

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

204078Orig1s000

PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: 204078
Supporting document/s: SDN 1, SDN 14, SDN 16
Applicant's letter date: July 31, 2012, Feb 21, 2013, Mar 18, 2013
CDER stamp date: July 31, 2012, Feb 21, 1013, Mar 18, 2013
Product: (b) (4) (neostigmine methylsulfate
Injection)
Indication: Reversal of the effects of nondepolarizing
neuromuscular blocking agents after surgery
Applicant: Éclat Pharmaceuticals
Review Division: Division of Anesthesia, Analgesia, and Addiction
Products
Reviewer: Huiqing Hao, Ph.D.
Supervisor/Team Leader: R. Daniel Mellon, Ph.D.
Division Director: Bob Rappaport, M.D.
Project Manager: Allison Meyer

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 204078 are owned by Éclat Pharmaceuticals or are data for which Éclat Pharmaceuticals has obtained a written right of reference. Any information or data necessary for approval of NDA 204078 that Éclat Pharmaceuticals does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 204078.

TABLE OF CONTENTS

1	EXECUTIVE SUMMARY	6
1.1	INTRODUCTION	6
1.2	BRIEF DISCUSSION OF NONCLINICAL FINDINGS	7
1.3	RECOMMENDATIONS	8
2	DRUG INFORMATION	12
2.1	DRUG	12
2.2	RELEVANT INDs, NDAs, BLAs AND DMFs	13
2.3	DRUG FORMULATION	14
2.4	COMMENTS ON NOVEL EXCIPIENTS	16
2.5	COMMENTS ON IMPURITIES/DEGRADANTS OF CONCERN	19
2.6	PROPOSED CLINICAL POPULATION AND DOSING REGIMEN	29
2.7	REGULATORY BACKGROUND	30
3	STUDIES SUBMITTED	31
3.1	STUDIES REVIEWED	31
3.2	STUDIES NOT REVIEWED	31
3.3	PREVIOUS REVIEWS REFERENCED	31
4	PHARMACOLOGY	31
4.1	PRIMARY PHARMACOLOGY	31
4.2	SECONDARY PHARMACOLOGY	32
4.3	SAFETY PHARMACOLOGY	33
5	PHARMACOKINETICS/ADME/TOXICOKINETICS	34
5.1	PK/ADME	34
5.2	TOXICOKINETICS	36
6	GENERAL TOXICOLOGY	36
6.1	SINGLE-DOSE TOXICITY	36
6.2	REPEAT-DOSE TOXICITY	37
7	GENETIC TOXICOLOGY	38
7.1	<i>IN VITRO</i> REVERSE MUTATION ASSAY IN BACTERIAL CELLS (AMES)	38
7.2	<i>IN VITRO</i> ASSAYS IN MAMMALIAN CELLS	39
7.3	<i>IN VIVO</i> CLASTOGENICITY ASSAY IN RODENT (MICRONUCLEUS ASSAY)	40
7.4	OTHER GENETIC TOXICITY STUDIES	40
8	CARCINOGENICITY	40
9	REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY	40
10	SPECIAL TOXICOLOGY STUDIES	40
11	INTEGRATED SUMMARY AND SAFETY EVALUATION	40

APPEARS THIS WAY ON ORIGINAL

Table of Tables

Table 1: Labeling Recommendations	9
Table 2: Drug Product Formulation	14
Table 3: Comparison of Tonicity and Dosing Instructions for Neostigmine and Nimbex .	15
Table 4: Container Closure System	16
Table 5: Comparison of Phenol Exposures from Neostigmine and Anzemet	17
Table 6: Summary of Intravenous Phenol Nonclinical Toxicology Data	18
Table 7: Drug Substance Impurities	20
Table 8: Drug Product Degradants.....	22
Table 9: Extractables That May Exceed the TTC of NMT 1.5 mcg/day.....	26
Table 10: pKa Values of Solvents, Phenol, and Predicted Leachable.....	27
Table 11: 12-Month Stability Data Impurities and Leachables	29
Table 12: FDA-approved Neostigmine NDAs.....	30
Table 13: Adverse Effects Associated with Excessive Cholinergic Receptor Stimulation (Ecobichon, 2001)	33

Table of Figures

Figure 1: Chemical Structure of [REDACTED] (b) (4)	28
Figure 2: Metabolism of Neostigmine	35
Figure 3: Cumulative Urinary Excretion of Neostigmine and Metabolites after IM Injection.....	36

1 Executive Summary

As discussed in the preIND/preNDA meetings with the Sponsor beginning in June of 2011, given the long clinical history of neostigmine use, no new nonclinical pharmacology or toxicology studies for the drug substance were required to support approval of this NDA. The pharmacology toxicology review therefore focused on the safety of the drug substance impurities and drug product degradants, the container closure system, and the drug product excipients. Upon review of these data, we conclude that there are no safety concerns with respect to the container closure system, the drug substance impurity specifications, or the drug product degradant specifications. In terms of excipient safety qualification, the total daily dose of the preservative phenol via this drug product formulation exceeds that of currently FDA-approved drug products that are administered as a single bolus injection; however, we recognize that previous clinical experience exists with the marketed unapproved drug products that may justify the safety in the phenol exposure via this product (see medical officer review).

As noted in the preIND meeting minutes from 2011, the Sponsor was also informed that the standard battery of genetic toxicology studies and reproductive and developmental toxicology studies would be required to be completed post-marketing if adequate data could not be identified in the published literature to appropriately inform labeling. A single in vitro bacterial reverse mutation assay was submitted for neostigmine and the results predict negative mutagenic potential. However, based on the lack of adequate data in the published literature to inform labeling with respect to in vitro clastogenicity, the in vivo genetic toxicity, and the reproductive and developmental toxicity of neostigmine, these studies are recommended as post-marketing requirements.

From a nonclinical pharmacology toxicology perspective, NDA 204078 may be approved pending agreement on labeling and with the recommended post-marketing requirements (PMRs).

1.1 Introduction

Neostigmine methylsulfate is a cholinesterase inhibitor. Éclat Pharmaceuticals has submitted NDA 204078 for neostigmine injectable seeking an indication for the reversal of the effects of nondepolarizing neuromuscular blocking agents after surgery.

According to archival records at the FDA, neostigmine in various dosage forms has been marketed in the United States since 1932 for a variety of uses including as a stimulant of the gastrointestinal tract and for the symptomatic treatment of myasthenia gravis. It was first approved by the FDA as an effective drug substance via the DESI process in 1939 (see Table 12 for NDAs submitted to the Agency for drug products containing neostigmine). Neostigmine has been used clinically to reverse the effects of nondepolarizing neuromuscular blocking agents used during surgical procedures. Inhibition of the enzyme acetylcholinesterase results in increased levels of acetylcholine in the neuromuscular synapse which can then compete with and displace

neuromuscular blocking agents. To date, a single entity injectable neostigmine drug product has not been previously approved by the FDA. All products on the market as of the date of this review are deemed marketed-unapproved drug products by the FDA.

The Applicant, Éclat Pharmaceuticals, does not currently market this drug product. The Agency has had numerous meetings with Éclat regarding the data that would be deemed necessary to submit an NDA for the proposed intravenous neostigmine drug product. Based on the long history of clinical use, the Division agreed that no nonclinical studies for neostigmine would be required to be completed for NDA approval. The NDA should include a literature review of the existing data, and, if upon review, the data were not deemed adequate to inform drug product labeling, additional studies may be required to be completed as Post-Marketing-Requirements (PMR) (preIND 111853, meeting minutes for meeting dated 6/30/2011). Additionally, the NDA must adequately address the blood compatibility for the formulation, provide justification for safety of the excipients, particularly the phenol, evaluate and justify the safety of the leachables/extractables, and justify the safety of impurities/degradants the levels exceed the ICH qualification threshold limits (preIND 111853, meeting minutes for meeting dated 06/30/2011 and 05/12/2012).

1.2 Brief Discussion of Nonclinical Findings

There were no new toxicology studies submitted in support of this NDA application. Based on published literature, the existing toxicology information is summarized below:

The toxicity of neostigmine in animals as reported in the literature is consistent with excessive nicotinic and muscarinic receptor activation. The toxic effects include skeletal muscle weakness and fasciculations, pupillary constriction, increased lacrimation, salivation and airway secretions, rise in colonic pressure, colonic spasms, defecation, flatulence, diarrhea, and, at higher doses, convulsions, dyspnea, bradycardia, and death. Death is usually caused by respiratory failure due to constriction of the bronchiolar musculature and excess bronchiolar secretions. The main toxicities are observed shortly after dosing (e.g., 2-4 minutes after a single subcutaneous dose of 0.1 mg in rats) and decrease in intensity as neostigmine is cleared from the circulation (Aeschlimann and Reinert, 1931). Toxicities after repeated doses were similar to the acute toxicities but tolerance develops after a few doses.

The Applicant submitted an in vitro bacterial reverse mutation assay for neostigmine indicating no concern for mutagenic potential. Adequate data with respect to the complete characterization of the genotoxic potential based on current standards and reproductive and developmental toxicology studies were not available in the published literature. Therefore, we recommend that these studies be completed as Post-Marketing-Requirements. In the mean time, this drug will be labeled as a Pregnancy Category C drug due to lack of adequate nonclinical data.

Carcinogenicity studies are not required for the proposed acute use. There are no adequate carcinogenicity data in the published literature.

1.3 Recommendations

1.3.1 Approvability

From a nonclinical pharmacology toxicology perspective, NDA 204078 may be approved, pending agreement on the drug product labeling and with the recommended post-marketing requirements (PMRs) as listed below.

1.3.2 Additional Non Clinical Recommendations

There are no adequate reproductive and developmental toxicity data available in the published literature and only one of the standard battery of genotoxicity studies has been completed to date. To allow adequate drug product labeling, post-approval requirements for the full standard batteries of reproductive and developmental toxicology and genetic toxicology studies (excluding the completed Ames test) are recommended.

