosing. Changes were limited to a decrease in body weight gain associated with decreased feed consumption in the first week(s) following the injection. This was accompanied by a reduction in the weight of the injected gluteus muscles and myocyte atrophy, both considered to be related to an exaggerated pharmacological action of the toxin. There were no compound-related lesions of the muscle groups distant to the injection sites or of the peripheral or central nervous systems.

A complete set of reproductive studies (fertility, embryo-fetal and pre- and postnatal studies) was conducted to detect the potential effects of *Clostridium botulinum* toxin type A hemagglutinin complex on reproduction. Although not statistically significant from controls, treatment with botulinum was associated with an increase in pre-implantation loss during the fertility study conducted in rats. Rabbits treated daily with botulinum toxin during embryofetal development also had an increase (not statistically significant) in pre-implantation loss and a tendency for an increase in incomplete ossification (2nd and 4th sternebrae) relative to controls, which achieved statistical significance when does were treated intermittently. A tendency for an increase in pre-implantation loss/early resorption was also noted in rats as was a tendency for increases in unossified 6th sternebrae. Additionally there was an increase in the percent of dams with stillbirths at the high dose in the pre- and post-natal development study. As such, it seems appropriate for Reloxin® to carry Pregnancy Category C labeling, as does BOTOX® (Botulinum toxin type A, marketed by Allergan). Although botulinum toxin was not clearly associated with teratogenesis in either species, skeletal malformations were noted in the rabbit study and confirmation requires repeating the study with a high dose of 20 SU/day.

The sponsor has conducted a study in female rats to evaluate the effects of chronic dosing at the nerve terminals. This study consisted of three monthly intramuscular injections at two dose levels (0.1 and 2 U/injection) followed by a 13-week or 26-week recovery period. This treatment regimen induced a reduction of the fiber size in the gastrocnemius muscle and was observed clinically as an apparent shrinkage of the injected muscle and reduced locomotory activity. Most of the fibers had returned to their normal size after a 13-week recovery period. Recovery of the muscle fiber was essentially complete after 26 weeks, with full recovery of locomotory activity at 17 weeks post treatment.

2.6.6.10 Tables and Figures N/A

2.6.7 TOXICOLOGY TABULATED SUMMARY N/A

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: From a pharmacology/toxicology perspective, Reloxin® for patients with moderate to severe glabellar lines is approvable, but will require a post-marketing commitment to perform a definitive rabbit embryofetal study to confirm the finding of skeletal malformations in rabbits.

Unresolved toxicology issues: The rabbit embryofetal study is considered inadequate, due to deaths at the high dose which was tolerated in the preliminary study. Consequently the current rabbit embryofetal data will not be included in the label and the sponsor will be required to repeat the study using 20 SU/rabbit/day as the high dose as a post-marketing commitment. It is appropriate to require this as a post-marketing commitment rather than pre-approval because although the data is needed for inclusion in the label, it does not effect how women of child bearing potential would be informed (i.e., Pregnancy Category). This course of action was reviewed/approved by Dr. Abby Jacobs (1-22-09). At present (1-23-09) the sponsor is seeking a — flagellin specification for the drug product. The maximum level of flagellin contamination qualified by the reproductive toxicology studies is — The maximum level of flagellin qualified in the repeat-dose study to examine the effects at the NMJ (AA60533) is no more than —6. This may be an overestimation of the relative flagellin contamination because flagellin is measured by SDS-PAGE and co-elutes with other small peptides, including nontoxic nonhemagglutinin. Therefore the sponsor will be asked to perform the repeat rabbit embryofetal study with drug product containing at least — , 'flagellin' contamination. The pharmacology/toxicology recommendation for the final drug specification should be no higher than the maximum amount qualified nonclinically (i.e., —), but definitely less than —

b(4)

Recommendations: The sponsor-proposed labeling has been modified and the recommended labeling appears below.