Based on the data submitted to date, the following studies are recommended as post-marketing requirements (PMRs) should this NDA be approved:

1. Conduct an in vitro or in vivo assay using mammalian cells for chromosomal damage for neostigmine methylsulfate.
2. If you conducted an in vivo assay to address Item 1 above, conduct a second in vivo assay for chromosomal damage for neostigmine methylsulfate; otherwise conduct an in vivo assay for chromosomal damage for neostigmine methylsulfate. NOTE: To address PMRs 1-2, you may refer to the options outlined in ICH S2(R1) titled "Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use" and propose an adequate battery of genetic toxicology studies.
3. Conduct a fertility and early embryonic development toxicology study in the rat model for neostigmine methylsulfate.
4. Conduct an embryo-fetal developmental toxicology study using the rat model for neostigmine methylsulfate.
5. Conduct an embryo-fetal developmental toxicology study using the rabbit model for neostigmine methylsulfate.
6. Conduct a peri- and post-natal developmental toxicology study in the rat model for neostigmine methylsulfate.

1.3.3 Labeling

Table 1: Labeling Recommendations

Sponsor's Proposed Labeling	Recommended Labeling	Rationale/Comment
<p>Highlights Indication and Usage NEOSTIGMINE METHYLSULFATE INJECTION, USP is a cholinesterase inhibitor indicated for ...</p>	<p>Highlights Indication and Usage Neostigmine Methylsulfate Injection USP, a cholinesterase inhibitor, is indicated for ...</p>	<p>Acceptable. Although neostigmine has not been previously given an FDA Established Pharmacological Class (EPC) designation, edrophonium has been designated "Cholinesterase inhibitor." The EPC must be included in the highlights. The language regarding the final indication will be determined by the clinical review team.</p>
<p>Use in (b) (4) Populations</p>	<p>Use in (b) (4) Populations Pregnancy: No human or animal data. Use only if clearly needed.</p>	<p>The Sponsor proposed nothing in the highlights, as there currently are no data, this is technically a Pregnancy Category C drug and the language proposed is CDER standard for this category.</p>
<p>8 USE IN SPECIFIC POPULATIONS</p> <p>8.1 Pregnancy</p> <p>Pregnancy Category C. (b) (4)</p> <p>(b) (4)</p> <p>It is not known whether neostigmine can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity. NEOSTIGMINE METHYLSULFATE INJECTION, USP should be given to a pregnant woman only if (b) (4)</p> <p>(b) (4)</p>	<p>8 USE IN SPECIFIC POPULATIONS</p> <p>8.1 Pregnancy Teratogenic effects. Pregnancy Category C</p> <p>(b) (4)</p> <p>It is not known whether Neostigmine Methylsulfate Injection can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity. Neostigmine Methylsulfate Injection should be given to a pregnant woman only if clearly needed (b) (4)</p> <p>(b) (4)</p> <p>Animal reproduction studies have not been conducted with neostigmine.</p>	<p>Statement of human data is listed first, as requested by the Maternal Health Staff in anticipation of finalization of proposed Pregnancy Labeling and Lactation Rule (PLLR).</p> <p>Language modified to accurately reflect the CFR requirements.</p>
<p>10. OVERDOSAGE</p> <p>(b) (4)</p>	<p>(b) (4)</p>	<p>The information is not deemed useful to the physician.</p>

Sponsor's Proposed Labeling	Recommended Labeling	Rationale/Comment
<p>(b) (4)</p>	<p>(b) (4)</p>	
<p>12 CLINICAL PHARMACOLOGY 12.1 Mechanism of Action Neostigmine is a cholinesterase inhibitor. (b) (4)</p> <p>(b) (4)</p> <p>Neostigmine does not readily cross the blood-brain barrier and therefore does not significantly affect cholinergic function in the central nervous system.</p>	<p>12 CLINICAL PHARMACOLOGY 12.1 Mechanism of Action Neostigmine is a competitive cholinesterase inhibitor (b) (4)</p> <p>By reducing the breakdown of acetylcholine, neostigmine-induces an increase in acetylcholine in the synaptic cleft which completes for the same binding sites as nondepolarizing neuromuscular (b) (4) and reverses the neuromuscular blockade. (b) (4)</p> <p>—Neostigmine does not readily cross the blood-brain barrier and therefore does (b) (4) not significantly affect cholinergic function in the central nervous system.</p>	
<p>12.2 Pharmacodynamics (b) (4)</p> <p>(b) (4)</p>	<p>12.2 Pharmacodynamics (b) (4)</p> <p>(b) (4)</p>	

Sponsor's Proposed Labeling	Recommended Labeling	Rationale/Comment
	<p>Neostigmine-induced increases in acetylcholine levels results in the potentiation of both muscarinic and nicotinic cholinergic activity. The resulting elevation of acetylcholine competes with nondepolarizing neuromuscular blocking agents to reverse neuromuscular blockade.</p>	
<p>13 NONCLINICAL TOXICOLOGY</p>	<p>13 NONCLINICAL TOXICOLOGY</p>	
<p>13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility</p> <p><i>Carcinogenesis:</i> (b) (4)</p> <p><i>Mutagenesis:</i> (b) (4)</p> <p><i>Impairment of Fertility:</i> Studies on the effect of neostigmine methylsulfate on fertility have not been performed.</p>	<p>13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility</p> <p><i>Carcinogenesis:</i> Long-term animal studies have not been performed to (b) (4)</p> <p>(b) (4) -evaluate (b) (4) the carcinogenic potential of neostigmine.</p> <p><i>Mutagenesis:</i> (b) (4)</p> <p>Neostigmine was not mutagenic in an in vitro bacterial reverse mutation assay (Ames test).</p> <p><i>Impairment of Fertility:</i> Studies on the effect of neostigmine methylsulfate on fertility have not been performed.</p>	
<p>(b) (4)</p>	<p>(b) (4)</p>	<p>These data are covered in Section 8 and do not need to be repeated here.</p>

Sponsor's Proposed Labeling	Recommended Labeling	Rationale/Comment
(b) (4)	(b) (4)	

The above labeling recommendations are being made prior to negotiation with the Applicant and discussion with the team. For final labeling of this product, the reader is referred to the action letter.

2 Drug Information

2.1 Drug

CAS Registry Number: 51-60-5

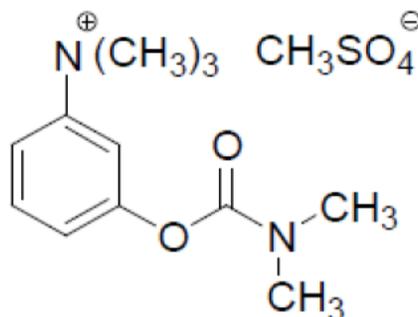
Generic Name Neostigmine methylsulfate

Code Name None

Chemical Name (m-hydroxyphenyl) trimethylammonium methylsulfate dimethylcarbamate
or
Benzenamimium, 3[[[(dimethylamino) carbonyl]oxy]-N,N,Ntrimethyl-, methylsulfate

Molecular Formula/Molecular Weight $C_{13}H_{22}N_2O_6S$ / 334.39 g/mol

Structure



Pharmacologic Class Cholinesterase inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND#	Drug	Status	Division	Indication	Stamp Date	Sponsor
111853	Neostigmine methylsulfate Injections, USP	Presubmission	DAAAP	Reversal of Neuromuscular Blockade	3/28/2011	Éclat Pharmaceuticals

NDA#	Drug Name	Division	Strength (route)	Marketing Status	AP Date	Indication	Company
20624	Anzemet (dolasetron mesylate) injection	DGIEP	20 mg/mL	Approved	9/11/1997	Prevention of nausea and vomiting associated with chemotherapy	sanofi aventis US LLC
20551	Nimbex (cisatracurium besylate) injection	DAAAP	2 mg/mL; 10 mg/mL	Approved	12/15/1995	Intermediate duration neuromuscular blocking agent	Abbvie Inc

The Applicant submitted patent certification statements for both of these NDAs noting that to the best of their knowledge, there are no unexpired patents for Anzemet and that this NDA application does not infringe upon Patent No. 5453510 for Nimbex. The company is referencing Anzemet to justify the levels of phenol in the drug product. They are referencing Nimbex to justify the tonicity of the drug product.

DMF#	Subject of DMF	Holder	Submit Date	Reviewer's Comment
(b) (4)	Neostigmine methylsulfate as manufactured in (b) (4)	(b) (4)	3/21/2011	Active, reviewed by Dr. Edwin Jao on 12/21/2012 who deemed the MF adequate.
	(b) (4)	(b) (4)	01/15/1995	Active, review updated as of 01/28/2011 and deemed acceptable for pharmaceutical usage; referenced by many approved ANDAs
	(b) (4)	(b) (4)	06/20/1994	Active, the DMF has been deemed acceptable for numerous ANDAs. According to DARRTs, there are no reviews for the particular stopper being employed for this drug product. The DMF contains data on many different rubber stoppers; however, the data for the specific stopper used in this drug product have been reviewed for this NDA.

2.3 Drug Formulation

The table below depicts the quantitative drug product formulation.

Table 2: Drug Product Formulation

Component	Function	Quality Standard	Quantity (mg/mL)	
			1:2000 Conc.	1:1000 Conc.
Neostigmine methylsulfate	API	USP, Ph. Eur., JP	0.5	1.0
Phenol	Preservative	USP-NF, Ph. Eur., JP	4.5	4.5
Sodium acetate trihydrate	(b) (4)	USP-NF, Ph. Eur., JP	0.2	0.2
Acetic acid/Sodium hydroxide	pH adjustment	USP-NF, Ph. Eur., JP	q.s. to pH 5.5	q.s. to pH 5.5
Water for Injection	(b) (4)	USP, Ph. Eur., JP	(b) (4)	

The osmolality of the solution is approximately 53-59 mOsmol, which is hypotonic (isotonic solutions are ~290 mOsmol). Although this drug is not isotonic, the Applicant notes that the FDA approved drug Nimbex (cisatracurium besylate) is also indicated for intravenous use and that drug has an osmolality of 8 mOsmol/L and is injected in the same volume as that proposed. Therefore, there is an FDA previous finding of safety for an intravenous hypotonic drug product, as summarized in the table below from the Sponsor.

Table 3: Comparison of Tonicity and Dosing Instructions for Neostigmine and Nimbex

	Neostigmine Methylsulfate (Éclat Pharmaceuticals)	Nimbex (Abbott Laboratories)
Indication	<i>Reversal of effects of non-depolarizing blocking agents</i>	As an adjunct to general anesthesia, to facilitate tracheal intubation
Concentration of drug in drug product	1 mg/mL	2 mg/mL
Dose of drug	5 mg (maximum dose)	0.2 mg/kg
Volume of drug administered at the above dose	5 mL	5 mL for a 50 kg adult; larger volumes for heavier individuals
Osmolality	55 mOsm/L	Approximately 8 mOsm/L
Route of administration	Intravenous	Intravenous
Rate of administration	Slow intravenous injection*	Over 5 to 10 seconds
Reference	Formulation and dosing recommendations based on current marketed unapproved products (e.g., Neostigmine methylsulfate package insert 04/2008)	Nimbex package insert 12/2010 MHRA 2011

*Over at least 1 minute

The container closure components for this drug product include a Type I clear glass tubular vial (b) (4) and an aluminum seal with a (b) (4) flip-off lid (b) (4) as summarized in the table below, reproduced from the Sponsor's submission.

(b) (4)

Table 4: Container Closure System

Component	Manufacturer/Supplier	DMF	Specification	Certificate of Analysis
(b) (4)				CofA
				CofA
				CofA

To the best of this review team’s knowledge, the particular (b) (4) has not been previously employed in an FDA-approved parenteral drug product. See discussion below regarding the extractable/leachable safety assessment.

2.4 Comments on Novel Excipients

Sodium acetate has been used in numerous IV drug products with a maximum potency of 59.4% as per the FDA Inactive Ingredients Database (IID). This excipient is not deemed novel by the Agency under the conditions of use proposed.

During the preIND/preNDA meetings, the Sponsor was specifically asked to address the safety of the proposed level of phenol in the drug product. The current product contains 4.5 mg/mL phenol as a preservative, with the same concentration employed in both the 0.5 mg/mL and 1.0 mg/mL strengths of neostigmine. Based on the maximal clinical dose of 5 mg neostigmine, the total dose of phenol is expected to be 45 mg if the 0.5 mg/mL neostigmine is used or 22.5 mg if the 1.0 mg/mL neostigmine drug product is employed. The Agency’s risk assessment must be based on the potential that up to 45 mg of phenol could be administered via this product as labeled. Currently, numerous FDA-approved IV drug products contain up to 5 mg/mL phenol, therefore, the concentration of phenol in this drug product is less than other FDA-approved intravenous drug products and the total daily dose of intravenous phenol is also less than other FDA-approved intravenous drug products. From these perspectives, phenol is not novel. However, in all other identified FDA-approved drug products, the drugs are administered several times a day rather than as a single bolus injection. Therefore, the

use of phenol in this drug product is novel in the sense that it likely results in a higher C_{max} than any other identified FDA-approved drug product to date based on current labeling.

The Sponsor did find historical data to indicate that the drug Anzemet (dolasteron mesylate), which contains phenol, was originally labeled for dosing up to 100 mg (20 mg/mL solutions) for the treatment of prevention of chemotherapy-induced nausea of vomiting, as outlined in the table below and reproduced from the submission:

Table 5: Comparison of Phenol Exposures from Neostigmine and Anzemet

	Neostigmine Methylsulfate (Éclat Pharmaceuticals)	Anzemet (sanofi-aventis)
Indication	<i>Reversal of effects of non-depolarizing blocking agents</i>	Prevention of chemotherapy-induced nausea and vomiting
Concentration of drug in drug product	1 mg/mL	20 mg/mL
Dose of drug	5 mg (maximum dose)	100 mg (standard dose)
Volume of drug administered at the above dose	5 mL	5 mL
Concentration of phenol in the drug product	4.5 mg/mL	5 mg/mL
Maximum daily dose of phenol	22.5 mg	25 mg
Route of administration	Intravenous	Intravenous
Rate of administration	Slow intravenous injection*	Can be safely infused intravenously as rapidly as 100 mg/30 seconds
Reference	Formulation and dosing recommendations based on current marketed unapproved products (e.g., Neostigmine methylsulfate package insert 04/2008)	Anzemet package insert 01/2008

*Over at least 1 minute

The Applicant acknowledges that the indication and dosing regimen cited in the table above are not longer in the approved product labeling. This indication was removed in 2010 based on concerns that the drug product resulted in QTc prolongation. As discussed with the Applicant at the time of the preNDA meeting, the challenge faced by the Agency is that the removal of this indication was based on data obtained after administration of the drug product, and the adverse effect of QTc prolongation may have been due to the drug substance dolastetron or the formulation which contained phenol. That being said, there are data in the published literature that suggests that dolasetron and other 5HT₃ antagonist drugs can interact with cardiac ion channels (Kuryshv et al., 2000). However, we cannot definitively rule out the possibility that the phenol in this formulation contributed to the AEs.

The Applicant provided a comprehensive literature review for phenol pharmacology and toxicity. Phenol has concentration-dependent effects. It is considered to have bacteristatic properties at concentrations of $\geq 0.2\%$, bactericidal properties at $> 1\%$, fungicidal properties at $\geq 1.3\%$. These effects are believed to be mediated by the ability of phenol to denature protein (Harvey, 1975). When used in concentrations of 1-2% it is believed to have topical anesthetic properties. Consistent with this pharmacodynamic effect, phenol appears to inhibit voltage-gated sodium channels at concentrations ranging from 0.09 to 1.4% (Zamponi and French, 1993;Harrold et al., 1996). Concentrations of phenol between 0.5% and 1.5% appear to be legally marketed as oral mucosal analgesics via tentative final monograph. For example, the marketed over-the-counter Chloraseptic[®] sore throat spray contains 1.4% phenol. At higher concentrations (5%), phenol can have adverse local tissue effects resulting from protein denaturation and even tissue necrosis. There are also some data to suggest that phenol's local anesthetic effects can occur in the absence of denatured proteins, possibly via nonspecific interaction with plasma membrane lipids and proteins (Sikkema et al., 1995).

The acute toxicity of phenol has been fairly well characterized, but the data do not define a NOAEL. The existing IV toxicity data are summarized in the table below:

Table 6: Summary of Intravenous Phenol Nonclinical Toxicology Data

Species	Dose	Concentration	Findings	Comment	Reference
Mouse	50, 100, 150, 200 mg/kg	10 mg/mL	LD ₅₀ = 112 mg/kg No mortality at 50 mg/kg Deaths preceded by muscle tremors, respiratory depression and coma	- HED of 546.34 mg - No NOAEL established	(Wein, 1939)
Rabbit	80, 120, 180, 280, 420 mg/kg	2 - 5%	LD ₅₀ = 180 mg/kg Clinical signs of muscle tremors and twitching & respiratory depression preceded deaths	- HED 3484 mg - No NOAEL established	(Deichmann and Witherup, 2013)
Dog	30 mg/kg	Not reported	Significant decreased blood pressure, generalized muscle twitching, convulsions	- HED 1000 mg - No NOAEL established	(Pilcher and Stollman, 1915)
Dogs	100 mg/kg	Not reported	Neuromuscular irritability, coma, convulsions, blood hemolysis, kidney lesions	- HED 3333 mg - No NOAEL established	(Oehme and Davis, 1970)

In response to the Division's concern, Éclat provided the following rationale for the safety of phenol in this formulation:

- The vasculature exposure to phenol is expected to be less than 0.1% (1:4 dilution from the concentration of 4.5 mg/mL) due to the blood flow through the cephalic and basilic veins in the upper arms (40-95 mL/min) and the 10 mL of maximal dosing volume of neostigmine. With mixing in the blood beyond the injection site, the effective concentration of phenol in the blood would be further diluted.
- Studies of the effects of phenol on the nervous system indicate that injection of 5% phenol or greater directly onto neuronal tissue is required to produce neurolytic effect (Wood, 1978). Degenerative effects on downstream organs are not expected at a concentration of 0.1% phenol should blood flow deliver this concentration to a tissue. Coan and colleagues demonstrated that injection of 0.1% phenol to the kidney had no adverse renal effects either on renal function or when examined histopathologically (Coan et al., 1982). At low concentrations, phenol is recognized to produce electrophysiological effects. Studies showed that concentration of phenol at 0.1% are capable of blocking sodium channels (Zamponi and French, 1993;Harrold et al., 1996). A potential for a small transient adverse effect on cardiac electrical activity with low concentration of phenol in the blood cannot be ruled out. However, the patient population for which neostigmine is indicated would be receiving concomitant muscarinic blockade and would be under the close watch of an anesthesiologist.
- Phenol at a concentration of 0.1% is only marginally hemolytic (<2% of blood cells were lysed by 1 hour of incubation) in vitro (Bukowska and Kowalska, 2004).

The above information, although generally supportive of the safety for the local tissue effects of phenol, do not provide definitive safety justification. There are no adequate intravenous toxicology studies for either phenol or this specific neostigmine drug product formulation that can define a NOAEL for phenol; therefore, there are technically inadequate nonclinical data to justify the safety of the proposed bolus dose of phenol.

However, the Division recognizes that this formulation has been marketed by other companies in the U.S. and overseas for over 20 years, and considerable human experience appears to exist which may be deemed adequate upon review to justify the safety of the phenol in this drug product formulation. The reader is referred to the clinical review by Dr. Simone for further discussion. Assuming adequate clinical experience exists to justify the safety of the phenol in this product, no further nonclinical studies will be required to support approval of this NDA.

2.5 Comments on Impurities/Degradants of Concern

Drug Substance Impurities

The drug substance specifications are listed in the table below.

Table 7: Drug Substance Impurities

Impurity	Structure	Proposed specification	Reviewer Comment
(b) (4)			

Impurity	Structure	Proposed specification	Reviewer Comment
(b) (4)			

We requested a computational toxicology Qualitative Structure Activity Relationship (QSAR) evaluation to evaluate the mutagenic potential of these impurities via in silico analysis. The report is reproduced in the Appendix of this review. Neostigmine and all of the impurities were predicted to be positive in the Ames assay and one of the compounds actually has been reported in the literature to be positive in this assay. (b) (4)

Among the three impurities, only (b) (4) was proposed to be NMT (b) (4) which exceeds the ICH Q3A(R2) qualification threshold of 0.15%. However, (b) (4) the proposed (b) (4) in the drug substance as an impurity is acceptable. In conclusion, the proposed drug substance specifications are acceptable.

Drug Product Degradants

The drug product stability specifications are listed in the table below:

Table 8: Drug Product Degradants

Degradant	Structure	Proposed	Reviewer
(b) (4)			

As per the Applicant, there is only one identified drug product degradant, (b) (4), which was also discussed as a drug substance impurity. The proposed specification for (b) (4) of NMT (b) (4) is acceptable as it is below the ICH Q3B(R2) qualification threshold of NMT 0.5% or 200 mcg, whichever is lower, for a drug with a total daily intake of 10 to 100 mg.