The Pharmacology/Toxicology suggested labeling provided below is consistent with wording proposed by the Division of Neurology Products (DNP) as of the date of this review. The only differences are the multiple of human dose values due to different clinical doses for the cervical dystonia indication (1000 Units/dose) compared to the glabellar lines indication (50 Units/dose). The Pharmacology/Toxicology suggested labeling is subject to changes depending on the label proposed by DNP for Dysport. DNP is expected to require the sponsor to perform a definitive rabbit embryofetal study as a post-marketing commitment. Once a time-line has been established for this commitment a memo review will be written to capture this information, as well as any significant labeling changes.

Suggested labeling:

8 USE IN SPECIFIC POPULATIONS

8.1 PREGNANCY: CATEGORY C

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b(4)

b(5)

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Reviewer's comment: A conservative estimate of the average weight (50 kg as opposed to the usual 60 kg) has been chosen for dose calculation comparison for this indication (cosmetic) to protect smaller individuals. Nonclinical dose information has been expressed on a daily basis (as opposed to total dose) because a specific critical time for botulinum toxin administration is not known.

13 NONCLINICAL TOXICOLOGY

13.1 CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY

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b(4)

b(5)

└

Signatures (optional):

Reviewer Signature



Supervisor Signature _____

Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

REFERENCES

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Pearce LB, Borodic GE, Johnson EA, First ER, MacCallum R (1995) The median paralysis unit: A more pharmacologically relevant unit of biologic activity for botulinum neurotoxin. Toxicon 33(2):217-227

Sponsor's suggested labeling:

2 Page(s) Withheld

 Trade Secret / Confidential (b4)

X Draft Labeling (b4)

 Draft Labeling (b5)

 Deliberative Process (b5)

Part C – Non-Clinical Pharmacology/Toxicology Reviewer(s)

CTD Module 2 Contents	Present?	If not, justification, action & status
Overall CTD Table of Contents [2.1]	N	No separate ToC [2.1]
Introduction to the summary documents (1 page) [2.2]	Y	
Non-clinical overview [2.4]	Y	
Non-clinical summary [2.6]	Y	
<input type="checkbox"/> Pharmacology	Y	
<input type="checkbox"/> Pharmacokinetics	Y	
<input type="checkbox"/> Toxicology	Y	

CTD Module 4 Contents	Present?	If not, justification, action & status
Module Table of Contents [4.1]	N	No separate ToC [4.1]
Study Reports and related info. [4.2]	Y	Pharmacokinetics not applicable for this BLA
<input type="checkbox"/> Pharmacology	Y	
<input type="checkbox"/> Pharmacokinetics	N	
<input type="checkbox"/> Toxicology	Y	
Literature references and copies [4.3]	Y	

Examples of Filing Issues	Yes?	If not, justification, action & status
content, presentation, and organization sufficient to permit substantive review?	Y	
<input type="checkbox"/> legible	Y	
<input type="checkbox"/> English (or translated into English)	Y	
<input type="checkbox"/> compatible file formats	Y	
<input type="checkbox"/> navigable hyper-links	Y	
<input type="checkbox"/> interpretable data tabulations (line listings) & graphical displays	Y	
<input type="checkbox"/> summary reports reference the location of individual data and records	Y	
<input type="checkbox"/> protocol-specified (as opposed to a different, post-hoc analysis) and other critical statistical analyses included	Y	
<input type="checkbox"/> all electronic submission components usable	Y	
data demonstrating comparability of product to be marketed to that used in clinical trials (when significant changes in manufacturing processes or facilities have occurred)	N	No significant changes have occurred
for each non-clinical laboratory study, either a statement that the study was conducted in compliance with the good laboratory practice requirements set forth in 21 CFR Part 58 or, if the study was not conducted in compliance with such regulations, a brief statement justifying the non-compliance	Y	Overall statement re: GLP in 2.6.6.1

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ON ORIGINAL**