Container Closure Leachables/Extractables

The container closure system includes a glass vial and rubber stopper that will come in contact with the drug solution. The (b) (4) product code is (b) (4). The stopper is the (b) (4) gray stopper.

During the preIND and preNDA meetings, the Division requested that specific extractable/leachable studies be completed, particularly since this drug product formulation contains phenol that may alter the extractable/leachable profile compared to other aqueous solutions.

An extractable/leachable assessment of the (b) (4) has been conducted (b) (4). According to the actual extraction study report (found in Module 4 of the submission), the following conditions were examined:

(b) (4)

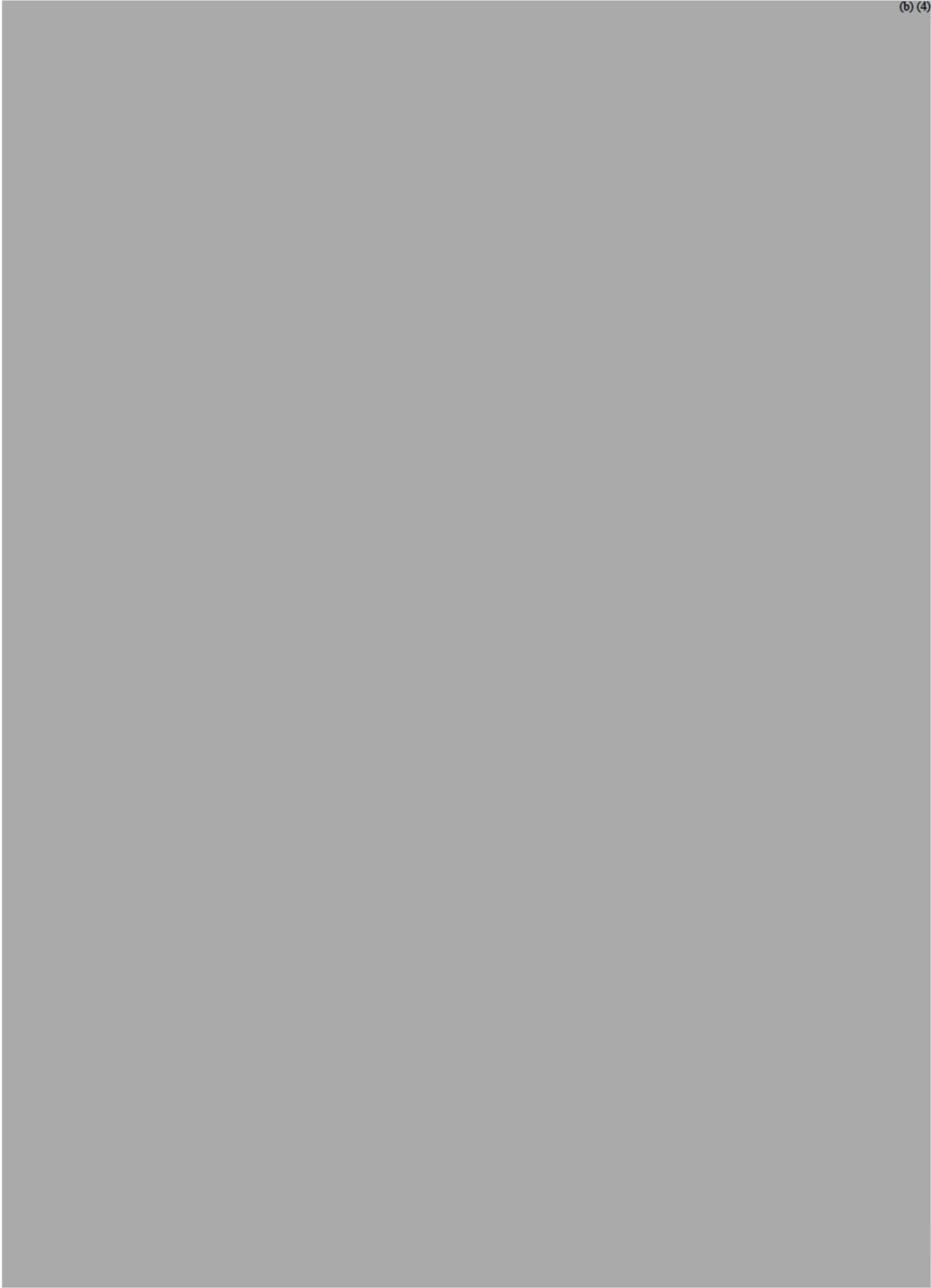
(b) (4)

(b) (4)

Numerous compounds were identified in these extraction studies, as summarized in the tables below:



(b) (4)



(b) (4)



The source of these compounds was attributed to [redacted] (b) (4)
[redacted]

Of the compounds identified in the extraction studies, only a few were actually found at levels that would exceed the TTC for genotoxic substances of 1.5 mcg/day via use of a single vial of this product. These are summarized in the table below, reproduced from the submission.

Table 9: Extractables That May Exceed the TTC of NMT 1.5 mcg/day

The content of Table 9 is completely redacted with a solid grey fill. In the top right corner of this redacted area, the text "(b) (4)" is printed in a small font.

[Redacted] (b) (4)

Table 10: pKa Values of [Redacted] (b) (4) Predicted Leachable

[Redacted] (b) (4)

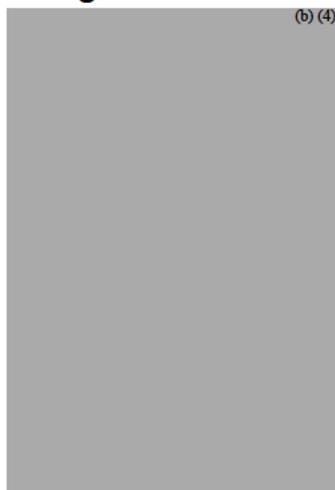
This conclusion was supported by our CMC review team; therefore, the extraction conditions are acceptable.

The Sponsor notes that the leachable [Redacted] (b) (4)

[Redacted] (b) (4)
[Redacted] (b) (4)
[Redacted] (b) (4)-related exposures to these substances are so low as to present negligible systemic non-carcinogenic toxic effects. Direct local effects are also not expected since concentrations in the drug product are in every case below normal levels circulating in plasma, with the exception of [Redacted] (b) (4). The slightly higher concentration of [Redacted] (b) (4) in the drug product than in plasma is not expected to have a significant safety impact due to the mixing in the blood and the natural physiological mechanisms used to control plasma [Redacted] (b) (4) levels within a tight range [Redacted] (b) (4). Therefore, these ions were not evaluated as potential leachables in the stability samples.

The findings and study plan for the extractable and leachable analysis were preliminarily discussed at the EOP-2 meeting in May of 2012. The Agency agreed with the Applicant's proposed plan to only monitor the drug product stability samples for [Redacted] (b) (4). The chemical structure of [Redacted] (b) (4) is provided below:

Figure 1: Chemical Structure of [redacted] (b) (4)



Based on a worst-case extraction data scenario, a person could be exposed to up to [redacted] (b) (4)

This would exceed the PQRI recommended Threshold of Toxicological Concern (TTC) of NMT 5 mcg/day for inhalation products. There do not appear to be any toxicology data for this chemical identified in any of the standard databases available. If the risk assessment were based on extraction data alone, further safety data would be required.

The results from the 6- and 12-month leachable analysis of the drug product samples on stability indicate that the level of this chemical are below the limit of quantitation [redacted] (b) (4) as noted in the table below from the Sponsors submission.

1 [redacted] (b) (4)

Table 11: 12-Month Stability Data Impurities and Leachables

(b) (4)



The Applicant originally proposed a specification of NMT (b) (4) for all leachables, which could result in a total daily dose of up to (b) (4) of any given leachable via use of a single vial of drug product. The Applicant believes that (b) (4) although above the PQRI recommended qualification threshold of NMT 5 mcg/day, is acceptable as the drug product is not used chronically. They provide no other safety justification other than a DEREK analysis for genotoxicity and carcinogenicity. The Agency does not accept QSAR data for anything other than the Ames assay. Their data would appear to support a specification of NMT (b) (4) which would result in exposure to up to (b) (4). During the course of the review, the Agency requested that rather than set a leachable specification of NMT (b) (4) they revise the specification to be NMT (b) (4). In their submission dated 3/18/2013, they revised the specification to NMT (b) (4), which is acceptable. There are no concerns with the container closure system.

2.6 Proposed Clinical Population and Dosing Regimen

Neostigmine Methylsulfate Injection is being proposed for the indication of reversal of neuromuscular blocking effects of non-depolarizing muscle relaxants after surgery. The recommended dosing regimen is intravenous bolus injection at initial dose of 30 mcg/kg and additional doses up to a total of 70 mcg/kg (or 5 mg whichever is less) when needed based on neuromuscular activities. Therefore, the maximum daily dose is 5 mg/day.

2.7 Regulatory Background

From 1939 to 1958, Hoffman-LaRoche Inc. received approvals for several neostigmine products (see the table below).

Table 12: FDA-approved Neostigmine NDAs

NDA#	Drug Name	Division	Strength (route)	Marketing Status	AP Date	Indication	Company
654	Prostigmin (Neostigmine bromide 5%) ophthalmic solution	DAIP	5% (ophthalmic)	Approval was withdrawn in 1995	1939	Glaucoma	Valeant Pharmaceuticals International
2449	Neostigmine methylsulfonate & Atropine sulfate	Unknown	1.5 mg/mL & 0.6 mg/mL (Injection)	Discontinued in 1954	5/9/1940	Intestinal peristalsis stimulant and diagnostic for Myasthenia gravis & related disorders	Hoffman-La Roche, Inc.
2574	Morphine sulfate & Neostigmine methylsulfonate	Unknown	8 mg morphine & 0.5 mg Neostigmine (hypodermic tablet)	Discontinued 1948	6/4/1940	Analgesic/ local anesthetic	Hoffman-La Roche
2575	Hydrochlorides of opioid alkaloids & Neostigmine methylsulfonate	Unknown	0.5 mg (hypodermic tablet)	Approved but never marketed; withdrawn?	6/13/1940	Analgesic	Hoffman-La Roche

(b) (4)

None of the above NDAs are being used as a referenced product for this NDA application, as none of the products are currently marketed. The above list is only provided for historical purposes, to document the first FDA approval date for the drug product labeling, and to document why neostigmine is not considered a new chemical entity.

As evident from the table above, the FDA has not previously approved a single entity intravenous neostigmine drug product. However, several companies including General Injectables and Vaccines Inc., American Regent Inc., Cardinal Health, and Fresenius Kabi, and Westward Pharmaceuticals Corp. have been marketing unapproved neostigmine injection (National Drug Code Directory Database). Éclat has not been marketing a neostigmine drug product.

Several preIND/preNDA meetings with Éclat Pharmaceuticals regarding the development plan for this drug product formulation beginning in 2011.

3 Studies Submitted

3.1 Studies Reviewed

There were no studies submitted. All nonclinical information was obtained from published literature.

3.2 Studies Not Reviewed

NA

3.3 Previous Reviews Referenced

None

4 Pharmacology

4.1 Primary Pharmacology

Reversal of neuromuscular blocking effects of nondepolarizing muscle relaxants:

Neostigmine is a cholinesterase inhibitor first reported in the literature in 1931 (Aeschlimann and Reinert, 1931). Neostigmine binds to acetylcholinesterase (AChE) and is also a substrate for acetylcholinesterase; however, the carbamate group on the neostigmine molecule forms an ester bond at the active site of the enzyme thereby producing a labile covalent bond. This ester bond is slow to be hydrolyzed and therefore, the neostigmine is frequently referred to as a “reversible” inhibitor as the AChE enzyme will regain activity in time. A similar process occurs with acetylcholine itself. However, acetylcholine is hydrolyzed rapidly; whereas the carbamylating agents such as neostigmine, the duration of inhibitions is between 3 and 4 hours (Taylor, 2001). By occupying acetylcholinesterase, neostigmine prevents the enzyme from hydrolyzing acetylcholine (ACh) and therefore increases ACh concentration in the neuromuscular junction in the synaptic cleft. For the proposed indication, the increased ACh competes with nondepolarizing muscle relaxants for acetylcholine receptors (nicotinic receptors) to reverse the muscle relaxation.

Numerous studies reported that neostigmine reverses neuromuscular blockade produced by nondepolarizing muscle relaxants *In vivo* (e.g., pancuronium, atracurium, curare, mivacurium) in rats, dogs, cats, and sheep (Aeschlimann and Reinert, 1931; Randall and Lehmann, 1950; Miller and Roderick, 1977b; Hennis et al., 1984; Jones, 1990). *In vitro*, neostigmine inhibited red blood cell acetylcholinesterase with IC_{50} of 6.9 nM (Harada et al., 2010).

The major metabolite, 3-hydroxyphenyltrimethylammonium (HPTMA) also has pharmacological activity as a cholinesterase inhibitor, but is 6.1-fold less potent than neostigmine (ED_{50} of 40 mcg/kg versus 6.5 mcg/kg) at antagonizing pancuronium-induced decreases in the force of contraction of the anterior tibialis muscle of the dog. Also, the characteristics of the time course of the effect was different from that of neostigmine as that with the equipotent doses, the onset of action was quicker (1 minute for HPTMA versus 10 minutes for neostigmine) and action duration was shorter (14 minutes for HPTMA versus 88 minutes for neostigmine) (Hennis et al., 1984).

Several authors have published data suggesting that neostigmine may also bind to muscarinic and nicotinic cholinergic receptors directly (Seifert and Eldefrawi, 1974; Sadoshima et al., 1988; Lockhart et al., 2001; Harada et al., 2010); however, these effects are believed to occur only at high concentrations that are not likely to occur in the clinical setting.

4.2 Secondary Pharmacology

Inhibition of acetylcholinesterase causes an increase in acetylcholine concentration in all cholinergic synapses, resulting in undesired stimulation of muscarinic and nicotinic ACh receptors in other tissues, e.g., the smooth muscles in the respiratory and gastrointestinal tract (Taylor, 1996). The table below summarizes the adverse effects of excessive cholinergic receptor stimulation (reproduced from Applicant's submission, as cited below):

Table 13: Adverse Effects Associated with Excessive Cholinergic Receptor Stimulation (Ecobichon, 2001)

Receptor Type	Site Affected	Manifestation
Muscarinic	Exocrine glands	Salivation, lacrimation, perspiration
	Eyes	Miosis, ptosis, blurred vision, conjunctival injection, bloody tears
	Gastrointestinal tract	Nausea, vomiting, abdominal tightness, swelling and cramps, diarrhea, tenesmus, fecal incontinence
	Respiratory tract	Excessive bronchial secretions, rhinorrhea, wheezing, edema, tightness in the chest, bronchospasm, bronchoconstriction, cough, bradypnea, dyspnea
	Cardiovascular system	Bradycardia, decreased blood pressure
	Bladder	Urinary frequency, incontinence
Autonomic nicotinic	Cardiovascular system	Tachycardia, pallor, increased blood pressure
Somatic nicotinic	Skeletal muscles	Muscle fasciculation, cramps, diminished tendon reflexes, generalized muscle weakness in peripheral and respiratory muscles, paralysis, flaccid or rigid tone

Pretreatment with or concomitant administration of muscarinic receptor antagonists, such as atropine or glycopyrrolate, can be used to reduce the unwanted stimulation of muscarinic acetylcholine receptors (Taylor, 2001).

4.3 Safety Pharmacology

Dedicated safety pharmacology studies were not complete for this NDA application given the long clinical history of use.

CNS effects:

As neostigmine does not readily cross the blood-brain barrier, significant CNS effects are expected when neostigmine is administered intravenously as proposed. There are no studies examining CNS effects after intravenous administration of neostigmine.

Cardiovascular effects:

Neostigmine can produce bradycardia. In addition to cholinesterase inhibition, neostigmine has been reported to directly interact with cardiac muscarinic ACh receptors and nicotinic receptors (Dunlap and Brown, 1983; Sherby et al., 1985). In an isolated guinea pig right atrium model, neostigmine decreased the spontaneous beating rate in a concentration-dependent manner up to 10 mcM (Endou et al., 1997), but returned to pre-drug levels with higher concentration (1 mM). The bradycardia was also abolished by atropine.

Respiratory effects:

Intravenous infusion of neostigmine to rabbits at 2.5 mcg/kg/min, but not 1 mcg/kg/min caused respiratory stimulation, accompanied by fasciculations and concurrent lactic acidosis. This effect was thought to be peripheral nicotinic receptor mediated as it was abolished by hexamethonium (Weinstock et al., 1981). In contrast to the respiratory stimulating effects, neostigmine given to rats that had fully recovered from neuromuscular blocking agents (vecuronium and rocuronium), showed dose-related impairment of respiration: IV dose of 0.03 to 0.12 mg/kg, impaired upper dilator muscle activity, genioglossus muscle function, diaphragmatic function and minute volume (Eikermann et al., 2007;Eikermann et al., 2008).

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

No new ADME studies were completed in support of this NDA. The data below were summarized from the published literature.

Neostigmine is poorly absorbed following oral administration. In rats given ¹⁴C labeled neostigmine 250 mcg orally, approximately 50% of dose was detected in the intestinal contents and feces, and about 20% of the dose was excreted in the urine by 24 hours postdosing (Roberts et al., 1966).

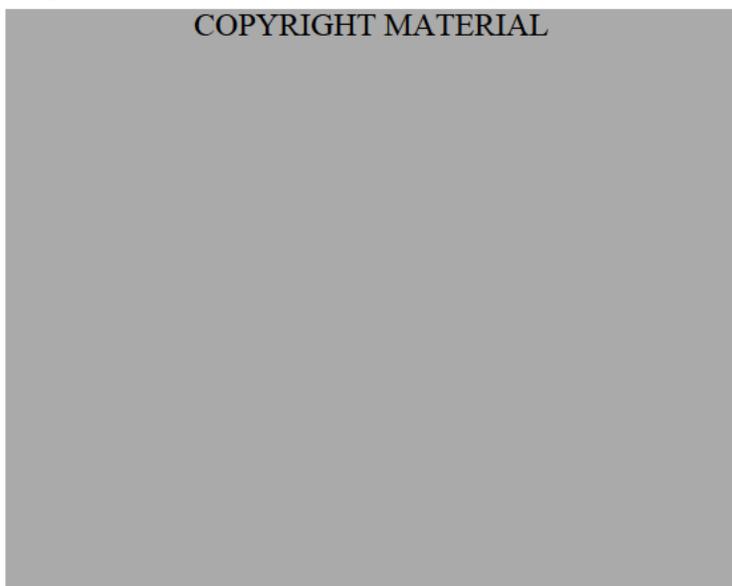
Tissue distribution studies show that neostigmine can be found in the liver, muscle, heart, and kidney. Following a single subcutaneous administration of ¹⁴C-neostigmine (1.68 mcmol/kg) to rats, $t_{1/2}$ was 10 minutes in plasma, 33 minutes in liver and 1.5 hours in muscle. Levels of radioactivity in the liver and kidneys were highest at 15 minutes. Radioactivity in the liver remained higher than in other tissues from 30 minutes until 48 hours postdosing (Somani, 1975). Some muscle may have higher levels of neostigmine than others. Following IV injection of 100 mcg/kg ¹⁴C-neostigmine iodide, plasma radioactivity rapidly disappeared, with 1% remained by 120 minutes post injection. At this time point, ¹⁴C concentration was 2-fold higher than plasma in the diaphragm, but only 1/3-1/2 of plasma level in other muscles including quadriceps, sternomastoid and intercostal muscles (Christensen and Helleberg, 1974).

Neostigmine does not readily cross the blood brain barrier in a significant amount. Cats were given either positively charged neostigmine or uncharged physostigmine (intravenously with initial dose of 1 mg and maintenance dose of 0.25 mg/20 min). Analysis of successive 40-minutes samples of cerebral ventricles effluent for cholinesterase activity demonstrated approximately 20-fold lower amount of neostigmine than its uncharged analog physostigmine in intracisternal fluid from the brains of these cats (Bhattacharya and Feldberg, 1958).

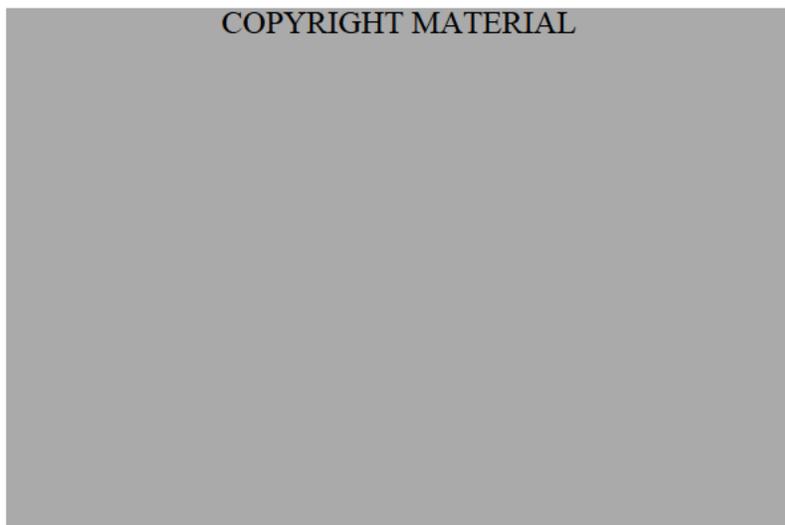
Neostigmine is metabolized in liver and eliminated in urine. Incubation with rat liver microsomes exhibited rapid hydrolysis of neostigmine to 3-hydroxyphenyltrimethylammonium (HPTMA) (Roberts et al., 1968) and the process was greatly enhanced in the presence NADPH₂ (Burdfield et al., 1973). A slow formation of

glucuronide (G-HPTMA) was also reported in isolated perfused rat livers (Somani and Anderson, 1975). Other metabolites detected in urine including HPTMA conjugate, 3-hydroxyphenyldimethyl amine (3-OH PDMA), and other two unidentified metabolites (M4 and M5) following subcutaneous administration for 7 days were reported without quantitative analysis (Somani et al., 1970). The metabolic pathways as proposed by Somani et al. is depicted below:

Figure 2: Metabolism of Neostigmine



Following oral administration of ^{14}C -neostigmine, HPTMA accounts for 90% of radioactivity of urine in rats (Roberts et al., 1966). Urine elimination as unchanged neostigmine was greater following parenteral administration compared to oral administration. Rats given 25 mcg of ^{14}C -neostigmine intramuscularly exhibited about 30% of dose excreted in urine as unchanged neostigmine and this occurred mainly within the first hour postdosing. After the first hour, very little unchanged neostigmine was excreted; after two hours the excretion of free HPTMA declined while its glucuronide conjugate continued to rise so that by 24 hours about equal proportions of the dose of neostigmine had been excreted as free and conjugated HPTMA (see Fig 4 below, (Husain et al., 1969)). Ligation of the renal pedicles of cats resulted in enhanced duration of action of an intravenous bolus dose (5, 10 or 20 mcg/kg) of neostigmine in antagonizing tubocurarine-induced tibial muscle twitch depression (Miller and Roderick, 1977a). Thus, renal excretion of parent drug plays a role in the elimination of neostigmine given parenterally.

Figure 3: Cumulative Urinary Excretion of Neostigmine and Metabolites after IM Injection**Figure 4: Cumulative Urinary Excretion of Neostigmine and Metabolites after a Single Intramuscular Injection**

Neostigmine (+), PTMA (×), PTMA-glucuronide (●) (Husain et al. 1969).

The elimination half-life of neostigmine was 7.3-23.5 minutes in animals including rats (Yamamoto et al., 1995), dogs (Baker et al., 1978) and guinea pigs (Fossati et al., 1990), and 53 minutes in humans (Taylor, 1996).

5.2 Toxicokinetics

There are no toxicokinetic data available.

6 General Toxicology

6.1 Single-Dose Toxicity

There were no GLP acute toxicology studies completed in support of this NDA. None of these studies provide adequate information to define a NOAEL. The following information was summarized from the published literature.

The toxicity of neostigmine is associated with excess nicotinic and muscarinic receptor activation. The toxic effects are marketed by skeletal muscle weakness and fasciculations, pupillary constriction, bloody lacrimation, salivation and increased airway secretions, rise in colonic pressure, colonic spasms, defecation, flatulence, diarrhea and convulsions, dyspnea, bradycardia, and death. Death is usually caused by respiratory failure due to constriction of the bronchiolar musculature and excess bronchiolar secretions. The main toxicities are observed shortly after dosing (e.g., 2-4 minutes after

a single subcutaneous dose of 0.1 mg in rats) and decrease in intensity as neostigmine is cleared out from the circulation.

The Intravenous LD₅₀ values of neostigmine were 0.16 mg/kg in mice and 0.165 mg/kg in rats (Haley and Rhodes, 1950; Randall and Lehmann, 1950).

6.2 Repeat-Dose Toxicity

No repeat-dose toxicology studies were completed to support this NDA. There are several repeat-dose pharmacology studies reported in the published literature. Although these studies are not adequate to define a NOAEL, they do shed some light on the repeated effects of the drug and therefore are briefly summarized here.

Buckley and Heading report that in an anesthetized rat model, rats receiving neostigmine methylsulfate (0.4 mcmol/kg) via in IP injection demonstrated expected muscle fasciculations and salivation. However, these effects were significantly reduced in rats that were given neostigmine bromide in the drinking water for a period of 4 weeks prior to the injection, suggesting that tolerance develops to neostigmine effects after repeated dosing. Tolerance was maintained for up to 3 weeks after cessation of treatment even though cholinesterase levels were back to normal within one week suggesting the tolerance may be do to changes in post-synaptic receptors (Buckley and Heading, 1970).

Buckley and Heading then treated male rats with increasing doses of neostigmine bromide via the drinking water for a total of 35 days. Diarrhea, lacrimation and muscle fasciculations were noted during the first three days, but these effects did not occur thereafter. Body weight gain was also reduced by neostigmine treatment. Due to the muscle tremors, treated rats were not able to walk or rear. These authors also reported that repeated exposure to neostigmine resulted in increased rate of cholinesterase synthesis and decreased sensitivity of cholinergic receptors (Buckley and Heading, 1971).

Ward and colleagues treated female Sprague-Dawley rats with 1 mg/kg neostigmine methylsulfate for 7, 30 or 100 days via SC injection. Neostigmine treatment resulted in reduced miniature endplate potentials (MEPP) via electrophysiological recordings from the diaphragm muscle. Evaluation of the motor endplates via electron microscopy revealed treatment-related ultrastructural changes described as simplified endplates, reduction in the number of postjunctional folds and widening of the synaptic cleft after only 6 days of treatment and were also noted after 100 days treatment. Widening of the synaptic cleft may signal atrophy of the nerve terminal or loss of neurotrophic factors required to maintain the connections. In addition, the authors describe some muscle fibers showing multiple endplates and reduced size axon terminals (Ward et al., 1975).

Gillies and Allen also reported that treatment of rats with neostigmine for 3 to 7 days resulted in the reduction in the quantal output of the nerve end. A hemidiaphragm -

phrenic nerve preparation from rats given 7-15 days of neostigmine at 0.8 mg/kg/day showed that number of quanta released by each nerve impulse was reduced to 52% of normal (at a stimulus rate of 1/sec) and the amplitude of miniature end-plate potential was reduced to 81% of normal (Gillies and Allen, 1977). In this study, the rats were less active and appeared to have muscular weakness as their resistance to the applied pressure was reduced.

Tiedt and colleagues treated rats with 0.1 mg neostigmine subcutaneously twice daily for 3 days or 22-25 days. They noted generalized tremor, muscle fasciculation, ruffling of the fur, excessive salivation, tachypnea, decreased voluntary activity, and apparent weakness 2-4 minutes after a single subcutaneous injection. These symptoms lasted 30-60 minutes post dosing and the severity declined after 4-6 days of dosing at 0.1 mg. Additionally, a resting tremor that persisted for several hours postdosing was frequently observed during the first 1-2 weeks of treatment. By 4-6 weeks of continued dosing, the acute signs observed after dosing were largely absent (Tiedt et al., 1978).

Costa et al. treated mice with neostigmine bromide in drinking water at daily increasing concentrations (20, 100, 200, or 1000 ppm) along with atropine (20 mg/kg) for four days. These mice exhibited limited toxicities (hyperlacrimation, without deaths) when challenged with neostigmine (4.2 mg/kg intraperitoneally). In contrast, treatment of naïve mice with neostigmine caused 30% mortality. This study also showed that muscarinic receptors were decreased in the small intestine of the neostigmine-treated mice (Costa et al., 1981).

Gwilt and Way report that chronic neostigmine treatment causes an adaptive reduction in the number of functional acetylcholine receptors at the endplate without otherwise affecting single channel properties themselves. Rats given 0.86 mg/kg neostigmine methylsulfate (SC) daily for 9-11 days, microelectrode recordings for the extensor digitorum longus muscle showed that neostigmine treatment significantly reduced ACh induced channel opening frequency without affecting single channel open time and conductance (Gwilt and Wray, 1986).

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Bacterial Reverse Mutation Assay

Study no.:	8267125
Study report location:	SDN1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	06/11/2012
GLP compliance:	Yes, a signed GLP compliance statement was included in the study report
QA statement:	Yes
Drug, lot #, and % purity:	Neostigmine, batch 6064983, purity

100.3%

Key Study Findings

Neostigmine methylsulfate was negative in the bacterial reverse mutation assay when tested up to 5000 mcg/plate

Methods

Strains: TA98, TA100, TA1535, TA1537 and WP2*uvrA*
 Concentrations in definitive study: 5.00, 16.0, 50.0, 160, 500, 1600, 3330 and 5000 mcg/plate with and without S9
 Basis of concentration selection: 5000 mcg/plate
 Negative control: Cell culture grade water
 Positive control:

Tester Strain(s)	S9	Positive Control	Dose (µg/plate)	Lot No.
TA98	-	2-nitrofluorene	1.0	S43858
TA100, TA1535	-	sodium azide	2.0	MKBF6507V
TA1537	-	ICR-191	2.0	110M1173V
WP2 <i>uvrA</i>	-	4-nitroquinoline-N-oxide	1.0	A0305157
TA98	+	benzo[a]pyrene	2.5	090M1400V
TA100, TA1535, TA1537	+	2-aminoanthracene	2.5	STBB1901
WP2 <i>uvrA</i>	+	2-aminoanthracene	25.0	STBB1901

Formulation/Vehicle: Deionized water
 Incubation & sampling time: Plate incorporation method, with 52±4 hours incubation at 37 ±2°C were employed.

Study Validity

This study was deemed valid based on the following:

- All doses were evaluated in triplicate plates
- All positive and vehicle control values were in expected ranges
- Adequate doses were tested

Results

No dose-related cytotoxicity was observed with any of the strains tested in the presence or absence of S9. There were no increases in the mean number of revertants/plate observed with any of the tester strains in the presence or absence of S9 mix.

7.2 *In Vitro* Assays in Mammalian Cells

No adequate studies were identified. As noted in the preIND/preNDA meetings, if there are no adequate data to inform labeling, we recommend that these studies be required to be completed as post-marketing requirements.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

No adequate studies were identified. As noted in the preIND/preNDA meetings, if there are no adequate data to inform labeling, we recommend that these studies be required to be completed as post-marketing requirements.

7.4 Other Genetic Toxicity Studies

None

8 Carcinogenicity

Not available and not applicable to this NDA due to the indicated acute use.

9 Reproductive and Developmental Toxicology

There are no adequate reproductive and developmental toxicology studies reported in literature. As noted in the preIND/preNDA meetings, if there are no adequate data to inform labeling, we recommend that these studies be required to be completed as post-marketing requirements.

10 Special Toxicology Studies

None.

11 Integrated Summary and Safety Evaluation

Introduction

Currently there are no FDA-approved injectable neostigmine products in the market. However, the proposed product Neostigmine Methylsulfate Injection has been marketed by other companies as an unapproved product for decades. Based on the long history of clinical use, nonclinical studies for neostigmine were not required for approval of this NDA. The following nonclinical information was provided based on published literature.

Pharmacology

Neostigmine is a cholinesterase inhibitor indicated to reverse the neuromuscular blocking effects of non-depolarizing neuromuscular blocking agents. By reducing the hydrolysis of acetylcholine, neostigmine increases the levels of acetylcholine in the synaptic cleft which competes with the neuromuscular junction blocking agents and therefore facilitates neuromuscular transmission.

ADME

The proposed route of administration is intravenous. Systemically, neostigmine is distributed widely and most significantly in the plasma, muscles, liver, and kidney, but

not brain as neostigmine does not readily cross the blood-brain-barrier. Following an intravenous dose, neostigmine is rapidly cleared from plasma. It is metabolized in the liver and excreted in the urine unchanged, as the major metabolite 3-hydroxyphenyltrimethylammonium (HPTMA), and as the glucuronide conjugate of HPTMA. The amount of parent compound excreted in urine accounts for 30% of dose in rats given 25 mcg neostigmine intramuscularly. Kidney elimination of unchanged neostigmine suggests the need for dose adjustment in patients with severe renal function impairments. The elimination $t_{1/2}$ is in a range of 7.3-23.5 minutes in the rat, dog, and guinea pig and 53 minutes in the human.

Toxicology

The toxic effects of neostigmine in animals are attributable to excessive nicotinic and muscarinic receptor activation. The toxic effects include skeletal muscle weakness and fasciculations, pupillary constriction, lacrimation, salivation, increased airway secretions, increased colonic pressure, colonic spasms, defecation, flatulence, diarrhea, convulsions, dyspnea and bradycardia, and death. Death is usually caused by respiratory failure due to constriction of the bronchiolar musculature and excess bronchiolar secretions. The main toxicities are observed shortly after dosing (e.g., 2-4 minutes after a single subcutaneous dose of 0.1 mg in rats) and decrease in intensity as neostigmine is cleared from the circulation. Toxicities after repeated doses were similar to the acute toxicities but tolerance develops after a few doses.

The intravenous LD₅₀ in mice is 0.16 mg/kg in mice and 0.165 mg/kg in rats (Randall 1950; Haley 1950).

Neostigmine is negative in the in vitro bacterial reverse mutation assay (Ames assay). Data with respect to in vitro genetic toxicity in a mammalian cell line or in vivo genetic toxicity are not available and will be required to be completed post-marketing.

There are no reproductive and developmental toxicology data available for product labeling. The drug will be considered a Pregnancy Category C drug at this time. Based on the long history of human use, the Division has informed the Sponsor that such studies were not required for approval but forewarned that they would likely be required as Post-Marketing Requirements (meeting minutes for pre (b) (4) meeting dated 12/22/2009).

Additionally, there are no carcinogenicity data available. However, based on the proposed indication of acute use, carcinogenicity studies are not necessary.

Formulation and quality control

Excipients: The proposed product formulation consists of neostigmine methylsulfate, phenol, sodium acetate, water, (b) (4) and sodium hydroxide. Most of the inactive ingredients, except phenol are also found endogenously and there are of no safety concerns. The proposed use of 0.45% phenol in this drug product formulation would produce up to 45 mg bolus exposure via the maximum human dose of neostigmine (5

mg). The concentration of 0.45% and total daily dose of 45 mg are within the range of approved uses of phenol for intravenous administrations. It is noted that the proposed bolus dose of 45 mg phenol would produce 2-4 fold higher C_{max} compared to the approved uses of 80 mg given as 4-8 doses in a day. However, considering the extensive clinical experience with similar marketed unapproved drug products, this magnitude of higher C_{max} is not likely a significant clinical concern. See the medical officer review for further details.

Leachables/extractables: The Sponsor has provided adequate extractable data for the container closure and has agreed to set a specification of NMT (b) (4) for the only identified potential leachable in the drug product, (b) (4). At this specification, a person could be exposed to NMT (b) (4) of this chemical. This is below the PQRI proposed qualification threshold for a nongenotoxic leachable in inhalation products and therefore deemed acceptable.

Drug substance impurities: There are three specified impurities in the drug substance including (b) (4). All of these contain a structural alert moiety, which is also found in neostigmine. The specification for (b) (4) of NMT (b) (4) would result in exposure to (b) (4). This specification is acceptable as this is below the threshold of toxicological concern for a genotoxic compound. The specification of NMT (b) (4) for (b) (4) is acceptable (b) (4).

Drug product impurity/degradant: The proposed specifications list (b) (4) as a single specified drug product degradant at NMT (b) (4). This specification is acceptable as it is below the qualification thresholds defined in the Guidance ICH Q3B(R2).

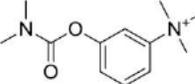
12 Appendix/Attachments

To: Huiqing Hao
 cc: Dan Mellon
 From: CDER/OPS/OTR/DDSR: The CDER Computational Toxicology Group

Date: April 3, 2012

Six compounds were evaluated by CDER/OPS/OTR/DDSR for bacterial mutagenicity using *Salmonella* mutagenicity (quantitative) structure-activity relationship [(Q)SAR] models. Three software programs were used: Derek Nexus 2.0.2 (DX), Leadscope Model Applier 1.3.3-3 (LMA), and MC4PC 2.4.0.7 (MC). The results of the predictions from the software programs were weighted equally and the analysis was optimized for sensitivity (minimizing false negatives) to reach the overall conclusion.

Salmonella Mutagenicity Predictions for Neostigmine¹

	Software	Salmonella Mutagenicity
	Derek Nexus	NSA
	Leadscope	-
	MC4PC	+
	Overall Prediction	+

Neostigmine is predicted to be positive for *Salmonella* mutagenicity.

Salmonella Mutagenicity Predictions

(b) (4)

(b) (4)	Software	Salmonella Mutagenicity
	Derek Nexus	A
	Leadscope	A
	MC4PC	A
	Overall Prediction	A

(b) (4)

¹ + = positive; - = negative; Eqv = equivocal; NSA = no structural alerts are identified by DX (Derek Nexus cannot differentiate between a negative call and the inability to make a call because of no coverage); NC = test chemical features are not adequately represented in the model training data set, leading to a no call; A = test chemical is experimentally active; = test chemical is experimentally inactive

Salmonella Mutagenicity Predictions (b) (4)

(b) (4)

Software	Salmonella Mutagenicity
Derek Nexus	NSA
Leadscope	-
MC4PC	+
Overall Prediction	+

(b) (4) is predicted to be positive for *Salmonella* mutagenicity.

Salmonella Mutagenicity Predictions (b) (4)

(b) (4)

Software	Salmonella Mutagenicity
Derek Nexus	NSA
Leadscope	NC
MC4PC	+
Overall Prediction	+

Reference List

- Aeschlimann JA and Reinert M (1931) The Pharmacological Action of Some Analogues of Physostigmine. *J Pharmacol Exp Ther* **43**:413-444.
- Baker PR, Calvey T N, Chan K, Macnee C M and Taylor K (1978) Plasma Clearance of Neostigmine and Pyridostigmine in the Dog. *Br J Pharmacol* **63**:509-512.
- Bhattacharya BK and Feldberg W (1958) Perfusion of Cerebral Ventricles: Assay of Pharmacologically Active Substances in the Effluent From the Cisterna and the Aqueduct. *Br J Pharmacol Chemother* **13**:163-174.
- Buckley GA and Heading C E (1970) Tolerance to Neostigmine. *Br J Pharmacol* **40**:590P-591P.
- Buckley GA and Heading C E (1971) The Effects of Prolonged Neostigmine Treatment. *J Physiol* **219**:6P-7P.
- Bukowska B and Kowalska S (2004) Phenol and Catechol Induce Prehemolytic and Hemolytic Changes in Human Erythrocytes. *Toxicol Lett* **152**:73-84.
- Burdfield PA, Calvey T N and Roberts J B (1973) In Vitro Metabolism of Neostigmine and Pyridostigmine. *J Pharm Pharmacol* **25**:428-429.
- Christensen CB and Helleberg L (1974) Uptake of ¹⁴C-Neostigmine in Rat Striated Muscles. *Acta Pharmacol Toxicol (Copenh)* **35**:58-64.
- Coan ML, Baggs R B and Bosmann H B (1982) Demonstration of Direct Toxicity of Phenol on Kidney. *Res Commun Chem Pathol Pharmacol* **36**:229-239.
- Costa LG, Schwab B W, Hand H and Murphy S D (1981) Decreased Muscarinic Binding Sites in Small Intestine From Mice Treated With Neostigmine. *Life Sci* **29**:1675-1682.
- Deichmann WB and Witherup S (2013) Phenol Studies VI: the Acute and Comparative Toxicity of Phenol and O-, M-, and P-Cresols for Experimental Animals. *J Pharmacol Exp Ther* **80**:233-240.
- Dunlap J and Brown J H (1983) Heterogeneity of Binding Sites on Cardiac Muscarinic Receptors Induced by the Neuromuscular Blocking Agents Gallamine and Pancuronium. *Mol Pharmacol* **24**:15-22.
- Ecobichon DJ (2001) Toxic effects of pesticides, in *Goodman & Gilman's The Pharmacological Basis of Therapeutics* (Hardman JG, Limbird LE and Gilman AG eds) pp 763-810, McGraw-Hill, New York.
- Eikermann M, Fassbender P, Malhotra A, Takahashi M, Kubo S, Jordan A S, Gautam S, White D P and Chamberlin N L (2007) Unwarranted Administration of

Acetylcholinesterase Inhibitors Can Impair Genioglossus and Diaphragm Muscle Function. *Anesthesiology* **107**:621-629.

Eikermann M, Zaremba S, Malhotra A, Jordan A S, Rosow C and Chamberlin N L (2008) Neostigmine but Not Sugammadex Impairs Upper Airway Dilator Muscle Activity and Breathing. *Br J Anaesth* **101**:344-349.

Endou M, Tanito Y and Okumura F (1997) A Comparison Between Chronotropic Effects of Neostigmine and Edrophonium in Isolated Guinea Pig Right Atrium. *J Pharmacol Exp Ther* **282**:1480-1486.

Fossati A, Vimercati M G, Bandi G L and Formenti A (1990) Pharmacokinetic Study of Neostigmine After Intranasal and Intravenous Administration in the Guinea Pig. *Drugs Exp Clin Res* **16**:575-579.

Gillies JD and Allen J (1977) Effects of Neostigmine and Pyridostigmine at the Neuromuscular Junction. *Clin Exp Neurol* **14**:271-279.

Gwilt M and Wray D (1986) The Effect of Chronic Neostigmine Treatment on Channel Properties at the Rat Skeletal Neuromuscular Junction. *Br J Pharmacol* **88**:25-31.

Haley TJ and Rhodes B M (1950) A Note on the Acute Toxicity of Neostigmine Methyl Bromide in the Rat. *J Am Pharm Assoc Am Pharm Assoc* **39**:701.

Harada T, Fushimi K, Kato A, Ito Y, Nishijima S, Sugaya K and Yamada S (2010) Demonstration of Muscarinic and Nicotinic Receptor Binding Activities of Distigmine to Treat Detrusor Underactivity. *Biol Pharm Bull* **33**:653-658.

Harrold JA, Elliott J R and Elliott A A (1996) Inhibition of Oocyte-Expressed Rat Brain Ila Na⁺ Channels by Phenol, P-Cresol and N-Pentanol. *J Physiol* **497**.P:127P.

Harvey SC (1975) Antiseptics and disinfectants; fungicides; ectoparasitocides, in *The Pharmacological Basis of Therapeutics* (Goodman LS and GILMAN A eds) pp 987-1017, Macmillan Publishing Co., New York, NY.

Hennis PJ, Cronnelly R, Sharma M, Fisher D M and Miller R D (1984) Metabolites of Neostigmine and Pyridostigmine Do Not Contribute to Antagonism of Neuromuscular Blockade in the Dog. *Anesthesiology* **61**:534-539.

Husain MA, Roberts J B, Thomas B H and Wilson A (1969) Metabolism and Excretion of 3-Hydroxyphenyltrimethylammonium and Neostigmine. *Br J Pharmacol* **35**:344-350.

(b) (4)

(b) (4)

(b) (4)

Jones RS (1990) Reversal of Atracurium Neuromuscular Block With Neostigmine in the Dog. *Res Vet Sci* **48**:96-98.

Kuryshv YA, Brown A M, Wang L, Benedict C R and Rampe D (2000) Interactions of the 5-Hydroxytryptamine 3 Antagonist Class of Antiemetic Drugs With Human Cardiac Ion Channels. *J Pharmacol Exp Ther* **295**:614-620.

Lockhart B, Closier M, Howard K, Steward C and Lestage P (2001) Differential Inhibition of [3H]-Oxotremorine-M and [3H]-Quinuclidinyl Benzilate Binding to Muscarinic Receptors in Rat Brain Membranes With Acetylcholinesterase Inhibitors. *Naunyn-Schmiedeberg's Arch Pharmacol* **363**:429-438.

Miller RD and Roderick L (1977a) Ligated Renal Pedicles and Duration of Action of Neostigmine and Pyridostigmine. *Br J Pharmacol* **60**:555-558.

Miller RD and Roderick L L (1977b) Pancuronium-Induced Neuromuscular Blockade, and Its Antagonism by Neostigmine, at 29, 37, and 41 c. *Anesthesiology* **46**:333-335.

Oehme, F. W. and Davis, L. E. The comparative toxicity and biotransformation of phenol. *Toxicol Appl Pharmacol* 17, 283. 1970.
Ref Type: Abstract

Pilcher JD and Stollman T (1915) Studies on the Vasomotor Centre. X. The Action of Phenol. *J Pharmacol Exp Ther* **6**:377-380.

(b) (4)

Randall LO and Lehmann G (1950) Pharmacological Properties of Some Neostigmine Analogs. *J Pharmacol Exp Ther* **99**:16-32.

Roberts JB, Thomas B H and Wilson A (1966) Excretion and Metabolism of Oral 14-C Neostigmine in the Rat. *Biochem Pharmacol* **15**:71-75.

Roberts JB, Thomas B H and Wilson A (1968) Metabolism of Neostigmine in Vitro. *Biochem Pharmacol* **17**:9-12.

Sadoshima J, Tokutomi N and Akaike N (1988) Effects of Neostigmine and Physostigmine on the Acetylcholine Receptor-Ionophore Complex in Frog Isolated Sympathetic Neurones. *Br J Pharmacol* **94**:620-624.

Seifert SA and Eldefrawi M E (1974) Affinity of Myasthenia Drugs to Acetylcholinesterase and Acetylcholine Receptor. *Biochem Med* **10**:258-265.

Sherby SM, Eldefrawi A T, Albuquerque E X and Eldefrawi M E (1985) Comparison of the Actions of Carbamate Anticholinesterases on the Nicotinic Acetylcholine Receptor. *Mol Pharmacol* **27**:343-348.

Sikkema J, de Bont J A and Poolman B (1995) Mechanisms of Membrane Toxicity of Hydrocarbons. *Microbiol Rev* **59**:201-222.

Somani SM (1975) Distribution of Neostigmine and Its Metabolites in Rat Tissues After Acute and Chronic Administration. *Eur J Pharmacol* **30**:336-343.

Somani SM and Anderson J H (1975) Sequestration of Neostigmine and Metabolites by Perfused Rat Liver. *Drug Metab Dispos* **3**:275-282.

Somani SM, Roberts J B, Thomas B H and Wilson A (1970) Isolation and Characterisation of Metabolites of Neostigmine From Rat Urine. *Eur J Pharmacol* **12**:114-119.

Taylor P (1996) Anticholinergic agents, in *Goodman and Gilman's The Pharmacological Basis of Therapeutics* (Hardman JG, Limbird LE, Molinoff PB, Ruddon RW and Gilman AG eds) pp 161-176.

Taylor P (2001) Anticholinesterase agents, in *Goodman & Gilman's The Pharmacological Basis of Therapeutics* (Hardman JG, Limbird LE and Gilman AG eds) pp 175-191, McGraw-Hill, New York.

Tiedt TN, Albuquerque E X, Hudson C S and Rash J E (1978) Neostigmine-Induced Alterations at the Mammalian Neuromuscular Junction. I. Muscle Contraction and Electrophysiology. *J Pharmacol Exp Ther* **205**:326-339.

(b) (4)

Ward MD, Forbes M S and Johns T R (1975) Neostigmine Methylsulfate. Does It Have a Chronic Effect As Well As a Transient One? *Arch Neurol* **32**:808-813.

(b) (4)

Wein R (1939) The Toxicity of Parachlorometacresol and of Phenylmercuric Nitrate. *Quarterly J Pharmacy Pharmacol* **12**:212-229.

Weinstock M, Roll D and Zilberman Y (1981) An Analysis of the Respiratory Stimulant Effect of Physostigmine and Neostigmine in the Conscious Rabbit. *Clin Exp Pharmacol Physiol* **8**:151-158.

Wood KM (1978) The Use of Phenol As a Neurolytic Agent: a Review. *Pain* **5**:205-229.

Yamamoto K, Sawada Y and Iga T (1995) Comparative Pharmacokinetics of Four Cholinesterase Inhibitors in Rats. *Biol Pharm Bull* **18**:1292-1295.

Zamponi GW and French R J (1993) Dissecting Lidocaine Action: Diethylamide and Phenol Mimic Separate Modes of Lidocaine Block of Sodium Channels From Heart and Skeletal Muscle. *Biophys J* **65**:2335-2347.

Zeiger E, Anderson B, Haworth S, Lawlor T and Mortelmans K (1988) Salmonella Mutagenicity Tests: IV. Results From the Testing of 300 Chemicals. *Environ Mol Mutagen* **11 Suppl 12**:1-157.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

HUIQING HAO
04/03/2013

RICHARD D MELLON
04/03/2013

I concur with Dr. Hao's recommendation that, from the nonclinical pharmacology toxicology perspective, NDA 204078 may be approved with the recommended PMRs and labeling.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA

NDA Number: 204-078

**Applicant: Eclat
Pharmaceuticals**

Stamp Date: July 31, 2012

Drug Name: Neostigmine

NDA/BLA Type: 505(b)2

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	√		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	√		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	√		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	√		As per the preNDA meeting minutes, given the marketed unapproved nature of this drug substance, some studies may be required as post-marketing requirements (PMRs), pending literature review.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).		√	(b) (4) All toxicology information in the NDA is based on published literature. Based on the historical use of neostigmine, the Division decided not to require nonclinical studies for approval of this NDA. Additional studies may be required as PMRs, pending literature review
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?		√	See above. Not applicable

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA**

	Content Parameter	Yes	No	Comment
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?		√	See above. Not applicable.
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	√		Leachable study for the container closure system was provided. The adequacy of this study will be a review issue.
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?		√	No nonclinical data is listed in the proposed labeling. Carcinogenicity studies are not needed for the proposed indication of single/acute use. Reproductive toxicology information is limited and will be stated as lacking.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	√		Impurity justification appeared to be adequate
11	Has the applicant addressed any abuse potential issues in the submission?		√	Not applicable
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?		√	Not applicable

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

Based on preliminary review, there are no comments for the 74-day letter.

Huiqing Hao, Ph.D.	Sept. 13, 2012
Reviewing Pharmacologist	Date
Dan Mellon, Ph.D.	Sept. 13, 2012
Team Leader/Supervisor	Date

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

HUIQING HAO
09/14/2012

RICHARD D MELLON
09/14/2012